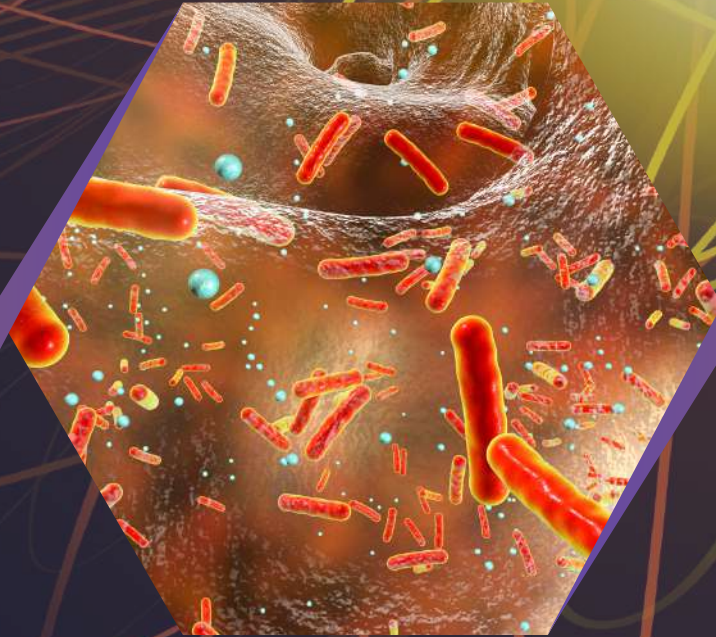


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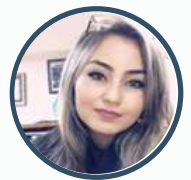
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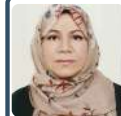
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The background of the entire page is a microscopic view of numerous green, rod-shaped bacteria. The bacteria are oriented in various directions, some appearing in sharp focus while others are blurred in the background, creating a sense of depth. The lighting is soft, highlighting the texture of the bacterial surfaces.

**ISOLATION AND IDENTIFICATION OF PARASITIC NEMATODES (CUCULLNUS
LONGICOLLIS) THAT INFECTS (MULLUS SURMULETUS) FISH IN THE MARINE
WATERS OF SIRTE-LIBYA**

AHMED. A . ALMASHAY

ISOLATION AND IDENTIFICATION OF PARASITIC NEMATODES (CUCULLANUS LONGICOLLIS) THAT INFECTS (MULLUS SURMULETUS) FISH IN THE MARINE WATERS OF SIRTE-LIBYA

Ahmed. A. ALMASHAY¹

Abstract:

Due to the important geographical location that characterized the city of Sirte-Libya, and given the importance of fish wealth in this region and the negative impact that may occur as a result of infecting fish with parasites, and the lack of research studies on this subject in this region, so this research study was conducted to contribute to the to clarify this issue.

A number of (70) fish specimens were collected from Sirte - Libyan coast facing Mediterranean Sea from January 2021 to March 2021 , and the measurements were made on them all marine fish examined externally in the laboratory, after that they were dissected by the scientific methods used and examined internally, as well as the digestive system was examined and conducted on parasites extracted by all the processes related to study. The discovered parasitic worms were carefully extracted, laboratory operations were conducted on them, and they were examined and photographed under a normal light microscope as well as an electron microscope.

The results showed that (2) of these fish were infected with nematode worms of the species *Cucullanus longicollis* , which were extracted from the large intestines of these fish. This parasite was recorded for the first time in the marine waters of the city of Sirte-Libya. The general shape of the parasite has been described, supported by pictures.

This research aims to identify the parasitic nematodes that infect *Mullus surmuletus* fish and to know the extent of environmental pollution in the study area and the rate of fish infestation, as well as to clarify and studying the general form of these parasitic worms under study.

Key words: *Mullus surmuletus*, *Cucullanus longicollis*, Waters of Sirte.

Introduction:

Fish meat of high nutritional value because it contains a high percentage of amino acids and minerals essential for human nutrition as well as it contains vitamins and few saturated fats (Salman , 2000).

Some parasites cause more mechanical damage to fish by migrating through tissues and may also cause an increase in growth in the connective tissues, which hinders growth and development processes. A number of fish parasites or their larval stages are transmitted to humans by eating raw or poorly cooked fish. Such as tapeworm, *Diphyllobothrium latum*, human liver worm *Clonorchis sinensis*, and nematodes worm *Gnathostoma spinigerum*. (Hoffmann ,1999).

Paraguassu *et al.*, (2002) were interested in studying parasitic diseases that affect the productivity of fish wealth as well as parasitic diseases that are transmitted to humans and animals. fish are considered to be intermediate hosts for them or as part of their life cycle.

Fish parasites are important biological indicators indicating the separation and location of fish for example, in the case of fish migration in the seasons of the year from one place to another, every place visited by fish acquires a type of parasite that indicates the environment and thus parasites have become evidence of annual migrations and knowledge of habits, behavior, reproduction and life cycles for fish (Marcelo and Cormona ,2001) .

Fish are infected with parasites as a result of eating intermediate hosts crustaceans the feeding of these fish increases in seasons in which the numbers of these intermediate hosts increase (Karawan *et al.*, 2012).

This research aims to identify the parasitic nematodes that infect *Mullus surmuletus* fish and to know the extent of environmental pollution in the study area and the rate of fish infestation, as well as to clarify and studying the general form of these parasitic worms under study.

Materials and Methods:

Fish samples:70 fresh *Mullus surmuletus* were collected from the shore of the city of Sirte – Libya in the period from January to March 2021.

Various measurements were taken for all samples from measuring length and weight in severe infestations , nematodes are prominent outside , after that ,the dissection of the fish begins using special dissecting tools and the cutting starts from the outlet opening towards the head of the fish , then we ascend by cutting to the top along the gills ,then we open the fish ,the fishes digestive system was removed and divided ,and each part was cut inside a Petri dish containing a saline solution at a concentration of 5% after that, the body cavity was examined with the naked eye and with a magnifying glass.

The contents of each pate were examined with a magnifying glass and examined under a light microscope ,after that ,the detected nematodes were extracted using an affine brush and placed in lactophenol for minutes after which it was placed on a glass slide and covered with the slide cover and examined under a light microscope with different enlargement powers to complete the studies on it.

The different images of the parts of the nematode (the anterior end, the posterior end, and the middle region) were taken for both males and females using a normal light microscope and electron microscope.

Phylum: Nematoda

Class: Phasmida

Order: Ascaridida

Family: Cucullanidae Cobbold, 1864.

Genus: *Cucullanus* Muller, 1777.

Species: *Cucullanus longicollis* Stossich, 1899.

Results

These worms were collected from the large intestines of the *Mullus surmuletus* fish, and the infection rate with this parasite is low.

The results showed that two fish were infected out of a total of (70) fish that were dissected.

Body slender with smooth cuticle , oral opening dorsoventrally elongate which contains within it a number of 25 to 28 triangular teeth which is located inside the bucal cavity, and the body is surrounded by prominent transverse striation Figure (1,7,8,10).

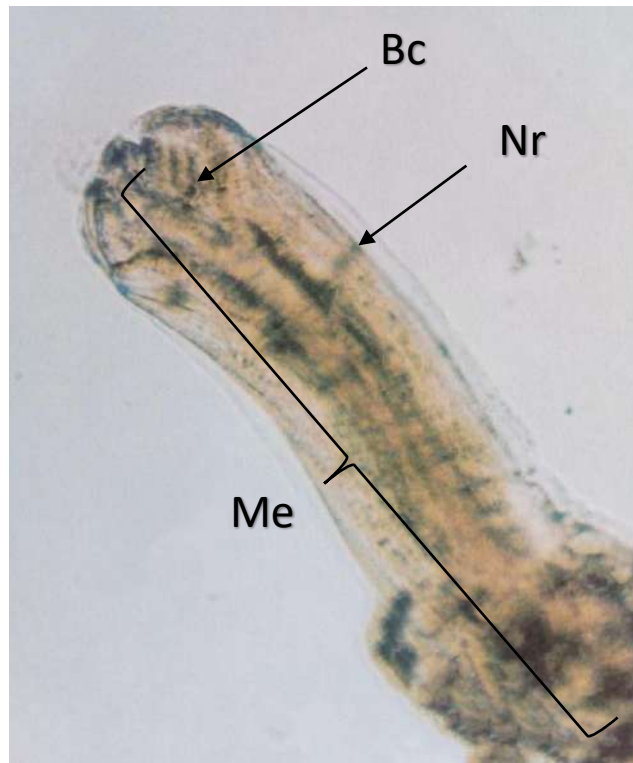
The muscular esophagus is two parts, anterior part in front of the worm and forms a pseudocapsule and this part narrows at its end after the nerve ring and posterior part , excretory pore situated posterior to esophago-intestinal junction, tail conical with two phasmids Figure (1).

The male:

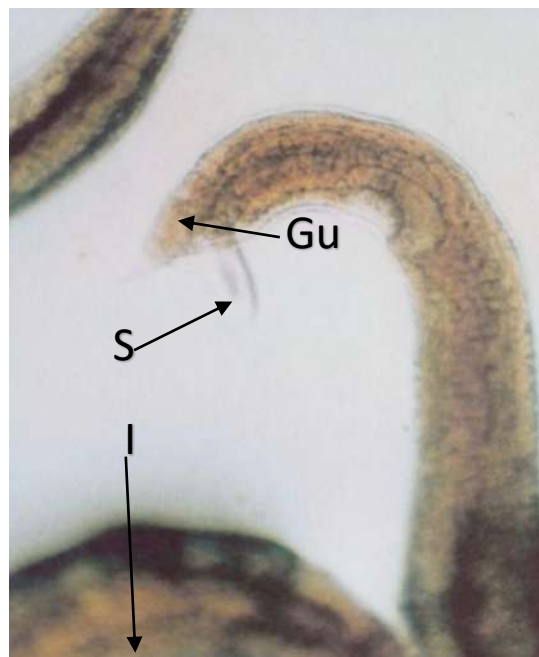
Shorter than the female, the neural ring is the excretory pore is located at the anterior end of the body. The posterior end is curved toward the ventral side and contains two equal spicules that stick out from gubernaculum at the back of the body and it touches the female during mating , and also contains a number of caudal papillae 5 pairs preanal ,1pair adanal and 5pairs postanal Figure (1,2,8).

The female:

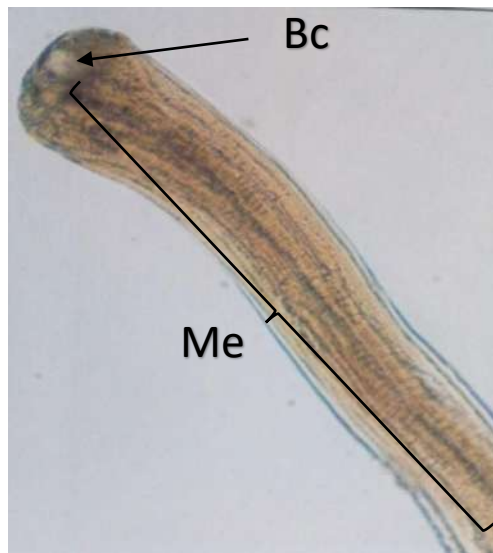
Longer than the male and the neural ring is located at the front end of the body. the vulva is located in the middle of the body and leads to a muscular vagina. The uterus is long and extends from the back to the middle of the body and is filled with eggs. The tail is pointed and the anus is located at the back end of the body Figure (3,4,5, 6,9,10).



(Fig. 1). Anterior region of male *Cucullanus longicollis*.
Bc: Buccal cavity. Nr: Nerve ring. Me: Muscular esophagus.



(Fig. 2). Posterior region of male *Cucullanus longicollis*.
Gu: Gubernaculum S: Spine. I: Intestine.



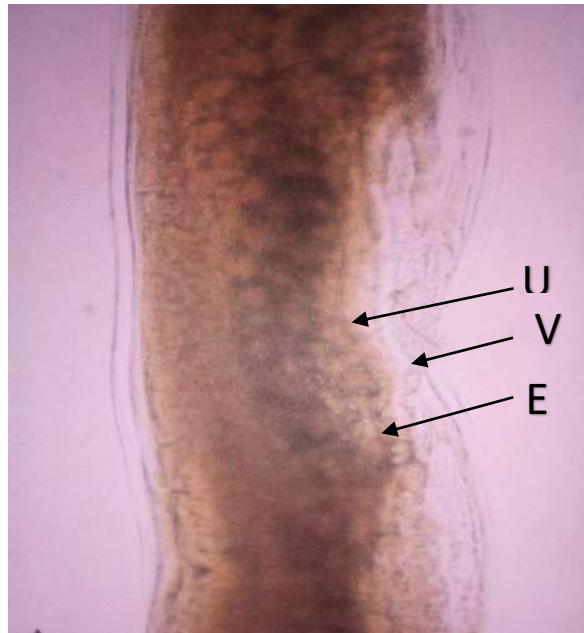
(Fig. 3). Anterior region of female *Cucullanus longicollis*.

Bc: Buccal cavity. Me: Muscular esophagus.



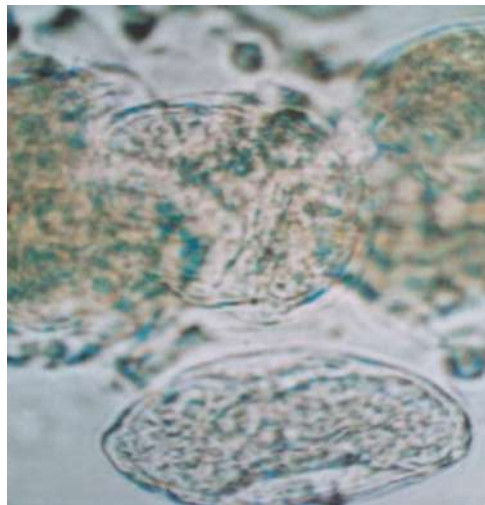
(Fig. 4). Posterior region of female *Cucullanus longicollis*.

S: Spine.



(Fig. 5). Median region of female *Cucullanus longicollis*.

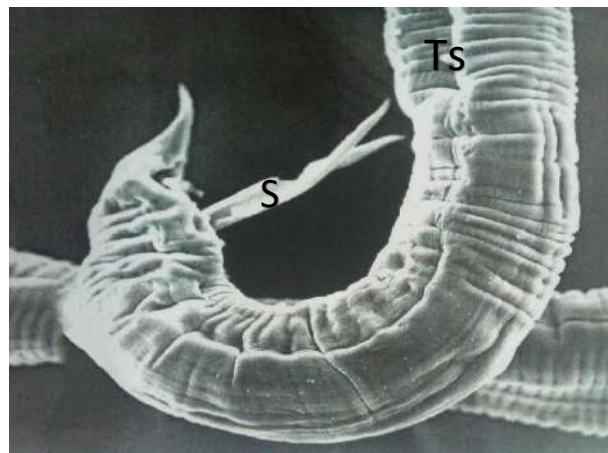
U: Uterus. V: Vulva. E: Egg.



(Fig. 6). Eggs of *Cucullanus longicollis*.



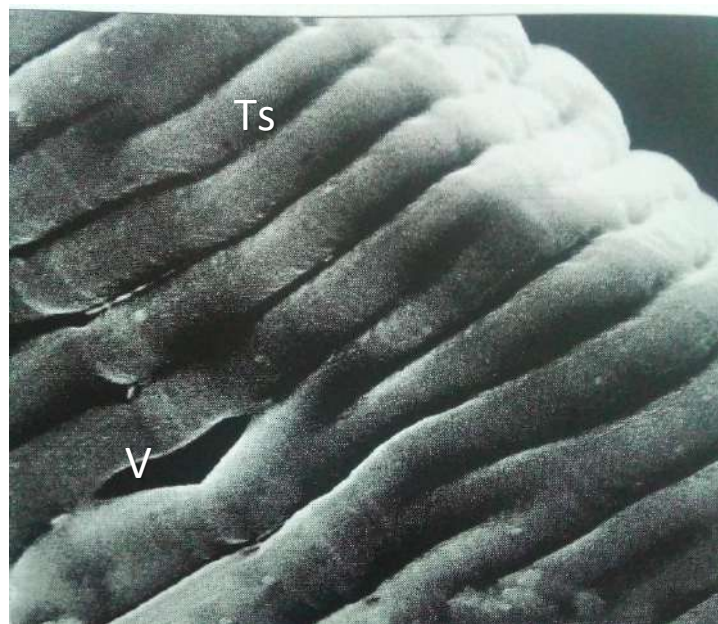
(Fig. 7). Scanning electron microscope of anterior region of *Cucullanus longicollis*
Bc: Bucal cavity. T: Tooth.



(Fig. 8). Scanning electron microscope of posterior region of male *Cucullanus longicollis*
Ts: Transverse striation . S: Spine .



(Fig. 9). Scanning electron microscope of posterior region of female *Cucullanus longicollis*
S: Spine.



(Fig. 10). Scanning electron microscope of median region of female *Cucullanus longicollis*.
V: Vulva. Ts: Transverse striation .

Discussion

Janiszewski has investigated the nematode parasites of 18 species of fish from the Adriatic Sea near Split, Yugoslavia. He describes and draws for the first time the male of *Cucullanus longicollis* from the intestine of *Mullus barbatus*. It is smaller than the female, the gubernaculum is short and the distance between the sucker and the anus is from 0-4-0-43mm. (Janiszewski, 2022).

Cucullanus carettae is found in the upper intestine of loggerhead turtles in the Mediterranean sea and Adriatic sea, causing infections in the turtles digestive system. (Renzo *etal.*, 2021).

Recent examinations of cucullanid nematodes (Cucullanidae) from marine fishes off New Caledonia collected in the years 2004-2005 revealed the presence of the five new species of *cucullanus*. (Moravic and Justine, 2020).

Anisakids nematodes are common parasites of animals including human causing economic losses and different parasitic diseases, the nematode *Dujardinascaris* spp. (Anisakidae) was described from the body cavity and small intestine of *Mullus surmuletus* in the Alexandria, Mediterranean Sea. R. (Hassan, 2019).

Two new species of parasitic nematodes are described from marine perciform fishes off New Caledonia: *Cucullanus epinepheli* sp. n. (Cucullanidae) from the intestine of the brownspotted grouper *Epinephelus chlorostigma* *Cucullanus epinepheli* sp. n. differs from its congeners mainly in possessing a unique structure of the anterior, elevated cloacal lip with a large posterior outgrowth covering the cloacal aperture and in the presence of cervical alae and two small preanal papillae on the median dome-shaped precloacal elevation. (Moravic and Justine, 2017).

In a study on parasitic worms that infect *Mullus surmuletus* fish, which were collected from northwest (Algeria), the results showed that these fish were infected with 14 species of parasites, including *Cucullanus longicollis* with an infection rate of 8%. (Hassani *etal.*, 2015).

Three new species of the genus *Cucullanus* have been extracted from marine fish in the shores of Brazil, which are the *Cucullanus gastrophysi* n.sp. parasite in *Lophius gastrophysus* and *Cucullanus protrudens* n. sp. from *Pagrus pagrus* and *Cucullanus pseudopercis* n. sp. From *Pseudopercis semifasciata* (Cuvier). (Vieira *etal.*, 2015)

Based on light and scanning electron microscopical observations, a new species of *Cucullanus*, is described from the intestine of marine edible fishes *Otolithus ruber*, were collected (February 2006 to July 2007) from fresh landing of Karachi coast, Pakistan. (Akhtar and Mujib, 2012).

Cucullanus dodsworthi was originally described from the checkered puffer fish, *Sphoeroides testudineus* from Brazilian waters. It can be concluded that *C. dodsworthi* is the only species of marine *Cucullanus* from the Americas that possesses lateral body alae. (Madrid H and Macedo M, 2011).

A new species of nematode, *Cucullanus costaricensis* n.sp. is described from the Red Sea catfish *Bagre pinnimaculatus* from Rio Tempisque, Costa Rica. (Caballero *etal.*, 2009).

14 species of parasites were extracted from *Mullus surmuletus* fish, including nematode worms of species *Capillaria gracilis* and *Anisakis simples*.(Klimpel *etal.*, 2008).

Six species of freshwater fish were examined for parasites, as they were collected from the waters of different areas of the outskirts of Kirkuk. Among the results obtained, it was found that *Cyprinus carpio* fish were infected with one species of nematode worms (*Cucullanus cyprinid*). (Ibrahim *etal.*, 2008).

200 Fish were collected, belonging to 7 species collected from the Khazar River passing through Nineveh Governorate (about 37 km east of the city of Mosul) during the period from October 2006 to April 2007. The fish were examined to investigate the nematodes that infect them, and the study showed that 12 fish were infected with nematodes out of the total examined fish, where the percentage of nematode infection was 6%. In this study, types of nematodes that infect freshwater fish were recorded, including the genera *Raphidiscaris sp.*, *Anisakis sp.* and *Eustrongylides sp.*, in addition to the species *Cuculleanelets minutes* (Al-tae and Zangana2011).

The main distinguishing characters of *Cucullanus heliomartinsi* are markedly short spicules that correspond to 2.5% of the total body length, deirids and excretory pore situated posterior to the oesophago-intestinal junction, and oesophagus divided in two distinct portions. (Moreira *etal.*, 2000).

Sharples and Evans (1995) recorded *Cucllanus cnidoglanis* from *Pagrus auratus* fishes in New Zealand and *C.sheardi* in *P.auratus* fishes from Australia and *C.pluronectid* in *P.auratus* fishes from Japan.

The genus *Cucullanus* includes several species that bear many similarities. The original descriptions of these are often poor, making comparisons between them difficult..(Moravec *etal.*, 1993).

References

- Akhtar Y & Mujib B. (2012). A new species of *cucullanus* (Nematoda: Cucullanidae) from marine edible fish *Otolithus ruber* (sciaenidae) based on light and Scanning Electron Microscopy. *Journal of Biological Research and Applied Sciences*, 3 (1) , 9-16. scholar.google.com
- Al-taee A and Zangana M. (2011). Detection of parasitic nematodes in some fresh water fishes in khazir river in Ninevah governorate. *Iraqi Journal of Veterinary Sciences* , 25(1) , 29-38. <https://www.velmedmosul.org/ijv>
- Caballero L., Prieto J., & Sarabia L. (2009). *Cucullanus costaricensis* n.sp.(Nematoda:Cucullanidae), a parasite of *Bagre pinnimaculatus* (Siluriformes:Ariidae) from Rio Tempisque, Costa Rica. *Journal of Parasitology*, 95 (2) , 413-423. <https://www.jstor.org/stable/27735590>
- Hassan G. (2019). Morphological characterization of *Dujardinascaris* spp. (Nematoda: Anisakidae) from the striped red mullet *Mullus surmuletus* in the Mediterranean Sea. *Journal of Sciendo, Helminthologia*, 56 (4) ,338-346. <https://doi.org/10.2478/helm-2019-0032>
- Hassani M., Kerfoul A., & Boutiba Z. (2015). Checklist of helminth parasites of Striped Red Mullet, *Mullus surmuletus* (Linnaeus, 1758) (Perciformes: Mullidae), caught in the Bay of Kritel , Algeria (western Mediterranean). Check list , 11(1) , 1-3. <https://doi.org/10.15560/11.1.1504>
- Hoffmann GL. (1999). Parasites of North American freshwater fishes, 2nd edition. *Journal of Aquatic Animal Health*, 11(2) , 198. <https://doi.org/10.1577/1548>
- Ibrahim Z., Rahimo F., & Nawab F. (2008). Freshwater fish worms caught from the waters of rural areas of Kirkuk Governorate, Iraq. *Kirkuk University Journal* , 3(2). www.iasj.net
- Janiszewski J. (2022). Some fish nematodes from the Adriatic Sea , *Journal of Zoologica Poloniae*, 5(2) , 7-30. www.Scholar.google.com
- Karawan A., Alaa A., & Jadaan M. (2012). A study of some internal parasites isolated from freshwater fish ,Coarse type ,in Diwanayah city , *Journal of Veterinary Sciences* , v. 5 , n. 2 , p.143. www.Scholar.google.com
- Klimpel S., Kleinertz S., & Palm H. (2008). Distribution of parasites from red mullets (*Mullus surmuletus* L., Mullidae) in the North Sea and the Mediterranean Sea. *Bulletin of Fish Biology* , 10 (112) , 25-38. scholar.google.com
- Madrid H & Macedo M.(2011). redescription and genetic characterization of *cucullanus dodsworthi* (nematode:cucullanidae) from the checkered puffer *sphoeroides testudineus* (pisces: tetraodontiforms). *Journal of Parasitology*, 97(4) , 695-706. <https://doi.org/10.1645/GE-2664.1>
- .Marcelo K., Carmona C., Roberto P., & Delir G. (2001).Nematodes of Elasmobranch fishes from the southern coastal of Brazil. *Journal of Oswaldo Cruz,Rio de Janeiro*, 96 (1) , 81-87. <https://doi.org/10.1590/S0074-02762001000100009>
- Moravec F & Justine J. (2020). New records of cucullanid nematodes from marine fishes off New Caledonia, with descriptions of five new species of *Cucullanus* (Nematoda, Cucullanidae). *Parasite –Journal*, 27 (37) , 23. <https://doi.org/10.1051/parasite/2020030>

- Moravic F & Justine J. (2017). Two new species of nematode parasites, *Cucullanus epinepheli* sp. n. (Cucullanidae) and *Procamallanus* (*Spirocamallanus*) *sinespinis* sp. n. (Camallanidae), from marine serranid and haemulid fishes off New Caledonia. *Journal of Folia Parasitologica*, 64 (11) , 1. <https://doi.org/10.14411/fb.2017.011>
- . Moravic F., Kohn A., & Fernandes M. (1993). Nematode parasites of fishes of the Parana River , Brazil. *Folia Parasitologica* , 40 (2) , 115-134. folia.paru.cas.cz
- Moreira N IB., Rocha G N., & Costa H M. (2000). A new nematode species (Cucullanidae) parasitizing *Parauchenipterus striatulus* (Steindachner , 1876) in Brazil. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 95 , 39-41. <https://doi.org/10.1590/S007402762000000100005>
- Paraguassu A., Luque J., & Alves D. (2002). Community ecology of metazoan parasites of red porgy , *Pagrus pagrus* (L., 1758) (Osteichthyes. Sparidae), from the coastal zone, state of Rio de Janeiro, Brazil. *Maringa Journal*, 24 (2), 461-467. 61_LIVRO <ufrjr.br
- Renzo L., Gialleonardo L., Marchiori E., Francesco G., Curini V., Cocco A Guccione S., Ferri N., Marcer F., Camma C & Pascucci I. (2021). *Cucullanus carettae* Baylis, 1923, in a loggerhead sea turtle (caretta) from the Adriatic sea: first detection and molecular characterization. *Journal Parasitol*, 120 , 341-345. <https://DOI.ORG/10.1007/s00436-020-06936-5>
- Salman , M. H. M. A., (2000). Basics of fish breeding and production ,Dar Alhekma printing and publishing –AL- Mosul , 392. scholar.google.com
- Sharples D., & Evans W. (1995). Taxonomy of the metazoan parasites of the snapper, *Pagrus auratus* in New Zeland. *New Zeland Journal of Zoology* , 22 , 163-174 <https://DOI.ORG/10.1080/03014223.1995.9518031>
- Vieira F., Pereira F., Pantoja C., Soares I., Pereira A., Timi J., Scholz T., & Luque J. (2015). A survey of nematodes of the genus *Cucullanus* Muller, 1777(Nematoda,Seuratoidea) parasitic in marine fishes off Brazil, including description of three new species. *Journal of Zootaxa*, 4039 (2:5)..www.biotaxa.org

USE OF BEET ROOT FOR CULTURING GRAM NEGATIVE BACTERIA IN LABORATORY

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Abstract:

Microbes were grown in the laboratory for different purposes, by supporting their needs for growth. Nutrient Agar is a universal medium used for growing a wide spectrum of bacteria. The need to make a medium which could replace the used commercial media by using materials which is available at the local market and cheap, especially using these media at scientific search laboratories cost so much due to high needs and use. This led to think about using grains and plant roots as substitute materials in order to prepare new media to cultivate bacteria.

This study used Beet root to prepare a medium to replace the traditional ready media which used to cultivate bacteria. Detection of the growth of Gram negative bacteria on Beet root Agar and Broth was done, preparation of Differential Beet Root media by adding lactose, bile salts and neutral red stain.

The Differential Beet root media efficacy in growth of some Gram negative and positive pathogenic bacteria were not significant at 0.05, if compared with the Nutrient and MacConkey media. Differentiation between lactose fermenter and non-lactose fermenter was done on differential Beet root media. It had the ability to inhibit the growth of *Staphylococcus aureus* in comparison with other culture media at 0.05 level.

This study showed the possibility of using available, cheap and simple materials as a medium which could replace the traditional ready media, after adding nourishing and mineral salt materials which was convenient for bacteria without any change in metabolism or the morphology.

Key words: Beet Root, Ready Media, Substitute Media.



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Introduction:

Microbial studies depend on culturing microbes under laboratory conditions on suitable medium which in turn supplies a suitable media providing the microbe by the optimum condition for growth.

Prepared nutrients in the laboratory for growing bacteria called cultured medium. Microbes can get energy source directly from sun light while its needs of carbon could be available in media as organic compounds e.g. carbohydrates or an organic materials e.g. CO_2 , H_2O . The nutrient agar usually used as general medium to cultivate wide range of bacteria and it's a basic medium composed of peptic digest of animals tissue ,beef extract , yeast extract, NaCl_2 and agar (Uthayasooryan *et al.*,2016).

Nutrient Agar is a medium used for culturing bacteria in scientific laboratories and schools but by high cost (Adesemoye and Adedire, 2005), this represent a big problem for many developing countries, This led many searchers to try different replacement materials in order to minimize the cost of using these media. Agar is solidifying material in culture media and few studies concentrate on substitution it , So, Researchers focus on using materials to prepare culture media which must be available easily and low cost compared by nutrient agar (Ravathie *et al.*,2016).

The aim of the present study to substitute the nutrient source present at the ready commercial media by local materials, cheap and available represented by beet roots which contain enough amounts of protein and starch which is needed to cultivate bacteria in the laboratory. This medium was more developed to be differential by adding lactose , bile salts and neutral red. Then was used to differentiate between lactose and non-lactose fermenter.

Materials and methods

Samples collection

Local Beet roots (*Beta vulgaris*) were used in Baghdad and its taxonomy was confirmed at Mustansiriyah University , College of Science , Biology Dep. .The beet roots were washed carefully by tap water to remove the soil , then sliced into small parts and put at distill water for 24 hr ,then blended by electric blender then used fine sieve in order to get the syrup , and filtered by filter paper Whatman no.

1. Agar-agar was prepared by 125 ml volume , by adding 2.25 gm of Agar-Agar to distill water , then sterilized by autoclave at 121 C for 20 min under 15 pounds per square inch , then let it to cool until it reach 42 C, then added 125 ml of Beet roots syrup prepared formerly and its pH was adjusted to reach 6 by using NaOH, the medium was solidified when put in sterilized petridishes . Nutrient agar media was used as a control (Uthayasooryan *et al.*,2016).The liquid medium which was prepared from beet roots by the same steps used to prepare the solid medium except Agar-Agar addition.

Preparation of Bacterial isolates

A new bacterial culture of (*Pseudomonas* , *Escherichia coli* , *Klebsiella*, *Staphylococcus aureus* and *Proteus mirabilis*),which were collected from higher studies at Biology Dep. , College of Science- Mustansiriyah University . The bacteria was streaked on Muller Hinton Agar and incubated at 37 C for 24 hr.

Bacterial culture on Beet roots media

Bacterial isolates were prepared as serial dilutions in order to reach final concentration 1.5×10^8 CFU/ml, then 0.1 ml was transferred by sterilized fine pipette, and cultured on the solid and liquid media prepared from beet roots under sterilized conditions and incubate at 37 C for 24 hr.

Differential beet roots Broth

The former medium was prepared by the same way(1L), in addition to some materials in order to be differential: 10g lactose, 5g NaCl, 5 g bile salt no.3 and 7ml of Neutral red (1%). pH was adjusted to 7.2, then sterilized by autoclave.

Differential beet roots agar

The former medium with the same materials, in addition to agar-agar (1.8 %) ,and medium subjected for boiling to melt agar-agar . pH was adjusted to 7.2, then sterilized by autoclave.

Detection of *E.coli* growth on Differential beet root Broth and Nutrient broth by spectrophotometric method

1-Preparation of Bacterial suspension

E.coli was inoculated on nutrient broth at 37C for 24 hr, then compared the growth with MacFarland tube which was prepared from 1 % BaCl₂ and H₂SO₄ to get 2×10^8 CFU/ml (Macfaddin, 2008).

2- Inoculation of the study media

Prepare 20 ml of Differential Beet root Broth and Nutrient broth in flasks (Triples). All flasks were inoculated by 0.5 % of the bacterial suspension prepared before. The readings were done at wavelength 470 nm for each flask, and given zero time for these readings. Incubate all the flasks aerobically at incubator at 37 C, and continue to record readings every 12 hr, then readings were recorded at a table and growth curve was drawn.

Comparing the growth of *E. coli* on Differential Beet root broth and MacConkey broth by spectrophotometric method

The same mentioned method was done but for 24 hr, then readings were recorded at a table and growth curve was drawn.

Comparing the growth of *E.coli* and *S. aureus* on Differential Beet root agar and MacConkey Agar by Viable Count

1- Prepare each of *E.coli* and *S. aureus* on nutrient broth then comparing the growth of cells with MacFarland tube (4) to get Cells No. 2×10^8 CFU/ml.

2- Prepare 50 ml flasks of MacConkey broth and Differential Beet root agar, and inoculated by 0.5 % of bacterial inoculum prepared before. Then incubated by needed time in a shaker in triples.

3- Dilutions of bacteria were made depending of incubation time.

4- After specific time intervals , 0.1 ml of liquid bacterial suspension on the surface of MacConkey agar and Differential Beet root agar by spreading method , then incubated at 37 C for 24 hr. the colonies were studied by calculating no. by colony counter.

Comparing the growth of Gram positive and negative bacteria Differential Beet root agar and Nutrient Agar and MacConkey Agar

Prepare plates of Differential Beet root agar, then they were inoculated by the bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae* , *Pseudomonas aeruginosa* and *E.coli*) which were refreshed at broth medium for 24 hr. These bacteria were grown also on Nutrient Agar and MacConkey agar as control.

Statistical tests:

They were done by using t test to compare the average of *E. coli* growth (Zar , 1984).

Results and Discussion

All bacterial isolates used at this study showed obvious growth on the solid and liquid beet root medium prepared from Beet roots.

Beet root agar
before culturing



Figure (1) Growth of bacteria on beet root agar

(1: *Klebsiella*, 2: *Pseudomonas*, 3: *Klebsiella* 4: *Escherichia coli*)

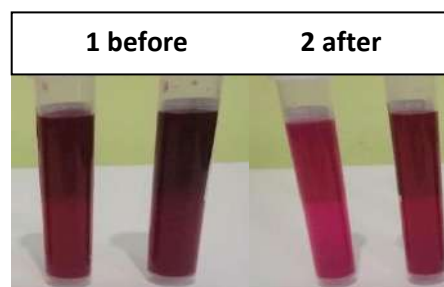


Figure (2) Appearance of *Klebsiella* bacterial growth on Beet root broth

Bacterial growth at the laboratory environment needs a suitable medium composed from combination of nutrients , moisture and other chemical materials which could be used by bacteria in order to grow, by using specific composition and nutrition base for bacterial growth depending on specific demands of specific bacteria and for specific purposes. Wide spectrum of these media were developed for different purposes and different uses. These media contained different sources of organic carbon, nitrogen, phosphor , sulfur , metals ions and finally iron (Fox, 2010).

Depending on biochemical and functional properties of bacteria , many media were developed in order to identify , isolate and preserve bacteria as pure culture (Engelkirk and Duben-Engelkirk, 2007).

Many techniques were used to cultivate bacteria depending on the purpose which bacteria were grown for it.

Media could be solid (e.g. Gelatin agar) or liquid , which enables cultivation of bacteria in test tubes (Dubey and Maheshwari, 2009), liquid media had different composition and used for cultivation of bacteria as pure culture , they composed of animal tissues or liquids like nutrition liquid ,or serum liquid ,or carbohydrates and milk liquid ,or it could be derived from fruits tissues or malt extract and yeast extract or fruits juices and finally fermented fruits juices(Engelkirk and Duben-Engelkirk, 2007).

Solid media in contrast were used more for isolation of pure culture, estimation of bacterial viability and different other purposes . The usual solidifying agent is agar- agar in solid and semisolid media which is isolated from green algae (Engelkirk and Duben-Engelkirk, 2007).

Generally there are three types of media : Natural, synthetic and semisynthetic. The chemical composition of natural media is unknown because it contains many of natural products e.g. milk , vegetables juices , animal cells ,urine , tissues and organs, while synthetic media had specific chemical composition (Madigan *et al.*,2006).

The previous studies studied the capacity of substitution of nutrient agar by plant materials , in order to cultivate isolated bacteria from different sources , e.g. one of the studies cultivate bacteria selectively on Vigna radiate Agar (Annan-Prah *et al.*,2010) , and they used it as substitute media (Kapilan and Thavaranjit , 2008). Legumes seeds could be used as substitute media (Ravathie *et al.*,2012).

Agar- Agar is a solidifying agent used to solidify the substitute media, because it is much easier to spread the microbial inoculum, count colonies and study the colonies characters on solid media. The nutrient Agar is commonly used media for different types of bacteria cultivation , the cost of preparation of 1 kilo of nutrient agar almost 93 € compared by low cost of prepared media of substitute materials which could be prepared by simple capacities and available in most of simple laboratories , even it could prepare these components at the time it needed or storage it for month or more at room temperature (Uthayasooriyan *et al.*,2016).

Plants were used in activation and modification of bacterial growth e.g. Beet root juice (Klewicka *et al.*,2015), also beet root juice used for cultivation of Tubercelli bacteria (Mohammed *et al.*,2018).

Beet root contain nutrition components e.g. carbohydrates, protein , high levels of important vitamins, nutrients which are needed by microbes by small amounts , low quantities of fatty acids as shown at figure (3) (Cecluand Nistor, 2020).

Different preparations of beet root had no effect on nutritional value, e.g. boiling it leads to increase its content from carbohydrates and protein than that on crude ordinary beet root, so the energy will increase , one ten of beet root content is a glucose, vegetables fruits which present as pure sugars, also gum and starch which form one third of beet root weight (Grieve, 2010).

Beet root juice had an important nutritional value due its contents of carbohydrates , sugars , fibers, fats, B₁,B₂,B₃,B₅,B₆,B , vitamin C , calcium, iron , magnesium , phosphor, potassium , sodium, zinc ,chlore, manganese , low amounts of selenium which could be available in prepared media, in addition to low amounts of biotin and vitamin E and betaine which is a nitrogenous compound present in beet root by a form resembling that of the amino acid (Grubben and Denton, 2004).

Water (g)	91.3 ± 4.29
Protein (g)	1.89 ± 0.3
Carbohydrate, by difference (g)	7.23 ± 2.33
Fiber, total dietary g	3.25 ± 0.55
Sugars, total (g)	6.76 ± 1.23
Total lipid (fat) (g)	0.15 ± 0.05
Ash (g)	1.08 ± 0.72
α-Carotene (µg)	22.0 ± 2.0
β-Carotene (µg)	0 11.64
Lycopene (µg)	30 ± 0.3
Lutein + zeaxanthin (µg)	0
Betaine (µg)	128.7 ± 22.0
Folate (µg)	109
Niacin (mg)	0.334
Vitamin A, IU	0
Vitamin B6	0.067
Vitamin C (mg)	7.2 ± 2.5
Sodium, Na (mg)	78.0 ± 5.0
Potassium, K (mg)	325 ± 4.5
Phosphorus, P (mg)	40.00
Magnesium, Mg (mg)	23.0 ± 2.0
Calcium, Ca (mg)	16 ± 3.5
Manganese, Mn (mg)	0.359 ± 0.04
Zinc, Zn (mg)	0.365 ± 0.015
Copper, Cu (mg)	0.075
Iron, Fe (mg)	0.80

Figure (3) Beet Root contents for each 100 g according to (Ceclu and Nistor,2020)

Using *Escherichia coli* with other types of bacteria is so important, because this bacteria is usually used in all microbial studies in all laboratories due to its short generation time and complete sequence of their genome, So, knowledge of chemical structure of these substituted nutrition sources and biochemical properties of bacteria in these substituted nutritional sources is so important , also it demands knowing how much these substituted nutritional sources is suitable as substituted nutritional sources and use different bacterial isolates to test these substituted media (Uthayasooriyan *et al.*,2016).

Results of *E.coli* growth on Differential Beet root broth and nutrient broth

The results showed at Table (1), a low increase on *E. coli* growth on nutrient broth compared by Differential Beet root broth, the spectrophotometric readings were 1.12 nm on Nutrient broth compared by 0.84 on Differential Beet root broth after 12 hr growth.

The statistical analysis by t test showed no significant differences between the two media at 0.01 and 0.05, So the Differential Beet root broth was equivalent to ready prepared media , due to beet root containing of nutritional materials protein(1.89 %) ,glucose, metals (e.g. K^+ , Ca^{+2} , Na^{+2}) and vitamins (A, B₆ and C) which facilitate the bacterial growth (Pellet and Shadarevian, 1976).

Table 1: Comparing *E.coli* growth on Differential Beet root broth and nutrient broth

Time (hr)	Spectrophotometric readings (nm)	
	Nutrient Broth	Differential Beet root broth
0	0.07	0.08
2	0.18	0.12
4	0.33	0.26
6	0.65	0.45
8	0.79	0.61
10	1.06	0.80
12	1.12	0.84

Results of *E.coli* growth on Differential Beet root broth and MacConkey broth

The results at table 2 showed a little increase of *E.coli* growth on MacConkey broth (1.4) nm compared by the Differential Beet root broth (1.1). After 14 hr and 24 hr, there were increase in growth of bacteria (1.83 nm in MacConkey broth and 1.78 nm in Differential Beet root broth), there were not any significant difference at 0.01 and 0.05, This confirmed that Differential Beet root broth could substitute the MacConkey broth, especially that *E.coli* grown at Differential Beet root broth fermented Lactose sugar by changing the media color just like its growth at MacConkey broth, due to presence of lactose sugar of both of them.

The presence of neutral red of both medium, which is an indicator used to detect the fermentation by lowering pH of media , So, this add a feature for the medium to use it as a differential medium to distinguish between fermenter and non-fermenter of lactose.

Table 2: Comparing *E.coli* growth on Differential Beet root broth and MacConkey broth

Time (hr)	Spectrophotometric readings (nm)	
	MacConkey Broth	Differential Beet root broth
0	0.20	0.19
2	0.29	0.22
4	0.36	0.31
6	0.46	0.45
8	0.66	0.79
10	1.20	0.89
12	1.29	1.10
14	1.40	1.10
24	1.83	1.78

Results of *E.coli* and *S. aureus* growth on Differential Beet root agar and MacConkey agar

Viable count (by 0.17×10^8) CFU/ml was used to compare the growth on Differential Beet root agar and MacConkey agar, the results showed at table 3, the aim of the study was done when No. of *E.coli* cells increased at zero time (0.17 and 0.18) $\times 10^7$ CFU/ml on Differential Beet root agar and MacConkey agar (80.3 , 90.2) $\times 10^7$ CFU/ml after 24 hr for both media respectively. The statistical tests showed no significant differences between the two media at 0.01 and 0.05. Also results showed that differential Beet root agar in growing Gram negative bacteria than of Gram positive bacteria by inhibiting growth of *S.aureus*, which in turn on MacConkey Agar by low no. comparing *E.coli*.

Table 3: Comparing *E.coli* and *S. aureus* growth on Differential Beet root agar and MacConkey agar by viable count

Time (hr)	Spectrophotometric readings (nm)			
	<i>E. coli</i>		<i>S. aureus</i>	
	MacConkey Agar	Differential Beet root agar	MacConkey Agar	Differential Beet root broth
0	0.17	0.18	0.02	-
4	0.18	0.18	0.04	-
8	8.50	9.20	0.15	-
12	30.0	30.0	0.20	-
16	50.2	45.1	0.30	-
24	80.3	90.2	0.38	-

Results of different Gram negative bacteria on Differential Beet Root Broth and Differential Beet Root agar

Different Gram negative bacteria (included *Salmonella typhi*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *E.coli* grown so well on both Differential Beet Root Broth and Differential Beet Root agar as shown table 4. The bacteria that was lactose fermenter were differentiated from non-lactose fermenter on Differential Beet Root agar.

At the same time colonies of *E.coli* and *K. pneumoniae*, whereas *Ps. aeruginosa*, *Proteus mirabilis* and *S. typhi*, which agreed with researcher results (Holt *et al.*, 1994).

Table 4 : Growth of different Gram negative bacteria on Differential Beet Root Broth and Differential Beet Root agar

Bacteria	Media		
	Differential Beet Root agar	Differential Beet Root broth	Lactose sugar fermentation
<i>E.coli</i>	+	+	+
<i>Salmonella typhi</i>	+	+	+
<i>Proteus mirabilis</i>	+	+	+
<i>K. pneumoniae</i>	+	+	+
<i>Ps. aeruginosa</i>	+	+	+

Colonies of *E.coli* on Differential Beet root agar were circular, convex and pink due to fermentation of lactose sugar available in the medium, this was exactly showed by the study of (Zegarra Montes *et al.*, 2008).

To make the medium differential and selective, MacConkey media was used. Due to its contents of bile salts which improves the growth of *E. coli* and *S.aureus* (Edwards and Ewing, 1970).

References

- Adesemoye, A. O. and Adedire, C. O. (2005) *World J. Microbiology and Biotechnology*, 21; 329-336.
- Annan-Prah., A. ; Akorli, S. Y. and Sedofia, K. B. (2010). *African J. Microbiology Research*, 4: 2626-2628.
- Ceclu, L. and Nistor, O. (2020) Red Beetroot: Composition and Health Effects . A Review Ceclu and Nistor. *J. Nutri. Med. Diet. Care*, 6(1).
- Dubey, R. C. and Maheshwari, D. K. (2009) “Practical Microbiology”, 6th ed., S. Chand & Company LTD, India, pp. :24-25.
- Edwards , P.R and Ewing , W.H. (1970) *Identification of Enterobacteriaceae* .3d-ed. Minneapolis, Burges publishing com.
- Engelkirk, P. G. and Duben-Engelkirk, J. (2007) “Laboratory Diagnosis of Infectious Diseases: Essentials of Diagnostic Microbiology”, Lippincott Williams and Wilkins, USA, pp. :133-134.
- Fox, A.(2010). “Microbiology and Immunology”. on-line, <http://pathmicro.med.sc.edu/book/bact-sta.htm>.
- Grieve, M. (2010) *Botanical.com* A modern herbal. <http://botanical.com/botanical/mgmh/b/beetro28.html>.
- Grubben, G. J. H. and Denton, O. A. (2004) *Plant Resources of Tropical Africa 2, Vegetables*. Backhuys, Leiden CTA, PROTA Foundation, Wageningen.
- Holt , T.G ; krieg, N.R and Sneath , P.H. (1994) *Berge's Manual of Determinative Bacteriology*. 9th-ed. Williams and Wilkins.
- Kapilan, R. and Thavaranjit , A. C. (2008). *Journal Science of the University of Kelaniya*, 4: 11-14.
- Klewicka, E ; Zduńczyk, Z. ; Juśkiewicz, J. and Klewicki, R. (2015) Effects of lactofermented beetroot juice alone or with N-nitroso-N-methylurea on selected metabolic parameters, composition of the microbiota adhering to the gut epithelium and antioxidant status of rats. *Nutrients*, 7(7): 5905–5915.
- Macfaddin , J.F.(2008) *Biochemical Tests for Identification of Medical Bactria*. 3d-ed. The Williams and Wilkins. Baltimore , USA.
- Madigan, M. T. ; Martinko, J. M. and Brock, T. D. (2006) “Brock Biology of Microorganisms”, 11th ed., Pearson Prentice Hall, USA.
- Mohammed, A.A. ; Al-Mugdadi, S.F. ; Al-Sudani1,B.T. and Kamel, J. K. (2018) Beetroot juice as an alternative growth and maintenance medium for six isolates of *Mycobacterium* spp. Ahmed A Mohammed et al .*J. Pharm. Sci. & Res.* 10(8): 1881-1884
- Pellet, P.L and Shadarevian, S. (1976) *Food Composition-2 nd-ed.* American University of Beriut , Lebanon.
- Ravathie, A. ; Sevvel, P. ; Nirmala, R. and Kularajany, N. (2012). *Journal Natural Product and Plant Resources*, 2: 697-700.

III. International Scientific Congress of Pure, Applied and Technological Sciences (Minar Congress)

- Uthayasooryan, M. ; Pathmanathan, S. ; Ravimannan, N. and Sathyaruban, S. (2016) Formulation of alternative culture media for bacterial and fungal growth. *Der. Pharmacia. Lettre.*, 8 (1):431-436.
- Zar , J.H. (1984) *Biostatistical Analysis* , 2nd- ed. Prentice hall inc , Englewood cliffs , N.I.
- Zegarra Montes, L.R. ; Sanchez Mejia , A.A. ; loza Munarriz ; C.A. and Celis Gutierrez , E. (2008) Semen and urine culture in the diagnosis of chronic bacterial prostatitis . *International Braz.J. Euro.* : 1(1),30-40.



**EFFECT OF SMOKING CIGARETTES AND HOOKAH ON SOME PHYSIOLOGICAL AND
BIOCHEMICAL VARIABLES AMONG THE STUDENTS OF THE AL-DOOR TECHNICAL
INSTITUTE**

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**EFFECT OF SMOKING CIGARETTES AND HOOKAH ON SOME
PHYSIOLOGICAL AND BIOCHEMICAL VARIABLES AMONG THE STUDENTS
OF THE AL-DOOR TECHNICAL INSTITUTE**

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Abstract:

The aimed of the study the effect of smoking cigarettes and hookah on some hematological and biochemical variables. Hematological parameters were measured the volume of (PCV), hemoglobin concentration (Hb), biochemical variables, lipid concentration in blood serum, glucose concentration in blood serum and ALP concentration in blood serum for some students of the Technical Institute, the role of smokers. 60 blood samples were collected from 20 cigarette smokers, 20 hookah smokers and 20 non-smoking students. Where there a significant increase at ($P < 0.05$) in the concentration of triglyceride in the blood of cigarette smokers compared to the control group and a significant decrease at ($P < 0.05$) in the concentration of Glucose and Hb compared with control group.

Key words: Cigarettes, Hookah, The Students of The Al-Door Technical Institute.



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Introduction:

Smoking: a process in which a substance is burned, which is often tobacco, and then the smoke is tasted or inhaled. This process is carried out primarily as an exercise for recreation through the use of drugs, as it results from the combustion of the active substance in the drug. (Francis *et al* ,2017) Smoking: a process in which a substance is burned, which is often tobacco, and then the smoke is tasted or inhaled. This process is carried out primarily as an exercise for recreation through the use of drugs, as it results from the combustion of the active substance in the drug. (Ahmed,2016)

Each year it kills more than 8 million people around the world, including more than 7 million who use it directly and about 1.2 million non-smokers are exposed to its smoke against their will. All forms of tobacco are harmful, and there is no safe level of exposure to tobacco. (Fadiel & Hasan ,2016)

Cigarette smoking remains the most common type of tobacco use worldwide. Other tobacco products include hookah tobacco Smoking shisha, hookah, or hookah is one of the very bad habits, which has spread widely among the youth of our country, and has become a fashion among young people, and it is a method of smoking that depends on the passage of smoke produced by burning coal and passes through the water and then the smoker inhales it, and unfortunately this habit has become not limited For young people only (Albacker *et al*,2020).

But some girls also smoke, and they smoke different types of hookah, under different names, It is strange that this habit is becoming more popular and attracts new victims every day, and some smokers believe that it is less harmful than smoking cigarettes, and it is a safe alternative to cigarettes, and they sometimes use it as a means of entertainment.(Bilito,2015) For entertainment at other times, they began to acquire them inside homes; To use it when necessary instead of a cigarette, but the danger of this habit is beyond the imagination of these smokers, as it is more harmful to health than cigarettes, and in the coming lines we will present some research results in this regard, with the damage and diseases caused by this bad habit (Hammal *et al*, 2015). .

Materials and methods:

Blood samples collected from the Technical Institute students, smokers and non-smokers, where it was chose 60 students. the students divided to groups as bilow

- 1- First group G1: non-smokers (control) 20 students
- 2- The second group G2: hookah smokers 20 students
- 3- The third group G3: cigarette smokers 20 students

After the blood drawn from the vein, (2) ml placed in plastic tubes containing an anticoagulant substance for the purpose of measuring some blood variables, and (5) ml was placed in plastic tubes free of anticoagulant for the purpose of obtaining blood serum to measure the biochemical tests, and the blood was kept The blood serum was measured by freezing it at a temperature of (-20) C. Then the hemoglobin (Hb) and the compacted cell volume (PCV), Glucose, Cholestrol, ALP Triglyceride were measured according to the categorization prepared by the company biolabo.

Results and discussion:

Table (1) Some hematological and biochemical variables for smokers and non-smokers for some students of the Technical Institute / AL-Door students

Variables	ALP	Glucose	Triglyceride	Cholestrol	Hb	pcv
Non-smokers ((control	11.40±1.54 a	98.85±8.85 a	40.61±5.57 b	132.40±15.18 a	16.20±1.88 a	48.00±4.32 a
hookah smokers	12.0143±1.64 a	102.85±2.54 a	54.57±11.35 a	122.50±4.88 a	14.185±1.70 b	44.28±3.72 a
cigarette smokers	12.68±1.30 a	88.14±4.70 b	42.24±4.50 b	136.8±39.288 a	15.45±0.86 ab	48.71±5.58 a

- The number of students is 60 for each group.
- Vertically different letters mean there is a significant difference at the level (P < 0.05).

results of Table (1) showed that there were no significant differences in the group of hookah and cigarette smokers in ALP concentration, serum cholestrol concentration and PCV compared with the non-smokers group (control group).

It shown through the results of Table (1) that there a significant increase at the level of probability (P≤0.05) in the group of hookah smokers in the concentration of triglyceride in the blood serum compared with the non-smokers (control group) and the group of cigarette smokers.

was noticed from the results of Table (1) that there a significant decrease at the probability level (P≤0.05) in the group of cigarette smokers in the concentration of glucose in the blood serum compared with the group of non-smokers (the control group) and the group of hookah smokers.

The results of Table (1) showed that there was a significant decrease at the probability level ((P≤0.05) in the hookah smokers group in the concentration of Hb compared with the group of non-smokers (the control group) other way it doesn't find significant differences in the cigarette smokers in the concentration of Hb compared with the group of non-smokers (control group) and hookah smokers group.

The Wannamethee S G and Shaper A G (2010) study reported that cigarette smoking associated with increased levels of GGT and ALP, but was inversely associated with AST. This study showed the relationship between smoking, liver and hepatitis The study of Deepa (2106) showed an increased in the Cholesterol concentration of and Triglyceride in the blood

Cigarette smoking has been associated with adverse effects on lipids, increasing the risk of atherosclerosis and coronary heart disease. Smoking is a prominent risk factor for coronary artery disease, atherosclerosis and peripheral vascular disorders. (Münzel,*et al*,2020)

The results of Table (1) showed a significant decreased in the probability level ((P≤0.05) in the hookah smokers group in the concentration of Hb compared with the group

of non-smokers (the control group) and there were no significant differences in the group of cigarette smokers in the concentration of Hb compared with the group of non-smokers (control group) and hookah smokers group.

Wannamethee S G and Shaper A G (2010) reported that cigarette smoking was associated with increased levels of GGT and ALP, but was inversely associated with AST. This study showed the relationship between smoking, liver and hepatitis. The study Deepa (2106) showed an increase in the concentration of Cholesterol and Triglyceride in the blood.

Cigarette smoking has been associated with adverse effects on lipids, increasing the risk of atherosclerosis and coronary heart disease. Smoking is a prominent risk factor for coronary artery disease, atherosclerosis and peripheral vascular disorders. (Tan *et al.*2018)

The results of the study of Mahsid *et al* (2018) showed that exposure to cigarette smoke leads to a significant decrease in the level of HDL and triglyceride, but has no effect on the level of total cholesterol and LDL in children and adolescents.

The study of Nwaokoro *et al* (2014). indicated that smokers are more likely to develop diabetes than non-smokers. Type 2 diabetes is the most common type of diabetes, accounting for at least 90% of cases globally.

Type 2 diabetes is closely related to some lifestyle factors, including smoking. In fact, smokers are 30 to 40% more likely than non-smokers to develop diabetes. People who already have diabetes and who smoke are more likely to develop uncontrolled diabetes (Malenica *et al*,2017)

Smoking damages cells and tissues, increasing inflammation. It also causes oxidative stress, which occurs when molecules called free radicals damage cells. These two conditions are associated with an increased risk of diabetes. It can cause other health problems, including cardiovascular disease. (Shah & Anzar,2020)

Research also indicates that heavy smoking increases abdominal fat. Even in people who are not obese or overweight, excess abdominal fat is a risk factor for diabetes.

The health risks are numerous, and researchers are constantly discovering new health concerns associated with smoking. The smoking habit is the leading cause of preventable death in the United States, while more than 16 million Americans suffer from a smoking-related illness. (Hameed *et al*,2019)

Smoking risks include cardiovascular disease, including coronary heart disease (CHD), heart attack, and stroke. (Qasim,*et al*,2019)

Lung diseases, such as chronic obstructive pulmonary disease (COPD) and chronic bronchitis

Sudden infant death syndrome (SIDS) in infants exposed to secondhand smoke
Autoimmune diseases, such as Crohn's disease
Damage to the eyes and optic nerve that can lead to blindness
Fertility problems, such as ectopic pregnancy, stillbirth, and preterm birth
Low birth weight and birth defects
Smoking during pregnancy increases osteoporosis for cancer, including lung, oral, bladder, colon, pancreatic, and kidney cancers. (Scott-Sheldon and Stroud, 2019)
Oral health problems, such as gum disease and tooth loss
Weakened immune system that increases susceptibility to infections (*et al*, 2018 Mahsid)

The study of Maja *et al* (2017) showed an increase in red blood cells, white blood cells, hemoglobin, and PCV in male smokers compared to female smokers that continuous smoking has severe adverse effects on blood variables and increases red blood cell count, hemoglobin concentration, PCV, white blood cell count, and may They are associated with the greatest risk of developing atherosclerosis, COPD or cardiovascular disease.(Li,et al,2018& Herath *et al*.2021& Sandhya *et al*,2015)

References:

- Abou Almakarem S, et al. (2020)** (Does hookah smoking carry less cardiovascular risks than cigarette smoking in patients presenting with myocardial infarction? *J Nat Sci Med* ;3:48-52.
- Albacker TB, Barghouthi RA, Al Saadan Y, Tokhta M, Alkorbi A, Abou Almakarem S, et al.(2020)**Does hookah smoking carry less cardiovascular risks than cigarette smoking in patients presenting with myocardial infarction? *J Nat Sci Med* ;3:48-52.
- Ahmed OA. (2016).**Effects of smoking cigarette on white blood cell and platelet parameter on a sample of normal subject in Rania city. *Imp J Interdiscip*;2:887-92.
- Bilto YY.(2015).** Effects of cigarette smoking on blood rheology and biochemistry. *Int J Sci Res*;4:107-12.
- Deepa S (2016).** Effect of Cigarette Smoking on Serum Lipid Profile in Male Population of Udaipur. *J. Biochem Anal Biochem* 5(3)1-3.
- Fadiel FE, Hasan HM. A.(2016).** comparative study on some haematological parameters of some smokers and non smokers blood samples. *World J Pharm Res*;5:146-54.
- Francis DB, Hall MG, Noar SM, Ribisl KM, Brewer NT. (2017).**Systematic review of measures used in pictorial cigarette pack warning experiments. *Nicotine & Tobacco Research.*;19(10):1
- Hameed R M, Mehdi W A, Mehde A A. (2019).**Biochemical significance of ecto 5'neucleotidase, xanthine oxidase and glutathione S-transferase determinations in sera of cigarette and water pipe young men smokers. *Med J Babylon*;16:70-6.
- Hammal F, Chappell A, Wild TC, Kindziarski W, Shihadeh A, Vanderhoek A, et al. (2015)**'Herbal' but potentially hazardous: An analysis of the constituents and smoke emissions of tobacco-free waterpipe products and the air quality in the cafés where they are served. *Tob Control*;24:290-7.
- Herath P , Wimalasekera S , Amarasekara T,Fernando M , Turale Sue (2021).** Effect of cigarette smoking on smoking biomarkers,blood pressure and blood lipid levels among Sri Lankan male smokers. *J.Postgrad Med* . 1136(10) 1-7
- Li J, Haardörfer R, Vu M, Windle M, Berg CJ.(2018).** Sex and sexual orientation in relation to tobacco use among young adult college students in the US: a cross-sectional study. *BMC Public Health*. 2018;18(1):
- Mahshid A, Mahmoud Y, Mostafa H, Alireza O, Gholamreza H, Mehdi K-D, Hooman S, Zahra H, Maryam H (2018).** Effect of Active and Passive Exposure to Cigarette Smoke on Lipid Profile of Children and Adolescents; A Systematic Review and Meta-Analysis *J. Pediatr* 6(5) 7575-7588.
- Maja M, Besim P, Tamer B, Tanja D, Sabina S,Selma S, Amar G, Ajla H, Adlija C (2017).** Effect of Cigarette Smoking on Haematological Parameters in Healthy Population *J. Med Arch*. 71(2): 132-136.
- Malenica M, Prnjavorac B, Bego T, Dujic T, Semiz S, Skrbo S, Gusic A, Hadzic A, and Causevic A (2017).** Effect of Cigarette Smoking on Haematological Parameters in Healthy Population *.J. Med Arch* 71(2): 132–136
- Münzel T, Hahad O, Kuntic M, Keaney J F, Jr, Deanfield J E, Daiber A.,(2020).** Effects of tobacco cigarettes, e-cigarettes, and waterpipe smoking on endothelial function and clinical outcomes *J. European Heart Journal*. 41(41). 4057–4070

Nwaokoro J C , Ede A O , Emerole C, Ibe S , Amadi , I.N.S. Dozie , S.C. Iwuji2 , A.A. N.

(2014). Study on the effect of smoking on Type 2 Diabetic Patients in Federal Medical Center Owerri, Southeastern Nigeria *Journal of Medical Sciences* 5(3) 63-71.

Qasim H , Alarab A B. , Alzoubi K H., Karim Z A., Alshbool F Z. , and Khasawneh F T. (2019). The effects of hookah/waterpipe smoking on general health and the cardiovascular system.*J. Environmental Health and Preventive Medicine* 24(58) 1-17.

Shah M A W, Anzar B A., (2020). .Correlation between anemia and smoking: Study of patients visiting different outpatient departments of Integral Institute of Medical Science and Research, Lucknow. *Journal of Physiology, Pharmacy and Pharmacology* 10(2)149-154.

Sandhya M, Satyanarayana U, Mohanty S, Basalingappa DR.(2015). Impact of chronic cigarette smoking on platelet aggregation and coagulation profile in apparently healthy male smokers. *Int J Clin Exp Physiol*;2:128-33.

Tan J, Zhang X, Wang W, et al.(2018). Smoking, blood pressure, and cardiovascular disease mortality in a large cohort of Chinese men with 15 years follow-up.*Int J Environ Res Public Health* 2018;15. doi:10.3390/ijerph15051026. [Epub ahead of print: Available.

Wannamethee S G and Shaper A G (2019). Cigarette smoking and serum liver enzymes: the role of alcohol and inflammation *J. Ann Clin Biochem* 47: 321–326.



**MOLECULAR CHARACTERIZATION OF UNKNOWN FUNGAL ISOLATES FROM SOIL
SAMPLES IN GARDENS OF MUSTANSIRIYAH UNIVERSITY /BAGHDAD CITY-IRAQ**

NOOR T. HAMDAN

**MOLECULAR CHARACTERIZATION OF UNKNOWN FUNGAL ISOLATES
FROM SOIL SAMPLES IN GARDENS OF MUSTANSIRIYAH UNIVERSITY
/BAGHDAD CITY-IRAQ**

Noor T. HAMDAN¹

Abstract:

The botanical gardens of Al-Mustansiriyah University are very rich, containing a wide variety of microorganisms which have not been recognised before. As a result, there are seven fungal isolates were molecular identified via Internal Transcribed Spacer (ITS) regions from these gardens in spring 2021 and including as *Fusarium boothii*, *Talaromyces* sp, *Gliocladium viride*, *Sordariomycetes* sp, and three of *Fusarium oxysporium*. This document is done to evaluate the molecular characterization of some unknown fungal isolates from soil samples in gardens of Mustansiriyah University /Baghdad city-Iraq.

Key words: Molecular Characterization, Unknown Fungal Isolates, Soil, Iraq.



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Introduction:

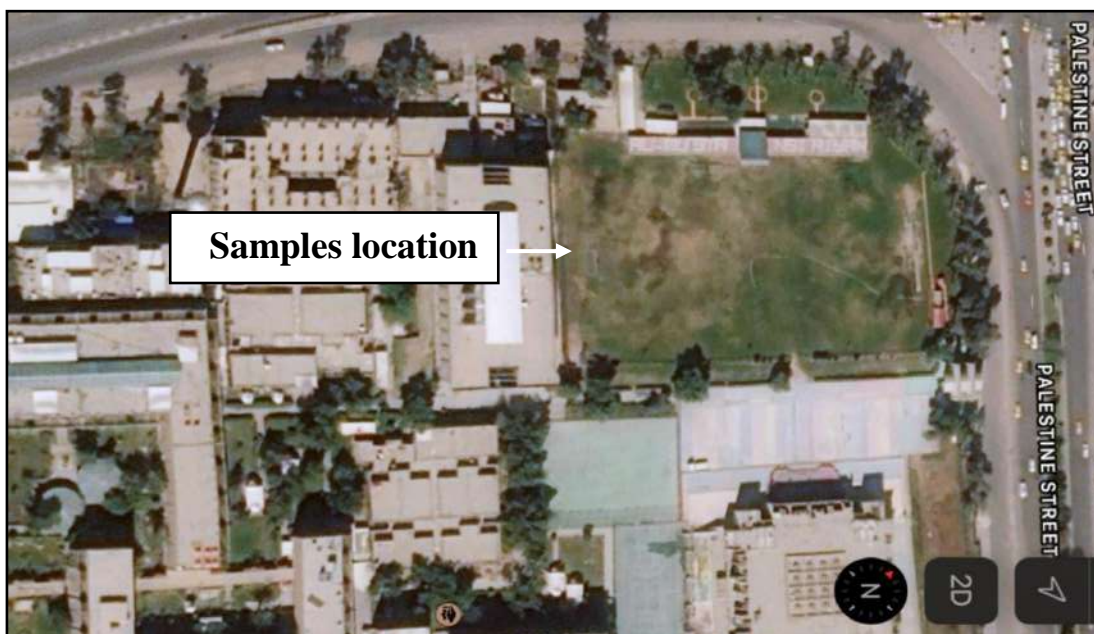
Soil fungi play an important role in biocontrol and fungicides. Environment always have new strains and genes as results of continuous changes of climate, as the organisms need to adapt with these changes in order to survive and conserve progeny (Yang *et al.*, 2016). For example, the environment of the botanical gardens of Mustansiriyah University is very rich, as it contained a wide variety of microorganisms which have not been recognised before. DNA Barcoding approach which provides limited and defined DNA zones with specific trend is regarded as one of the highest efficient and fast approach to classifying unknown endophytes (Schoch *et al.*, 2012). For example, the Internal Transcribed Spacer (ITS) region was widely employed since it takes into consideration the greatest sequenced zone for endophytic classification of species. The ITS region was highly variable of the non-coding in plenty of phylogenetic elements for allowing species level sequences isolation (Haque *et al.*, 2020).

Therefore, the samples were collected from the soil of the botanical gardens at Mustansiriyah University in Baghdad city- Iraq for the detecting of the varieties of fungi.

Materials and Methods

Fungal isolation

The botanical gardens that rises at Mustansiriyah University's Science College which is located on Palestine Street in Baghdad, Iraq, as shown in Figure 1. The samples were collected from these garden by means of sterile polyethylene bags. Soil samples were analysed for the presence of fungal new isolates by using the plates dilution method. A volume of 1 g from each sample was added to a plate and then the sterilized Potato Dextrose Agar (PDA) medium with chloramphenicol (0.05g/L) were decanted on it after cooling to 45 ° C. Then, the cultures were kept around 28 ± 2 °C throughout a period of 5 days (Beeck *et al.*, 2014).



Figure(1): The satellite picture of the sampling location.

DNA extraction

The dominance fungi (7 isolates) were collected from the plate and transferred to a new PDA medium plate to obtain single colony of fungi for DNA extraction. The powdered fungi were used to extract DNA by using Genetic DNA isolation kit (Doctor protein INC, Korea), followed by manufactures instructions. The absorbance of diluted DNA solution at 260 and 280 nm was measured using Nanodrop to estimate DNA concentrations (25-100 ng/rxn) and purity (1.6-1.8) (Thermo Scientific, Germany). The electrophoresis on a 1% agarose gel stained with ethidium bromide was used to determine the purity of the DNA. The solutions were kept at -20 °C until they were needed.

Fungi identification by PCR

Two universal primers, ITS1 (forward): 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 (reverse): 5'-TCCTCCGCTTATTGATATGC-3', were used to amplify the ITS region of the fungi (White *et al.*, 1990). For PCR experiments, we used DNA Polymerase (Doctor protein INC, Korea) according to the manufacturer guidelines. The following were the PCR amplification circumstances: Denaturation was started at 95° C for 5 minutes, then 35 cycles of 95° C for 30 seconds, 55° C for 30 seconds, and 72° C for 1 minute, followed by a final extension at 72° C for 7 minutes. Electrophoresis is then used to assess the integrity and quantity for PCR product at 1.5 percent agarose gel. At the Macrogen sequencing laboratory, the gene sequences have been verified from both strands of PCR amplification products (Macrogen Inc., Seoul, Korea). The gene sequence analyses were performed using the sequences available in the National Center for Biotechnology Information (NCBI) online database (GenBank) for identification of species via the BLAST tool.

Results and Discussion

Molecular characterization of fungal isolates

The PCR amplification products of ITS region size were around 375 bp on 1.5% agarose gel. Fig. 2 shows that all fungal isolates of *Fusarium boothii*, *Talaromyces*, *Gliocladium viride*, *Sordariomycetes*, three of *Fusarium oxysporium* gave 375bp amplified bands respectively. The BLAST results were obtained by searching with partial nucleotide sequences at the GenBank database. Furthermore, the percentage similarity was revealed by Blast analysis. Sequence for the identified species include *Fusarium boothii*, *Talaromyces*, *Gliocladium viride*, *Sordariomycetes*, and three genus of *Fusarium oxysporium* which were then submitted online in the GenBank with the accession numbers [(KY263546.1,%identity100),(MW619990.1, %identity97), (Gu903311.1 ,%identity 96), (MW530060.1,%identity 96), (MW775868.1,%identity94), (MW76587.1, %identity99), (MW535851.1, %identity94), respectively] , as illustrated in figure(3, 4, 5, 6).

Despite the benefits of specificity and repeatability, the systematic classification of fungi using these genotypic approaches is difficult and rather time-consuming, but also requiring specific tools and, in some cases, previous experience of nucleotide sequences in the particular species. Internal transcribed spacer (ITS) regions, for example, were extensively employed to reconstitute the evolutionary origins of many types of fungus (Bellemain *et al.*, 2010 ; Avin *et al.*, 2014). Understanding in biological complexity is very important to study

the activity and development of microorganisms in parallel with environmental changes. Such an understanding is necessary to keep these organisms under control, as they may cause some epidemiological problems(Sheth and Wang, 2018).

Soil is a very rich environment for many components which play an important role in changing the DNA sequences of many organisms as a result of expose to pollution and mutagen elements like heavy metals, antibiotic, nutrient and other sources of antagonisms of other living organisms(Cheek *et al.*, 2020).

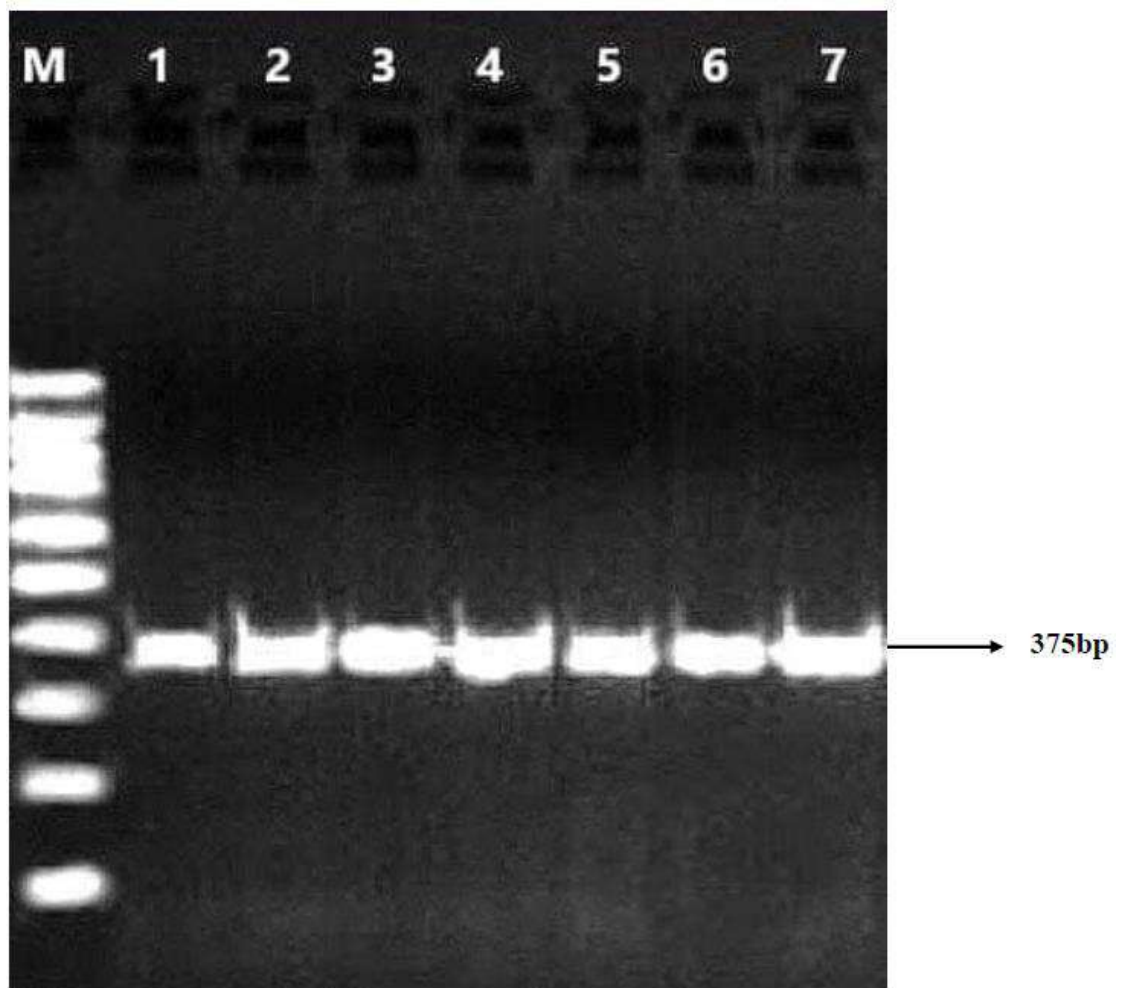
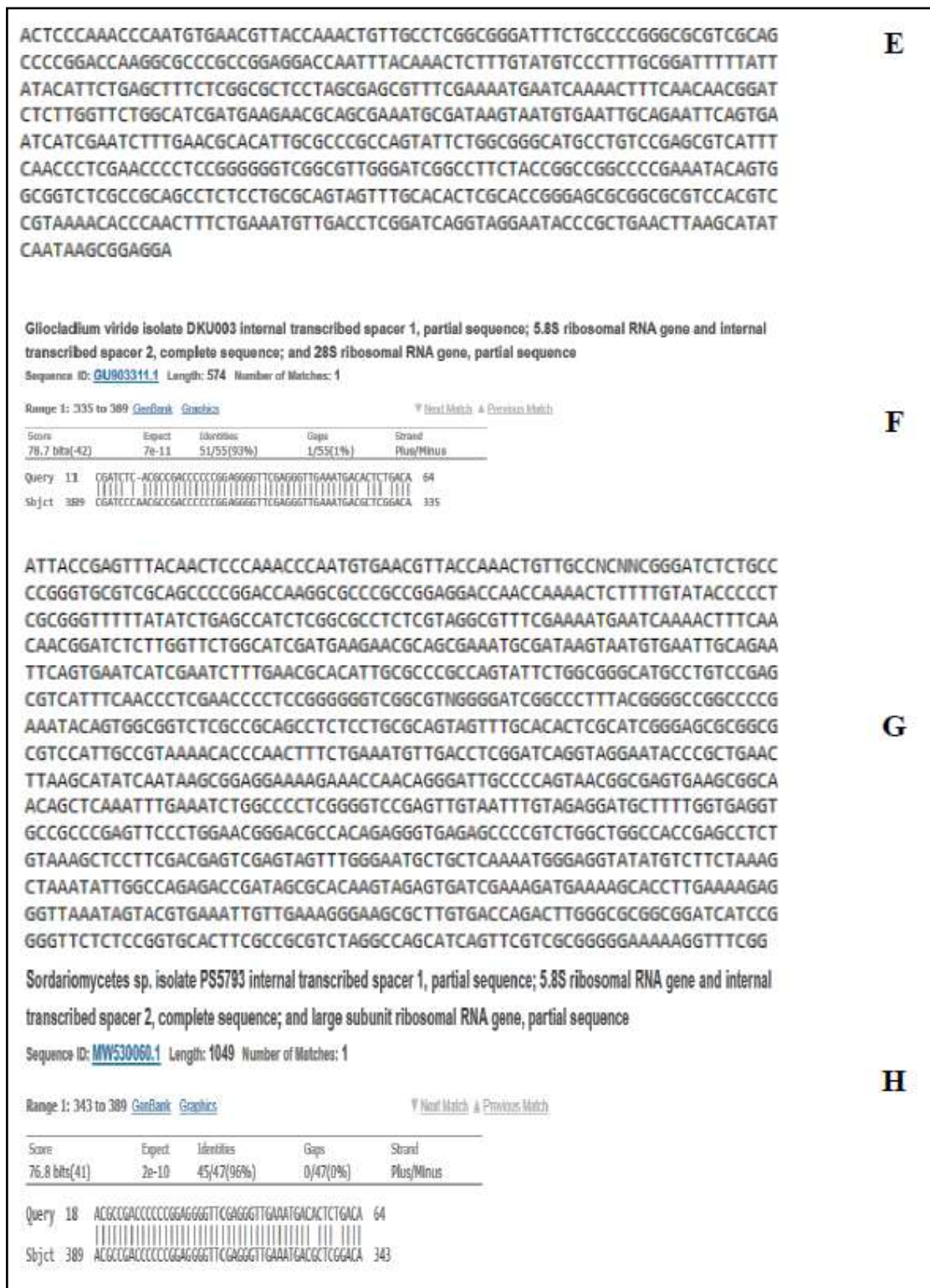
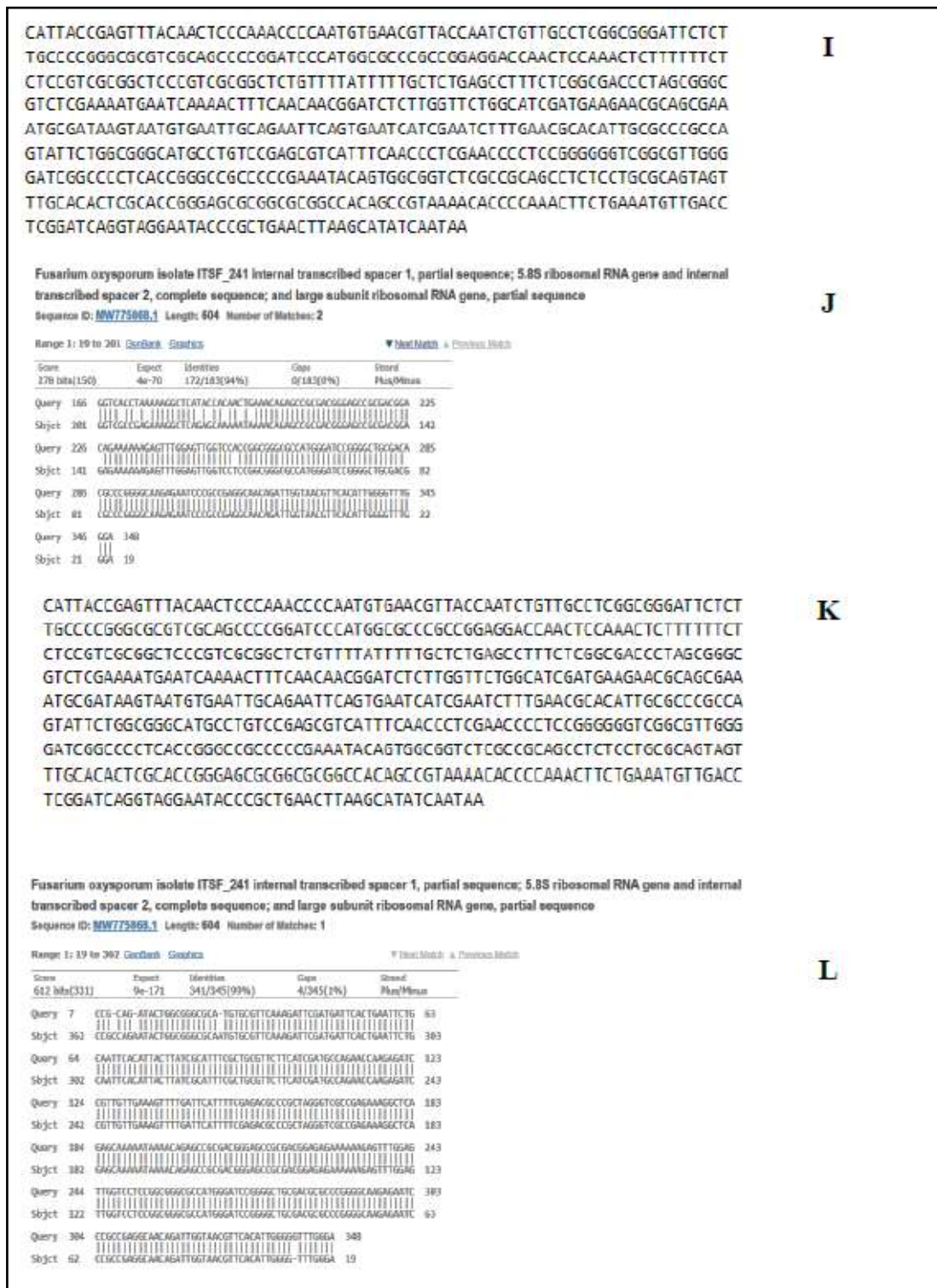


Figure (2): PCR amplified products using agarose gel electrophoresis for ITS regions of *Fusarium boothii*(1), *Talaromyces*(2), *Gliocladium viride*(3), *Sordariomycetes*(4) and three of *Fusarium oxysporium*(5,6,7). M=DNA marker (100 bp).



Figure(3): A,C: The nitrogen bases of the gene 18srRNA for *Fusarium boothii*(1) and *Talaromyces*(2). B, D: The matching of nitrogen sequences bases of gene 18 sRNA for *Fusarium boothii*(1), *Talaromyces*(2) nitrogen bases sequences in genes bank NCBI for *Fusarium boothii*(1), *Talaromyces*(2).





Figure(5): I,K: The nitrogen bases of the gene 18srRNA for *Fusarium oxysporium*(5,6). J, L: The matching of nitrogen sequences bases of gene 18 sRNA for *Fusarium oxysporium*(5,6) nitrogen bases sequences in genes bank NCBI for *Fusarium oxysporium*(5,6).

References

- Avin, F.A.; Yee, S.B.; Tan, S.; Shahbazi, P.; Vikineswary, S. (2014). Molecular Divergence and Species Delimitation of the Cultivated Oyster Mushrooms: Integration of IGS1 and ITS. *Sci. World J.*, 793414.
- Beeck, O. D. M., Lievens, B., Busschaert, P., Declerck, S., Vangronsveld, J., & Colpaert, J. V. (2014). Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies." *PLOS ONE*, 9(6).
- Bellemain, E.; Carlsen, T.; Brochmann, C.; Coisacc, E.; Taberlet, P.; Kausrud, H. (2010). ITS as an environmental DNA barcode for fungi: An in silico approach reveals potential PCR biases. *BMC Microbiol.* 10, 189.
- Cheek, M, Nic Lughadha, E, Kirk, P. (2020). "New scientific discoveries: Plants and fungi," *Plants, People, Planet.* 2: 371-388.
- Haque, Z, Iabal, M.S., Ahmad A., Khan M. S and Prakash, J. (2020). Molecular Characterization of *Trichoderma* spp. Isolates by Internal Transcribed Spacer (ITS) Region Sequencing Technique and its Use as a Biocontrol Agent. *The Open Biotechnology Journal*, 14: 70-77.
- Schoch, C. L.; Seifert K. A.; Huhndorf S.; Robert, V.; Spouge, J. L.; Levesque, C. A. and Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi, *Proceedings of the National Academy of Sciences of the United State of America.* 109 (16): 6241-6246.
- Sheth, R. U., & Wang, H. H. (2018). "DNA-based memory devices for recording cellular events," *Nature reviews. Genetics*, 19(11): 718-732.
- White, T. J., Bruns T., Lee, S. and Taylor, J.(1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR- Protocols and Applications - A Laboratory Manual.* Academic Press. pp. 315-322.
- Yang, P., Shi, W., Wang, H., & Liu, H. (2016)."Screening of freshwater fungi for decolorizing multiple synthetic dyes," *Brazilian Journal of Microbiology*, 47(4): 828-834.

**EVALUATION THE NEEDS OF PLAY SPACES IN KINDERGARTEN FROM A
BIOPHILIC PERSPECTIVE (MOSUL AS A CASE STUDY)**

MAYSAA MOFFEQ ALOBAIDI



EVALUATION THE NEEDS OF PLAY SPACES IN KINDERGARTEN FROM A BIOPHILIC PERSPECTIVE (MOSUL AS A CASE STUDY)

Maysaa Moffeq ALOBAIDI ¹

Abstract:

Childhood represents one of the important stages in human life, in which the child's abilities develop and his talents open, and the child can influence, direct and form both physically and mentally. Playspaces are the best place in which the child can meet the needs of his body and psyche, so it has become necessary to take care of this space, which represents a good environment for the development of the child's potential and improving his social behavior, and recent studies have proven that the interaction of the child with nature in this type of space is one of the positive aspects That can contribute to the child's development, health and behavior, hence the importance of researching the needs of indoor and outdoor play spaces. In kindergarten buildings, which are the category of children aged (4-5) years, the research aims to evaluate the needs of indoor and outdoor play spaces for kindergartens from A Biophilic perspective, after surveying the opinions of mothers about the needs of their children in those places, which contributes to promoting Child health and behavior, and evaluation of the reality of indoor and outdoor play spaces in kindergartens in Mosul from a biophilic perspective, which achieves interaction and harmony with the environment and contributes to improving his health, behavior, and psyche.

Key words: Play Spaces, Kindergarten, Biophilic, outdoor, indoor, child space..



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Introduction:

Our children, in their different age stages, need to provide great care and attention that will enable them to grow up in a healthy environment, both physically and psychologically, which will be reflected in the future on their social and health behavior. Scientific, and that the possibility of exploiting the landscape can contribute to the development of the child's personality and enhance focus in children, and contribute to improving his behavior with others, especially in the play spaces of kindergarten buildings in which the child shares play with others, the research deals with assessing the needs of play spaces in kindergarten buildings Children in the city of Mosul, through the research methodology, in two phases. The first is to conduct a questionnaire to clarify the needs of mothers in the integration of their children's play space with nature in the kindergarten, and the second is to analyze the playing spaces for a group of kindergarten buildings in the city of Mosul from a biophilic perspective and to develop solutions and design treatments that contribute in the treatment and improvement of these spaces.

2. Child And Nature

Many studies have referred to the child's relationship with the external environment and its importance and direct impact on the child's performance, development, physical and emotional, the Contact with nature is important for a child's development, regardless of the children's race or socioeconomic status [1][2] Many experiments and studies have shown the many benefits of nature on the health of the child through lower rates of respiratory diseases and obtaining vitamin D from sunlight. They have shown an improvement in the child's motor activity and have provided him with appropriate weight rates due to the possibility of walking and sports in those areas that allow him to explore the environment and use Its different senses, in addition to social interaction and the participation of others[3].amid nature to discover the environment around it[4]. Therefore, we can conclude that nature affects the health, psyche, behavior, and development of the child, which is shown in Figure 1

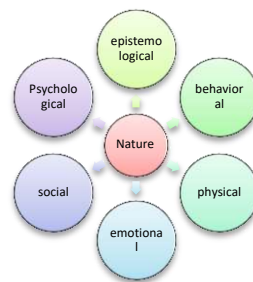


Fig1. The effect of nature on child (the researcher).

3. Children's Spaces From A Biophilic Perspective

It can be said that it is self-evident that the first years of a child's life witness physical, intellectual and psychological growth and development, in which play contributes a lot through the child's learning in these stages of a set of motor, intellectual and behavioral skills[5] An outdoor play space should be seen as a place where children can run and play and a therapeutic environment where it can meet the varying needs of different children[6], The children's play spaces include two types, indoor and outdoor, and when conducting an experiment about the time the child spends indoors with outdoors, the results were similar, as indoor play (63%) with outdoor play was play (59%) [7], A Comprehensive study conducted in Canada showed that it is preferable that the outdoor space allocated for each child's play in the outdoor play space is 13.5 square meters[4], In order for the playing spaces to be well-designed, there are a set of principles and conditions that must be fulfilled, represented by

(their location is good - available to all children of all kinds - fulfilling the child's psychological and kinetic needs - giving the possibility of sharing in games between children - safe for children - interactive and benefiting from the elements different nature)[8]. From the perspective of the interaction between the child and nature and the importance of using the elements of nature in these spaces known as biophilic spaces, experiments showed that children who frequently play spaces that interact with nature and contain natural elements have better motor skills, balance and coordination compared to children who play in traditional playgrounds.[9], And Kellert outlined the most important principles of biophilic design, which are: "Environmental Features-Natural shapes and forms- Natural patterns and processes- light and space- Place-based relationships-The development of relations between man and nature"[10], Show fig 2.

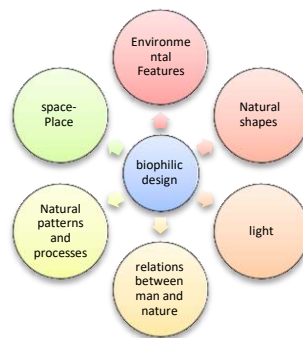


Fig 2. principles of biophilic design (the researcher).

4. Previous literature

Many recent studies have dealt with the relationship between the design of children's spaces and its relationship with nature and its impact on children and within more than one direction, Cengiz and Ozge Boz emphasized the importance of interaction between the child and the natural environment and the role of this in motivating the child, developing his skills and improving his performance with others, A comparison was made that showed that children who play in playgrounds designed according to biophilic principles are healthier, energetic and have better behavior.[9] McGee presented a set of biophilic features that can be designed based on play spaces in centers for the care of sick children, which can contribute to the healing of children and raise their immunity, which is a positive aspect in the interior design of these spaces represented by their natural features and their relationship with natural forms such as plants, animal forms, natural light and harmony spatial[11] and Nogueira gave a set of design criteria for the children's play floor through biophilic design, in a way that enhances the idea of harmony with the nature between man and the external environment and identified it through (contact with nature - materials - colors - safety and order - cultural and ecological connecting) [12], while Woodward and Zari indicated the importance of the education space being an enjoyable space for children by highlighting the importance of designing the natural spaces that exist in children's schools. It attracts them because of its importance in positively affecting their psyche and behavior.[13] It is the same design elements that came to it Park and Lee when analyzing a group of childcare buildings according to biophilic design principles by identifying biophilic design patterns and conducting a field survey of childcare facilities in Japan and the characteristics of their design patterns by clarifying the positive impact of those patterns on children's health and the most important design elements in those facilities and buildings [14].

All studies confirmed the importance of the relationship between the child's spaces and nature, which is expressed by the principles and elements of biophilic design in a way that

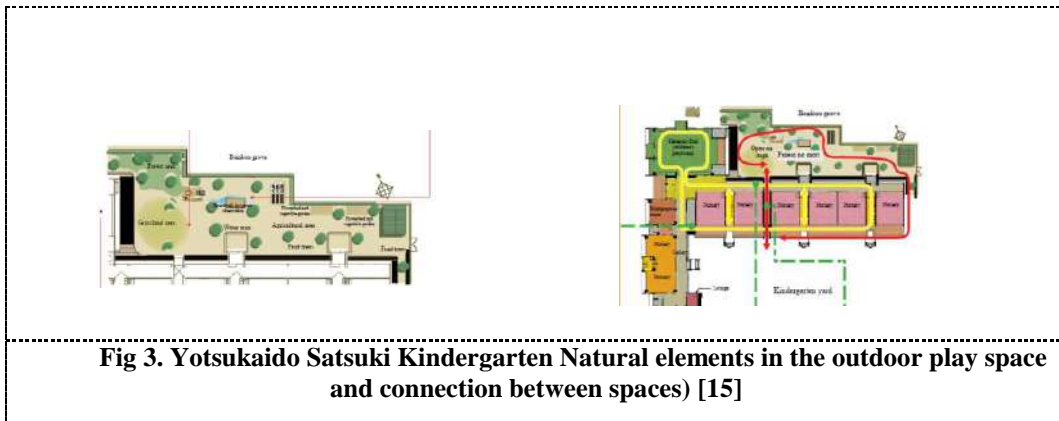
meets the child's health, psychological and behavioral needs and through what can be achieved when these elements interact in the design of play spaces in kindergarten.

5. Biophilic design for kindergarten buildings

In this paragraph, the research will discuss and analyze a set of international models of kindergarten buildings, which were designed in a way that deliberately integrates the building and its interaction with nature and according to the principles of biophilic design to deduce the most important mechanisms adopted by the designers in the spaces of the kindergarten in a biophilic style.

5.1. *Yotsukaido Satsuki Kindergarten- Yotsukaido City –japan 2010*

The designer's goal in Yotsukaido Satsuki Kindergarten is to develop children's sense of the natural environment and enhance their belonging to it by investing the space for outdoor play by planting plants, grass, fruits, and vegetables, and adding sand in the form of an external hill around which children run, In addition to the direct connection between the indoor and outdoor play space [15]see fig 3.



5.2. *Seiwa Kindergarten- Machida- japan 2019*

The building is an addition to a kindergarten erected in 1960. What is noticeable is the addition of a corridor between the two buildings fig 6., and this corridor was relied upon by the designer to make it an important space for children to play and gather, and a roof that allows the introduction of sunlight and natural light with the use of natural wooden furniture and planting plants inside it and the presence of birds to give a feeling of integration with nature As for the outdoor spaces They are natural and contain natural elements such as plants, sand, and water , see fig 4 [16]



5.3. Ombú Afuera Kindergarten- chilli -2021

The idea of designing outdoor spaces for playing is based on the principle of interaction with nature fig 8, which can provide a healthy and educational environment, in addition to adopting natural materials for furnishing such as bricks, while providing places for planting plants and vegetables, The design was used in organic fig 5, not geometric, and basket-like forms with different types of local tree [17].



From the examples presented in the previous paragraph, it becomes clear to us a set of mechanisms adopted by the designers of kindergarten buildings and from a biophilic perspective, these mechanisms will contribute to meeting the physical, health, psychological and behavioral needs of children, which we see in Table 1.

Table 1. The mechanisms of achieving biophilic design in the design of children's play spaces in kindergarten buildings

	Using and employing the various elements of nature (plant - animal - rocks - sand - water) .
Connection with nature	Diversity in the natural elements used (adding local trees - making water pools - adding sand hills) Adoption of natural materials in furnishing seating areas and umbrellas and adding stairs and ropes)
Inspired by natural shapes	Simulation of natural forms and elements of the natural environment (adding plant shapes and boxes for planting plants) Employing natural light in the design (direct – indirect) Inspiring natural shapes and bodies such as plants and animals by drawing them on walls and ceilings - being inspired by unnatural materials
Positive feelings of belonging	Use shapes that give a positive feeling and a sense of containment and safety Change through transition between natural elements

6. practical study

In this part of the study, a questionnaire was conducted by directing a set of questions to a group of mothers who have children in kindergarten to ask them about what are the elements that their children need in the playgrounds in kindergarten, which can contribute to achieving interaction between the child and his natural environment And shown in Table 3, the sample included 100 mothers. This questionnaire allowed us to identify what are the elements and forms that can be available in the children's play spaces and through which we can achieve satisfaction of the child's psychological, health, kinetic, and behavioural needs and their importance in those spaces.

Table 2. Questionnaire: Questions directed to mothers

Please answer the following questions for mothers who have children in kindergarten

child's age:

Kindergarten name:

1. Do you find it necessary to provide suitable playing spaces in the kindergarten that your child attends?	yes	no
2. Do you think that the play spaces in the kindergarten that your child frequents are suitable for his (kinetic) needs?		
3. Do you think that the play spaces in the kindergarten that your child frequents are suitable for his (psychological) needs?		
4. Do you think that the play spaces in the kindergarten that your child frequents are suitable for his (behavioral) needs?		
5. Determine according to importance. What are the elements that should be focused on when designing the indoor play space for your child's kindergarten? (Please arrange the answer in order of importance: ceilings / floors / walls / furniture / colors / openings		
6. What elements do you prefer to include in the interior spaces of children's play (which includes walls, ceilings and floors)? Determine according to the importance of the following (vegetal shapes (trees - flowers, etc.) - animal shapes (pictures or statues of animals) - organic shapes (mountains - rivers, etc.).		
7. Do you think that the indoor play space should have access to natural light and natural air?	yes	no
8. Do you prefer a connection between the indoor and outdoor play spaces in your kindergarten? (Through openings such as doors and windows)		
9. What do you prefer in designing the play space for your child's kindergarten?	internal external Part of the classroom	
10. What shape do you think will be most loved by your child in the play space?	organi c	geometri c
11. What material is the most suitable furniture for your child?	Natura l	unnatural
12. What is the most important feature that should be available in the play space for your child?	safe healthy Enjoyable	

In the second part of the practical study, a group of kindergarten buildings in the city of Mosul was analyzed, as a number of these buildings located in the city were visited by a researcher, and photographs were taken and the outdoor and indoor play areas were drawn in those buildings and evaluated from a biophilic perspective according to the research vocabulary collected in The theoretical framework of the research and the outcome of previous studies and global examples and determining what are the local needs for play spaces in kindergartens in the city of Mosul and the principles and mechanisms possessed by

these spaces according to the biophilic design, which can contribute to improving the child’s environment, growth and development, The following kindergartens are included:

Table 3. Research samples(Kindergartens covered by the researcher)

Kindergartens name	Outdoor space	Indoor space		
Alnebras				
Shumue al noor				
Alruwaad				
Ahabab allah				
Qamar alzeman				
Alashbal				
Aihbab Allah				
Almubdie Alsaghir				
Mays Alreem				

awl khutwata



Zomoreda

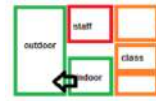


Table 4. Evaluation The Needs Of Play Spaces In Kindergarten From A Biophilic Perspective (researcher)

		I	O
X1 Connection with nature	X1.1 employing the various elements of nature	X1.1.1.growing plants	
		X1.1.2.Animals	
		X1.1.3.sand basin	
		X1.1.4.Water	
		X1.1.5.rocks	
	X1.2 Connecting with the outside nature	X1.2.1. direct	
		X1.2. 2.indirect	
	X1.3 not connected		
	X1.4 natural materials		
X1.5 Simulation of natural forms and elements of the natural environment	X1.5.1.adding local trees		
	X1.5.2.making water pools		
	X1.5.3. adding sand hills		
X1.6 natural light	X1.6.1.direct		
	X1.6.2.Indirect		
X2 Inspired by natural shapes	X2.1 space shape	X2.1.1.Organic	
		X2.1.2 geometric	
	X2.2 natural shapes	X2.2.1.Add drawn plant shapes	
		X2.2.2.Add drawn animal shapes	
	X2.3 furniture shape	X2.3.1.simulation of botanical shapes	
		X2.3.2.simulation of animal shapes	
X3 Positive feelings of belonging	X3.1 Positive feelings of belonging	X3.1.1.Safe(Furniture and flooring suitable for children)	
		X3.1.2.Healthy(Natural lighting and ventilation)	
		X3.1.3.Enjoyable(Variety of games and colors)	
	X3.2 Change through transition	Variety of floor treatments	
		X3.2.1.	
		X3.2.2.Variety of color	
	X3.2.2.Make transitions between spaces		

Table 5. Checked values (researcher)

K in d er g ar te ns	X1					X2										Pl a y S p a c es					
	X1.1					X1.2	X1.3	X 1. 4	X1.5			X1.6		X2.1			X2.2		X2.3		
	X 1. 1 1	X 1. 1 2	X 1. 1 3	X 1. 1 4	X 1. 1 5	X1 .2. 1.1	X1 .2. 1.2	X 1. 5 1	X 1. 5 2	X 1. 5 3	X1 .6. 1	X1 .6. 2	X 2. 1 1	X 2. 1 2	X 2. 2 1		X 2. 2 2	X 2. 3 1	X 2. 3 2		
A	*					*			*		*		*								O i
B	*					*		*		*		*		*	*	*	*	*	*	*	O i
C	*					*		*		*		*		*	*	*	*	*	*	*	O i
D	*					*		*		*		*		*	*	*	*	*	*	*	O i
E	*					*		*		*		*		*	*	*	*	*	*	*	O i
F	*		*	*		*		*	*	*		*		*	*	*	*	*	*	*	O i
G	*					*		*		*		*		*	*	*	*	*	*	*	O i
H	*					*		*	*	*		*		*	*	*	*	*	*	*	O i
I	*					*		*		*		*		*	*	*	*	*	*	*	O i
J	*					*		*		*		*		*	*	*	*	*	*	*	O i
k	*					*		*		*		*		*	*	*	*	*	*	*	O i

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Kindergartens	X3						Play Spaces
	X3.1			X3.2			
	X3.1.1	X3.1.2	X3.1.3	X3.2.1	X3.2.2	X3.2.3	
A	*	*			*		O i
B	*	*	*	*	*	*	O i
C		*			*		O i
D	*	*			*		O i
E	*	*	*			*	O i
F	*	*			*	*	O i
G		*	*				O i
H	*	*	*		*		O i
I					*		O i
J	*	*	*		*	*	O i
k	*	*	*	*	*	*	O i

7. Discuss the results

5.4. The results of the first part of the practical study, represented by a set of questions that the researcher asked a group of mothers, showed the following: 48% of the mothers considered that the play spaces in their kindergartens are not suitable for their children's motor needs, due to the small area of space, especially the indoor play spaces, while 66.7% of them considered it suitable for their children's behavioral needs, and that For the possibility of participation in play between the child and other children when playing, which is appropriate by 77.8% for his psychological needs.

- the mothers agreed 100% on the importance of providing lighting and ventilation in these indoor and outdoor spaces for the health of children and the importance of achieving direct contact between the indoor and outdoor play space by 74.1%, and they differed Their opinions about the type of connection between the indoor and outdoor play space with other spaces, as 40% considered it important that it be connected and close to the classes and even part of it, and 40.8% preferred it to be outdoor, while only 10.2% preferred it to be indoor.

- When asked about the design of the indoor play spaces, the mothers' opinions differed in the arrangement of its elements, but most of the answers focused on the design of the floors and giving them priority as they are the most elements that can provide a safe environment when children fall when playing, and that plant shapes come to the fore in the details that can excite their children Then animal, organic

- And 55.6% said that the organic shape would be closer to their children than the geometric shape chosen by 44.6%. As for the nature of the furniture, the research found that 66.7% of the mothers.

- 60% of the mothers agreed that the playing space should be safe first, then healthy and then enjoyable to reduce the risk of injuries to the child while playing and moving.

5.5. The results of the analysis of samples of kindergarten buildings in the city of Mosul, which the researcher visited to assess the indoor and outdoor play spaces from a biophilic perspective:

- Connection with nature: The results of the analysis showed that the outdoor play spaces achieved 100% of contact with nature, but they relied on employing the outdoor garden as an outdoor play space and planting local plants, while most of them lacked the use of water, rocks and sand elements, and the researcher noted the lack of all spaces to use toys made of natural materials It is limited to games made of metal and plastic, which are spaces that enjoy 100% natural lighting. As for the internal spaces, 30% of them are not directly connected with the external spaces, and they represented internal spaces within the building, so they lacked the necessary natural lighting for children's spaces.

- Inspired by natural shapes: The results showed that all external and internal spaces took geometric shapes, and kindergarten buildings lacked an organic form in the playing spaces, and the spaces relied on adding natural shapes inspired by animal shapes, especially in the interior spaces, and 90% of the selected samples, while 50% of the outer spaces The shapes of furniture were inspired by animal shapes in most of their forms. In the interior spaces, 70% of the samples represented the shapes of furniture simulating the shapes of animals, and only 2% in the outdoor spaces.

- Positive feelings of belonging: 80% of the external spaces are safe and 100% healthy, enjoying light and natural ventilation, 50% are pleasant, while 70% of the internal spaces are safe spaces whose floors have been treated, 60% are healthy for their lack of light sources and natural ventilation, and 70% are diverse pleasant spaces The shapes and colors in it, most of the external and internal spaces lacked diversity between floor treatment and were limited to the diversity of colors in the internal playing spaces.

Conclusions


In the early stages of development, the child needs to provide an appropriate healthy environment that meets his health, psychological and behavioral needs. The biophilic theory is one of the theories that can achieve these needs, especially for the play spaces, which represent one of the most important spaces in kindergartens, and recent studies have proven that the interaction between the child and nature in this The spaces can contribute significantly and effectively to meeting the needs of the child and his development, and the research has identified these needs and their values from the samples dealt with in the research in the practical study, as the researcher found the importance of emphasizing on providing an interactive environment with the child in the indoor and outdoor play spaces because of the importance of this for the child and his development And that the space enjoys adequate lighting and ventilation, healthy for children, taking into account the direct contact between the interior and the outside, and that the various elements of nature such as water, rocks and even some pets such as birds are available in these spaces because of its positive repercussions on the health of the child, and the roof can be employed to establish gardens and play spaces in case There was not enough space for these buildings, the research suggests creating a natural environment and employing natural elements within the indoor play spaces. By adding elements such as sand and rocks basin that invest in children's toys, as the research noted that most of the samples of kindergarten buildings in the city of Mosul did not invest these elements in the interior spaces, and these spaces also need treatments at the floor level first through the use of appropriate and protective tiles when the child falls And the treatment of walls with a diversity of colors and shapes, and the research suggests that the importance of kinesthetic and visual communication between the internal and external play spaces because the communication between the spaces creates a diversity in movement, colors and transition and is important for the child's psyche.

References

1. Chawla L *Significant life experiences revisited: A review of research on sources of environmental sensitivity*. Environmental Education Research. 1998;4:369–382
2. Faber Taylor A Kuo FE 2006. *Is contact with nature important for healthy child development? State of the evidence*. In: Spencer C, Blades M, editors. *Children and their environments: Learning, using and designing spaces*. Cambridge (UK: Cambridge University Press) pp 124–140.
3. Chawla L 2015 *Benefits of Nature Contact for Children*. *Journal of Planning Literature* Vol. 30(4) pp 433-452.
4. Dudek M 2007 *A DESIGN MANUAL: Schools and Kindergartens* Birkhauser Verlag AG Basel- Boston –Berlin .
5. Dudek M 2005 *Children's Spaces*(Architectural Press An imprint of Elsevier Linacre House) Jordan Hill, Oxford OX2 8DP pp198 .
6. Owens P 2009 *Designing for Children's Needs: Creating an Outdoor Play Environment for the Yolo Crisis Nursery* .
7. Storli R and Beate E 2019 *Children's play, well-being and involvement: how children play indoors and outdoors in Norwegian early childhood education and care institutions*. international journal of play 2019, vol. 8, no. 1, 65–78 <https://doi.org/10.1080/21594937.2019.1580338> .
8. Shackell A Butler N Doyle P and Ball D 2008 *Design for Play: A guide to creating successful play spaces* (DCSF Publications-Crown)Play England Big Lottery Fund copyright.
9. Cengiz C and Ozge Boz A 2019 *Biophilic Playgrounds as Playscapes in Child-Nature Interaction* International Journal of Scientific and Technological Research . Vol.5, No.12 .
10. Kellert S and Calabrese E 2015 *The Practice Of Biophilic Design*. <https://www.biophilic-design.com/> .
11. McGee B 2012 *An Inventory Of Biophilic Design Attributes Within Child Life Play Spaces*. A Thesis Submitted to the Faculty of the Graduate School at The University of North Carolina at Greensboro in Partial Fulfillment of the Requirements for the Degree Master of Science.
12. Nogueira P 2017 *Biophilic Playspace Design: Bringing Together Children And Nature In Urban Honolulu* A Darch Project Submitted To The Graduate Division Of The University Of Hawai'i At Mānoa In Partial Fulfillment Of The Requirements For The Degree Of Doctor Of Architecture
13. Woodward E and Zari M 2018 *Reconnecting Children With Nature: Biophilic Primary School Learning Environments* 2018 ZEMCH International Conference, Melbourne, Australia.
14. Park S and Lee H 2019 *Spatial Design of Childcare Facilities Based on Biophilic Design Patterns*. <https://www.mdpi.com/journal/sustainability>, Sustainability 2019, 11, 2851. doi:10.3390/su11102851.

III. International Scientific Congress of Pure, Applied and Technological Sciences (Minar Congress)

15. A Collection of Exemplary Design of Kindergarten Facilities, Based on the Revision of Guidelines for Designing Kindergarten Facilities.2010
16. <https://www.designdaily.net/project/seiwa-kindergarten/>
17. <https://www.archdaily.com/966642/ombu-afuera-kindergarten-andres>



**DETERMINATION OF THE ACTUAL INTENSITY BY CORRECTION OF THE EMISSION
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**AHMED ALI AKBAR
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DETERMINATION OF THE ACTUAL INTENSITY BY CORRECTION OF THE EMISSION SPECTRUM LINES OF HEAVY METALS CONTAINED IN CRUDE OIL USING LASER INDUCED PLASMA –TECHNIQUE

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Abstract:

Laser induced plasma spectroscopy (LIPS) is a novel technique for elemental analysis based on laser-generated plasma. In this technique, laser pulses are applied for ablation of the sample, resulting in the vaporization and ionization of sample in hot plasma which is finally analyzed by the spectrometer. The elements are identified by their unique spectral signatures. The plasma was produced using the fundamental harmonic (1064 nm) of the Nd: YAG laser and the emission spectra were recorded at 3.3 μ sec. detector gate delay to study the capabilities of LIPS as a rapid tool for material analysis. LIPS method was developed for elemental analysis of the crude oil. Because of the special features of crude oil as a liquid organic material which makes the analysis process difficult and inaccurate. This difficulty is concentrated due to the effect of the self- (absorption and stimulation), so the properties of the spectral analysis lines of the laser induced plasma spectrum (LIPS) are severely affected, and thus affecting the evaluation process accuracy. In this effort, there was an ability to determine the qualitative evaluation of the various heavy metals in a crude oil sample. For the large number of these metals, three minerals (Fe, Ni and V) were identified for the purpose of clarifying the method, After determining the optimal experimental conditions to improve the sensitivity of the developed LIPS system through the optimal selection of the most important parameters. Here, an analysis method based on global reference line selection was proposed for optimal estimation of the analytic lines properties of the plasma spectrum (actual intensity I_c , optimum temperature T_e , highest stability score S_s and without self-strain f_s), to improve the analysis accuracy of a crude oil sample using LIPS - technical. The optimum stability score (OSS) method was used to estimate the plasma temperature based on the calculation results from the Boltzmann diagram. Furthermore, the internal reference line for each specific mineral was considered while the correction was made for the analytical lines.

Key words: Heavy Metals Assessment, Crude Oil, LIPS-Technique, Correction The Self-Strain Effect.



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Introduction:

Laser Induced Plasma Spectroscopy (LIPS) is a useful technique that requires optical access for the sample surface and can be used as a fast method for the qualification and quantification of the contaminants in various categories of samples. It is an emerging technique for elemental determination in a wide range of environmental samples, metallurgical, metallic and non-metallic, sewage sludge, liquid, aerosol, gases and biological samples etc [1-7]. Several elements can be measured with this technique simultaneously even at parts per million concentration level. Recent developments towards improving analytical capabilities of this technique have led to more applications of LIPS. However, still more efforts are required to improve the precision and the sensitivity of this technique. Some improvement in methodology is needed for its wide applications in terms of its efficiency and limit of detection [11-13]. The assessment and characterization of large variety of samples of various natures require special technique like LIPS. Some capabilities of LIPS as a rapid tool for analysis of sample are pollution monitoring in air, soil, sewage sludge, ground and wastewater, determination of ore composition during mining, chemical analysis of explosive materials, forensics and biochemical applications, detection of radioactive contamination and hazardous materials. This technique can also be used for rapid identification of metals and alloys during recycling of scrap materials, on-line compositional analysis of the molten metals, on-line compositional analysis of liquid glass for process control (iron, lead etc), depth-profiling and compositional analysis of surface coatings (galvanized steel, plastic coating and heavy-metals in paint), on-line monitoring of particulates in air (stack emission monitoring etc.) [14].

In LIPS minimal sample preparation is required which reduces worker exposure to hazardous radioactive or toxic substances. LIPS requires small amount (μgm) of sample, and element detection limit can be achieved in the range of ppm/ppb. In addition to its potential benefits, there are some inherent disadvantages of this technique: [15-16]. The qualification and quantification of heavy metals within liquid samples are pertinent to industrial processing, environmental monitoring and waste treatment.

In the last decade laser-induced breakdown spectroscopy has become a prime area of scientific and technological interest for researchers in the various fields of science. To-date, laser-induced breakdown spectroscopy (LIPS) has been tested with limited success for liquid samples.[17]

The technique is based on the generation of laser-induced plasma on a solid, liquid or gaseous sample by focusing a high-power pulsed laser beam onto the sample and the collection of light emitted from the plasma to be used to determine the elemental composition of the sample. Since its inception, the LIPS-technique method has been used by several research groups across the world to analyze metallic alloys, such as Al-based [19,20], Fe-based [20], Cu-based [21–25] and Au-based [23,26] alloys as well as non-metallic samples, such as soil, rock and glass [23,25,27–29].

In the present work appropriate choice of experimental parameters and plasma conditions are to be applied to obtain accurate assessment results using the LIPS technique and out -coming results should be presented and discussed.

2. Experimental Details

The experimental arrangement of the LIPS set-up which was built is shown in the Figure (1). Where the system of experimental work was designed and included the followings:

1. A pulsed laser source (Nd: YAG) Laser that generates the powerful optical pulses with beam energy of 2000 mj, the wavelength of (1064 , 532) nm, and the pulsed duration of 10 ns which are used to form the plasma plume. The laser spot on sample is 400 μm in radius using a lens of focal length of 10 cm with a pulse energy of 500 mj, where the irradiance is 63.69 j/cm^2 .
2. A lens is used in order to direct and focus the laser beam on the aquatic material (target).
3. A target holder for the optical insulator.
4. X-Y-Z stage is used to remove the target when needed.
5. A light collection system was used to collect and direct the spark light into a fiber optical cable (FOC), which is used to collect the light and send it to the spectrometer.
6. A detection system (spectrometer/detector).
7. A computer (PC) is applied to control both the laser and the detector to store the spectrum.

The optical emission of crude oil sample is collected by the lens and is focused on to optical fiber which delivers the plasma light to the entrance slit of spectrum analyzer (with wavelength range (320-740) nm , which serves to deflect light according to wavelength and then reversed by mirrors, then focused to detect and convert optical signals to digital, and then transport the digital signal for application. The spectral lines of the sample is then analyzed. Finally, the information is transmitted to a PC where it is processed with the Spectra Suite software. This is to be done in order to obtain better quality assessment of heavy metals (HMs) in the crude oil.

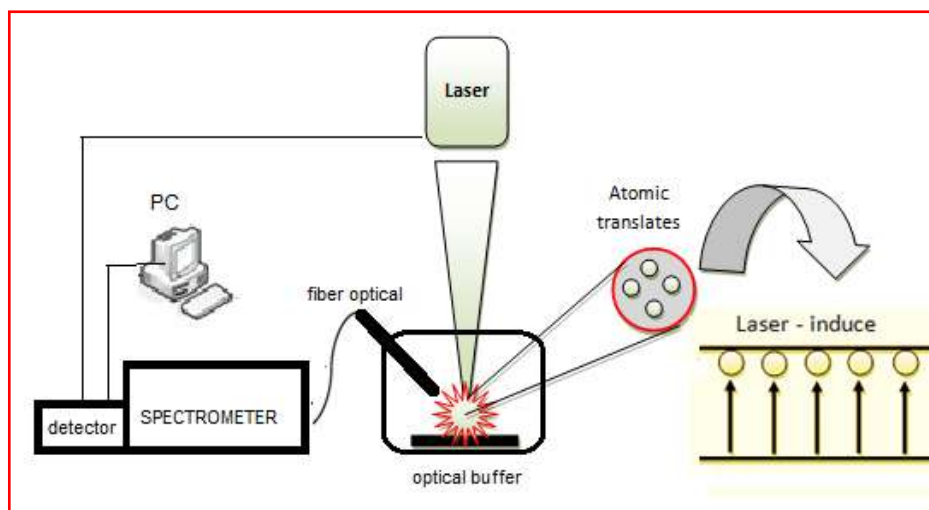


Figure 1 Schematic diagram of the experimental set-up.

3. Results And Discussion

3.1. Selection and Correction of the Emission Spectra Lines

Figure 2 below represents the emission spectra of all the elements and minerals present in the crude oil with the spectral range between 320 and 740 nm. It consists of many strong and weak emission lines of the components found in crude oil. Typically, the strong emission lines belong to the components with a higher (main) concentration, while the weak lines come from the elements of lower (weak) concentration. The emission spectra analysis was performed using the software based on linear correlation with the NIST atomic database.

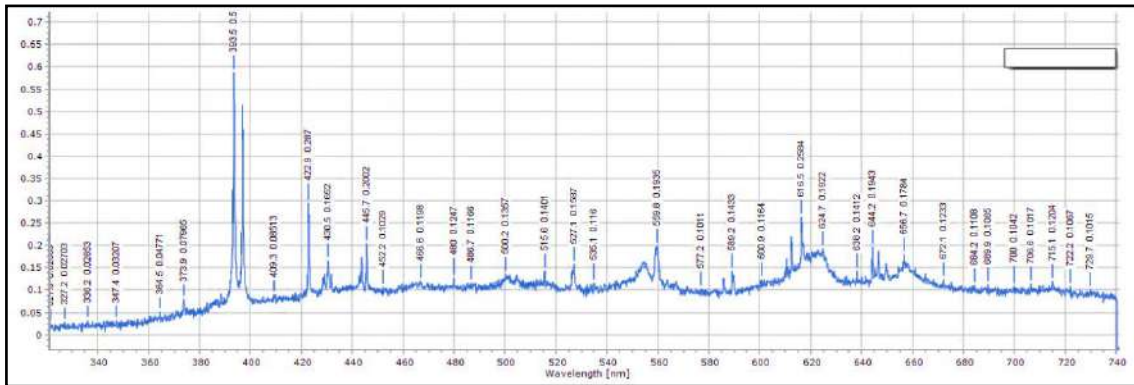


Figure (2) The emission lines spectrum of plasma by using laser pulse energy of 500 mj and 1064 nm of the Nd:YAG laser

Selected heavy metals were "discovered" and had many spectral lines in the original ore spectrum. The original full spectrum from 320 to 740 nm was consisting of hundreds of spectroscopic lines, and it was analyzed and about 33 heavy metals were found as shown in table (1). Three minerals in crude oil, Fe, Ni, and V were chosen as an illustration of the analysis process. In the LIPS technique, the intensities of the emission lines are used to determine and calculate plasma properties such as temperature and electron density to identify heavy metals above in the crude oil.

Table (1) the heavy metals present in the analyzed crude oil sample

O.	N									0	1	2	3	4	5	6	7
etal	M	<i>e</i>	<i>i</i>	<i>r</i>	<i>u</i>	<i>o</i>	<i>e</i>	<i>n</i>	<i>o</i>	<i>c</i>	<i>b</i>	<i>d</i>	<i>i</i>	<i>a</i>	<i>m</i>	<i>a</i>	

O.	N	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3
etal	M	<i>e</i>	<i>r</i>	<i>m</i>	<i>h</i>	<i>u</i>	<i>h</i>	<i>i</i>	<i>f</i>	<i>r</i>	<i>u</i>	<i>y</i>	<i>d</i>	<i>g</i>	<i>b</i>		

The criterion for checking whether LIPS plasma is optically thin, is necessary for quantitative analysis of components and metals, using LIPS spectra of a sample. This is obtained from the ratio of the two line densities of a given element in the same charge state Z, where they should be the same as the ratio of their corresponding transmission probabilities.

These assumptions must be validated before performing a quantitative LIPS analysis. The optically thin state of the plasma is necessary to ensure that other atoms in a lower energy state do not re-absorb the radiation emitted by an excited atom and to avoid the so-called self-absorption effect. In the absence of self-absorption, the intensity ratio of two emission lines of the same type, having the same higher energy level, should be the same as the ratio of their corresponding transmission probabilities. The experimentally observed intensity ratio of different spectral lines of Fe I, Ni I and VI was used and compared with their transition odds ratio.

Table (2) displays the intensity ratio and transition probability ratio between the two Fe lines of 479.91 nm and 559.84 nm, Ni lines of 450.81 nm and 527.05 nm, and V lines of 412.23 and 569.30 nm. The intensity ratio of FeI/FeI was 0.57 and their transmission ratio was different. The density ratio of NiI/NiI was 0.663 and their transmission ratio was 0.838. The density ratio of VI/VI was 0.803 and their transmission ratio was 5.89. A mismatch is observed between the intensities of Fe, Ni, V and their corresponding transition probability. This confirms the presence of self-absorption by examining the sample.

Table (2) Comparison between the intensity ratio of two emission lines of Fe (I), Ni (I), V (I) and the ratio of their corresponding transition probabilities before correction procedure.

No.	Metal	I	Wa (nm)	E (eV)	A	Ratio of I	ratio of A
1	Fe I	0.110704	479.91	6.86610	9.83E+0	0.57	5.4*10-3
2	Fe I	0.192639	559.84	6.86610	1.82E+0		
3	Ni I	0.1053	450.81	6.292711	7.08E+0	0.663	0.838
4	Ni I	0.1587	527.05	6.292711	8.44E+0		
5	V I	0.078815	412.23	5.27597	2.41E+0	0.803	5.98
6	V I	0.09812374	569.30	5.27597	4.03E+0		

Since the material used in the analysis process is a liquid and organic (hydrocarbon) crude oil, the examination process was difficult due to the presence of the problem of self-absorption in the profile of the analysis lines, which led to a reduction and a difference from the actual value of the intensity of the spectral lines.

Thus, it leads to an inaccurate quantitative analysis process. Self-absorption appeared when the plasma light was re-absorbed by cold atoms along the optical path making the line shape flat or in extreme cases showing a dip in the center of the line.

Therefore the true values of the integrated densities of the selected emission lines are to be determined and discovered, through several important steps.

3.2. Selection of the Global and Internal Reference Line (GRL & IRL)

For all emitted spectral lines, the origin line is specified as the reference for all spectral lines. This line is chosen because it has a high degree of stability, which depends on it in the process of correction for other spectral lines that have a lower degree of stability, and the

selection process depends on two important parameters of the properties of spectral lines, namely (lower level of energy and the probability of transmission). According to Equation 1, the degree of stability of the spectral lines is calculated, where the line with the highest excitation energy for the lowest energy level with the lowest transmission probability is less affected by the absorption and self-excitation problems, so it will be chosen as the reference line for the spectroscopy lines and shown in table (1).

$$\text{Stability score} = E/A \dots \dots \dots (1)$$

Where: E = low energy level and A = transition probability

In figure (3) the red line represents the peak power that runs between the upper and lower point. The green line represents the average power, which represents the largest triangle area under this curve for these points, where the touch point of this green line is called the curve (touch point). The point of intersection of this green line with the temperature axis, gives the highest peak in the triangle under the curve, and represents the heat content of all spectral lines (actual plasma temperature).

The area between the point of intersection and the touch point is represented by the full wave half maximum (FWHM) area, where the spectral line with the highest stability score corresponding to the point of intersection of the average power line (green line) with the temperature axis is the line that will represent GRL. Where GRL is the strong spectral line with the highest degree of stability and is located at the peak of the triangle representing heat content, located at the top of the FWHM region.

For this reason, focusing on GRL was done in the process of calculating the plasma parameters for property correction (intensity, temperature, electron density and skew degree) of other emitted spectral lines that have lower stability.

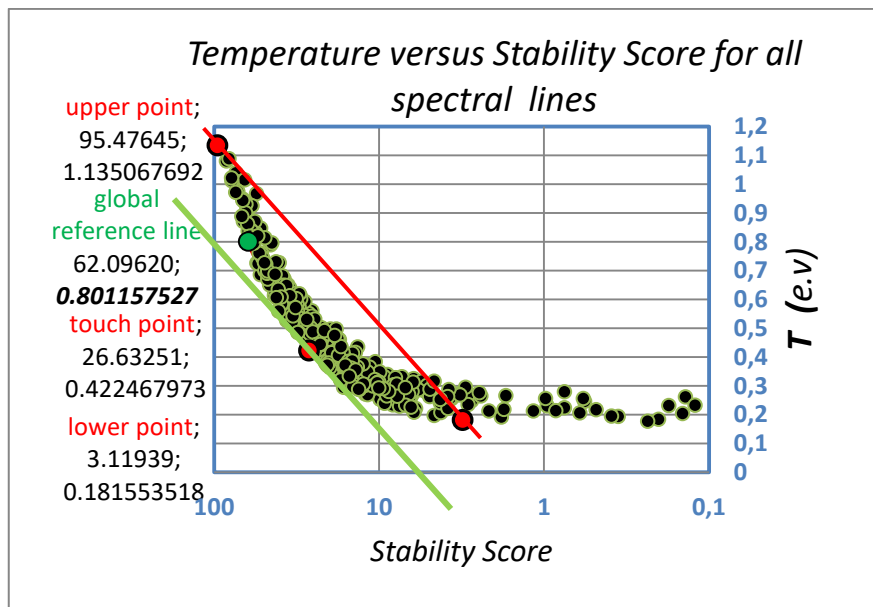


Figure (3) the stability score with temperature of each analytical lines

The following figures (4A, B and C) show the value (Sc) of Fe, N and V through which the IRL of each metal is determined, that represents the strong spectral line for each metal, and it is less affected by the problem of self-absorption compared with other spectral lines for the same metal, since it is characterized by the highest degree of stability and the lowest absorption coefficient for the spectral analysis lines of the specific metal.

For each plasma spectrum, the spectral line with the highest stability is determined as the global reference line based on equation (1), where there is one line for each plasma spectrum. While the specific internal reference lines. One internal reference was chosen for each mineral present in the specified crude oil sample, while there was more than one internal reference line. As shown in the figures below, the internal reference lines for the same selected sample are different. Because the samples contain many different metals, but three metals Fe, Ni and V were chosen as examples.

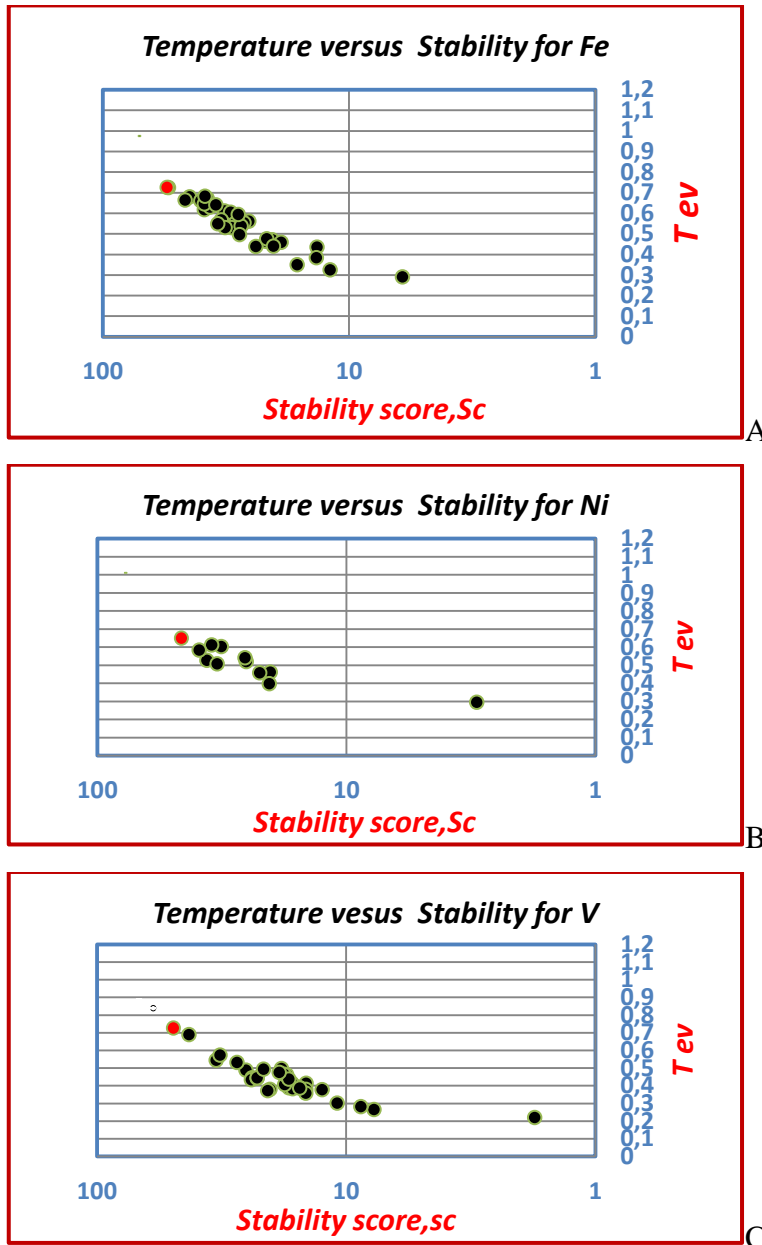


Figure (4 A,B and C) show the stability score with temperature of analytical Lines of Iron, Nickel and Vanadium

According to the stated stability score (Sc) which was calculated by the mathematical selection of reference lines in the section before, a number of analytical lines for heavy metals in the crude oil sample were selected from the specified spectrum. Some of these analytical lines are (Cr), (Fe), (Sc), (Ti) and (V), which are shown in Table (3) where the first ten lines that have a high degree of stability for all types of components of the crude oil sample. Table

(3) shows the spectral parameters of the specific GRL extracted from Figure (5) below, which represents the spectral lines emitted by the generated plasma.

Table (3) The top ten lines have highest stability score.

NO.	element	W	E _i	A	Stability Score	Type
1	Cr I	606.09	6.006451	1.59E+04	62.09620	GRL
2	Cr II	713.11	6.577529	4.02E+04	62.03830	AL
3	Cr II	577.60370	8.30844	7.24E+05	61.57990	AL
4	V II	678.0857	9.418584	5.54E+06	60.66024	AL
5	Fe II	669.321	7.128263	1.58E+05	59.54933	AL
6	Fe II	669.321	7.128263	1.58E+05	59.54933	AL
7	Sc II	476.6061	8.011334	1.45E+06	56.45828	AL
8	Ti II	596.71640	8.10827	1.33E+06	57.51798	AL
9	V II	654.56	9.340436	6.65E+06	59.45316	AL
10	V II	654.57750	9.34044	6.65E+06	59.45316	AL

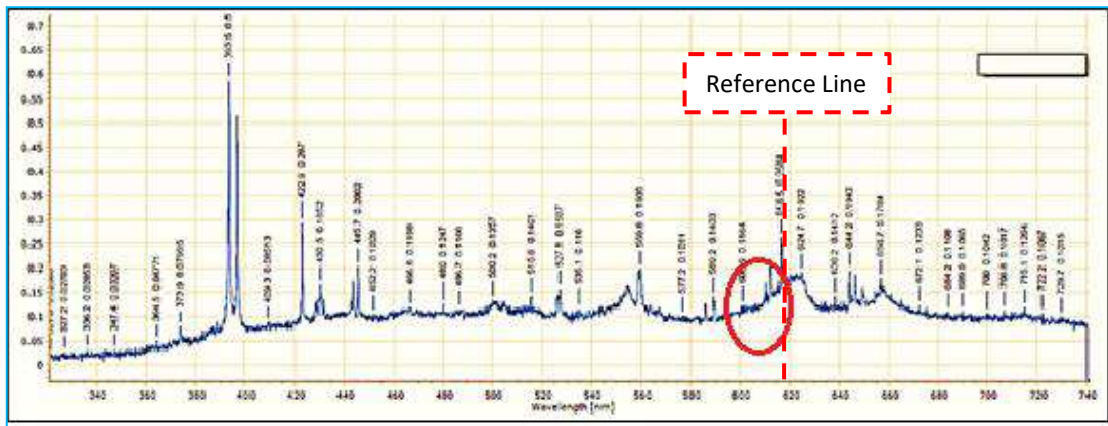


Figure (5) The emission spectra lines of the plasma generated

However, in this procedure, only the properties of the analytic lines are corrected. The slopes of the spectral lines of all components are inconsistent, scattered and random indicating that the analytical lines of each component are differently affected by the effect of self-induce and absorption.

The initial Boltzmann diagram is shown in Figure (6) which is based on the specific emission lines of the components prior to the rectification process. It can be seen that the points are randomly distributed with a very weak linear relationship. It is caused by a decrease in the intensity of the analytic lines, and is the result of the effect of self-(induce and absorption). Moreover, the analytic lines of the excitable for different components and metals in the initial Boltzmann diagram are not parallel, indicating that the effects of self-induction in all analytic species led to different degrees of intensity decrease in their spectral lines as shown below.

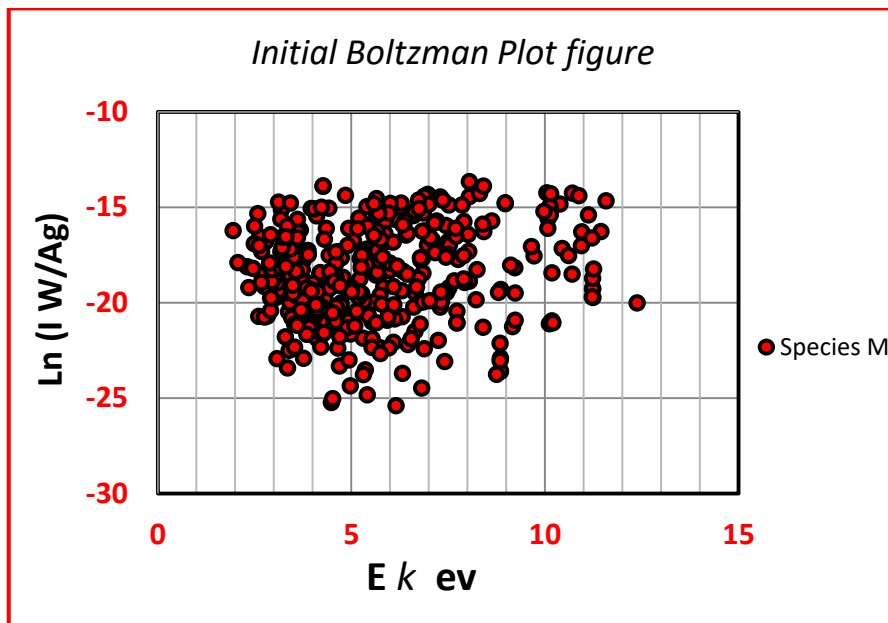


Figure (6) showing the Boltzmann plot before correction

3-3-Self-(Strain coefficient) Correction of the Internal Reference Line and Analytical Lines

In Figure (7) the optimum temperature is searched for the range of [1.2-0.2] e.v which was obtained from the Boltzmann diagram. This represents the temperature of the distorted spectral lines before the correction process (shown in Figure (7)), and after calculating the optimum plasma temperature and correcting the self-skewness of the spectral analysis lines by choosing the global reference line, the calculated plasma temperature is (0.801), which is the same as the global reference line temperature.

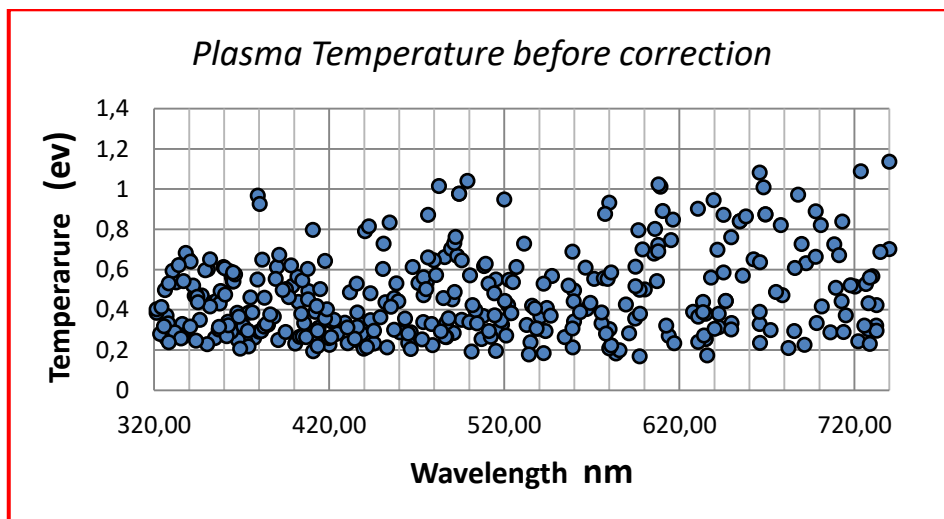


Figure (7) The extracted temperature before correction of the self-absorption of heavy metals lines (HMs).

The plasma temperature corresponding to the best stabilization value is the optimum temperature, Where the steps for determining the optimum stability score (OSS) were shown in Figure (3), Where the fitness value stabilizes at a corresponding plasma temperature of (0.801) e.v as given in figure (8) below.

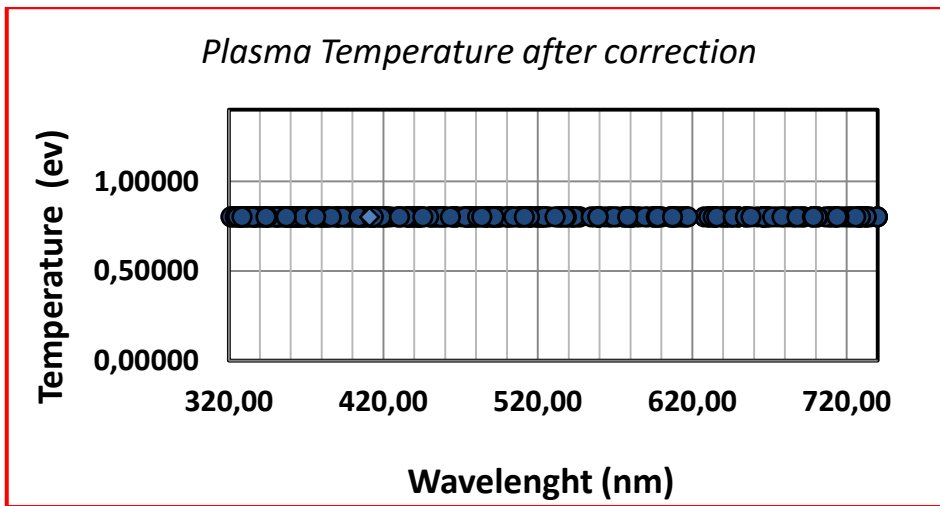


Figure (8) The extracted temperature after correction of the self-absorption of heavy metals (HMs) by PSO method .

Thus, a Boltzmann diagram is drawn using intensity-corrected analysis lines as shown in Figure (9) and optimally, temperature was estimated by OSS algorithm. Points for each type of component and metal found are closely distributed over the fitted line. In addition, the lines fitted to the different components tend to be parallel and identical to each other as shown before.

The Boltzmann diagram was constructed using a set of emission lines defined by drawing the left-hand side (LHS) of the equation (2) as a function of the upper state energy E_k and the linear fit over these data points extracted the corrected intensity values after determining the optimum plasma temperature, which is extracted from the slope ($1/K_B T_e$) in equation(2).

$$I/A_k g_k = \{C_s F / U_s(T)\} \exp(-E_k/K_B T) \dots\dots\dots (2)$$

$$y_k = \ln \frac{I_k^{ki}}{A_{ki} g_k}, \quad x_k = E_k, \quad m = -\frac{1}{k_B T}, \quad q_s = \ln \frac{C_s F}{U_s(T)}$$

$$y_k = m x_k + q_s$$

Figures (6) and (9) show Boltzmann plots building using specific emission lines before and after self-(absorption and induce) correction. It is evident from Figure (6) that the Fe, Ni, and V data points are relatively more dispersed due to self-induction and, therefore, did not yield the true values of plasma temperature and metal concentration.

Boltzmann plots were reconstructed by the data points of the Boltzmann plot after correction and emission intensity correction as shown in Figure (9) which shows good correlation, with data points span along the fitted lines without scattering without the effect of self-induction in the emission lines thus, the intensity of the extracted analytic lines and the optimum plasma temperature and inclination were relatively more accurate when extracted from the slope ($1/k_B T_e$).

Figures (6) to (9) showed that the Fe, Ni and V data points are relatively smoother due to self-(induce and absorption) evaluation and correction, thus, yielding the true values of the properties of the lines of analysis.

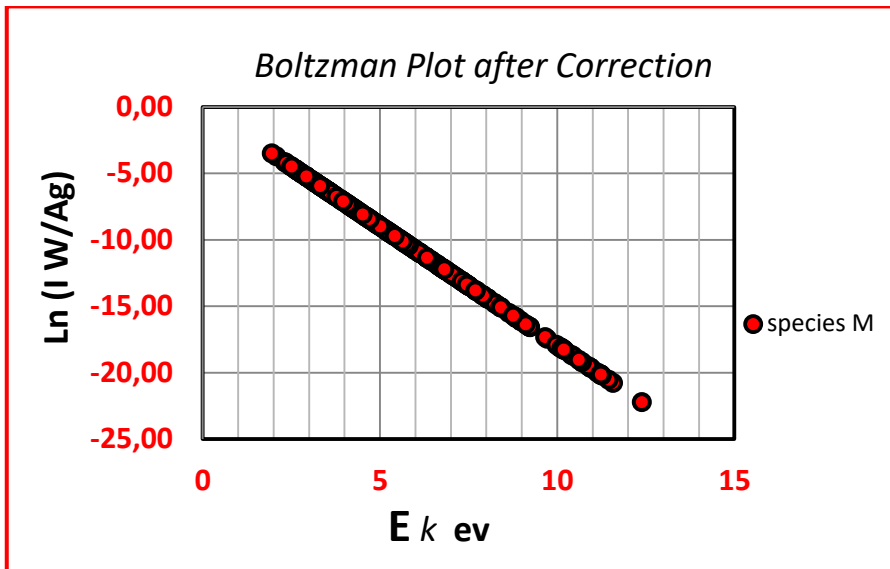


Figure (9) The Boltzmann plot after correction of the reference line of all heavy metals (HMs) by OSS method

Using $\ln(IW/Ag)$ and replacing the new intensity which was obtained by the correction process, figure (10)A is obtained below, which represents the emitted spectrum lines of the plasma without the effect of corrected self-(induce and absorption) Figure (11) A,B,C show the intensity after correction of self-absorption for each limited heavy metals(Fe,Ni and V).

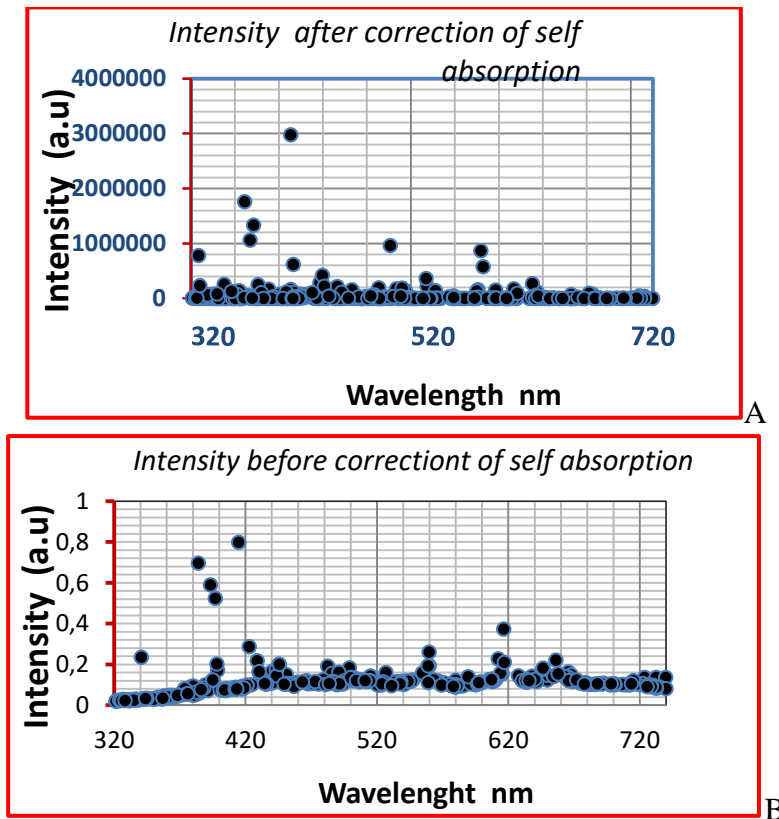


Figure (10) A: The emission spectrum lines of the plasma generated after correction of all heavy metals lines ,B: before correction

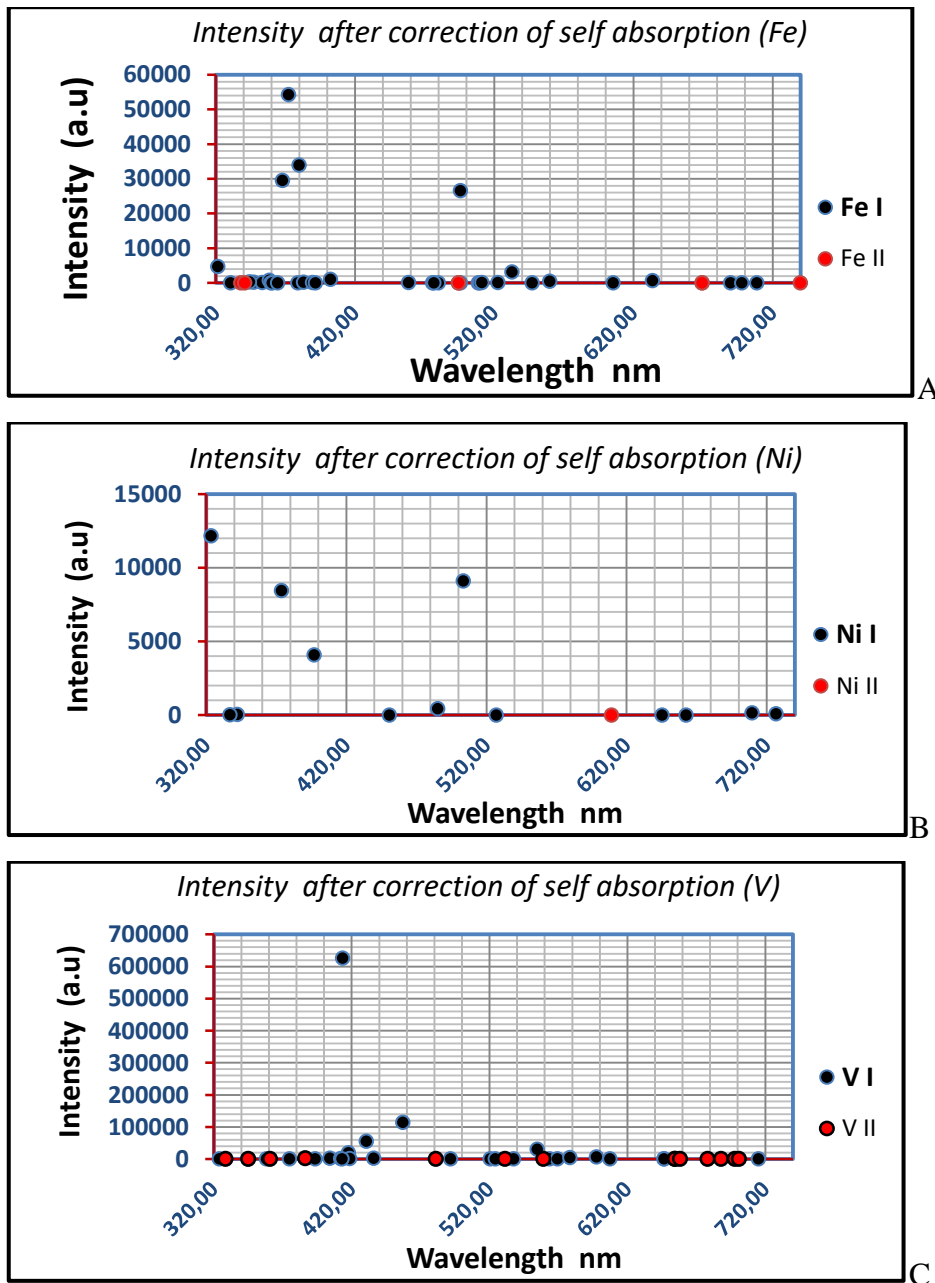


Figure (11) showing the emission spectrum lines of the plasma generated after correction of all Iron, Nickel and Vanadium metals lines

In the present work and as mentioned above the optimum stability score (OSS) method was applied in the selection of the global reference line (GRL) by selecting high stability values to evaluate and correct the temperature and self-(induce and absorption) for the selected analytical lines.

For the determination of the strain modulus (self-induce and absorption), the intensity values and temperature of the analytical lines after and before correction were used, and by extracting from the Boltzmann diagram. Thus It was constructed using corrected optimum plasma temperature, and the corrected analytic lines intensities.

The strain coefficients (S_f) of the analyzed lines due to self-(induce and absorption) are extracted using equation (3) as shown in Fig. (12) A, B, C and D for all components and metals. Fe, Ni, and V metals were identified as illustrative examples.

$$S_f = I_1/I_2 \dots\dots\dots (3)$$

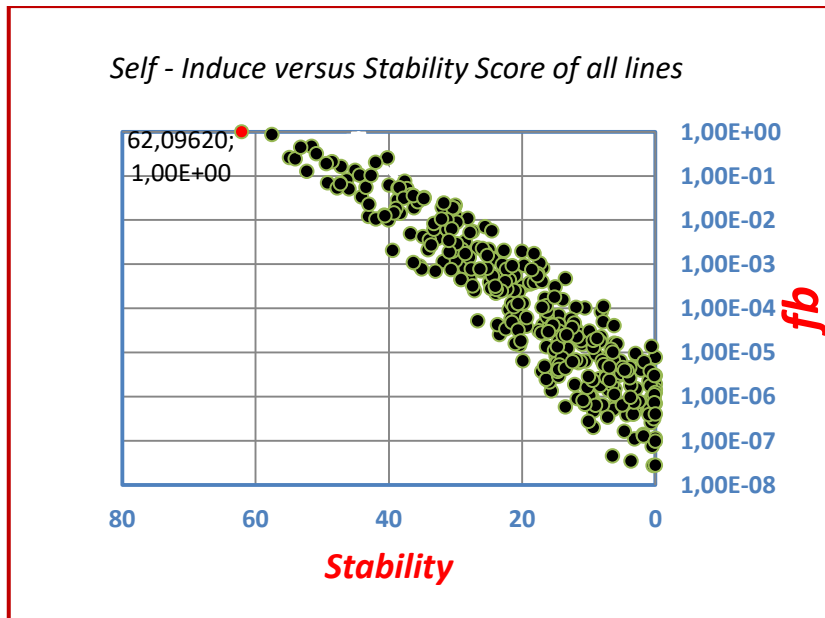
Where: I_1 = Intensity after correction and I_2 =Intensity before correction

The emission lines for each component are assumed to have an approximately high specific stability (Ss) in the FWHM region, which has little distortion and skew when the strain modulus is close to or equal to (1) and are used as optimal analysis lines (Reference line RL). Since it has a high landing angle (high cooling rate) and a smaller different excitation energy so there was no effect of reabsorption of heat from the cold surrounding atoms as shown in Figure (13) (A), (B) and (C) and D)for Fe, Ni and V arrangements. Using the method presented in the figure below, the obtained self-strain coefficients differed from Fe(I) to Ni(I) and to V(I) (from 1 to $1 * 10^{-7}$).

Theoretically, the degree of influence of the self-strain lines of these minerals is related to the atomic properties of these lines. This means that an element with a high degree of stability is often not strongly affected by self-absorption or stimulation.

Figure (13) shows that each point represents an analytical line for a particular mineral, where the points located after the FWHM region have a small angle of inclination (α) because of low cooling rate, meaning that the lines with a small degree of stability (S) have a distortion effect and a larger deflection, because it has a small slope angle (i.e. a small cooling rate). It is shown in figure (13) that the self-induce temperature is low and the impact time is large, allowing the distortion of analytical lines intensity to be of lower stability score S_s , Where the following numbers represent the regression angle of the analysis lines with respect to the specific mineral as shown in Figure (13),B, C and D before.

Therefore, low self-strain coefficients of Fe are of higher relative error than those with high self-strain coefficients of Fe.



(A) The self-induce with the stability score by use OSS method for all analytical lines of heavy metals presented in crude oil

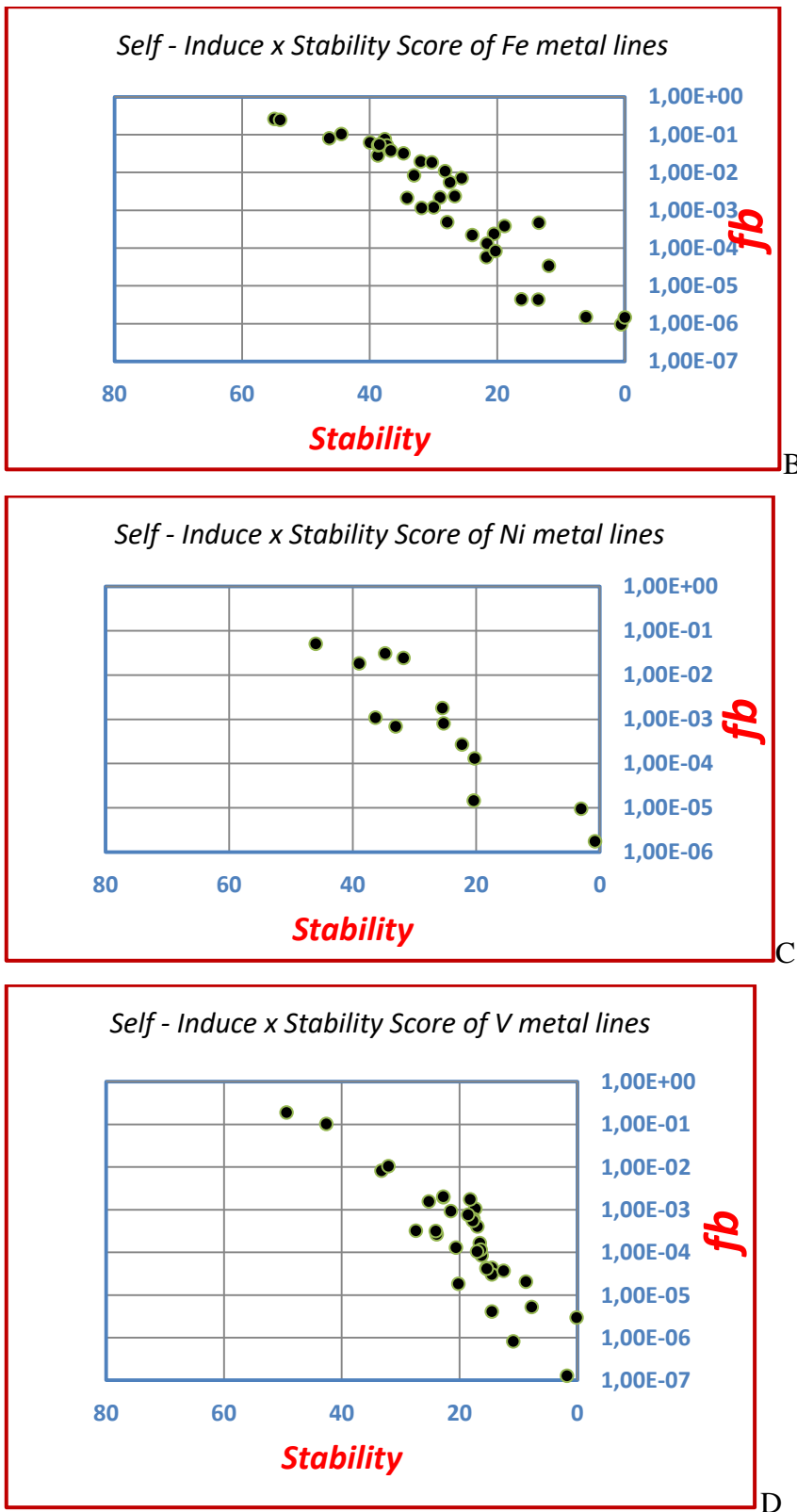
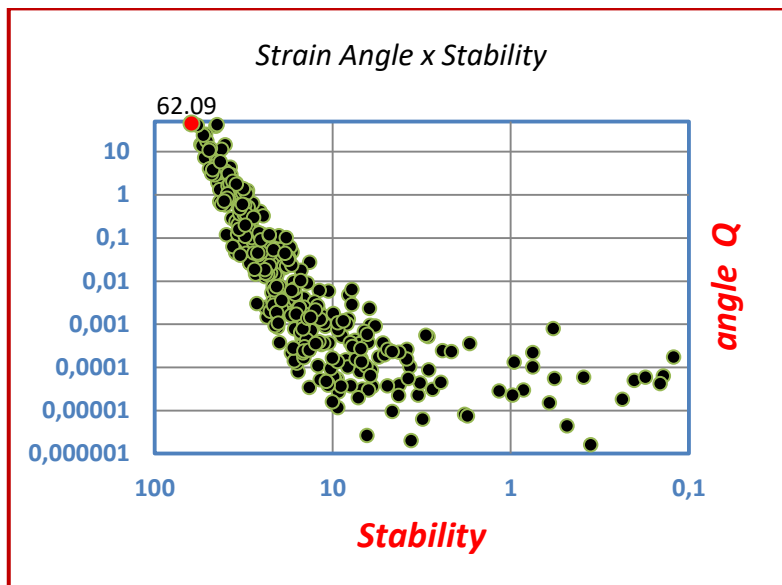
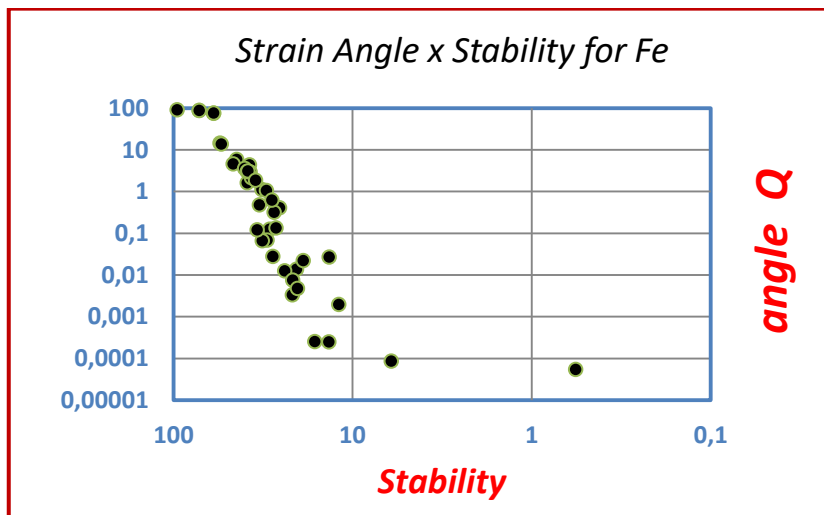


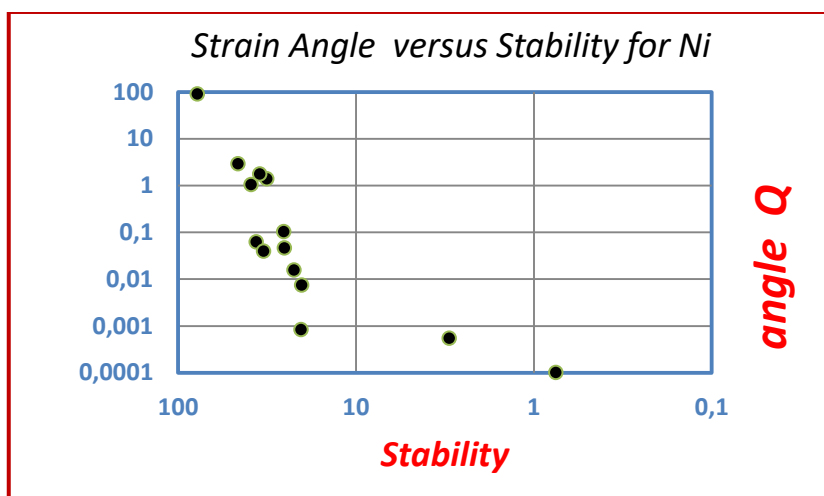
Figure (12 A,B,C and D) Self-induce with the stability score by use OSS method for each analytical lines of Iron , Nickle and Vanadium metal presented in crude oil



(A) The landing angle before correction with the stability score by use OSS method for each analytical lines of heavy metals presented in crude oil



B



C

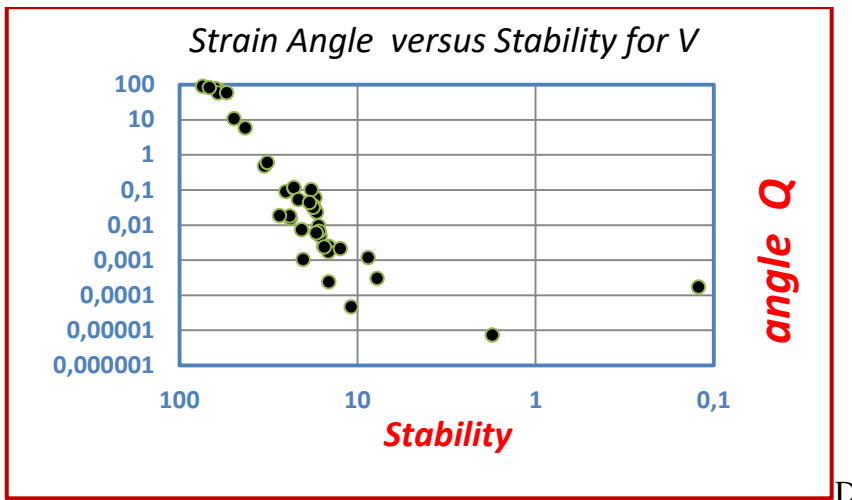


Figure (13 A,B,C and D)The landing angle before correction with the stability score by use OSS method for each analytical lines of Iron ,Nickel and Vanadium metal presented in crude oil

Figure (14) shows the relationship between the intensity of the analysis lines before the correction process and its stability score. The intensity of the analysis lines before the correction process was scattered and irrational due to the self-induce and absorption which resulted in self-strain that caused the decrease in the of land angle (low cooling rate).

On the other hand, after correction process by OSS method, the data points that represent the intensity of the analysis lines with the of stability score with a linear correlation becomes more reasonable and acceptable, which shows that the intensity (Ic) increases with the degree of stability (Ss) which is shown in figure (15).

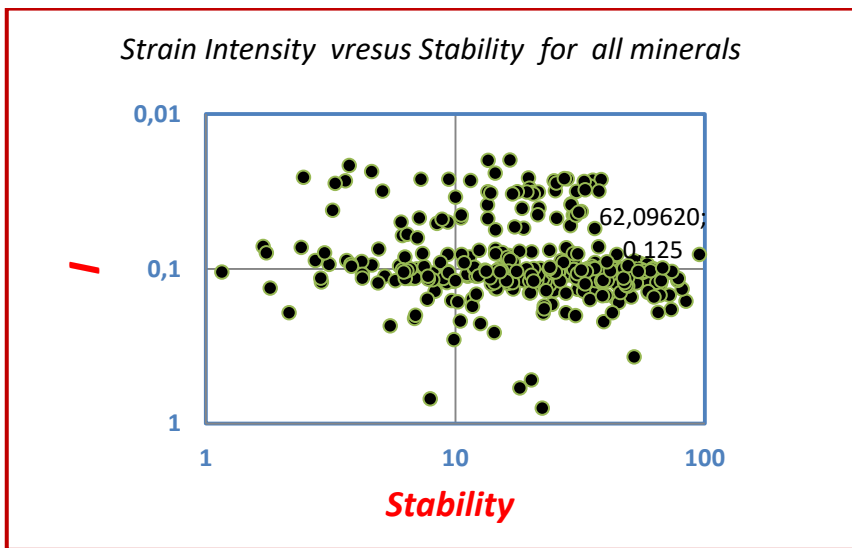


Figure (14) The strain intensity before correction with the stability score by use of OSS method for each analytical line of heavy metals presented in crude oil

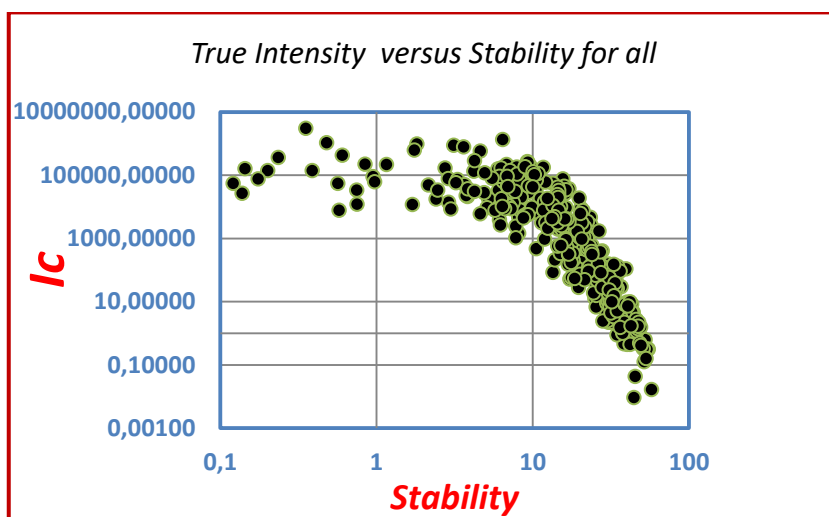


Figure (15) The corrected intensity with the stability score by use OSS method for each analytical line of heavy metals presented in crude oil

Through the above correction procedures and the results obtained, which represent the actual values of the emission line for the metals displayed in the selected crude oil, table (2) reveals the intensity ratio and transition probability ratio of the two Fe lines of 479.91 nm and 559.84 nm, the Ni lines of 450.81 nm and 527.05 nm, and V lines of 412.23 and 569.30 nm. The intensity ratio of FeI/FeI was 0.00541 and their transmission ratio was similar. The intensity ratio for NiI/NiI was 0.838 and their transmission ratio was 0.838. The density ratio for V I/V I was 5.977 and their transmission ratio was 5.89. The intensities of Fe, Ni, V and their corresponding transmission potential were observed, which are clearly supporting the assumption of optically thin laser-induced plasma [14].

Table (1) Comparison between the intensity ratio of two emission lines of Fe (I), Ni (I), V (I) and the ratio of their corresponding transition probabilities after correction procedure.

No.	Metal	Ic	Wa (nm)	E (eV)	A	Ratio of I	ratio of A
1	Fe I	2.19047	479.91	6.86610	9.83E+0	5.41*10-4	5.4*10-3
2	Fe I	404.40424	559.84	6.86610	1.82E+0		
3	Ni I	4.41649	450.81	6.292711	7.08E+0	0.838	0.838
4	Ni I	5.26447	527.05	6.292711	8.44E+0		
5	V I	1865.8962	412.23	5.27597	2.41E+0	5.977	5.98
6	V I	312.19668	569.30	5.27597	4.03E+0		

IV. Conclusion

In this study, LIPS technology was applied to perform accurate analysis of the quality of heavy metals present in the selected crude oil. Plasma was produced under optimized experimental conditions by using Nd YAG lasers with specific parameters. For a more accurate analysis of all the heavy metals present, the analytical lines representing those metals

were selected and their actual intensity value was determined. Parameters of the spectral lines selected were used in Boltzmann plots and the actual intensity values of these spectral lines were extracted and used in LIPS calculations to evaluate all components in the selected crude oil. In order to correct the self-(induce and absorption) problem, an improved quality method for LIPS calculations was proposed by relying on (stability score), through two stages.

First, analytic lines possess a different range of degree of stability. Where the line with the highest stability score was chosen as the global reference line, and the stability was determined by a mathematical formula through easily accessible parameters (transition probability A and low energy level E) of the analytic line, the internal reference line for each component (metal) was considered and selected Before correcting for other analytical lines.

Second, an algorithm (OSS) was used for more accurately evaluation of the plasma temperature between the minimum and maximum temperature when the calculated plasma temperatures for different species are unequal.

Experimentally, after applying the proposed method, the points on the Boltzmann diagram were closer to the line, regular and without scattering. In addition, the lines fitted for the different components (minerals) in the crude oil sample were nearly parallel. With the improvement of the proposed method, the LIPS method becomes more feasible, practical and accurate in the actual application of heavy metal analysis tasks in (liquid organic state) and will be compatible with the requirements of on-site and real-time applications.

References

- Ying M, Xia Y, Sun Y, Lu Q, Zhao M and Liu X 2003 Study of the plasma produced from laser ablation of a KTP crystal *Appl. Surf. Science* 207:227-235
- Russell S H, Frank C D, Andrzej W M, Kevin L M, Roy A W and Patrick D F 2005 Laser-induced breakdown spectroscopy (LIBS) – an emerging field-portable sensor technology for real-time, in-situ geochemical and environmental analysis *Environment Analysis* 521-28
- Lazic V, Colao F, Fantoni R, and Spizzicchio V 2005 Laser-induced breakdown spectroscopy in water: Improvement of the detection threshold by signal processing *Spectrochimica Acta Part B* 60:1002-1013
- Gondal M A, Hussain T, Yamani Z H and Bakry A H 2007 Study of hazardous metals in iron slag waste using laser-induced breakdown spectroscopy *J. Environment Science and Health Part A* 42 (6):767-775
- Gondal M A, Hussain T, Yamani Z H and Baig M A 2007 The role of various binding materials for trace elemental analysis of powder samples using laser-induced breakdown spectroscopy *Talanta* 72(2) 642-649
- Gondal M A, Hussain T, Ahmad Z and Bakry A 2007 Detection of contaminants in ore samples using laser-induced break down spectroscopy *Journal of Environmental Sciences and Health Part A* 42:879-887
- Hussain T and Gondal M A 2008 Detection of toxic metals in waste water from dairy products plant using laser-induced breakdown spectroscopy *Bulletin of Environmental Contamination & Toxicology* 80(6):561-565
- Gondal M A, Hussain T, Yamani Z H and Ahmad Z 2007 Determination of toxic metals in petroleum, cultivated land and ore samples using laser-induced breakdown spectroscopy *Bull. Environ. Contam. Toxicol* 78:270-274
- Hussain T, Gondal M A, Yamani Z H and Baig M A 2007 Measurement of nutrients in green house soil with laser-induced breakdown spectroscopy *Environmental Monitoring and Assessment* 124:131-139
- Gondal M A, Hussain T, Yamani Z H and Baig M A 2006 Detection of heavy metals in Arabian crude oil residue using laser-induced breakdown spectroscopy *Talanta* 69:1072 -1078
- Gondal M A, Hussain T and Yamani Z H 2008 Optimization of the LIBS parameters for detection of trace metals in petroleum products *Energy Sources Part A-Recovery, Utilization and Environmental Effects* 30 441-451
- Radziemski L J and Cremers D A 1989 *Laser-induced plasma and applications* (New York: Marcel Dekker) pp 295-325
- Natrella M G 1963 *Experimental Statistics* NBS Handbook vol 91 (National Institute of Standards and Technology, Gaithersburg, MD)
- Cho H H, Kim Y J, Soo Jo Y, Arai N and Lee Y 2001 Application of laser-induced breakdown spectrometry for direct determination of trace elements in starch-based flours *J. Anal. At. Spectrum* 16:622-627
- Radziemski L J 2002 From LASER to LIBS, the path of technology development *Spectrochimica Acta part B Atom Spectrosc* 57:1109-1113

- Rusak D A, Castle B C, Smith B W and Winefordner J D 1998 Recent trends and the future of laser-induced breakdown spectroscopy. *Trends in Anal. Chem.* 17453-461
- T Hussain and M A Gondal, Laser Research Laboratory, Physics Department, King Fahd University of Petroleum & Minerals (KFUPM), Box 5047, Dhahran 31261, Saudi Arabia, *Journal of Physics: Conference Series*439(2013) 012050.
- D A Cremers and L J Radziemski, *Handbook of laser-induced breakdown [18] spectroscopy*(John Wiley & Sons Ltd, West Sussex, 2006)
- A Ciucci, V Palleschi, S Rastelli, A Salvetti and E Tognoni, *Appl. Spectrosc.*53, 960 (1999)
- E Tognoni, G Cristoforetti, S Legnaioli, V Palleschi, A Salvetti, M Mueller, U Panne and I Gornushkin, *Spectrochim. Acta*B62, 1287 (2007)
- F Colao, R Fantoni, V Lazic, I Caneve, A Giardini and V Spizzichino, *J. Anal. At. Spectrom.* 19, 502 (2004)
- I Fornarini, F Colao, R Fantoni, V Lazic and V Spizzichino, *Spectrochim. Acta*B60, 1186(2005)
- V S Burakov and S N Raikov, *Spectrochim. Acta*B62, 217 (2007)
- J A Aguilera, C Aragon, G Cristoforetti and E Tognoni, *Spectrochim. Acta*B64, 685 (2009)
- K K Herrera, E Tognoni, N Omenetto, B W Smith and J D Winefordner, *J. Anal. At. Spectrom.* 24, 413 (2009)
- M V Belkov, V S Burakov, V V Kiris, N M Kozhukh and S N Raikov, *J. Appl. Spectrosc.*72,376 (2005)
- F Colao, R Fantoni, V Lazic, A Paolini, F Fabbri, G G Ori, L Marinangeli and A Baliva, *Planet. Space Sci.*52, 117 (2004)
- B Salle, J I Lacour, P Mauchien, P Fichet, S Maurice and G Manhes, *Spectrochim. Acta*B61,301 (2006)
- A De Giacomo, M Dell'Aglio, O De Pascale, S Longo and M Capitelli, *Spectrochim. Acta* B62, 1606 (2007).



**EFFECT OF ETHIDIUM - BROMIDE ON ANTIBIOTIC RESISTANT OF
UROPATHOGENIC E. COLI ISOLATES**

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EFFECT OF ETHIDIUM - BROMIDE ON ANTIBIOTIC RESISTANT OF UROPATHOGENIC *E. COLI* ISOLATES

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Abstract:

Antimicrobial Resistance among commonly –acquired uropathogens is an emerging concern over the past decades that warrants a continuing reevaluation of the appropriateness of recommended empiric antimicrobial Regimens for treatment of Urinary Tract Infections (U.T.I.s). Most of the Antibiotic Resistance Genes were plasmid determined, so it was, the first attempt to study the effect of curing agent (Ethidium-Bromide) on Antibiotic Resistance of Uropathogenic *E. coli* isolates. (106) samples were collected from patients suffering from Urinary Tract Infection (U.T.I.). Samples were implanted on the culture media Eosin-methylene Blue (EMB) medium and MacConkey agar to isolate the bacteria and to diagnose them using microscopic, culture and biochemical tests and confirmed by the Vitck-2 system. Of the total, 45(42%) isolates were selected which belong to *Escherichia coli*. The susceptibility test towards eight antibiotics were carried out and the results showed that Ciprofloxacin, Erythromycin, Ampicillin, Norfloxacin, Ceftriaxon and Amikacin were the most effective antibiotics and their resistance percentages were 20%, 20%, 20%, 20%, 30% and 30% respectively, Co-trimazole and Chloramphenicol were less effective and their resistance percentage were 90% both of them. Three isolates of *E. coli* (5,8,17) were selected depending on results of antibiotic sensitivity tests as showed multiple –antibiotic resistance (100%). First attempt made on the effect of Ethidium –Bromide (0.1%) as a curing agent on these three –multi-drug resistance (MDR) isolates which used at concentration (0, 10^{-1} , -----, 10^{-10}) and the results showed *E.coli* (MDR) were sensitive to a (Ciprofloxacin, Norfloxacin and ceftriaxone) at Et.-Br. of concentration at (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6}) while normal activity were observed at concentration of (10^{-7} , 10^{-8} , 10^{-9} , 10^{-10}) Of Et.-Br. The results of Agarose – Gel Electrophoresis of both normal *E. coli* (MDR) and cured isolates showed the presence of chromosomal and plasmide DNA bands in the normal case while only chromosomal DNA bands with *E. coli* isolate no.(8) treated with an Ethidium –Bromide at concentration of (10^{-2} , 10^{-5}).

Key words: Ethidium-Bromide, Uropathogenic *E. coli* , Antibiotic Resistance.



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Introduction:

A disease urinary tract infection (UTI) defined as the presence of microorganism in a properly collected specimen of Urine bacteriuria more than 10⁵ bacteria per ml of urine [1]. The symptoms of UTI are frequent urination, flank pain, dysuria, burning while urination and sometimes fever. In general bacterial infection of urinary tract are the commonest cause of both community acquired and nosocomial infection in patients admitted to hospital. Secondly after respiratory tract infection [2] The infection are a common source of morbidity as much as twelve per 1000 consultation in general practice are on account of them [3].

• The Genus *Escherichia*:

The genus *Escherichia* is related to the family Enterobacteriaceae, is a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). *E. coli* and other facultative anaerobes constitute about 80% of gut flora, [4] and fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. A growing body of research, though, has examined environmentally persistent *E. coli* which can survive for extended periods outside of a host [4].

• **Uropathogenic *E. coli* (UPEC)** is one of the main causes of urinary tract infections.[5] It is part of the normal flora in the gut and can be introduced in many ways. In particular for females, the direction of wiping after defecation (wiping back to front) can lead to fecal contamination of the urogenital orifices. Anal intercourse can also introduce this bacterium into the male urethra, and in switching from anal to vaginal intercourse, the male can also introduce UPEC to the female urogenital system [5].

• Antibiotic Resistance

E. coli shows Resistance to a diversity of antibiotics, including β -lactams, aminoglycosides, quinolones and the mechanisms that contribute to resistance in UPEC strains are as follows: (1) inactivation of hydrolytic enzymes by β -lactamases; (2) no hydrolytic enzyme inactivation by aminoglycoside acetyl transferase enzymes; (3) permeability alteration through active efflux pumps; (4) inactivation of the target site; and (5) resistance acquired by the horizontal transfer of genetic elements, such as insertion sequences, gene cassettes, integrons, and transposons [6]. Integrons generally constitute an integrase gene (*intI*), an attachment site, and a promoter that induces the expression of any suitable integrated gene(s). Additionally, two classes of integrons (classes 1 and 2) have been identified and characterized in MDR-UPEC clinical strains [7, 8]. These integrons carry several genes that encode proteins that participate in antibiotic resistance. These integrons are frequently embedded in mobile DNA elements, such as transposons and conjugative plasmids, to spread horizontally throughout bacterial populations [9,10]. This study was viewed on the effect of Ethidium-Bromide on antibiotic resistance in which it is (Et.-Br.) an intercalating agent commonly used as a fluorescent agent (nucleic acid stain) in Molecular Biology Laboratories for techniques such as agarose – gel electrophoresis, when exposed to ultraviolet, it will fluoresce with

orange colour ,intensifying almost 20 fold after binding to DNA under the name (homidine). Ethidium-Bromide may be a mutagen, a carcinogen, or a teratogen, although this depend on the organism exposed and the circumstances of exposure [11].

• **Materials and Methods**

• **Samples collection**

All bacterial samples used in this study were obtained from midstream urine of UTI Patients of different hospitals in Baghdad.

• **Bacterial diagnosis**

Initial diagnostic depending on Gram reaction and morphological characteristic of the colonies based on bacterial growth on macConkey agar, blood agar, and EMB (Eosine methylene blue medium), as well as a number of biochemical test [12], and VITEK 2 Compact system.

• **Molecular Identification of *E. coli* isolates**

• **Extraction of Genomic DNA**

Bacterial genomic DNA was extracted by using a commercial purification system (Wizard and Genomic DNA purification Kit (Promega,USA), and the primers (Alpha DNA, Canada) were provided in lyophilized form [13].

• **Polymerase Chain Reaction (PCR) Amplification**

Technique A PCR technique was used with primers[14].Primer Design A. Primers Selection The primers (IDT DNA, USA) used in PCR amplification were specific for 16S rRNA Primer of E.coli database of DNA sequences in NCBI gave the basic information to design specific primers. Primer sequence. 5→3 F5'GGAGTTAGCCGGTGCTTCTT3' and R5'GATGACCAGCCCACTGGAA3'.

• **Antibiotic susceptibility test**

Disk agar diffusion according to Kirby Baur standardized antimicrobial susceptibility single disk method was carried out toward eight antibiotics (Ciprofloxacin, Norfloxacin, Erythromycin, Ampicillin, ceftriaxone, Amikacin, Chloramphenicol and Cotrimoxazole), Bioanalyse (Turkey).

The antibiotic resistance percentage detected to each antibiotic as = number ofresistance isolate to a specific antibiotic /Total number of tested isolates ×100% [15].

• **Effect of curing agent (Ethidium- Bromide).**

In this part of study the highly *E. coli* resistant isolate were used for studying the effect of the curing agent Ethidium - Bromide concentrations of (0,10⁻¹,10⁻²,10⁻³,10⁻⁴,10⁻⁵,10⁻¹⁰) by dissolving 0.1mg of Et.- Br. in 10ml D.W. then the above concentrations were prepared and added to a plate of nutrient agar seeded with a lawn of nutrient broth (18-24h.) culture of isolates (5, 8 and 17). Then, the antibiotic susceptibility were measured at each concentration [16].

• **DNA profile by gel electrophoresis**

Bacterial plasmid DNA was extracted from cultured cells using the alkaline SDS method with promega DNA kit described by [17] as follow: Centrifuge 25 ml of culture,

resuspended pellet in 0.5ml of lysozyme solution containing (0.3M NaOH, 2% SDS), mix and incubated at 70°C for 15min, add 0.08ml of acid phenol/chloroform (1:1) and mix gently, separate phases by centrifugation at 10000xg for 10min and transfer the upper aqueous phase to a new Ependorff tube containing 0.07M sodium acetate and 0.7ml isopropanol, centrifuge again for 2min , the pellet dissolved in 0.05ml TE buffer, stored at 4°C and samples were electrophorated using 0.7% agarose with 5v/cm for 2h, after ending of electrophoresis process, the gel was exposed to U.V. light with 340nm to observe plasmid bands.

• Results and Discussion

The present study included (45) positive samples from U.T.I. patients with different ages and sexes from different hospitals and laboratories. Bacterial isolates were identified to the level of subspecies using the traditional biochemical and morphological test described by [12] and then confirmed by VITEK 2 system.

• Molecular Identification by PCR Technique

Molecular diagnosis of bacterial Isolates revealed that all isolates (100%) were *Escherichia coli* which showed expected amplicons size 956bp for housekeeping gene 16S rRNA as in figure (1).



Figure (1) Gel electrophoresis (1% agarose, 7 V/Cm2) and ethidium bromide staining to detect 16S rRNA gene size product (956 bp). *E. coli* for isolates obtained *E. coli* (1-11) sample showed positive PCR., and the run lasted for 13 min. /100V.

• Antibiotics Susceptibility

The Susceptibility tests toward eight antibiotics were carried out and results are illustrated in figure (2) showed that Ciprofloxacin , Norfloxacin, Erythromycin, Ampicillin, Ceftriaxone and Amikacin were the most effective antibiotics and their resistance percentage were 20%, 20%, 20%, 30% and 30% respectively, While Chloramphenicol and Co-trimoxazole were less effective and their resistance percentage were 90% both of them. Three isolates *E. coli* (5, 8, 17) were selected depending on results of antibiotics sensitivity tests as showed multiple –antibiotics resistance(100%).

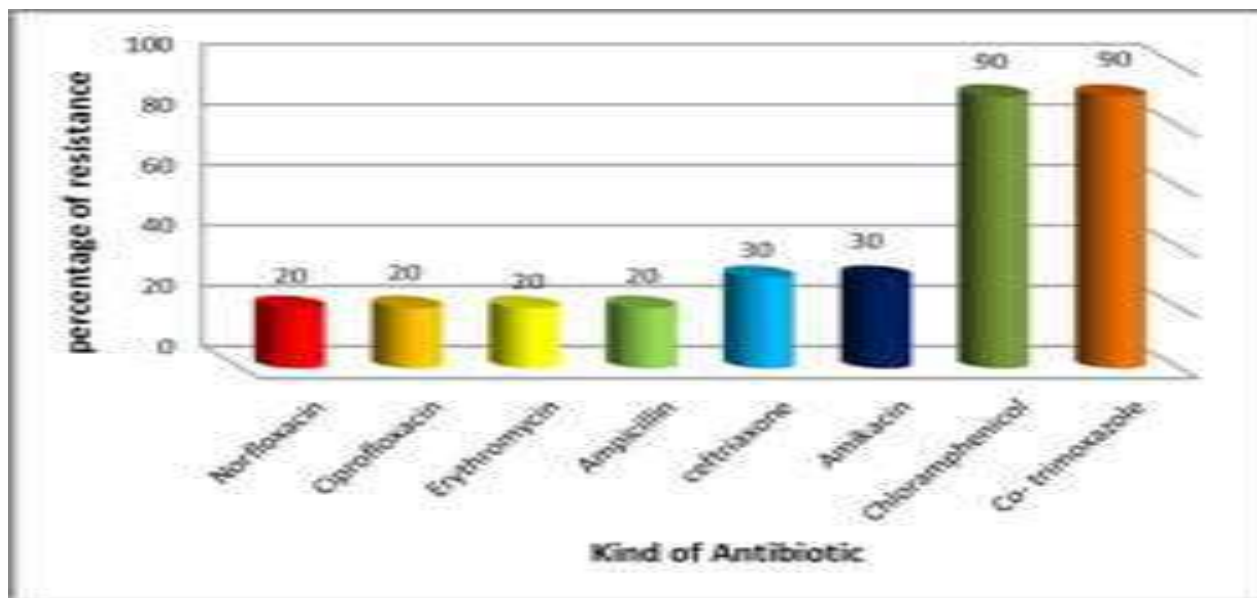


Figure (2): The resistance percentage of *E. coli* isolates to antibiotics.

Results agreed with (Peleg and Hooper 2010) who found that the *E. coli* isolates showed a high sensitivity to each of Ampicillin, Ciprofloxacin, Erythromycine , Tetracycline, Gentamicin and Cephalothin, and showed a low resistance (10%) and (20%) to Cefotaxime and Amikacin respectively, but disagreed with them results when they found that (80%) of the environmental isolates were resistance to Amikacin [18].

• The effect of Ethidium-Bromide concentrations on Antibiotic sensitivity of three highly resistance *E. coli* isolates.

The results of the effect of Ethidium- Bromide on a three higher resistance *E. coli* (5, 8, 17) were studied and shown as in table (1). These results showed that three *E. coli* isolates became sensitive to Ciprofloxacin, Norfloxacin and Ceftriaxone till 10^{-6} concentration of Et.-Br. While still resistance to the remained five antibiotics under this study.

Table (1): The effect of Ethidium -Bromide concentrations on Antibiotic sensitivity of three highly resistance *E. coli* isolates (5, 8, 17) .

Antibiotics Eth. Br. Con.	ip. C	or. N	ry. E	mp. A	eft. C	mk. A	hlora. C	o-tri. C
0	R RR	R RR	R RR	R RR	R RR	R RR	R RR	R RR
10 ⁻¹	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻²	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻³	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻⁴	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻⁵	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻⁶	S SS	S SS	R RR	R RR	S SS	R RR	R RR	R RR
10 ⁻⁷	R RR	R RR	R RR	R RR	R RR	R RR	R RR	R RR
10 ⁻⁸	R RR	R RR	R RR	R RR	R RR	R RR	R RR	R RR
10 ⁻⁹	R RR	R RR	R RR	R RR	R RR	R RR	R RR	R RR
10 ⁻¹⁰	R RR	R RR	R RR	R RR	R RR	R RR	R RR	R RR

These results were in agreement with Hopwood *et al.*, 1995 and pang *et al.*, 2007 who study the role of many antibiotic resistance properties of different bacterial isolates either *Streptomyces* and *E. coli* it may be effected if it was plasmid determined in *E. coli* whereas the antibiotic production by *Streptomyces* spp. remained at the same rate after curing by Et.-Br. because it was chromosomally determined antibiotic while many of antibiotic resistance genes were plasmid determined and affected when the producers organisms treated with curing agents [19,20].

Also, the results of agarose electrophoresis were shown in figure-3- revealed the presence of plasmid DNA bands of (472, 670, 520 and 950) bp in (3,5,6,7) isolates

respectively and chromosomal band in normal cases, while the presence of one chromosomal band with isolate no.(8) which treated with Ethidium-Bromide at concentration of (10^{-2} to 10^{-5}). The effect of keylating agent (Ethidium-Bromide) may cause a genetic mutations due to the changes in antibiotics resistance types of curing isolates after one hour of electrophoresis which were plasmid determined and affected by many mutagenic agents [21, 22]. Our results were in contrast with Pavlov *et al.*, 2006. who found that genetics of actinorhodine – production by *Streptomyces coelicolor* was chromosomally determined and curing isolates treated with Ethidium- Bromide still actinorhodine producers [21]. Another study showed the presence of only one mega plasmid band of *Bacillus thuringensis* after 1:30h. of electrophoresis while, there is a complex plasmid profile with different molecular weights occur within the same species. This reason may related to the differences of plasmid properties between strains related to the same species or even sub species level [22, 23].

Bora *et al.*, 1994 found that these differences in plasmid profile may caused by the differences in the method of DNA separation, the molecular weights of plasmid and its copy number found in bacterial cells [24].

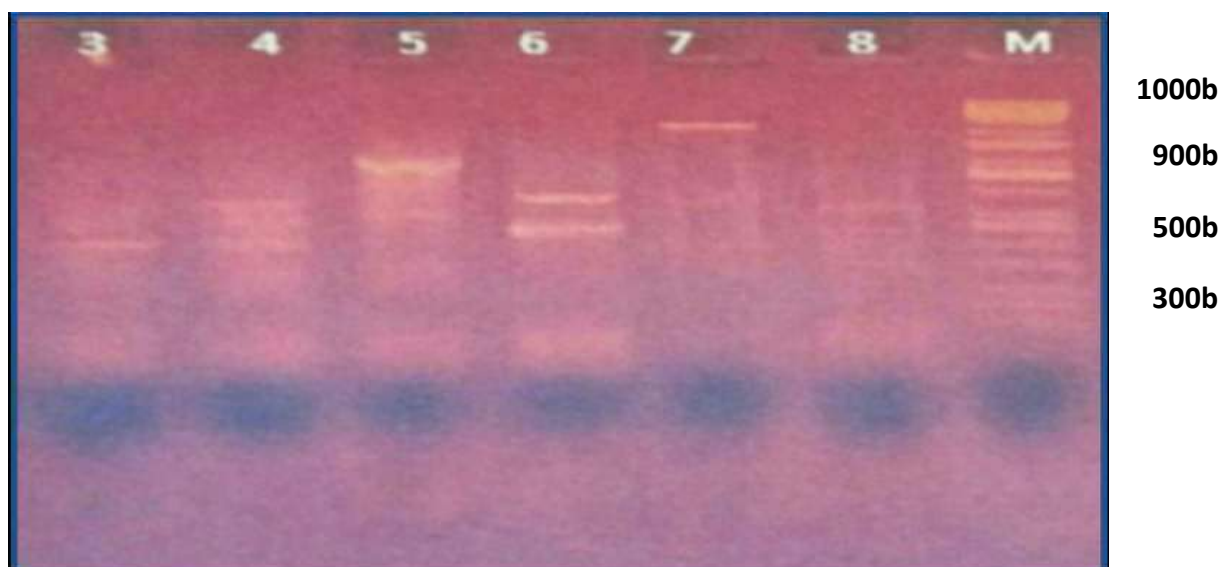


Figure (3): Agarose gel electrophoresis of both chromosomal and plasmid DNA isolated from *E. coli* isolates. Lane 3, 4, 5, 6, 7 represent the normal isolates; . Lane 8 represents treated isolate with Ethidium-Bromide at concentration (10^{-2}); lane M represents a marker.

• Conclusion

Our results may help to investigate the effect of Ethidium-Bromide on another pathogenic bacteria, also it was important to determined the specific gene sequence of cured isolate and the effect of Ethidium-Bromide on other virulence factors of more epidemiological agents in Iraq.

• References

- [1] AcostaPérez, G.; IbáñezCervantes, G.; BelloLópez, J. M.; Hernández, J. M.; Hernández-Montaez, Z.; GionoCerezo, S. *etal.*, (2015). Structural diversity of class I integrons in multiresistant strains of *Escherichia coli* isolated from patients in a hospital in MexicoCity. *Curr.Microbiol.* 71,501–508. doi :10.1007/s00284-015-0876-9.
- [2] Tozuvelckis, L.S; Tazule, P.E.; Tassias, P.T. and Legakis, N.J., (2000). CTX-beta lactamase an emerging group of extended spectrum enzymes .*International J.Antimicrobial Agents* . 14 : 2:137 -142.
- [3] Shanson ,D.C.(1980). *Microbiology in clinical practice* (2nd) ed. Tohn Right , London.
- [4] Bhakdi, S. Mackman, N, Menstrina, G, Gray, I., (1988). The hemolysin of *Escherichia coli*. *Eur. J. Epidemiol.* 4(2):135-43.
- [5] Stipevic ,T.; Piljac, T., and Isserof, R. R., (2005). Di-rhamnolipid from *Pseudomonas aeruginosa* display differential effects on human 7 keratinocyte & fibroblast cultures.*J. Dermatol Sci.*40(2): 141-3.
- [6] Peleg, A.Y., and Hooper, D. C., (2010).Hospital-acquired infections Due to Gram-negative bacteria. *N. Engl. J. Med.* 362,1804–1813. doi :10.1056/NEJMra0904124.
- [7] Stalder, T.; Barraud, O.; Casellas, M.; Dagot, C., and Ploy, M.C., (2012). Integron Involvement in environmental spread of antibiotic resistance *Front. Microbial.* 3:119.doi:10.3389/fmicb.2012.00119.
- [8] Deng,Y.; Bao, X.; Ji, L.; Chen, L.; Liu, J.; Miao, J.; *etal.*, (2015). Resistance integrons: class I, 2 and 3 integrons. *Ann. Clin. Microbiol. Antimicrob.* 14:45. doi :10.1186/s12941-015-0100-6.
- [9] Najjar, N. G.; Farah, M. J.; Hashwa, F. A., and Tokajian, S. T., (2010). Antibiotic resistance patterns and sequencing of class I integron from uropathogenic *Escherichia coli* in Lebanon. *Lett. Appl. Microbiol.* 51,456–461. doi:10.1111/j.1472-765X.2010.02926.x.
- [10] Ferjani, S.; Saidani, M.; Amine, F. S.; Boutiba Ben Boubaker, I., and Boubaker, I., (2015). A comparative study of antimicrobial resistance rates and phylogenetic groups of community-acquired versus hospital-acquired invasive *Escherichia coli*. *Med. Mal. Infect* 45,133–138.doi:10.1016/j.medmal.2015.01.012.
- [11] Staley, J. T. and William, S. S.T., (1994). *Bergys Manual of Determinative Microbiology* .9th ed .William sandwilkins company Baltimore, Maryland, U.S.A.
- [12] Baron, E. J.; Peterson, L. R., and Finegold, S. M., (1994). In *Baily & Scott's. Diagnostic microbiology* (9 th ed) (Mosby. St. Louis, Baltimore.:Pp 429- 30.
- [13] Zhaohui, L., (2008). *Effects of Downregulations of microglial CD11B expression in normal and neurofilament light chain knockout mice* (Doctoral dissertation).
- [14] Ranjzad, F.; Mahban, A.; Shemirani, A. I.; Mahmoudi, T.; Vahedi, M.; Nikzamir, A. and Zal, M. R., (2011). Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. *Journal Assist Reprod Genetic*, 28:225–232.
- [15] Magray, M. S. D.; Kumar, A.; Rawat, A. K., and Srivastava, S., (2011). Identification of *Escherichia coli* through analysis of 16S rRNA and 16S-23SrRNA internal transcribed space region sequence. *Bio information* .6(10):370-371.

- [16] Kirby, A. S.; Posplech, A. K., and Neuman, E. D., (1995). Laboratory Manual of Expermatal Microbiology.(2nd ed.). Mosby. st. Louis, Baltimore.pp 429-430.
- [17] Prescott, L. M.; Harly, J. P., and Klien D. A., (2005). Microbiology: antimicrobial chemotherapy .6th ed..McGraw Hill, Boston, New York. Pp: 430-439.
- [18] Hopwood, D. A.; Chater, K. F., and Bibb, M. J., (1995). Genetic regulation of Antibiotic production. Ciba. found. Symp. 1995;118: 64-74.
- [19] AL- Obidi, I. M., (2005). Determination of some epidemiological markers in locally isolated *Vibrio cholerae*. M.Sc. Thesis, College of Science, University of AL-Mustansiriah.2005.
- [20] Pang , B; Yan , M.; Cui, Z.; Zhang, L., and Kan , B., (2007). Genetic diversity of toxigenic and not toxigenic *V. cholera* serogroups O1 and O139 revealed by array-based comparative genomic hybridization.J. Bacteriol . 2007 ; 189:4837 - 49.
- [21] Taher, N. A., (2003). Astudy on two Enzymes (IgA Protease and Urease) by Proteus miabilisN28 caused urinary tract infection ph. D. thesis, college of science , Al-Nahrain University, Baghdad. Iraq.
- [22] Pavlov, A. R.; Pava, N. V.; Kozyavkin, S. A., and Slesarev, A. I., (2006). Thermostable DNA polymerase for a wide spectrum of applications : comparison of a Robust hybrid Topotaq to other enzymes .In Kieleczawa . J. DNA Sequencing II : optimizing preperation and cleanup.2006: pp. 241-57.ISBN.
- [23] Mohammed, A. J.; Al- Kazaz, A., and Ali, S. M., (2013). plasmid Role in crystal protein production in Locally isolated *Bacillus thuringesis* Iraqi J. of science. 2013: P301-6.
- [24] Bora, R. S.; Mutry M. G., and sekar, V., (1994). Introduction to lepidopteran- specific insecticidal crystal protein gene of *Bacillus thuringesis* subspecies Kurstaki by conjugal transfer in cotton phyllo sphere. Appl. Environ .Microbiol. 1994; 60: 214-22.

**DETECTION OF PREVALENCE OF INTESTINAL PARASITES IN FRESHWATER FISH
LIZA ABU FROM EUPHRATES RIVER, AL-DIWANIYAH CITY – IRAQ**

**IKHLAS ABBAS MARHOON
MARWA SAMI ALWAN**



DETECTION OF PREVALENCE OF INTESTINAL PARASITES IN FRESHWATER FISH LIZA ABU FROM EUPHRATES RIVER, AL-DIWANIYAH CITY – IRAQ

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Marwa Sami ALWAN²

Abstract:

A 176 samples of freshwater fish *Liza abu* were fishing from Euphrates river in the Al-Diwaniyah city – Iraq , to investigate of some intestinal parasite , identify the rate of infection with this parasites and then determining the relationship between temperature and prevalence of parasitic infection rates , for the period between October 2020 and March 2021. All fish examined by using laboratory methods to detected intestinal parasitic infection.

The results revealed that the total infection rate of prevalence of intestinal parasites in *Liza abu* was 75.56%, and the results recorded presence of five different species of parasites were isolated from freshwater fish which are as follows: Three species belonging to two classes of protozoan : Sporozoa (*Cryptosporidium* sp. and *Eimeria* sp.) and one species belonged to Ciliata which is *Tetrahymena* sp. with an infection rate 55.68% , 35.79% and 17.61% respectively. Two species belong to parasitic helminthes : *Eustrongylides* sp.(Nematodes) with an infection rate 22.73%, and the other species is related to *Neoechinorhynchus* sp. (Acanthocephalans) with an infection rate of 44.88%.

Also results showed a significant difference in infection rates during the months of the study, the highest percentage of infection was appeared during March and February which reached 93.94% and 91.67% respectively, while the infection rate decreased in January (45.0%) .

Key words: *Cryptosporidium* sp., *Eimeria* sp. , intestinal parasites, freshwater fish.



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Introduction:

Fish are described as cold-blooded animals (temperature of body is variable) and it's represent the largest vertebrate group, found in all types of water such as freshwater like rivers ; marine like seas , oceans and lakes , fish differ in their shapes, sizes, weights and colors, as their numbers are estimated at more than 40,000 species (1,2). Meat of fish is characterized by being of a high nutritional value because it contains a high percentage of amino acids and a lot of essential minerals such as phosphorous, calcium and potassium(3), as well as containing a number of vitamins which necessary for growth of the body such as vitamin A , E and D3 and omega 3 , fish is characterized by containing a small amounts of saturated fat. (4,5). Because of their advantages , Fish wealth have received a great interest through maintain their productivity to compensate for some nutritional requirements in a balanced manner with the continuous increasing in the world's population (6),and to provide food resources, especially protein ones. Therefore, many people depends on fish to provide near by 50% of its animal protein needs (7,8).

Fish, like other animals, are exposed to many risk factors like parasitic infection , fish have a great ability to resist diseases if it's present in a good conditions(9). Sometimes the parasitic infection don't cause obvious damages and the infected fish appear to be healthy look like the non infected one, and sometimes parasites become pathogenic (10). Some parasites are pathogenic to fish themselves , and some are pathogenic to other animals, whether to other fish or to invertebrates of carnivores , including humans (11). The damages of parasitic infection varies between two types which are : mechanical and chemical damages , the first one mechanical damage such as tissue rupture, intestinal canal obstruction, or the host's participation in its food, as well as feeding on body tissues and fluids, the second one chemical damage resulting from toxic substances secreted from parasite as a response to the resistance of the host's tissues or as a result to the parasite performing its usual vital actions (9,12). The *Liza abu* , like other fishes , infected with different types of parasites, such as protozoa, Trematoda , Cestoda , Nematodes , Acanthocephalans and crustaceans (12,13,14).

Many studies found that *Liza abu* fish were infected with internal parasites , including *Cryptosporidium* sp. , *Eimeria* sp. and the larval stage of the tapeworm *Ligula intestinalis*, *Capillaria* spp. , *Neoechinorhynchus iraqensis* (12,13,15). And in other studies which specialized in identifying the parasitic worms of freshwater fish , many parasitic worms were diagnosed (16,17,18). Worldly , several studies indicated a high rate of mortality in fish community as a result of infection with various types of parasites, especially nematodes and tapeworms (10,19,20)

Because of the *Liza abu* is one of the important freshwater fish as a good food for humans (a protein source) with a distinctive taste and an appropriate cost, so this study came to identifying the types of parasites that infect this type of fish, and on the other hand, the infected fish are a source of infection of other types of fish through polluted of river water with infectious phases which are excreted with feces.

Therefore, the objectives of this study aimed to determination of total infection rate of different types of intestinal parasites and detection of prevalence of intestinal parasites in freshwater fish *Liza abu* from Euphrates river.

Material and Methods

A total of 176 specimens of fish were fishing from Euphrates river in the Al-Diwaniyah city – Iraq , for the period between October 2020 and March 2021. All fish were brought to parasitic laboratory , To preparation and examination of samples , fish has been dissected from ventral side , firstly checked visually ,and then examined by using direct smear method according to (21)., as well as stool samples were also stained by using a modified Ziehl-Neelsen stain technique to detected the presence of oocysts of *Cryptosporidium* sp. according to (11). The results were statistically analyzed by using chi-square at $p \leq 0.05$ (22).

Results and Dissection

The results revealed that the total infection rate of prevalence of parasites in *Liza abu* was 75.56%, and the results recorded presence of five different species of parasites were isolated from freshwater fish which are as follows: Three species belonging to two classes of protozoan : Sporozoa (*Cryptosporidium* sp. and *Eimeria* sp.) and one species belonged to Ciliata which is *Tetrahymena* sp. with an infection rate 55.68% , 35.79% and 17.61% respectively. Two species belong to parasitic helminthes : *Eustrongylides* sp.(Nematodes) with an infection rate 22.73%, and the other species is related to *Neoechinorhynchus* sp. (Acanthocephalans) with an infection rate of 44.88%. The results showed a significant difference between the highest infection rate, which was with *Cryptosporidium* sp.(55.68%) and the lowest was *Tetrahymena* sp. (17.61%) at $P \leq 0.05$.

The results which shown in Table (1) revealed that the total infection rate was 75.56% , which is represent a rather high rate of infection , it give as an important indicator for infection in freshwater fish with various types of intestinal parasites and their role in contamination of water with infective stages of parasites and then spread of infection to other fishes.

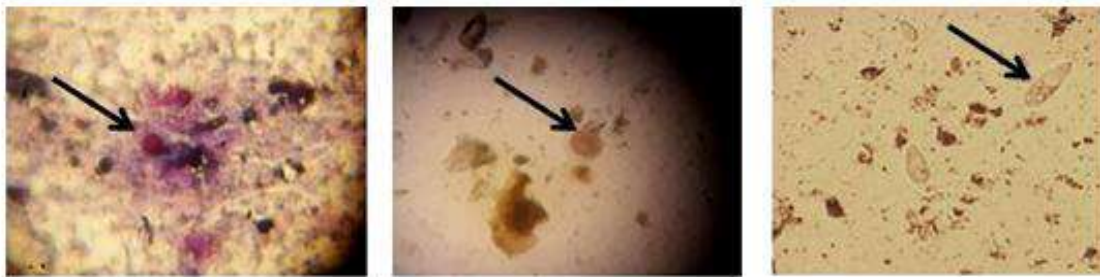
Table (1):Types of intestinal parasites which isolated from *liza abu* and the infection percentage for each of them

Groups of parasites	Species of parasites	No. of infected fish	Percentage of infection
Protozoan	<i>Cryptosporidium</i> sp.	98	55.68
	<i>Eimeria</i> sp.	63	35.79
	<i>Tetrahymena</i> sp.	31	17.61
Nematodes	<i>Eustrongylides</i> sp	40	22.73
Acanthocephalans	<i>Neoechinorhynchus</i> sp.	79	44.88
Total infection		133	75.56

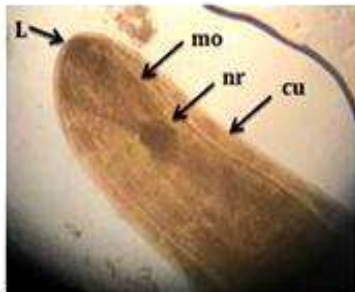
The result of current study agrees with many studies that recorded infection of freshwater fish , including *Liza abu* , with different types of parasites (13,15,18). The presence of intestinal parasitic infections indicates to importance of freshwater fish as a

source of infection for other hosts of local fish , the infected fish shed infectious stages (nematode eggs , oocysts of *Eimeria* sp. and *cryptosporidium* sp.) without appearance of clear clinical symptoms, therefore it contribute to pollution of aquatic environment with these parasites (11,23).

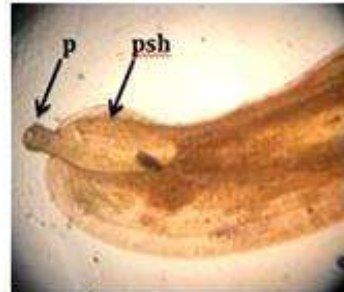
Also results show that *Liza abu* was infected with five types of parasites with different rates of infection (Table 1), three species belonged to protozoan where two species of coccidia were recorded: *Cryptosporidium* sp. (Pic.1) and *Eimeria* sp. (Pic.2), with an infection rate 55.68% and 35.79%, respectively, and the third protozoan was *Tetrahymena* sp. (pic.3) with infection rate 17.61% ; While the current study recorded infection with one type of nematode *Eustrongylides* sp. (Pic.4) was observed inside the body cavity of the fish (22.73%), and *Neoechinorhynchus* sp. (Pic.5) belong to Acanthocephalans (44.88%), which was found in large numbers inside small intestine, and the statically analysis showed a significant difference between the highest and lowest infection rate at $P \leq 0.05$.



Pic.(1): oocyst of *Cryptosporidium* sp.(X60) Pic.(2): oocyst of *Eimeria* sp.(X60) Pic.(3): Trophozoite of *Tetrahymena* (X40)



Pic.(4): anterior end of *Eustrongylides* sp.
L= Labiate, mo= muscular esophagus
nr = nervous ring , cu = cuticle (X40)



Pic.(5): anterior end of *Neoechinorhynchus* sp.
P = proboscis , psh = proboscis sheath (X40)

The high percentage of Coccidian infection (*Cryptosporidium* sp. and *Eimeria* sp.) in current study may be attributed to the difference in river water contamination with oocysts of these parasites, as well as the susceptibility of oocysts in maintaining its ability to infect with the possibility of staying in the water for a long time (24), the reasons may also be due to the long period of exposure to infection with oocysts, the large ages fish are exposed to infection more than young ages and therefore it more likely to acquire the infection (25).the ciliated *Tetrahymena* sp. was found with the lowest infection rate (17.61%), it is a free-living ciliates, but it can parasitize on different hosts, it inhabits in large intestine, especially the colon, causing in some severe cases damage and erosion in the mucous layer of the intestine, where it appears in the form of white spots representing areas of necrosis (26), infection with this parasite occurs through drinking water, this parasite found in fresh river water, and several

infections have been recorded in freshwater fish, where it is isolated from the gills , skin and intestinal lumen of fish (27)

The nematode *Eustrongylides* sp. was recorded , it was seen as a red worms present on the outer surface of the internal viscera. This result agrees with (15), infection with *Eustrongylides* sp. may be due to the fact that the life cycle of this worm is indirect, it needs its intermediate hosts to complete its life cycle, where fish are infected when they eat oligochaeta , then larvae migrate inside the fish's body and settle on outer surface of the internal organs (28).

While the *Neoechinorhynchus* sp. needs small crustaceans, which are the first intermediate host where crustaceans eat the eggs, then it hatch to form acanthor (the larval stage) comes out and penetrates the intestine and develops to form the acanthellae, which has all the characteristics of adult worms, then the fish (represent the second host) feed on small crustaceans that carry the infective stage (14) , fish become infected with the larvae and can transmit the parasite to humans and/or mammals that feed on it, or the acanthella becomes adult worm inside the intestines of fish that fed on infected fish(9). *Neoechinorhynchus* sp. had ability to infect a wide range of many species of fish (16,28) and this explains the infection rate in our study, as it is possible for transmitted of infection from other river fish to *Liza abu*.

Table (2): the changes in parasitic infection rate during the months of study

months of study	No. of examined fish	No. of infected fish	Percentage of infection
October	34	29	85.29
November	28	18	64.29
December	25	13	52.00
January	20	9	45.00
February	36	33	91.67
March	33	31	93.94
Total	176	133	75.56

The results in Table (2) showed a different in the rates of infection during the months of study, the highest percentage of infection was during February and March which reaching to 91.67% and 93.94% respectively, and the lowest percentage of infection was during January (45.00%). A significant differences between the highest and lowest incidence of infection at $P < 0.05$ was found.

This result agree with several studies that recorded a high incidence of infection in the spring months, it is due to the increase of fish feeding that occurs at the beginning of spring as a result of reproduction of fish (15,18,23).

The temperate months are characterized by the abundance of intermediate hosts such as crustaceans and Oligochaeta , in addition to the fact that the maturation of some infectious stages requires a high temperatures to complete their maturatin and become infectious (12),

and this leads to an increase in environmental pollution , with the infectious stages of many parasites , such as rivers (18)

The high incidence of infection during the spring season comes as a result of diversity of animal species such as insects, crustaceans, and mollesca , and these organisms play an important role as a food for a number of aquatic animals , including fish, while the decrease infection rate during January, may occurred as a result of lack of food , or as a result of environmental or physiological factors related with the host itself, such as lack of activity and movement during cold months (28).

References

- (1) Huet, M. (1970), Textbook of Fish Culture: Breeding and Cultivation of Fish, Fishing News Books Ltd.
- (2) Al-Faluji, S. J. (2011), The Comparative Advantage of Fish Production in Iraq for the Period (1980-2008), Master Thesis, Faculty of Agriculture, Baghdad University.
- (3) Begum A, Nurul Amin MD, Kaneco S, Ohta K (2005) Selected elemental composition of the muscle tissue of three species of fish *Tilapia nilotica*, *Cirrhina mrigala*, and *Clarius batrachus*, from the fresh water Dhanmondi Lake in Bangladesh. *Food Chem.*, 93:439–443.
- (4) Belitz, H.D; Grosch, W and Schieberle, P.(2004). Fish, whales, crustaceans, mollusks. In: *Food chemistry*. Springer, Berlin/Heidelberg, pp 619–642
- (5) Bendik, I.; Friedel, A.; Roos, F.; Weber, P. and Eggersdorfer, M. (2013). Vitamin D: a critical and essential micronutrient for human health. *Front Physiol* 5(article 248):1–14.
- (6) Coppes, Petricorena, Z.L.; Pavlisko, A. and De Vecchi, S. (2002). Texture measurements in fish and fish products. *J Aquat Food Prod Technol* 11(1):89–105.
- (7) Elagba-Mohamed, H.A., Al-Maqbaly, R. and Mohamed, M. H. (2010). Proximate composition, amino acid and mineral contents of five commercial Nile fishes in Sudan. *Afr J Food Sci* 4(10):650–654
- (8) Kawarazuka, N. and Béné, C.(2013). The potential role of small fish species in improving micronutrient deficiencies in developing countries: building evidence. *Public Health Nutr* 14(11):1927–1938
- (9) Mhaisen, F.T; Al-Niaeem, K.S. and Jassim, A.A.R. (2010), Parasites and Disease Agents of Cultured Fishes of Basrah Province, *Basrah Journal of Agriculture Sciences*, 23 (2), p.92-106.
- (10) Santos, M.A.; Jerônimo, G.T. ; Cardoso, L. ; Tancredo, K. R. ; Medeiros, P.B. ; Ferrarezi, J.V. ; Gonçalves, E. L. ; Assis, G.C. and Martins, M. L.(2017). Parasitic fauna and histopathology of farmed freshwater ornamental fish in Brazil. *J. Aquacul.*,470(1): 103-109.
- (11) Ali, A. H. and Al-Mahmood, S.S. (2009). Pathological effect of *Cryptosporidium mansonii* oocysts isolated from fish and treated with infrared microwave and ozone in common carp. *Iraq J. Vet. Sci.*, 23(1):187-192.
- (12) Al- Awadi , H. M. ; Mhaisen , F.T. and Al-Joborae , F. F. (2010).Parasitic fauna of fishes in bhar Al-Najaf depression , Mid. Iraq.,11(1):1-9.
- (13) Bilal, S.J. (2013). Ultra- and molecular study of some cestodes and nematodes parasitizing some freshwater fishes in Kurdistan region-Iraq. PhD. Thesis Coll. Edu., Univ. Salahaddin.
- (14) Amin, O.M. (2011). First report of *Neoechinorhynchus* (Acanthocephala : Neoechinorhynchidae) from marine fish of the eastern seaboard of Vietnam with the description of six new species. *J. Parasitol.*, 18: 21-34.
- (15) Al-Tae, A.F. and Zangana, M. Gh. (2011).Detection of parasitic nematodes in some fresh water fishes in khazir river in Ninevah governorate. *IRAQI JOURNAL OF VETERINARY Medicine, UNIVERSITY of MOSUL, IRAQ.* 25(1):29-38.

- (16) Iqbal, Z. and Hussain, U.(2013).Parasitic infection of an ornamental fish, Shubunkin, *Carassius auratus* L. imported to Pakistan. *Biol.*,59(2): 281-286.
- (17) Florindo, M. C. ; Jerônimo, G.T. ; Steckert, L. D. ; Acchile, M. ; Figueredo, A. ; Gonçalves, E. ; Marchiori, N. C. and Martins, M. L.(2017). Metazoan parasites of freshwater ornamental fishes. *Lat. Am. J. Aquat. Res.*,45(5): 992-998.
- (18) Al-Jawda, J.M. and Asmar,K.R. (2013). Myxosporeans (Phylum Myxozoa) Parasitic on Some Fishes from Tigris River at North, Mid and South of Baghdad Province, Iraq. *Basrah J. Agric. Sci.*, 26 (1).
- (19) Heins, D.C. ; Barry, K. A. and Petrauskas, L. A.(2014). Consistency of host responses to parasitic infection in the three-spined stickleback fish infected by the diphyllbothriidean cestode *Schistocephalus solidus*. *Biol. J. Linn. Soc.*,113(4): 958-968.
- (20) Portz, L. ;Antonucci, A.M. ; Ueda, B. ; Dotta, G. ; Guidelli, K. Roubedakis, M.L. ; Martins, M.K. ; Carniel and Tavechio, W. (2013). Parasitos de peixes de cultivoe ornamentais. *Parasitologia de peixes de água doce do Brasil.*, Edu. Maringa, 85-114 pp.
- (21) Baron, E. J. ; Peterson, I. and Finegold, S.M.(1994). *Diagnostic microbiology*, 9th ed. mosby-year book .Inc. St. Louis, P:792.
- (22) SAS. Statistical analysis system, Users Guide (2012) Statistical.Version 9. 1th ed. USA: SAS. Inst. Inc. Cary.
- (23) Al-Nasiri, F.S. (2013). Protozoan parasites of five fish species from the Tigris River in Salah Al-Deen province, Iraq. *J. Tikrit Univ. Agri. Sci.*, 13(1): 355-359.
- (24) Soave, R. and Armstrong, D.(1986). *Cryptosporidium* and Cryptosporidiosis . *J. Infect. Dis.*, 8:1012-1023.
- (25) Al- Chaysh, A. & Mahdy, O. S.(1998). Studies on cryptosporidium nasorum in fish (*Tilapia zellii*) in Egypt . *Asia Vet. Med. J.*, 39: 201- 207.
- (26) Hoffman G. L.; Landolt, M. ; Camper, J. E.; Coats, D. W.; Stokey, J.L. and Burek J. D. (1975) A disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. *J. Parasitol.*,61:217-223.
- (27) Jerome C. A.; Simon, E. M. and Lynn, D. H. (1996). Description of *Tetrahymena empidokyrea* n. sp., a new species in the *Tetrahymena pyriformis* sibling species complex (Ciliophora, Oligohymenophorea), and an assessment of its phylogenetic position using small-subunit rRNA sequences. *Can. J. Zool.*,74:1898-1906.
- (28) Abdullah, S.M.A. (2002). Ecology, taxonomy and biology of some parasites of fishes from Lesser Zab and Greater Zab rivers in north of Iraq. Ph. D. Thesis, Coll. Educ. (Ibn Al-Haitham), Univ. Baghdad: 153pp.

A petri dish containing a bacterial culture. The agar surface is covered with numerous yellow, glistening colonies. Some colonies are arranged in streaks, while others are in small clusters or individual spots. A yellowish, rectangular object, possibly a piece of paper or a small block of agar, is placed in the center of the dish, partially overlapping the colonies.

**DETECTION OF SOME VIRULENCE FACTORS AND ANTIBIOTICS RESISTANCE
OF KLEBSIELLA PNEUMONIAE**

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IHSAN ALI RAHEEM
ALAA LAEBI ABDULLAH
BATOOl ABD AL AMEER BAQER**

**DETECTION OF SOME VIRULENCE FACTORS AND ANTIBIOTICS
RESISTANCE OF *KLEBSIELLA PNEUMONIAE***

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Ihsan Ali RAHEEM²

Alaa Laebi ABDULLAH³

Batool Abd Al Ameer BAQER⁴

Abstract:

Background: Infections of *Klebsiella pneumoniae* can include; diarrhea, septicemia, pneumonia, urinary tract infection and infections of soft tissues. Many factors are donated to *K. pneumoniae* pathogenicity particularly production of enzymes and formation of biofilm. **Objective:** find the relationship between the resistance of *K. pneumoniae* bacteria to antibiotics of quinolones and their ability to produce enzymes of beta lactamase. **Materials and Methods:** The Study included isolation and identification of (51) isolate of *K. pneumoniae* and (94) isolates of other bacteria from different clinical sources in some Baghdad hospitals. **Results:** The isolation and diagnosis of (51) isolates of *K. pneumoniae* from infection of urinary tract were 49.1%, infection of wounds were 31.3% and infection of burns were 19.6%. All bacterial isolates were identified by the biochemical, cultural and microbial characteristics and confirmed by Api E20 System. I showed of β -lactamase test of *Klebsiella pneumoniae* revealed that (35) 68.6% isolates were positive. While 16 (31.4%) isolates were able to produce urease. Four groups of quinolones were tested by done the sensitivity test of isolates and results revealed the following percentage of resistant to Norfloxacin, Ofloxacin and ciprofloxacin consequently were (50.1%), (44.5%), (39.4%). whereas, the lower percent of resistant to Levofloxacin was (26.8%). In contrast, the β -lactamase positive *K. pneumoniae* exhibited a high resistance in compare to isolates that negative for β -lactamase. The minimum inhibitory range concentrations of ciprofloxacin were arranged between (4-512 $\mu\text{g/ml}$). From isolates that resistant to Ciprofloxacin, the DNA plasmid was determined. Single plasmid bands were included in two isolates with same size and other isolates were confined free plasmid.

Key words: *Klebsiella pneumoniae*, Virulence Factors, Biofilm, β -lactamase.



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Introduction:

The genus of *Klebsiella pneumoniae* is; a non fermentive bacillus, Gram negative bacteria, because of anti-phagocytosis capsule, it is considered as a main pathogen amongst nosocomial infections [1]. Species of *Klebsiella* are habitually found as anormal flora in the human mouth, nose and gastrointestinal tract; nevertheless, they can similarly act as opportunistic pathogens of human [2]. Infections of *Klebsiella* can include; diarrhea, septicemia, pneumonia, urinary tract infection and infections of soft tissues [3]. Many factors are donated to *K. pneumoniae* pathogenicity particularly production of enzymes and formation of biofilm. Additionally, ability of adhesion, siderophores, antigen of capsule and others such as protease, proteimase or any enzyme that makes proteolysis that is initiates catabolism of protein via the peptide bands hydrolysis that linkage amino acids together in a chain of poly peptide[4]. Protease shear progress multiple times and diverse classes of protease can complete the same reaction via entirely altered catalytic mechanisms. Proteases can be found in bacteria, archaea, animals, plants and viruses. The immunoglobulins and immune cells can be attacked by proteases [5,6]. The mode of growth (biofilm) is another important virulence factor contributing to the *K. pneumoniae* pathogenesis in clinical settings which is involved in acute and chronic infections [7]. Biofilm is defined as an exopolysaccharied-surrounded bacterial complex on the biotic or abiotic surfaces and is made from planktonic cells (individual free-floating) .In the biofilm bacterial cells often show a diversity of phenotypic alterations from those in the culture of planktonic .These include some phenotypic changes such as motility, production of extacellular polysaccharide and increased resistance to antibiotic and host defense system [8].

The aim of the study find the relationship between the resistance of *K. pneumoniae* bacteria to antibiotics of quinolones and their ability to produce enzymes of beta lactamase.

Materials and methods

Samples collection

The isolation and diagnosis of fifty one of *K. pneumoniae* isolates were constituted the following percentages; from urinary tract infection (25) 49.1%, Wound infection (16)31.3 % and Burn infection (10) 19.6% from total isolation at several hospitals in Baghdad. All bacterial isolates were identified by the biochemical, cultural and microbial characteristics and confirmed by Api E20 System [10].

Production of β -lactamase test

Rapid lodometric method is used for the identification of production of β -lactamase enzyme to the isolates (9).

Production of urease test

In this test, slant solid urea substrate was used for all *K. pneumoniae* (isolates9)

Biofilm Formation

The bacterial activity for biofilm formation was measured by culturing on congo –red agar media, in which cells were incubated for 18 h to 36h at 37 c, dark ends of growing determined the biofilm production (10).

The sensitivity of isolates against four tested quinolones groups

Fifty one isolates of *K. pneumoniae* were tested for the identification on sensitivity to (Ofloxacin , Norfloxacin , Ciprofloxacin and Levofloxacin) by using of Mueller-Hinton agar and depending on inhibition zone CLSI (11).

Determination of the minimum inhibitory concentrations of ciprofloxacin

Two fold dilution methods were used on Mueller-Hinton agar for determinate MIC_s of Ciprofloxacin [12].

Plasmid DNA extraction

Plasmid DNA extraction was isolated from resistant bacteria for Ciprofloxacin and the extraction results was determinate by agaros gel (%0.8).

Result and discussion

Diagnosis of *K. pneumoniae*

The results revealed that 51 out of 145 isolates (35.2%) were *K. pneumoniae*, whereas 94 out of 145 isolates (64.8%) for other bacteria from diverse clinical source are displayed in table (1) and figure (1).

Table (1) Isolated bacteria combined according to source and the percent of infection

Isolat ed bacteria	Other		<i>K. pneumoniae</i>		total
	N O.	%	N O.	%	
urinary tract infection	46	48.9	25	49.1	71
Wound infection	28	29.8	16	31.3	44
Burn infection	20	21.3	10	19.6	30
Total number	94	100	51	100	145

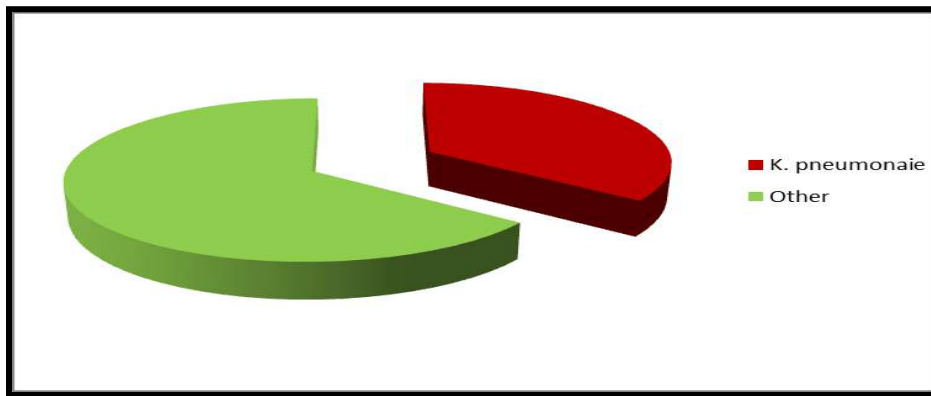


Figure (1): Percent of bacteria spp and *K. pneumoniae* from clinical samples

β -lactamase enzyme Production

Quinolones are inhibited by β -lactamase enzyme and this enzyme was identified via method of rapid iodometric. The positive test showed that the color of iodine was converting from deep color to colorless. The results exhibited that (35) isolates produce this enzyme whereas, (16) isolates were not produce the enzyme and β -lactamase enzyme production goods not existence in all isolates were study. The results in stability with the results of (1) which stated that *K. pneumoniae* isolates produce β -lactamases enzyme.

Urease enzyme production

The urea is uses by urease enzyme and release of alkaline pH of the basal ammonium and alters for example a substrate the phenol red to pink .This study displayed that (35) isolates were producer of the enzyme in percent of (%68.62) whereas, (16) isolates in percent of (%31.38) were not producers of this enzyme.

Formation of biofilm

After incubation at 37⁰C on Congo red agar media, the biofilm was formed. Whereas, our results exhibited that wholly the (38) isolates of *Klebsiella* revealed complete formation of biofilm via forming colonies of deep darken on Congo-red agar medium figure(2).These consequences are covenant with that of [13], which they can isolate a high incidence of Germany clinical isolates that have analogous activity of virulence factors like siderophores, resistance to serum, production of capsule and formation of biofilm.



Figure (2): Biofilm producer on Congo- red agar by *K. pneumoniae* isolate

Quinolones sensitivity test

The result indicated that (% 49.1) of the isolates were resistant to Norfloxacin, %43.1 were resistant to Ofloxacin and %39.2 were resistant to Ciprofloxacin whereas, the lower percent of resistant to Levofloxacin %23.5 were exposed in figure (3).

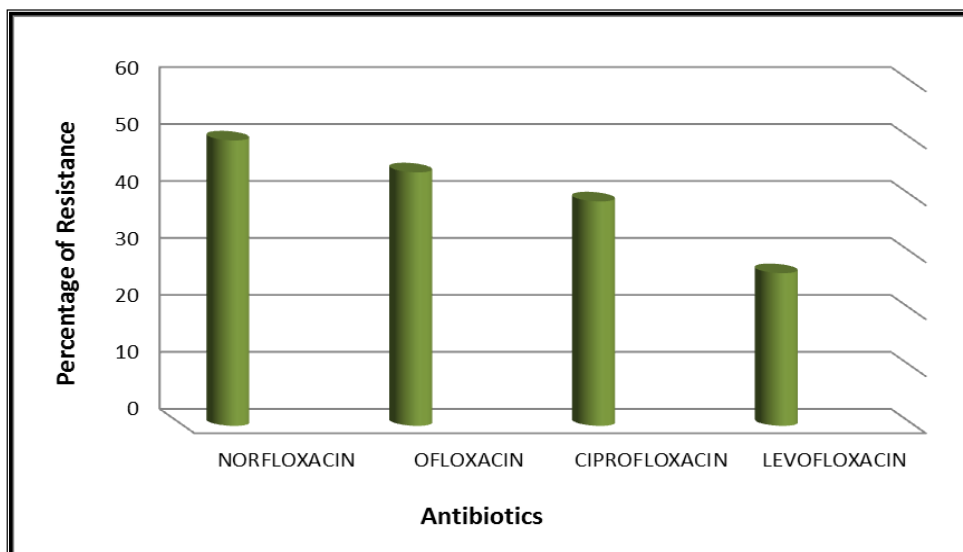


Figure (3) the percent of resistant to Quinolones for *K. pneumoniae*

The results agrees with the results of [14] which establish that the resistant percent to Ciprofloxacin was %35 (from 110 isolate) along with in figure (4) displayed that positive isolates to β -lactamase enzyme were hyper resistant equaled with negative isolates to β -lactamase enzyme. This may be because of using of these antibiotics in treatment infection of *K. pneumoniae* which resistant to other antibiotics. The percent of resistant in which study to positive isolates to β -lactamase enzyme were %80 to Levofloxacin, %72.6 to Ciprofloxacin and %66.4 to Ofloxacin although, the percent of resistant to negative isolates to β -lactamase enzyme were %36.8 to Norfloxacin, %33.6 to Ofloxacin, %27.4 to Ciprofloxacin and %20 to Levofloxacin.

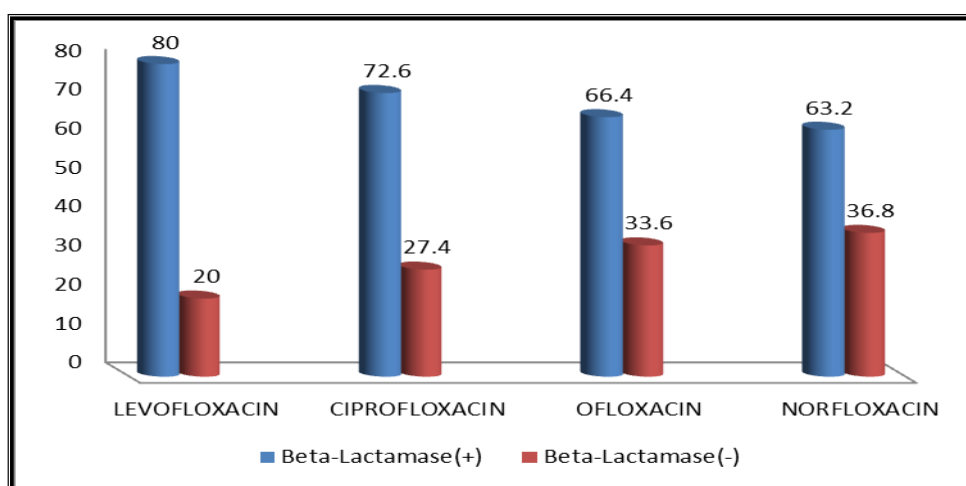


Figure (4) Percent of resistant to antibiotics for positive and negative isolate to β -lactamase

The isolated *K. pneumoniae* from diverse infections resistant to altered types of antibiotics and hyper using of these antibiotics in treatment of infection result in the resistant due to bacterial modification in addition to presence of plasmid of resistant or collective between chromosome and plasmid of bacteria through conjugation and transformation [15].

The recent agree study displayed %51.6 from the isolates were resistant to Norfloxacin and these results agreed with results in India which revealed that *K. pneumoniae* are resistant to these antibiotics and Vancomycin [16]. These antibiotics used broadly from production 1986 in treatment of systemic infection counter to Gram positive and Gram negative bacteria [16].

Determination of MIC to Quinolones

This study exhibited (Table 2) that MIC of Norfloxacin to *K. pneumoniae* isolates were between (16-512) Mg/ml and there were (5) isolates that resistant of (512) Mg/ml. Whereas, MIC of Ofloxacin range between (8-512) Mg/ml and there were (3) isolates that resistant of (512) Mg/ml in addition to, the MIC of Ciprofloxacin to *K. pneumoniae* isolates were between (4-512) Mg/ml and there were (2) isolates that resistant of (512) Mg/ml. Finally, the MIC of Levofloxacin to *K. pneumoniae* isolates were arranged among (4-512) Mg/ml and there were (0) isolates that resistant of (512) Mg/ml. These results were agree with the results in (17) which displayed that *K. pneumoniae* isolates were hyper resistant to Norfloxacin and the difference of range MIC between (8-512) Mg/ml may be due to the quality in collection of sample from difference clinical source and from diverse ecology due to alteration of isolates in range of exposure to antibiotic and in altered concentrations.

Table (2): MIC of isolate to Quinolones

MIC Mg/ml	Norfloxacin	Ofloxa cin	Ciprofloxa cin	Levofloxacin
	Number of isolates			
4	-	-	6	10
8	-	2	8	9
16	8	9	13	18
32	10	7	10	8
64	16	13	4	4
128	5	12	6	2
256	7	5	2	-
512	5	3	2	-

Plasmid DNA extraction for *K. pneumoniae*

Extraction of plasmid DNA was isolated from resistant bacteria of Norfloxacin and the results of extraction presented existence of single bands of plasmid in two isolates (Kp₂₈, Kp₄₂) as revealed in figure (5). Other study [18] supposed that resistant to Norfloxacin of *K. pneumoniae* was chromosomal. The plasmids double circle strands DNA presence in cytoplasm contain genes that responsible for resistant property can be transfer between one genus types or between different types of genus, the gene can be transfer through plasmid between bacterial types for example between *Enterobacteriaceae* and *P.aeruginosa*, these property give the bacteria resistant to quinolones and these type of resistant very little between clinical isolate (19). Quinolones resistant by plasmid give low level resistant compared with other types of resistant mechanism (20).

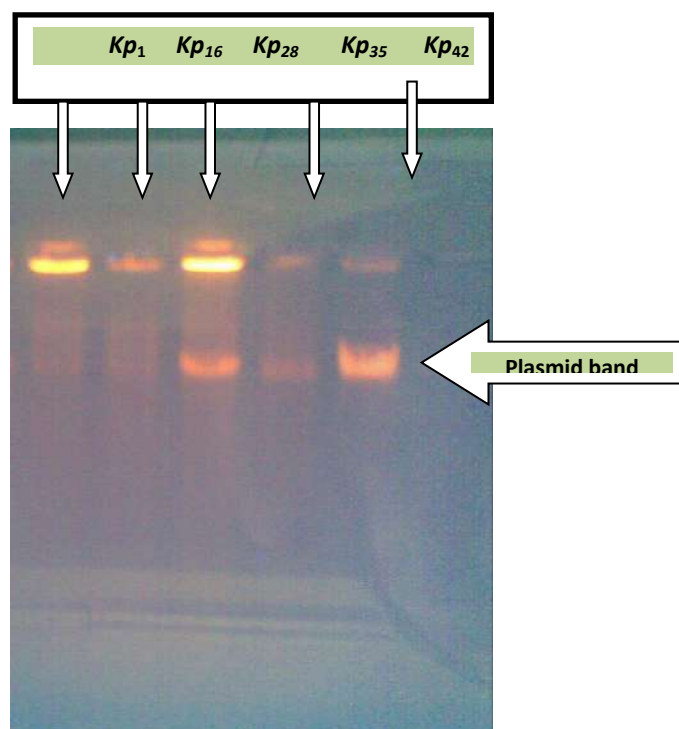


Figure (5) Plasmid contents for some *K. pneumoniae* isolate resistant to Norfloxacin on agarose gel (%0.8)

References

- (1) L.G. Harris^{1,2*}, S.J. Foster², and R.G. Richards¹ (2002) . an introduction to staphylococcus aureus, and techniques for identifying and quantifying s. aureus adhesins in relation to adhesion to biomaterials. EUROPEAN CELL AND MATERIAL vol . 4 : pp39-60 .
- (2) Ban A . Mohammed , Ali M . Hassan and Ashraf S. Hassan (2012) . In Vitro Antimicrobial Activity of Thymus vulgaris, Origanum vulgare and Rosmarinus officinalis Against Dental Caries Pathogens , Ibn al-Haitham journal for pure & applied science , VOL: 25 , No:2 , ISSN: 1609-4042 .
- (3) Khatoon A , kamal M, Syed F. hussain , wasi alam , omair rauf and syed M. shahid (2010) antimicrobial susceptibility patters and identification of plasmid – borne methicillin resistant Staphylococcus aureus . American –Eurasian j . agaric & environ 7(2) 139-145
- (4) Ryan ,K.J. and Ray , C.G.(2004). Sherni,s Medical Microbiology (4th ed) .McGraw Hill.P.396-401.
- (5) Madigan, M.T.; Martinko, J.M. and Parker, J. (2003). Prock Biology of Microorganisms. Tenth edition. Prentice- Hall, Inc. London, Sydney, pte. Ltd. Hong, Kong, Toronto, S.A. dec.v. Tokyo, pte, Ltd, Uper Saddle River, New jersey.
- (6) Mohammed F. AL-Marjani , Kadhim A. Kadhim , Afraa A. Kadhim and Ahmad AB Yosef Kinani (2015) . Ciprofloxacin Resistance in Staphylococcus aureus and Pseudomonas aeruginosa Isolated from Patients in Baghdad . International Journal of Pharma Sciences and Research (IJPSR) . Vol. 6 No.2 Feb 2015 .
- (7) Henry F. Chambers¹ and Frank R. DeLeo² . (2009) .Waves of Resistance: Staphylococcus aureus in the Antibiotic Era . blished in final edited form as: Nat Rev Microbiol. 2009 Sep; 7(9): 629–641.
- (8) Kenneth Todar, Ph.D .(2012) Staphylococcus aureus and Staphylococcal Disease . Todar's Online Textbook of Bacteriology www.textbookofbacteriology.net
- (9) WHO (1978) Techniques for the detection of B-Lactamase producing strains of *Neisseria gonorrhoea.*, 616:137-43 .
- (10) Hassan, A.; Usman, J.; Kaleem, F.; Omair, M .;Khalid, A. and Iqbal ,M.(2011). Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz. J. Infect. Dis.; 15(4):305-311.
- (11) CLSI (Clinical & Laboratory Standards institute) (2009). Performance Standards for Antimicrobial Susceptibility Testing, 19th supplement, CLSI document M100-S19. 29 (3). CLSI, Wayne, Pennsylvania, USA.
- (12) Alalem ,A.M. (2008). Antibiotic resistant *S.aureus* infection studies in hospitals .Ph.D, Middle East Technical University.Turkey.
- (13) Podschun ,R.;Fischer,A; and Alluman,U.(2000).Expression of putative virulence factors by clinical isolates of Klebsiella planticola .J.Med.Microbiol.49(2):115-119.
- (14) Paul, R.; Das, N.K.; Dutta, R. and Banerjee, A.K. (2011). Bacterial contamination of the hands of doctors: Astudy in the medicin and dermatology wards. Original Article, 77(3):307-313.
- (15) Tiwari,H.K. and Sen,M.R.(2006). Emergence of vancomysin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Diseases .6:156-161.

- (16) Bearden, D.T. and Danziger, L.H. (2001). Mechanism of action and resistance to quinolones. *Pharmacotherapy* .21(10): 224-232 .
- (17) Swedres (2010). A Report on Swedish Antibiotic utilization and Resistance in human Medicine. SMI.
- (18)- Islam, S. (2008) chromosomal antibiotic resistance mechanisms in *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae* . from department of Laboratory medicine Karolinska institute, Stockholm, Sweden .
- (19) Jacoby, A. G.; Nancy C. and Ken, B.W.(2003) . prevalence of plasmid-mediated Quinolone resistance . *Antimicrobial Agents Chemotherapy* , 47(2):559.
- (20) Farra, A. (2007) antibiotic resistance and antibiotics consumption in Sweden with focus on *E.coli* and *Pseudomonas aeruginosa* from the department of Medicine infection Speisease unit Karolinska instate, Stacham. Sweden.



SPECTROSCOPIC ANALYSIS OF MAGNESIUM OXIDE PLASMA PRODUCED BY LASER

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SPECTROSCOPIC ANALYSIS OF MAGNESIUM OXIDE PLASMA PRODUCED BY LASER

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Abstract:

My proceeding prepared magnesium oxide nanoparticles using pulsed laser deposition in an air environment. At which deposition of pure Mg and MgO powders prepared as bult with thickness 1 cm under pressure 6 ton and Using a Q-switched Nd: YAG laser with a wavelength of 9nsec of 1064 nm, the influence of energy laser on size material of particle as well as optical characteristics and surface morphology as a result of laser pulses were examined. The absorption spectra of MgO exhibited a redshift in UV-visible measurements. X-ray diffraction (XRD) investigation showed the Mg metal powder percentage purities. The properties of plasma are studied using optical emission spectroscopy (OES).

Key words: Magnesium (Mg), Magnesium oxide (MgO), Pulse Laser Deposition (PLD), Optical Emission Spectroscopy (OES).



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Introduction:

Plasmas created by a laser resulting due to the interactions with solid objects have a lot of potential for both basic and practical study. Many criteria that characterize the qualities of the target influence the characteristics of these plasmas, including medium of the environment properties, Pulse duration and laser wavelength, and so on[1]. The ablation process is broken into three steps when utilizing long pulse duration lasers (> 1 ns). The laser light interacts with the solid in the first stage, creating rapid ionization of the target surface into plasma in a short period relative to the pulse duration. The plasma absorbs the laser light successfully in the second stage and expands isothermally. Finally, the produced a plasma plume grows quasi-adiabatically in a medium with or without applied fields, which can be a vacuum or a background gas [2]. Because of the mechanisms of optical absorption commence LIBS sampling, it is possible to analyze solids, liquids, and gases[3]. A plasma is generated when Warms, The energy from the laser pulse ablates, atomizes, and ionizes the sample material.

MgO is a crystalline solid with a high ionic content, crystallizing into a building made of rock salt. There are fcc Mg⁺ and O Sublattice cleavage planes, as well as reduced Cleavage planes that are energy neutral (100). MgO has a lattice constant of 4.212\AA , a refractive index of 1.72, and a dielectric constant of 9.83, respectively. Magnesium oxide shows great promise choice in terms of bulk properties: a large bandgap (7.8 eV) [4].

Because of because to its low dielectric constant and dielectric loss, MgO has gained a lot of attention as a platform for a superconductor that can withstand high temperatures layer formation [5]. MgO has a variety of applications in microwave devices because to any of these qualities. MgO is an excellent buffer for epitaxial optical waveguide films because of its low refractive index[6].

A spectrograph and detector are used to detect the plasma light once it has been spectrally resolved. The resultant plasma spectrum can provide information that is both quantitative and qualitative, such as constituent composition. The widths, shapes, and shifts of emission lines may be used to figure out how hot the plasma is and how dense the electrons are [7]. The capacity of plasma temperature to define and predict other plasma features, such as relative populations of energy levels and particle speed distribution, makes it a significant thermodynamic parameter. The ratio approach with two Hydrogen lines was employed in this laboratory experiment, which presupposes that local thermodynamic equilibrium (LTE) is achieved inside the plasma. In a vacuum, at a pressure of 2.5×10^{-2} mbar, Using LIBS with irradiances more than 108 W/cm^2 and estimates, it has been demonstrated that LTE is frequently satisfied after a few hundred nanoseconds following plasma creation. Two spectral lines from the same ionization stage's intensity ratio of an atom or ion. may be determined using the ratio technique, which is a standard approach of measuring plasma temperature [8].

$$T = \frac{(E_2 - E_1)}{k \ln \left(\frac{I_1 \lambda_1 A_2 g_2}{I_2 \lambda_2 A_1 g_1} \right)} \quad (1)$$

Where T_e is electron temperature, E is the level energy of these spectral lines, I is intensity, λ is laser wavelength, g The is the likelihood of a transition, and is the statistical weight. The electron concentration was determined using a variety of approaches, including Stark widening of spectral lines in a linear fashion, which is a well-known technique. Doppler width and the Stark effect are the primary causes of line broadening in LIBS plasmas. Width of Doppler is determined by the emitting species' temperature and atomic mass; however,

because the hydrogen line's adverbial adverbial a Doppler width in this experiment is normally between 0.04 and 0.07 nm, this sort of widening is neglected in this experiment. Radiators and nearby particles generate the Stark effect, which causes pressure widening. Electron-ions collisions in plasmas produce these interactions to a large extent. The widening of the hydrogen line employed in this experiment is mostly due to the Stark effect [7,9].

In The Saha-Boltzmann equation is used, as well as spectral lines of the same element and ionization intensity; the equation for Saha-Boltzmann is as follows:[8]:

$$n_e = \frac{I_1}{I_2^*} 6.04 \times 10^{21} (T)^{3/2} e^{\frac{(E_1-E_2-X_z)}{kT}} \quad (cm^{-3}) \quad (2)$$

$$\text{Where: } I_2^* = \frac{I_2 \lambda_2}{g_2 A_2} \quad (3)$$

X_z is the species ionization energy in z level ionization in eV, I_z is the line intensity, λ is the wavelength of the associated transition, g is the statistical weight, and is the probability of the transition, and T_e is the electron temperature.

Plasma frequency (f_p) is calculated from the equation [8]

$$f_p \approx 8.98 \sqrt{n_e} \quad (\text{Hz}) \quad (4)$$

Plasma frequency is affected by plasma density, Because of this, the plasma frequency is quite high. the smallness of m of the electron [9].

The reaction of charged particles to minimize the effect of local electric fields is known as Debye shielding, and it is this shielding that gives plasma its quasi-neutrality feature. D , often known as the Debye length, is defined as [10].

$$\lambda_D = \left(\frac{\epsilon_0 k T_e}{n_e e^2} \right)^{1/2} = 743 * (T_e / n_e)^{1/2} \quad (5)$$

where: D stands for Debye length (cm), L stands for system dimension (cm), n_e stands for electron density, e stands for electron charge, while T_e stands for electron temperature (C) Electron density and temperature control the number of particles in the Debye sphere., and is denoted by N_D . The second requirement for plasma presence is $N_D \gg \gg 1$, which is as follows: [12].

$$N_D = \frac{4\pi}{3} n_e \lambda_D^3 \quad (6)$$

2-Setup for testing

A design representation of the LIBS setup is shown in Fig. (1). The LIBS setup consists of:

- ❖ Under vacuum ($P = 2.5 \times 10^2$ mbare)
- ❖ The Nd: YAG laser has a wavelength of 1064 nm, a pulse duration of 9 ns, and a repetition rate of 6 Hz, 10 mm focal length, and energy range (500 to 1000) mJ.
- ❖ To create a plasma plume, a Nd: YAG laser with a wavelength of 1064 nm was focused on a target placed under vacuum. The laser light was aimed at the object at a 45o angle to form a beam of radiation. An optical fibre attached to a spectrometer has been used to

capture the optical emission spectra of Mg and MgO plasma. The analyzer has a high resolution and responds to wavelengths from 200 and 900 nm with 3648 pixels, depending on the grating used. To establish the plasma characteristics, the produced spectra sections were studied and Data from the National Institute of Standards and Technology was compared (NIST) [13].

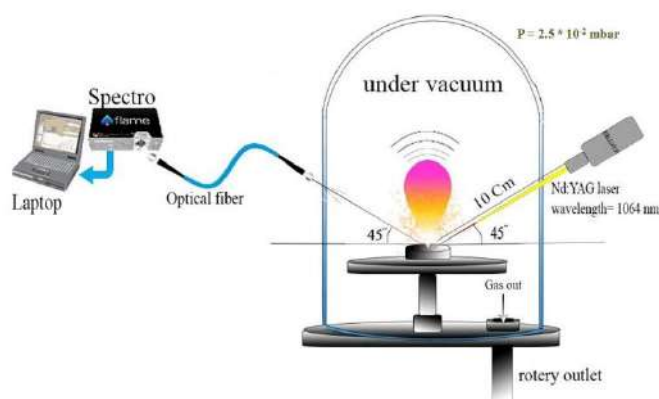


Figure 1: Setup of the beam plasma spectroscopy technology.

3-Discussion of the Findings

Fig. (2) and (3) display the spectrum of optical emission of MgO and Mg plasma, respectively. MgO and Mg plasma produced by laser with different pulsed laser energy (400,600,800,1000) mJ, which is confined in a vacuum, as shown in Fig. (3). The optical emission spectra were recorded in the spectral range (400-1000) nm. The lines emission related to Mg and O. In contrast, Fig. (3) appear the optical emission spectra of Mg plasma, the lines emission also related to Mg and O. From figures, the intensity of plasma increases with increased laser energy.

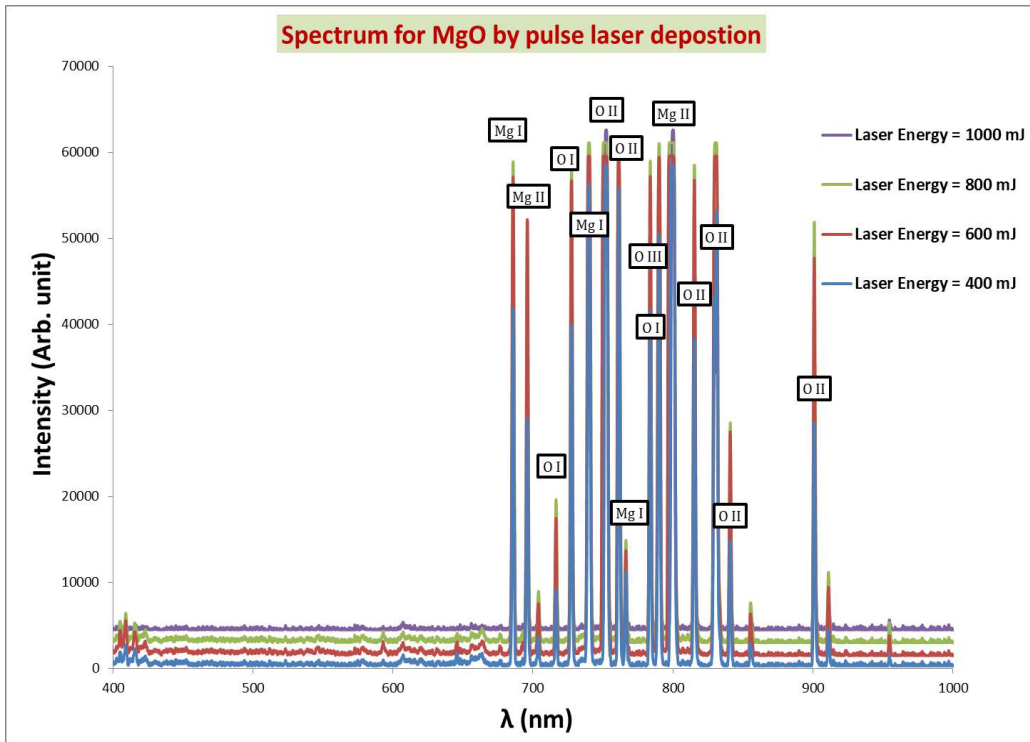


Figure 2: The intensity of MgO plasma in a Different laser energies in a vacuum.

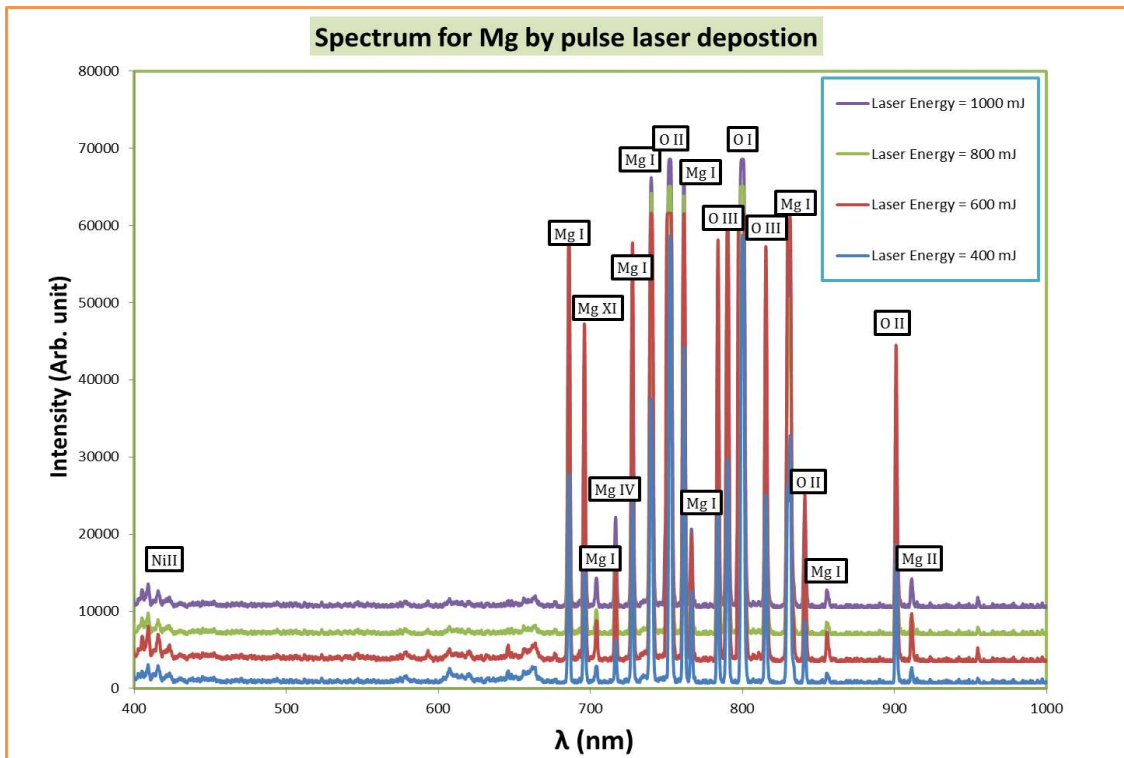


Figure 3: The intensity of Mg plasma in a vacuum with various lasers energies

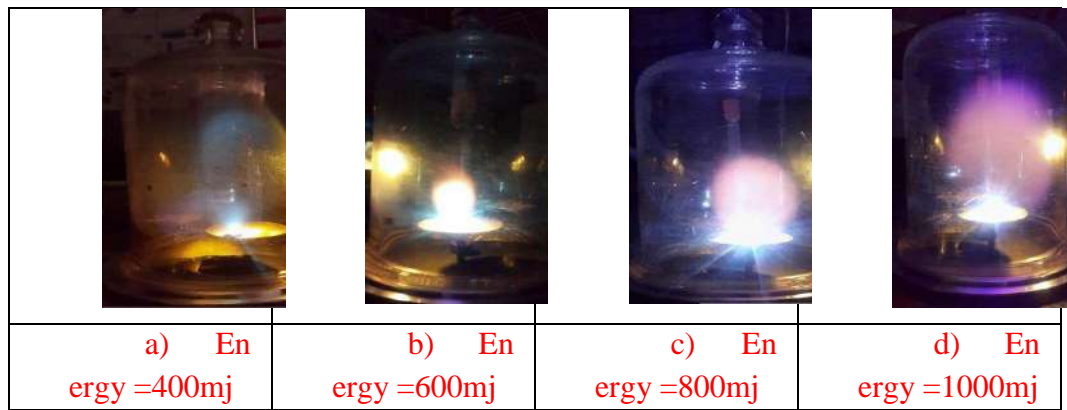


Figure 4: plume plasma Mg Different laser intensities are used to hit a target in a vacuum.

The plasma properties were estimated using optical emission spectra. The computed Temperature of electrons (T_e), density of electrons (n_e), frequency of plasma (f_p), and Debye length (D), and Debye number (N_D) for MgO and Mg plasma, respectively, lasers pulse energies are shown in Tables (1) and (2). Figures (4) and (5) depict the electron temperature and density of MgO and Mg plasmas, respectively. T_e and n_e increase as laser energy increases. Because free electrons in plasma have a high kinetic energy relative to collisions in other media, energy transfer to species is possible in a variety of ways and the presence of secondary charged particles is linked to the elevated electron temperature T_e in the vacuum for MgO and Mg.

The plasma requirements are met based on the findings of plasma parameters (λ_D , f_p and N_D). Because f_p is proportional to n_e , the findings reveal that f_p increases with laser intensity, but λ_D and N_D decrease, as seen in Figure 1. (Nek. M. Shaikh et al.) [14]

Table 1: characteristics of plasma for MgO in vacuum using different laser intensities

Laser energy (mJ)	T_e (eV)	$n_e \cdot 10^{24}$ (cm^{-3})	f_p (Hz) $\cdot 10^{16}$	$\lambda_D \cdot 10^{-13}$ (cm)	N_d	Plasma idelity
1000	1.219	7.0	2.380	2.874	698833.988	0.365
800	1.123	3.7	1.724	3.809	852946.279	0.319
600	1.053	2.2	1.321	4.812	1010851.986	0.285
400	0.851	0.3	0.487	11.734	1990635.919	0.181

Table 2 shows the plasma characteristics for Mg in vacuum using different laser intensities (=1064)nm.

Laser energy (mJ)	T_e (eV)	$n_e \cdot 10^{24}$ (cm^{-3})	f_p (Hz) $\cdot 10^{16}$	$\lambda_D \cdot 10^{-13}$ (cm)	N_d	Plasma idelity
1000	0.992	8.0	0.025	242.693	48000013.111	0.022
800	0.975	6.9	0.024	260.000	50549072.780	0.021
600	0.968	6.4	0.023	267.785	51686152.769	0.021
400	0.961	6.1	0.022	274.998	52734532.728	0.020

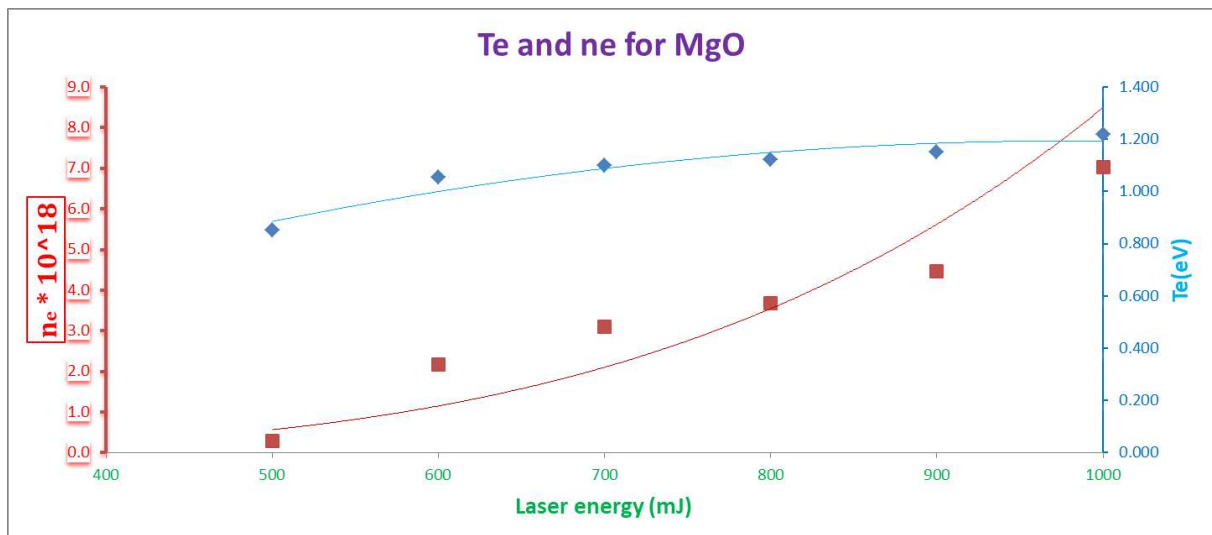


Figure 4: For MgO plasma in vacuum, the change of (T_e) and (n_e) vs energy of laser.

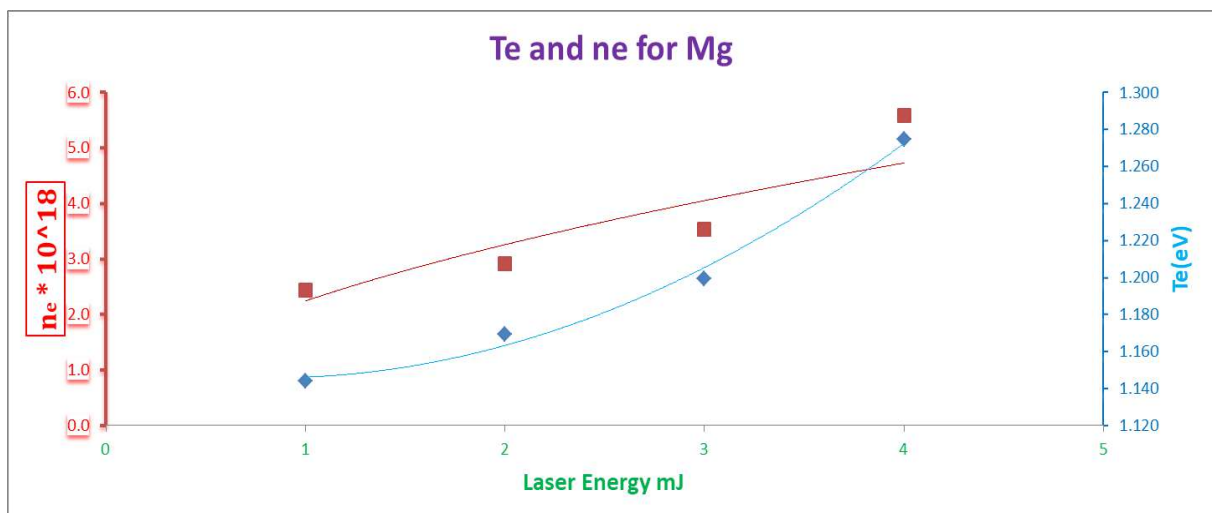


Figure 5: For Mg plasma in vacuum, the fluctuation of (T_e) and (n_e) vs energy of laser.

4-Conclusions

The laser energy had a considerable influence on the MgO and Mg plasma Intensity of spectral lines. The intensity of the plasma is shown to rise when the laser energy is increased. Fundamental plasma properties The temperature of electrons, their density, Debye length, the number of particles in the Debye sphere, and plasma frequency are all affected by laser energy. With increasing laser intensity, The temperature of electrons, the number density of electrons, and the frequency of the plasma all increase, whereas other plasma properties such as λ_D and N_D drop.

References

- [1] A. A. I. Khalil, “Spectroscopic studies of UV lead plasmas produced by single and double-pulse laser excitation,” *Laser Phys.*, vol. 23, no. 1, 2013.
- [2] N. M. Shaikh, Y. Tao, R. A. Burdt, S. Yuspeh, N. Amin, and M. S. Tillack, “Spectroscopic Studies of Tin Plasma Using Laser Induced Breakdown Spectroscopy,” *J. Phys. Conf. Ser.*, vol. 244, no. PART 4, pp. 2–5, 2010.
- [3] M. L. Najarian and R. C. Chinni, “Temperature and electron density determination on Laser-Induced Breakdown Spectroscopy(LIBS) plasmas: A physical chemistry experiment,” *J. Chem. Educ.*, vol. 90, no. 2, pp. 244–247, 2013.
- [4] K. Nashimoto, D. K. Fork, and T. H. Geballe, *Appl. Phys. Lett.* 60, 1199 (1992).
- [5] F. Ahmed, K. Sakai, H. Ota, R. Aoki, N. Ikemiya, and S. Hara, *J. Low Temp. Phys.* 105,1343 (1996).
- [6] X. L. Guo, Z. G. Lu, X. Y. Chen, S. N. Zhu, S. B. Xiong, W. S. Hu, and C. Y. Lin, *J. Appl. Phys.* 29, 1632 (1996).
- [7] S. Z. H. R. and J. A. Kashif Chaudhary, “Laser-Induced Plasma and its Applications,” *RFID Technol. Secur. Vulnerabilities, Countermeas.*, 2016.
- [8] S. S. Harilal, C. V. Bindhu, R. C. Issac, V. P. N. Nampoori, and C. P. G. Vallabhan, “Electron density and temperature measurements in a laser produced carbon plasma,” *J. Appl. Phys.*, vol. 82, no. 5, pp. 2140–2146, 1997.
- [9] M. C. Chen and E. C. Chen, *Introduction To Plasma Physics And Controlled Fusion*, vol. 1. Los Angeles, 1983.
- [10] A. M. El Sherbini, “Measurement of Plasma Parameters in Laser-Induced Breakdown Spectroscopy Using Si-Lines,” *World J. Nano Sci. Eng.*, vol. 2, pp. 206–212, 2012.
- [11] BORIS M. SMIRNOV, *Physics of Ionized Gases*, vol. 16, no. 1. 2001.
- [12] Suresh Chandra, *textbook of plasma physics*, 1ed ed. india, 2010.
- [13] version 5., “National Institute of Standards and Technology (NIST) Atomic spectra database,” 2017.



**ATOMISTIC SIMULATION STUDY ON THE CORROSION INHIBITION BY
SOME PHTHALIMIDE DERIVATIVES**

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ATOMISTIC SIMULATION STUDY ON THE CORROSION INHIBITION BY SOME PHTHALIMIDE DERIVATIVES

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Abstract:

In this study, we used both the semi empirical PM3 and the molecular dynamics simulation methods based on the density functional theory to pursue an accurate description of the corrosion inhibition behavior of some phthalimide derivatives on the copper in the nitric acid solution which have been studied previously by another researchers work, via determine the relationship between the molecular structure of phthalimide derivatives and inhibition efficiency. Experimental work by using the weight loss method and polarization techniques suggests that these derivatives can be used a inhibitor. First, We have modeled these three derivatives using the PM3 and DFT to get the most stable structure to describe the electronic parameters which are allied with inhibition efficiency such as the *EHomo*, *E LUMO*, the charge distribution, absolute electronegativity (χ) values. However, the theoretical study was in agreement with previous experimental stud, by considering that all these derivatives can be used as inhibitor.

Key words: Corrosion, Copper, DFT, IP, HNO₃.



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Introduction:

Copper is widely used in various industrial operations because of its good properties, for example, it is used in the production of wires, sheets, tubes, as well as to form alloys[1-5]. As know that the copper shown a resistant toward the effect of the atmosphere and different types of chemicals, but, in corrosive media such as the acidic media it is susceptible to corrosion. However, the investigation of the corrosion behavior of copper in the aggressive media especially in the acidic media is still a subject of many research work, and that because of, there is no formed of any protective passive layer on the copper surface, and based on that the protection of copper from the corrosion still attracted many researchers by using different techniques and different types of inhibitor, However, the selectivity of specific kinds of inhibitor to reduce the corrosion effects is considered one of the most effective methods for the metal protection in against the corrosion [6-12].

Generally, corrosion inhibitors are molecules that have the capability of slow down the corrosion rate of the system under investigate, and to get that these substances should had an interaction with the surface of the metal or alloys which be usually via the adsorption of these molecules on the metal/or alloy surfaces which will lead to block the active sites that are presence on the metal or alloy surface by displacing water molecules, and a results of that will lead to form of compact barrier layer on the metal/or alloy surface [11, 13]. In general, the compounds or substances that used to act as corrosion inhibitor is divided to, organic and inorganic inhibitors, and this due to the chemical constituents of the inhibitor [14-16], and from the literature is found that some of the inorganic inhibitors should be avoided such as, phosphate, arsenates and dichromate, and that because of the cost, degradation behaviors and the toxicity of these inhibitors.

Over the years, there are many significant efforts to find the most suitable corrosion inhibitors, and from the literature shown that the most substances which can be used as inhibitors are the organic molecules, which usually containing in their molecular structure a heteroatoms such as, nitrogen, oxygen, sulphur, multiple bonds and/or aromatic ring and these can be used because the capability of donate single pairs of electron to the surface of the metal or alloy and prevent or reduce the corrosion form start [14, 17] and allow an adsorption on the metal or alloy surface especially in the acidic corrosive media [13, 18-20], however, the inhibition efficiency will follow the sequence $O < N < S < P$. On the other hand, there are several factors need to be in our consideration to choose the inhibitor, for instance, the cost, availability of the materials and its amount, but the more important factor is the safety of the substances toward the environments. Recently, most researcher focus on using inhibitors which are eco-friendly to the environments, for instance, banan peel, henna, rosemary leaves delonix regia extracts[21-23] and many others Also the corrosion protection of copper have been investigated by several studies using the green inhibitors, for instance, using the natural honey[24], as well as the *Rosmarinus officinalis* are used to act as green inhibitor for different types of metals namely, aluminum, zinc, iron, and the copper as well[25].

Although all these experimental works to find the suitable way to prevent or reduce from the corrosion effects on the metal and alloys, when the experiment is achieved under well-defined all the conditions but, still the understanding of the adsorption mechanism of how the molecules can adsorbed onto the metal/alloy surface and the type of formed bond not clear. In this respect, the computational tools are play an important role *via* applied the

Density Functional theory or the semiempirical methods, and that because the simulation methods for instance, the density functional theory is considered as inexpensive and one of the most reliable modeling methods that can be used to examine the interactions that can be happened between the adsorbate molecules and the adsorbent also describe the molecular structure reactivity and all that can give an idea about the inhibition efficiency, as a result, this can help us to develop a new efficient and cost-effective corrosion inhibitors.

However, in the last decade, in the surface science field have been used the computational simulation methods to understand more about the different types on interactions that can be occurred between the molecules and the surface of the metal or alloy aid on the previous experiment results. For this reason, this paper aims to use and apply a series of different modeling techniques which are included both PM3 and DFT to extend the investigation of using three different phthalimide derivatives, which are used in previous experimental work as inhibitor for the copper corrosion [26], and discuss the relationship between the simulation calculations and experimental results by determining and calculating the most common electronic parameters which are related to the inhibition efficiency such as, the energies of highest occupied molecular orbital (E_{HOMO}) and the lowest unoccupied molecular orbital (E_{LUMO}), the band gap, and the dipole moment (μ). And all this because copper is considered one of the most technology important metals, also is an example of noble metals that have the d-band completely full.

1. Computational Details

In this study, and in order to find the most stable conformations of the corrosion inhibitor and to speed up our calculations, therefore, the structure of the molecules have been firstly optimized by using the semi-empirical calculation with PM3 [27, 28], then used the molecules structures that obtained from the PM3 simulations to re-optimize it again and that by using the DFT simulation method to get the full optimized conformations and evaluate the quantum chemical parameters. In this work, the calculation depends on using the DFT as method are implemented by using two different basis sets 6-311G(d,p) with using the Becke-3-Lee-Yang-Parr (B3LYP) functional [29-32] and this methodology shows a favorable geometries for a wide range of compounds. In this work, all the simulations are done using the GAUSSIAN 09 W software.

2. Results and Discussion

To understand the nature of interaction between the different adsorption centers of phthalimide derivatives that are shown in Figure 1, and the copper surface, IP then DFT have been used to fully optimize of the supposed inhibitors structures, and all the quantum chemical parameters for all the three different derivatives have been calculated and illustrated in table 1.

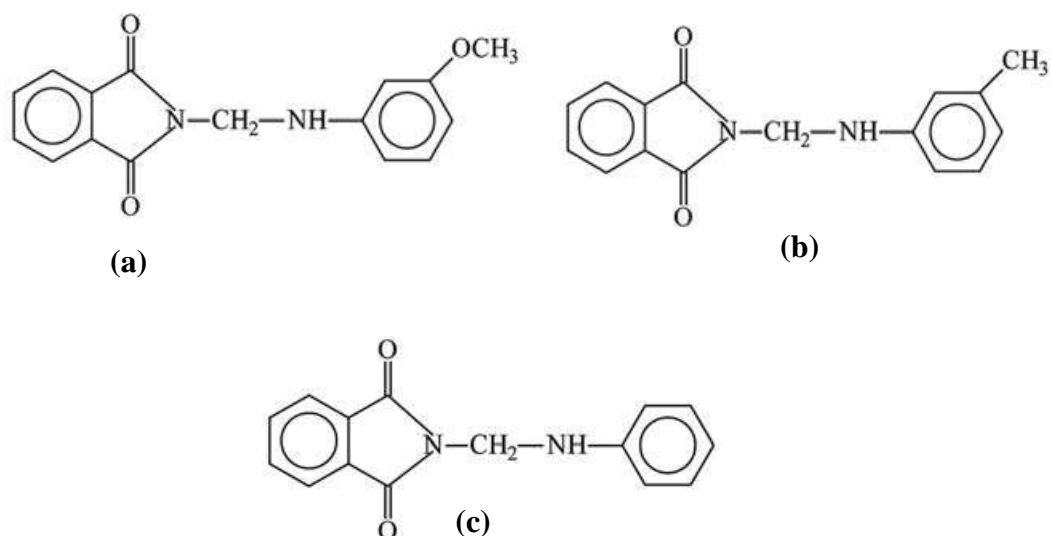


Figure 1: Phthalimide derivatives used as inhibitors [26]

Table 1:- The quantum chemical parameters of Phthalimide derivatives.

Parameters	Inhibitors		
	(a)	(b)	(c)
$\Delta E = E_{HOMO} - E_{LUMO} $	3.0477	3.0212	3.0771
$I = -E_{HOMO}$	5.3269	5.3517	5.4208
$A = -E_{LUMO}$	2.2792	2.3304	2.3437
$\chi = (I+A)/2$	3.8030	3.8410	3.8823
$\eta = (I-A)/2$	1.5240	1.5107	0.7693
$s = 1/\eta$	0.6562	0.6619	1.2990

According to the MOs theory can be investigate the tendency of the molecule to donate the electron to the copper surface. Firstly, based on the value of band gap, the lower value can be referring to presence of strong interaction between the copper surface and the inhibitors under investigation. From the results in table 1 can be see that compound (b) has the lower of ΔE which can be consider is much preferable as inhibitor if compared with other two different derivatives of phthalimide, but if based on the calculated of E_{HOMO} found that compound (c) is much better because the higher of E_{HOMO} is consider the one which has the high ability to donate the electrons to the empty orbital of the copper. Based on that, our results are not in agreement with previous study [26] which supposed that compound (a) is the most preferable inhibitor. In our opinion, it should be as found from the experimental work, because it has more than heteroatoms if compared with (b) and (c), but may the design of these compounds is based just on the change the atom that attach with the -CH₃, which could be not affect the distribution of the electrons as shown in Figure 2-4, respectively.

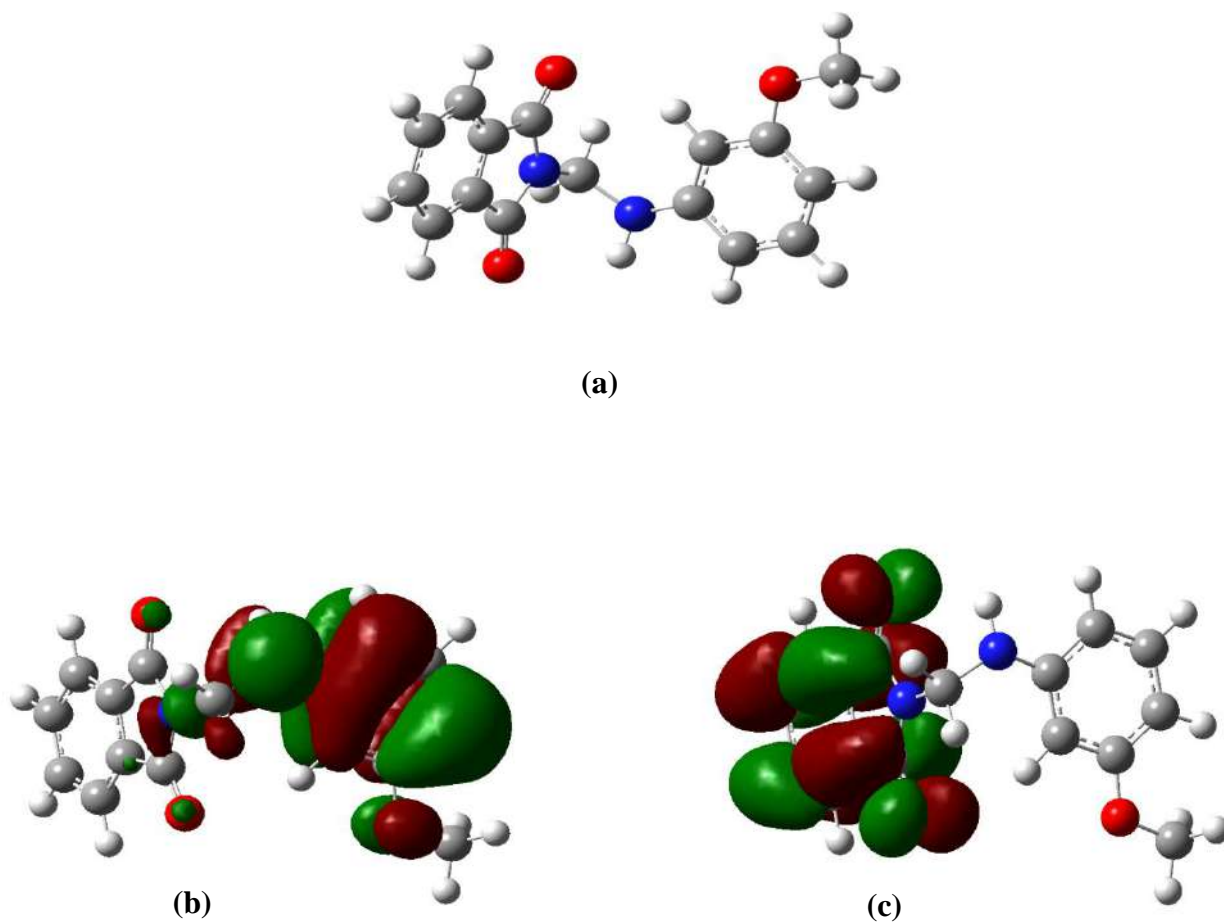


Figure 2: Optimized structure of compound (a), whereas, a is the full optimized geometry, b is the HOMO and c is represent the LUMO.

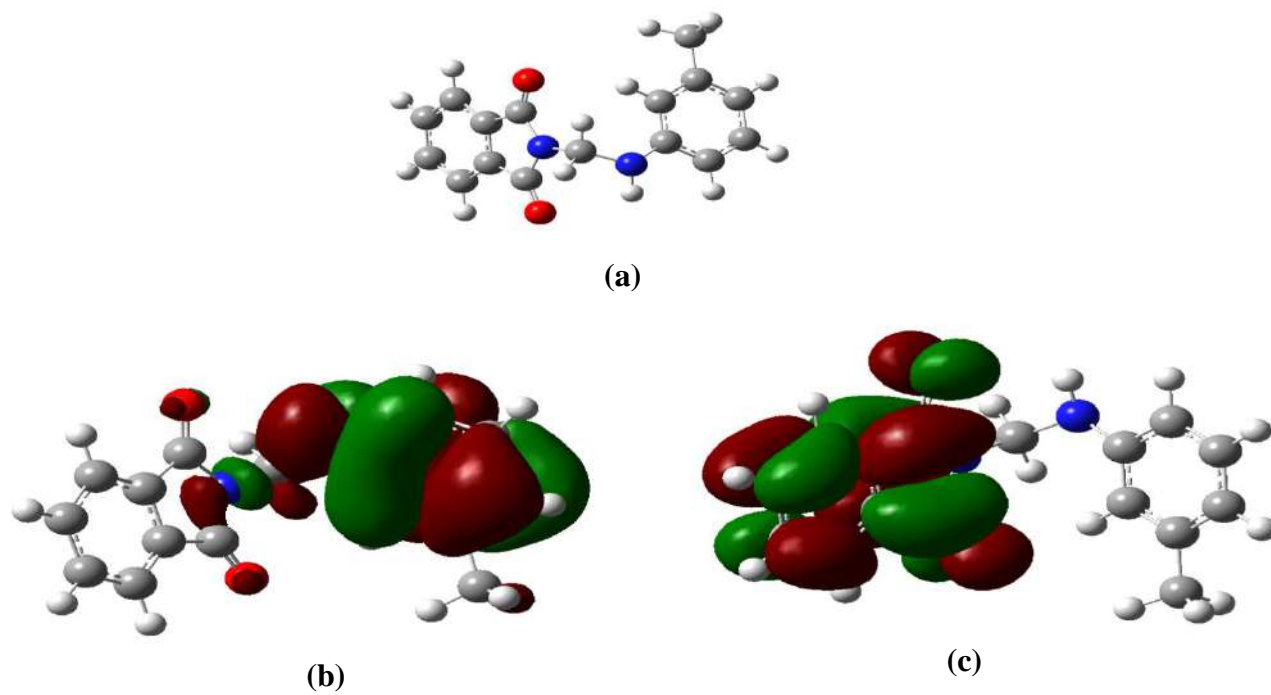


Figure 3: Optimized structure of compound (b), whereas, a is the full optimized geometry, b is the HOMO and c is represent the LUMO

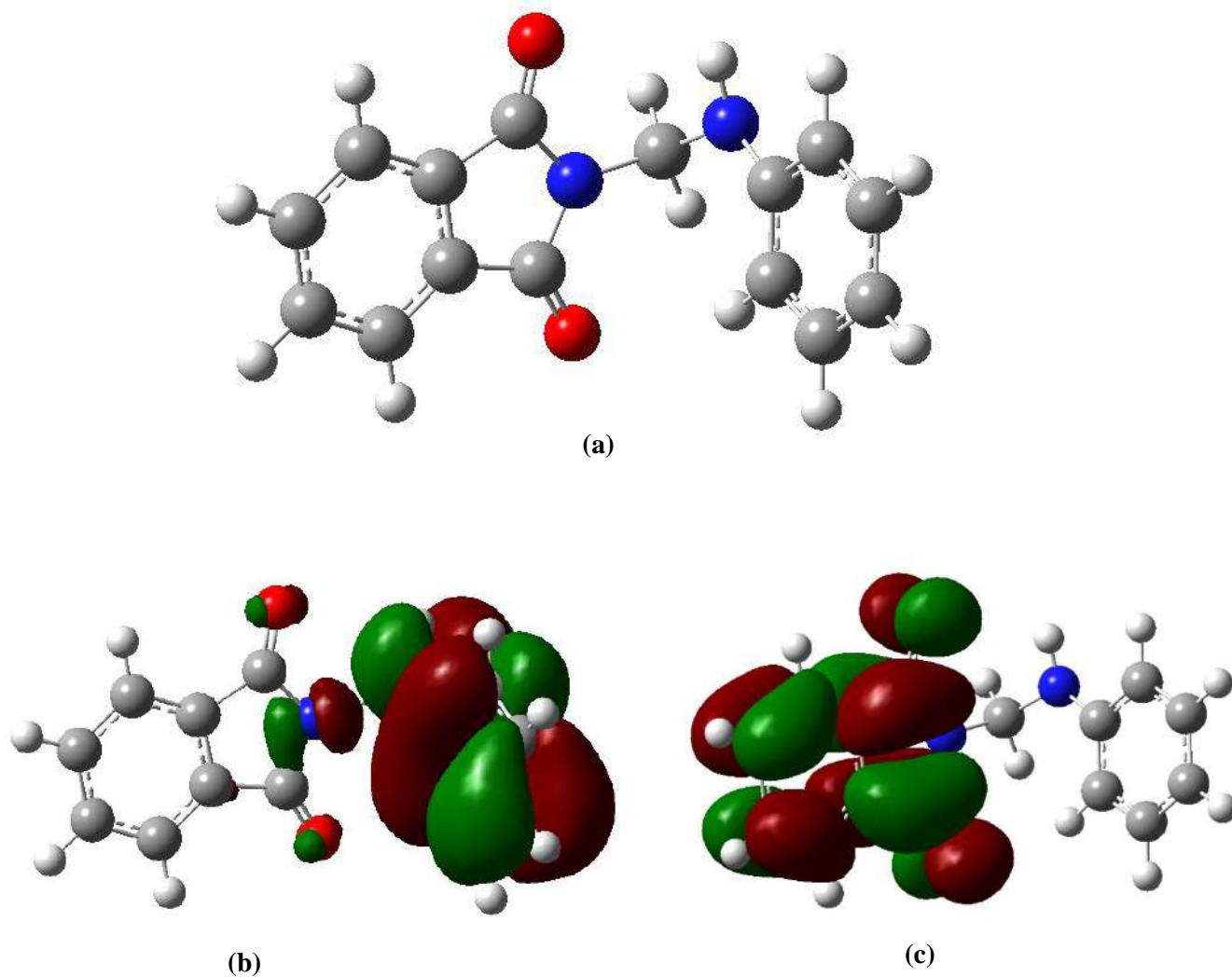


Figure 4: Optimized structure of compound (c), whereas, a is the full optimized geometry, b is the HOMO and c is represent the LUMO.

However, there are other parameters such as the chemical hardness and electronegativities our results shown that compound (a) has the lowest value of electronegativity which mean that occurring of donating and flow of electrons from it and here, our results can be explain the high inhibition efficiency of (a) what presented by previous work[26]. On the other hand, all the molecules are shown a higher chemical hardness if compared with the iron, which means that all these molecules are consider a good inhibitors.

3. Conclusions

In conclusions both the theoretical and experimental work were shown that these molecules can be used to inhibited the corrosion of copper in the acidic aggressive media (HNO_3), And consider compound (a) is the more reactive one toward donate the electrons to the copper surface and has the strong interaction by formed a film over the surface and that due to it has the lowest electronegativity.

References:

- [1] Z. Tao, G. Liu, Y. Li, R. Zhang, H. Su, S. Li, Electrochemical Investigation of Tetrazolium Violet as a Novel Copper Corrosion Inhibitor in an Acid Environment, *ACS Omega* 5(9) (2020) 4415-4423.
- [2] Å. Björkbacka, C.M. Johnson, C. Leygraf, M. Jonsson, Role of the Oxide Layer in Radiation-Induced Corrosion of Copper in Anoxic Water, *The Journal of Physical Chemistry C* 120(21) (2016) 11450-11455.
- [3] J.S. Daubert, G.T. Hill, H.N. Gotsch, A.P. Gremaud, J.S. Ovental, P.S. Williams, C.J. Oldham, G.N. Parsons, Corrosion Protection of Copper Using Al₂O₃, TiO₂, ZnO, HfO₂, and ZrO₂ Atomic Layer Deposition, *ACS Applied Materials & Interfaces* 9(4) (2017) 4192-4201.
- [4] H. Gerengi, M. Mielniczek, G. Gece, M.M. Solomon, Experimental and Quantum Chemical Evaluation of 8-Hydroxyquinoline as a Corrosion Inhibitor for Copper in 0.1 M HCl, *Industrial & Engineering Chemistry Research* 55(36) (2016) 9614-9624.
- [5] Y. Wang, Y. Yu, J. Zhang, L. Gao, L. Feng, D. Zhang, Click-assembling triazole membrane on copper surface via one-step or two-steps and their corrosion inhibition performance, *Applied Surface Science* 427 (2018) 1120-1128.
- [6] G. Gece, Drugs: A review of promising novel corrosion inhibitors, *Corrosion Science* 53(12) (2011) 3873-3898.
- [7] N. Dheer, R. Kanojia, C. Chandra, H. Kim, G. Singh, 4-(2-pyridylazo)-resorcinol as effective corrosion inhibitor for mild steel in 0.5M sulphuric acid, *Surface Engineering* 23(3) (2007) 187-193.
- [8] A.Y. Musa, R.T.T. Jalgham, A.B. Mohamad, Molecular dynamic and quantum chemical calculations for phthalazine derivatives as corrosion inhibitors of mild steel in 1M HCl, *Corrosion Science* 56 (2012) 176-183.
- [9] J.Z. Ai, P. Mei, W. Chen, X.P. Guo, Investigation on imidazoline amide adsorption and electrochemical behaviour of coupled steels, *Surface Engineering* 23(1) (2007) 62-67.
- [10] H. Ju, Z.-P. Kai, Y. Li, Aminic nitrogen-bearing polydentate Schiff base compounds as corrosion inhibitors for iron in acidic media: A quantum chemical calculation, *Corrosion Science* 50(3) (2008) 865-871.
- [11] A. Kokalj, Ab initio modeling of the bonding of benzotriazole corrosion inhibitor to reduced and oxidized copper surfaces, *Faraday discussions* 180 (2015) 415-38.
- [12] F. Li, M. Bai, S.a. Wei, S. Jin, W. Shen, Multidimension Insight Involving Experimental and in Silico Investigation into the Corrosion Inhibition of N,N-Dibenzyl Dithiocarbamate Acid on Copper in Sulfuric Acid Solution, *Industrial & Engineering Chemistry Research* 58(17) (2019) 7166-7178.
- [13] N. Kovačević, A. Kokalj, Chemistry of the interaction between azole type corrosion inhibitor molecules and metal surfaces, *Materials Chemistry and Physics* 137(1) (2012) 331-339.
- [14] K.F. Al-Azawi, S.B. Al-Baghdadi, A.Z. Mohamed, A.A. Al-Amiery, T.K. Abed, S.A. Mohammed, A.A.H. Kadhum, A.B. Mohamad, Synthesis, inhibition effects and quantum chemical studies of a novel coumarin derivative on the corrosion of mild steel in a hydrochloric acid solution, *Chem Cent J* 10 (2016) 23-23.

- [15] A. Pardo, M.C. Merino, A.E. Coy, R. Arrabal, F. Viejo, E. Matykina, Corrosion behaviour of magnesium/aluminium alloys in 3.5wt.% NaCl, *Corrosion Science* 50(3) (2008) 823-834.
- [16] E.S.H.E. Ashry, A.E. Nemr, S.A. Essawy, S. Ragab, Corrosion inhibitors part V: QSAR of benzimidazole and 2-substituted derivatives as corrosion inhibitors by using the quantum chemical parameters, *Progress in Organic Coatings* 61(1) (2008) 11-20.
- [17] M.I. Farah, M.A. Saifaldeen, Performance of Schiff Bases Metal Complexes and their Ligand in Biological Activity: A Review, *Al-Nahrain Journal of Science* 24(1) (2021).
- [18] L. Guo, I.B. Obot, X. Zheng, X. Shen, Y. Qiang, S. Kaya, C. Kaya, Theoretical insight into an empirical rule about organic corrosion inhibitors containing nitrogen, oxygen, and sulfur atoms, *Applied Surface Science* 406 (2017) 301-306.
- [19] B.E.A. Rani, B.B.J. Basu, Green Inhibitors for Corrosion Protection of Metals and Alloys: An Overview, *International Journal of Corrosion* 2012 (2012) 380217.
- [20] J. Zhang, G. Qiao, S. Hu, Y. Yan, Z. Ren, L. Yu, Theoretical evaluation of corrosion inhibition performance of imidazoline compounds with different hydrophilic groups, *Corrosion Science* 53(1) (2011) 147-152.
- [21] M. Pourriahi, M. Nasr-Esfahani, A. Motalebi, Effect of henna and rosemary extracts on the corrosion of 304L stainless steel in 3.5% NaCl solution, *Surface Engineering and Applied Electrochemistry* 50(6) (2014) 525-533.
- [22] M. Kliškić, J. Radošević, S. Gudić, V. Katalinić, Aqueous extract of *Rosmarinus officinalis* L. as inhibitor of Al-Mg alloy corrosion in chloride solution, *Journal of Applied Electrochemistry* 30(7) (2000) 823-830.
- [23] O.K. Abiola, N.C. Oforka, E.E. Ebenso, N.M. Nwinuka, Eco-friendly corrosion inhibitors: the inhibitive action of *Delonix Regia* extract for the corrosion of aluminium in acidic media, *Anti-Corrosion Methods and Materials* 54(4) (2007) 219-224.
- [24] A.Y. El-etre, Natural honey as corrosion inhibitor for metals and alloys. i. copper in neutral aqueous solution, *Corrosion Science* 40(11) (1998) 1845-1850.
- [25] Y.J. Yee, Green inhibitors for corrosion control: a Study on the inhibitive effects of extracts of honey and *rosmarinus officinalis* L. (Rosemary), Institute of Science and Technology, University of Manchester, 2004.
- [26] A.S. Fouda, A.A. El-Aal, A.B. Kandil, The effect of some phthalimide derivatives on corrosion behavior of copper in nitric acid, *Desalination* 201(1) (2006) 216-223.
- [27] J.J.P. Stewart, Optimization of parameters for semiempirical methods I. Method, *Journal of Computational Chemistry* 10(2) (1989) 209-220.
- [28] G. Gece, S. Bilgiç, Quantum chemical study of some cyclic nitrogen compounds as corrosion inhibitors of steel in NaCl media, *Corrosion Science* 51(8) (2009) 1876-1878.
- [29] A.D. Becke, Density-functional thermochemistry. III. The role of exact exchange, *The Journal of Chemical Physics* 98(7) (1993) 5648-5652.
- [30] C. Lee, W. Yang, R.G. Parr, Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density, *Physical Review B* 37(2) (1988) 785-789.
- [31] S.G. Zhang, W. Lei, M.Z. Xia, F.Y. Wang, QSAR study on N-containing corrosion inhibitors: Quantum chemical approach assisted by topological index, *Journal of Molecular Structure: THEOCHEM* 732(1) (2005) 173-182.

- [32] M. Lashgari, M.R. Arshadi, G.A. Parsafar, A Simple and Fast Method for Comparison of Corrosion Inhibition Powers between Pairs of Pyridine Derivative Molecules, CORROSION 61(8) (2005) 778-783.



**GENOTYPING AND SEROPREVALENCE OF TOXOPLASMA GONDII IN ABORTION
WITH ELEVATION OF INTERLEUKIN-6 LEVEL**

AYA R. ABDUWAHHAB ALHEANY

**GENOTYPING AND SEROPREVALENCE OF *TOXOPLASMA GONDII* IN
ABORTION WITH ELEVATION OF INTERLEUKIN-6 LEVEL**

Aya R. Abduwahhab ALHEANY¹

Abstract:

Toxoplasmosis is a harmful microorganism that resides inside the cells of the host and produces serious symptoms such as abortion. A total of (48) blood samples were taken from aborted women in this study, with (40) healthy persons serving as a control group. Between the 15th of January and the 30th of September 2021, the patients were treated at Al-Karkh Maternity Hospital. The infection rate was highest in the age range (18-25) years for both the infected women and the control group, according to the findings. Among aborted mothers with Toxoplasmosis, the IgG antibody positivity rate was 40 percent higher than the IgM antibody positivity rate, while the negative rate was 8 percent (16.6 percent). When compared to healthy persons, there was a considerable increase in the frequencies of positive Anti-Toxoplasmosis IgM antibody (69.8%) and IL-6 (75%) when compared to healthy individuals. IL-6 had a greater prevalence of 36 (66.7%) than IgM (30%). (73.4 percent). The ROC test was utilized to evaluate those two parameters. The fragment 96bp of the B1 gene of the DNA isolated was inflated using nested PCR test amplification. Ordinary PCR was performed to amplify the IL-6 gene in 431 bp of isolated DNA.

Key words: Genotyping, *Toxoplasma gondii*, abortion, Interleukin-6.



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Introduction:

Toxoplasmosis has been demonstrated over decades to be a dangerous illness that causes major health problems and may even result in death (1). *Toxoplasma gondii* parasite interferes inside cells (2), producing harm within those cells, which emerges in circumstances of host immunological weakness, particularly in pregnant women and chronic illness patients (3). Toxoplasmosis may produce thyroid and sex hormone imbalances (4), and these hormones create changes in the physiological condition of patients, either evident or concealed instances (5). The presence of high amounts of cytokines in the sera of infected persons indicates the existence of damage inside the patient's body (6), which helps to identify the course of the disease caused by this parasite (7). Previous research has found a significant increase in interleukin-6 in the partners of persons infected with this parasite, and this cytokine has been linked to the presence of the immune system at the site of infection (8). There is a particular gene for each cytokine that can be determined by genotyping, and this gene may occur in mutations in the sequences of the genetic sequence, which may directly impact the illness state or infection in the host body (9). Interleukin-5 and interleukin-beta-1 are two more interleukins that play an immunological function in toxoplasmosis infections (9). Interleukin-6 is thought to be a guide for some bacterial and parasite illnesses (10). We can assess the clinical status of infected individuals, particularly toxoplasmosis, by using serological analyses (11) and quantifying its height in such infections (12). The goal of our research is to genotype and profile interleukin-6 in patients with toxoplasmosis.

Materials and methods

A total of (48) blood samples were taken from aborted women and (40) healthy persons as a control group in the current investigation. The patients were seen at Al-Karkh Maternity Hospital between the 15th of January and the 30th of September 2021. Venous blood samples were collected from each participant (patients and controls) and allowed at room temperature for 15-30 minutes to clot before being centrifuged at 3000 RPM to extract serum samples, which were stored at -20 C until use. Toxo, Bioelisa The IgM and IgG-capture immuno-enzymatic test was used to detect *T. gondii* IgG and IgM antibodies in serum or plasma. Diluted samples were incubated in microplate wells coated with rabbit Ab antihuman IgG and IgM for the assay. The EIASA approach was used to quantify IL-6 (Dianova-Immunotech, Hamburg, Germany).

For IL-6 amplification, F: CAGAACTCAGATGACTG and R: GTGGGGCTGATTGGAAACC were employed. The primers F:GGAAGTGCATCCGTTTCATGAG and R: TCTTTAAAGCGTTCGTGGTC were used to amplify the B1 gene.

Statistical analysis

The Chi-Square test and the t-test were among the statistical approaches utilized to assess and evaluate our findings. The scatter plot chart and the receiver operating characteristic (ROC) curve were employed. Sensitivity, specificity, and accuracy were the validity tests.

Results

The prevalence of patients based on their age was presented in the table (1). In both the infected women and the control group, the infection rate was highest (18(37.5 percent) within the age bracket (18-25) years.

Table (1): Prevalence of the studied groups according to age groups

Age (years)		Studied groups		Total	Chi-Square
		Healthy control	Miscarriage		
18 - 25	N	22	18	40	P=0.004 HS
	%	55.0%	37.5%	45.5%	
26 - 35	N	10	14	26	
	%	25.0%	29.2%	29.5%	
36 - 46	N	8	16	22	
	%	20%	33.3%	25.0%	
Total	N	40	48	88	
	%	100.0%	100.0%	100.0%	

Acute Toxoplasmosis increased the rate of IgM positive. As seen in the table (2), persistent infection increased the rate of IgG positive . This table demonstrated that the majority of the aborted females were positive for T. gondii (33(68.8 percent), with a highly significant difference (P=0.001) when compared to the control group.

Table (2): Toxo IgM positivity rate among the studied groups

Toxo. IgM		Studied groups		Chi-Square (P-value)
		Control	Aborted women	
Negative	N	40	15	P=0.001 HS
	%	100.0%	31.2%	
Positive	N	0	33	
	%	0%	68.8%	
Total	N	40	48	
	%	100.0%	100.0%	

As indicated in table (3), IgG antibody exhibited a greater positive rate than IgM 40(83.3 percent) among aborted Toxoplasmosis women with 8 (16.6 %) negativity rate compared to healthy controls (100.0 %) negative rate, with a highly significant difference (P0.01).

Table (3): IgG anti *T. gondii* positivity rate among the studied groups

Toxo. IgG		Studied groups		Chi-Square
		Healthy pregnant controls	Aborted women	
Negative	N	20	8	P=0.00 HS
	%	100.0%	16.6%	
Positive	N	0	40	
	%	0%	83.3%	
Total	N	20	48	
	%	100.0%	100.0%	

When compared to the healthy group, there was an increase in the frequency of positive Anti-Toxoplasmosis IgM antibody (69.8 percent) and IL-6 (75 %), with highly significant differences (0.0 % and 26.6 % for IgM Toxo and IL-6, respectively). Toxo IgM concentration and IL-6 were assessed in ng/ml for patients when compared to healthy controls, as well as (mean SD) for (IL-6) and Toxo IgM when compared to the healthy group and (10.65 5.2 and 0.427 0.2 ng/ml for IL-6 and Toxo IgM) as shown in table (4).

Table (4): Levels of Toxo. IgM and IL-6 among the studied groups

Parameters	Patients	No.	Mean	Std.	Error	t-test (P-value)
Toxo-IgM	Healthy	40	0.43	0.20	0.022	HS (P<0.01)
	Aborted	48	1.294	0.559	0.057	
	Total	88				
(IL-6)	Healthy	40	10.65	5.2	0.585	HS (P<0.01)
	Aborted	48	129.23	16.76	17.103	
	Total	88				

Table (5) demonstrated that there was a harmony between both markers, while IL-6 percent was larger than IgM, which may be related to activity, as any elevation in IL-6 is regarded a deformity and congenital abnormalities.

Table (5): Toxo. IgM and IL-6 positivity rates among the studied groups

Toxo IgM		Studied groups		Total	Chi-Square
		Control	Miscarriage		
Negative	N (%)	40(100.0%)	12(33.3%)	52(59.0%)	HS (P<0.01)
Positive	N (%)	0 (0.0%)	36(66.7%)	36(31.0%)	
Interleukin-6 (IL-6)					
Negative	N (%)	30 (73.4%)	12(22.0%)	42 (46.9%)	P=0.00 HS (P<0.01)
Positive	N (%)	10 (26.6%)	36(78.0%)	46 (53.1%)	
Total	N	40	48	88	
	%	100.0%	100.0%	(100.0%)	

To assess those two toxoplasmosis variations may help in diagnosing the malformation fetal loss; the following findings were displayed in figure utilizing ROC (1).

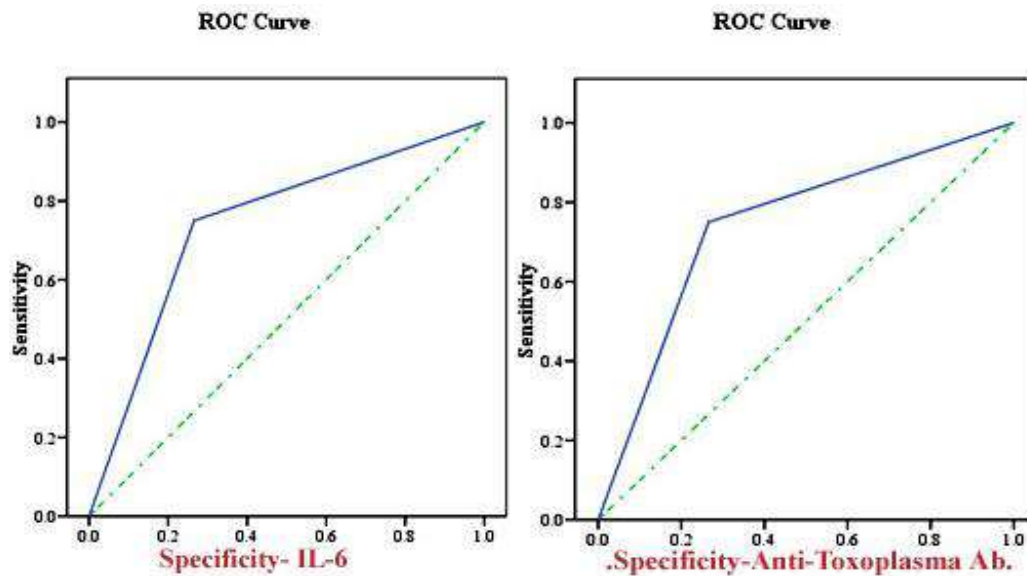


Figure (1): ROC curves for assessment of IL-6 & Toxo. IgM

Following the completion of the reaction phase, use PCR to inflate the piece 96bp of B1 gene of the DNA isolated by ready kit. The interior (electrotropic) was migrated using an agarose gel at a concentration of 2.5 percent and the DNA volumetric guide, ladder (M) 100bp size, as shown in figure (2).

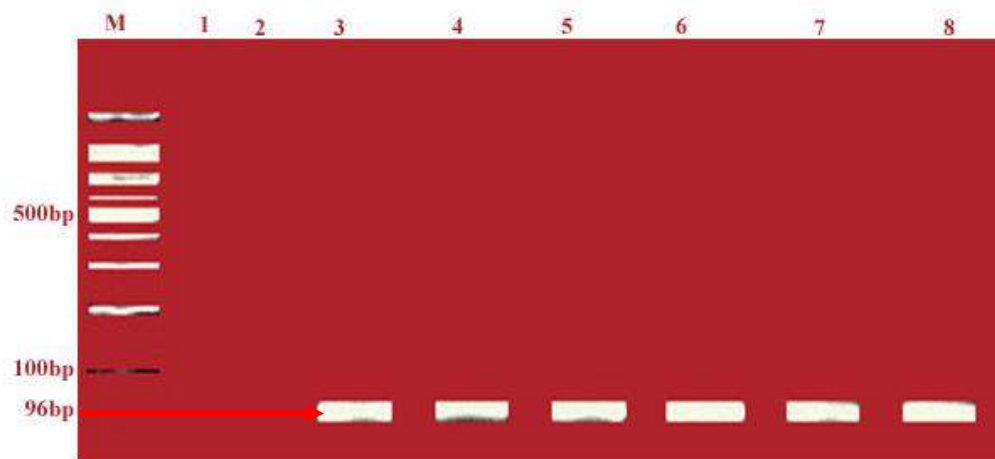


Figure (2): Detection of *Toxoplasma gondii* genome by nested PCR using *Toxoplasma gene B1* primers, M : 100 bp DNA ladder (M).

Lane3: Reference *Toxoplasma* strain Lanes: (2,4,5, and 6) positive isolates (96 bp).

Lane: (1) negative isolate.

Following the completion of the reaction phase, the IL-6 gene was amplified using conventional PCR in 431 bp of the DNA recovered by the ready kit. The interior (electrotropic) was migrated using agarose gel at a concentration of 2.5 percent and the DNA volumetric guide, DNA ladder (M) 500bp size, as shown in figure (3).

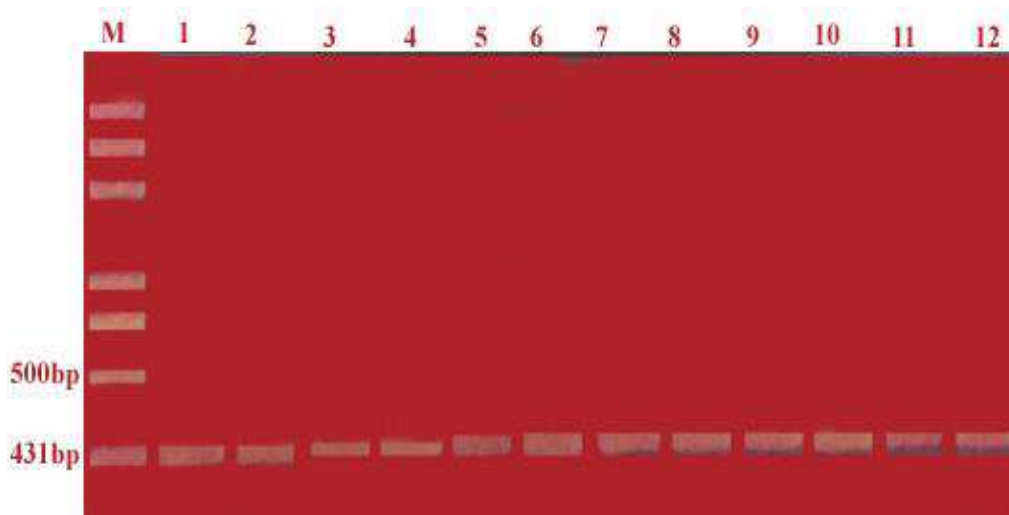


Figure (3): Detection of *IL-6* by ordinary PCR using *gene IL-6* primers, in 431 bp, M : 100 bp DNA ladder (M).

Lane1: Reference *IL-6* gene Lanes: (2-12) positive isolates (431 bp).

Discussion

Toxoplasmosis is a dangerous illness that can cause major health problems and even death. In this study, the age group (18-25) years 18 (37.5 percent) was shown to be greater in both the infected and control groups. Florence Toxoplasma infection is more prevalent in adults in their twenties, according to Abdulwahhab, A.R et al (2020).(13). The current study's findings were consistent with those of Kheirandish et al. (2019), who observed that the case group's seropositivity rate for Toxoplasma IgM was greater than the control group's (14). When compared to the healthy group, there was a significantly significant increase in the frequency of positive Anti-Toxoplasmosis IgM antibody cases as well as IL-6. These findings were corroborated by (Smith, et al., 2021) (15). It is apparent that Igm and IL-6 worked well together. Although the rate of IL-6 positive was greater than that of Toxoplasma IgM, this may be related to activity, as any increase in IL-6 was regarded a deformity and a congenital anomaly (16) . According to Adams, J. H. (2015), IL-6 plays both a protective and a detrimental effect in the regulation of toxoplasma as well as retinal integrity in the case of reinfections (17). These two measures are evaluated in regular Toxoplasmosis work, perhaps as markers of prenatal abnormalities and abortion(18). Hajian-Tilaki, K. (2013) defined the (ROC) curve and area under the curve (AUC) as an efficient measurement of accuracy and was recognized as relevant explanation(19) .The detection of toxoplasmosis using PCR gene targeting B1 indicated that the pregnant women who participated in the study were infected with toxoplasmosis among pregnant women who had T. gondii B1 gene in their placental tissue samples after delivery (20). (21) reported the similar finding. The conventional PCR of the IL-6 gene in 431 bp of the DNA isolated using a ready kit was used to determine genotypes by amplification (22). This cytokine was found in order to establish its importance in the treatment of toxoplasmosis (23). According to Wujcicka, W. et al. (2018), Interleukin-6 is a significant cytokine in determining Toxoplasma gondii infection, and its genotyping was determined by PCR at locus 431bp (24).

References

- (1) Samojłowicz D, Twarowska-Małczyńska J, Borowska-Solonyanko A, Poniowski ŁA, Sharma N, Olczak M. Presence of *Toxoplasma gondii* infection in brain as a potential cause of risky behavior: a report of 102 autopsy cases. *Eur J Clin Microbiol Infect Dis*. 2019;38(2):305–17.
- (2) Leroux L-P, Dasanayake D, Rommereim LM, Fox BA, Bzik DJ, Jardim A, et al. Secreted *Toxoplasma gondii* molecules interfere with expression of MHC-II in interferon gamma-activated macrophages. *Int J Parasitol*. 2015;45(5):319–32.
- (3) Bourgonje AR, Abdulle AE, Timens W, Hillebrands J, Navis GJ, Gordijn SJ, et al. Angiotensin-converting enzyme 2 (ACE2), SARS-CoV-2 and the pathophysiology of coronavirus disease 2019 (COVID-19). *J Pathol*. 2020;251(3):228–48.
- (4) Alvarado-Esquivel C, Ramos-Nevarez A, Guido-Arreola CA, Cerrillo-Soto SM, Pérez-Álamos AR, Estrada-Martínez S, et al. Association between *Toxoplasma gondii* infection and thyroid dysfunction: a case-control seroprevalence study. *BMC Infect Dis*. 2019;19(1):1–5.
- (5) Horowitz R. *How Can I Get Better?: An Action Plan for Treating Resistant Lyme & Chronic Disease*. St. Martin's Griffin; 2017.
- (6) Lathe R, St. Clair D. From conifers to cognition: Microbes, brain and behavior. *Genes, Brain Behav*. 2020;19(8):e12680.
- (7) Godoi APN, Bernardes GCS, Nogueira LS, Alpoim PN, de Barros Pinheiro M. Clinical Features and Maternal-fetal Results of Pregnant Women in COVID-19 Times. *RBGO Gynecol Obstet*. 2021;43(5):384–94.
- (8) Arcanjo A, Logullo J, Menezes CCB, Giangiarulo TC de SC, Dos Reis MC, de Castro GMM, et al. The emerging role of neutrophil extracellular traps in severe acute respiratory syndrome coronavirus 2 (COVID-19). *Sci Rep*. 2020;10(1):1–11.
- (9) Schmid-Hempel P. *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. Oxford University Press; 2021.
- (10) Ríos-Toro J-J, Márquez-Coello M, García-Álvarez J-M, Martín-Aspas A, Rivera-Fernandez R, Saez de Benito A, et al. Soluble membrane receptors, interleukin 6, procalcitonin and C reactive protein as prognostic markers in patients with severe sepsis and septic shock. *PLoS One*. 2017;12(4):e0175254.
- (11) Safarpour H, Cevik M, Zarean M, Barac A, Hatam-Nahavandi K, Rahimi MT, et al. Global status of *Toxoplasma gondii* infection and associated risk factors in people living with HIV. *Aids*. 2020;34(3):469–74.
- (12) Dard C, Swale C, Brenier-Pinchart M-P, Farhat DC, Bellini V, Robert MG, et al. A brain cyst load-associated antigen is a *Toxoplasma gondii* biomarker for serodetection of persistent parasites and chronic infection. *BMC Biol*. 2021;19(1):1–26.
- (13) Abdulwahhab AR, Nooruldeen MY, Muhsin SS. Genetic change and biochemical variations occurrence in patients with *Toxoplasma gondii* infections. *EurAsian J Biosci*. 2020;14(1):1309–13.
- (14) Kheirandish F, Ezatpour B, Fallahi S, Tarahi MJ, Hosseini P, Rouzbahani AK, et al. *Toxoplasma* serology status and risk of miscarriage, a case-control study among women with a history of spontaneous abortion. *Int J Fertil Steril*. 2019;13(3):184.
- (15) Smith NC, Goulart C, Hayward JA, Kupz A, Miller CM, van Dooren GC. Control of

- human toxoplasmosis. *Int J Parasitol.* 2021;51:95–121.
- (16) Lima MC, Mendonça LR de, Rezende AM, Carrera RM, Aníbal-Silva CE, Demers M, et al. The transcriptional and protein profile from human infected neuroprogenitor cells is strongly correlated to zika virus microcephaly cytokines phenotype evidencing a persistent inflammation in the CNS. *Front Immunol.* 2019;10:1928.
- (17) Pittman KJ, Knoll LJ. Long-term relationships: the complicated interplay between the host and the developmental stages of *Toxoplasma gondii* during acute and chronic infections. *Microbiol Mol Biol Rev.* 2015;79(4):387–401.
- (18) Peyron F, L'ollivier C, Mandelbrot L, Wallon M, Piarroux R, Kieffer F, et al. Maternal and congenital toxoplasmosis: diagnosis and treatment recommendations of a French multidisciplinary working group. *Pathogens.* 2019;8(1):24.
- (19) Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Casp J Intern Med.* 2013;4(2):627.
- (20) Silva CM, Silva ALP, Watanabe KFC, Chaves Bezerra NP, Bezerra DC, Gomes HM, et al. First report of detection of *Toxoplasma gondii* DNA in oysters (*Crassostrea* sp.) in the state of Maranhão. *Rev Bras Parasitol Veterinária.* 2020;29(3).
- (21) Li T, Cui L, Xu X, Zhang H, Jiang Y, Ren L, et al. The Role of Tim-3 on dNK Cells Dysfunction During Abnormal Pregnancy With *Toxoplasma gondii* Infection. *Front Cell Infect Microbiol.* 2021;11:47.
- (22) Mousa NM, Jasim HM. Association of IL-1 β + 3954 G> A and IL-6-174 G/C polymorphisms in congenital toxoplasmosis. *Vaccimonitor.* 2021;30(3):125–32.
- (23) Greigert V, Bittich-Fahmi F, Pfaff AW. Pathophysiology of ocular toxoplasmosis: Facts and open questions. *PLoS Negl Trop Dis.* 2020;14(12):e0008905.
- (24) Wujcicka W, Wilczyński J, Śpiewak E, Nowakowska D. Genetic modifications of cytokine genes and *Toxoplasma gondii* infections in pregnant women. *Microb Pathog.* 2018;121:283–92.



**VISIBLE QUANTITATIVE ASSAY OF PEPCID IN PHARMACEUTICAL DOSAGE
FORMS VIA SAFRANIN REAGENT**

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VISIBLE QUANTITATIVE ASSAY OF PEPCID IN PHARMACEUTICAL DOSAGE FORMS VIA SAFRANIN REAGENT

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Abstract:

Due to the medical importance of Pepcid, a spectrophotometric method had been suggested for the determination of Pepcid in its pure form as well as in some of its pharmaceutical preparations. The proposed method relies on two important steps; the first one, is the oxidation-reduction reaction between Pepcid and an amount of cerium IV (oxidizing agent) in presence of acidic medium, followed by the second step that occurs between cerium (III) with safranin, at the selected wavelength 514 nm. The proposed method obeyed Beer's law within the range (2-40) $\mu\text{g.mL}^{-1}$, with good sensitivity relative to the molar absorption coefficient value $3.21 \times 10^4 \text{ l.mol}^{-1}\text{.cm}^{-1}$ and the Sandal value equal to $0.00105 \mu\text{g.cm}^{-2}$. The proposed method has been successfully applied to quantify Pepcid in pure form and its pharmaceutical preparations.

Key words: Pepcid, Pharmaceutical Preparations, Safranin, Cerium IV.



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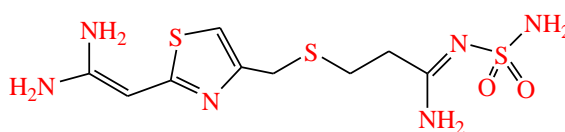
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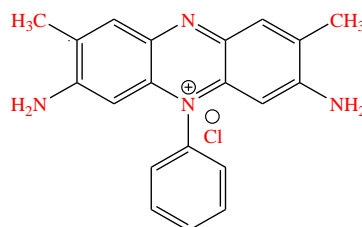
Introduction:

Pepcid (Pep.) Scheme 1, is an antihistamine receptor drug, its most important medicinal uses in the treatment of gastro-esophageal reflux disease, Zollinger-Ellison syndrome and peptic ulcer disease as it reduces the production of stomach acids. Pep chemically named as 3-[(2-[(diaminomethylidene)amino]-1,3-thiazol-4-yl)methyl)sulfanyl]-N-sulfamoylpropanimidamide [1-3]. Through the literature survey of methods used for estimating Pep, a number of methods have been reported, and these methods have been established on different techniques for Pep quantification, including spectrophotometric, spectrofluorometric and Ultraviolet spectrophotometric methods [4-13]. Other methods are HPLC combined with ultraviolet methods (Spectrophotometric and HPLC methods) used for determination of Pep in pharmaceutical dosages [14-17], also titrimetric method was used for the quantitative determination of Pep in pure and medical dosages [18]. Finally, Voltammetric method was used to estimate Pep on graphite electrode [19].



Scheme 1. Chemical structure of Pep.

The organic [fluorescent](#) reagent used in this work was Safranin (Scheme 2), with the chemical name 3,7-Diamino-2,8-dimethyl-5-phenylphenazin-5-ium chloride. Safranin is a crystalline solid that exhibit a characteristic green metallic luster; easily soluble in water and can be dyed blue or violet. They are strong bases and form stable mono salts. Their alcoholic solution shows yellow-red fluorescence.



Scheme 2: Chemical structure of Safranin

The amount of cerium (IV) is proportional inversely to the Safranin amount, therefore, the increasing of cerium (IV) was decreased the absorption of Safranin. Depending on this principle, The first step of the proposed method include the addition of an excess amount of cerium (IV) to the Pep solution in presence of acidic medium, then, the remaining amount of cerium (IV) reduces the absorption of Safranin dye which was measured at 514 nm.

Methodology:

Apparatus and chemical materials:

Double beam JASCOV-630UV-Visible spectrophotometer with 1 cm matched cells was used for all absorbance measurements. pH measurements have been measured using HANA pH meter.

The chemical solutions prepared with an analytical reagent grade of chemical materials. Pepcid solution, $100 \mu\text{g.mL}^{-1}$ (SDI), was prepared by dissolving 0.1 g of Pep in 100 mL distilled water using a suitable volumetric flask. The reagent Safranin solution 0.002% (BDH), prepared when 0.002 g of Safranin was dissolved in distilled water using a 100 mL volumetric flask. The oxidizing agent solution $500 \mu\text{g.mL}^{-1}$ was prepared by dissolving 0.2255 g of ammonium ceric sulphate (Fluka) in 5ml of concentrated sulphuric acid, and completed to the mark with distilled water using a 100 mL volumetric flask. Finally, Sulphuric acid solution, 1%, was prepared with an appropriate dilution of concentrated Sulphuric acid with distilled water in 250 mL volumetric flask.

Preparation of pharmaceutical dosages:

1. Gastrofam tablets 40 mg, (Turkey).
2. Ulceran tablets 20 mg, (Cyprus).
3. Famodar tablets 20 mg, (Jordan).

Ten tablets of each brand have been weighed with an approved value of 40 or 20 mg and ground into a fine powder, weighed, then filtered through Whatman No. 42 filter paper, the filter solution was diluted to obtain $100 \mu\text{g ml}$ as the concentration suitable for analysis

Results and discussion:

Using the oxidation-reduction reaction to estimate Pep, the optimal quantities of each component of the reaction were studied and the optimal amount was selected to obtain a stable color complex using $100 \mu\text{g}$ of Pep, as follows:

The optimum type and quantity of acid:

Several types of acids were studied, including (acetic acid, hydrochloric acid and sulphuric acid) with a concentration of 3%, where different volumes were added with an amount ranging between (0.1-3.0) mL of these acids as shown in Figure 1.

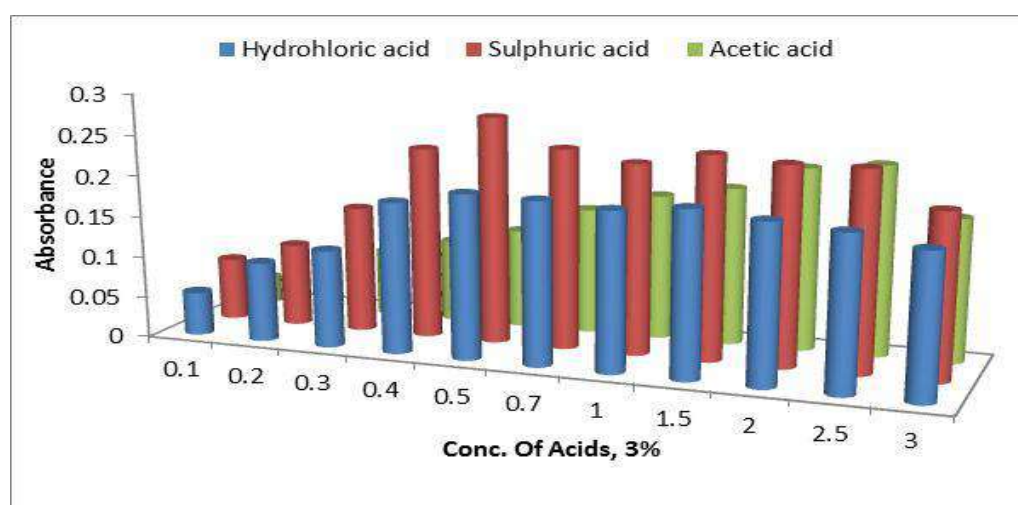


Figure 1: Type and quantity of acids

The results shown in Figure 1 indicate that the volume of 0.5 ml of 3% sulphuric acid was chosen as the best depending on the highest value of absorbance.

The optimum quantity of cerium (IV):

In order to estimate the optimal amount of oxidizing agent, Ammonium Ceric Sulphate, prepared at a concentration of 0.7×10^{-2} M, which are required for oxidation of Pep, various amount between (0.5-3) mL of Ce(IV) have been added to volumetric flasks of 25 mL containing (50-500) μg of Pep, then add 0.5 ml of 3% sulphuric acid. Leave this mixture for 20 minutes to complete the oxidation process, and then add the reagent Safranin at a concentration of 1.8×10^{-3} M. After diluting all the volumetric flasks to the mark with distilled water, the absorbance intensity was measured at the selected wavelength 514 nm. The experimental results proved that 1 ml of the oxidizing agent Ce(IV) gave the best value for absorbance and also the value correlation coefficient was (0.97054), therefore, 1 ml of Ce(IV) was adopted for the subsequence experiments.

Time of oxidation process:

The time required to complete the oxidation process between each of Pep and the oxidizing agent Ce (IV) ions was studied as shown in Table 1.

Table 1: Effect of time on oxidation process

Oxidation time	5	10	15	20	25	30
Absorbance	0.122	0.167	0.211	0.256	0.251	0.248

The optimum amount of Safranin:

Volumes ranging from (0.5-4) milliliters of reagent safranin at a concentration of 1.8×10^{-3} M were added to volumetric flasks of 25 mL containing different quantities ranging from (50-500) μg of Pepcid, and then the optimal quantities of sulphuric acid, oxidizing agent, cerium ion were added. Waiting for 20 minutes for the purpose of completing the oxidation process, the absorption intensity was measured at the selected wavelength of 514 nm, where the practical results showed that a volume of 2 mL of the safranin reagent at a concentration of 1.8×10^{-3} M gave the best values for the absorbance and the correlation coefficient. Therefore, this volume was adopted in the subsequent experiments.

The effect of surfactants:

Several types of different surface tension factors (positive, negative and neutral) have been studied, where Sodium dodecyl sulphate (SDS) was used as an example of the negative type, while Cetylpyridinium chloride CPC was used as an example of the positive type, and Triton X-100 was used as an example of the neutral type, and through the laboratory results it was noted that the use of surface tension factors of all kinds, it had a negative effect on the nature of the reaction, so this study was neglected from later experiments.[22]

Order of addition:

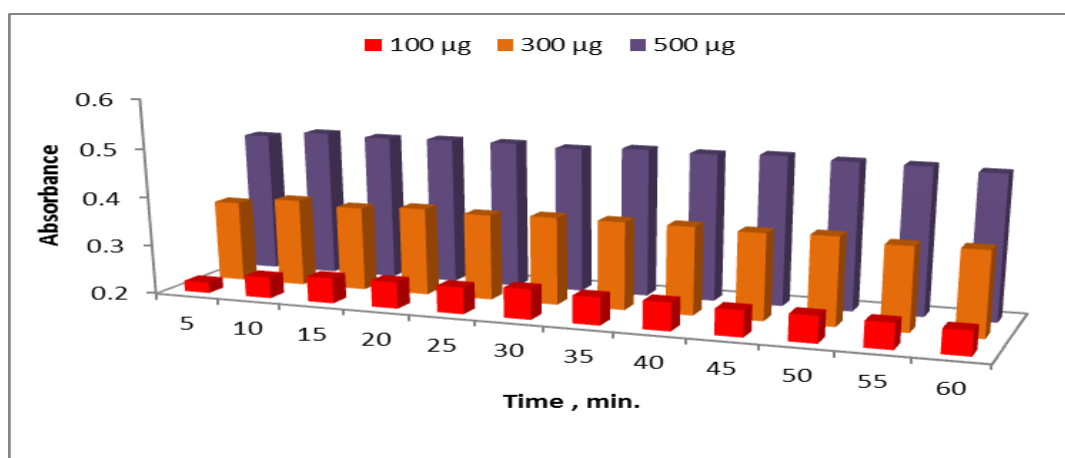
The additive sequences were studied using the redox reaction to quantify Pep, and Sequence No. I was considered the optimum as it gives the highest value of absorbance as shown in Table 2.

Table 2: The sequence of addition

Reaction component	Sequence	Absorbance
Pep + H ₂ SO ₄ + Ce(IV) + Safranin	I	0.253
Pep + Safranin + Ce(IV) + H ₂ SO ₄	II	0.038
Pep + H ₂ SO ₄ + Safranin + Ce(IV)	III	0.024
Pep + Ce(IV) + H ₂ SO ₄ + Safranin	IV	0.219

Stability of the resulted color:

The effect of time on the color intensity as well as absorbance intensity was measured at the selected wavelength 514 nm. Under the optimum conditions, when the absorbance was recorded at various intervals of time, which indicated that the resulted colored product was remained constant after 10 min. more than an hour as shown in Figure 2.


Figure 2: effect of time on the colored product

Beer's law and final spectrum:

The standard curve of Pep as well as final absorption spectrum were studied after fixing the optimal conditions for the determination of Pep. Quantities ranging between (50-1000) µg of Pep were added to 25 mL volumetric flasks, then an excess amount (1 mL) of the oxidizing agent cerium ion Ce(IV) 0.7×10^{-2} M, followed by the addition of 0.5 ml of 3% sulphuric acid, have been added. After waiting period of 20 minutes to complete the oxidation process, Safranin was then added to the reaction components, and the intensity of absorption was measured after diluting the solutions in all volumetric flasks to the mark using distilled water at the wavelength of 514 nm. As shown in (Fig.3 and 4)[23].

The proposed method follows Beer's law within the concentration range (50-1000) µg. Whereas, Sandel's significance was within limits $0.00105 \mu\text{g}\cdot\text{cm}^{-2}$ and the molar absorption coefficient is within limits $3.21 \times 10^4 \text{ l}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$. The present method for determination of Pep was applied in its pharmaceutical preparations[24].

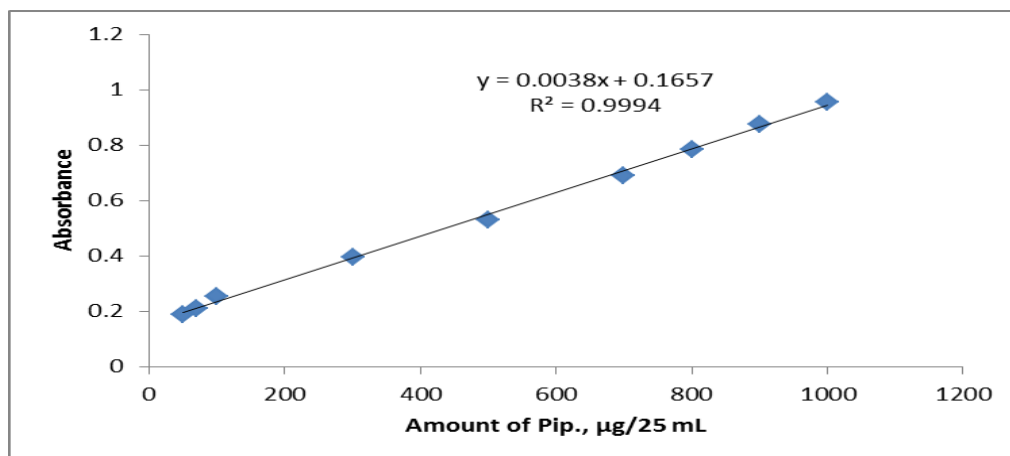


Fig.3: Calibration Curve of Pepcid

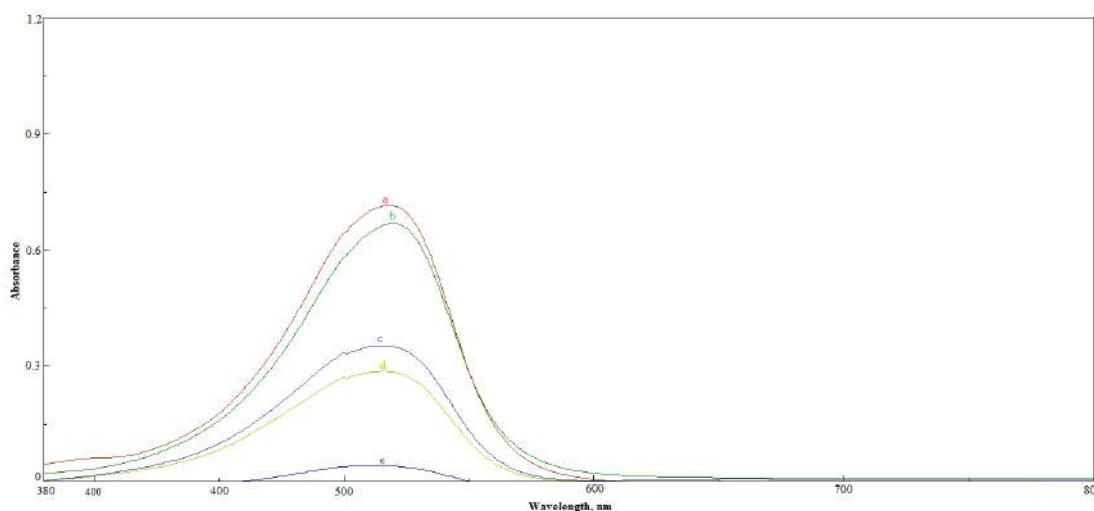


Fig. 4: Final absorption spectrum of (a=28, b=24, c=8, d=4 and e=2 $\mu\text{g.mL}^{-1}$) of Pep measured against reagent blank

Accuracy and precision:

The compatibility of the current method of calibration curve for the determination of Pep and for (4) concentrations has been studied as shown in Table 3.[25]

Table 3: The sequence of addition

Statistical values	Amount of Pep taken, $\mu\text{g}/25\text{ml}$			
	100	300	500	750
Recovery*, %	100.25	99.83	99.74	99.89
RSD*, %	± 0.376	± 0.344	± 0.287	± 0.234

The results in Table 3 show that the accuracy as well as the precision was reliable.

Nature of the reactions:

The reaction ratio for Pep:Ce(IV), and Ce(IV):Safranin have been studied using Job's method (continuous variations method), the obtained results was shown in Figure 5 illustrate that 1:1 was the ratio of Pep to Ce(IV).

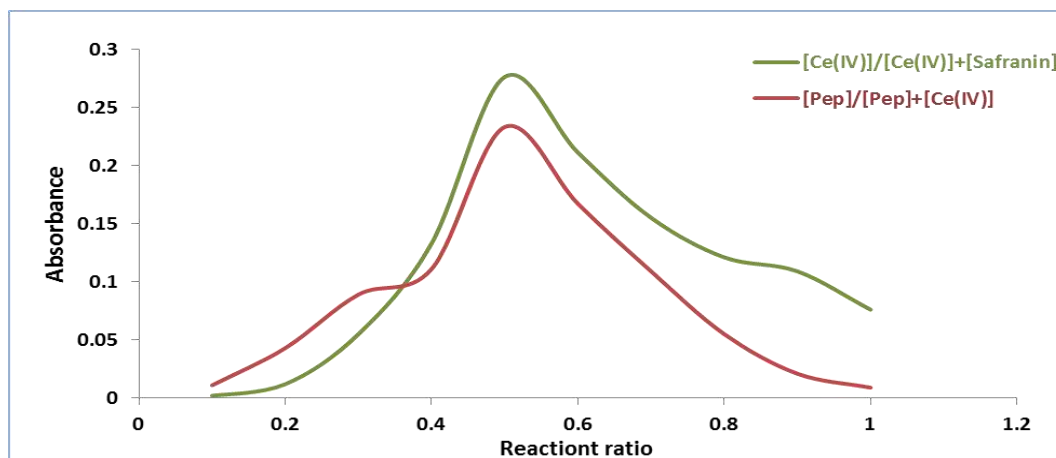
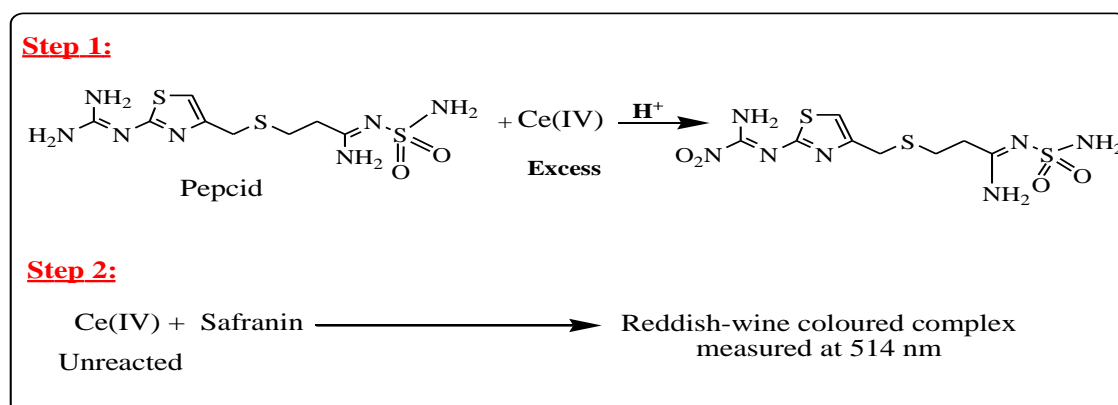


Figure 5: Job's plot for Pep –Ce(IV)

So that, the suggested equation was:



Interferences:

During the drug's manufacturing process, there are chemicals that are added in specific proportions to the medicine in order to improve the taste, smell and appearance of the medicine, including for example (gum acacia, glucose, sodium chloride, fructose, starch, and menthol). The effect of these substances on the proposed method for quantifying Pep in its pharmaceutical preparations was studied as shown in Table 4.[26]

Table 4: effect of foreign species

Interferences	Recovery(%) of 100 µg Pep / µg of interference added			
	100	250	500	1000
Menthol	99.41	99.74	99.04	99.76
Glucose	99.57	99.49	99.41	99.82
Starch	99.49	99.46	99.57	99.74
Acacia	100.19	100.19	100.19	99.89
Lactose	100.27	100.27	100.27	99.67

We concluded from the experimental results shown in Table 4 that the foreign substances studied did not interfere with the current method for determination of Pep.

Application of method:

The present method was applied to some of the Pepcid preparations shown in Table 5 which shows good recovery rates for Pepcid when applying the current proposed method. Some well-known Pepcid preparations are included

Table 5: Application of method

Amount of Pep, µg	Recovery(%) of Pep*		
	Gastrofam tablets 40 mg, (Turkey)	Ulceran tablets 20 mg, (Cyprus)	Famodar tablets 20 mg, (Jordan)
100	99.34	99.93	100.11
250	99.21	99.89	100.28
500	99.17	99.91	100.19

* Average of five determinations

The present method was applied to some of the Pepcid preparations shown in Table 5 which shows good recovery values for Pepcid when the present proposed method is applied. The t-test is one of the important statistical values and the values of the t-test [21] were calculated, by comparing the current proposed method with a modern spectral method proven in the literature [12] as shown in Table 6, which indicates that the t-test did not exceed the theoretical values at the level of 95% confidence in eight degrees of freedom ($N_1+N_2-2=8$).

Table 6: The t-test calculations

Drug	Pharmaceutical preparation	t-test	Tabulated t-test
Famodar tablets 20 mg, (Jordan)	Tablet	1.7357	2.571

Comparison of the method:

Table 7 illustrates a comparison of the current spectral method with two modern spectroscopic methods for estimating Pep that have been proven in the literature [5,12] indicating that the proposed method is sensitive and can be successfully applied to identify Pep in its pharmaceutical preparations.

Table 7: Comparison of the present method with Literature method

Analytical parameters	Present method	Literature method	
		[5]	[12]
Reaction	Oxidation reduction	Ion-pair formation	Oxidation reduction
λ_{\max} (nm)	514	410	528
Reagent	Safranin	Bromophenol blue	Acriflavine
Medium	Aqueous	Organic	
Beer's law range ($\mu\text{g/ml}$)	2-40	1-12	0.1-3.8
Molar absorptivity ($\text{l.mol}^{-1}.\text{cm}^{-1}$)	3.21×10^4	2.28×10^4	-----
Reaction time (min)	20	-----	10
Color of the product	Red	Yellowish orange	Red
Sandell's sensitivity ($\mu\text{g.cm}^{-2}$)	0.00105	0.015	----
R.S.D. (%)	$\pm 0.234 - \pm 0.376$	1.04	2.26
Application of the method	Pharmaceutical preparation	Pharmaceutical preparation	Pharmaceutical preparation

Conclusion:

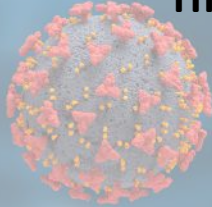
A spectroscopic method has been proposed to determine Pep in its pure form and in its pharmaceutical preparations, as this method is characterized by the ease, accuracy and high stability of the formed color complex. The proposed method depends on oxidation-reduction reaction, where Pep is oxidized using Ce(IV) in presence of sulphuric acid, which in turn reduces the intensity of the color of the Safranin dye, the amount of decrease in the intensity of the dye color was measured, which is proportional to the amount of the Ce(IV), which in turn is proportional to the amount of the medicine Pep, the method was applied successfully for the determination of Pep in its pure form and in its pharmaceutical preparations.

References:

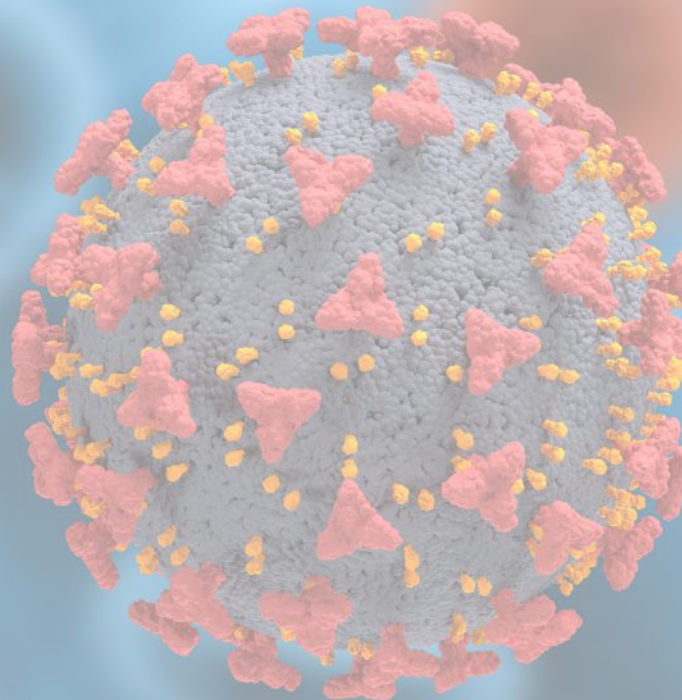
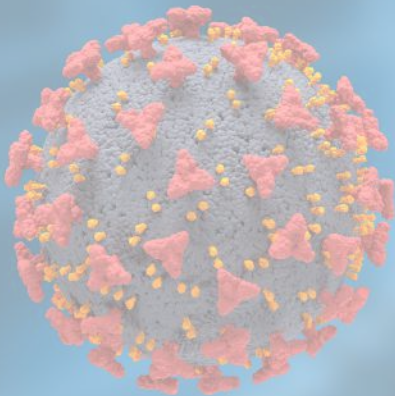
- [1] British Pharmacopoeia on CD-ROM 2001 Version 5, 3rd Ed., Vol. 1, Copyright by System Simulation Ltd, The Stationery Office Ltd., London.
- [2] Fischer J, Ganellin CR (2006). [Analogue-based Drug Discovery](#). John Wiley & Sons. p. 444.
- [3] Hu FL, Jia JC, Li YL, Yang GB (2003). "Comparison of H₂-receptor antagonist- and proton-pump inhibitor-based triple regimens for the eradication of *Helicobacter pylori* in Chinese patients with gastritis or peptic ulcer". *The Journal of International Medical Research*. 31 (6): 469–74.
- [4] S. Hassan, E. Zaheer, I. N. Muhammad, A. Hassan, M Ali and M.Qadri, "Determination of Chemical Stability of Various Pepsid Dosage Forms by UV –Visible Spectrophotometric Method and Data Analysis by R-GUI Stability Software", *Journal of Basic & Applied Sciences*, 2015, 11, 232-239.
- [5] K. Basavaiah and O. Zenita, "Spectrophotometric Determination Of Pepsid Using Sulphonphthalein Dyes", *Quim. Nova*, 34(5), 735-742, 2011
- [6] A.Malik , K.M. Yar ,M.S. Q. ,H.Amir , Z.R. Ali, "Spectrofluorimetric analysis of pepsid in pharmaceutical preparations and biological fluids by derivatization with benzoin", *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy* ; 134 ; 449-452 ; 2014
- [7] Elbaramawi S., El-Sadek M., Baraka M., Abdel-Aziz L., & Sebaiy M. (2020). Instrumental analysis of some anti-ulcer drugs in different matrices. *Chemical Reports*, 2(1), 156-172.
- [8] M. H. Soliman , Iodometric determination of pepsid, rantidine HCl and gliclazide in pharmaceutical preparations, *J. Drug Res. Egypt*. 2005; 26 (2-Jan): 150-153
- [9] S. S. Abid, and R.M.Ali, "Determination of Pepsid in Pharmaceutical Preparations by Flow Injection Spectrophotometric Method and using organic reagent (Pyro catecho), *Der Pharma Chemica*, 2016, 8(15): 11-19.
- [10] R.V Rele, J.M. Parab, V.V. Mhatre, C.B. Warkar. Simultaneous RP-HPLC Determination of Diclofenac Potassium and Pepsid in Pharmaceutical Preparations. *Research J. Pharm.and Tech*. 4(4): April 2011; Page 638-641.
- [11] Ahirrao V.K.,R.P. Pawar , "Simultaneous Quantification of Pepsid and Ibuprofen in Pharmaceutical Dosage by Using Validated Stability Indicating LC Method", *Res. J. of Pharmaceutical Sci.*, Volume 2, Issue (4), Pages 1-9, May,30 (2013).
- [12] Abdussamed M. A. Saeed1 Elham S. Salih, " Indirect Spectrofluorometric Method for the Determination of Cefotaxime Sodium, Ciprofloxacin Hydrochloride and Pepsid in Pharmaceuticals Using Bromate-Bromide and Acriflavine Dye", *Baghdad Science Journal*, 2020, 17(1):66-77
- [13] R. Kumar, A. Chandra, P. K. Gautam, "Development And Validation Of Ultraviolet Spectrophotometric Method For Quantitative Estimation Of Pepsid In Bulk And Tablet Dosage Form", *Asian J Pharm Clin Res*, Vol 10, Issue 8, 2017, 381-385
- [14] A. Nita, D.M. Tit, L. Copolovici, C. E.Melinte, "D. M. Copolovici, S. Bungau, C. Iovan, "HPLC-UV method for determination of pepsid from pharmaceutical products", *Rev.Chim.(Bucharest)*,69(2)2018, 297-299.

- [15] E. Atrey, P. Shende and Gaud RS, "RP-HPLC Method Development and Validation for Simultaneous Estimation of Ondansetron Hydrochloride and Complexed Pepcid in Bulk and Dosage Form", *J. Anal. Pharm. Res.*, 2017, 5(2): 00138.
- [16] S. Abo El Abass, M. I. Walash and F. Ibrahim, "Development and Validation of Spectrophotometric and Pre-column Derivatization HPLC Method for Determination of Pepcid in Pharmaceuticals by Reaction with Sodium Nitroprusside; Application to Combined Tablets", *Pharm Anal Acta*, 2016, 7(5), 1000476.
- [17] M. S. Arayne, N. Sultana, M. H. Zuberi, and F. A. Siddiqui, "Simultaneous determination of metformin, cimetidine, pepcid, and ranitidine in human serum and dosage formulations using HPLC with UV detection", *Journal of Chromatographic Science*, Vol. 48, 2010, 721-725.
- [18] B. M. Yevstahiyovych, S.Y. Yuriivna, K. S. Pavlivna, D. L. Osypivna, " Kinetic investigation of Pepcid S-oxidation reaction using potassium caroate. Development and validation of the titrimetric method for the quantitative determination of Pepcid in pure substance and medical preparation", *Ars Pharm.* 2018; 59(2): 69-76
- [19] I. G. David , D. E. Popa, A. Calin, M. Buleandra, E. Elena, "Voltammetric determination of pepcid on a disposable pencil graphite electrode" , *Turk J Chem* (2016) 40, 125 – 135.
- [20] Zucca, Paolo; Vinci, Carla; Rescigno, Antonio; Dumitriu, Emil; Sanjust, Enrico (2010). "Is the bleaching of phenosafranine by hydrogen peroxide oxidation catalyzed by silica-supported 5,10,15,20-tetrakis-(sulfonatophenyl) porphine-Mn(III) really biomimetic?". *Journal of Molecular Catalysis A: Chemical*. 321 (1–2): 27–33
- [21] Christian, G D., (2004) *Analytical chemistry* John (USA : Wiley and Sons) 6th Ed. p 83-99.
- [22] Bernhard, W (November 2016). "Lung surfactant: Function and composition in the context of development and respiratory physiology". *Annals of Anatomy*. 208: 146–150.
- [23] Harris D. C., (2007) *Quantitative Chemical Analysis*, China Lake, California, 8th Ed., p.393.
- [24] Mikkelsen S.R.,(2016) *Bioanalytical Chemistry*, 2nd Ed., Wiley&Sons, Inc., Hoboken, New Jersey, p.3.
- [25] BS ISO 5725-1: "Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions.", p.1 (1994).
- [26] Jafar, Syed A. (2010). "Interference Alignment — A New Look at Signal Dimensions in a Communication Network". *Foundations and Trends in Communications and Information Theory*. 7 (1): 1–134

**LONG-TERM PATHOLOGICAL SIDE EFFECTS OF INFECTION WITH
THE COVID-19 VIRUS IN THE MOSUL CITY**



ARWA ADRESS ALNUIMY



LONG-TERM PATHOLOGICAL SIDE EFFECTS OF INFECTION WITH THE COVID-19 VIRUS IN THE MOSUL CITY

Arwa Adress ALNUIMY ¹

Abstract:

Background: The world witnessed a crisis that swept out almost all of humanity's future, namely the spread of a coronavirus virus known as Covid19. Although it is a microorganism that cannot be seen with the naked eye and parasitizes on humans, animals and plants, it is the cause of a universal crisis in which people have been forced to stop their lives and stay in homes. Just as it has been disastrous for public health, it has disrupted health care systems and daily life. COVID-19 had spread at the end of 2019 in Wuhan, Hunan Province, where the seafood market is. The virus is spread quickly and later reach to all parts of the world. The impact of Covid-19 was not only severe, but it also caused complex long-term complications as a result of the complexity of the virus itself, and the virus had lasting effects on physical and psychological public health. Despite the complex negative complications caused by Covid-19, we can understand some of the mechanisms that cause these complications from the repercussions of the long-term negative effects caused by the types of Corona viruses that preceded Covid-19, namely SARS and MERS. But this requires raising awareness and conducting more studies to find out how to effectively manage the severe consequences caused by COVID-19 and to benefit from previous lessons in order to achieve the best results. **Objectives:** The study was conducted to identify long-term negative effects of Covid19 infection in the city of Mosul, the center of the Iraqi governorate of Nineveh. **Methods:** The number of sample members from whom the data was collected was 510 people of both sexes and of all ages, recording as many statistics as possible that illustrate the side effects of a covid - 19 infection. When we finished collecting the data and verifying that there are no errors, we conducted an analysis of these data and this statistical analysis of the data comes for the purpose of extracting and interpreting the results. The Statistical Package for Social Sciences (SPSS) v.20 was used to find tables and columns for frequencies and percentages. **Results:** The study has shown that Covid19 infection has many long-term negative effects on many organs and systems in the body and it is one of the most important results of our study. The study found that the highest age group involved in the study was between 36-45, with a percentage of 39.2%. The percentage of females is higher than males at 68.6%. As for the negative effects, Covid-19 caused damage to the lungs and chronic bronchitis had the highest rate of 29.4%. As for the long-term effects of Covid-19 infection on the kidneys, Cystoureteropyelitis was the highest with a rate of 44.1%. While Tachycardia had the highest percentage of cardiovascular diseases with a rate of 67.1%. On the side of neurological diseases, chronic headache was the highest, and it was 60.6%. As for skin diseases, the percentage was 38%, the largest was Lipsotrichia. In Ophthalmology, 69.9% of the share of Blurred vision, and ear diseases Oxyacoia has 51.2%. As for mental illnesses, there were many diseases, and the highest percentage was Anxious 22.1%. As for other



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diseases, Nervous colon was the highest with a rate of 15.9%, and for many other diseases, with different rates. **Conclusion:** The results of the study conducted in the city of Mosul showed that Covid19 infection caused many long-term illnesses that must concerted efforts to get the best results in their treatment.

Key words: Covid – 19, Long- Term Disease, Mosul.

Introduction:

Coronavirus disease 2019 (COVID-19) first appeared in Wuhan, China And the infected people suffered from acute respiratory disease and the cause of acute respiratory syndrome, and the causative agent of the disease was the virus scientifically called "the emerging coronavirus" (SARSCoV2) (1,2), Corona viruses (CoVs) are a large group of viruses that are common among many animals, including humans (1) It was discovered in 1960 AD and were given the name coronaviruses by the International Committee for Taxonomy of Viruses because they took on a crown-like shape. Cleavages of the viral spike (S-type proteins) form a aura that fills the virus's surface and causes cell infection (3) The emerging corona virus is the third corona virus to cause a large-scale epidemic in the twenty-first century, after corona virus (acute respiratory syndrome) SARS-CoV in 2003, and Middle East Respiratory Syndrome (MERS CoV) in 2012 (4) , In December 2019, a new epidemic of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) began in Wuhan, China. This new epidemic spread rapidly worldwide, affecting 76,769 people in 27 countries (5) There are types of strains spreading for this virus, certainly considered one among them is the deadly "S" strain, which constitutes 70% of the analyzed lines and spreads in no time and is answerable for the outbreak of the epidemic, and the alternative is much less virulent "L" wherein a mutation with pairs of nucleotides become found (6), The rising corona virus is associate enveloped virus with one and undivided nuclear material, RNA (7,8), Outside the body of the organism, it is called a virus, and since it is a protein and nucleic acid only, it is not considered a living organism, as it does not have the characteristics of nutrition and self-reproduction due to its lack of organelles such as bacteria, Scientifically, the Covid virus is called a "pathological factor" or an agent, as it is affected by temperature, as it is surrounded by a fatty envelope that melts at high temperature it dies when exposed to 56 degrees Celsius for half an hour and can live at minus 60 degrees, and alcohol can kill it, Inside the body, it is called a virus because it contains mitochondria and other vital organelles that help in its activity, reproduction and maintenance with an ideal pH of 7.2 (9), Clinical signs and symptoms of COVID-19 include fever, dry or productive cough, fatigue, shortness of breath, muscle pain, dizziness, confusion, headache, sore throat, loss of appetite, runny nose, chest pain, diarrhea, nausea and vomiting, and loss of sense of smell (10,11), The mortality rate associated with COVID-19 is significantly higher compared to seasonal influenza (5).

Methods and Data analysis

The study was carried out in many areas of the city of Mosul in the form of a questionnaire, communicating directly with patients to answer the paragraphs of the questionnaire, and by distributing it on websites, the study started in September 2020, and data collection stopped in September 2021. The number of sample members from whom the data was collected was 510 people of both sexes and of all ages, recording as many statistics

as possible that illustrate the side effects of a covid - 19 infection, When we finished collecting the data and verifying that there are no errors, we conducted an analysis of these data and this statistical analysis of the data comes for the purpose of extracting and interpreting the results. The Statistical Package for Social Sciences (SPSS) v.20 was used to find tables and columns for frequencies and percentages.

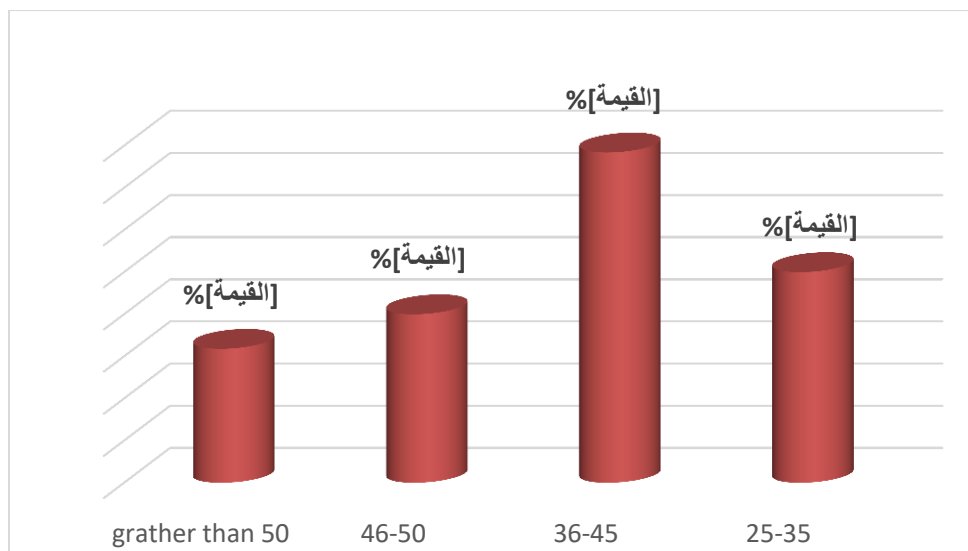
Results

First: - Distribution of sample members according to age

The current study found that the very best proportion of the age group within the sample is 39.2% (200) people and it absolutely was for people aged between 36-45 years, and 25% (127) people aged between 25-35 years, whereas 20% (102) people for the age group that ranges between 46-50 years, and also the lowest percentage of age in the study sample were those over fifty years previous (81) people Table (1)

Age	Repetition	percentage
45-36	200	%39.2
35-25	127	%25
50-46	102	%20
greater than 50	81	%15.9

Table (1) shows the distribution of the sample by age group



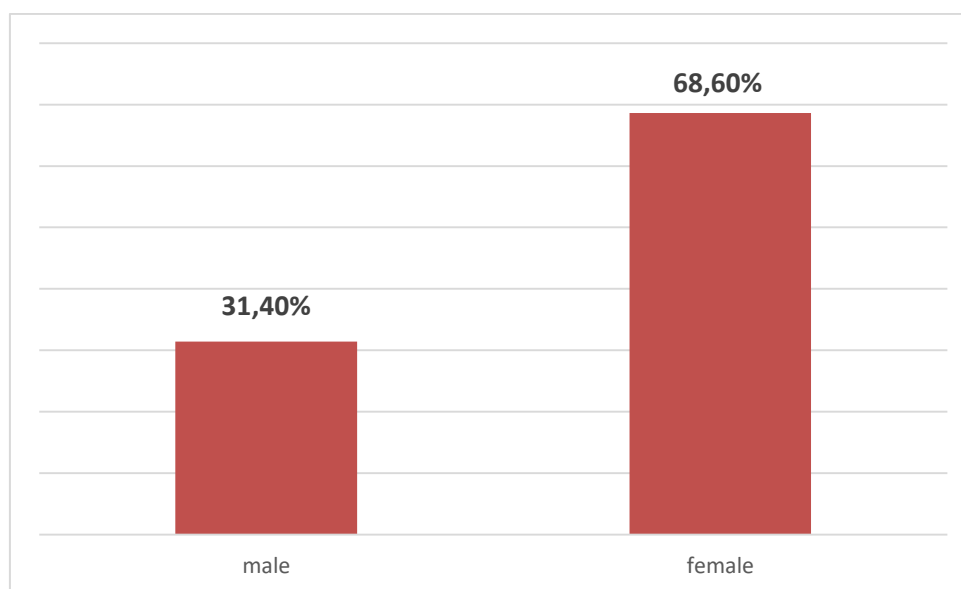
Scheme (1) Shows distribution of the sample according to age

Second: - Distribution of the sample members by gender

Table (2) shows the distribution of individuals in the sample by gender. We note through the study sample that the percentage of females reached 68.6% (350) persons, which is higher than the percentage of males, which was 31.4% (160) persons.

Gender	Repetition	percentage
Male	160	% 31.4
Female	350	% 68.6

Table (2) shows the distribution of the members of the sample by gender



Scheme (2) Shows distribution of the sample members according to gender

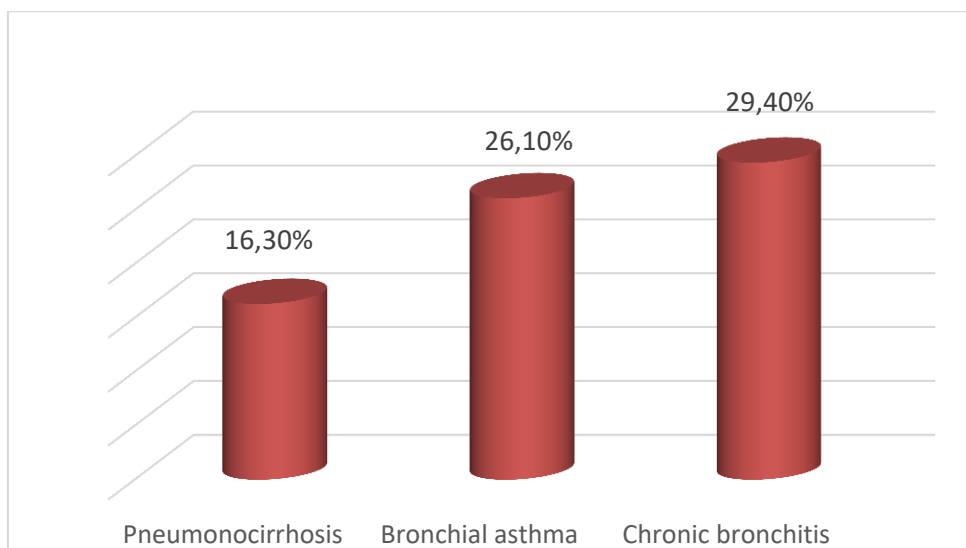
Third: distribution of sample members according to collateral illnesses occurring after the infection

1- pulmonary diseases

The so-called coronavirus targets the lungs in most cases, and only a few cases of infections that are classified as simple to medium are infections that did not directly affect the lungs, that being said, the lungs were the virus' first target, so as a result of the virus attack, the lungs can cause severe damage, whether it be infections that can be treated or pulmonary fibrosis, which can lead to most cases of death, the results of our study indicated that the highest rate of lung infection as a result of infection with the Corona virus was for chronic bronchitis with a rate of 29.4%, bronchial asthma at a rate of 26.1%, and lung fibrosis at a rate of 16.3% , Although our study has so far not found an incidence of lung cancer, this has two reasons, either that the virus does not cause the disease or that it is a long-term sequela, the symptoms of which only after a period of time longer than the period in which our study took place has been done, that has been a year, and this matter requires further study. Table (3)

pulmonary diseases	Repetition	percentage
Chronic bronchitis	150	% 29.4
Bronchial asthma	133	% 26.1
Pneumonocirrhosis	83	% 16.3

Table (3) shows lung diseases



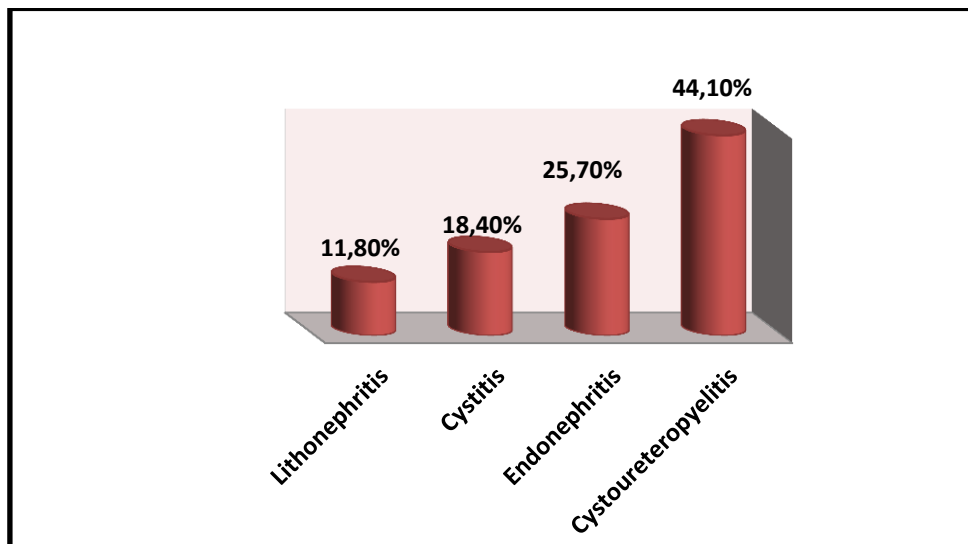
Scheme (3) showing lung diseases

2- Kidney diseases:

The Corona virus has proven that it has the ability to target many body systems in addition to the respiratory system. Studies have proven that the kidneys and urinary system are one of the most important organs targeted by the virus, the study found that the most common side diseases caused by infection with the virus were Cystoureteropyelitis infection with a percentage of 44.1%, followed by inflammation of the kidneys with a percentage of 25.7%, cystitis with a rate of 18.4% and infections due to kidney stones at a rate of 11.8%. The study did not indicate any infection with urinary system cancer. It may be for the same reasons mentioned above as shown in Table (4).

Urinary tract diseases	Repetition	percentage
Cystoureteropyelitis	225	% 44.1
Endonephritis	131	% 25.7
Cystitis	94	% 18.4
Lithonephritis	60	% 11.8

Table (4) shows the diseases of the urinary system



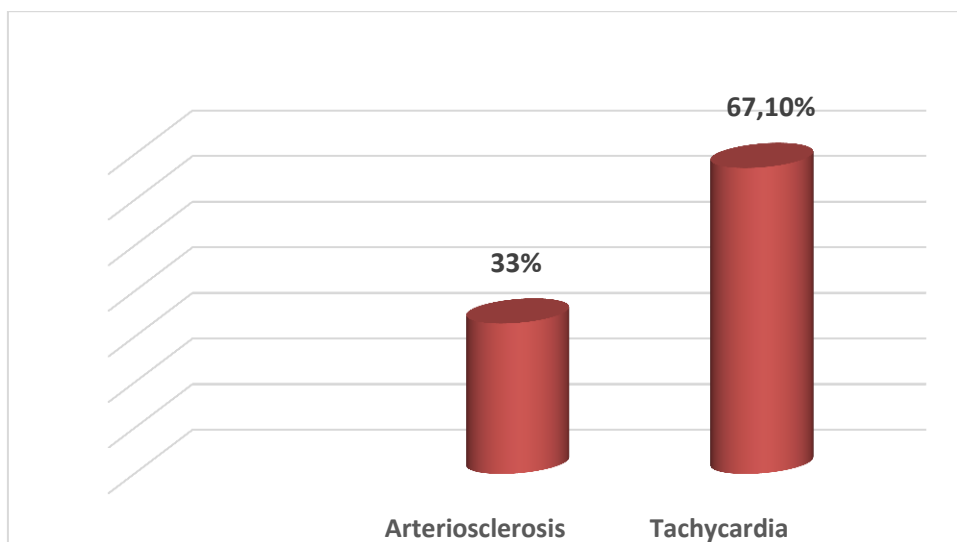
Scheme (4) showing the incidence of urinary system diseases/

3- Circulatory system diseases:

It has been determined amongst maximum of these infected with the Corona virus that an acceleration within side the heartbeat happens after recovery, and that is because of both a coronary heart harm or a lung harm, and it's miles essential to decide the motive of this acceleration so one can take suitable remedy steps , The results of our study showed that the acceleration of heart rate among the study samples was 67.1%, while arteriosclerosis came after that, and by 33% , The majority of patients with atherosclerosis were over the age of 45, and although it is not always the age at which atherosclerosis occurs, infection with the virus has caused the age of infection with this serious disease, while the study did not clarify the incidence of other heart diseases. Table (5)

Circulatory system diseases	Repetitio n	percentage
Tachycardia	342	%67.1
Arteriosclerosis	168	%33

Table (5) shows diseases of the cardiovascular system



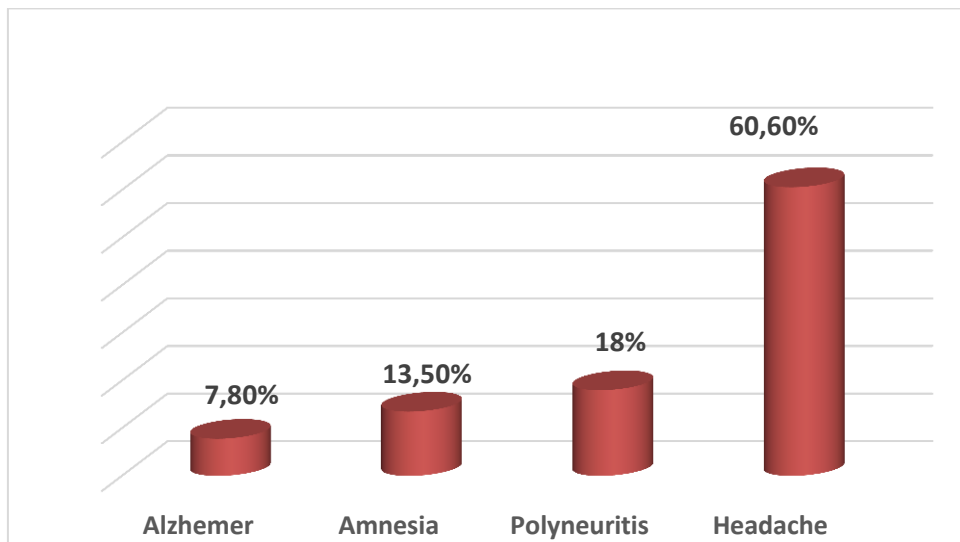
Scheme (5) showing diseases of the cardiovascular system

4- Nervous system diseases:

He was also “one of the most targeted organs of the body” by the virus, and among the symptoms caused by infection with the virus was chronic headache, and as it was one of the most important symptoms during infection, the headache was continuous for many people after infection and those who were not infected. And our results showed that the incidence of chronic headaches was 60.6%, which is a high and dangerous rate because this headache may cause other related diseases. Then came peripheral neuritis with a rate of 18%, and unlike other diseases that could be caused by any other disease, infection with the Corona virus caused temporary memory loss (Amnesia), and its rate was 13.5%, which is also a “high percentage compared to other causes that lead to temporary memory loss and Alzheimer’s by 7.8%”. Also, this percentage is considered high compared to other diseases that lead to Alzheimer's disease. All Alzheimer's patients were elderly people over the age of 60 years, and their data was taken from their families. Table (6)

Nervous system diseases	Repetition	percentage
Chronic headache	309	%60.6
Peripheral neuritis	92	%18
Amnesia	69	%13.5
Alzheimer	40	%7.8

Table (6) shows diseases of the nervous system



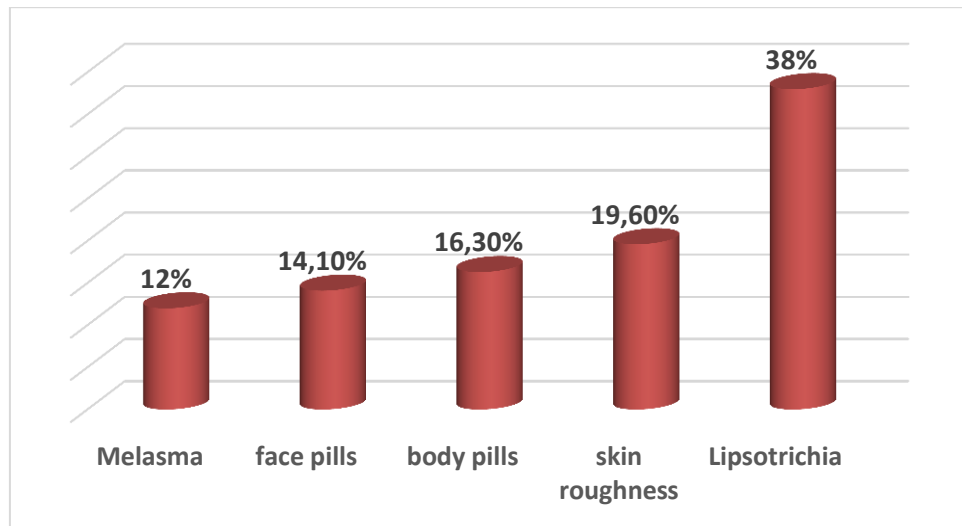
Scheme (6) showing diseases of the nervous system

5- Skin diseases:

Table (7) indicated that Corona virus infection has caused a number of skin diseases, the most prominent of which was hair loss, which affected a large number of women, and this may be due to several reasons, including anemia, thyroid dysfunction, hormonal disorders, or as a result of increased hair dryness and several reasons that can to cause this precipitation , The rate of hair loss in our study was 38%, which is considered a high percentage, but it is not surprising that hair in women is always influenced by other factors that lead to its loss, not to mention the virus, which has caused great confusion among women. Scientists and other skin diseases caused by the virus are roughness of the skin in 19.6%, pimples on the body in 16.3%, facial pills in 14.1% and spots on the skin in 12%.

Skin diseases	Repetition	percentage
Lipsotrichia	194	%38
skin roughness	100	%19.6
body pills	83	%16.3
face pills	72	%14.1
Melisma	61	%12

Table (7) shows skin diseases



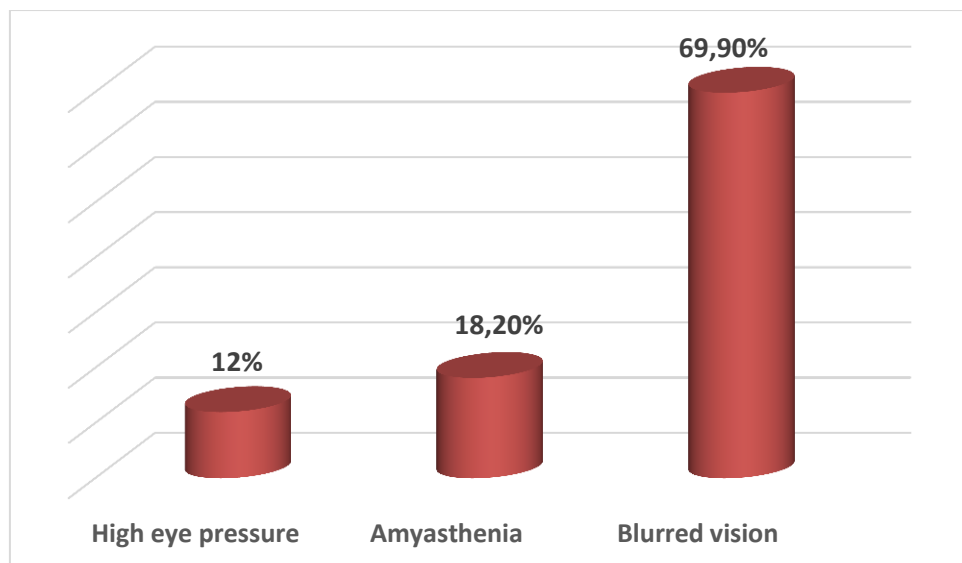
Scheme (7) showing the incidence of skin diseases

6- Eye illnesses:

It absolutely was discovered that the virus caused eye diseases that weren't restricted to the old only, however it affected several ages and caused blurred vision, and therefore the the} proportion was 69.9%. It also caused weakness within the muscles of the eyes, at a rate of 18.2%. This disease ends up in many alternative symptoms from Feeling pain and inflammation in the eye, and an increase in force per unit area occurred among the cluster that wasn't infected with this disease before exposure to the Corona virus, and our results showed that it was 12%. Table (8)

Ophthalmology	Repetition	percentage
Blurred vision	356	%69.9
Amyasthenia	93	%18.2
High eye pressure	61	%12

Table (8) shows eye diseases



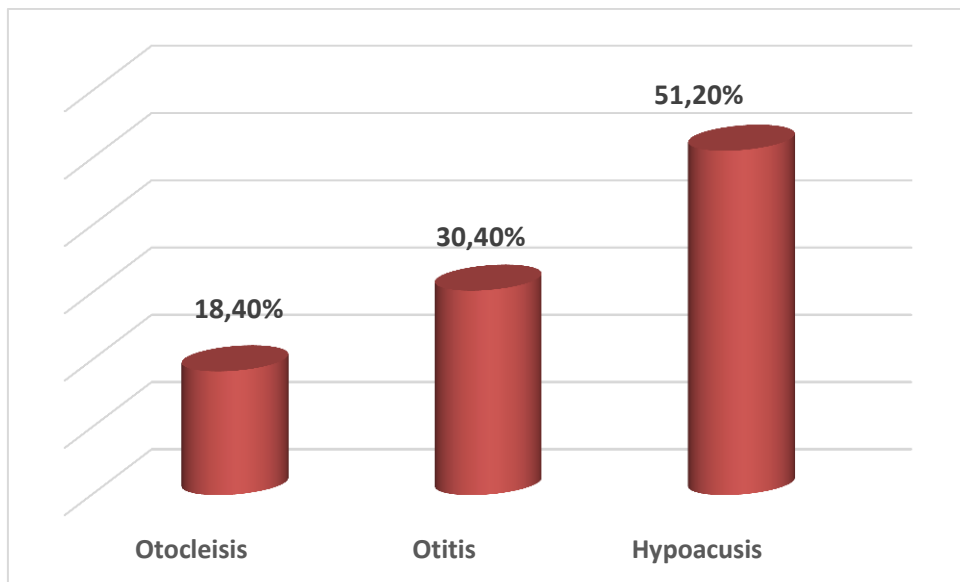
Scheme (8) showing the extent of Ophthalmology

7- Ear diseases:

It had been not expected that infection with the Corona virus would cause harm to the ear as a result of it's not associate entry space for the virus, and because it isn't one in all the members expected to be affected by the virus, it is not within the general course of the virus, however we have a tendency to found that there are damages that occurred in the ear among the samples of our study. The virus infected the bulk of the aged with Oxyacoia at a rate of 51.2%, that may be a high percentage, and there have been ear infections by 30.4% and ear obstruction by 18.4%. Table (9)

Ear diseases	Repetition	Percentage
Oxyacoia	261	%51.2
Otitis	155	%30.4
Otocleisis	94	%18.4

Table (9) shows ear diseases



Scheme (9) showing ear injuries

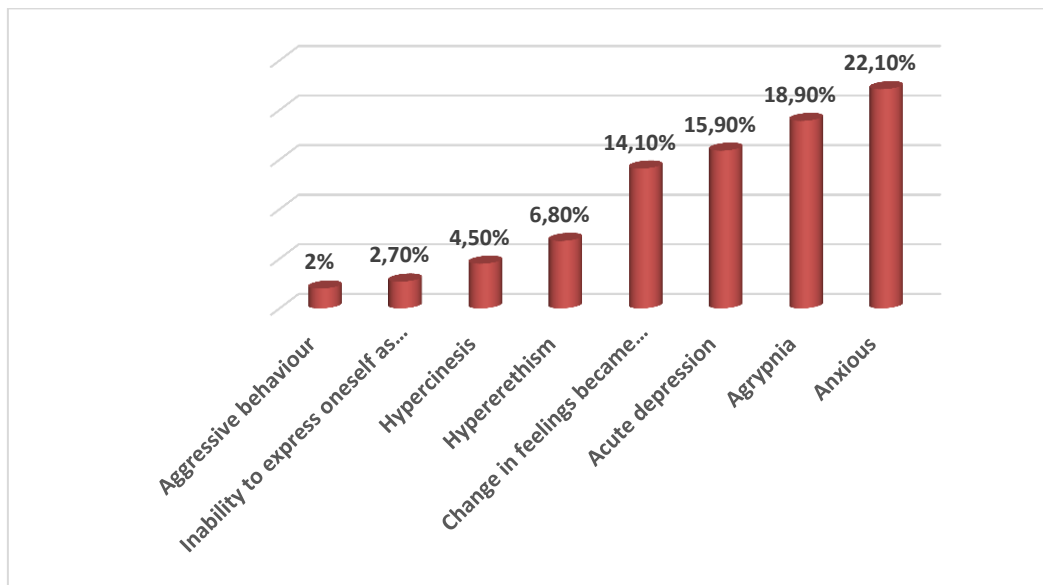
8- Mental diseases:

it's no secret to everybody that infection with the virus has caused a bearing on the status thanks to this mysterious virus and therefore the several variations it caused in terms of infection, and infection might cause sudden death, the person may be very ill and lying in the hospital, but he recovers after a while, and the person may be infected, but without symptoms and another infected, and the symptoms are different from one person to another. Causing mental illness, for example, that the injured person, even after recovery, has constant anxiety, this is what our study found, the table (10) showed it was 22.1%, while others suffered from insomnia and excessive thinking, and it was 18.9%. From my question to some people about what they think, some indicated that the thoughts are dark in most cases, although some of them said that I did not have any kind of insomnia and thinking at night, but After the injury, this symptom started , Entering the other dark tunnel, which is depression, and it was at a rate of 15.9%, and most of the sufferers were women , Others also indicated a change in their

feelings and they became more cruel than before, and that was 14.1%, and the rate of excessive nervousness was 6.8%, while others indicated that they had hyperactivity, any incomprehensible movement of the hands accompanied by a loss of focus and forgetting in most cases, and the rate was 4.5 % , Some of the infected indicated that when ill from infection with the virus, they were unable to specific themselves as before. Of course, this kind of psychological harm is in the middle of alternative diseases, as a result of the loss of the power to express oneself results in psychological disturbance and access to other psychological diseases, and also the proportion of them was 2.7%, the study indicated that The infection with the virus caused "aggressive" behavior after recovery for a few of the infected, and this behavior is extremely dangerous to society and should result in other a lot of dangerous behaviors, and it had been 2%,All the aforementioned psychiatrically unwellness need medical intervention, just like the case of organic diseases, however it's nearly a lot of as a result of those with organic diseases solely hurt themselves within the event of their illness, whereas patients with psychological conditions harm those around them and cause a danger to society. this needs treatment as shortly as possible. Reaching depression in standard cases requires loads however once infected with the virus for a brief amount of time, the reason behind this share of depression and alternative mental illnesses, all of this makes it a significant disease for public health.

Psychological diseases	Repeti tion	Percentag e
Anxious	113	%22.1
Agrippina	96	%18.9
Acute depression	81	%15.9
Change in feelings became more cruel	72	%14.1
Hypererethism	35	%6.8
Hyperkinesia	23	%4.5
Inability to express oneself as before	14	%2.7
Aggressive behavior	10	%2

Table (10) shows Psychological diseases



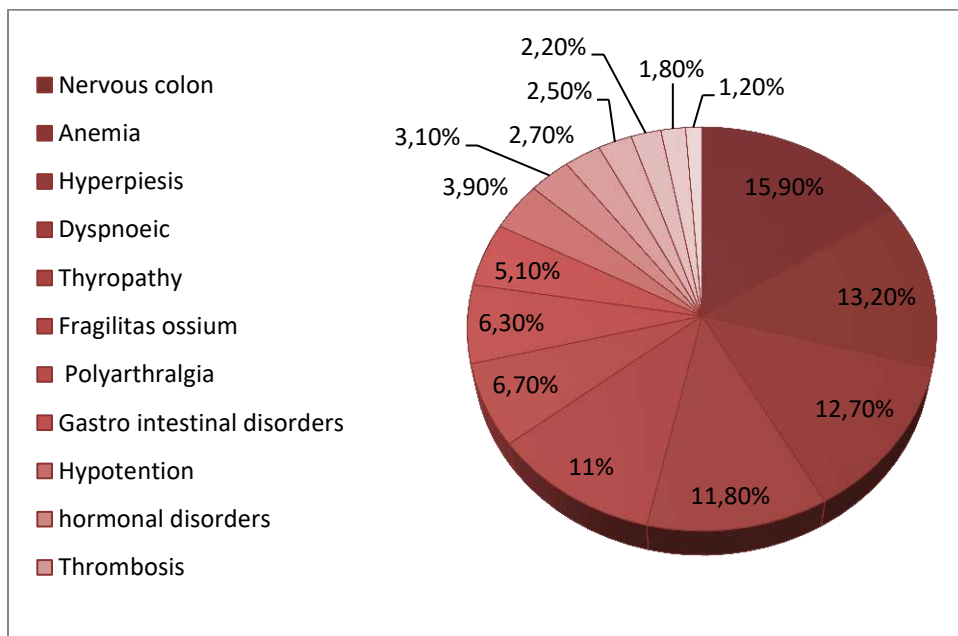
Scheme (10) showing mental illness that occurred after infection with Covid-19

9- Other diseases:

The highest incidence of alternative post-injury diseases was Irritable viscus Syndrome, and presumably this can be because of the unhealthy status that accompanies the injury and its aftermath, and it had been 15.9% , The study also indicated that those infected with the virus, after their recovery, found that they developed anemia, and this is due to several reasons, including a lack of appetite to eat, or it may be the result of physiological damage caused by the virus, and it was 13.2% , Corona virus infection after recovery led to high blood pressure in cases that were not infected with this high previously, and it was 12.7% , There were also cases of shortness of breath, and this symptom, which is one of the symptoms of infection with the virus, continues to exist even after the end of the infection and recovery, and it occurs as a result of injury to the lung or respiratory passages, and its percentage in the study was 11.8%, While the study showed that the infection caused thyroid disorders after infection with Covid-19, this means that the infection caused a “hormonal imbalance.” The percentage of thyroid disorders in our study samples was 11%, The study showed that people recovering from Covid-19 had osteoporosis, at a rate of 6.7%, and there are other diseases with different rates, including joint pain, at a rate of 6.3%, Gastrointestinal disorders by 5.1%, low blood pressure by 3.9%, hormonal disorders by 3.1%, blood clotting by 2.7%, and the infection also led to a high percentage of obesity and was 2.5%, while the percentage of blood viscosity was 2.2%, clots by 1.8%, and ulcers stomach by 1.2% Table (11).

Other diseases	Repetition	Percentage
Nervous colon	81	%15.9
Anemia	69	%13.2
Hyperpiesia	65	%12.7
Dyspneic	60	%11.8
Thyropathy	54	%11
Fragilities osmium	34	%6.7
Polyarthralgia	32	%6.3
Gastro intestinal disorders	26	%5.1
Hypotension	20	%3.9
hormonal disorders	16	%3.1
Thrombosis	14	%2.7
Overweight	13	%2.5
Blood viscosity	11	%2.2
Clot	9	%1.8
Gastrohelcosis	6	%1.2

Table (11) shows the diseases that can be caused by infection with Covid-19



Scheme (11) showing the various diseases that can be caused by infection with Covid-19

Discussion

The effect of COVID-19 has been a general medical condition that has disturbed "daily existence and medical services frameworks ,Because of the intricacy of this infection, it is normal that the adverse consequences it causes will be intricate and unquestionably long-term impacts on public, physical and mental health , It requires increased awareness and research to know how to effectively manage these consequences , The justifications for why patients experience long-term manifestations after infection are not explicitly known , It is either because age, hereditary qualities, method of infection, and the presence of synchronous infection (12) Although the mechanisms that cause these effects are not precisely understood, we can comprehend the post-infection effects of the Covid-19 virus if we follow what was caused by the other coronaviruses that preceded it: SARS 2003 and MERS 2012 (13) , We can likewise take lessons from them to figure out how to manage crises and decrease the seriousness of these adverse consequences , Coronaviruses of the family enter the body mainly through the mucous layer of the nose and pharynx Some of them are deposited in the lungs and other organs, including the heart and kidneys, and these organs express angiotensin-converting enzyme (ACE2) receptors on the surface of their cells , ACE2 receptors in particular aminopeptidase Membrane-bound are the places most likely to bind SARS-Cov2 molecules to the host cell , This chemical is high in mature cells contrasted with youthful cells, which makes them more vulnerable to this virus (14,15) What prompts the most effect on the tissues and organs of the body and its openness to numerous entanglements after infection is the cellular explosion in the immune system by activating white blood cells through the IL-6 pathway(16) Also, infection with COVID-19 leads to exhaustion of natural killer cells and cytotoxic T cells (17) Thus, Covid-19 survivors are more likely to develop many diseases such as chronic obstructive pulmonary disease and autoimmune diseases (18) , Among the other damage caused by Covid-19 is the deformity that influences the lining of blood vessels In one study, elements of the Covid-19 virus were found in the endothelial cells of the blood vessels of the lung, kidney, and heart, and it caused endothelial inflammation in the blood vessels Since the lining of blood vessels is present throughout the body, complications may occur by activating the function of this lining and causing inflammation, edema, thrombosis and organ failure (19)

COVID-19 is principally a respiratory disease, and studies are gathered on the extent and seriousness of the respiratory complications it causes (20) Particularly "pneumonic, for example, powerless lung capacity, fibrosis and lung diseases overall (21) These respiratory side effects are similar to those caused by the SARS virus (22) Concerning the comprehension of these difficulties and the way of deal with them, it appears to be unclear and requires further studies , The explanations behind this and the pathophysiology of these respiratory sicknesses may be due to the role of inflammation and injury that occurs to the alveolar epithelial cells, which leads to lung infections (21) Or , pneumonia might be because of harm brought about by injury to the cranial nerve (23) Lung fibrosis may be caused by immune response reactions associated with CD4 cells (24) Long-term fibrosis in the lungs is caused by an immune pathway that involves activation of an associated inflammatory response with CCL2 The chemokine (C-C motif) ligand 2 (25,26) (CCL2) is also referred to as monocyte chemoattractant protein 1 (MCP1) and small inducible cytokine A2. CCL2 is a small cytokine that belongs to the CC chemokine family. CCL2 recruits monocytes, memory T cells, and

dendritic cells to the sites of inflammation produced by either tissue injury or infection and CXCR2 are closely related receptors that recognize CXC chemokines that possess an E-L-R amino acid motif immediately adjacent to their CXC motif, They are both expressed on the surface of neutrophils in mammals (27,28), The pathway that drives the CXCR2 / CCL2 axis activates the expression of TNF, iNOS, which in turn changes the oxidative balance in the lungs and causes tissue damage in the lungs (29) Researchers note that lung harm might be because of direct alveolar harm and cellular storm (30).

While the results of another study conducted on 62 patients out of 138 were transferred to intensive care It was found that these patients developed acute respiratory distress syndrome (ARDS) in the rate of 61%, Irregular heartbeat 44%, Hypertension 31%, cardiovascular disease and blood vessels 14.5% (31) and coronary heart disease (1)

Studies have indicated the occurrence of heart diseases after infection with SARS, including myocardial infarction and coronary artery disease (32,33), Another study showed an acceleration of the heartbeat and a percentage of 72% (34), Studies demonstrate that complications that affect the heart as a result of infection with Covid-19 have unsure mechanisms, In spite of its reality, the specific systems of its event are obscure and unknown, Some studies indicate that it may be occur due to lack of blood supply (35,36,37), Or on the other hand it very well might be because of the inflammatory response and cause inflammation of the blood vessels (38,39) Angiotensin-converting enzyme 2 might assume a crucial part as a connection between the heart and blood vessels on the one hand, and Covid-19 on the other (40).

Studies have indicated that one of the complications caused by Covid - high blood pressure 14.9% This study attributed these complications to the occurrence of cellular storms (1), While another study indicated a decrease in blood pressure and a percentage of 50% (34) The imbalance in pressure level could also be because of the loss of (ACE2) within the heart, blood vessels, and brain, as a result of the death of neurons, there is a loss of function and control of the autonomic nervous system, which regulates blood pressure (41), Worsening blood pressure leads to changes in pressure reflex receptors and an increase in sympathetic outflow (42,43).

Covid-19 causes a clot due to the lung damage it causes, which comes down on the heart and prompts to cardiac arrest (44) Clots were one of the most important complications after recovering from SARS, A Singaporean report demonstrated that strokes led to death for those recuperating from SARS, and 8 deaths occurred, 4 of whom died due to pulmonary embolisms and 4 died due to venous thrombosis (33), Even small clots can interfere with blood flow and cause harm to patients (45), Therefore, it is preferable to use anticoagulants, as in addition to their anticoagulant role, they have some immunomodulatory and anti-inflammatory properties (46).

As for injuries that occur in the nervous system in patients recovering from Covid-19, One review demonstrated the event of numerous complications, including neurodegenerative disorders and accelerated brain aging, and harm to the nervous system could also be because of the high level of inflammatory cytokines and also the negative effects of immune reactions (47), Another study indicated that those recovering from Covid-19 had a headache rate 44% (48,49).

After recovery, COVID-19, in addition to physical and public health ailments, causes many mental illnesses, including stress, anxiety, depression, anger and fear.(50) As observed after infection with Covid-19 Chronic fatigue syndrome (CFS) Which is represented by the presence of severe stress that leads to cognitive impairment, lack of sleep and sometimes "hyperactivity" (51-55) All of them need health care after and during infection and return to the deadly nature of the virus, The psychological and neurological reasons for Covid-19 sickness are usually complex and multiple, as they are related to the direct impact of infection or cerebrovascular diseases, including hypercoagulability (56) And the fears of a deadly disease (57) Other studies have found that this could be due to financial insecurity and fear of job loss from the pandemic (12)

Studies have indicated that infection with Covid-19 is associated with fatigue and muscle and joint pain after recovery This is similar to what happened after SARS, which caused avascular necrosis of the femoral head (22), It is believed that it occurred due to the dexamethasone drug used during the infection (13) , Another study showed the injury had caused other complications, including sudden weight loss, ear pain, eye problems, dizziness, confusion, and tachycardia (59,60) And hair loss, which can occur due to early follicular transformations, and although this loss is not permanent, it creates anxiety in patients (61).

Conclusions

Covid-19 is a deadly disease that produces several of the medical conditions during infection and continues until after recovery. In this investigation, we discovered that survivors of the disease experienced a variety of other adverse effects, the virus is destructive, whether it is in the respiratory system, which is the virus's recipient, or in other body systems, and precautions must be made to avoid infection in order to live a long and healthy life. The individual before the viral infection is not the same as the person after the virus infection.

References

- (1). Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., ... Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet (London, England)*, 395(10223), 497–506. [https://doi.org/10.1016/S0140-6736\(20\)30183-5](https://doi.org/10.1016/S0140-6736(20)30183-5)
- (2). Zhou, P., Yang, X. L., Wang, X. G., Hu, B., Zhang, L., Zhang, W., Si, H. R., Zhu, Y., Li, B., Huang, C. L., Chen, H. D., Chen, J., Luo, Y., Guo, H., Jiang, R. D., Liu, M. Q., Chen, Y., Shen, X. R., Wang, X., Zheng, X. S., ... Shi, Z. L. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature*, 579(7798), 270–273. <https://doi.org/10.1038/s41586-020-2012-7>.
- (3). Loon, S. C., and Lun, K. (2013). SARS: a timely reminder. *The British journal of ophthalmology*, 97(9), 1217–1218. <https://doi.org/10.1136/bjophthalmol-2013-303596>.
- (4). Buchholz, U., Müller, M. A., Nitsche, A., Sanewski, A., Wevering, N., Bauer-Balci, T., Bonin, F., Drosten, C., Schweiger, B., Wolff, T., Muth, D., Meyer, B., Buda, S., Krause, G., Schaade, L., & Haas, W. (2013). Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October–November 2012. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin*, 18(8), 20406.
- (5). Organisation WH. Infection prevention and control during health care when novel coronavirus (nCoV) infection is suspected. [https://www.who.int/publications-detail/infection-preventionand-control-during-health-care-when-novel-coronavirus-\(ncov\)-infection-is-suspected-20200125](https://www.who.int/publications-detail/infection-preventionand-control-during-health-care-when-novel-coronavirus-(ncov)-infection-is-suspected-20200125). 2020.
- (6). Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China 2019. (2020).. [New England Journal of Medicine](https://doi.org/10.1056/NEJMoa2001017) 382(8) DOI: [10.1056/NEJMoa2001017](https://doi.org/10.1056/NEJMoa2001017)
- (7). Su, S., Wong, G., Shi, W., Liu, J., Lai, A., Zhou, J., Liu, W., Bi, Y., & Gao, G. F. (2016). Epidemiology, Genetic Recombination, and Pathogenesis of Coronaviruses. *Trends in microbiology*, 24(6), 490–502. <https://doi.org/10.1016/j.tim.2016.03.003>.
- (8). Zheng, Y. Y., Ma, Y. T., Zhang, J. Y., and Xie, X. (2020). COVID-19 and the cardiovascular system. *Nature reviews. Cardiology*, 17(5), 259–260. <https://doi.org/10.1038/s41569-020-0360-5>.
- (9). Abdul-Hussein, Fouad Razzaq, (2020). Comprehensive scientific guide to the emerging corona virus, Ministry of Higher Education and Scientific Research, University of Al-Muthanna, Department of Scientific Affairs.
- (10). Chen, N., Zhou, M., Dong, X., Qu, J., Gong, F., Han, Y., Qiu, Y., Wang, J., Liu, Y., Wei, Y., Xia, J., Yu, T., Zhang, X., and Zhang, L. (2020). Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet (London, England)*, 395(10223), 507–513. [https://doi.org/10.1016/S0140-6736\(20\)30211-7](https://doi.org/10.1016/S0140-6736(20)30211-7).
- (11). Wu, J., Liu, J., Zhao, X., Liu, C., Wang, W., Wang, D., Xu, W., Zhang, C., Yu, J., Jiang, B., Cao, H., and Li, L. (2020). Clinical Characteristics of Imported Cases of Coronavirus Disease 2019 (COVID-19) in Jiangsu Province: A Multicenter Descriptive Study. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 71(15), 706–712. <https://doi.org/10.1093/cid/ciaa199>.

- (12). Iyengar KP, Vaishya R. Long-term health consequences of COVID-19 disease. *Apollo Med* 2020;17:S32-5. DOI:[10.4103/am.am_109_20](https://doi.org/10.4103/am.am_109_20)
- (13). 13- Carfi, A., Bernabei, R., Landi, F., & Gemelli Against COVID-19 Post-Acute Care Study Group (2020). Persistent Symptoms in Patients After Acute COVID-19. *JAMA*, 324(6), 603–605. <https://doi.org/10.1001/jama.2020.12603>.
- (14). South, A. M., Diz, D. I., and Chappell, M. C. (2020). COVID-19, ACE2, and the cardiovascular consequences. *American journal of physiology. Heart and circulatory physiology*, 318(5), H1084–H1090. <https://doi.org/10.1152/ajpheart.00217.2020>.
- (15). Ciaglia, E., Vecchione, C., & Puca, A. A. (2020). COVID-19 Infection and Circulating ACE2 Levels: Protective Role in Women and Children. *Frontiers in pediatrics*, 8, 206. <https://doi.org/10.3389/fped.2020.00206>
- (16). 16- Tufan, A., Avanoğlu Güler, A., and Matucci-Cerinic, M. (2020). COVID-19, immune system response, hyperinflammation and repurposing antirheumatic drugs. *Turkish journal of medical sciences*, 50(SI-1), 620–632. <https://doi.org/10.3906/sag-2004-168>.
- (17). 17- Gosain, R., Abdou, Y., Singh, A., Rana, N., Puzanov, I., and Ernstoff, M. S. (2020). COVID-19 and Cancer: a Comprehensive Review. *Current oncology reports*, 22(5), 53. <https://doi.org/10.1007/s11912-020-00934-7>.
- (18). Osman, M. S., van Eeden, C., and Cohen Tervaert, J. W. (2020). Fatal COVID-19 infections: Is NK cell dysfunction a link with autoimmune HLH?. *Autoimmunity reviews*, 19(7), 102561. <https://doi.org/10.1016/j.autrev.2020.102561>.
- (19). 19- Varga, Z., Flammer, A. J., Steiger, P., Haberecker, M., Andermatt, R., Zinkernagel, A. S., Mehra, M. R., Schuepbach, R. A., Ruschitzka, F., & Moch, H. (2020). Endothelial cell infection and endotheliitis in COVID-19. *Lancet (London, England)*, 395(10234), 1417–1418. [https://doi.org/10.1016/S0140-6736\(20\)30937-5](https://doi.org/10.1016/S0140-6736(20)30937-5).
- (20). Madjid, M., Safavi-Naeini, P., Solomon, S. D., & Vardeny, O. (2020). Potential Effects of Coronaviruses on the Cardiovascular System: A Review. *JAMA cardiology*, 5(7), 831–840. <https://doi.org/10.1001/jamacardio.2020.1286>
- (21). Fraser E. (2020). Long term respiratory complications of covid-19. *BMJ (Clinical research ed.)*, 370, m3001. <https://doi.org/10.1136/bmj.m3001>
- (22). Zhang, P., Li, J., Liu, H., Han, N., Ju, J., Kou, Y., Chen, L., Jiang, M., Pan, F., Zheng, Y., Gao, Z., and Jiang, B. (2020). Long-term bone and lung consequences associated with hospital-acquired severe acute respiratory syndrome: a 15-year follow-up from a prospective cohort study. *Bone research*, 8, 8. <https://doi.org/10.1038/s41413-020-0084-5>.
- (23). Komatsu, R., Okazaki, T., Ebihara, S., Kobayashi, M., Tsukita, Y., Nihei, M., Sugiura, H., Niu, K., Ebihara, T., and Ichinose, M. (2018). Aspiration pneumonia induces muscle atrophy in the respiratory, skeletal, and swallowing systems. *Journal of cachexia, sarcopenia and muscle*, 9(4), 643–653. <https://doi.org/10.1002/jcsm.12297>
- (24). Yang, L.; Liu, S.; Liu, J.; Zhang, Z.; Wan, X.; Huang, B.; Chen, Y.; Zhang, Y. COVID-19: Immunopathogenesis and Immunotherapeutics. *Signal Transduct. Target. Ther.* **2020**, 5, 1–8. [[CrossRef](#)]
- (25). Sun, L., Louie, M. C., Vannella, K. M., Wilke, C. A., LeVine, A. M., Moore, B. B., & Shanley, T. P. (2011). New concepts of IL-10-induced lung fibrosis: fibrocyte recruitment and M2 activation in a CCL2/CCR2 axis. *American journal of physiology. Lung cellular and molecular physiology*, 300(3), L341–L353. <https://doi.org/10.1152/ajplung.00122.2010>.

- (26). Liao, M., Liu, Y., Yuan, J., Wen, Y., Xu, G., Zhao, J., Cheng, L., Li, J., Wang, X., Wang, F., Liu, L., Amit, I., Zhang, S., and Zhang, Z. (2020). Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nature medicine*, 26(6), 842–844. <https://doi.org/10.1038/s41591-020-0901-9>.
- (27). Carr, M. W., Roth, S. J., Luther, E., Rose, S. S., and Springer, T. A. (1994). Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proceedings of the National Academy of Sciences of the United States of America*, 91(9), 3652–3656. <https://doi.org/10.1073/pnas.91.9.3652>.
- (28). Xu LL, Warren MK, Rose WL, Gong W, Wang JM (September 1996). "Human recombinant monocyte chemotactic protein and other C-C chemokines bind and induce directional migration of dendritic cells in vitro". *Journal of Leukocyte Biology*. 60 (3): 36571. doi: 10.1002 /jlb.60.3.365 PMID 8830793. S2CID 24481789.
- (29). Wu J. (2020). Tackle the free radicals damage in COVID-19. *Nitric oxide : biology and chemistry*, 102, 39–41. <https://doi.org/10.1016/j.niox.2020.06.002>.
- (30). Spagnolo, P., Balestro, E., Aliberti, S., Cocconcelli, E., Biondini, D., Casa, G. D., Sverzellati, N., & Maher, T. M. (2020). Pulmonary fibrosis secondary to COVID-19: a call to arms?. *The Lancet. Respiratory medicine*, 8(8), 750–752. [https://doi.org/10.1016/S2213-2600\(20\)30222-8](https://doi.org/10.1016/S2213-2600(20)30222-8).
- (31). Wang, D., Hu, B., Hu, C., Zhu, F., Liu, X., Zhang, J., Wang, B., Xiang, H., Cheng, Z., Xiong, Y., Zhao, Y., Li, Y., Wang, X., and Peng, Z. (2020). Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA*, 323(11), 1061–1069. <https://doi.org/10.1001/jama.2020.1585>.
- (32). Peiris, J. S., Chu, C. M., Cheng, V. C., Chan, K. S., Hung, I. F., Poon, L. L., Law, K. I., Tang, B. S., Hon, T. Y., Chan, C. S., Chan, K. H., Ng, J. S., Zheng, B. J., Ng, W. L., Lai, R. W., Guan, Y., Yuen, K. Y., and HKU/UCH SARS Study Group (2003). Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. *Lancet (London, England)*, 361(9371), 1767–1772. [https://doi.org/10.1016/s0140-6736\(03\)13412-5](https://doi.org/10.1016/s0140-6736(03)13412-5).
- (33). Chong, P. Y., Chui, P., Ling, A. E., Franks, T. J., Tai, D. Y., Leo, Y. S., Kaw, G. J., Wansaicheong, G., Chan, K. P., Ean Oon, L. L., Teo, E. S., Tan, K. B., Nakajima, N., Sata, T., & Travis, W. D. (2004). Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. *Archives of pathology & laboratory medicine*, 128(2), 195–204. <https://doi.org/10.5858/2004-128-195-AODDTS>.
- (34). Yu, C. M., Wong, R. S., Wu, E. B., Kong, S. L., Wong, J., Yip, G. W., Soo, Y. O., Chiu, M. L., Chan, Y. S., Hui, D., Lee, N., Wu, A., Leung, C. B., and Sung, J. J. (2006). Cardiovascular complications of severe acute respiratory syndrome. *Postgraduate medical journal*, 82(964), 140–144. <https://doi.org/10.1136/pgmj.2005.037515>.
- (35). Xu, Z., Shi, L., Wang, Y., Zhang, J., Huang, L., Zhang, C., Liu, S., Zhao, P., Liu, H., Zhu, L., Tai, Y., Bai, C., Gao, T., Song, J., Xia, P., Dong, J., Zhao, J., & Wang, F. S. (2020). Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *The Lancet. Respiratory medicine*, 8(4), 420–422. [https://doi.org/10.1016/S2213-2600\(20\)30076-X](https://doi.org/10.1016/S2213-2600(20)30076-X).
- (36). Shi, S., Qin, M., Shen, B., Cai, Y., Liu, T., Yang, F., Gong, W., Liu, X., Liang, J., Zhao, Q., Huang, H., Yang, B., and Huang, C. (2020). Association of Cardiac Injury With

- Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. *JAMA cardiology*, 5(7), 802–810. <https://doi.org/10.1001/jamacardio.2020.0950>.
- (37). Guo, T., Fan, Y., Chen, M., Wu, X., Zhang, L., He, T., Wang, H., Wan, J., Wang, X., & Lu, Z. (2020). Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). *JAMA cardiology*, 5(7), 811–818. <https://doi.org/10.1001/jamacardio.2020.1017>.
- (38). Vardeny, O., and Solomon, S. D. (2017). Influenza vaccination: a one-shot deal to reduce cardiovascular events. *European heart journal*, 38(5), 334–337. <https://doi.org/10.1093/eurheartj/ehw560>.
- (39). Corrales-Medina VF, Madjid M, Musher DM. (2010). Role of acute infection in triggering acute coronary syndromes. *Lancet Infect Dis*. 10(2):83-92. doi:10.1016/S1473-3099 (09) 70331-7
- (40). Li, M., Dong, Y., Wang, H., Guo, W., Zhou, H., Zhang, Z., Tian, C., Du, K., Zhu, R., Wang, L., Zhao, L., Fan, H., Luo, S., and Hu, D. (2020). Cardiovascular disease potentially contributes to the progression and poor prognosis of COVID-19. *Nutrition, metabolism, and cardiovascular diseases : NMCD*, 30(7), 1061–1067. <https://doi.org/10.1016/j.numecd.2020.04.013>.
- (41). Wrapp, D., Wang, N., Corbett, K. S., Goldsmith, J. A., Hsieh, C. L., Abiona, O., Graham, B. S., and McLellan, J. S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science (New York, N.Y.)*, 367(6483), 1260–1263. <https://doi.org/10.1126/science.abb2507>.
- (42). Xu, P., Sriramula, S., and Lazartigues, E. (2011). ACE2/ANG-(1-7)/Mas pathway in the brain: the axis of good. *American journal of physiology. Regulatory, integrative and comparative physiology*, 300(4), R804–R817. <https://doi.org/10.1152/ajpregu.00222.2010>
- (43). Alenina, N., & Bader, M. (2019). ACE2 in Brain Physiology and Pathophysiology: Evidence from Transgenic Animal Models. *Neurochemical research*, 44(6), 1323–1329. <https://doi.org/10.1007/s11064-018-2679-4>.
- (44). Bikdeli, B., Madhavan, M. V., Jimenez, D., Chuich, T., Dreyfus, I., Driggin, E., Nigoghossian, C., Agha, W., Madjid, M., Guo, Y., Tang, L. V., Hu, Y., Giri, J., Cushman, M., Quéré, I., Dimakakos, E. P., Gibson, C. M., Lippi, G., Favaloro, E. J., Fared, J., ... Global COVID-19 Thrombosis Collaborative Group, Endorsed by the ISTH, NATF, ESVM, and the IUA, Supported by the ESC Working Group on Pulmonary Circulation and Right Ventricular Function (2020). COVID-19 and Thrombotic or Thromboembolic Disease: Implications for Prevention, Antithrombotic Therapy, and Follow-Up: JACC State-of-the-Art Review. *Journal of the American College of Cardiology*, 75(23), 2950–2973. <https://doi.org/10.1016/j.jacc.2020.04.031>.
- (45). Iba, T., and Levy, J. H. (2018). Inflammation and thrombosis: roles of neutrophils, platelets and endothelial cells and their interactions in thrombus formation during sepsis. *Journal of thrombosis and haemostasis : JTH*, 16(2), 231–241. <https://doi.org/10.1111/jth.13911>.
- (46). Li, X., and Ma, X. (2017). The role of heparin in sepsis: much more than just an anticoagulant. *British journal of hematology*, 179(3), 389–398. <https://doi.org/10.1111/bjh.14885>.

- (47). Heneka, M. T., Golenbock, D., Latz, E., Morgan, D., and Brown, R. (2020). Immediate and long-term consequences of COVID-19 infections for the development of neurological disease. *Alzheimer's research & therapy*, 12(1), 69. <https://doi.org/10.1186/s13195-020-00640-3>
- (48). Kingstone, T., Taylor, A. K., O'Donnell, C. A., Atherton, H., Blane, D. N., and Chew-Graham, C. A. (2020). Finding the 'right' GP: a qualitative study of the experiences of people with long-COVID. *BJGP open*, 4(5), bjgpopen20X101143. <https://doi.org/10.3399/bjgpopen20X101143>.
- (49). Maury, A., Lyoubi, A., Peiffer-Smadja, N., de Broucker, T., and Meppiel, E. (2021). Neurological manifestations associated with SARS-CoV-2 and other coronaviruses: A narrative review for clinicians. *Revue neurologique*, 177(1-2), 51–64. <https://doi.org/10.1016/j.neurol.2020.10.001>
- (50). Torales, J., O'Higgins, M., Castaldelli-Maia, J. M., and Ventriglio, A. (2020). The outbreak of COVID-19 coronavirus and its impact on global mental health. *The International journal of social psychiatry*, 66(4), 317–320. <https://doi.org/10.1177/0020764020915212>
- (51). Wostyn P. (2021). COVID-19 and chronic fatigue syndrome: Is the worst yet to come?. *Medical hypotheses*, 146, 110469. <https://doi.org/10.1016/j.mehy.2020.110469>.
- (52). Vink, M., and Vink-Niese, A. (2020). Could Cognitive Behavioural Therapy Be an Effective Treatment for Long COVID and Post COVID-19 Fatigue Syndrome? Lessons from the Qure Study for Q-Fever Fatigue Syndrome. *Healthcare (Basel, Switzerland)*, 8(4), 552. <https://doi.org/10.3390/healthcare8040552>.
- (53). Lamprecht B. (2020). Gibt es ein Post-COVID-Syndrom? [Is there a post-COVID syndrome?]. *Der Pneumologe*, 1–4. Advance online publication. <https://doi.org/10.1007/s10405-020-00347-0>.
- (54). Pallanti, S., Grassi, E., Makris, N., Gasic, G. P., and Hollander, E. (2020). Neurocovid-19: A clinical neuroscience-based approach to reduce SARS-CoV-2 related mental health sequelae. *Journal of psychiatric research*, 130, 215–217. <https://doi.org/10.1016/j.jpsy.2020.08.008>
- (55). Nath A. (2020). Long-Haul COVID. *Neurology*, 95(13), 559–560. <https://doi.org/10.1212/WNL.00000000000010640>
- (56). Baldini, T., Asioli, G. M., Romoli, M., Carvalho Dias, M., Schulte, E. C., Hauer, L., Aguiar De Sousa, D., Sellner, J., and Zini, A. (2021). Cerebral venous thrombosis and severe acute respiratory syndrome coronavirus-2 infection: A systematic review and meta-analysis. *European journal of neurology*, 28(10), 3478–3490. <https://doi.org/10.1111/ene.14727>.
- (57). Rogers, J. P., Chesney, E., Oliver, D., Pollak, T. A., McGuire, P., Fusar-Poli, P., Zandi, M. S., Lewis, G., and David, A. S. (2020). Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: a systematic review and meta-analysis with comparison to the COVID-19 pandemic. *The lancet. Psychiatry*, 7(7), 611–627. [https://doi.org/10.1016/S2215-0366\(20\)30203-0](https://doi.org/10.1016/S2215-0366(20)30203-0).
- (58). Horby, P., Lim, W. S., Emberson, J. R., Mafham, M., Bell, J. L., Linsell, L., Staplin, N., Brightling, C., Ustianowski, A., Elmahi, E., Prudon, B., Green, C., Felton, T., Chadwick, D., Rege, K., Fegan, C., Chappell, L. C., Faust, S. N., Jaki, T., ... Landray, M. J. RECOVERY Collaborative Group. (2021). Dexamethasone in Hospitalized Patients with

Covid-19. *The New England journal of medicine*, 384(8), 693–704. <https://doi.org/10.1056/NEJMoa2021436>.

- (59). Tenforde, M. W. *et al.*(2020). Symptom duration and risk factors for delayed return to usual health among outpatients with COVID-19 in a multistate health care systems network—United States,. *Morb. Mortal Wkly Rep.* **69**, 993–998. <https://doi.org/10.15585/mmwr.mm6930e1> (2020).
- (60). Carfi, A., Bernabei, R., Landi, F. & Gemelli Against, C.-P.-A.C.S.G. Persistent symptoms in patients after acute COVID-19. *JAMA* **324**, 603–605. <https://doi.org/10.1001/jama.2020.12603> (2020).
- (61). Mieczkowska, K., Deutsch, A., Borok, J., Guzman, A. K., Fruchter, R., Patel, P., Wind, O., McLellan, B. N., Mann, R. E., and Halverstam, C. P. (2021). Telogen effluvium: a sequela of COVID-19. *International journal of dermatology*, 60(1), 122–124. <https://doi.org/10.1111/ijd.15313>

**THE IMPACT OF MARINE TRANSGRESSION IN THE MARSHES OF
SOUTHERN IRAQ**

BUSHRA MAJEED ISSA



THE IMPACT OF MARINE TRANSGRESSION IN THE MARSHES OF SOUTHERN IRAQ

Bushra Majeed ISSA ¹

Abstract:

The marshes in southern Iraq are one of the most interesting phenomena to study because of their unique environment. Therefore, this region has received a lot of attention in various studies. It is known about the environment of the marshes that it represents a fresh water environment where it is supplied with river water. Therefore, it would be natural for the sediments to reflect that environment, especially in the surface areas of it. However, the location of the marshes in the southern part of Mesopotamia, which was affected by changes in the sea water level during the Holocene period, may raise interest to know the environmental status of the marshes during that period. To find out, three sites were chosen in the marshes of northern Basra province to represent the southern part of Iraq, where the depths of the samples varied between surface and subsurface samples, reaching a depth of three meters. The samples were subjected to grain size analysis in order to know the nature of the sediments and the extent of their differences or similarities through the depths, as well as the diagnosis of the shells present in them to define the environment in them at the time while they were following the same environment or there was a change in it with the varying depths. The study showed the dominance of silt deposits in the study area clearly over other types of sediments, in addition to the variation in the sources of those deposits. While the shells, which belong to different types of fauna, revealed the presence of the marine influence in addition to the well-known riverine influence in the region, which confirms the arrival of the impact of the change in the level of marine waters during the Holocene period to the marsh areas in southern Iraq.

Key words: Marine Transgression, Marshes, Southern Iraq, Holocene, Northern Basrah.



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Introduction:

The south of Iraq is characterized by marshes, which are called locally (Ahwar), and they are a unique ecosystem, classified as a type of wetland (Young et al., 2002). The marshes area is covered by sediments, including fluvial sediments carried by the Tigris and Euphrates rivers, and others by wind transport (Aqrawi & Evans, 1994). The repercussions of fluctuations in sea level, sedimentation and tectonic activities are factors that affected the development of the marshes in southern Mesopotamia (Aqrawi, 1993). The palynological evidence of climatic and environmental changes in the Quaternary period of southern Iraq showed the effect of marine transgression in the formation of marine sediments at the beginning of the Holocene (Al-Jibouri, 1997). The effect of marine waters was reflected in the sediments of southern Iraq through the presence of foraminifera species that indicated as marine (Issa, 2010), as well as marine species of ostracoda in it (Issa, 2016).

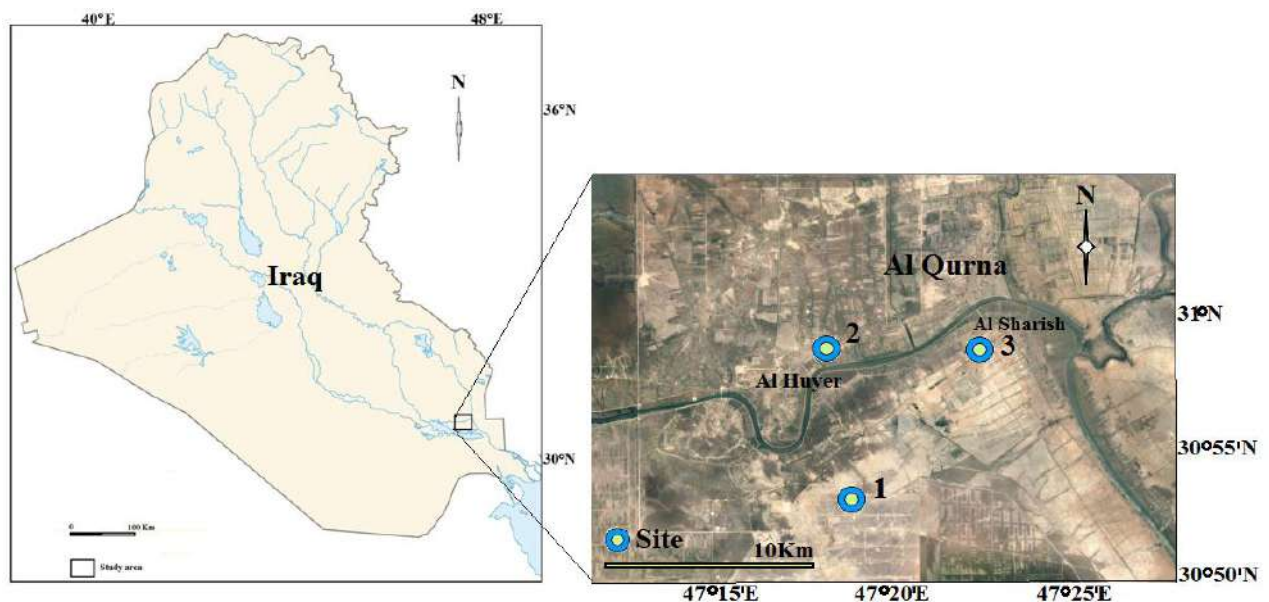
Methods

34 samples were selected from three sites distributed in the study area in the marshes area north of Basra Governorate (Figure 1). The sampling was carried out during November 9, 2009 by using shovel machine. The percentages of sand, silt and clay were determined for all samples using wet sieving on 0.0625 mm sieve in order to isolate sand from silt and clay. As for the particles finer than , pipette method was used depending on Folk (1980).

To identify the fauna shells existent in the samples, the sand remaining on the 0.0625 mm sieve was collected and dried. Thereafter the macrofauna shells were isolated from the microfauna shells, and each of them was placed on a slide appropriate to their size, to be diagnosed under binocular microscope, and then their species were determined. In order to classify the species, it was adopted Keen and Coan (1974) for gastropoda and categorization of Moore (1969) for pelecypoda, while the classification of foraminifera was used Loeblich and Tappan (1988), for determine the ostracoda species was used Moore and Bitrat (1961) (Peiris, 1969).

Figure 1

Location map of samples



Results and discussion

Sediments Texture Analysis

The results of the grain size analysis for the three sites according to Folk (1980) detected the percentage of sand, silt and clay (Table 1, 2 and 3). As it was found that 41% of the total samples fall within the type of silt deposit; 27% sandysilt sediment; 15% mud deposit; 11% clay sediment and the remaining 6% is represented by sandymud sediment 3% and the other 3% by sandyclay deposit.

Table 1:

Grain-size texture of Site 1 sediments

Texture of sediments		Sand%	Silt%	Clay%
Mud	Max.	8	56	57
	Min.	3	39	41
	Average	5	48	47
Sandy silt	Max.	18	79	16
	Min.	15	66	6
	Average	3	73	11
Clay	Max.	1	31	71
	Min.	0	29	68
	Average	1	30	70
Sandy clay	Max.	17	21	64
	Min.	12	18	62
	Average	15	20	61

Table 2:

Grain-size texture of Site 2 sediments

Texture of sediments		Sand%	Silt%	Clay%
Silt	Max.	10	80	32
	Min.	2	62	17
	Average	5	70	25
Sandy silt	Max.	19	64	24
	Min.	15	55	12
	Average	17	60	17
Sandy mud	Max.	16	55	34
	Min.	13	51	32
	Average	14	53	32

Table 3:

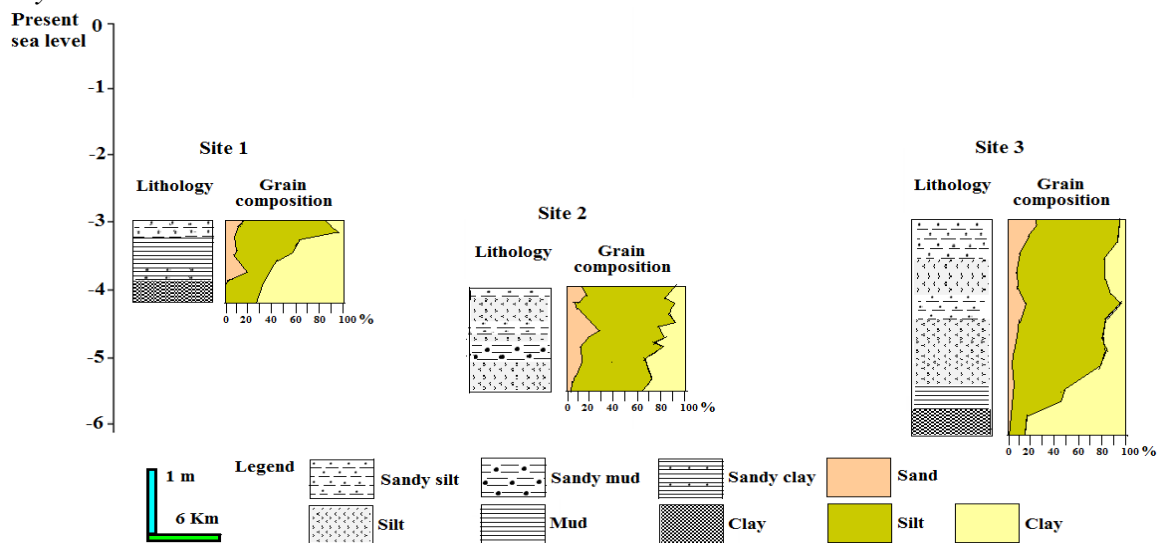
Grain-size texture of Site 3 sediments

Texture of sediments		Sand%	Silt%	Clay%
Silt	Max.	9	77	27
	Min.	2	71	14
	Average	6	74	21
Sandy silt	Max.	25	76	9
	Min.	17	72	3
	Average	20	74	6
Mud	Max.	5	44	55
	Min.	1	42	52
	Average	3	42	53
Clay	Max.	1	17	84
	Min.	< 1	16	82
	Average	< 1	16	82

It was observed that all sites were covered by sandsilt sediments and these sediments extended to depths ranging from 12 to 32 cm. As for the silt deposit, it formed the largest percentage in the second and third sites, with depths of 151 to 209 cm. While the depositions of mud was the most present in the first site. As for the clay deposit, it appeared in sites 1 and 3, but its appearance in site1 was shallower at a depth of 87 cm, while it only appeared at a depth of 280 cm in the third location (Figure 2). This may be due to the physiographic difference of the study area. The appearance of this sediment may also indicate a change in the sedimentation condition, which is closer to a marine water environment (i.e. an indication of marine transgression), which can be confirmed by the nature of the fauna shells accompanying the sediment. Issa (2016) indicated that the sedimentary environment in the marshlands could be fluvial mixed with marine sediments.

Figure 2

Lithological columns of the studied sites and the results of the complete sedimentological analysis



Fauna Shells

After identification the fauna shells for the three sites, the results showed the presence of two groups; macrofauna, whose shells appeared clearly on the study area surface (Figure 3), represented by the Mollusca phylum, the two classes Gastropoda and Pelecypoda. While microfauna represented Foraminifera and Ostracoda (Figure 4). By following the distribution and abundance of species' shells (Figure 5) in the depths of the three sites, three zones were distinguished (Figure 6):

Figure 3

Mollusca shells the apparent at surface of the site 1



(C) *Corbicula fluminalis* (Müller,1774)

(U) *Unio tigridis* (Bourguignat,1853)

(B) *Bellamyia bengalensis* (Lamarck,1822)

(Mt) *Melanoides tuberculata* (Müller,1774)

(Mp) *Melanopsis (Melanopsis) praemorsum* (Linnaeus,1758)

1- Zone 1

The zone was distinguished by the appearance of the five types of molluscs in the three sites, namely; *Corbicula fluminalis* (Müller,1774); *Unio tigridis* (Bourguignat,1853); *Bellamyia bengalensis* (Lamarck,1822); *Melanoides tuberculata* (Müller,1774) and *Melanopsis (Melanopsis) praemorsum* (Linnaeus,1758) except for the second species, which never appeared in the third site.

At site 1, mollusca species appeared along the zone. Where the five species appeared close in the abundance at the zone top, but the first species was the most abundant and prevalent in the sediments. The thickness of zone at site 1 is estimated to be approximately 45 cm.

In site 2, the zone thickness was estimated at 31 cm, where the five species appeared at the zone top sediments, and the species continued to appear, except for the second species, whose appearance was confined to the surface only of the zone. The first species also prevailed in its abundance in the zone within the second site.

As for the thickness of the zone in the third site, it reached 51 cm, the species *Corbicula fluminalis*; *Melanooides tuberculata* and *Melanopsis (Melanopsis) praemorsum* appeared throughout the zone except for *Bellamyia bengalensis*.

The distinction of Zone 1 was based on the environment of the five species of molluscs that coexisted together, which reflected the environment of the freshwater marshes.

Corbicula fluminalis, *Unio tigridis*, and *Bellamyia bengalensis* are known to exist in the marshland environment where the water is fresh with varying energy of this water for the first species (Plaziat & Younis, 2005), as for *Melanooides tuberculata* and *Melanopsis (Melanopsis) praemorsum* despite being freshwater species (Plaziat & Younis, 2005; Gutiérrez Gregoric et al. 2007), however, both species can be found in other environments. *Melanooides tuberculata* recorded its appearance in estuarine environments (Bolaji et al., 2011), as well as the type *Melanooides (Melanopsis) praemorsum*, which was indicated to exist in sediments affected by an estuarine environment (Al Ameri & Briant, 2018). However, these species grouped together and *Corbicula fluminalis* dominate, the freshwater marshes being a feature of this range.

2- Zone 2

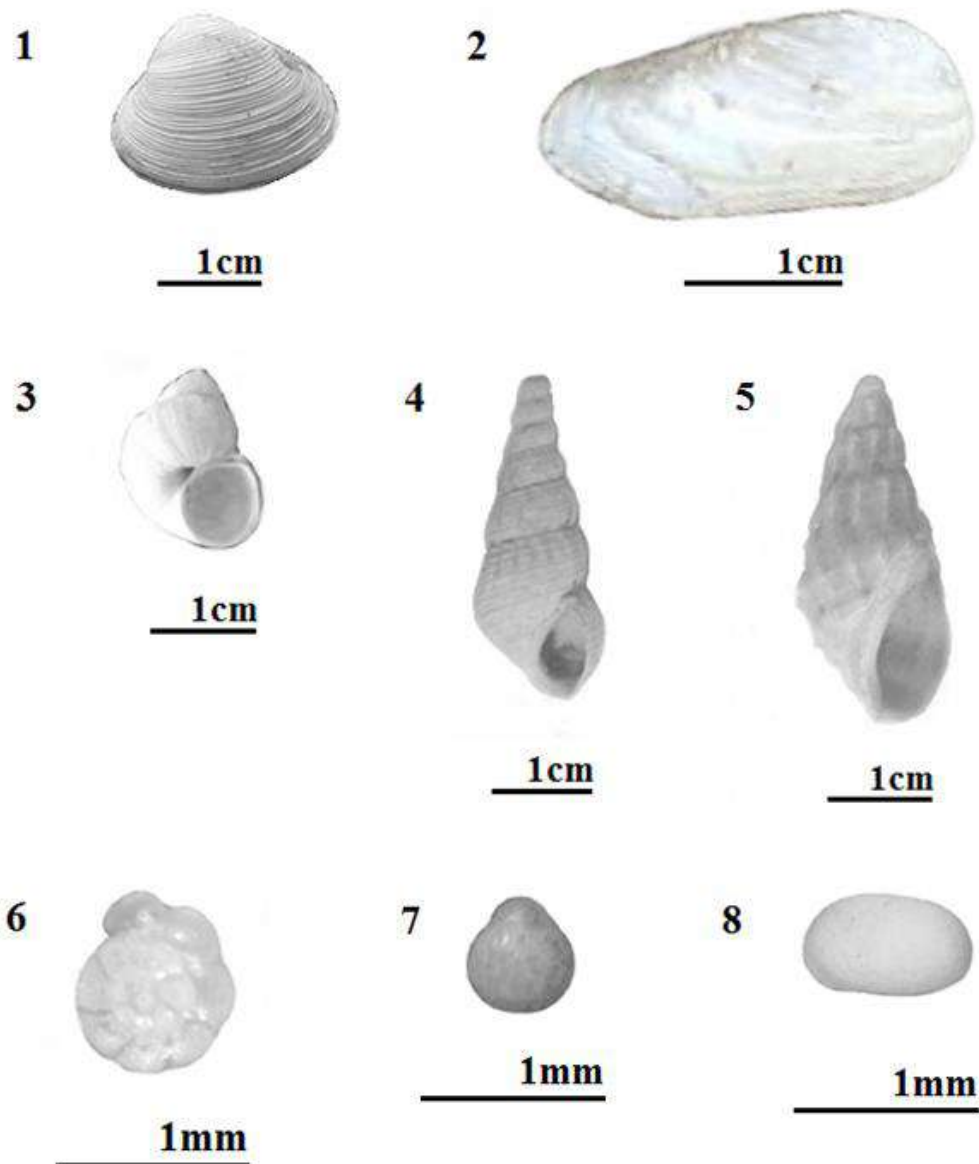
The zone was estimated to have a thickness of 30 cm at site 1, 29 cm in site 2 and the largest thickness of 89 cm at site 3. What distinguishes this zone is the regression of the appearance of molluscs species to two species or only one.

The zone at site 1 was determined by the presence of *Corbicula fluminalis* at the top of the zone with a gradual increase in the appearance of *Ammonia beccarii* (Linné, 1758). While *Corbicula fluminalis* and *Melanooides tuberculata* prevailed at the top of the zone at the second site, with *Ammonia beccarii* appearing at the bottom of the zone. As for the third site, the zone was distinguished from the presence of *Corbicula fluminalis* and *Melanooides tuberculata* with fragments of molluscs shells, which extended to the bottom of the zone where *Ammonia beccarii* appeared.

The presence of *Corbicula fluminalis* is a clear indication of the presence of the river influence and thus the fresh water, but the existence of *Melanooides tuberculata* with it and shell fragments as well as the appearance of *Ammonia beccarii* is an indication of an environmental change where salinity differs and the energy of the environment as well. *Ammonia beccarii* is one of the species indicated to exist in a brackish environment with marine influence (Murray, 1991). It also indicates the occurrence of *Melanooides tuberculata*, which was known to be able to tolerate variations in salinity. All species and the circumstance of this zone refer to being affected by saline water, so the range can be defined as representing a brackish environment.

Figure 4

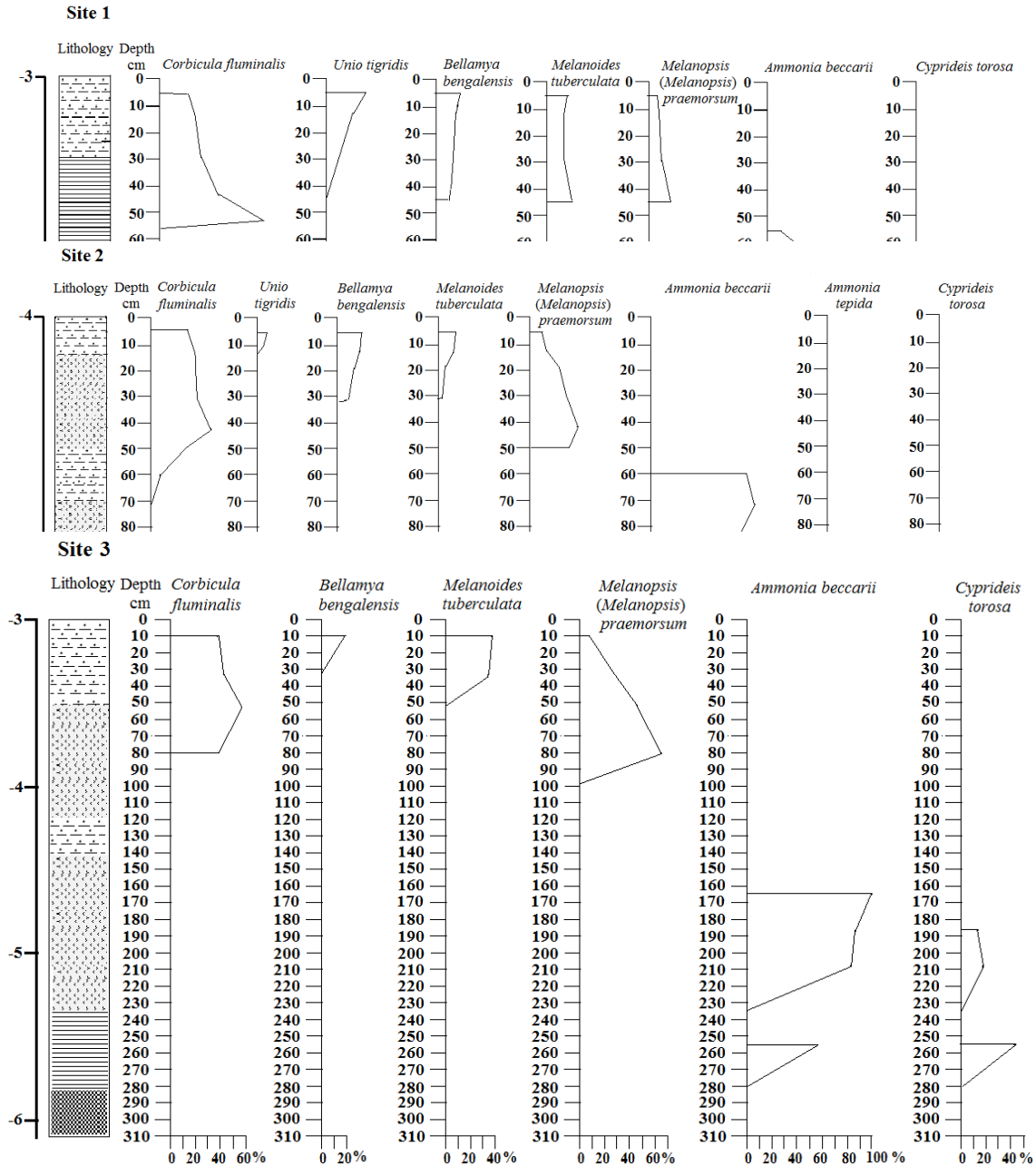
The identified species shells in the study area



Pelecypoda: 1. *Corbicula fluminalis* (Müller,1774); 2. *Unio tigridis* (Bourguignat,1853); Gastropoda: 3. *Bellamya bengalensis* (Lamarck,1822); 4. *Melanoides tuberculata* (Müller,1774); 5. *Melanopsis (Melanopsis) praemorsum* (Linnaeus,1758); Foraminifera: 6. *Ammonia beccarii* (Linné,1758); 7. *Ammonia tepida* (Cushman,1926); Ostracoda: 8. *Cyprideis torosa* (Jones, 1850).

Figure 5

Relative abundances of the mollusca, foraminifera and ostracoda species in site 1, site 2 and site 3



3- Zone 3

The zone thickness reached 36 cm in site 1, 91 cm in site2 and 170 cm in site3. The occurrence of *Ammonia beccarii*, *Ammonia tepida* (Cushman, 1926) and *Cyprideis torosa* (Jones, 1850) clearly dominated this zone. The first type was the most dominant and continued to appear throughout the zone. The third zone was distinguished as a brackish-marine environment.

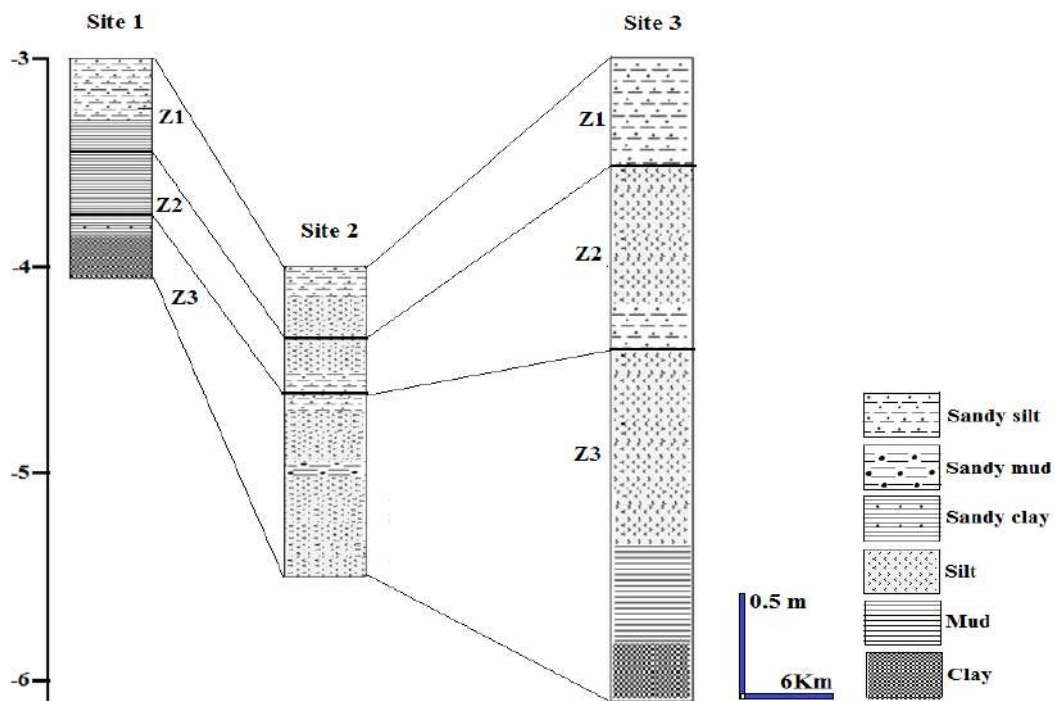
The brackish environment is characterized by the presence of *Ammonia tepida* and *Cyprideis torosa*. Whereas, *Ammonia tepida* is a species that spreads in brackish waters

(Murray, 2006) with salinity less than 33‰ (Le Campion, 1968; Rouvilois, 1970; Debenay, 1978; Redois, 1996 in Debenay, et al. 1998). The environment of *Cyprideis torosa* also indicates the brackish condition through its existence (Meisch, 2000).

It was noted that the presence of the two species was not continuous along the zone, with the existence of *Ammonia beccarii* which it complete dominance over the zone, it is known that this species dominates in marine environments (Debenay, et al. 1998). This imposes the possibility of the presence of marine influence as a result of the change in sea level and the arrival of the impact of marine transgression during the late Holocene period, which is reflected by the nature of the shells, and the age of sediments where these was previously referred to in the study of Aqrawi(2001).

Figure 6

Distribution of zones in the study area



Conclusions

- The types of sediments in the study area are six types; Silt, Sandy silt, Mud, Clay, Sandy mud, Sandy clay. However, the predominant sediment is Silt.
- The different types of sediments are due to the different sedimentation conditions. The sediments of the study area are mixed with fluvial and marine sediments.
- Depending on the distribution and abundance of shells of the species classified in the study area, three zones were identified that varied in their environment. Zone1 reflected the freshwater marsh environment. While Zone 2 represents the brackish environment influence in the marshes. As for Zone 3, it showed the effect of marine waters clearly.
- The effect of marine waters in the study area is likely due to the effect of marine transgression during the late Holocene, depending on the shells appearance nature.

References

- Al Ameri, I. & Briant, R. (2018). A late Holocene molluscan-based palaeoenvironmental reconstruction from southern Mesopotamia: implications for the palaeogeographic evolution of the Arabo-Persian Gulf. *Journal of African Earth Sciences*, 152, 1-9. ISSN 1464-343X.
- Al-Jibouri, B. S. (1997). *Palynological evidences of the climatic and environmental changes during the Quaternary period in the Mesopotamian plain southern Iraq*. [Unpublished M.Sc. dissertation]. University of Baghdad, (In Arabic).
- Aqrawi, A. A. M. (1993). Implication of sea-level fluctuations, Sedimentation and Neotectonics for the evolution of the marshland (Ahwar) of Southern Iraq. *Quaternary Proceedings*, 3, 17-25.
- Aqrawi, A. A. M. & Evans, G. (1994). Sedimentation in the lake and marshes (Ahwar) of Tigris-Euphrates delta, Southern Mesopotamia. *Sedimentology*, 41(4), 755-776.
- Aqrawi, A. A. M. (2001). Stratigraphic signatures of climatic change during the Holocene evolution of the Tigris –Euphrates delta, lower Mesopotamia. *Global and planetary Change*, 28, 267-283.
- Bolaji, D. A., Edokpayi, C. A., Samuel, O. B., Akinnigbagbe, R. O. & Ajulo, A. A. (2011). Morphological characteristics and salinity tolerance of *Melanoides tuberculata* (Müller, 1774). *World Journal of Biological Research*, 4(2), 1-11.
- Gutiérrez Gregoric, D. E., Núñez, V., Ferrando, N. S. & Rumi, A. (2007). First record of invasive snail *Melanoides tuberculata* (Müller) (Gastropoda: Prosobranchia: Thiariidae) for the Iguazú River Basin, Argentina–Brazil. *Comunicaciones Sociedad Malacológica del Uruguay*, 9(90), 109-112.
- Issa, B. M. (2016). Ostracoda and Charophyte as indicators of environmental variability in the marshland area of Southern Iraq. *Journal of Basrah Researches ((Sciences))*, 42(2) A, 148-159. ISSN 18172695.
- Issa, B.M. (2010). Depositional environments and biofacies of selected sediments, north Basrah. *Journal of Basrah Researches ((Sciences))*, 36 (5) A, 1-14.
- Plaziat, J-C. & Younis, W. R. (2005). The modern environments of Molluscs in southern Mesopotamia, Iraq: A guide to paleogeographical reconstructions of Quaternary fluvial, palustrine and marine deposits. *Carnets de Geologie, Carnets de Geologie*, (A01), 1-18. hal- 00142754.
- Keen, A.M. & Coan, E. (1974). *Marine Molluscan genera of western north America*. Stanford University Press.
- Loeblich, A. R. & Tappan, H. (1988). *Foraminiferal genera and their classification*. Von Nostrand Reinhold.
- Meisch, C. (2000). Freshwater Ostracoda of Western and Central Europe. *Süßwasserfauna von Mitteleuropa*, 8 (3), 1-522.
- Moore, R. C. (1969). *Treatise on invertebrate Paleontology, Part N: Mollusca 6, (Bivalvia)*, v.2. Kansas Press.
- Murray, J.W. (1991). *Ecology and Palaeoecology of Benthic Foraminifera*. Logman Scientific & Technical, London.
- Murray, J. W. (2006). *Ecology and Applications of Benthic Foraminifera*. Cambridge University Press.



**PREPARATION AND CHARACTERIZATION OF GRAPHENE OXIDE COMPLEXES FOR
TRIAZOLE SCHIFF BASES**

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PREPARATION AND CHARACTERIZATION OF GRAPHENE OXIDE COMPLEXES FOR TRIAZOLE SCHIFF BASES

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Abstract:

In this study, thiocarbohydrazide (TCH), triazole and various nano-derivatives of graphene oxide and imines were prepared through the following steps:

1- Preparation of Thiocarbohydrazide (TCH): Thiocarbohydrazide was prepared by heat sublimation of a mixture of carbon disulfide and aqueous hydrazine..

2- Preparation of 4-amino-5-pyridin-4-yl-4-hydroxy-4,2,1-triazole-3-thiol: Triazole was prepared by using thermal melting of a mixture of TCH and nicotinic acid, which gave a good yield.

3- Preparation of N-(3-mercapto-5-(pyridin-4-yl)-4,2,1-triazole-4-yl)hydrazine carbothiamide (A1): The preparation was made by dissolving triazole in ethanol and ammonium hydroxide with carbon disulfide in the presence of Aqueous hydrazine 80% cooled and recrystallized with ethanol and water.

4- Preparation of 3-(pyridin-4-yl)-4,2,1-triazol-4-yl-4,3-b [4,3,1]thiadiazole-6-amine: The preparation was made through the use of thermal melting of a mixture of N-(3-mercapto-5-(pyridin-4-yl)-4,2,1-triazole-4-yl)hydrazine, carbothiamide (A1) and benzaldehyde, which gave a good product and in a short time.

5- Preparation of graphene oxide (GO): through the interactions of active groups in the synthesis of graphite in the presence of oxidizing agents and acids to prepare graphene oxide. Where the molecules of acids and oxidizing agents penetrate between the layers within the graphite crystals to partially or completely separate the layers through the chemical peeling process using the modified Hummer method.

6- Preparation of nanocomposites (NGO) (A3): This was done by homogenizing graphene oxide in dioxane by ultrasound with the addition of the prepared triazole (A) under heat sublimation.

Key words: Graphene Oxide, Triazole Schiff, TCH.



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Introduction:

The word nano is derived from the ancient Greek (Nanos) meaning the infinitesimally small mythical dwarf world, and in the field of science, nano means a part of a billion (one thousandth of a million) (10^{-9})⁽¹⁾. There are two ways in which we can manufacture a nano-size of matter One of them is from top to bottom UP-DOWN⁽²⁾. This method starts with a palpable size of the material under study and gets smaller gradually until reaching the nanoscale. These techniques include photo-drilling, cutting, scraping, and milling.

The other method is BOTTOM-UP⁽³⁾, which starts with single molecules as the smallest unit and assembles into a larger structure. These methods are often chemical⁽⁴⁾, and are characterized by the small size of the products (one nanometer), less waste of the basic material and having a strong bonding between the resulting nanoparticles.

The nanoformations include quantumdots⁽⁵⁾, which are three-dimensional semiconductors with dimensions ranging from 2 to 10 nanometers, nanoballs⁽⁶⁾, which are nano-carbon belonging to the fluorine class of C60 material, and the diameter of the nanospheres may reach 500 nanometers or more. Microscopic atomic or partial atoms ranging in number from a few atoms (molecule) to a million atoms, connected to each other almost spherically and with a diameter of less than 100 nanometers, when the size of nanoparticles⁽⁷⁾ reaches the nanoscale in one dimension, it is called a quantum well⁽⁸⁾. When its nanoscale is in two dimensions, it is called a quantum wire. When these nano-sized particles are in three dimensions, they are known as (quantum dots ⁽⁹⁾. Nanotubes Nanotubes are sometimes made of inorganic materials such as metal oxides (vanadium oxide), manganese oxide, titanium oxide, boron sulfide and molybdenum), while the other type is carbon tubes that were discovered in 1990⁽¹⁰⁾. It rotates around an axis to take the cylindrical shape where the atoms of the two ends of the slide are connected to each other to close the tube. One end of the tube is mostly open and the other closed in the form of a hemisphere⁽¹¹⁾. Nanocomposites⁽¹²⁾ are materials to which nanoparticles are added during the manufacture of these materials, and as a result, the nanomaterial will greatly improve the properties of materials, as adding other types of nanoparticles will improve the optical properties and electrical insulation properties as well as mechanical properties such as hardness and strength. That the volume percentage of the added nanoparticles is very low (in the range of 0.5% to 5%) because the ratio between the surface area to the volume of nanoparticles is high. Research is currently being conducted to obtain new nanocomposites with properties and characteristics that differ from the original compounds. The now known nanoparticles are polymeric nanocomposites.

The graphene oxide is one of the carbon nanostructures, which is a single SLGO sheet or several sheets of graphite so that it does not exceed ten sheets, each on top of each other called FLGO. The hybridization of carbon atoms in graphene oxide is a tetrahedral SP3 structure. , carboxyl, carbonyl and alcohol(13), graphene oxide can be obtained through the modified Hummer method(14), and graphene oxide can be reduced to reduced graphene oxide, using hydrazine as a reducing agent(19).

Heterocyclic compounds contain one or more heterocyclic atoms in the ring such as nitrogen, oxygen and sulfur, in addition to the presence of carbon atoms, and many studies have proven that they are of great importance in the medical field(20), and as pesticides for herbicides, insects and tapeworms(28) in addition to being a substance A nerve sedative and a muscle relaxant(29) The 4-amino antipyrine compound is also one of the important heterocyclic compounds that have biological activity, as it is considered an effective analgesic and antipyretic(30).

Prepared-1,2,4- triazole was by solid-state reactions through thermal heating without using a solvent, and the yield was 80% (34). Using the base medium(35), and its derivatives were also prepared by condensation between monosubstituted hydrazine or diazylamine in the presence of a weak acid⁽³⁶⁾

The preparation chemistry of triazoles and their derivatives by fusing has received great attention due to their important biological and industrial applications. The triazole nucleus has attracted high interest in various fields, such as medical research and chemistry as well as in materials science due to its unique composition and properties(43), and it was also possible to obtain many antibiotics, drugs and dyes. By laboratory preparation of triazole and its derivatives(44).

practical part:

Material and instruments

1- Infrared measuring device (FT.I.R): Solids were formatted in the form of KBr discs with a range (400-4000cm⁻¹). The measurement was taken in the laboratory of Tikrit University - College of Education for Pure Sciences - Department of Chemistry.

2-Transmission electron microscope device. Transmission electron microscopy:TEM Use the scanning electron microscope to obtain information about the surface and the dimensions that are stacked in minutes with the dimensions of the particles themselves. Use the Czech Reputic/Belsorp Minill/TE SCAN) device from Kashan University/Iran.

3-Measuring the degree of melting (M.P) using a device: Electro thermal melting point apparatus 9300

4-Ultrasonic device Ultra Sonic Water bath: Ultrasonic Homogenizer model 300 V/T, 230 Volts/50 Hz)) in the Instrumentation Laboratory / Department of Life Sciences / College of Education for Pure Sciences / Tikrit University.

5- Electronic sensitive scale: Use the Germany, Sartorius Lab_BL219 device with an accuracy of up to +010.00 (Organic Chemistry Laboratory / Department of Chemistry / College of Education for Pure Sciences / Tikrit University)

Chemical Materials Used: All chemicals were from Flucka and BDH Chemical Ltd.

2-1-4-Experimental part

2-1-4-1-Preparation of thiocarbohydrazide (TCH)

20ml of 80% aqueous hydrazine was added to a round flask of 100 ml capacity in an ice bath, and at zero centigrade, 5 ml of carbon disulfide was gradually added over 10 minutes with magnetic stirring, and then the mixture was ascended for 30 minutes, until a yellow mixture was formed. The mixture was cooled in an ice bath. The precipitate was collected by filtration and washed with diethyl ether and then with ethanol until the precipitate turned white. It was recrystallized with distilled water and dried at 50° C. The melting point was from (170_172)° C, which is consistent with the literature(46) The percentage of the product is (66%). Figure (1) note the table (1).

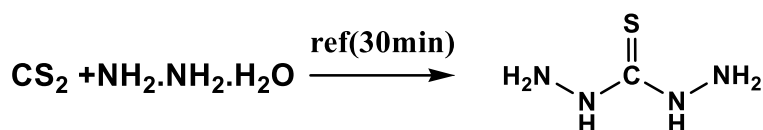
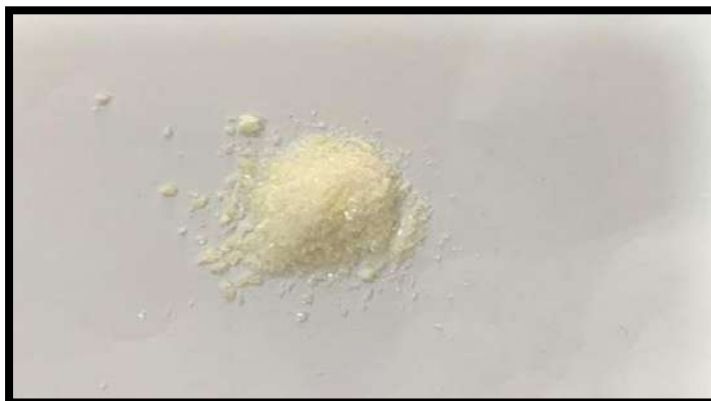


Table (1): Physical properties of the prepared compound

Com p. No	Molecul ar Formula	M.P (C)	Y ield	Color	Solvent
TCH	CH ₆ N ₄ S	174_ 172	6%	Pearl White	D.W



TCH Figure (1) of the compound

2-1-4-2-Preparation of 4-Amino-5-pyridin-4-yl-4,2,1-triazole-3-thiol (A)

Mix 0.01 mol (3 grams) of TCH with 0.01 mol (2.5 grams) of nicotinic acid without using a solvent, then put the mixture in a sand bath with stirring with a glass motor for 3-4 hours until the nature of the reaction mixture changed in terms of texture and color. The molten was treated with a 10% solution of sodium bicarbonate, then the precipitate was filtered, washed with distilled water, and dried at a temperature of 50°C⁽⁴⁷⁾. It was recrystallized with solvent and the preparation was done according to the following equation

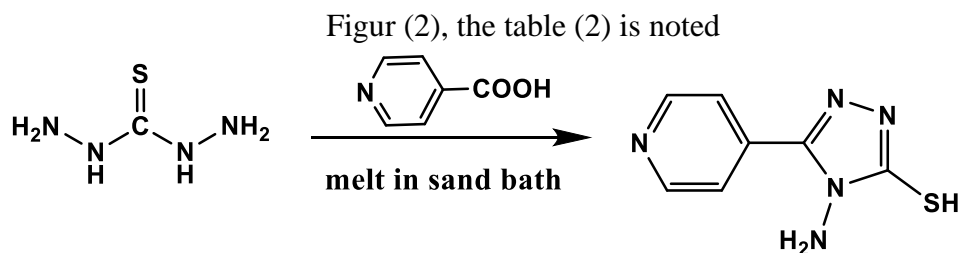


Table (2): Physical properties of the prepared compound (A).

Co mp No	Molecula r Formula	M .P	Yield %	Colour	Solvent
A	C ₇ H ₇ N ₅ S	208	60 %	Pink	EtOH



Figure (2) for compound A

2-1-4-3-Preparation of N-(3-mercapto-5-(4-pyridyl)-4,2,1-triazole-4-yl)hydrazine carbothiamide (A1)

Dissolve 0.004 mol (0.85 g) of the prepared triazole in 50 mL of 96% ethanol and 20 mL of ammonium hydroxide (NH₄OH), 20 mL of carbon disulfide (CS₂) were added gradually for 15 minutes with stirring. The solution was left for an hour, then added. To the mixture 40 ml of aqueous hydrazine (N₂H₄.H₂O) 80%, then the mixture was cooled in an ice bath, where a white precipitate was obtained, filtered and recrystallized from a mixture of ethanol and water at a ratio of (1:3) ⁽⁴⁸⁾ as in Figure (3), Table (3) notes. Preparation Equation

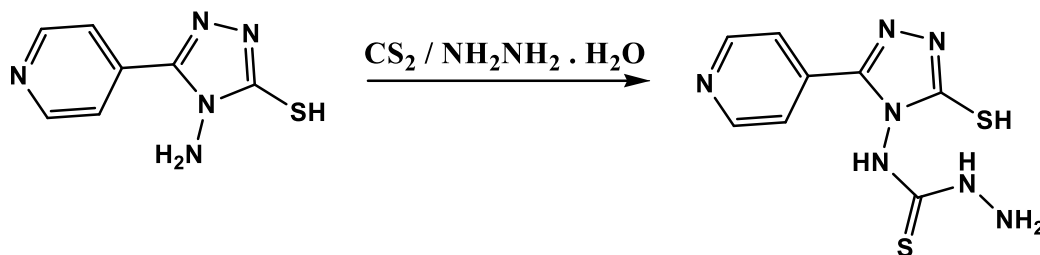


Table (3): Physical properties of the prepared compound (A1).

Com p. No	Molecular Formula	M.P (C)	Yield (%)	Color	Solvent
A1	C ₈ H ₉ N ₇ S	120-123	85	Light yellow	Dioxane



Figure(3)

2-1-4-4-Preparation 3-(pyridine)-[4,2,1,1]triazolol[3,4,b][1,3,4][thiadiazole-6-amine (A₂)

Mix 0.001 mol of the prepared derivative (A1) with (0.001 mol (0.149 g) of 4-Diamino

benzaldehyde in a suitable heat-resistant beaker by adding a drop of glacial acetic acid. The mixture was heated slowly and slowly for (5-10) minutes at the melting point. With stirring and mixing well until the nature of the molten reactants change in terms of color and texture. The product was collected and recrystallized from absolute ethanol⁽⁴⁹⁾ as in Figure (4), note the table (4). Preparation equation.

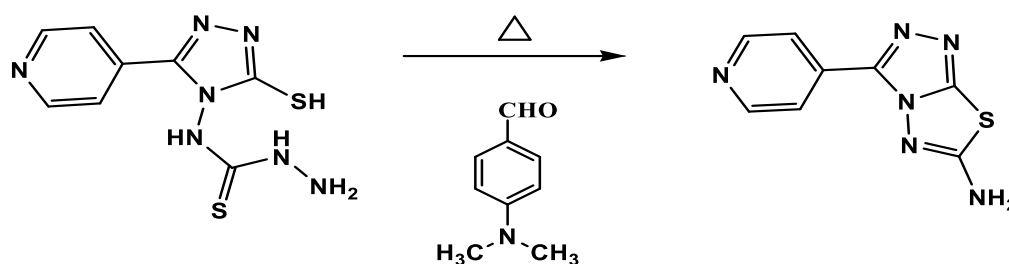


Table (4): Physical properties of the prepared compounds

Compound No	Molecular Formula	M.P (C)	Yield (%)	Color	Solvent
A ₂	C ₁₇ H ₁₈ N ₈ S	150-152	82	Orange	EtOH



Figure (4)

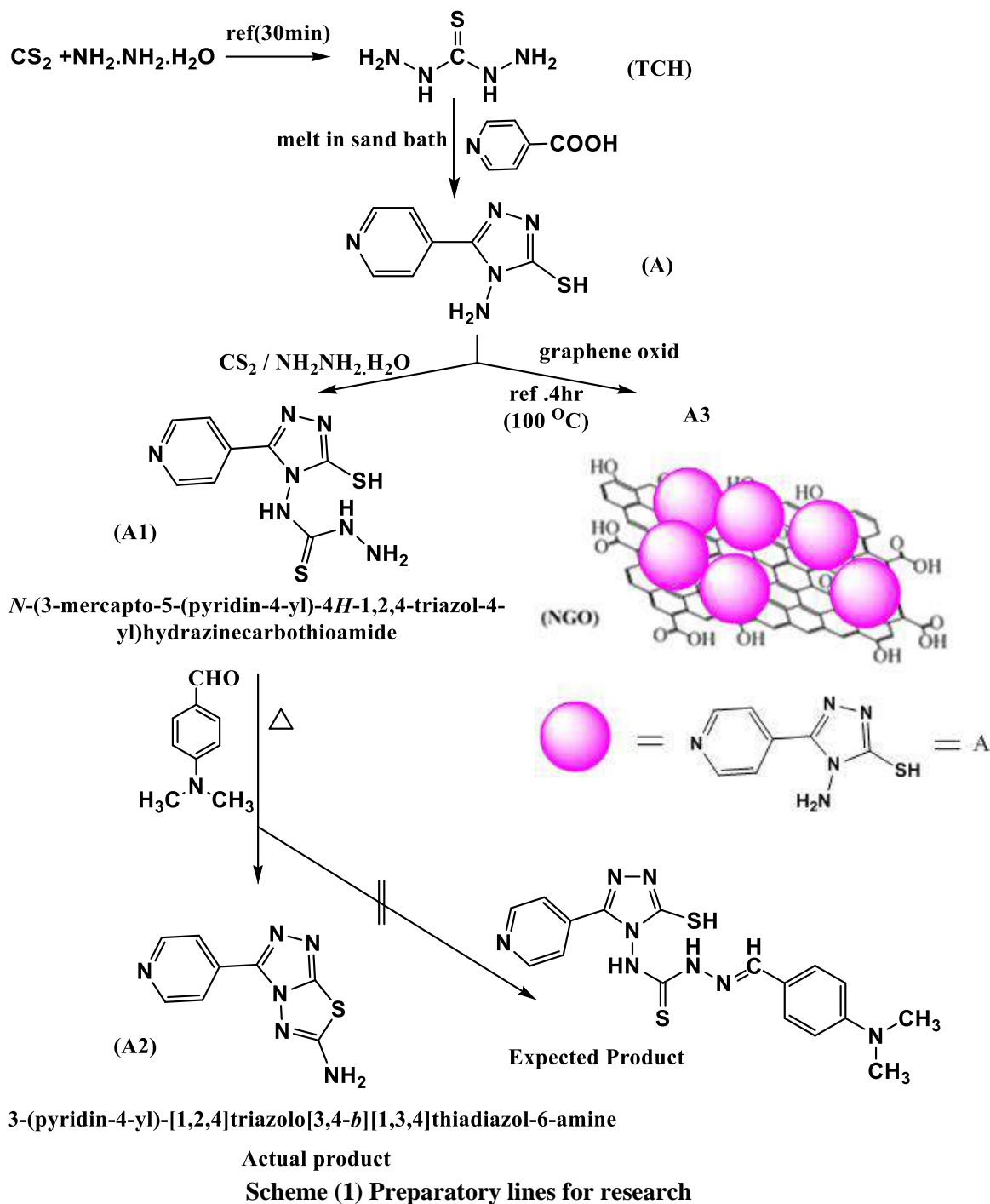
2-1-4-5-Preparation of graphene oxide GO by Hammer method

In an ice bath, a 600 ml beaker was placed and 46 ml of concentrated sulfuric acid (H_2SO_4) was added to it with continuous magnetic stirring and at $0^\circ C$, 5.1 g of sodium nitrate was gradually added with stirring, and after 15 minutes, 1 g of graphite was added during a period of 10 minutes, and then 6 g of potassium permanganate was added to the mixture. Slowly and carefully, gradually over 10 minutes, while maintaining the temperature below $10^\circ C$ for two hours, and then raising the mixture from the ice bath. Then, 46ml of distilled water was added drop by drop, stirring for 15 minutes, raising the temperature to $98^\circ C$, then adding 140ml of warm distilled water with a lower temperature. from 50 m and leave to stir for 10 minutes, then add 15 ml of hydrogen peroxide H_2O_2 with a concentration of 30% with stirring for 30 minutes and then divide the mixture into two parts and add 150 ml of distilled water to each part and leave to precipitate for 24 hours pour and wash with (HCl) 10% one time and five times In non-ionic water until reaching the acidic function $PH = 7$ and dried at a temperature of $(60-70) C$ ⁽⁵⁰⁾.

2-1-4-6-Preparation of nanocomposites 3- Thiol-4-amino-1,2,4-triazole/graphene oxide (NGO)

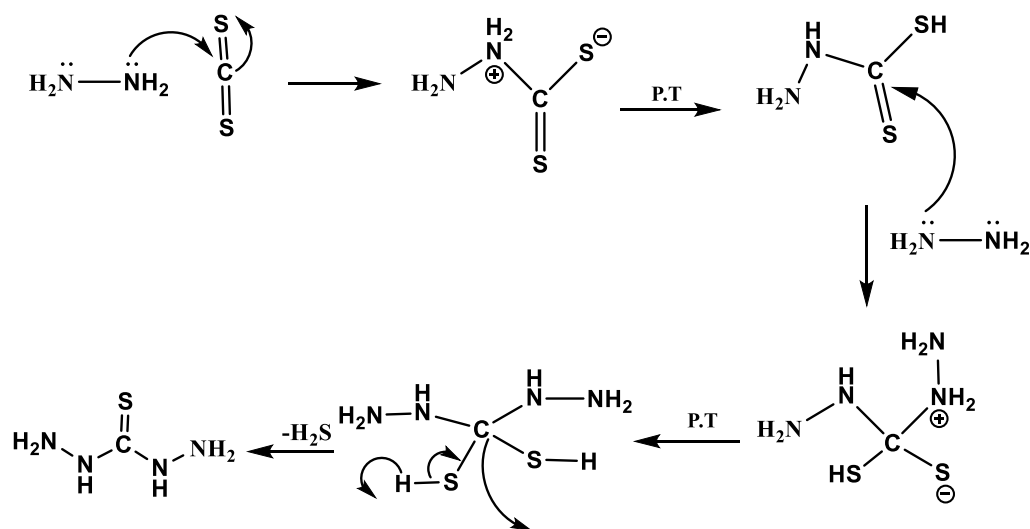
1 g of the prepared graphene oxide was dissolved in 15 ml of dioxane. The solution was homogenized by ultrasound until the aqueous became clear, then 1 g of triazole was added in an ice bath at $0C$, escalated for 30 minutes with magnetic stirring, then the precipitate was filtered, washed with distilled water and dried at $70C$ ⁽⁵¹⁾.

Results and discussion

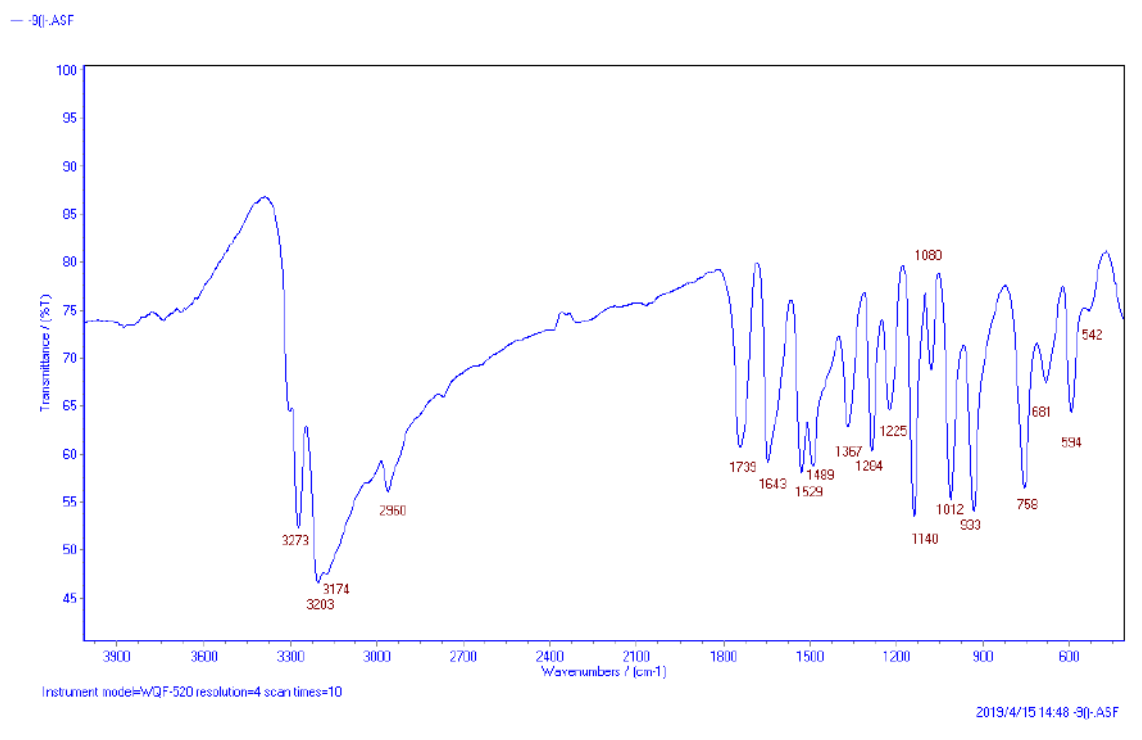


Characterization of thiocarbonylhydrazide TCH

Thiocarbonylhydrazide was prepared by heating a mixture of carbon disulfide and aqueous hydrazine, as distinct crystals were obtained with a yield of 66% according to the following mechanism⁽⁵²⁾.



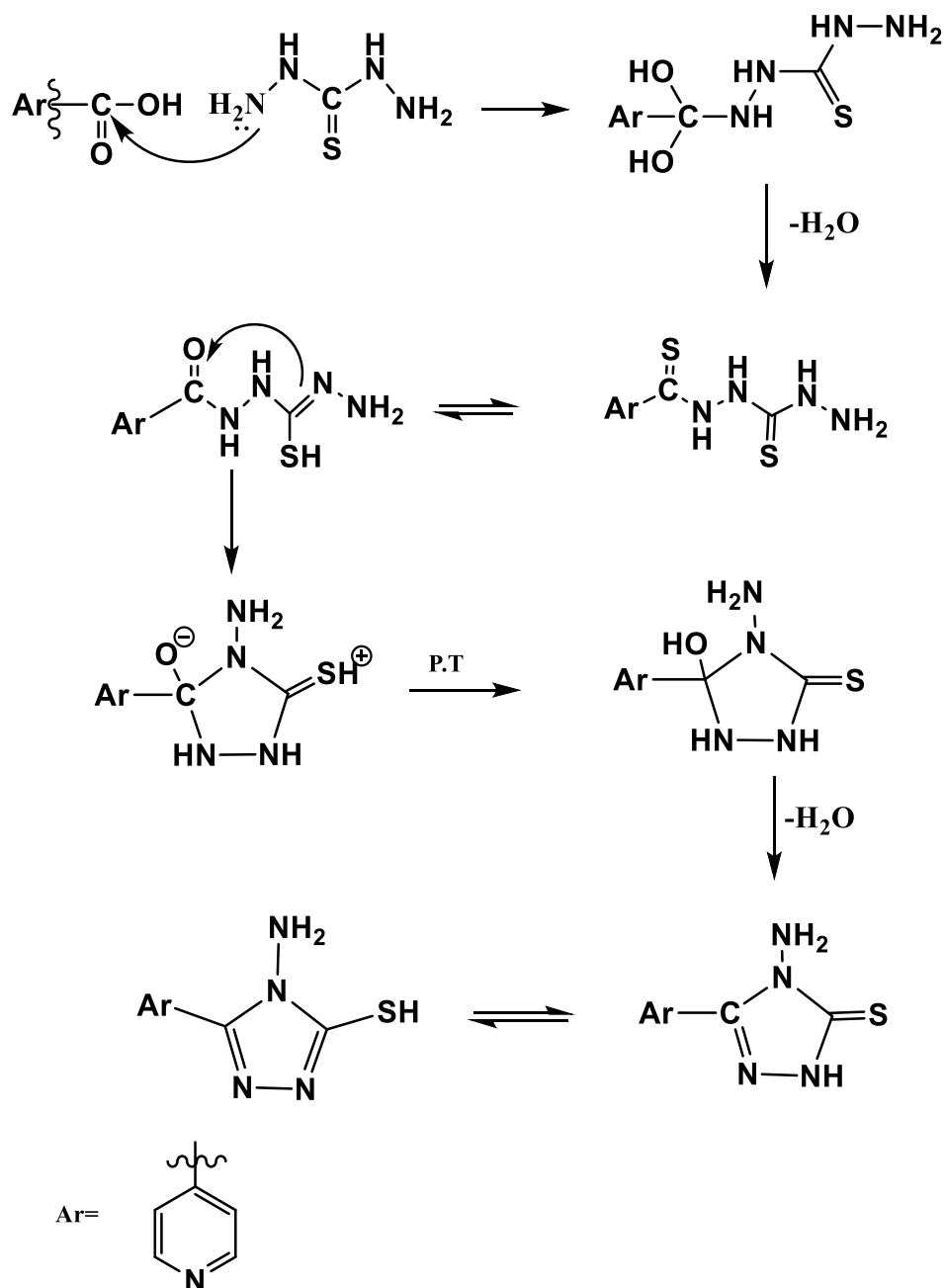
Some physical properties such as color and melting point were fixed, and the infrared spectrum of TCH showed a band at $(1284) \text{ cm}^{-1}$ due to the bond stretching ($\nu\text{C}=\text{S}$) in addition to the symmetric and asymmetric stretching frequency of the primary amine group (νNH_2) at $(3174, 3203) \text{ cm}^{-1}$, respectively, either the secondary amine band (νNH) appeared at $(3273) \text{ cm}^{-1}$, with the appearance of a stretching band ($\nu\text{C}-\text{N}$) at $(1529) \text{ cm}^{-1}$, as in Figure (1)



Figure(1)Infrared (FT-IR) spectrum of TCH

Characterization of the compound 4-amino-5-pyridin-4-yl-4hydrogbin-4,2,1-triazole-3-thiol (A).

The compound was prepared from thermal melting from the reaction of TCH with monocarboxylic acid, as it was observed that the physical properties of the product changed, such as color change and melting point, as the reaction proceeds according to the following mechanism⁽⁵³⁾.



The red spectrum showed two stretching and asymmetrical bands of the primary aromatic amine group (NH_2) at $(3222, 3267) \text{ cm}^{-1}$, and the stretching of the bond ($\nu\text{C}=\text{N}$) showed a band at $(1647) \text{ cm}^{-1}$, with a band appearing at $(1500) \text{ cm}^{-1}$ returns to ($\nu\text{C}=\text{C}$) of the aromatic ring of bardine, and the appearance of a band at the range $(952) \text{ cm}^{-1}$ back to ($\nu\text{C}-\text{S}$).

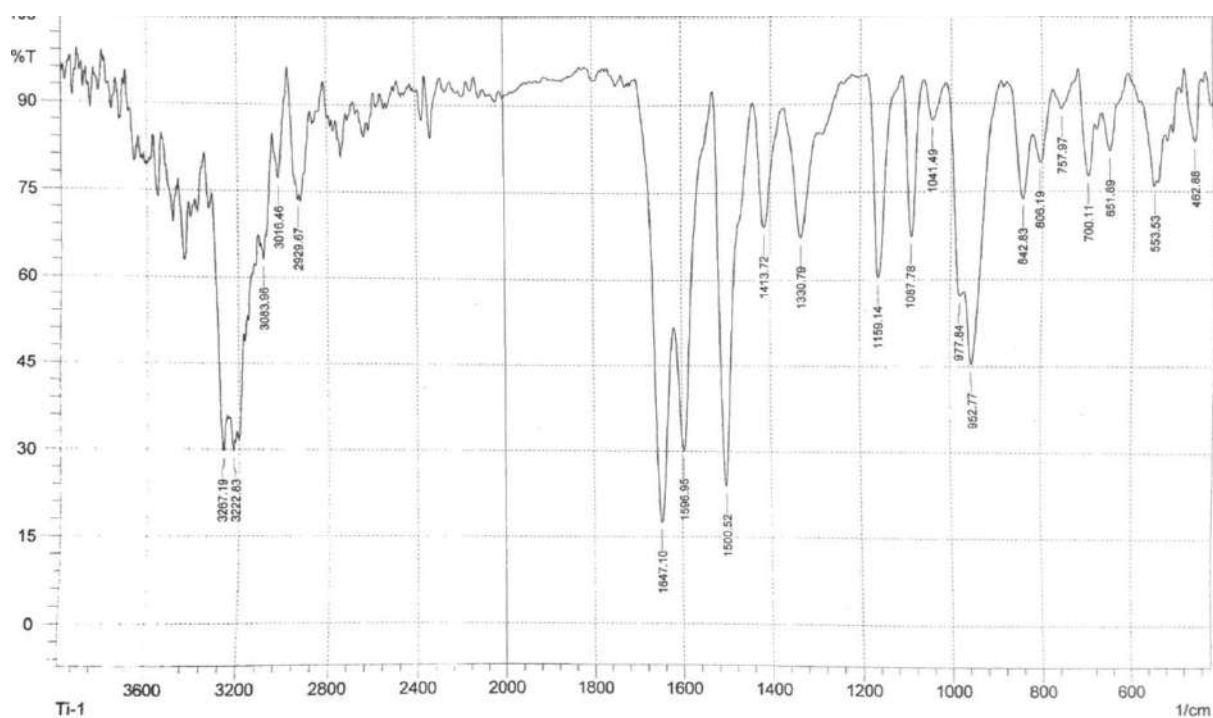


Figure (2) FT-IR spectrum of compound A

Characterization of N-(3-mercapto-5-(pri-yl)-4,2,1-triazole-4-yl)hydrazine carbothiamide (A1)

This compound was prepared by the reaction of triazole (A) with carbon disulfide and aqueous hydrazine(80%).

The red spectrum showed two stretching and asymmetrical bands of the primary aromatic amine group (NH₂) at (3415,3404) cm⁻¹, and the stretching of the bond (νC=N) showed a band at (1595) cm⁻¹, with a band appearing at (1515) cm⁻¹ returns to (νC=C) of the aromatic ring of bardine, and the appearance of a band at the range (1348) cm⁻¹ back to (VC=S).

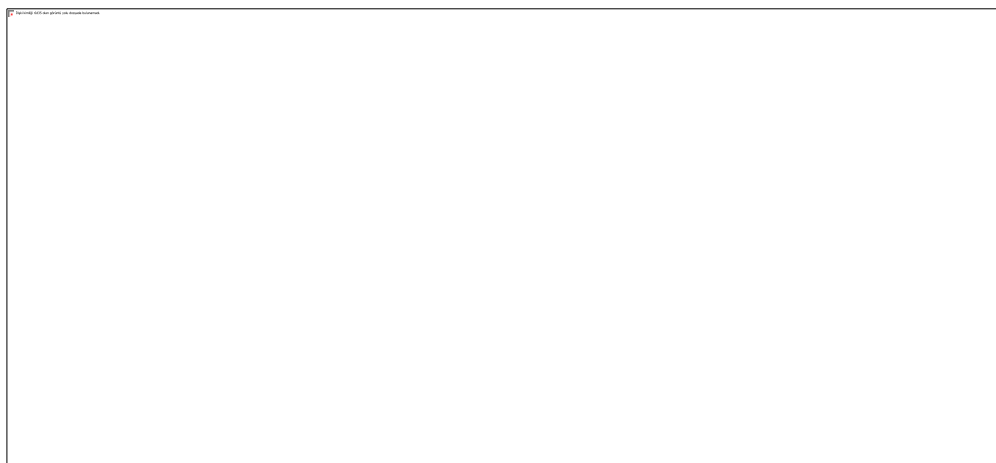


Figure (3) FT-IR spectrum of compound A1

Characterization of the compound 3-(pyridine)-[4,2,1,1]triazolo[4,3]-4,3,1][b[thiadiazole-6-amine (A2).

The infrared (FT-IR) spectrum showed two symmetrical and asymmetric stretching bands for the (NH₂) group within the range (3292-3388) cm⁻¹, as well as the appearance of a band stretching band (C=N) within (1604) cm⁻¹, with the appearance of Two stretch bands within the range (1488,1519)cm⁻¹ belong to the (C=C) spincter.

The nuclear magnetic resonance spectrum of the isotope carbon 13 (C¹³-NMR) was studied using the solvent DMSO-d₆ in tetramethylsilane (Tetramethylsilane: TMS) as an internal reference. The unexpected and resulting signals appeared from the potential thermal decomposition of the expected compound according to Scheme (1), where three signs were attributed to the pyridine ring, which are according to Figure (3) with the numbers (C3, C2, C1) each according to its effect on the electrical negative of the pyridine nitrogen atom that displaced C3 More than C1, C2 was affected by the isomethine group inside the combined triazole ring, and C4, C5, and C6 appeared according to their expected positions compared to the hypothetical spectrum, shifted more towards the low field by the effect of multiple heterogeneous atoms, and this interaction, which we did not find In the literature, what corresponds to and can be explained by the radical thermal decomposition during the thermal smelting reaction.

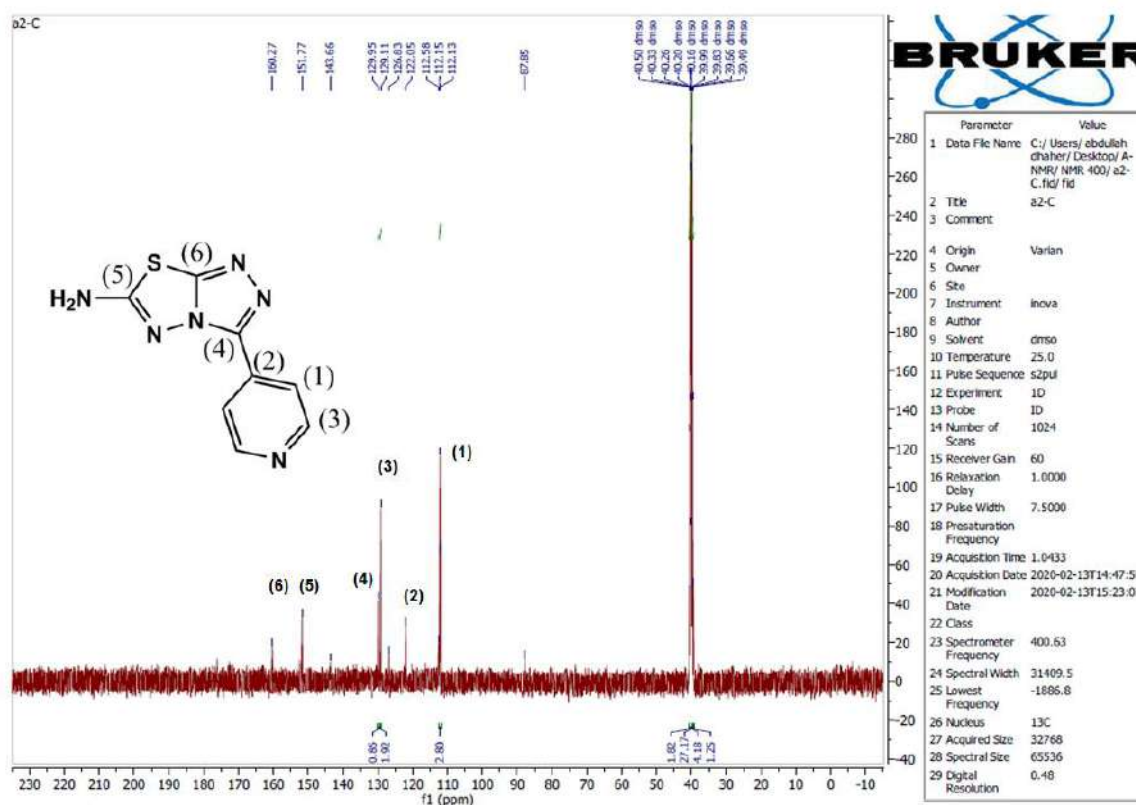
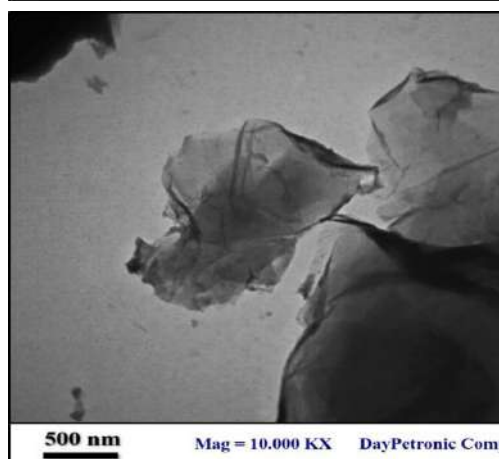
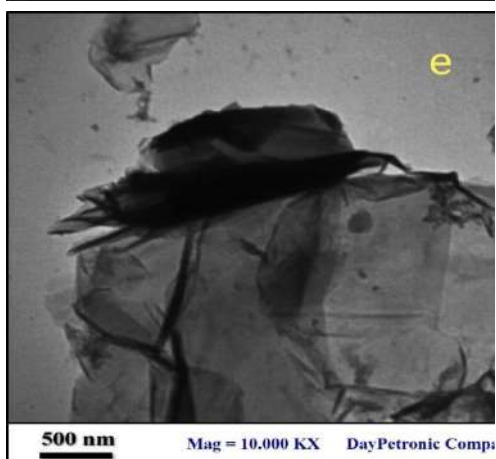
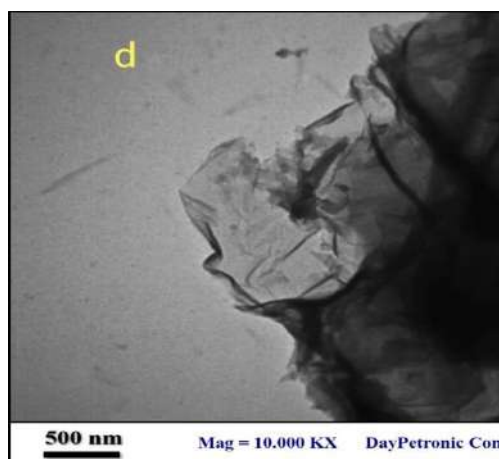
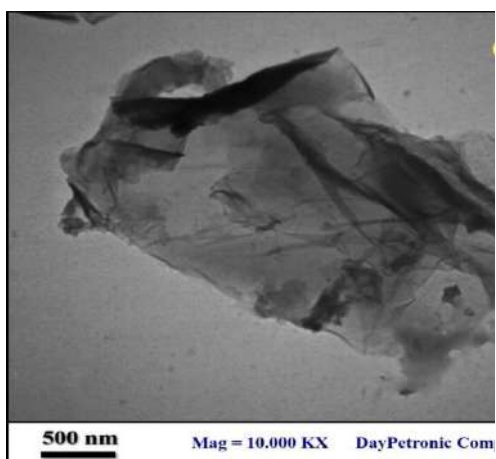
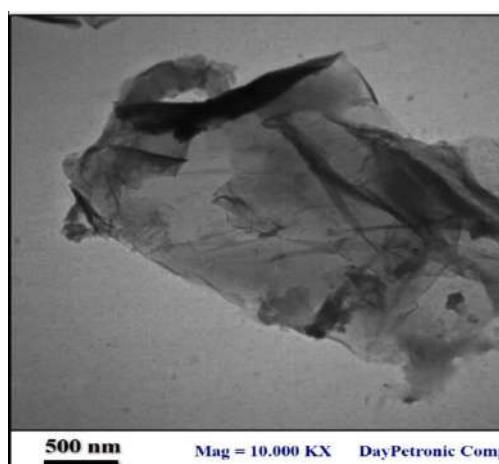
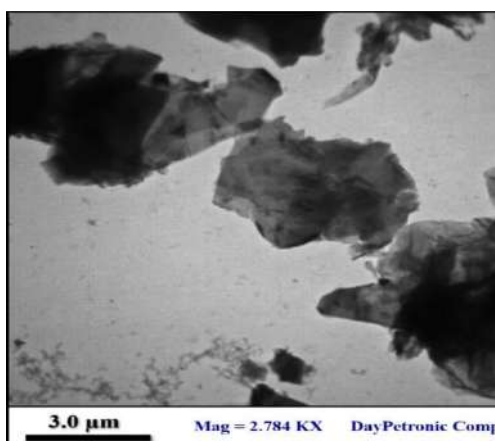


Figure (4) Nuclear resonance spectrum of the isotope carbon-13 for compound (A2).

Characterization of the compound 3-thiol-4-amino-1,2,4-trisol/graphene oxide (A3) (NGO) by transmission electron microscopy (TEM).

The transmission electron microscope images of the sample NGO showed that the clear crust remained on the layers even in the micro-images such as (a) and (b), and the nano-images showed the large flat area of the sheets with the presence of a layered grouping up to a three-layer (c) or monolayer (d) state. With clear nanoscale edges, high transparency for the layers (e) and (f), and the presence of some gaps on the surface (g), with an excellent spread of the compound material A on the surface and with nanoscale measurements up to 20 nanometers (h) with no concentration of the substance on the edges except partially. (c) and (i).



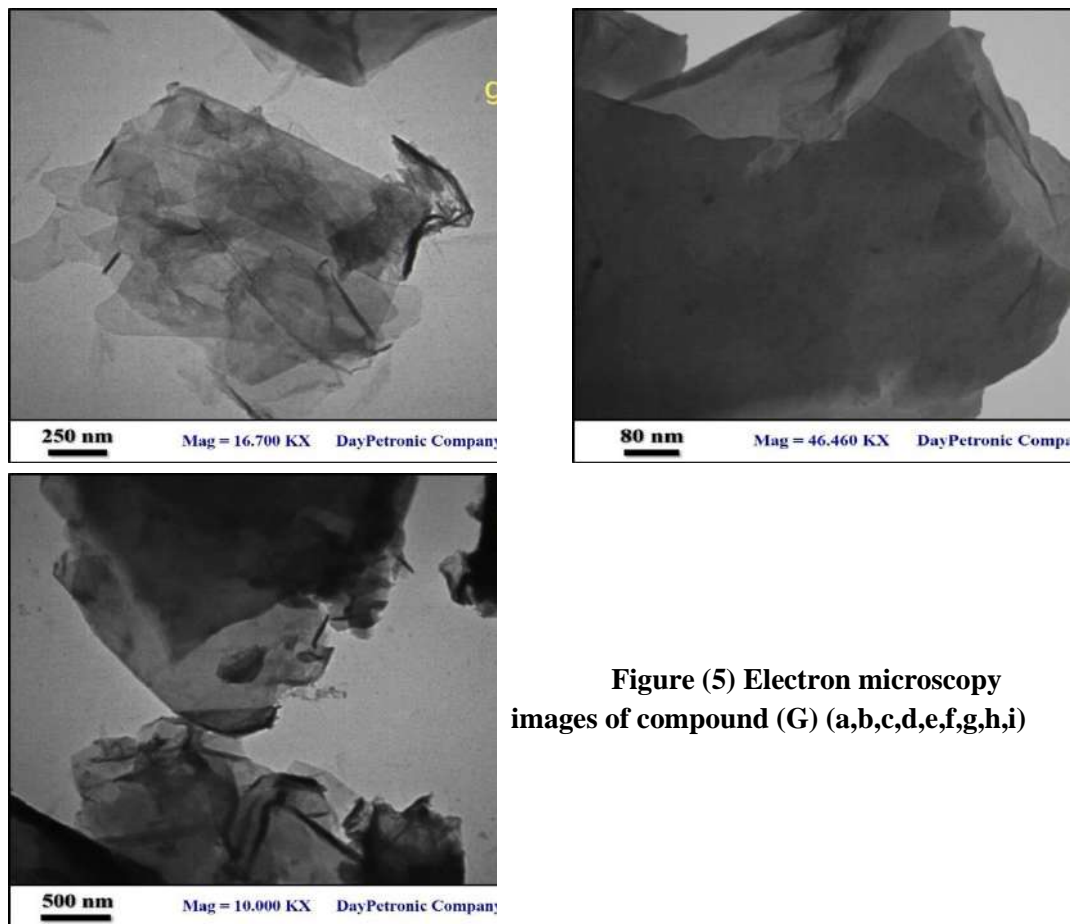


Figure (5) Electron microscopy images of compound (G) (a,b,c,d,e,f,g,h,i)

References

- [1]. Archer, "Stain films on silicon". *Journal of Physics and Chemistry of Solids* 14: 104. [Bibcode1960JPCS...14..104A](#). [doi:10.1016/0022-3697\(60\)90215-8R](#) (1960).
- [2]. C. Kittel, Introduction to Solid State Physics, 7th ed., John Wiley & Sons, Inc., New York, 1999
- [3]. Fei Wang and Akhlesh Lakhtakia. Selected Papers on Nanotechnology -- Theory & Modeling (Milestone Volume 182) . SPIE Press
- [4] Ekimov, A.I. & Onushchenko, A.A.. "Quantum size effect in three-dimensional microscopic semiconductor crystals". *JETPLett.* 34:345-349. [Bibcode](#)
- [5] Fritz Allhoff and Patrick Lin , *Nanotechnology & Society: Current and Emerging Ethical Issues* (Dordrecht: Springer, 2008).
- [6] William Sims Bainbridge: (*Nanoconvergence The Unity of Nanoscience, Biotechnology, Information Technology and Cognitive Science* , June P 27(2007)
- [7]. Fritz Allhoff, Patrick Lin, James Moor, and John Weckert ,(*Nanoethics: The Ethical and Societal Implications of Nanotechnology*) (Hoboken: John Wiley & Sons, 2007).
- [8] J. Clarence Davies, EPA and Nanotechnology: Oversight for the 21st Century, *Project on Emerging Nanotechnologies* , PEN 9, May 2007.
- [9]. Lynn E. Foster: *Nanotechnology: Science, Innovation, and Opportunity* , December 21, Prentice Hall, ISBN 0-13-192756-6 (2005).
- [10]. Hari Singh Nalwa, *Encyclopedia of Nanoscience and Nanotechnology*, American Scientific Publishers (1991)
- [11]. Michael Rieth and Wolfram Schommers, *Handbook of Theoretical and Computational Nanotechnology* (10-Volume Set), American Scientific Publishers. (2006).
- [12]. Jumana Boussey, Georges Kamarinos, Laurent Montès, (*Towards Nanotechnology*), "Nano et Micro Technologies", Hermes Sciences Publ., Paris, (2003) .
- [13]. A. H. Al-Agha, I. A. Latif, *DIYALA JOURNAL FOR PURE SCIENCES*. Vol: 12 No:3 , July(2016).
- [14]. S. Alam, N. Sharma, L. Kumar, Synthesis of Graphene Oxide (GO) by Modified Hummers Method and Its Thermal Reduction to Obtain Reduced Graphene Oxide (rGO). *Graphene*, 6 , 1- 2 (2017).
- [15]. L. Shahriary, and A. Athawale, Graphene oxide synthesized by using modified hummers approach. *International Journal. Renew. Energy Environmental Engineering* , 2, 58-63, (2014).
- [16]. W. S. Hummers, R. E. Offeman, *Journal Of The American Chemical Society*, 80, 1339, (1958).
- [16]. B. C. Brodie, *Justus Liebigs Annalen der Chemie*, 114(1), 6-24 (1860).
- [17]. B. C. Brodie, *Philos. Trans. R. Soc. London* , 149, 249, (1859).
- [18]. A. Saini, A. Kumar, V. K. Anand, S. C. Sood, *International Journal of Engineering Trends and Technology (IJETT)*. Volume-40 Number-2 - October (2016)
- [19]. Ahmad. H. A. Seliman, *M.Sc. Thesis*, College of Education for pure sciences – University of Tikrit (2018).
- [20]. K. Y. Grieme, T. Sharma, M. Raje, H. King, *Org. Lett.*; 3:4185-4187, (2001).

- [21].M.A.P.Martins,A.F.C.Flores,andG.P.Bastos,A.Sinhorin,(2002).
- [22].H.G.Bonacorso,N.Zanatta,*TetrahedronLett.*41:293-297, (2000).
- [23].T.W.Newton,*Chem.Abst.*129,119078, (1998).
- [24].V.N.Niu,D.H.Y.Wang,G.X.A.Amslerk,Phantand,OrYs:InterScienceConference on Antimicrobial Agents and Chemotheraby43,Chicago,111., (2003).
- [25].F.V, Malik Manvi., B. K Nanjwad, S. Sanjiv and P. Pankaj., *Der Pharmacia letter.*, 2(1), 347-359,(2010)
- [26].A.D.Towns., *Dyes Pigments* , 42, pp. 3-28, (1999).
- [27].*Ind . Chem. . Soc .* ,LIX,558-561 (1982) .
- [28].A.Maslat,and,M.Talib.J.P harma Col.54,55(2002).
- [29].J.JpialaandH.L.yaleU:s.patent.Chem, Abstract.16,83,176(1964).
- [30].M.Z.Thani,M.S,C,Thesis Mustansiryra university(2002).
- [31]. R. K. Al-Shemary, A. M. Al-khazraji and A. N. Niseaf, *The Pharma Innovation Journal*, ISSN: 2277-7695, Vol. (5), No. (1), pp. 81-86 (2016).
- [32]. P. Kaur, R. K., M. Goswami , "A Review on Methods of Synthesis of 1,2,4- Triazole Derivatives." *International Research Journal of Pharmacy*, (2018).
- [33] Jawad K. Shneine, Y. H. A. "Chemistry of 1, 2, 4-Triazole: A Review Article" *International Journal of Science and Research*, (2016).
- [34] S. A. M. Abdullah, M.Sc. Thesis, College of Education for pure sciences University of Tikrit, (2015).
- [35] K. M. Daoud, M. Y. Saleh, S. S. Ismael, *DIYALA JOURNAL FOR PURE SCIENCES*. Vol: 13 No:3, July (2017).
- [36] Hanif, M., et al. "Synthesis, urease Inhibition, antioxidant and antibacterial studies of some 4-amino-5-aryl-3H-1, 2, 4-triazole-3-thiones and their 3, 6- disubstituted 1, 2, 4-triazolo [3, 4-b] 1, 3, 4-thiadiazole derivatives." 23(5): 854-860. (2012).
- [37] R. Vaghasiya, H. Ghodasara, P. Vachharajani, and V. Shah, "International Letters of Chemistry," *Physics and Astronomy*, vol. 8, pp. 30-37, (2014).
- [38] Al-Khuzai, et al. "Synthesis, characterization and evaluation antimicrobial activity of some new substituted 2-mercapto-3-phenyl-4 (3H)- quinazolinone." 55(2Supplement): 582-593, (2014).
- [39] G. D. Gupta, D. K. Jain , *Journal of Advanced Pharmacy Technology Research*, 6(3), pp.141-146 (2015).
- [40].Miszczyk, P., et al. "Reaction of 3-amino-1, 2, 4-triazole with diethyl phosphite and triethyl orthoformate: acid-base properties and antiosteoporotic activities of the products." 22(2): 254. (2017).
- [41] H. M. Mohammed, J. of University of Anbar for pure science, Vol.11:N0.2 (2017).
- [42] S. Sari, et al, "Synthesis and anticonvulsant screening of 1, 2, 4-triazole derivatives," *Pharmacological Reports*, vol. 70, pp. 1116-1123, (2018)
- [43] A. Thakur, P. S. G., P. k. Shukla, A. Verma and P. Pathak. "1,2,4-triazole scaffolds: recent advances and pharmacological applications." Department of Pharmaceutical chemistry, Faculty of Health Sciences, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Naini, Allahabad-211007, (2016).
- [44]. Süleymanoğlu, N., et al. "1, 2, 4-triazole derivative with Schiff base; thiol- thione tautomerism, DFT study and antileishmanial activity." 1150: 82-87. (2017).

- [45]. Namratha, B., et al. "1,2,4-Triazoles: synthetic strategies and pharmacological profiles." 8: 73-80. (2014)
- [46] M.Namvari and H.Namazi,"Sweet grapheme I: toward hydrophilic grapheme nanosheets Via click grafting alkyne-saccharides onto azid-functionalized graphene oxid,"Carbohydrate research, vol.396,pp.1-8,(2014)
- [47]H.N.Moorthy,U.B.Vittal,C.Karthikeyan,V.Thangapandian,A,Venkadachallam,and,Trivedi,' 'Synthesis,antifungal evaluation and in silico study of novel Schiff bases derived from 4-amino-5(3,5-dimethoxy-phenyl)-4H-1,2,4-triazol,"Arabian Journal of Chemistry, Vol.10,pp.S3244-S3239,(2017)
- [48].S.M.S.Shariar, M.Jesmin and M.Jesmin and M.Ali., International Letters of Chemistry physics and Astronomy , Vol , 26pp 53-61 (2014).
- [49] Hamid. M . S. Al-Jubori,Ph.D.Thesis, Collge of Education for pure sciences_University of Tikrit (2015)
- [50] L-shahriary and A.A.Athawale,"Graphene oxide synthesized by using modieied hummers approach ,"Int J Renew Energy Environ Eng,Vol.2,pp.58-63.(2014)
- [51] Chitara, B., et al. "Solution processed reduced grapheme oxide ultraviolet detector."99(11):113114.(2011).
- [52] O. Rasheed, "Aspects of organonitroen and organosulphur chemistry in synthesis and recognition, "University of Manchester.(2016)
- [53] K. P. Barot, K. S. Manna, and M. D. Ghate, "Design, synthesis and antimicrobial activities of some novel 1,2, 4-triazole-5-thion and 1, 3-thiazolan-4-one derivatives of benzimidazole,"Journal of Saudi Chemical Society, vol. 21, pp. S35-S43. (2017).



DETERMINATION OF BIOFILM FORMATION GENES USING PCR TECHNIQUE FOR STAPH. SPP. ISOLATIONS FROM WOUND AND BURN INFECTIONS IN BAQUBA CITY

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DETERMINATION OF BIOFILM FORMATION GENES USING PCR TECHNIQUE FOR STAPH. SPP. ISOLATIONS FROM WOUND AND BURN INFECTIONS IN BAQUBA CITY

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Abstract:

The bacteria *Staphylococcus aureus* has been discovered to be a major source of community and hospital-acquired infections. The production of *ica*-dependent biofilms is critical in the persistence of infections in hospitalized patients. Between November 2017 & April 2018, the current study was conducted at Teaching Baquba Hospital's Bacteriology Laboratory in Baquba City and the laboratory of microbiology and polymerase chain reaction (PCR) unit in the Biology Department / College of Science/ Diyala University (2018). Materials and methods: We obtained 13(17.3%) *Staph.aureus* isolates from 100 clinical specimens (burns, wounds, urine, and blood) after identified them. Following by employed Congo Red Agar(CRA) and tissue culture plate method (TCP) to detect Biofilm development in isolates, as well as a PCR assay and particular primers to determine the presence of the *icaA* & *icaD* genes. The results showed *ica A/D* were found in 69 % (9/13) of cases, *icaA* gene is present at 7 (53.8%) and the *icaD* gene at 2(15.3%) in *Staph.aureus* isolates. CRA method found biofilm generation in 6 (46%) of thirteen *Staph. aureus* isolates, while TCP detected biofilm creation in 10 (76%) isolates. When phenotypic approaches compared to the detection of the *icaA* and *icaD* genes, only 5 (71%) of the *icaA* genes were found to be positive by TCP, while only 2 (1%) of the *icaD* genes were found to be positive by TCP. In short: The findings show the significance of *S. aureus*' virulence factors in clinical samples for the *icaA* and *icaD* genes and the phenotypic biofilm formation variety. The creation of in vitro slime using the CRA approach is not necessarily consistent even when the *icaA* and *icaD* genes exist. Although certain isolates lack the genes *icaA* & *icaD*, the ability to generate biofilms highlights the importance of the further gene research, and the absence of the *icaA* and *icaD* genes, the capability from certain isolates to create biopolymers emphasises the need for continuous genetic study into *icas* caused by variations in the number of genes associated with biofilms. When comparing phenotypic techniques, TCP is still the best tool for the screening of biofilms. The aim of this research though is that the biofilm forming potential should be actually linked to the presence of *icaA* and *icaD* genes in *S. aureus* isolates.

Key words: Staphylococcus Aureus, Biofilm, IcaA and IcaD Genes, CRA, MTP.



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Introduction:

Staphylococcus aureus is a potent human pathogen; it is very much associated with nosocomial diseases and community-acquired diseases. [1]. Septicemia, meningitis, pneumonia, and endocarditis are among the illnesses that range in severity from mild to severe life-threatening infections (2, 3). Health resources as well as an increased risk of morbidity and mortality have been expounded by infection [4]. A few of the factors of virulence produced by *Staph. aureus* are biofilms, hemolysins, leukocidins, proteases, enterotoxins, exfoliative toxins and immunological modulatory factors. [5, 6]. *Staph. aureus'* ability to manufacture biofilm helps bacterial resistance and is considered relevant to chronic illness and antibiotic resistance [7, 8]. Biofilm is a microbial community where cells attach on a surface enveloped by a matrix of Extracellular Polymeric Substances (EPS). Biofilm formation regulates the creation of biofilm by the expression of polysaccharide intracellular adhesion (PIA) [9], which mediates cell-to-cell adhesion and it's the gene product of *ica* types (*ica A*, *ica B*, *ica C*) [10]. The intercellular adhesion (*ica*) locus consisting of the genes *ica A*, *ica B*, *ica C* encodes proteins mediating the synthesis of PIA and polysaccharide/adhesin PS/A in staphylococci species [11]. Among *icaA* and *icaD* were found to play a role in the formation of biofilm in *Staph. aureus* and *Staph. Epidermidis* [12]. The *icaA* gene encodes the N-acetylglucosaminyl transferase as an enzyme involved in the synthesis of N-acetylglucosamine oligomers from UDP-N-acetylglucosamine. Further, *icaD* has been reported to play a role in the maximal expression of N-acetylglucosaminyl transferase, leading to the phenotypic expression of the capsular polysaccharide. [13]. Our aim was to determine whether there was a link between the potential to form biofilms in clinical *Staph.aureus* *icaA* and *icaD* genes.

Materials and Methods

• Specimens & Study Design:

From November 2017 to April 2018, the current investigation was carried out in Teaching Baquba Hospital's Bacteriology and Microbiology laboratories, as well as the PCR unit in the Biology department of the College of Science at Diyala University (2018). We collected and identified 13 *Staph. aureus* isolates from 100 clinical specimens (burns, wounds, urine, and blood).

• Identifying *Staph aureus*

Blood Agar (BA), MacConkey Agar (MA) and Mannitol salt agar (MSA) were used in all the samples to inoculate and cultivate at 37°C for 24 hours. The isolates were identified as *S. aureus*. by standard microbiological methods such as biochemical, slide and coagulase tube tests [14 & 15]

• Congo red agar (CRA) technique for biofilm formation

The biofilm-producing isolates were screened using the Congo Red Agar (CRA) procedure described by [16]. Congo red agar medium was made with 37 g/l brain heart infusion broth (BHI), 10 g/l agar, 50 g/l sucrose, and 0.8 g/l Congo red. A Congo red staining solution was prepared and autoclaved separately from the other media components. The test organisms were placed on plates and cultivated for 24 to 48 hours at 37°C. Positive polysaccharide intracellular adhesion (PIA) strains produced black colonies, while negative PIA strains produced red colonies.

• Biofilm formation detection by tissue culture plate method (TCP)

The TCP test is widely used and is deemed a standard technique for detecting biofilms. The TCP approach suggested [17] indicates that all isolates are able, according to [18], to make a biofilm with an incubation time change to 24 hours. Isolates from fresh agar plates were inoculated in trypticase soy broth with 1% glucose and incubated for 24 hours at 37°C in stationary condition and diluted (1 in 100) with fresh medium.

Individual wells of sterile, polystyrene, flat-bottom tissue culture plates were filled with 0.2 ml aliquots of the diluted cultures, and only broth served as a control to check sterility and non-specific binding of media. The tissue culture plates were incubated for 24 hours at 37°C

When the plates were tapped after incubation, the contents of each well were carefully extracted. The wells were cleansed four times with 0.2 ml of phosphate buffer saline (PBS) with pH(7.2) to remove free-floating planktonic bacteria in the wells.

Each well was then filled with a further 25microlitres of a 1 % crystal violet solution. For 15 minutes, the plates were completely and gently washed with water at room temperature. Adherent cells, which usually formed biofilm on all side wells, had been uniformly coloured with violet crystal. The crystal violet films were solubilized in 95 percent ethanol for 200 microliters(to extract violet color), and 125 microliters were transferred to a new polystyrene microtiter dish and read . Optical densities (OD) of stained adherent bacteria were determined with a micro ELISA auto reader (model 680, Bio-rad), and the wavelength of values was considered as an index of bacteria adhering to surface and forming biofilms. Experiments for each strain were performed in triplicate and repeated three times. To compensate for background absorbance, OD readings of wells with ethanol were used as blank and subtracted from all tests' values. Biofilm production is considered high, moderate, or weak 570 nm (OD570 nm) as shown in Table- 1.

Table 1. Classification of bacterial adherence according to the TCP method

<i>Biofilm formation</i>	<i>Adherence</i>	<i>Mean OD values</i>
<i>Non/weak</i>	<i>Non/weak</i>	<i>< 0.120</i>
<i>Moderate</i>	<i>Moderately</i>	<i>0.120 - 0.240</i>
<i>High</i>	<i>Strong</i>	<i>> 0.240</i>

• DNA extraction from *Staph. aureus* isolates:

The PCR genomic DNA template was extracted from Luria-Bertani broth bacterial isolates that had grown overnight. DNA was extracted by a certain modification using the method Cetyltrimethyl Ammonium Bromide (CTAB)[19]. About 1 ml of bacterial suspension was centrifuged and the supernatant was discarded. The pellet was suspended in Tris-Ethylene Diamine Tetra Acetic acid (EDTA) buffer (TE buffer). Then the lysis of the cell wall and proteins was done by adding 10% Sodium dodecyl sulfate (SDS) and 20 mg/ml proteinase K respectively followed by incubation at 37°C for up to 1 hour. After incubation, 5M sodium chloride (NaCl) solution followed by CTAB/NaCl (2% CTAB/0.7M NaCl) were added and incubated at 37°C for 10 minutes to remove proteins and polysaccharide complexes. An again equal volume of chloroform: isoamyl propanol (C: I) in the ratio (24:1) was added to the suspension to separate DNA from proteins and other cellular components. Then, an upper aqueous solution containing DNA was transferred to a new Eppendorf tube after centrifugation. A further pure form of DNA was obtained by treating the solution with isopropanol followed by 70% ethanol. Thus, obtained DNA was air-dried and re-suspended in 50µl of TE buffer.

• Detection of *icaA* and *icaD* genes by PCR

Polymerase chain reaction (PCR) employing forward and reverse primers was discovered in *icaA* and *icaD* genes (Table 2). The PCR reaction mixture (25µl) contained 12.5µl Master mix (MgCl₂, dNTPs, PCR buffer, and Taq polymerase), 2µl of DNA sample, 0.5µl of each primer, and 9.5µl sterile deionized distilled water. Initial PCR settings were initial dematuration (94°C for 5 minutes), followed by 30 cycles (30 seconds for 94°C), annealing (30 seconds for 55°C) and extension (45 seconds for 72°C) and then finishing cycling (72°C for 7 minutes)[13]. The amplified PCR product, together with the ladder, was passed over a 2 percent agarose gel stained with ethidium bromide to separate the PCR products (EtBr). The gel was viewed and photographed using a gel documentation system (UVTEC Cambridge, UK).

Table -2 indicates the primers used in this project

Primer	Oligonucleotide Sequence (5' - 3')	Size of Product, bp	Temperature of Annealing	Reference
<i>icaA</i> -F	TCTCTTGCAGGAGCAATCAA	188	55C	13
<i>icaA</i> -R	TCAGGCACTAACATCCAGCA			
<i>icaD</i> -F	ATGGTCAAGCCCAGACAGAG	198	55C	13
<i>icaD</i> -R	CGTGTTTTCAACATTTAATGCAA			

Analytical Statistics

Standard statistical procedures were used to calculate data frequencies and percentages, and Pearson's Chi-square test was used to compare them. The P-value was set at 0.05, which was statistically significant.

Results:

Seventy five (75%) of 100 samples showed positive growth, while 25 (25%) showed negative growth. In addition, 13 (17.3 %) of the samples tested positive for *S. aureus*, with the percentages of other bacteria listed in Table -3. The clinical samples used in this study (burns, wounds, urine, and blood) showed in Table -4

In addition, *Staph. aureus* was found in 17.3% (13/100) of the collections, with the percentages of other bacteria indicated in Table 3. The clinical samples utilised in this study are listed in Table 4. (burns, wounds, urine, and blood).

Table 3 shows the prevalence of several bacteria obtained from various patients

Microorganisms	Number	Percentage %
<i>Staphylococcus aureus</i>	13	17.3%
<i>Staphylococcus epidermidis</i>	11	14.6%
<i>Acinetobacterbaumannii</i>	8	10.6%
<i>Pseudomonas aeruginosae</i>	9	12%
<i>E. coli</i>	11	14.6%
<i>Proteus Vulgaris</i>	10	13.3%
<i>Proteus mirabilis</i>	4	5.3%
<i>Serratia marcescens</i>	6	8%
<i>Providencia rettgeri</i>	3	4%

Table 4 shows the distribution of Staphylococcus aureus isolates from various clinical sources

Source of samples	No. of <i>Staph.aureus</i>	Percentage%
Wounds	4	30%
Burns	4	30%
Urine	3	23%
Blood	2	15%
Total	13	100%

The CRA method found biofilm generation in 6 (46%) of 13 *S. aureus* isolates, while the microtiter plate assay TCP detected biofilm production in 10 (76%) isolates. among which 6(46.2%) were strong, 4(30.7%) were moderate and 3(23.1%) were biofilm nonproducers. In 46 percent of isolates, both approaches revealed a connection. In *Staph. aureus* isolates, the *icaA* gene was discovered in 7 (53%) and the *icaD* gene was detected in 2 (15%), according to genomic analysis. PCR findings are presented in figure (1). When phenotypic approaches were compared to the detection of the *icaA* and *icaD* genes, only 5 (71%) of the *icaA* genes were found to be positive by TCP, while only 2 (1%) of the *icaD* genes were found to be positive by TCP.

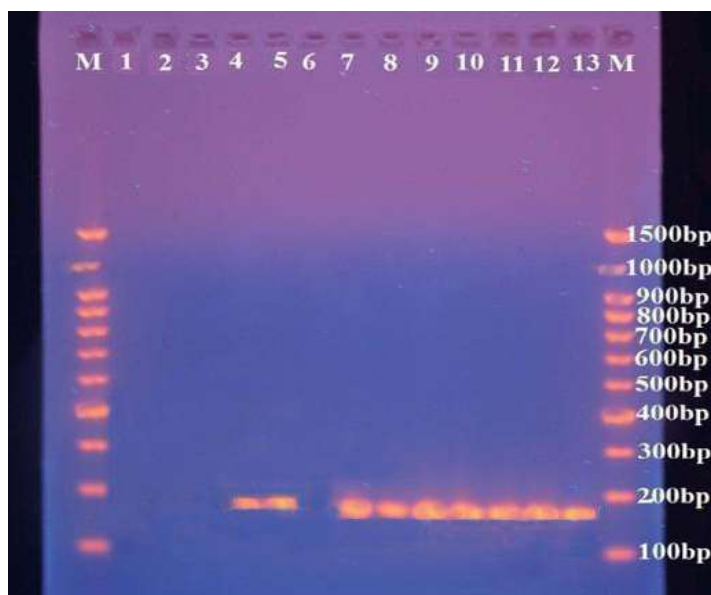


Figure 1: *icaA* and *icaD* primers were used to amplify isolated DNA from *S. aureus*. 100bp is M. Maker, Lanes 4, 5, show 198bp PCR products for the *icaA* gene. PCR products of 188bp for the *icaD* gene are shown in Lanes 7–13.

Discussion

Staph. aureus has a virulence component called biofilm development. The ability to create extracellular slime and form a biofilm is a crucial pathogenic feature, making clinical therapy extremely difficult. The *ica* locus, which is essential for the synthesis of Staphylococcus polysaccharide intracellular adhesion (PIA), has a function in cell to cell interactions during biofilm formation and is typically prevalent in clinical isolates [20].

The goal of this study was to find out how common the *icaA* and *icaD* genes are in *S. aureus* strains that can produce biofilms. In our investigation, 17.3 percent (13/75) of the samples revealed *Staph. aureus* growth, which is similar to Karki researcher and practically identical to Belbase who found 20.37 % *Staph. aureus* growth [21]. Other studies, however, found 51.52 % and 30.40 % of *Staph. aureus*, respectively, which were higher rates than the current study, possibly due to the large sample size and extended study duration [4, 22].

In this work, all *Staph. aureus* isolates were tested for biofilm generation using two methods (CRA and TCP), and then PCR was used to identify and validate biofilm forming bacteria by detecting the *icaA* and *icaD* genes. All biofilm-producing *Staph.aureus* isolates tested positive for the *icaA* or *icaD* genes using the TCP approach, however only 6 (46%) were recognised as biofilm producers using the CRA method. These findings are in close accordance with those of [23]. The production of biofilms is common among *Staph. aureus*. by TCP technique, was found to be 35.5 %, which contradicts our findings [24].

Another study found that 12 (30%) of 40 *Staph.aureus* isolates included the *icaA* gene, 8 (20%) isolates were positive for the *icaD* gene, and five (12.5%) isolates contained both genes [25].Mirzaee *et al.*,[26], discovered that twelve isolates (38.7%) were excellent biofilm producers. According to their findings, 18 (80.6 %) Staph . The *icaD* gene was found in 51.6 % of aureus isolates, while *icaA*, *icaB*, and *icaC* were found in 45.1 %, 45.1%, and 77.4 percent of isolates, respectively [16]. Gad *et al.* observed a greater rate of biofilm formation, with 83.3 % of *Staph.* The Media Transfer Protocol assay identified aureus obtained from urine catheterized individuals as the biofilm producer [17].

According to Nasr and colleagues, the presence of the *icaAD* gene in *Staph. aureus* isolates is not always associated with the development of slime or biofilm in vitro [7]. Cafiso and colleagues [24] discovered that 35% of *Staphylococcus spp.* possessed both the *icaA* and *icaD* genes, with others having only the *icaD* gene. The *icaA* and *icaD* genes were both part of one operon, according to Mahmoud Gad and associates [27], and the whole operon was either present or absent in all *Staphylococci* isolated from catheter segments, and all *Staphylococci* isolated from urine samples formed more biofilm.

Our research has a few drawbacks. First, we did not test *S. aureus* isolates for antibiotic sensitivity to detect antibiotic resistance, and additional genes implicated in the slime generation process were not tested in this work.

Conclusion

Finally, our findings demonstrate the importance of the *icaA* and *icaD* genes, as well as the phenotypic heterogeneity of biofilm formation, as virulence factors in *Staph. aureus*. clinical sources .It demonstrates the intricacy and diversity of biofilm regulating systems. Even when the *icaA* and *icaD* genes are present, in vitro slime formation using the CRA technique does not necessarily correspond. The ability of one isolate to generate biofilms in the absence of the *icaA* and *icaD* genes emphasises the importance of large genetic studies into *ica*-independent biofilm production processes and other *ica* genes. This could be the result of a difference in the way things are done.

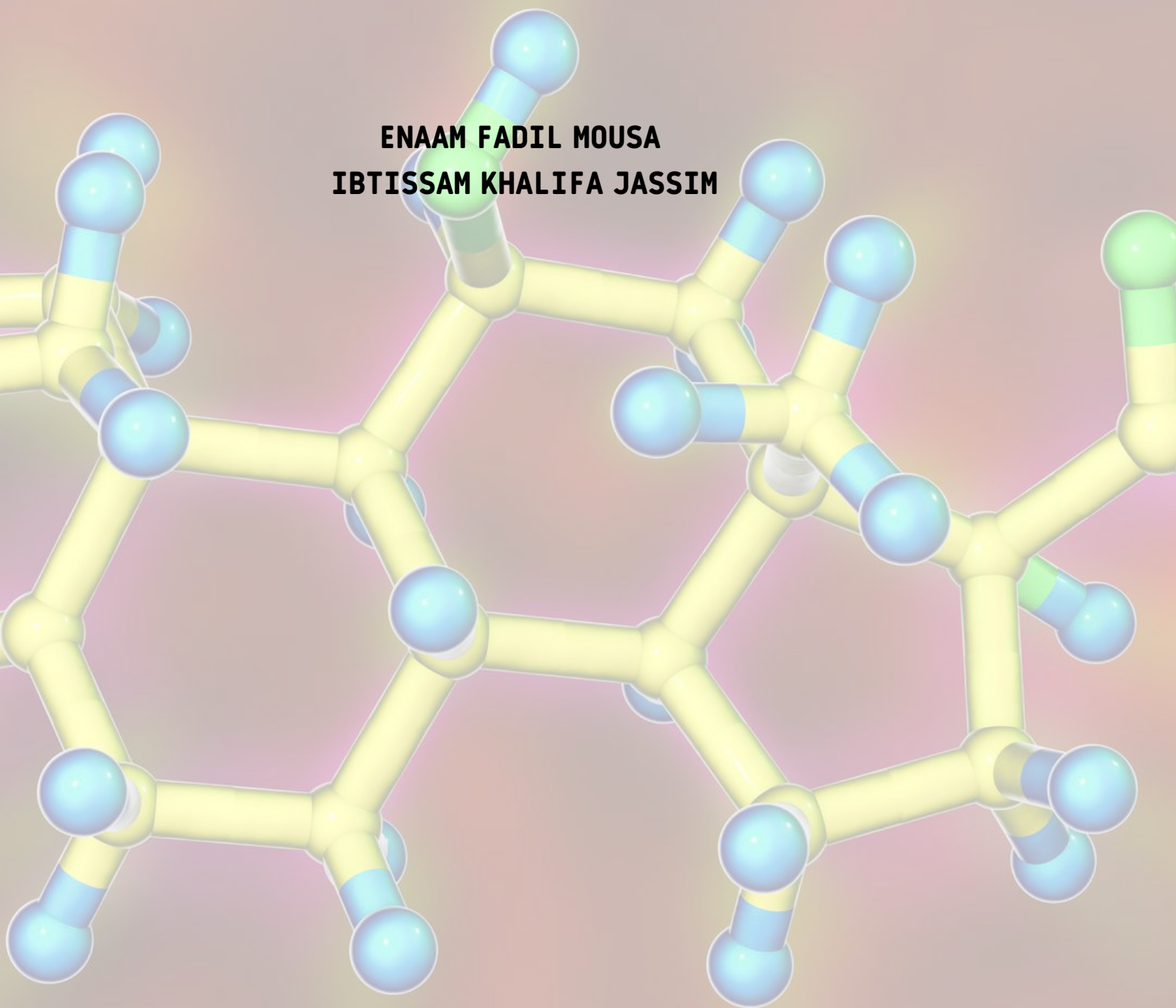
References

- [1] Rajbhandari, R., Manandhar, S. P., & Shrestha, J. (2003). Comparative study of MRSA and its Antibiotic Susceptibility pattern in indoor and outdoor Patients of Bir Hospital. *Nepalase J Microbiol*, 1, 62-5.
- [2] David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical microbiology reviews*, 23(3), 616-687
- [3] Popovich, K. J., & Hota, B. (2008). Treatment and prevention of community-associated methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections. *Dermatologic Therapy*, 21(3), 167-179.
- [4] Bhatta, D. R., Cavaco, L. M., Nath, G., Gaur, A., Gokhale, S., & Bhatta, D. R. (2015). Threat of multidrug resistant *Staphylococcus aureus* in Western Nepal. *Asian Pacific Journal of Tropical Disease*, 5(8), 617-621.
- [5] Oogai, Y., Matsuo, M., Hashimoto, M., Kato, F., Sugai, M., & Komatsuzawa, H. (2011). Expression of virulence factors by *Staphylococcus aureus* grown in serum. *Applied and environmental microbiology*, 77(22), 8097-8105.
- [6] DeLeo, F. R., Diep, B. A., & Otto, M. (2009). Host defense and pathogenesis in *Staphylococcus aureus* infections. *Infectious disease clinics of North America*, 23(1), 17-34.
- [7] Nasr, R. A., AbuShady, H. M., & Hussein, H. S. (2012). Biofilm formation and presence of *icaAD* gene in clinical isolates of staphylococci. *Egyptian journal of medical human genetics*, 13(3), 269-274.
- [8] Subramanian, P., Shanmugam, N., Sivaraman, U., Kumar, S., & Selvaraj, S. (2012). Antibiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. *The Australasian medical journal*, 5(7), 344.
- [9] Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: from the natural environment to infectious diseases. *Nature reviews microbiology*, 2(2), 95-108.
- [10] Ammendolia, M. G., Di Rosa, R., Montanaro, L., Arciola, C. R., & Baldassarri, L. (1999). Slime production and expression of the slime-associated antigen by staphylococcal clinical isolates. *Journal of clinical microbiology*, 37(10), 3235-3238.
- [11] O'GARA, J. P., & Humphreys, H. (2001). *Staphylococcus epidermidis* biofilms: importance and implications. *Journal of medical microbiology*, 50(7), 582-587.
- [12] Yazdani, R., Oshaghi, M., Havaei, A., Pishva, E., Salehi, R., Sadeghizadeh, M., & Forouhesh, H. (2006). Detection of *icaAD* gene and biofilm formation in *Staphylococcus aureus* isolates from wound infections. *Iranian J Publ Health*(2006) 35: 25-28.
- [13] Arciola, C. R., Baldassarri, L., & Montanaro, L. (2001). Presence of *icaA* and *icaD* genes and slime production in a collection of staphylococcal strains from catheter-associated infections. *Journal of clinical microbiology*, 39(6), 2151-2156.
- [14] Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries Part 2* Cambridge University Press. New York, 300-301.
- [15] Collee, J. G., Duguid, J. P., Fraser, A. G., Marmion, B. P., & Simmons, A. (1996). Laboratory strategy in the diagnosis of infective syndromes. *Mackie and McCartney practical medical microbiology*, 14, 53-94.
- [16] Freeman, D. J., Falkiner, F. R., & Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. *Journal of clinical pathology*, 42(8), 872-874.
- [17] Christensen, G. D., Simpson, W. A., Younger, J. J., Baddour, L. M., Barrett, F. F., Melton, D. M., & Beachey, E. H. (1985). Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *Journal of clinical microbiology*, 22(6), 996-1006.

- [18] O'Toole, G. A., & Kolter, R. (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. *Molecular microbiology*, 28(3), 449-461.
- [19] Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12, 13-15.
- [20] Ando, E., Monden, K., Mitsuhashi, R., Kariyama, R., & Kumon, H. (2004). Biofilm formation among methicillin-resistant *Staphylococcus aureus* isolates from patients with urinary tract infection. *Acta Medica Okayama*, 58(4), 207-214.
- [21] Belbase, A., Pant, N. D., Nepal, K., Neupane, B., Baidhya, R., Baidya, R., & Lekhak, B. (2017). Antibiotic resistance and biofilm production among the strains of *Staphylococcus aureus* isolated from pus/wound swab samples in a tertiary care hospital in Nepal. *Annals of clinical microbiology and antimicrobials*, 16(1), 1-5.
- [22] Acharya, J., Mishra, S. K., Kattel, H. P., Rijal, B., & Pokhrel, B. M. (2008). Bacteriology of wound infections among patients attending tribhuvan university teaching hospital, kathmandu, Nepal. *Journal of Nepal Association for Medical Laboratory Sciences P*, 76, 80.
- [23] Zalipour, M., Sedigh Ebrahim-Saraie, H., Sarvari, J., & Khashei, R. (2016). Detection of biofilm production capability and *icaA/D* genes among staphylococci isolates from Shiraz, Iran. *Jundishapur Journal of Microbiology*, 9(12).
- [24] Aboelnour, A., Sayed Zaki, M. E., & El-Kannishy, S. M. H. Kheir NAEI (2018) Molecular Study of Intracellular Adhesion Genes (*ica*) and Fibronectin Binding Protein Genes (*FnB*) in Clinical Isolates of *Staphylococcus aureus* Isolated from Patients Under Chemotherapy. *Int J Drug Dev & Res* 10: 8, 12, 8-12.
- [25] Keikhaie, K. R., Sargazi, A., Hassanshahian, M., & Shahi, Z. (2017). Detection of Intercellular Adhesion Genes (*icaA* and *icaD*) in *Staphylococcus aureus* Clinical Isolates in Zabol-Iran. *Res Mol Med*, 5(1), 40-43.
- [26] Cafiso, V., Bertuccio, T., Santagati, M., Campanile, F., Amicosante, G., Perilli, M. G., ... & Stefani, S. (2004). Presence of the *ica* operon in clinical isolates of *Staphylococcus epidermidis* and its role in biofilm production. *Clinical Microbiology and Infection*, 10(12), 1081-1088.
- [27] Gad, G. F. M., El-Feky, M. A., El-Rehewy, M. S., Hassan, M. A., Abolella, H., & Abd El-Baky, R. M. (2009). Detection of *icaA*, *icaD* genes and biofilm production by *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from urinary tract catheterized patients. *The Journal of Infection in Developing Countries*, 3(05), 342-351.

**SYNTHESIS , CHARACTERIZATION AND BIOLOGICAL ACTIVITY
STUDY OF SOME HETEROCYCLIC COMPOUNDS**

**ENAAM FADIL MOUSA
IBTISSAM KHALIFA JASSIM**



SYNTHESIS ,CHARACTERIZATION AND BIOLOGICAL ACTIVITY STUDY OF SOME HETEROCYCLIC COMPOUNDS

Enaam Fadil MOUSA ¹
Ibtissam Khalifa JASSIM ²

Abstract:

Heterocycles are an important class of organic compounds because of their applications in medicines and industrial fields. Therefore our study included preparation of these compound such as oxazepine and quinazoline rings, which were prepared through two steps: The first step included the reaction of the Schiff bases derived from sulfamethaxazole (1-4) with each of phthalic anhydride and 3- nitrophthalic anhydride for the preparation of oxazepines (5-12) .While the second step included the preparation of quinazoline compounds (13-16) from the reaction of Schiff bases (1-4) with anthranilic acid using dry benzene as a medium and solvent for the reaction. All prepared compounds were characterized by using infrared,proton- nuclear magnetic resonance, mass techniques and melting points, and their purity was determined by thin layer chromatography technique also screened the biological activity of some of these prepared compounds by using two types of bacteria Gram-positive and negative . The results showed that these compounds have a good inhibition against these organisms.

Key words: Heterocyclic compounds, 1,3-Oxazepine, Quinazoline, Antibacterial Activity.



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Introduction:

Heterocyclic systems are widespread occurrence in nature, especially in such natural products as nucleic acids, plant alkaloids and chlorophyll[1,2]. Heterocyclic compound is one which possesses a cyclic structure with at least two different of hetero atoms in the ring. Nitrogen, Oxygen and Sulphur are the most common heteroatoms. Heterocyclic compounds are very widely distributed in nature and essential to life in various ways. Most of the sugars and their derivatives, including vitamin C, for instance, exist in the form of five-membered (Furan) or six-membered (Pyran) rings containing one oxygen atom. Most members of vitamin B group possess heterocyclic ring containing nitrogen. One example is vitamin B6 (Pyridoxine), which is a derivative of Pyridine, essential in amino acid metabolism. [3-6].

Oxazepine is seven member heterocyclic compound ring that contains Oxygen and Nitrogen heteroatoms[7]. Oxazepine derivatives have diverse biological pharmacological activities [8] like enzyme inhibitors [9] analgesic [10] antidepressant [11] and psychoactive drugs [12,13].

Quinazolines are heterocyclic compounds with a ring two nitrogen atoms and four carbon atoms, which have been synthesized for their uses in medicine fields such as antimalarial and anticancer agent [14,15].

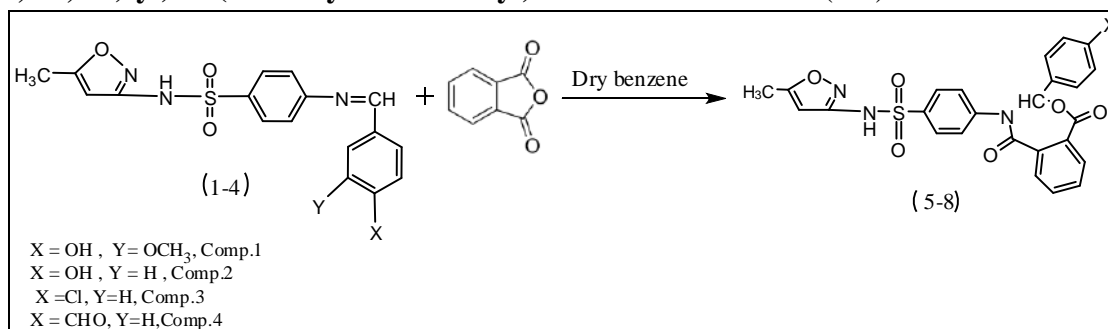
2. Materials and Methods

Melting Points (M.P.) were measured by a hot stage Gallenkamp Melting Point apparatus and they were uncorrected. Some of Infra Red Spectra were recorded by [F.T.I.R.] Fourier Transform infrared SHIMADZU 8300 Infrared Spectrophotometer. KBr disc or thin film were performed at Ibn Sina state company and others were registered by FTIR. SHIMADZU 8400 infra red spectrophotometer.

Spectra ¹H NMR were obtained with Fourier transform Varian Spectrometer at 400 MHz in Dimethyl sulfoxide (DMSO) solution with the Tetramethyl silane (TMS) as internal standard. Mass spectra were recorded using Mass Spectrometer, Agilent Technology (HP) at Tehran University, Central Lab, Iran. The biological activity study was tested at biology department, college of science - University of Baghdad.

2.1. Preparation methods

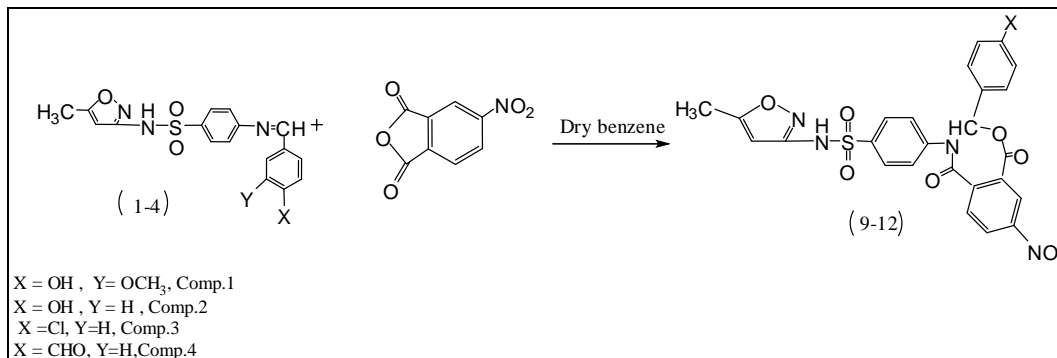
1- synthesis of 4-(3-(4-Substitutedphenyl)-1,5-Dioxobenz[e][1,3]Oxazepin-4(1H,3H,5H)-yl)-N-(5-Methylisoxazol-3-yl)Benzenesulfonamide (5-8)⁽¹⁶⁾



A mixture of (1-4) Schiff base at 0.0005 mol with phthalic anhydride (0.0005 mol, 0.08g) dissolved in dry benzene (15 mL), reflux the mixture at (18 -20) hrs, the mixture was evaporated to remove the excess of solvent. After that the mixture was filtered and purified

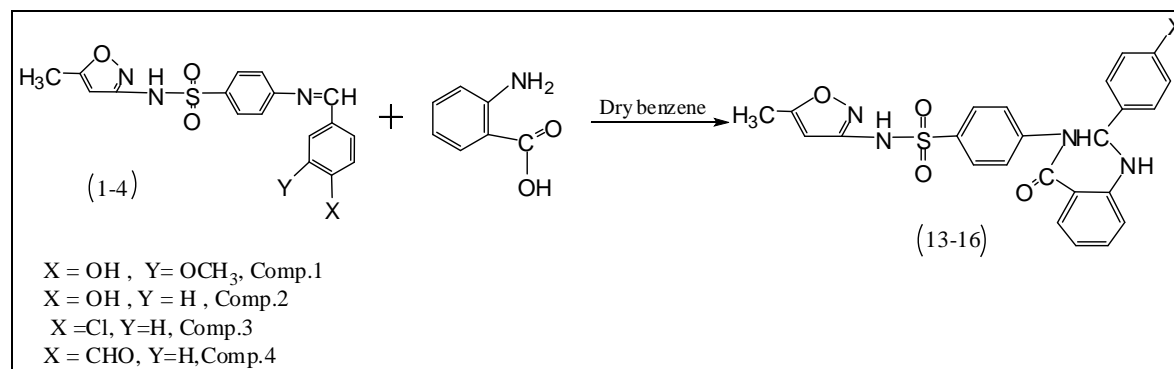
by crystallization process using ethanol to give product m.p.for (5) is (154-156⁰C), for (6) is (237-239⁰C), for(7) is (146-148⁰C) and for (8) is (240-242⁰C) while the yields are(74% ,76%, 70% and 73% respectively.

2- Preparation of 2-(4-substituted phenyl)-3- amino)N-(5-methylisoxazol-3-yl)benzenesulfonamide2,4-oxazepin-1,5-dione (9-12) ⁽¹⁶⁾



A mixture of (1-4) Schiff base at 0.0005mol with (0.0005mol,0.09g) of 3-nitrophthalic anhydride dissolved in dry benzene (15mL) reflux the mixture at (18-20) hrs, the mixture was evaporated to remove the excess of solvent. After that the mixture was filtered and purified by crystallization process using ethanol ,m.p.for (9) is (189-191⁰C),for (11) is (147-149⁰C) and for (12) is (176-178⁰C), while the yields are(75% ,71% ,70% and 72%) respectively.

3- Synthesis of 4-(2-(4-substituted phenyl)-4-oxo-1,2-dihydroquinazolin-3(4H)-yl)-N-(5-methylisoxazol-3-yl)benzenesulfonamide (13-16) ⁽¹⁷⁾



A mixture of (1-4) Schiff bases at 0.0005mol with anthranilic acid (0.0005 mol,0.07g) was refluxed for 20 hours in 15 ml Dry Benzene, then the solvent evaporated ,filtered off and recrystallized from ethanol, m.p. for(13) is (138-140⁰C), for (14) is (161-163⁰C) and for (16) is (111-113⁰C),while the yields are (71% , 74% , 74% and 72%) respectively.

3.Results and Discussion

3.1.Synthesis

In the current study series of heterocyclic compounds based on Schiff bases were synthesized using Schiff bases (1-4) as starting materials for the preparation. Physical properties for prepared compounds (5-16) are listed in table (1) .

1- Characterization of compounds (5-8)

Compounds (5-8) were prepared from refluxed the compounds 1-4 and Phthalic Anhydride in the solvent(Dry Benzene).These synthesized compounds received a satisfactory analysis of their proposed structures , which was confirmed using FT.IR 1HNMR and Mass Spectra.

The FT-IR spectra of compound (5-8) were confirmed from the appearance of carbonyl group(lactone) bands for at (1693,1720,1685 and 1693)⁽¹⁸⁾ and C-H aliphatic bands at[(2993,2839), (2939,2885), (2959,2893) and(2985,2848)] and bands at (1267, 1249 ,1269 and 1265 cm^{-1}) belongs to (C-O-C) band for these compounds respectively and hydroxyl groups at (3511 and 3537 cm^{-1}) for compounds (5) and (6) respectively and (798 cm^{-1}) band for (C-Cl) of compound (7)⁽¹⁸⁻²⁰⁾ .

The ¹ H-NMR spectrum of compound (5) exhibited the peak at δ (2.24) ppm due to (CH₃) while a singlet signal at δ (3.79) ppm due to (OCH₃), the singlet signal at δ (6.08) ppm. suggested the attribution of the proton of isoxazol and protons of aromatic rings appeared at (δ 6.53-7.87)ppm , proton of (NH) appeared at (δ 10.90) and proton of (OH) group appeared at (δ 9.73).Figure(1) the ¹ H-NMR spectrum of compound (4).The mass spectrum of this compound, Figure (2) , displayed the molecular ion at $m/z = 536.10$.

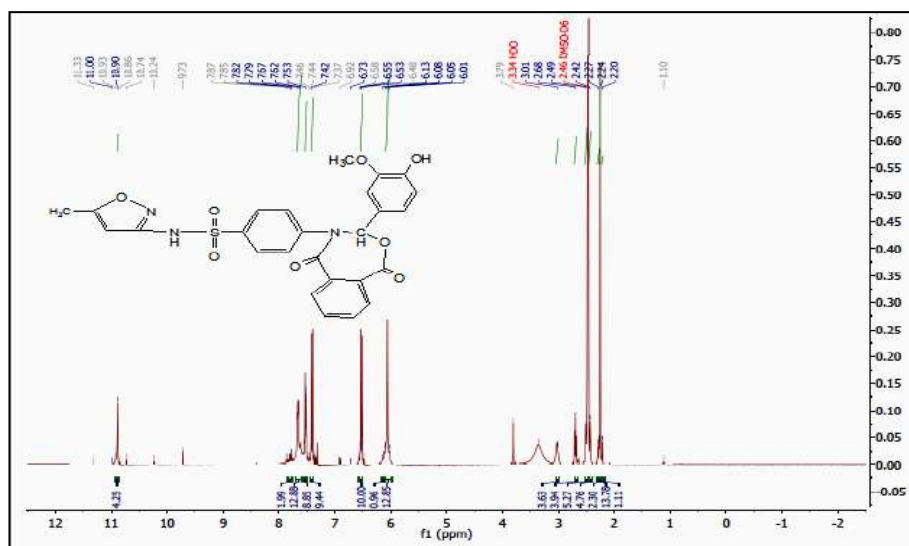


Fig 1 :¹H-NMR Spectrum of compound (5).

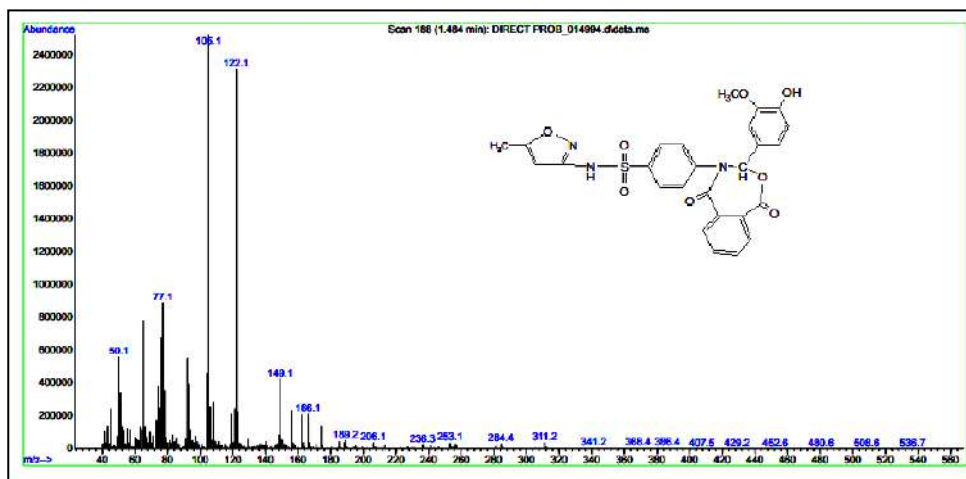


Fig 2: Mass Spectrum of compound (5).

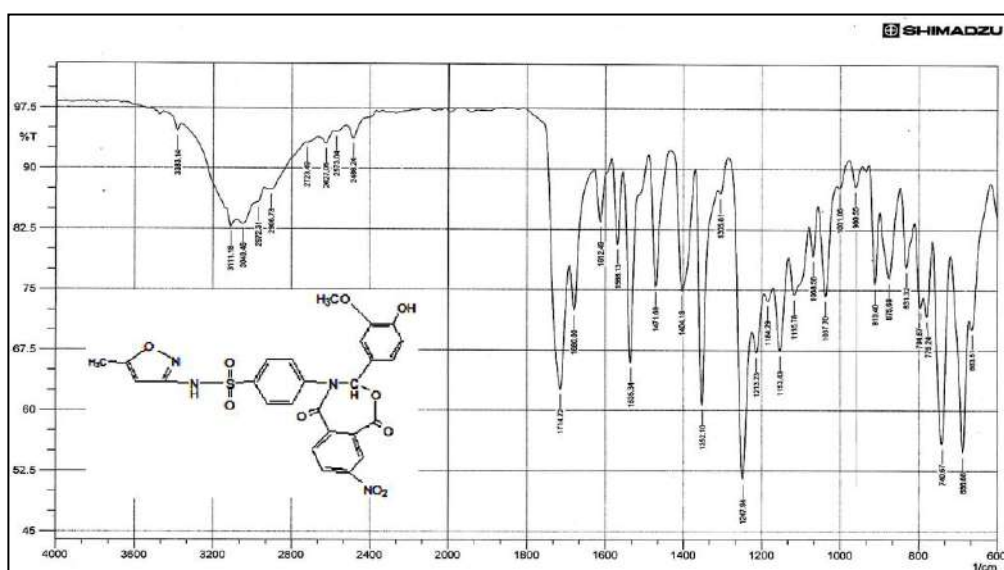
2- Characterization of compounds (9-12)

Compounds (9-12) were prepared from the reaction of the compounds (1-4) with 3-Nitro phthalic anhydride in dry benzene. These synthesized derivatives received a satisfactory analysis of their proposed structures, which was confirmed using FT.Ir, ¹HNMR and Mass Spectra.

FTIR Spectra for 9-12 derivatives were confirmed from the appearance of carbonyl group (lactam) bands at (1680, 1639, 1695 and 1681) and C-H aliphatic bands at (2972, 2839), (2981, 2839), (2924, 2850) and (2981, 2885) and bands at (1247, 1249, 1244 and 1253) belongs to (C-O-C) band and Nitro groups (1535, 1539, 1531 and 1539) for these compounds respectively figure 3 explain FT IR spectrum for derivative 9.

for derivative (11) ¹HNMR spectrum exhibited peak at δ (2.27) ppm due to (CH₃) while the singlet signal at δ (6.07) ppm. suggested the attribution of the proton of isoxazol with

Protons of Aromatic Rings appeared at 7.43-8.35 ppm and proton of (NH) appeared at (δ 11.39) ppm, figure(4).



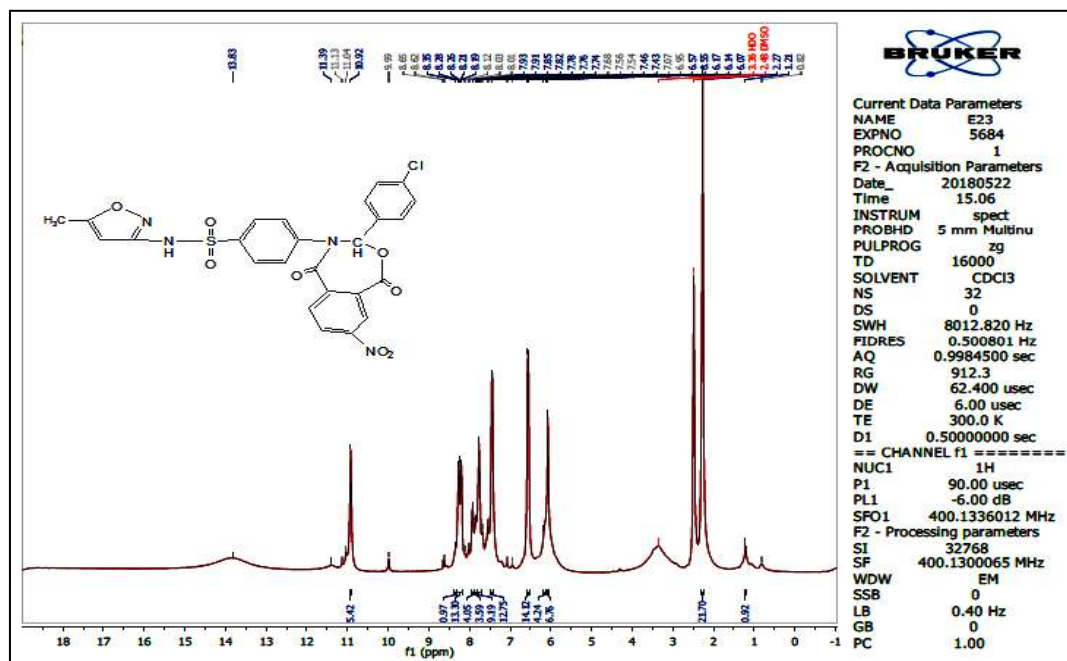


Fig. 4: ¹H-NMR chart derivative (11).

3- Characterization of compounds (13- 16)

Derivatives 13-16 were prepared from refluxed the derivatives(1-4) with 2-aminobenzoic acid in dry benzene. These synthesized derivatives received a satisfactory analysis of their proposed structures, which was confirmed using FT-IR, ¹H-NMR and Mass Spectra.

were characterized by their melting points and FT-IR, ¹H-NMR and mass spectra and checked by T.L.C.

FT-IR spectra of 13-16 derivatives were confirmed from the appearance of carbonyl group bands at (1679, 1666, 1666 and 1668 cm⁻¹) and OH group (3475 cm⁻¹) for each of the compounds (10 and 12) and (825 cm⁻¹) band for (C-Cl) of compound (15) C-H aliphatic bands at [(2993, 2839), (2939, 2885), (2959, 2893 and (2926, 2846))] and bands at (3257, 3263, 3209 and 3209 cm⁻¹) belongs to (N-H) band for these compounds respectively. Figure(5) shows the FT-IR spectrum of (13) compound.

The ¹H-NMR spectrum of compound (14) exhibited the peak at δ (2.36) ppm due to CH₃ while the singlet signal at δ (6.17) ppm. suggested the attribution of the proton of (NH) group for pyrimidine ring and protons of aromatic rings appeared at 7.30 -7.48 ppm, proton of (NH) appeared at δ 11.42 and proton (OH) group showed at (δ 9.43). Figure(6) the ¹H-NMR spectrum of derivative 14.

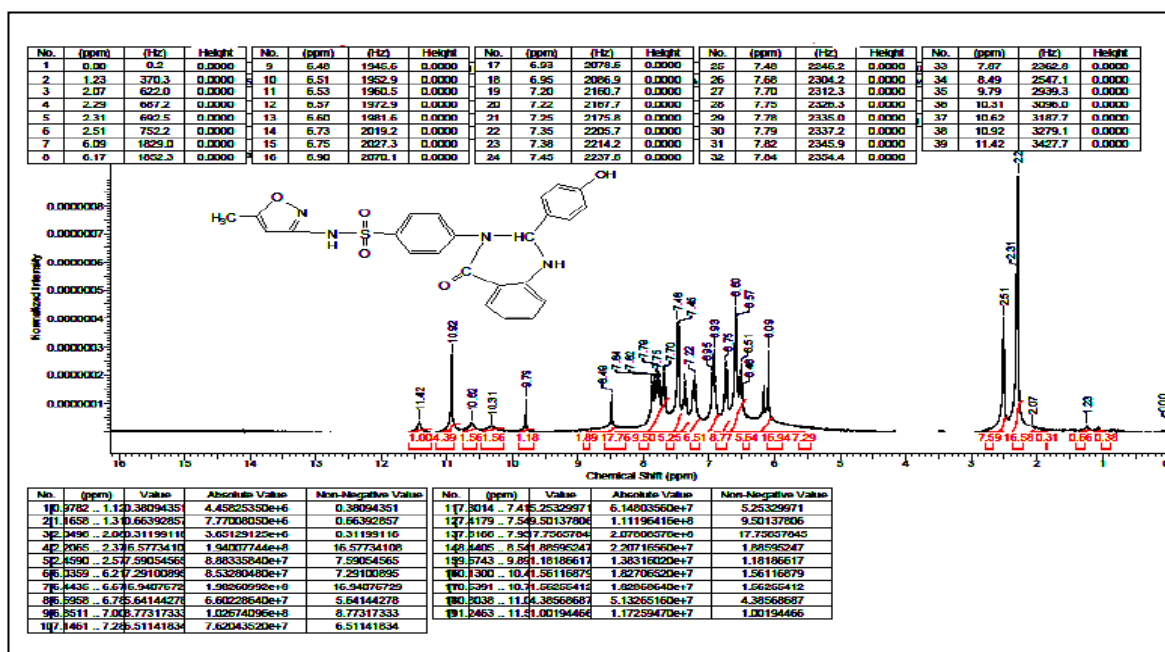


Fig.5: FT-IR spectrum of compound (13)

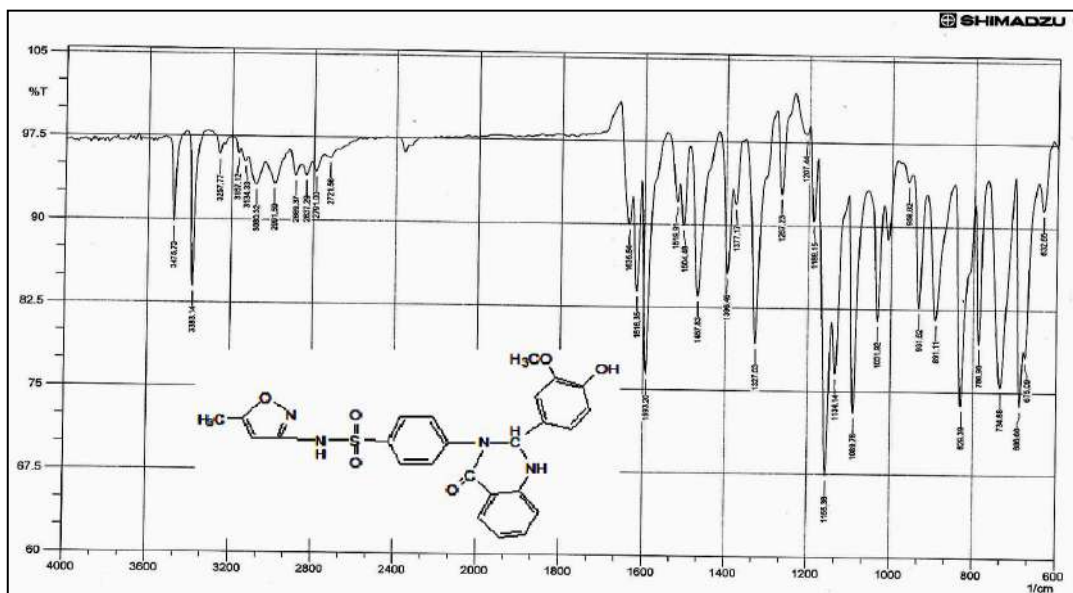
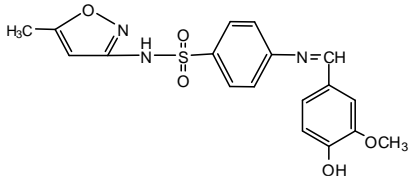
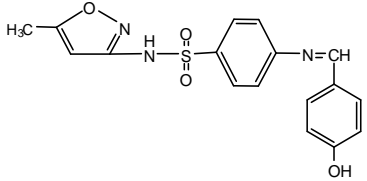
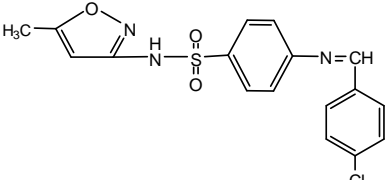
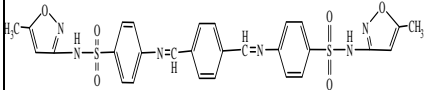
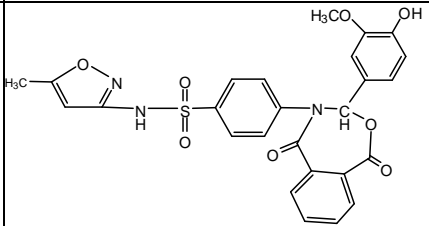
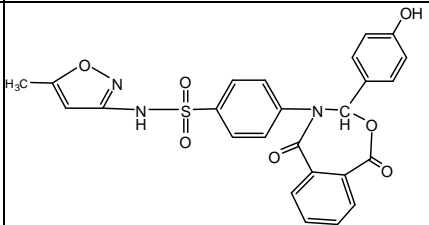
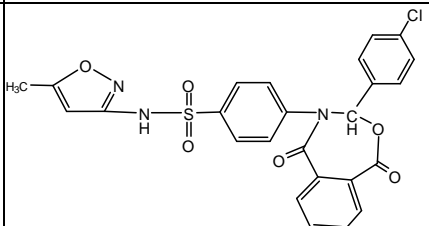
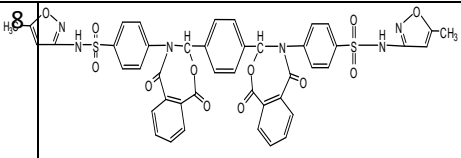
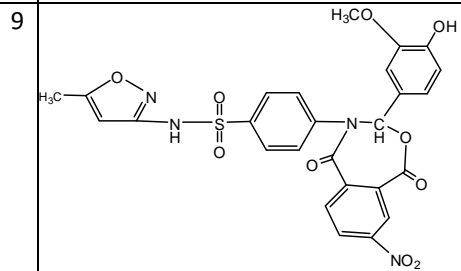
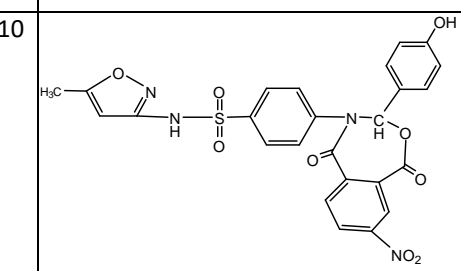
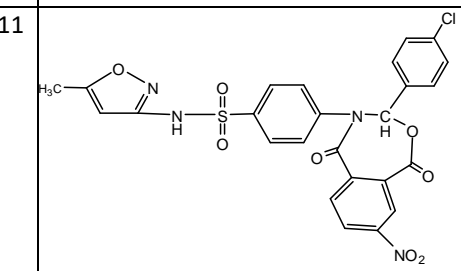
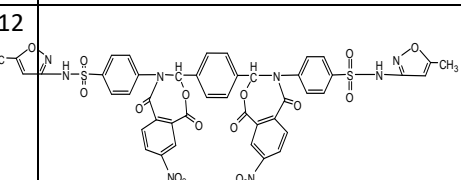
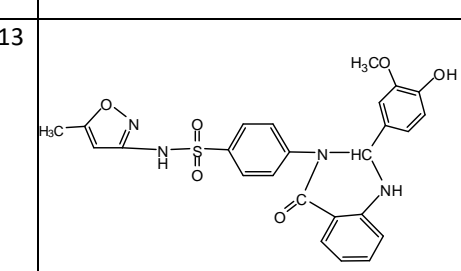
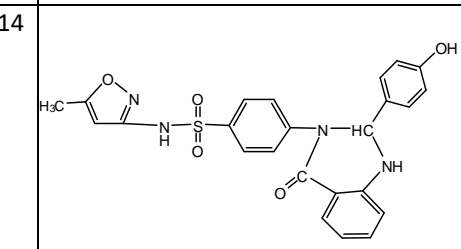


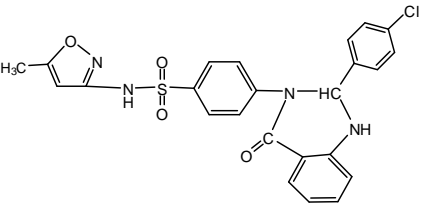
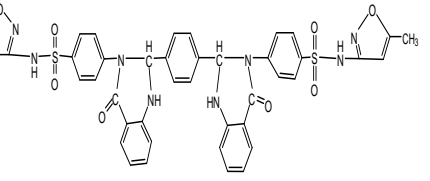
Fig.6 : ¹³C-NMR chart for derivative (14).

Table (1): Physical data and yields of the prepared compounds.

Com p. No.	Compound Structure and Nomenclature	Molecular Formula	Molecular Weight (g/mol e)	Yield (%)	M.P. (°C)	Colour	Rf
1		$C_{18}H_{17}N_3O_5S$	387.40	76	172-174	Dark yellow	0.74
2		$C_{17}H_{15}N_3O_4S$	357.40	80	192-194	Dark yellow	0.82
3		$C_{17}H_{14}N_3O_3SCl$	375.78	86	137-139	Pale Yellow	0.81
4		$C_{28}H_{24}N_6O_6S_2$	604.56	85	250(dec)>	Orange	0.58
5		$C_{28}H_{24}N_6O_6S_2$	604.56	85	250(dec)>	Orange	0.58
6		$C_{25}H_{19}N_3O_7S$	505.52	76	237-239	Pale Yellow	0.79
7		$C_{25}H_{18}N_3O_6SCl$	523.90	70	146-148	Orange	0.79

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8		$C_{44}H_{32}N_6O_{12}S_2$	752.68	73	240-242	Yellow	0.68
9		$C_{26}H_{20}N_4O_{10}S$	565.51	75	189-191	Orange	0.62
10		$C_{25}H_{18}N_4O_9S$	550.52	71	----	gummy	0.72
11		$C_{25}H_{17}N_4O_8SCl$	568.90	70	147-149	Yellow	0.61
12		$C_{44}H_{30}N_8O_{16}S_2$	797.68	72	176-178	Yellow	0.56
13		$C_{25}H_{24}N_4O_7S$	430.98	71	138-140	Orange	0.76
14		$C_{24}H_{20}N_4O_5S$	476.40	74	161-163	Orange	0.72

15		$C_{25}H_{19}N_5O_6Cl$	505.28	74	gummy	Brown	0.73
16		$C_{42}H_{34}N_8O_8S_2$	842.56	72	111-113	Yellow	0.51

3.2. Antimicrobial activity

The antibacterial activity of some prepared compounds were screened against gram positive (*Staphylococcus aureus* (G+)) and gram negative bacteria (*Pseudomonas aeruginosa* and *E.coli* (G-)) and fungicidal activity against *Candida Albicans* by the disc diffusion technique⁽²⁰⁻²²⁾. Muller Hinton agar was used as culture media for antibacterial activity. Recommended concentration 25,50,100 μ g / ml of the these compounds in DMSO solvent, the results are illustrated in table 2. In addition, the finding towards inhibition of microorganisms was compared with that of positive controls, Amoxicillin drug and DMSO as a negative control, compounds 11 and 14 exhibited high growth inhibitory effect against *Staphylococcus aureus* bacteria and *Candida Albicans* fungal whereas compound 8 does not have any activity against all bacteria except *Candida Albicans* fungal, it displayed good activity against it, as shown in Table 2.

Table 2. Antibacterial and Antifungal Activities of some prepared derivatives

deriv. .	<i>Staphylococcus</i>	E.coli	<i>Pseudomonas</i>	<i>Candida Albicans</i>
	11	33	23	---
14	35	22	+	-
8	-	-	-	38
DMSO	-	-	-	-
Amoxicillin	30	-	8	-
Nystatine	-	-	-	42

References

- (1). Al-Mulla A.(2017).A review: biological importance of heterocyclic compounds. *Der Pharma Chemica.*;9(13):141-172.
- (2). Balaji K, Bhatt PR, Mallika D, Jha AN. (2015), Design, synthesis and antimicrobial evaluation of some mannich base derivative of 2 (2-substituted)-5- amino-thiadiazoles.*International Journal of Pharmacy and Pharmaceutical Sciences.*;7:145-149.
- (3). R. O. C. Norman (1974). Principles of Organic Synthesis , *John Wiley & Sons, Inc. New York*, 313.
- (4). Hilal. M. Abdullah , Ibtisam K.Jassim and Malak N. Safi . (2012), Synthesis and characterization of new heterocyclic compounds with studying its biological activity, *Kerbala Journal of Pharmaceutical Sciences*, 4 ,119- 135.
- (5). Kawther A. Alheety ,Wissam Khalifa Jassim and Ibtissam Khalifa Jassim. (2019). Synthesis and characterization of Oxadiazole compounds derived from Naproxen, *Journal of pharmaceutical Sciences and Research* ,11(4), 1582-1588.
- (6). Ibtisam K.Jassim: Ghazwan H.A.AL-Sumaidaie and Sarah S.K.AL-Shejary, (2016). Synthesis and Characterization of Some New heterocyclic derivatives and Study Their antibacterial activity , *Kerbala journal of pharmaceutical sciences* . No (11) , 61-80.
- (7). Zainab, A . S., and Hasan, T.G.(2018). Synthesis and Identification of New Oxazepine Derivatives bearing Azo group in their structures. *Iraqi Journal of Science*, 59 (1A): 1-8.
- (8). Ramesh, L.S., Mahesh, S.M. and Jyoti, B.W. (2012). Anticoagulant potential of schiff bases of 1, 3- oxazines. *International Journal of Pharmacy and Pharmaceutical Sciences*, 4(4): 320-323.
- (9). Moawad, E. B. (1989). Synthesis of some new hetrocyclic compounds with expected potential biological activity. *Journal of Islamic Academy of Sciences*, 2(4): 237-240.
- (10). Sawant, R., Bhangale, L., Wadekar, J. and Gaikwad, P. (2012).Substituent selection for design and synthesis of antimicrobial 1, 3 oxazines. *ATopliss Modified Approach*, 32-39.
- (11). Jiu, J., Mizuba, S. and Hribar, J. (1977). Microbial transformation of -Chloro-10, 11-dihydrobenz(b,f)(1,4) Oxazepine by fungi. *American Society For Microbiology*, 33(1): 26-30.
- (12). Bera, M. and Roy, S. (2009). Triggering ring-opening and endo-selective ring-closing in a cascade. *J. Org. Chem.*, 74: 8814-8817.
- (13). Hamak, K.F. and Eissa, H.H.(2013).Synthesis, Characterization, Biological Evaluation and Anti Corrosion Activity of Some Heterocyclic Compounds Oxazepine Derivatives from Schiff Bases. *Organic Chem Curr Res* , 2(3): 3-7.
- (14). Mosaad S. M., Mohamed K. I., Alafify A. M., AbdelHamide S. G. and Mostafa A. M.(2005).Synthesis and anti-inflammatory evaluation of some new quinazoline derivatives. *International Journal of Pharmacology*. 1(3)261-266.
- (15). Gharib A., Vojdanifard L., Noroozi P. N., Hashemi P., Khorasani B.R., Jahangir M. and Roshani M.(2014). Catalytic synthesis of bis-2,3-dihydroquinazolin4(1H)-ones and 2,3-dihydroquinazolin-4(1H)-ones derivatives with the aid of silica-supported preyssler nanoparticles (SPNP).*Bulgarian Chemical Communications*. 46(4) 667-679.

- (16). Teba abraham and M N Mohammed.(2019).Synthesis and Biological of new Oxazepine - 4 , 7- dione of pyrazine, IOP Conf. Series: **Journal of Physics: Conf. Series** 1294.
- (17). Fadhil L. Faraj; Maryam Z.; Mohammadjavad P.;Chung Y. Looi;Nazia A.Majid; Hapipah M. Ali; Noraini A. Nura S. Gwaram and Mahmood A. Abdulla.(2014). Synthesis, Characterization, and Anticancer Activity of New Quinazoline Derivatives against MCF-7 Cells. **Hindawi Publishing Corporation □e Scientific World Journal** Pp1-15.
- (18). Zainab Amer Sallal and Hasan Thamer Ghanem.(2018).Synthesis and Identification of New Oxazepine Derivatives bearing Azo group in their structures. **Iraqi Journal of Science**, 59(1A) 1-8.
- (19). Tawfiq,M.T., Jassim,I.K. and Hadi,F. A.(2018).Synthesis and Characterization of Some New 1,3- Oxazepine-4,7-dion Derivatives Via Schiff s' Bases . **Journal of Education and Scientific Studies**,12(3),2413-4759.
- (20). Sallal, Z. A., & Ghanem, H. T. (2018). Synthesis and Identification of New Oxazepine Derivatives bearing Azo group in their structures. **Iraqi Journal of Science**, 59(1A), 1-8.
- (21). Muhsen, T. R.; Tomma, J. H.; Mukhlus, A. J. A.(2017). Synthesis and Characterization of Novel Compounds Containing of Imidazolidinone and Oxazepine Rings. **Ibn AL-Haitham Journal For Pure and Applied Science**, 25(3), 276-283.
- (22). Oliveira W. F., "Staphylococcus aureus and staphylococcus epidermidis infections on implants",*Journal of Hospital Infection*,V. 98,N.2, P.111-117, 2018.
- (23). Entesar.O ; Enaam F. M.,(2017). Synthesis New of Cyclic Imide Compounds for AmpicillinDrug and Study Their Biological Activity.**Baghdad Science Journal**, 14 (4), 756-764.
- (24). Enaam F. M.; Sundus Hadi Merza. (2021). Synthesis, Characterization and Biological Activity of heterocyclic compounds derived from Amoxcilline drug. **Annals of R.S.C.B** ,25, (6), 5087 – 5102 .



**IMMUNOLOGICAL AND SOME BIOLOGICAL BIOMARKERS
INVESTIGATION IN PATIENTS WITH SYSTEMIC LUPUS
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IMMUNOLOGICAL AND SOME BIOLOGICAL BIOMARKERS INVESTIGATION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Abstract:

Immunological and some Biological biomarkers have evolved to aid in the diagnosis and assessment of systemic lupus erythematosus (SLE) pathophysiological processes, with the ultimate objective of enhancing disease management. The aim of this study was to investigate the potential biochemical and immunological indicators for SLE diagnosis and assessment of the pathophysiological process. The samples of this study were divided into two groups: thirty-five SLE patients (10 male and 25 female) with ages ranging from 25 to 34, and thirty-five healthy controls with ages ranging from 25 to 36, and the biomarkers of this current study were included the Antinuclear Antibody (ANA) (IU/ml), Anti-double-stranded DNA (dsDNA) (IU/ml), Antinuclear Antibody (ANA) (IU/ml), Antinuclear Antibody (ANA) (IU/ml), Antinuclear Antibody (ANA) (IU/ml), Antinuclear Antibody (ANA) (IU/ml), Anti-Cyclic Citrullinated Peptide (IU/ml), Rheumatoid Factor Latex (IU/ml), C-Reactive Protein (CRp) (mg/l), erythrocyte sedimentation rate (ESR) (mm/h) and body weight (kg/ B.W.). The results was showed a significantly higher percentage in discoid rash (21%), malar rash (29%), photosensitivity (19%) anemia (26%), Proteinuria (21%) and Leukopenia (18%) in patients with SLE; hair loss (5%) and nervous system (4%) was none significantly higher; Anti-dsDNA was the most prevalent presenting characteristic in this research (33%), followed by blood urea (13%), hematuria (12 percent), and renal involvement (12%) in all individuals with SLE. Conclusion: All biomarkers and clinical features studied were found to be elevated in patients with SLE, particularly ANA and Anti-dsDNA, which were the most common autoantibodies found in this study, as well as other immunological and some biological markeres. As a result, For SLE, no one biomarker can be sensitive or specific enough.

Key words: Erythematosus, Immunological and Some Biological Markeres, Antinuclear Antibody, Anti-Double-Stranded DNA, and Anti-Cyclic Citrullinated Peptide.



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Introduction:

Systemic lupus erythematosus is a systemic autoimmune illness defined by abnormal immune system activity (Kiriakidou and Ching 2020). It has a wide spectrum of clinical manifestations, including renal, dermatological, neuropsychiatric, and cardiovascular symptoms (Kiriakidou and Ching 2020). (Tsokos 2011). SLE has an annual incidence of 0.3–31.5 per 100,000, and the adjusted prevalence is approaching, if not exceeding, 50–100 per 100,000. (Gergianaki *et al.*, 2017). Unfortunately, there appears to be a rising tendency in the prevalence of SLE over time (Rees *et al.*, 2017). SLE-related healthcare expenses are linked to the severity of the illness and the organ(s) affected (Bertsias *et al.*, 2016).

SLE is a disease with several causes. Quantifying the prevalence of SLE in different nations can help to elucidate the role of genetic, environmental, and other causal variables in the disease's natural history, as well as its clinical and societal implications (Danchenko, Satia and Anthony 2006). Environmental risk factors include UV radiation exposure, Epstein–Barr virus (EBV) infection, endogenous retroviral sequences, and numerous medications (AL-Atabi 2020; Alexander *et al.*, 2021). SLE is a major socioeconomic and public health problem since current treatments, such as drugs and a multidisciplinary approach, can only control symptoms and halt the progression of the illness, not cure it (Kiriakidou and Ching 2020). It is critical to improve the ability to identify SLE early in order to provide effective treatment. As a result, biomarkers, especially immunological biomarkers, have emerged to help in the diagnosis of SLE and the evaluation of its pathophysiological processes, with the ultimate goal of improving disease control (Yu, Nagafuchi and Fujio 2021).

A biomarker is a measurable indicator of a normal biological function, pathogenic activity, or a response to medicine or intervention (Szeffler *et al.*, 2012; Ramsey-Goldman *et al.*, 2021). Biomarkers it has recently been redefined to include a distinguishing physical sign or cellular, biochemical, molecular, or genetic feature, as well as the ability to recognize and/or observe a biologic process or morbid condition using a qualitative and/or quantitative test (González, Ugarte-Gil, and Alarcón 2021). Blood, urine, and tissues can all include biomarkers (Bertolo *et al.*, 2020). Biomarkers have been widely used in the detection, prediction, evaluation, and control of a variety of diseases in recent years, including SLE, diabetes, heart disease, and cancer (Narendra, Blixt and Hanania 2019). Only a few indicators can be utilized to diagnose skin lesions, which are common clinical symptoms of SLE (Yu *et al.*, 2019).

"A feature that is objectively tested and analyzed as an indication of normal biochemical processes, pathogenic processes, or pharmacological reactions to a therapeutic intervention" is what a biological marker, or biomarker, is (Biomarkers 2001; Hui-Yuen *et al.*, 2018). Many people may have autoantibodies for several years before the first clinical symptom arises. People of all genders, ages, and ethnic backgrounds are equally vulnerable (Varsha Bhatt *et al.*, 2020). SLE includes complicated laboratory results, a wide range of symptoms, and an unpredictable course, making diagnosis and monitoring disease activity challenging and frustrating for doctors. The aim of this study was to look into potential immunological and some biological indicators for SLE diagnosis and assessment of the pathophysiological process.

Material and methods

This research included seventy samples of both genders who were randomly assigned to Baghdad-Iraq. The samples were divided into two groups: 35 SLE patients (10 male and 25 female) with ages ranging from 25 to 34 years, and 35 control individuals (20 female and 15 male) with ages ranging from 25 to 36 years. The biomarkers were used to diagnose patients with SLE and the control group were assessment. Five milliliters of venous blood were drawn from patients, three milliliters were transferred to a sterile gel tube, and the sample was centrifuged for 15 minutes at 3000 rpm. The serum was divided into several aliquots and immediately frozen at -20°C before being tested for immunological and biochemical markers: Antinuclear Antibody (ANA) (IU/ml), Anti-dsDNA (IU/ml) was measured by using the commercially available Human ELIZA KIT (Liason Diasorin for detection of ANA and Anti-dsDNA Ab/ Genus HY-PRO 54 Specific Protein Analyzer/ semi-automated/ Germany), Anti CCP (IU/ml) by Liason Diasorin for detection of ANA and Anti and ds-DNA Ab (Analyzer/ Full-automated/ Italian), Latex Rf (IU/ml), CRp (mg/l) by Human ELIZA KIT /semi-automated/ Germany) according to the manufacturer's instructions, ESR (mm/h) was identified by techniques used to measure the ESR according to the Westergren method (1921), Weight (kg/ B.W.) was transferred to EDTA tubes for hematological analysis.

Statistical Analysis:

The data was statistically analyzed using SPSS (version 23). An independent t test was used to examine the significance of variations in means. A P value of less than 0.05 is considered statistically significant. (IBM Corporation, 2015).

Results

1. Assessment of clinical features and certain laboratory findings in SLE patients

Seventy samples from both genders, the patients have been grouped into two categories: thirty five SLE patients and thirty five as control as described in methodology. Patients with SLE group were clinically and laboratory diagnosed. Table 1 show complete history with presenting clinical features and certain laboratory findings was recorded. Numbers of parameters were examined as: Discoid rash, Malar rash, Photosensitivity was a significantly higher in patients with SLE; Hair loss and Nervous system was none significantly higher in patients with SLE. Laboratory findings such as Anti-dsDNA was most common presenting feature in this study, anemia, blood urea, Hematuria, Proteinuria and Leukopenia and Renal involvement investigated in all patients with SLE.

Table 1: Clinical features* and certain laboratory findings in SLE patients (N=35)

Clinical features	N (%)
Arthritis	20
Malar rash	29
Photosensitivity	19
Renal involvement	12
Discoid rash	21
Hair loss	5
Nervous system involvement	4
Laboratory findings	
Anti-dsDNA	33
Anemia	26
Increased blood urea	13 (above 45) mg/dl
Hematuria	12
Proteinuria	21
Leukopenia	18

2. Immunological and some biological biomarkers in patients with SLE

A total of 70 people were involved in the study: 35 healthy controls and 35 SLE patients with illness. The ACR criteria were used to diagnose all of the cases. Clinical and laboratory evaluations were performed as shown in table 1; the results in table 2 showed a significant (P0.05) increases in mean \pm SE values of ANA, Anti ds DNA, Anti CCP, Latex Rf, ESR, and h-CRP, and a significant (P0.05) decrease in weight in the SLE patients group as compared to the control individuals.

Table 2: Immunological and some biological biomarkers in patients with SLE

Biomarker	Group	N	Mean \pm SE
Anti DNA	Control	35	4.69 \pm 0.29b
	Patients	35	27.23 \pm 2.56a
ANA	Control	35	0.36 \pm 0.02b
	Patients	35	2.17 \pm 0.29a
Anti CCP	Control	35	4.30 \pm 0.16b
	Patients	35	37.89 \pm 2.95a
Latex Rf	Control	35	3.89 \pm 0.19b
	Patients	35	33.43 \pm 3.56a
CRp	Control	35	5.53 \pm 0.33b
	Patients	35	18.50 \pm 1.99a
ESR	Control	35	13.94 \pm 1.14b
	Patients	35	47.44 \pm 2.27a
Weight	Control	35	83.50 \pm 1.90a
	Patients	35	84.72 \pm 1.88a

Means with different letter in each group are significantly different (P<0.05)

Discussion

Immunologists, biologists, and clinicians have been challenged by systemic lupus erythematosus (SLE). The results described in tables (1 and 2) demonstrated that changed immunological and some biological of several biomarkers play a key part in an idea that was supported by SLE research. The current study's scientific contribution is to determine the usefulness of several biochemical and immunological biomarkers that might be used to identify patients with SLE. Table 2 shows substantial (P0.05) increases in ANA, Anti-dsDNA, Anti-CCP, Latex Rf, ESR, and CRP values in the SLE patients group compared to the control group, but a significant (P0.05) drop in weight in the SLE patients group compared to the control group.

For diagnostic reasons, prognosis, and therapeutic treatment, antinuclear antibody (ANA) assays were frequently performed on the blood of individuals with various connective tissue disorders, including systemic lupus erythematosus (SLE). These findings are consistent with those of Pisetsky, Gilkeson, and Clair (1997) and Saeed *et al.*, (2017), who found that the latex agglutination test for ANA detection is reliable and specific in active SLE, and that the presence of these antibodies is the second most common manifestation of SLE (Teija Kuna *et al.*, 2021). Anti-dsDNA elevation is associated with an increase in interferons (IFN), indicating long-term immunological dysregulation and the development of SLE (Munroe *et al.*, 2016).

Furthermore, anti-dsDNA positivity corresponds with acute sickness (ter Borg *et al.*, 1990; Elsayed and Mohafez 2020), disease severity (Davis, Percy, and Russell 1977), and complement concentrations in SLE patients (ter Borg *et al.*, 1990; Elsayed and Mohafez 2020). Anti-dsDNA was also predictive of haematological or organ flare, as seen in table 1, with increases in anemia and hematuria percentages (Petri *et al.*, 2009; Shamim *et al.*, 2020). Anti-dsDNA antibody is extremely specific, but its sensitivity is only 57.3 %, according to Jeon *et al.*, (2010) and Kavanaugh and Solomon (2002), however Anti-dsDNA was the most prevalent positive antibody detected in sensitive investigations with patients with SLE, as shown in table 1 (33 %).

The results in table 1 showed high percentage of SLE patient's involvement 20% of arthritis and increasing levels of anti-CCP, Latex Rf and ESR in table 2. In SLE patients with and without arthritis, as well as in patients with rheumatoid arthritis, the combination of RF latex testing, CCP antibody, and ESR testing offers a highly specific screening test for rheumatoid arthritis with equivalent sensitivity for the diagnosis of rheumatoid arthritis (Abdul Wahab *et al.*, 2013; Chang *et al.*, 2016).

A significant ($P < 0.05$) increasing in C-reactive protein (CRP) is a protein that is created in the liver by pro-inflammatory cytokines such as tumor necrosis factor alpha, interleukin-6, and interleukin-1B, and these rising levels are consistent with the authors' findings (Kobak *et al.*, 2014; Saeed *et al.*, 2017), who demonstrated that a positive CRP test could be indicative of a wide range of conditions such as cancer, rheumatoid arthritis and rheumatic fever, myocardial infarction, pneumococcal pneumonia, and SLE and concerning the relation between CRP and the presence of auto-antibodies conclude that evaluated C-reactive protein and that there are major statistical limitations between CRP and autoantibodies that showed in SLE patients.

Conclusions

All biomarkers and clinical characteristics investigated were found to be increased in patients with SLE, especially ANA and Anti-dsDNA, which were the most common autoantibodies found in this study. There was also a high percentage of patients with Malar rash (29%) Photosensitivity (19%), Discoid rash (20%), and Proteinuria (18 %). Because no single biomarker is sensitive and specific enough, combining many biomarkers with mathematical models may be a viable strategy for diagnosing SLE.

References

- Abdul Wahab, A., Mohammad, M., Rahman, M. M., & Mohamed Said, M. S. (2013). Anti-cyclic citrullinated peptide antibody is a good indicator for the diagnosis of rheumatoid arthritis. *Pakistan journal of medical sciences*, 29(3), 773–777.
- AL-Atabi D.A., Bushra F. Hasan, Ali H. AL-Hafidh. (2020). Estimation of Some Immunological and Biochemical in the Patients with Systemic Lupus Erythematosus in Males and Females in Baghdad. *Medico Legal Update*, 20(3), 972–978.
- Alexander, R. V., Rey, D. S., Conklin, J., Domingues, V., Ahmed, M., Qureshi, J., & Weinstein, A. (2021). A multianalyte assay panel with cell-bound complement activation products demonstrates clinical utility in systemic lupus erythematosus. *Lupus science & medicine*, 8(1), e000528.
- Bertolo, M.; Baumgart, S.; Durek, P.; Peddinghaus, A.; Mei, H.; Rose, T.; Enghard, P.; Grützkau, A. (2020). Deep Phenotyping of Urinary Leukocytes by Mass Cytometry Reveals a Leukocyte Signature for Early and Non-Invasive Prediction of Response to Treatment in Active Lupus Nephritis. *Front. Immunol.* **2020**, 11, 256.
- Bertsias, G.; Karampli, E.; Sidiropoulos, P.; Gergianaki, I.; Drosos, A.; Sakkas, L.; Garyfallos, A.; Tzioufas, A.; Vassilopoulos, D.; Tsalapaki, C.; et al. (2020). Clinical and financial burden of active lupus in Greece: A nationwide study. *Lupus* **2016**, 25, 1385–1394.
- Biomarkers Definitions Working Group. (2001). Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clinical pharmacology and therapeutics*, 69(3), 89–95.
- Chang, P. Y., Yang, C. T., Cheng, C. H., & Yu, K. H. (2016). Diagnostic performance of anti-cyclic citrullinated peptide and rheumatoid factor in patients with rheumatoid arthritis. *International journal of rheumatic diseases*, 19(9), 880–886.
- Danchenko N, Satia JA, Anthony MS. (2006). Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus*. 2006 May;15(5):308-18.
- Davis P, Percy JS, Russell AS. (1977). Correlation between levels of DNA antibodies and clinical disease activity in SLE. *Ann Rheum Dis* 1977;36:157_9.
- Dr Varsha Bhatt, Dr Mitali Khadke*, Dr Arjun Kakrani, Dr Love Garg, Dr Manaswini Edara (2020). A Clinical, Biochemical and Immunological Profile of Systemic Lupus Erythematosus in Adult Patients in a Tertiary Care Centre. *JMSCR* Vol. 08, Issue; 01. Page 401-408
- Elsayed, S.A., Mohafez, O.M.M. (2020) . Autoantibodies spectrum in lupus nephritis in a cohort of Egyptian patients: relation to disease activity and prognostic value. *Egypt Rheumatol Rehabil* 47, 39.
- Gergianaki, I.; Fanouriakis, A.; Repa, A.; Tzanakakis, M.; Adamichou, C.; Pompieri, A.; Spirou, G.; Bertsias, A.; Kabouraki, E.; Tzanakis, I.; et al. (2017). Epidemiology and burden of systemic lupus erythematosus in a Southern European population: Data from the community-based lupus registry of Crete, Greece. *Ann. Rheum. Dis.* **2017**, 76, 1992–2000.

- González, L. A., Ugarte-Gil, M. F., & Alarcón, G. S. (2021). Systemic lupus erythematosus: The search for the ideal biomarker. *Lupus*, 30(2), 181–203.
- Hui-Yuen, J. S., Gartshteyn, Y., Ma, M., O'Malley, T., Conklin, J., Eichenfield, A. H., Imundo, L. F., Dervieux, T., & Askanase, A. D. (2018). Cell-bound complement activation products (CB-CAPs) have high sensitivity and specificity in pediatric-onset systemic lupus erythematosus and correlate with disease activity. *Lupus*, 27(14), 2262–2268.
- IBM Corp. Released (2015). IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.
- Jeon JY, Kim HA, Kim SH *et al.* (2010). Interleukin 6 gene polymorphisms are associated with systemic lupus erythematosus in Koreans. *J. Rheumatol.* 37(11), 2251–2258.
- Kavanaugh AF, Solomon DH. (2002). Guidelines for immunologic laboratory testing in the rheumatic diseases: anti-DNA antibody tests. *Arthritis Rheum.* 47(5), 546–555.
- Kiriakidou, M.; Ching, C. (2020). Systemic Lupus Erythematosus. *Ann. Intern. Med*, 172, ITC81–ITC96.
- Kobak S, Yilmaz H, Sever F, Duran A, Sen N, Karaarslan A. (2014). The prevalence of antinuclear antibodies in patients with sarcoidosis. ;2014:351852
- Munroe ME, Lu R, Zhao YD *et al.* (2016). Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann Rheum Dis* 2016;75:2014_21.
- Narendra, D.; Blixt, J.; Hanania, N. (2019). Immunological biomarkers in severe asthma. *Semin. Immunol.* **2019**, 46, 101332.
- Petri M, Singh S, Tesfayone H, Malik A. (2009). Prevalence of flare and influence of demographic and serologic factor on flare risk in systemic lupus erythematosus: a prospective study. *J Rheumatol*; 36:2476_80.
- Pisetsky DS, Gilkeson G, Clair EW. (1997). Systemic lupus erythematosus. Diagnosis and treatment. *Med Clin North Am.* 1997;81;113–128.
- Ramsey-Goldman, R., Alexander, R. V., Conklin, J., Arriens, C., Narain, S., Massarotti, E. M., Wallace, D. J., Collins, C. E., Saxena, A., Putterman, C., Brady, K., Kalunian, K. C., & Weinstein, A. (2021). A Multianalyte Assay Panel With Cell-Bound Complement Activation Products Predicts Transition of Probable Lupus to American College of Rheumatology-Classified Lupus. *ACR open rheumatology*, 3(2), 116–123.
- Rees, F.; Doherty, M.; Grainge, M.; Lanyon, P.; Zhang, W. (2017). The worldwide incidence and prevalence of systemic lupus erythematosus: A systematic review of epidemiological studies. *Rheumatology* **2017**, 56, 1945–1961.
- Saeed I, AlAmeri AM, Allah SA, Ali Z, Gazee I, Al-Ameer ZA. (2017). Association of ANA seropositivity with RF, CRP, Brucellosis test in patients with SLE, a comparison between immunofluorescence technique and latex agglutination. *Iraq Med J* 2017;1(4)98-100.
- Shamim, R., Farman, S., Batool, S., Khan, S., & Raja, M. (2020). Association of systemic lupus erythematosus disease activity index score with clinical and laboratory parameters in pediatric onset systemic lupus erythematosus. *Pakistan journal of medical sciences*, 36(3), 467–472. <https://doi.org/10.12669/pjms.36.3.1480>

- Swaak AJ, Aarden LA, Staius van Eps LW, Feltkamp TE. (1979). Anti-dsDNA and complement profiles as prognostic guides in systemic lupus erythematosus. *Arthritis Rheum* 1979;22:226_35.
- Szefer, S.J.; Wenzel, S.; Brown, R.; Erzurum, S.C.; Fahy, J.V.; Hamilton, R.G.; Hunt, J.F.; Kita, H.; Liu, A.H.; Panettieri, R.A., Jr.; et al. (2012). Asthma outcomes: Biomarkers. *J. Allergy Clin. Immunol.* **2012**, 129, S9–S23.
- Ter Borg EJ, Horst G, Hummel EJ, Limburg PC, Kallenberg CG. (1990). Measurement of increases in anti-double-stranded DNA antibody levels as a predictor of disease exacerbation in systemic lupus erythematosus. A long-term, prospective study. *Arthritis Rheum* 1990;33:634_43.
- Tešija Kuna, A., Đerek, L., Drvar, V., Kozmar, A., & Gugo, K. (2021). Assessment of antinuclear antibodies (ANA): National recommendations on behalf of the Croatian society of medical biochemistry and laboratory medicine. *Biochemia medica*, 31(2), 020502.
- Tsokos, G.C. (2011). Systemic lupus erythematosus. *N. Engl. J. Med.*, 365, 2110–2121.
- Westergren A. Studies of the suspension stability of the blood in pulmonary tuberculosis. *Acta Med Scand* 1921;54: 247–82.
- Yu, H.; Jiang, L.; Liu, R.; Yang, A.; Yang, X.; Wang, L.; Zhang, W.; Che, T. (2019). Association between the ratio of aryl hydrocarbon receptor (AhR) in Th17 cells to AhR in Treg cells and SLE skin lesions. *Int. Immunopharmacol.* **2019**, 69, 257–262.
- Yu, H.; Nagafuchi, Y.; Fujio, K. (2021). Clinical and Immunological Biomarkers for Systemic Lupus Erythematosus. *Biomolecules* **2021**, 11, 928.

**AMELIORATIVE EFFECT OF BETA CAROTENE AGAINST TITANIUM DIOXIDE
NANOPARTICLES REPRODUCTIVE TOXICITY ON TESTIS AND EPIDIDYMIS OF
MALE ALBINO MICE MUS MUSCULUS**

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AMELIORATIVE EFFECT OF BETA CAROTENE AGAINST TITANIUM DIOXIDE NANOPARTICLES REPRODUCTIVE TOXICITY ON TESTIS AND EPIDIDYMIS OF MALE ALBINO MICE MUS MUSCULUS

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Hala M.N. AL-SAILY³

Abstract:

The present study aimed to evaluate the improving or ameliorative effect of beta carotene on titanium dioxide nanoparticles induced testicular toxicity at the histological level. Forty adult healthy male albino mice weighting between 30-37gm and aged 12 to 15. Animals were randomly grouped in to four groups with ten mice in each: the first one was administered normal saline, whereas second group mice were administered 10 mg/kg body weight of beta carotene, third group were given 300 mg/kg body weight of titanium dioxide solution, last fourth group were administered 300 mg/kg body weight of titanium dioxide solution and 10 mg/kg body weight of beta carotene after two hours of every titanium uptake after ten days of pre protective administration of 10 mg/kg body weight beta carotene ; all for thirty five days. Results exhibited that the histological toxic effects induced by A 300 mg/kg titanium dioxide nanoparticles administered mice testis showing germinal epithelium sloughing and odema with irregular spermatogenesis .and showing That epididymal epithelium exhibiting lipid vacuoles in the supranuclear region of the principal cells, with papillary infoldings lined by columnar cells and occur hyperplasia of clear cells, Some lumens were devoid of sperm with few cellular debris, also showed atrophy in the smooth muscle between ducts with Inflammatory infiltrate in connective tissue. A group treated with 300 mg/kg titanium dioxide NPs plus 10 mg/kg body weight administered mice that testis and epididymis showing an intact of histologic architecture. And the above effects were ameliorated by the administration of beta carotene, and the pre protective effect of beta carotene increased that effect.

In conclusion Beta carotene has improving effects against the histological damage in testis and epididymis and the arrest in spermatogenesis that resulted from the toxicity of titanium dioxide nanoparticles as it repair the damaged parameters and obtaining control like features.

Key words: Tetanium Dioxide, Reproductive Toxicity, Beta Carotene, Histology.



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Introduction:

Nanotechnology, is a branch of scientific research deals with formation and control of tiny particles of nanoscale less than 100 nm characterized by original and unique physiological and biochemical properties. Recent research explain that mineral nanoparticles characterized by high bioavailability because of their novel features as they were have huge surface area and adsorbing ability and they reach to the deepest tissues and interact with cell membranes.(1,2).Titanium oxide nanomaterial is widely used in wide range of our life such as in painting printers, cosmetics, etc.(3) but it has side effects on human and animals.(4) the properties that been the cause of its adverse effects and intrinsic toxicity on environment and human health ;along with its ability of producing free radicles and can damaging DNA molecules ;and then alter the structure of many protiens causing cancer at the end(5).

Caratenoids, are tetraterpenoid structure pigments with red,orange, purpule and yellow colors; They are widely dispersed pigments in our nature as they are found in some types of bacteria, algae, fungi, animals and plants. There are two types of carotenoids: Xanthophylls and our focused in component ;carotenes. Caroteins themselves are of many types: α -carotene and β -carotene and others(6). Her we focus on the use of β -carotene. Beta carotene was functioned in body into retinol; a vitamin that body used it widely or it exhibit an antioxidant activity(7). So it exhibited biological and pharmaceutical functions like: radioprotection, antioxidation, antiepilepsy and cardiovascular protection(8,9,10).

Materials and methods

Titanium dioxide Nanoparticles were brought from:sigma Aldrich , German ;passed on privisions provided by company with purity of 99.9%.

Fourty adult healthy male albino mice weighting between 30-37gm and aged 12 to 15 week brought from university of Babylon animal house . mice were acclimatized for two weeks under controlled conditions of light and dark 12:12 hours, the study curried out in Babylon university animal house. Animals were randomly grouped in to four groups with ten mice in each: the first one was administered normal saline, whereas second group mice were administered 10 mg/kg body weight of beta carotene(Alfa Vitamins Labs ,INC.USA). , third group were given 300 mg/kg body weight of titanium dioxide solution, last fourth group were administered 300 mg/kg body weight of titanium dioxide solution and 10 mg/kg body weight of beta carotene after two hours of every titanium uptake after ten days of pre protective administration of10 mg/kg body weight beta carotene ; all for thirty five days. Blood withdrewd through heart puncture for attainment of serum for hormone levels estimation. Testis and epididymis were extruded, weighted and then putted in formaline for 24 houre and then histologically processed accorgind to (11,12). Data were analysed by using SPSS – version 20, SPSS,Inc, Chicago, Illinois, USA. Discriptive statistics mean \pm standard deviation , differences were compared by ANOVA.

Results

Table 1 : Weights of the bodies, testis and epididymis of control and the experimental groups

Weight group	Body weight	Testis	epididymis
	mean± SD		
Control	34.1± 0.08	0.193±0.03	0.139±0.02
Carotene 10mg\kg	33.6±0.06	0.196± 0.02	0.142±0.01
Titanium dioxide NPs300mg\kg	32.4±0.06	0.107±0.03	0.114±0.05
Titanium dioxide NPs 300mg\kg + carotene 10mg\kg	33.8±0.03	0.189± 0.01	0.185±0.02
L.S.D.	1.3	0.032	0.028

Level of the significance value p<0.05.

Results exhibit a non-significant difference in body weight of control , beta carotene alone and the mixed Ti2O+beta carotene groups, while a significant decrease in titanium group alone in comparison with control.

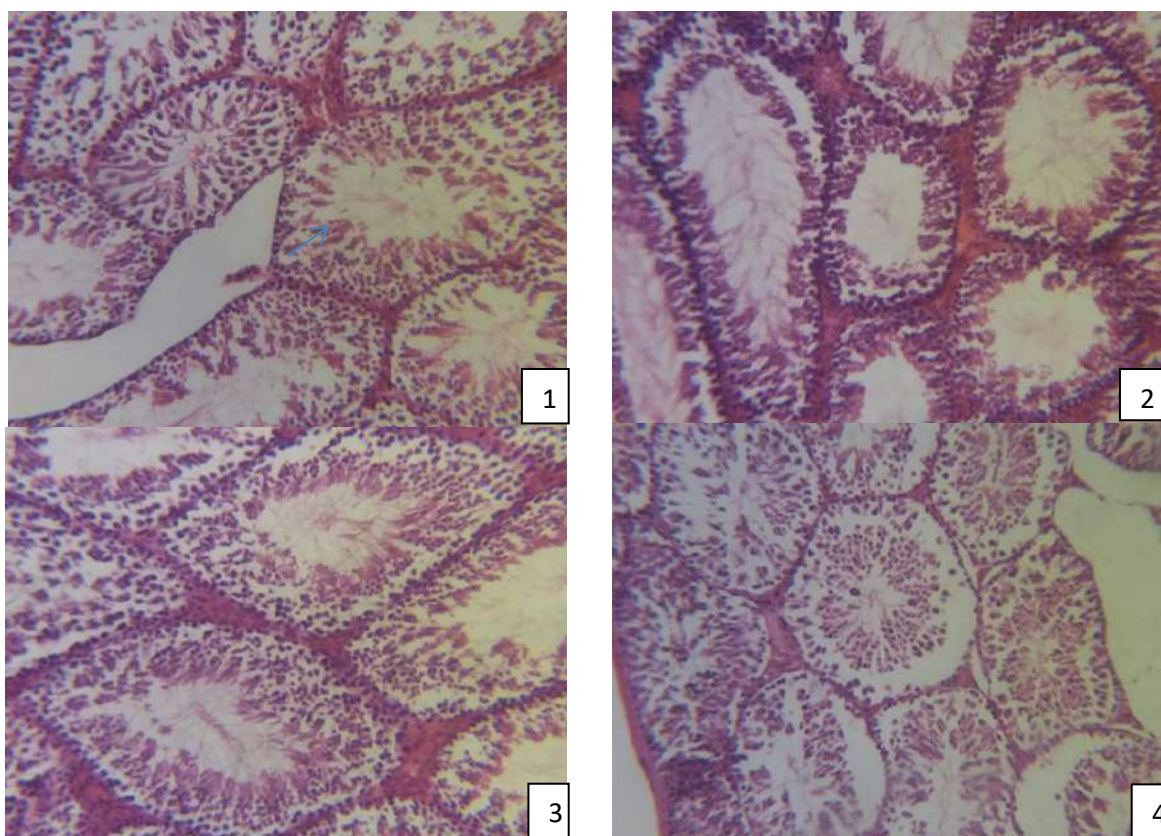


Figure 1: 1.Control mice testis showing an intact of histologic architecture with regular spermatogenesis (10/10). Tubular wall thickness 25.04 μm, tubular diameter: 103.5 μm. H&E. x100. 2. A 10 mg\kg body weight Beta carotene administered mice testis showing an intact of histologic architecture with regular spermatogenesis (10/10). Tubular wall thickness 34.4 μm, tubular diameter: 79.9 μm. H&E. x100. 3. A 300 mg\kg Tetanium dioxide NPs

administered mice testis showing germinal epithelium sloughing and odema with irregular spermatogenesis(9/10) blue arrow. Tubular wall thickness 25.42 μm , tubular diameter: 95.48 μm . H&E. x100 .4. A 300 mg/kg titanium dioxide NPs plus 10 mg/kg body weight administered mice testis showing an intact of histologic architecture with regular spermatogenesis (10/10). Tubular wall thickness 21.08 μm , tubular diameter: 81.1 μm . H&E. x100.

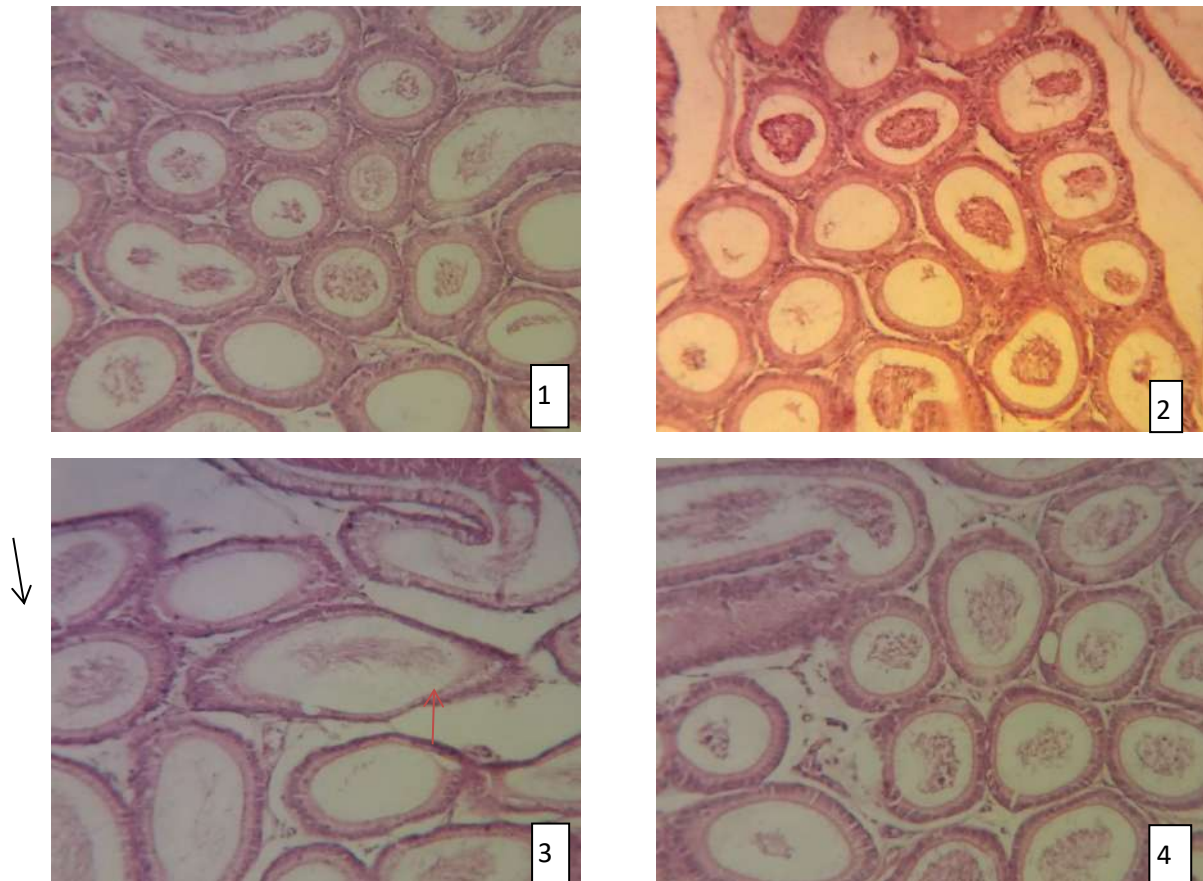


Figure 2: 1. Control mice epididymis showing an intact of histologic architecture Pseudostratified columnar epithelium tissue with stereocilia and spermatozoa were found in lumen, H&E. x100. 2. A 10 mg/kg body weight Beta carotene administered mice epididymis showing an intact of histologic architecture H&E. x100. 3. A 300 mg/kg Tetanium dioxide NPs administered mice epididymis showing The epididymal epithelium exhibiting lipid vacuoles in the supranuclear region of the principal cells black arrow, Intracystic papillary infoldings lined by columnar cells with clear cytoplasm and occur hyperplasia of clear cells red arrow, Some lumens were devoid of sperm with few cellular debris, also showed atrophy in the smooth muscle between ducts with Inflammatory infiltrate in connective tissue. H&E. x100 .4. A 300 mg/kg titanium dioxide NPs plus 10 mg/kg body weight administered mice epididymis showing an intact of histologic architecture. H&E. x100.

Discussion

The current study aim focused on the investigation of the protective effect of beta carotene against the reproductive toxicity which induced by titanium dioxide nanoparticles. Results were explained that there was a weight decreasing effects of titanium dioxide nanoparticles on body weight in comparison with control group, beta carotene group and the group of combination. Weight loss may be caused by the oxidative damage that cause an oxidative stress leading to the breakdown of the large body molecules, like proteins, fats (13). This decrease may indicates the potential toxic effects of NPs, Which causes physiological change in mice ,it can effects the appetite and food consumption with consequent effects on body weight, these results agreement with (14) A (18,19) were found that TiO₂ could penetrate the intestinal mucosa; and it could translocate through ileac epithelium and Peyers patches causing damage and chronic failure of the intestinal epithelium. Although several reports advises that the smaller is the size of nanoparticles, the higher is their toxicity (15). Nanoparticles have the surface chemistry due of nanoparticles has been known critical in its interaction and transport the biological membrane led to cause tissue damage or induce atrophy of the tests (16) , or it may be the reason for the low weight of the testicles is the low level of the hormone testosterone, because this hormone is necessary for the building and growth of the body's muscles and reproductive organs, the tissue structure of these organs and the performance of their function, as the decrease in this hormone may causes genital atrophy(17)

Histological observations exhibited an elevation in the histomorphological quality of the studied testis and epididymis in the groups treated with beta carotene in comparison with TiO₂ toxicated group; as the resulting sloughing is caused by the effects of those nanomaterials on intermediate filaments and microtubules of sertoli cells leading to allocating germ cells and disturbing the development of spermatids (20) the epididymis is an organ dependent on androgens, most important testosterone ,which its receptors were spread in the epithelial cells lining androgen Important in the differentiation and proliferation of epithelial cells in all areas of the epididymis(21).The pre protective administration of beta carotene could excellently re-repair the toxic effects of titanium on the histology of the reproductive organs. (22) were explained that the administration of beta carotene orally for ten days could led to Its accumulation in tissues and exerts a protective effect toward the oxidative stress. Also , the beneficial effects of pre protection of beta carotene on Sertoli function that would led to decrease in the sloughing of germ cells and vaculation of the germinal epithelium. The histological damage resulted from titanium might resulted by free radicals formed and the antioxidative effect of beta carotene reduced the free radicals stress and improving the histological damage caused in testicular and epididymal tissues and improve the spermatogenesis (23,24) . In conclusion Beta carotene has improving effects against the histological damage in testis and epididymis and the arrest in spermatogenesis that resulted from the toxicity of titanium dioxide nanoparticles as it repair the damaged parameters and obtaining control like features.

References

- (1). Siegrist M, Stampfli N, Kastenholtz H, et al. (2008). Perceived risks and perceived benefits of different nanotechnology foods and nanotechnology food packaging. *Appetite*. 52(2): 283–290.
- (2). Handford CE, Dean M, Henchion M, et al. (2014). Implications of nanotechnology for the agri-food industry: Opportunities, benefits and risks. *Trend Food Sci Technol* . 40(2): 226–241.
- (3). Kaida T, Kobayashi K, Adachi M, Suzuki F. (2004). Optical characteristics of titanium oxide interference film and the film laminated with oxides and their applications for cosmetics. *J Cosmet Sci*. 55(2):219–20.
- (4). Ma L, Liu J, Li N, Wang J, Duan Y, Yan J, et al. (2010). Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO₂ delivered to the abdominal cavity. *Biomaterials*. 31(1):99–105.
- (5). Shariat Sajad, Mehrdad Modaresi. (2016). The effects of titanium dioxide nanoparticles on liver histology in mice. *Journal of chemical and pharmaceutical Research* ,8(4):1313-1316.
- (6). Takashi Maoka. Carotenoids as natural functional pigments. *Journal of Natural Medicines*(2020) volume 74, pages 1–16 7. Martín JF, Gudiña E, Barredo JL. Conversion of β -carotene into astaxanthin: two separate enzymes or a bifunctional hydroxylaseketolase protein? *Microb Cell Factories*. 2008;7:3.
- (7). Schweiggert RM, Kopec RE, Villalobos-Gutierrez MG, Högel J, Quesada S, Esquivel P, et al. (2013). Carotenoids are more bioavailable from papaya than from tomato and carrot in humans: a randomised crossover study. *Br J Nutr*. 12:1–9.
- (8). Salvadori DM, Ribeiro LR, Xiao Y, Boei JJ, Natarajan AT. (1996). Radioprotection of beta-carotene evaluated on mouse somatic and germ cells. *Mutat Res*. 356(2):163–70.
- (9). 10. Gopal K, Nagarajan P, Jedy J, Raj AT, Gnanaselvi SK, Jahan P, et al. (2013). β -carotene attenuates angiotensin II-induced aortic aneurysm by alleviating macrophage recruitment in ApoE^{-/-} mice. *PLoS One*. 8(6):e67098.
- (10). 11. Bancroft, J.D.; Survana, K. and Layton, C. (2013). *Bancroft's theory practice of histological techniques*. 7th ED, Churchill Livingstone, Edinburgh, London. 662pp.
- (11). 12. Manar Mohammad Hasan AL-Murshidi. (2021). Histological study of testis tissue of male golden hamster (*Mesocricetus auratus*) in different time points of Diabetes. *Ann Trop Med & Public Health*;22(IV): SPe150. DOI: 10.36295/ASRO.2019.22047. https://www.journal.atmph-specialissues.org/abstract.php?article_id=6459
- (12). 13. AL-Ghazali, Fadhaa Abdulsada Adhab. (2021). Study the protective effect of cold aqueous extract of *Moringa Oleifera* seeds in the level of some reproductive hormones and Some physiological and histological parameters in the male white rats *Rattus norvegicus* treated with cadmium chloride. thesis. College of Education - Karbala University, Iraq.
- (13). 14. De Jong, W.H.; Van Der, V.; Leo, T.M.; Sleijffers, A.; Park, M.V.D.Z.; Jansen, E.H.J.M.; Van Loveren, H.; Vandebriel, R.J. (2013). Systemic and immunotoxicity of silver nanoparticles in an intravenous 28 days repeated dose toxicity study in rats. *Biomaterials*, 34, 8333-8343.

- (14). 15. Chen, X; Schluesener, H.J. (2008). Nanosilver: a nanoparticle in medical application. *Toxicol Lett.* 176:1–12.
- (15). 16. Chithrani, B.D.; Ghazani, A.A.; Chan, WC. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano. Lett.* 6(4):662-668.
- (16). 17. Maitra, S. K. & Mitra, A. (2008). Testicular functions and serum titers of LH and testosterone in methyl parathion-fed roseringed parakeets. *Ecotoxicology and environmental safety*, 71(1), 236-244.
- (17). 18. Ammendolia MG, Iosi F, Maranghi F, Tassinari R, Cubadda F, Aureli F, Raggi A, Superti F, Mantovani A, De Berardis B (2017) Short-term oral exposure to low doses of nano-sized TiO₂ and potential modulatory effects on intestinal cells. *Food Chem Toxicol* 102:63–75. <https://doi.org/10.1016/j.fct.2017.01.031>
- (18). 19. Brun E, Barreau F, Veronesi G, Fayard B, Sorieul S, Chanéac C, Carapito C, Rabilloud T, Mabondzo A, Herlin-Boime N, Carrière M (2014) Titanium dioxide nanoparticle impact and translocation through ex vivo, in vivo and in vitro gut epithelia. *Part Fibre Toxicol*
- (19). 20. Hess RA, Nakai M. (2000). Histopathology of the male reproductive system induced by the fungicide benomyl. *Histol Histopathol.* 15(1): 207–24.
- (20). 21. Mahsa Hamzeh and Bernard Robaire.(2013). Effect of Testosterone on Epithelial Cell Proliferation in the Regressed Rat Epididymis
- (21). 22. Lyama T, Takasuga A, Azuma M. Beta-carotene accumulation in mouse tissues and a protective role against lipid peroxidation. *Int J Vitam Nutr Res.* 1996;66(4):301–23. Ahotupa M, Huhtaniemi I. Impaired detoxification of reactive oxygen and consequent oxidative stress in experimentally criptorchid rat testis. *Biol Reprod.* 1992;46:1114–8.
- (22). 24. Aoss Moez Abed–Alhussian Alyassery and Manar Mohammad Hasan Al-Murshidi.Effects of silver nanoparticles on the histology of testis and some accessory sex glands of male albino mice. *Biochem. Cell. Arch.* Vol. 21, Supplement 1, pp. 2501-2506, 2021. DocID: <https://connectjournals.com/03896.2021.21.2501>



**DETERMINING THE MOST APPROPRIATE HARVEST DATE FOR CELERY, PARSLEY
AND CHAMOMILE. IT CONTAINS OF VOLATILE OIL AND TOTAL PHENOLS**

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ZIENA M. ABDUL-QADER**

**DETERMINING THE MOST APPROPRIATE HARVEST DATE FOR CELERY,
PARSLEY AND CHAMOMILE. IT CONTAINS OF VOLATILE OIL AND TOTAL
PHENOLS**

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Zainab J. AL-MOUSSWI²
Ziena M. ABDUL-QADER³

Abstract:

This Experiment was carried out in the fields of College of Agricultural Engineering Sciences, University of Baghdad during the autumn season 2014-2015 . Plants seeds (celery, parsley and Chamomile) planted in dishes, Seedlings transferred after month when seeds germination .The seedlings were planted in the field on lines between 75 cm and 40 cm between one plant and another . This experiment was carried out using the design RCBD on three replicates. The first factor represents different plants, While The second factor represents harvest dates .The results were showed that The harvest of leaves celery at three o'clock in the afternoon gave the highest percentage of volatile oil and penol, which was 0.75 ml / 100 g and 5 mg/100g. Parsley gave highest level of volatile oil at 6 pm, which was 0.5 ml / 100 g, While the harvest time at three in the morning produced the highest percentage of phenols, which reached 3.7 mg/100g. The amount of volatile oil in the flowers of chamomile plant increased when harvesting at 12 AM, which gave the highest amount of 0.063 ml / 100 g compared to the date of harvest at three in the afternoon, which produced the lowest amount of volatile oil amounted to 0.026 ml / 100 g. and harvest time at 9 pm in the content of total phenols.

Key words: Phenol, Volatile Oil, Celery, Parsley, Chamomile, Harvest Dates.



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Introduction:

Determining the time of harvesting medicinal plants is one of the important stages for the production of medicinal compounds. It is noted that there is a difference in the amount of active ingredients in it according to the different times of collection of the day and even in the different seasons of the year. Therefore, the appropriate time for harvesting should be chosen so that the amount of active ingredients in it is as large as possible. The content of Datura plant of alkaloids was doubled when collected in the early morning and before sunrise compared to noon. The essential oils in Lavender flowers, Cyclamen leaves and Myrrh flowers were found to be in the highest concentration when harvested at ten o'clock in the morning (Ozsoy, 1995). There are a lot of active substances transformed into secondary materials as a result of metabolic processes. Secondary metabolites increase vary in plants during the daylight hours of the night as Glycosides in Digitalis plant which is made up during the day and dissolved during the night until the morning, and the same applies to Steroidal Alkaloids in Poppy and Ergot, and the glycosides in Willow plant (Al-Maghazy, 2000).

Celery (*Apium Gravalens L.*) and Parsley (*Petroselinum crispum*) belongs to the family Apiaceae (Norman and Max, 2001). The original home is India and the USA (Nadkarni and Nadkarni, 1976). Its fruits are small in size, greenish-white, tentacle-shaped, grayish-brown in color and aromatic (Teng and et al., 1985). Celery contains the following substances, including isoimperatorin, isoquercitrin, linoleic acid, and volatile oil, which consists of d-limonene, Selinene, Santalol, Eudesmol, dihydrocarvone, Sedanenolide, Isopimpinellin, Apiumoside and Celeroside (Garg and et al., 1980). Also used as a laxative, carminative, diuretic, stimulant, tonic, lowers blood pressure, treats indigestion, tonics the uterus, and is used in the treatment of infections (Leung, 1980). It was found the volatile oil an effect on the nervous system, as it is analgesic and antispasmodic (Bisset and Wichtl, 1994), and it is also used in the treatment of Rheumatism (Yan and et al., 1998). It contains many Mineral and secondary compounds such as flavonoids, kaempferol, Quercetin, flavones, Apigenin and Luteolin (Pettersson and et al., 2006). It contains antioxidants and thus protects humans from various types of cancer. It is also a booster for the immune system due to the fact that it contains of vitamin C and flavonoids, especially Apignin, which works to reduce the division of cancer cells (Nielse and et al., 1999).

Chamomilla recutita L. is an important medicinal plant that is used in the treatment of many diseases. This plant grows and spreads abundantly in Europe and Temperate regions (Schilcher, 1978). German chamomile contains Terpenoids, Chamazulene, -bisabolol oxide A and B flavonoids (apigenin, luteolin, quercetin), Coumarins, Spiroethers, Anthemic acid, Choline, Tannin and Polysaccharides (Berry, 1995), and 0.24-2% blue-colored volatile oil, 8% Flavone, 10% Gum, 0.3% Choline and 0.1%. % Coumarins (Thorne, 2008). The volatile oil extracted from the flowers is used in the manufacture of cosmetics and many food industries (Schilcher and et al., 2005). It is used externally to treat wounds, Ulcers, Eczema, Dermatitis, Sciatica and Rashes in children (Blumenthal, 1998). It is used internally as a tonic and to treat some mental illnesses such as hysteria and convulsions (Martens, 1995).

This research was aimed to determine the best time to harvest celery, Parsley and chamomile to obtain the highest percentage of volatile oil and total phenols due to their medical and nutritional importance and the lack of studies in this field.

Materials And Methods

This Experiment was carried out in the fields of College of Agricultural Engineering Sciences, University of Baghdad during the autumn season 2014-2015 . Plants seeds (Celery, parsley and Chamomile) in dishes, Seedlings transferred after month when seeds germination .The seedlings were planted in the field on lines between 75 cm and 40 cm between one plant and another . This experiment was carried out using the design RCBD on three replicates. The first factor represents different plants, While The second factor represents harvest dates(3am, 6am, 9am, 12am, 3pm, 6pm, 9pm, 12pm). The service operations of watering, weeding and fertilizing were carried out. Samples were taken over the course of a whole day and every three hours.It was dried in ventilated and shaded rooms away from sunlight and preserved according to its content from volatile oil and total phenols.

Studied Characters

Determination the content of the vegetative parts from the volatile oil. ml / 100 g

After collecting the samples, they were dried in ventilated rooms, with the samples turned daily for 10 days, and after making sure that the samples were completely dry, they were ground by an electric grinder and kept in paper packages. 50 g for each harvest date and placed in a 1000 ml glass flask. 600 ml distilled water was added at a temperature of 90 ° C for 3-4 hours. The oil was separated from the water using diethyl ether and using a separating funnel. They were kept in sealed and opaque plastic containers inside the refrigerator at a temperature of 4°C.

Determination of phenols. mg/100g

The concentrations of total phenol content in the sample was estimated by taking 1 g of the crushed sample with 15 ml of ethyl alcohol of 96% concentration and put in a water bath at 95 °C for 10 minutes, Filter the solution using medical gauze and add 5 ml of ethyl alcohol to the remaining sample and heat in a water bath for 3 minutes and supplement the volume with alcohol to 20 ml. Then it was filtered with filter paper. Then taken is 1 mL of solution and 1 mL of hydrochloric acid, 1 ml of Arno reagent and 2 mL of sodium hydroxide are added and the volume is supplemented with distilled water to 15 ml and kept in opaque plastic containers. Phenols were determined according to the Arnows method by measuring optical absorption at a wavelength of 515 nm using a complex chromatography-spectrophotometer produced by special reactions of the Arno detector. (Mahadevan and Sridhar, 1982).

Results and Discussion

Results in Figure 1 reveal that the time of celery plant harvest Significant differences on the volatile oil content, where indicated significant increases in harvesting the leaves at three o'clock in the afternoon gave the highest value of volatile oil to 0.75 ml / 100 g, which did not differed significantly of harvest at three and six in the morning (0.7 and 1.4 ml / 100 g) compared to the date of harvest at 12 o'clock at night, which produced the lowest amount of 1.75 ml / 100 g. The results of Figure 2 show that significant differences in the content of celery from total phenols when collected at different times of the day, where indicated significant increases of the harvest time at 3 pm produced the highest value (5 m g /100 g) of total phenols, while the lowest value produced by the harvest at 12 pm obtained 1.85 m g /100 g.

Figure 1 : Effect of harvest time on Celery leaf content of volatile oil ml/100g

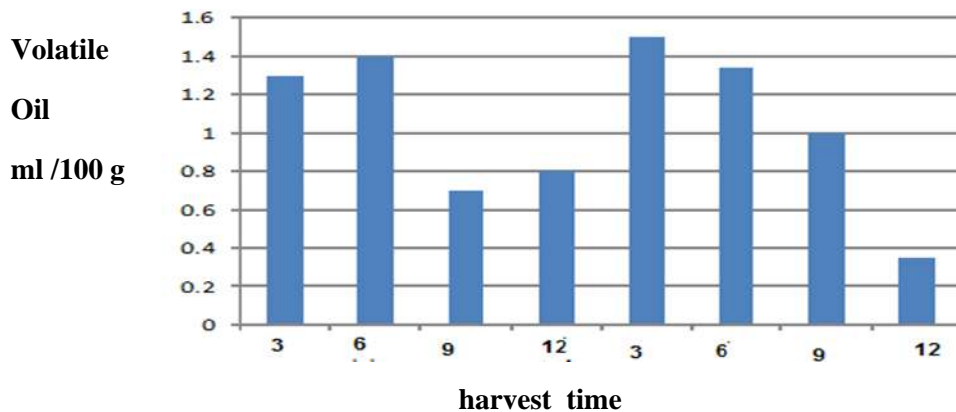
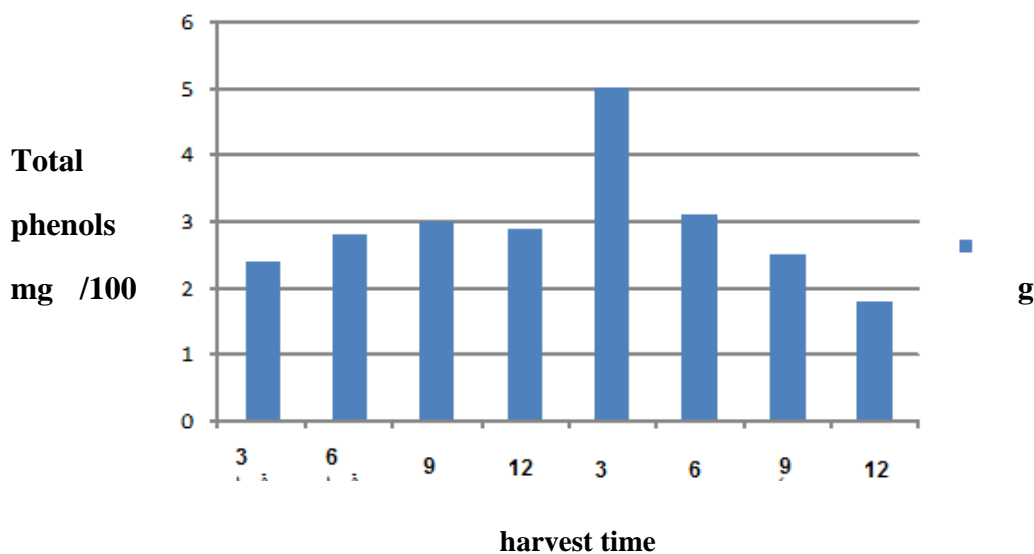


Figure 2: Effect of harvest time on Celery content of total phenols mg/100g



Results in Figure 3 reveal significant differences on the volatile oil content in Parsley Depending on the time of harvest, where indicated significant increases at 6 pm gave the highest value 0.5 ml / 100 g which did not differ significantly from the 9 pm 0.4 which did not differ significantly, while the lowest value produced by the 6 am gave 0.1 ml / 100 g. The results of Figure 4 show that harvest time at 3am gave the highest amount of total phenols which was 3.1 mg/100 gm compared to the twelfth hour at night, which gave the lowest amount of total phenols, while the results converged at the harvest dates at three, six and nine in the evening in the content of total phenols .

Figure 3: Effect of harvest time on the Parsley content of oil ml / 100 g

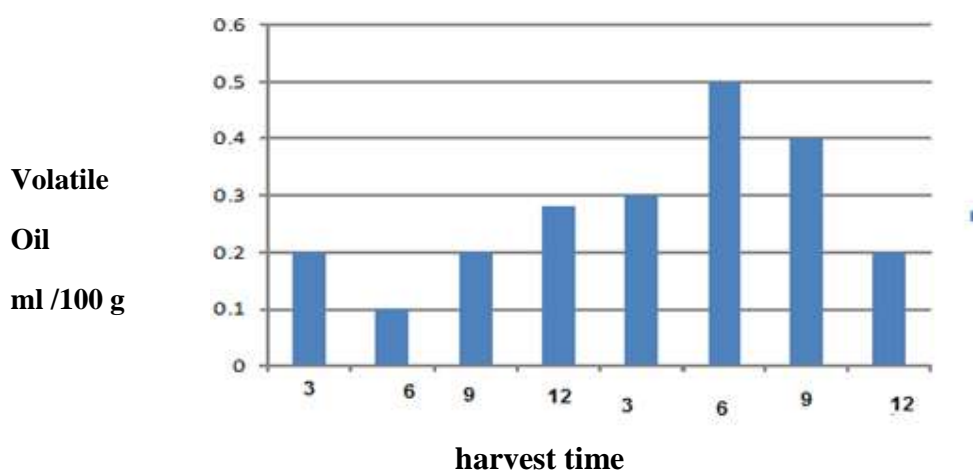
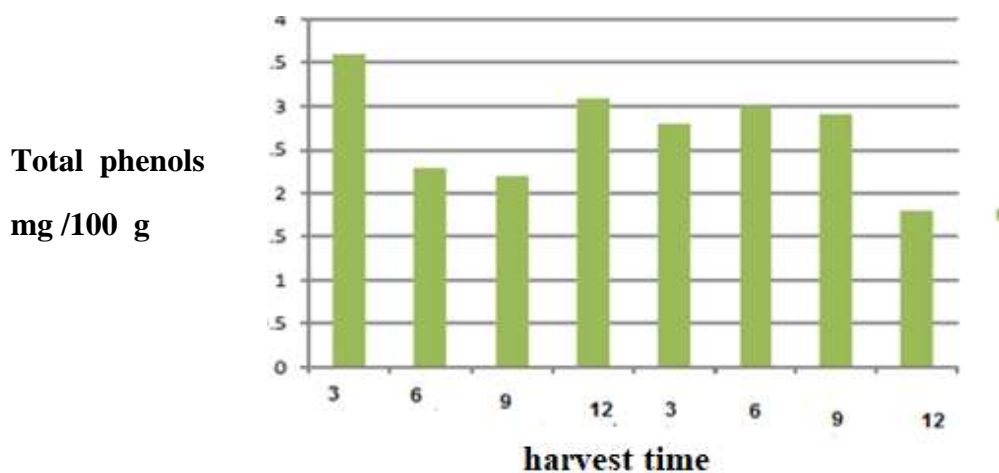


Figure 4: Effect of harvest time on the Parsley content of total phenols mg/gm



The amount of volatile oil in the flowers of the chamomile plant increased when harvesting at twelve noon, as it gave the highest amount of 0.063 ml / 100 gm, compared to the harvest at 3 pm, which gave the lowest amount of volatile oil amounted to 0.026 ml / 100 gm. The results also converged between the 6 am, 9 am and 12 pm, as shown in the figure 5. As for the total phenols in chamomile flowers as shown in the figure 6, It is noticeable that the harvest date at 9 pm 20 produced the highest amount of 20 mg/100g, which did not differ significantly from, 9am, and 12 pm compared to the harvest date at 12 am which

produced the lowest amount of total phenols 12 mg/100g. When comparing the plants of celery, Parsley and chamomile in terms of their content of total phenols, We are find the superiority of chamomile plant in its content of total phenols over celery and Parsley plants at all harvest dates, as indicated in Figure (7).

Figure 5: Effect of harvest time on the volatile oil content of the Chamomile plant

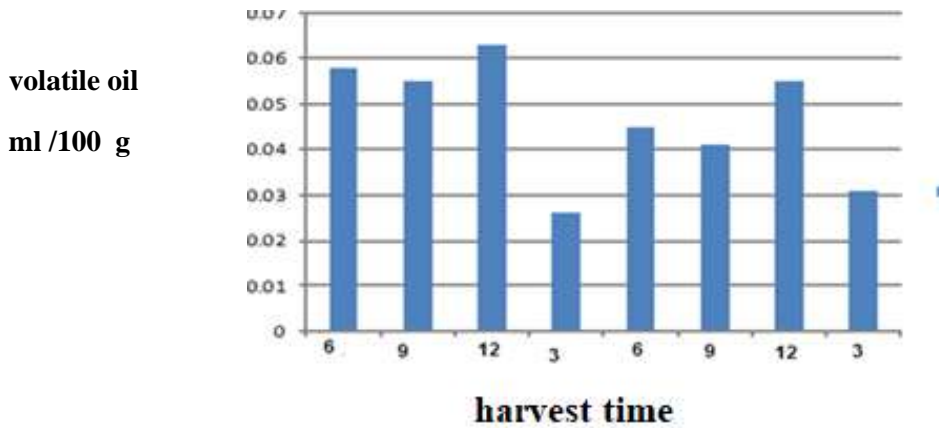


Figure (6) Effect of harvest time on the content of total phenols in the Chamomile plant

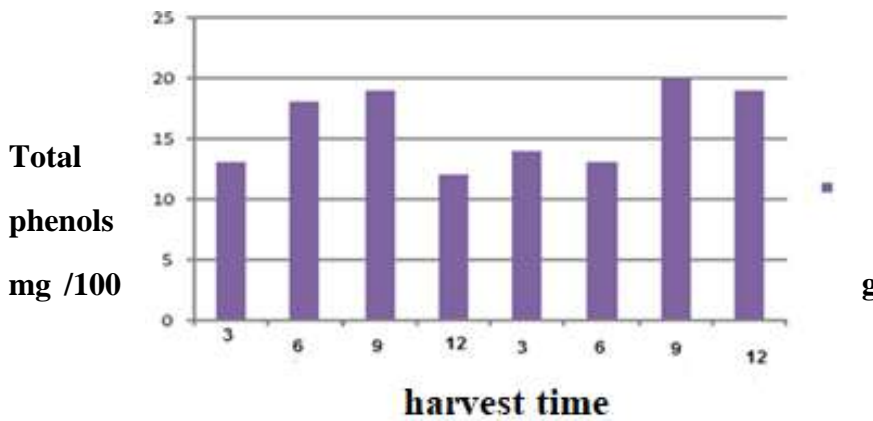
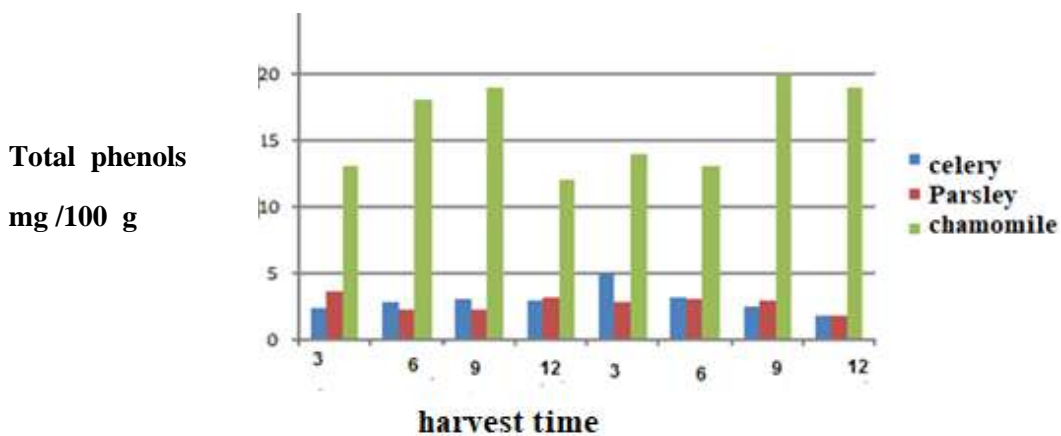


Figure 7 : Comparison of the effect of harvest time on the total phenols content of Celery, Parsley and Chamomile plants



The cause of the increase in volatile oil and total phenols may be due to the biosynthesis of active compounds in medicinal plants is controlled by the genetic factor, but the concentration of these compounds is affected by environmental factors and agricultural processes such as harvest time and type of agricultural process (Hadizadeh and et al. 2007). These factors affect the growth of plants and the quality and quantity of volatile compounds within plants. Environmental factor such as intensity, type and period of illumination in addition to temperature, humidity and soil properties, these factors individually or collectively affect the chemical composition and storage location of secondary compounds within medicinal and aromatic plants. (Ramezani and et al., 2009 and Boira and et al., 1998). This results agreed with the findings of (Esmailia and et al., 2012).The collection of the saffron plant at different times during the day had clear differences in the plant's content of active substances.

Conclusions and Recommendations

We conclude from this experiment that the plants' content of active substances, especially volatile oils, clearly affect the time of their harvest during the hours of the whole day, which was reflected on the amount of oil extracted and phenols in the plant, so we recommend that the harvest time be between three and six in the evening The content of Celery and Parsley plants from volatile oil and phenols is at their highest levels, While Chamomile flowers are preferred to be collected between 6 am to 12 pm, and We also recommend studying the dates of harvest in other plants because of this aspect of great importance in the amount of active substances. This is reflected positively in the amount of active substances that have important economic dimensions.

References

- Abu Zaid, A. N. (2001) Plants and Medicinal Herbs. First Edition, Madbouly Library, Cairo, PP. 150.
- Al-Maghazi, A. Mohamed (2000) The constitutional conditions and specifications that must be met when handling medicinal and aromatic plants. Assiut Journal of Environmental Studies - Nineteenth Issue. pg 27
- Bisset, N.G. and Wichtl, M. (1994) Herbal Drugs and Phytopharmaceuticals. Medpharm GmbH Scientific Publishers, Stuttgart, CRC Press, Boca Raton, 91-95.
- Blumenthal, M.(1998) The Complete German Commission E Monographs: Therapeutic Guide To Herbal Medicines. Austin: American Botanical Council. <https://doi.org/10.7326/0003-4819-130-5-199903020-00024>
- Boira H., A. Blanquer.(1998) Environmental factors affecting chemical variability of essential oils in *Thymus piperella* L. Biochem. Syst. Ecol., 1998, 26, 811-822.
- Esmailian, y., G. Mohammad, R. Mohammad and M. Masoud.(2012) Diurnal variability of stigma compounds of saffron (*Crocus sativus* L.) at different harvest times. Annals of Biological Research, 2012, 3 (3):1562-1568
- Fejes, S.Z., A. Blazovics, E. Lemberkovics, G. Petri, E. Szoke and A. Kery. (2000) Free radical scavenging and membrane protective effects of methanol extracts from *Anthriscus cerefolium* (Hoffm) L. and *Petroselinum crispum* (Mill) Nym. Ex A. W. Hill. *Phytother. Res.* 14: 362–365.
- Garg, S.K. (1980) Glucosides of *Apium graveolens*. *Planta Med.*, 38(4).
- Hadizadeh, F., M. Mahdavi, S.A. Emami, Z. Khashayarmanesh, M. Hassanzadeh, J. Asili, M. Seifi, H. Nassirli, A. Shariatimoghadam, R. Noorbakhsh.(2007) Evaluation of ISO Method in Saffron Qualification. *Acta Hort.* 739: 405-409.
- Leung, A.Y. (1980) Encyclopedia of Common Natural Ingredients Used in Food, Drug and Cosmetic. John Wiley and Sons, New York
- Mahadevan A. and Sridhar R. (1982) Methods in physiological plant pathology. II. Ed. Sivakami Publ. pp. 157-159
- Martens, D.(1995) Chamomile: The Herb and the Remedy. Prover, The Journal of the Chiropractic Academy of Homeopathy. 6:15-18.
- Montaner, C., E. Floris and J. Alvarez (2000) Is self-compatibility the main breeding system in borago (*Borago officinalis* L). Theoretical and Applied Genetics. 1:185-9.
- Nadkarni, K.M. and A.K. Nadkarni (1976) Indian Materia Medica. 3rd edition. Bombay: Dhoot Papeshwar Prakashan Ltd., pp. 119-120
- Nielsen, S.E., J. F. Young, B. Daneshvar, S. T. Lauridsen, P. Knuthsen, B. Sandstro and L. O. Dragsted.(1999) Effect of parsley (*Petroselinum crispum*) intake on urinary apigenin excretion, blood antioxidant enzymes and biomarkers for oxidative stress in human subjects. *British Journal of Nutrition.* 81:447-455.
- Norman GB and W. Max . (2001) Herbal Drug and Phytopharmaceuticals, A Handbook for practice on a scientific basis with reference to German Commissioner Second edition. Boca Raton : Medpharm Scientific Publishers, pp. 81-82.
- Ozsoy, U. E. (1995) Investigations on some agronomic and quality characteristics of the Izmir oregano (*Origanum onites* L.) populations collected from Mugla region. Master

- Thesis. Graduate School of Natural and Applied Sciences. Ege University. 67 p (in Turkish).
- Peterson,S., J. W. Lampe, T. K Bammler, K.Gross-Steinmeyer, D. L. Eaton. (2006) Apiaceous vegetable constituents inhibit human cytochrome P-450 1A2 (hCYP1A2) activity and hCYP1A2-mediated mutagenicity of aflatoxin B1. *Food Chem Toxicol.*44(9):1474-84.
- Pullaiah, T .(2006) *Encyclopedia of World Medicinal Plants*. Vol. I. New Delhi: Regency Publications, pp. 187-188
- Ramezani S, F. Ramezani, F. Rasouli, M. Ghasemi and M.Fotokian .(2009) Diurnal variation of the essential oil of four medicinal plants species in Central Region of Iran. *Research Journal of Biological Science* .4: 103–106
- Razzaghi-Abyaneh M., T. Yoshinari, M. Shams-Ghahfar, M. Okhi, M.B. Rezaee, H. Nagasawa and S. Sakudas .(2007) Dillapiof and Apiol as SpecificInhibitors of the Biosynthesis of Aflatoxin G1 in *Aspergillus parasiticus*. *Biosci Biotechnol.Biochem.*71(9):2329-32.
- Robbers, J.E.,M.K. Speedia, and V.E. Tyler.(1994) *Pharmacognosy and pharmacobiotechnology*. Baltimoreiwilliams and Wilkins.
- Schilcher, H.(1978)Influence of herbicides and some heavy metals on growth of *Matricaria chamomilla* L. and the biosynthesis of essential oil. *Acta Horticulture.*73:339–41
- Schilcher H, Imming P, Goeters S.2005 () Active chemical constituents of *Matricaria chamomilla* L. syn. *Chamomilla recutita* (L.) Rauschert. In: Franke R, Schilcher H, editors. *Chamomile-industrial profiles*. Boca Raton: CRC Press; 2005. pp. 55–76.
- Teng, C.M., L.G. Lee, S.N. Ko .(1985) Inhibition of platelet aggregation by Apigenin from *Apium graveolens*. *As. Pac. J. Pharmacol.*, 83: 85.
- Thorne Research, Inc. (2008). *Matricaria chamomilla*. *Alternative Medicine Review*, 13(1), 58- 62.
- Yan, C.H., Y.P. Feng and J.T. Zhang .(1998) Effects of dl-3-n-butylphthalide on regional cerebral blood flow in right middle cerebral artery occlusion rats. *Chung Kuo Yao Li Hsueh Pao.* 19: 117-20
- Zhang, H.,F. Chen, C.Wang and H.Y. Yao .(2006) Evaluation of antioxidant activity of porsely (*Petroselinum crispum*) essential oil an identification of its antioxidant constituents.*Food ResearchInternation*



**THE SIGNIFICANCE OF COMPLETE BLOOD COUNT AS
LABORATORY DIAGNOSIS IN COVID-19**

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THE SIGNIFICANCE OF COMPLETE BLOOD BLOOD COUNT AS LABORATORY DIAGNOSIS IN COVID-19

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Abstract:

The increased global prevalence of COVID-19 has resulted in a significant increase in the occupancy rate of healthcare facilities, resulting in overcrowding in some areas. In this situation, healthcare staff must efficiently assess patients' status and undertake risk stratification, particularly in a triage setting. The complete blood count is a easily available, cost-effective, and time-saving testing procedure that may be useful in supporting those processes. This review examines changes in complete blood count parameters in COVID-19 patients with the goal to identify the most common changes in the parameters and their relationship to illness progression and severity. Anemia, increased red blood cell distribution width, decreased white blood cell count, significant alterations in white blood cell differential count, and thrombocytopenia were all prevalent symptoms in COVID-19 patients. Changes in complete blood count values and their magnitude are thought to provide useful information regarding illness severity and prognosis. Finally, COVID-19 patients have a consistent pattern of abnormalities in complete blood count values that are linked to illness development, severity, and prognosis. Given the useful information offered by the test, it is envisaged that complete blood count evaluation would play a significant role in COVID-19 management.

Key words: COVID-19, The Complete Blood Count.



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Introduction:

The unique Covid-19 virus has spread over the world, and the number of cases continues to rise. SARS-CoV-2 is an enclosed, spherical, single-stranded RNA virus that is genetically linked to the earlier SARS-CoV and Middle East respiratory syndrome (MERS-Cov) coronaviruses, which caused a substantial number of respiratory infections worldwide in the previous two decades. The symptoms of coronavirus disease 2019 (COVID-19), which is caused by a viral infection, ranging from asymptomatic to severe pneumonia and multi-organ failure, resulting in a high rate of morbidity and fatality. Fever, a dry cough, and exhaustion are the symptoms. COVID-19 patients are becoming more common at a faster rate, putting a greater strain on healthcare facilities and staff. In many parts of the world, hospitals and intensive care units have seen a significant increase in patient volume, and some have even been overburdened with patients with mild to severe COVID-19. The current circumstance may need physicians and healthcare workers evaluating patients' situations and estimating their risk and prognosis as soon as possible to provide suitable and necessary procedures and treatments for the patients' well-being¹.

The complete blood count is a widely available, cost-effective, and time-efficient test that could be utilized to help with COVID-19 management. According to certain research, a full blood count can help doctors assess a patient's status and predict their prognosis. Healthcare staff, including those on the front lines such as emergency/field hospitals and emergency wards, can easily understand complete blood count test findings. The rising number of cases, combined with overburdened healthcare facilities, necessitates healthcare staff quickly assessing patients' conditions and performing risk stratification in order to treat them appropriately especially when dealing with a triage situation. This review examines changes in total blood count parameters (erythrocytes, leukocytes, and platelets) in COVID-19 patients to identify common changes in the parameters and their relationship to disease development and severity².

Complete blood counts are a common, generally inexpensive, time-efficient, and easily interpretable laboratory test that can provide reliable information on a patient's hematologic parameters. Routine complete blood counts using automated hematology analyzers can reveal the estimated quantity of red blood cells, white blood cells, and platelets in circulation, as well as their characteristics and differential count³. In the last few decades, technological advancements have enabled increasingly complex approaches to be employed in automated hematology analyzers. A blood sample is typically sucked by the machine, separated into numerous streams, and then combined with other substances. In the last few decades, technological advancements have enabled increasingly complex approaches to be employed in automated hematology analyzers. Typically, the machine aspirates a blood sample, which is subsequently separated into numerous streams and mixed with various buffers for examination. Differential lysis, which uses different strengths of detergent to separate distinct types of leukocytes, fluorescent dyes, and reagents to quantify hemoglobin and myeloperoxidase-containing leukocytes are just a few examples⁴. The following are some of the common principles utilized in automated hematology analyzers, but they are not exhaustive. To begin, light scatters from different angles to reveal cell size, nuclear lobulation, and cytoplasmic granularity. Second, electrical impedance and conductivity can be used to determine the size of blood cells, for example. Third, fluorescence or light

absorbance of labeled cells, as used in nucleic acid content measurements and reticulated blood cell analysis⁵. A complete blood count is commonly used to screen for disease and abnormalities, as well as to identify problems or disorders that require special care before undergoing a medical operation. They can also be used to help with diagnosis and to track the progress of patients through serial examinations⁶.

2. WBCs parameters in patients Covid-19 infected

This may cause compensation hyperplasia of the erythroid cell line, resulting in the release of Leukocyte differential count may provide further information and serve as a predictor for the severity of the patient's disease and prognosis. When compared to healthy people, COVID-19 patients have reduced eosinophil and lymphocyte levels and greater neutrophil and monocyte levels^{7,8}. (Tanni et al. 2020) from New York found that many COVID-19 patients had a low or absent eosinophil count, with 34% of the patients having a low absolute eosinophil count and 60% having no eosinophils.

During the follow-up, the group of patients discovered that many COVID-19 patients had eosinopenia, and that the dynamics of eosinophil levels may be used to predict and measure disease severity. Patients whose eosinophil levels increased quickly had a lower severity than those whose eosinophil levels increased slowly⁹. The pathophysiology of a fall with no eosinophil results in a higher fatality rate than a fall with eosinopenia, therefore an eosinophil count can help determine whether a patient has a more serious condition. (Sun *et al.* 2020) of COVID-19 eosinophil level white blood cells, also known as leukocytes, play an important part in immunity and the host's response to infection. Numerous studies have highlighted the importance of evaluating white blood cells, and the differential count serves an important role in confirming the COVID-19 diagnosis and predicting disease severity. Patients with COVID-19 had lower leukocyte levels than healthy people, according to research by Peng et al. (2020) and Sun et al. (2020). Patients with severe disease, on the other hand, have greater leukocyte counts during assessment than those with severe cases^{10,11}.

Increased neutrophils and lymphopenia are the differential count components that have been highlighted, increasing in the neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR). Researchers discovered that the alterations are statistically significant and are connected to the severity and outcome of the disease. Patients with significant lymphopenia and greater NLR and MLR have a higher risk of developing severe illness and developing acute respiratory distress syndrome (ARDS)¹². According to (Liu *et al.* 2020), the frequency of in-hospital deaths is much higher in patients with higher NLR, with an 8% increased risk of in-hospital mortality for each unit rise in NLR.

Infections also have a significantly decreased immune response and ability to fight infections, making them more susceptible to bacterial co-infection, allowing for a further rise in neutrophil levels, as well as other markers of inflammation and infection including C-reactive protein and pro-calcitonin¹³. It has now been identified as an independent risk factor for COVID-19 patients' in-hospital mortality¹⁴. Patients with higher NLR are more likely to acquire more disease severity and have a higher mortality rate than those with lower NLR, according to a meta-analysis conducted by (Chan *et al.*, 2020) , (Zeng *et al.* 2020)^{15,16}.

In bacterial and viral infections, increased neutrophils and monocytes are prevalent. Neutrophil proliferation is a common physiological response in infections and plays an

important role in infection protection¹². Patients who are suffering from Patients infected with MERS-CoV have a higher MLR, which may be related to the virus that causes COVID-19 to some extent. In MERS, the infection caused an increase in certain cytokines (IL-6 and IFN-) and chemokines (IL-8, CXCL-10, and CCL-5) that increased monocyte levels in some patients, although individuals with a higher severity have a lower percentage of monocytes¹⁷.

In COVID-19, lymphopenia is a common finding. the study by China, (Huang *et al.* 2020) discovered that about half of COVID-19 patients had lymphopenia⁵. The rise in NLR and MLR was largely attributed to the decrease in lymphocyte count. Although the underlying etiology of lymphopenia is unknown, various explanations have been proposed. This could be linked to the virus's capacity to infect T cells via the Angiotensin converting enzymes (ACE2) receptor and CD147-spike proteins. SARS-CoV-2 is anticipated to be capable of directly infecting and destroying lymphocytes. The virus may multiply and infect even more people as the infection progresses¹⁸.

This resulted in a long-term inflammatory response and constant T cell activation. T cell depletion will ultimately occur as a result of this. T cells that have been overworked will be unable to operate properly, limiting their ability to moderate and control infection and inflammation. Uncontrolled inflammation can trigger a cytokine storm, exacerbating the condition. The cytokine storm will eventually cause lymphocyte anti-proliferation and death, resulting in lymphopenia. COVID-19-induced damage to lymphatic organs, which results in spleen atrophy and lymph node necrosis in some patients, may also result in lymphopenia. Patients with severe COVID-19 may also have an increase in their blood pressure¹⁹. Leukocyte counting and differential counts are widely available, reasonably priced, and quick. Furthermore, it may provide numerous benefits in terms of supporting diagnosis, predicting and evaluating disease severity, and thus it may be helpful to begin using leukocyte assessment in the management of COVID-19²⁰.

3. CBC of the blood patients infected with Covid -19

Anemia was seen in a substantial number of COVID-19 patients, according to an analysis of erythrocyte parameters in COVID-19 patients. According to (Huang *et al.* , 2020) 38,2 % of COVID-19 patients their hemoglobin levels drop²¹. This finding is consistent with (Taneri *et al.*, 2020) meta-analysis' which indicated tht the mean hemoglobin level of COVID-19 patients meets the WHO's definition of anemia in men (130 g/L). Hemoglobin levels dropped with age, comorbidities, and illness severity⁶. The severity of COVID-19 disease is linked to the degree of hemoglobin depletion. Several investigations have discovered that hemoglobin COVID-19 patients with severe disease and those referred to Intensive Care Unite ICU had considerably lower levels than those with the mild and moderate diseases who did not require ICU admission. Patients who do not survive have much lower hemoglobin levels than those who do, allowing hemoglobin levels to be used as a prediction or warning system for illness severity through measurement and monitoring. In their investigation (Henry *et al.* 2020) discovered that some COVID-19 had a lower hematocrit level and a wider red blood cell distribution width RDW. Hematocrit and hemoglobin levels tend to drop as illness severity increases, whereas RDW rises in the opposite direction. Increasing in the opposite over time. Greater RDW was also linked to 9-fold increased risk of severe COVID-19 and a 16-fold increased risk of severe acute kidney injury, indicating

that RDW can be used as a predictor of disease severity and renal injury consequences⁹. The etiology of COVID-19's anemia is still unknown. Anemia may result from cytokine-mediated inhibition of bone marrow erythropoiesis activity and decreased erythrocyte lifespan in a variety of chronic illnesses¹⁰. COVID-19's anemia-causing mechanism is unknown, and numerous possibilities have been offered. Moreover, the hypoxia generated by the SARS-CoV-2 virus's activity in the lung tissue and its gas exchange structures may create systemic inflammation, affecting iron metabolism. Besides being inflamed, iron metabolism may be harmed as a result of the innate immune system's response to prevent viral reproduction. To multiply inside a human body, the virus requires a faster metabolism and a sufficient amount of iron. The innate immune system will respond by activating signaling pathways that cause the liver to create more hepcidin hormone, which inhibits the action of the transporter ferroportin, which transfers iron out of cells, resulting in less iron being absorbed from the diet in the digestive system. Long-term infection can reduce the amount of iron supplied for erythropoiesis, lowering hemoglobin levels⁶. Infection with SARS-CoV-2 can cause direct injury to the bone marrow, disrupting the erythropoiesis process even more⁹.

An increase in RDW indicates the presence of anisocytosis, a disorder in which the size and volume of red blood cells vary noticeably. Higher RDW is linked to worse outcomes in a variety of clinical disorders, including heart illness, sepsis, pneumonia, and renal disease. 4 A number of theories have been proposed to explain the cause of anisocytosis in COVID-19 patients, particularly those with severe disease. COVID-19 infection can damage bone marrow, impairing the erythropoiesis process. This could cause compensatory hyperplasia of the erythroid cell line, resulting in the discharge of immature red cells into the bloodstream. the disease can also cause aberrant lipid metabolism and fragmentation of structural protein components, which can lead to a breakdown of structural membrane homeostasis and circulating red blood cell structural integrity^{9,11}.

COVID-19 infection may cause compensatory hyperplasia of the erythroid cell line, resulting in the release of widely known coagulation disorder and formation of microthrombi in blood vessels, further disrupting the structural integrity of red blood cells, causing size variation, anisocytosis, and increased RDW. The production of microthrombi is suspected to be the cause of acute renal injury in some COVID-19 patients. Acute kidney injury may cause a disturbance in erythropoietin production, resulting in additional erythropoiesis deficiencies and, as a result, more severe anemia and anisocytosis⁹.

4. Platelets evaluations with covid-19 infected case

Platelet parameter assessment and evaluation may have a role in COVID-19 management. Thrombocytopenia is a condition that occurs as a result of a variety of viral infections and is common in critical illnesses. It could indicate significant organ dysfunction as well as the onset of intravascular coagulopathy¹⁹. (Guclu *et al.* 2020) discovered that 25.1 % of COVID-19 patients have thrombocytopenia at the time of admission to the hospital²⁰. Thrombocytopenia has also been linked to disease severity and patient survival in COVID-19, according to several studies. Patients with severe COVID-19 had a lower platelet count than individuals with a milder form of the disease, according to a meta-analysis by Lippi *et al.* (2020)¹⁹.

Platelet count in non-surviving patients is much lower than in survivors, according to

several studies. 20–22 Surviving patients had a considerably higher platelet count mean of (207.69 K/uL) than non-survivors (180.59 K/uL), according to (Guclu *et al.* 2020)²⁰. According to data from Wuhan, patients with thrombocytopenia had a greater mortality rate than those with a normal thrombocyte count (30.9 % vs. 8.9 % and a 40 %) reduction in mortality risk occurs with every (50 x 10⁹/L) elevated in platelet count²¹.

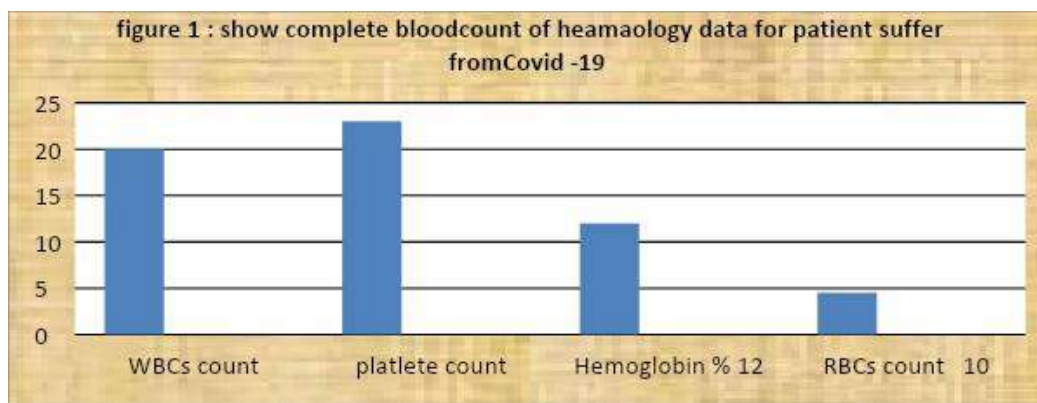
COVID-19 thrombocytopenia is likely multifactorial. It could be caused by SARS-CoV-2 attacking the bone marrow directly. ACE2 surface receptors are also found on hematopoietic stem cells in the bone marrow, allowing the virus to infect and reproduce the cells before inducing apoptosis²². ACE2 receptors can also be identified on stromal cells in the bone marrow and liver cells that produce thrombopoietin (TPO), a substance that promotes thrombopoiesis, megakaryocyte maturation, and differentiation. Thrombopoiesis may be inhibited as a result of this mechanism, resulting in thrombocytopenia. Viral infection can also cause the production and release of antibodies and immune complexes, which can bind to platelets and cause them to break down. Because some cytokines also block thrombopoiesis, prolonged inflammation and cytokine storm may exacerbate platelet loss. COVID-19 infection, as is well known, can harm lung tissue and pulmonary endothelial cells, resulting in endothelial cell death and vascular leakage. Damage to those tissues activates platelets, which causes platelet aggregation and encapsulation, resulting in coagulation and platelet exhaustion in the micro vascular lungs. In the lungs, megakaryocyte maturation, fragmentation, and platelet formation may occur in the capillary beds. Damage to lung tissue may reduce the availability of functioning capillary beds and lead to platelet production deterioration, exacerbating thrombocytopenia^{23,24}. In COVID-19 individuals, a lower platelet count combined with lymphopenia resulted in a greater platelet-to-lymphocyte ratio (PLR). In their study, (Peng *et al.* 2020) discovered that COVID-19 patients have a higher PLR than healthy subjects. PLR levels were also observed to be higher in a group of patients who had ARDS as compared to those who did not, and these differences were statistically significant¹².

The mean platelet volume (MPV) and platelet distribution width are two platelet indices that have recently received a lot of attention (PDW). Platelet activation is indicated by increased MPV and PDW. This could be due to damage to body organs and long-term inflammation, which causes an increase in cytokine and chemokine release. Platelets with a bigger volume create more thromboxane A₂, platelet thrombopoietin A, and beta-thromboglobulin, as well as more dense granule. These characteristics allow bigger platelets to be more reactive and prothrombotic. PDW elevation is common in coagulation disorders characterized by hyper-coagulation, such as cardiovascular disease²⁵. This could clarify why the evidence gathered was inconclusive.

This could explain why COVID-19 individuals have increased MPV and PDW, according to data from studies in China and Turkey. Furthermore, patients with higher illness

severity and mortality had higher MPV and PDW levels, suggesting that these parameters could be used to assess patients' health and predict disease severity and death^{11,22}.

A complete blood count (CBC) is a simple, inexpensive, time-saving, easily interpretable test that is generally available, even in remote regions. In COVID-19, complete blood count analysis may be useful for monitoring patients' status, prognosis, and/or risk stratification, as well as forecasting disease severity.



The literature on laboratory hematology showed a substantial growth between December 1, 2019, and July 3, 2020. Further analysis, however, uncovers deficiencies in research methods and subject matters²⁶. Retrospective research, letters to the editor, and narrative reviews are highly recommended. Unfortunately, in a review of the COVID-19 literature, both prospective and case-control studies were found to be underrepresented²⁷. Furthermore, the number of articles classified as recommendations or consensus statements remains low. Probably because of the pandemic's rapid global spread and the problems it poses to national and international institutions. Despite the enormous number of studies providing laboratory data, hematological parameters are often overlooked. Approximately 40% of research focuses primarily on clinical and epidemiological aspects of Sars-Cov-2 illness, with laboratory data being reported seldom. China has been by far the largest contributor, and many studies labeled as reviews and meta-analyses by academics from other parts of the world are based on data from China²⁸. This knowledge is difficult to generalize. Still, progress has been made. There has, however, been a rapid rise in the Covid-19 literature in the laboratory hematology disciplines, in particular coagulation, automated hematology, and cell morphology²⁹.

The clinical hematology laboratory, according to research conducted in China and abroad, plays a significant role by supplying the clinical team with a variety of valuable prognostic markers (Table 1). Although some information is based on limited data and should be confirmed via more research, the present evidence clearly establishes "the clinical hematology laboratory" as a key collaborator in the triage and care of impacted individuals. "Apart from RT-PCR testing for the organism, laboratory tests" for COVID-19 diagnosis have not been evaluated for sensitivity or specificity, despite their value as prognostic indications³⁰.

Table 1: Important hematologic indicators in Covid-19 infection.

hematological	Clinical
Thrombocytopenia	Consumptive coagulopathy
Leukocytosis	Bacteria superinfection
Neutrophilia	cytokine storm
Lymphopenia	Defective host response
Monocyte ,Eosinophilia	Elevated percentages

In conclusion, the COVID-19 pandemic has posed a substantial challenge to the

international hematology laboratory community. More than ever, hematology labtechnicians' professionalism and collegiality are vital to the mission's success in properly combating this risk. This paper has emphasized the important educational role that the professional societies play, as well as the significance of lab information in the treatment of COVID-19. A common CBC analysis result in COVID-19 patients is:

- Hemoglobin, hematocrit, and RDW levels are all lower in erythrocytes.
- Wbcs parameters and differential count: lower leukocyte level, but substantially greater in patients with severe disease, increased neutrophil, lymphopenia (and consequently elevated NLR and MLR), and eosinopenia up to eosinophil absence.
- Platelet parameters: Thrombocytopenia, elevated PLR, and elevated MPV and PDW are all significant.

To check the reliability and accuracy of the use of complete blood count evaluation in evaluating and monitoring patients' condition, risk stratification, and predicting disease severity and mortality in COVID-19, more research involving a larger number of samples and/or patients with more diverse demographical characteristics is needed. Despite the fact that complete blood counts are routinely performed in COVID-19 patients, the values of their parameters have not been consistently employed in disease severity categorization or outcome evaluation, to the researchers' knowledge.

5. Conclusion

COVID-19 individuals have a consistent pattern of abnormalities in complete blood count values that is linked to illness development, severity, and prognosis. It is hoped that this time-saving, cost-effective, readily accessible, and explainable technique will be effective in the management and treatment of the COVID-19 epidemic.

References

1. Kannan S, Ali Pakeer PSS, Ali AS, Hemalatha K. COVID-19 (Novel Coronavirus 2019)
2. Recent Trends. *Eur Rev Med Pharmacol Sci.* 2020;24(4):2006-2011.
3. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-To-Lymphocyte Ratio As An Independent Risk Factor For Mortality In Hospitalized Patients With COVID-19. *J Infect.* 2020;81(1):e6-12.
4. George-Gay B, Parker K. Understanding The Complete Blood Count With Differential. *J Perianesthesia Nurs.* 2003;18(2):96-117.
5. Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al. *Williams Hematology.* 9th Ed. New York: McGraw-Hill; 2015.
6. Huang Y, Tu M, Wang S, Chen S, Zhou W, Chen D, et al. Clinical Characteristics Of Laboratory Confirmed Positive Cases Of SARS-CoV-2 Infection In Wuhan, China: A Retrospective Single Center Analysis. *Travel Med Infect Dis.* 2020;36(February):1-3.
7. Taneri PE, Gómez-Ochoa SA, Llanaj E, Raguindin PF, Rojas LZ, Roa-Díaz ZM, et al. Anemia And Iron Metabolism In COVID-19: A Systematic Review And Meta-Analysis. *Eur J Epidemiol [Internet].* 2020;35(8):763-73. Available from: <https://doi.org/10.1007/s10654-020-00678-5>
8. Giacomelli A, Ridolfo AL, Milazzo L, Oreni L, Bernacchia D, Siano M, et al. 30-Day Mortality In Patients Hospitalized With COVID-19 During The First Wave Of The Italian Epidemic: A Prospective Cohort Study. *Pharmacol Res.* 2020;158(May).
9. Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGE, Tan GB, et al. Hematologic Parameters In Patients With COVID-19 Infection. *Am J Hematol.* 2020;95(6):E131-4.
10. Henry BM, Benoit JL, Benoit S, Pulvino C, Berger BA, de Olivera MHS, et al. Red Blood Cell Distribution Width (RDW) Predicts COVID-19 Severity: A Prospective, Observational Study From the Cincinnati SARS-CoV-2 Emergency Department Cohort. *Diagnostics.* 2020;10(9):1-9.
11. Leach M. Interpretation Of The Full Blood Count In Systemic Disease – A Guide For The Physician. *J R Coll Physicians Edinb.* 2014;44(1):36-41.
12. Wang C, Deng R, Gou L, Fu Z, Zhang X, Shao F, et al. Preliminary Study To Identify Severe From Moderate Cases Of COVID-19 Using Combined Hematology Parameters. *Ann Transl Med.* 2020;8(9):593-593.
13. Peng J, Qi D, Yuan G, Deng X, Mei Y, Feng L, et al. Diagnostic Value Of Peripheral Hematologic Markers For Coronavirus Disease 2019 (COVID-19): A Multicenter, Cross-Sectional Study. *J Clin Lab Anal.* 2020;(June):1-10.
14. Sun S, Cai X, Wang H, He G, Lin Y, Lu B, et al. Abnormalities Of Peripheral Blood System In Patients With COVID-19 In Wenzhou, China. *Clin Chim Acta [Internet].* 2020;507(April):174-80. Available from: <https://doi.org/10.1016/j.cca.2020.04.024>
15. Tanni F, Akker E, Zaman MM, Figueroa N, Tharian B, Hupart KH. Eosinopenia And Covid-19. *J Am Osteopath Assoc.* 2020;120(8):504-8.
16. Chan AS, Rout A. Use of Neutrophil-To-Lymphocyte And Platelet-To- Lymphocyte Ratios in COVID-19. *J Clin Med Res.* 2020;12(7):448-53.
17. Zeng F, Li L, Zeng J, Deng Y, Huang H, Chen B, et al. Can We Predict The Severity Of Coronavirus Disease 2019 With A Routine Blood Test? *Polish Arch Intern Med.* 2020;130(5):400-6.
- 18.
- 19.

20. Yang L, Liu S, Liu J, Zhang Z, Wan X, Huang B, et al. COVID-19: Immunopathogenesis And Immunotherapeutics. *Signal Transduct Target Ther* [Internet]. 2020;5(1):1–8. Available from: <http://dx.doi.org/10.1038/s41392-020-00243-2>
21. Fathi N, Rezaei N. Lymphopenia in COVID-19: Therapeutic Opportunities. *Cell Biol Int*. 2020;44(9):1792–7.
22. Lippi G, Plebani M, Henry BM. Thrombocytopenia Is Associated With Severe Coronavirus Disease 2019 (COVID-19) Infections: A Meta-Analysis. *Clin Chim Acta*. 2020;506:145–8.
23. Güçlü E, Kocayiğit H, Okan HD, Erkorkmaz U, Yürümez Y, Yaylacı S, et al. Effect Of COVID-19 On Platelet Count And Its Indices. *Rev Assoc Med Bras*. 2020;66(8):1122–7.
24. Zhao X, Wang K, Zuo P, Liu Y, Zhang M, Xie S, et al. Early Decrease In Blood Platelet Count Is Associated With Poor Prognosis In COVID-19 Patients—Indications For Predictive, Preventive, And Personalized Medical Approach. *EPMA J*. 2020;11(2):139–45.
25. Liu Y, Sun W, Guo Y, Chen L, Zhang L, Zhao S, et al. Association Between Platelet Parameters And Mortality In Coronavirus Disease 2019: Retrospective Cohort Study. *Platelets*. 2020;31(4):490–6.
26. Xu P, Zhou Q, Xu J. Mechanism Of Thrombocytopenia In COVID-19 Patients. *Ann Hematol*. 2020;99(6):1205–8.
27. Zhang Y, Zeng X, Jiao Y, Li Z, Liu Q, Ye J, et al. Mechanisms Involved In The Development Of Thrombocytopenia In Patients With COVID-19. *Thromb Res*. 2020;193:110–5.
28. Salamanna F, Maglio M, Landini MP, Fini M. Platelet Functions And Activities As Potential Hematologic Parameters Related To Coronavirus Disease 2019 (Covid-19). *Platelets*. 2020;31(5):627–32.
29. Lippi G, Plebani M. The critical role of laboratory medicine during coronavirus disease 2019 (COVID-19) and other viral outbreaks. *Clin Chem Lab Med*. 2020; 58(7): 1063- 1069.
30. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol*. 2020; 95(6): e131- e134.
31. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (Covid-19) infections: A meta-analysis. *Clin Chim Acta*. 2020; 506: 145- 148.
32. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med*. 2020; 58(7): 1021- 1028
33. Liu X, Zhang R, He G. Hematological findings in coronavirus disease 2019: indications of progression of disease. *Ann Hematol*. 2020; 99(7): 1421- 1428.

RADON CONCENTRATION AND ITS EXLATION RATE IN TEA SAMPLES

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RADON CONCENTRATION AND ITS EXHALATION RATE IN TEA SAMPLES

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Abstract:

In the various houses and cafes on the market, social spaces, and tourism, Iraqis are among the most tea-drinking people. Several experts in Iraq and around the globe are interested in investigating the radiation activity of tea in order to determine the radiation hazards linked with both the lives and health of tea addicts. In this research, A plastics nuclear path detector (CR-39) was used to explain the results of radon concentrations and Rn-222 exhalation rates in nine tea specimens. Specimen were taken from a variety of regional Iraqi markets. The chemical then drilling with NaOH solution at 70°C for 8 hours to identify the subsurface paths, which were subsequently analyzed with sufficient resolution by optical microscopes. The radon levels varied between (137.129 - 25.4092) Bq/cm³, with the mean being 72.687 Bq/cm³. The percentage of radon exhalation varied from (15.76981– 2.922053), with an average of 8.359 mBq/m³. h. Exhalation percentage and radon levels have a positive connection (R² = 0.893). The results of the specimen revealed that its health hazards were not harmful. The findings of research study were contrasted to the findings of other studies that contained other food products and concluded that they were within globally allowed limits and did not pose a hazard to human health and life.

Key words: Closed-Can Technique, Imported Tea Brands, Radon Exhalation Rate.



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Introduction:

Humans are introduced to varied quantities of radiation, which is either environmental or man-made for different causes. The human's body could be polluted with radioisotopes by consuming plants or the flesh of animals which eats plants that include radionuclide materials originating from the primary soil. The plant absorbs such materials alongside other organic components, which are categorized as production elements, including fluids and drinking water in which there is a modest dose in the water, breathing air, whose is considered principal source of radon gas present in the earth 's environment and is produced by the spontaneous decay of uranium, represents the major source of spontaneous radioisotope dose that penetrates into the human body. Tea is a fragrant beverage made by squeezing the therapeutic leaf of the tea plant with hot or boiling water. Several techniques and procedures for measuring the amount and activity of radioisotopes like uranium in natural samples have been published in the literature. These approaches can be categorized under three spectrums based on the types of radiation recorded: beta gamma, alpha, and. The actual type of the specimen, the specimen mass, and the specimen import must all be considered when selecting one of these spectrums. In the realm of radioactive pollution, the solid-state pathogenic approach can detect the intensity of any charged particles, including charged particles, and is categorized as alpha spectroscopic. Many scientists have utilized it to determine Uranium concentrations in ecological specimens [1,2]. Organic radionuclide concentrations in food are affected by varying foundations levels atmospheric conditions and agriculture conditions. The short half-life decay isotopes of radon (Rn-222) make up the majority of the exposure's levels [3]. The Rn-222 is a pale, unscented, and tasteless inactive gas that is imperceptible to the human senses. It is continuously generated via the decay of naturally occurring radioactive isotopes like Uranium-238, Uranium-235, and Thorium-232. Uranium is found in almost all minerals, plants, and soil. The radioisotope ^{222}Rn , produced by the breakdown of U-238, is the primary source of internal radon exposure to humans (about 55 percent) [4,5]. Radon gas rapidly travels from the earth into the air and decomposes into radon progeny or radon daughter, which are short-lived degradation particles. It decomposes through alpha emission with a half-life of 3.82 days and it has 5.5 MeV of energy [4-6]. Because radon levels in soil and water vary greatly from location to location, the general populations exposures to radon fluctuates greatly as well [7]. Scientists discovered in a latest report that the plant may absorb radon gas from the external environment. Radon can be absorbed by plants via their roots (indirect radioactivity) or via their airy organs (direct radioactivity) [8,9]. The transmission of radionuclides into people's dietary habits is facilitated through a few key mechanisms. Food, drink, and medicine are the main methods for radon to deliver a dose. Primitive radioisotopes as well as their progeny are conveyed to the human through the food supply chain according to their composition in soil and phosphoric composts [10]. To quantify prospective radiation exposure and assess the consumers subjected to radiation it is critical to test the radioactive material of food and water specimens. It is well established that long-term exposed to high concentrations of radon gas and its offspring could cause pathological effects such as pulmonary functioning alterations and the development of lung cancer [11]. To reduce these hazards, information of the expiration rate out from surface of construction substances allows one to assess their proportion to radon input into to the indoor area, thus radon concentrations and its impact on human health should be studied. For such assessment of public health

danger, measures of Ra-226 contents and Rn level and its clones are required. In Iraq, the daily overall intake of tea, specifically black tea, is around 0.5 liters, compared to roughly 2 liters of drinking water. As a result, special interest should be paid for the tea. Tea, like many other plants, is grown in soil that should include nutrients in order to produce a healthy and flavorful plant. A fertilizer includes certain uranium in its manufacturing process, that can be transferred into the tea plant as deposited in it. Therefore, under this research, the Rn-222 and Rn exhalation averages for nine various tea brand specimens inside the Iraqi market are estimated by using CR-39 SSNTD method for nine various kinds of tea that are frequently used by the Iraqi public.

Material and Method

Nine distinct tea specimens were acquired from the Iraqi market and analyzed. About 15 grams of tea was stored in little tubular containers that were labeled including an identifying code. With this research, the SSNTD, also referred as the CR-39 plastic sensor, has been used. These sensors were small square tiles 1 cm x 1 cm with such a width of 200µm as shown in Fig. 1. Nine specimens of indigenous and imported tea were acquired from local Iraqi markets to evaluate alpha contamination in tea. All tea specimens were kept for a month to achieve radioactive equilibrium between both the decay series of radium and radon. The sensors were taken from the containers after 30 days and chemically polished to reveal the alpha particle's nuclear routes utilizing a NaOH solution over seven hours and a 6.25 N in a boiling water at 70°C [11]. The detectors then cleaned using filtered water and instead dried via air after completing the chemical scaling procedure and checking the nuclear impacts. The impacts of alpha events onto the surface of the detectors are visually examined using a Nikon YS Alphhot Japan optical microscope with a resolution of x1000 (100X). In every detector, the alpha affects are recorded in at least 10 various positions Radon disintegration is a completely random statistical occurrence.

the CR-39 sensors were subjected to radon-affected specimens in the amount of air surrounding them. The continuity formula Eq.1 was used to relate the measured path density for radon as well as its daughter activity for each unit volume of space [13].

$$\rho = X * A \quad 1$$

where the ρ : the total for paths per square centimetre

X: a length-based fixed (cm)

The amount of alpha activity in a given volume is denoted by the letter A. (dissolution each unit time for cm²)

The sum of individual variations estimated for all isotopes is the amount of the fixed X (²¹⁴Po , ²²²Rn and ²¹⁸Po). The alpha-counting for radon activity and also its daughters is used to measure concentration for radon. The actual number for paths were estimated through processing an unprotected film detector CR-39 within similar etching conditions, and the path number was measured in terms of number for paths each mm². The output generated by etched sensor detection is integrated path density, (path. mm-2) reported on the detectors, The following formula Eq.2 is used to calculate the behavior of ²²²Rn concentration (CRn) Bq/m³

utilizing K_i , the mean value of the correction parameter of ^{222}Rn in $(\text{Bq} \cdot \text{day} \cdot \text{m}^{-3})$ per $(\text{paths} \cdot \text{mm}^{-2})$ where T is exposition period (day) [14]:

$$\text{CRn} = (\rho) / \text{TKi} \quad 2$$

in which K_i is the length calibrated factor, which is comparable to $(\text{tracks}/\text{m}^2/\text{d}$ per $\text{Bq}/\text{m}^3)$, while ρ is the record density.

All the radon concentrations are measured (Bq/m^3) in the SI units, as well as the daughters for radon were represented in Working Levels (WL), that is calculated as follows [15]:

$$\text{Cp(WL)} = (\text{F} * \text{CRn}) / 3700 \quad 3$$

F stands for the equilibrium variable, which should be set to $\text{FCRn} = 0.4$ [16].

Eq. 4 is also used to measure the annual effective dose in Working Level Month (WLM) units [16]:

$$\text{WLM} = (\text{F} * \text{t} * \text{CRn}) / (170 * 3700) \quad 4$$

Hence, the effective dose and amount of Radon are related as follows eq.:

$$E_{\text{ff}} = G C_{\text{Rn}} \quad 5$$

G is a constant in this equation.

Eq. 3 was used to quantify the effective alpha energy density (WL) for this analysis, while Eqs. 4 and 5 were used to measure annual effective dose equivalent (WLM/year) and effective dose, respectively.

By using Eq.6 the exhalation rate was calculated [17]:

$$E_x = \frac{C_t \lambda V}{S[t - 1/\lambda(1 - e^{-\lambda t})]} \quad 6$$

where E_x is Rn-222 exhalation rate in $(\text{mBq}/\text{m}^2 \cdot \text{h})$, C_t is mean Rn-222 concentration in (Bq/m^3) , V is size of plastic can in (m^3) , t is the time for exposure to Rn, λ is the rate of degradation and S stands for total area for surface where is Rn-222 is emitted into a sealed plastic can.

Results and discussion

Table 1 shows the total data for radon concentrations in Bq/m^3 , equilibrium equivalents Rn-222 concentrations (CEEC in Bq/m^3), and Average Effectiveness Dose Eff (in mSv/y) for nine tea specimens purchased for Iraqi market. The levels of radon were detected using a sealed can dosimeter, which is shown in Figure-1, this implies that the air was contained within the containers for the duration of the exposure.

Rn -222 concentrations in tea specimens averaged $72.687 \text{ Bq}/\text{m}^3$ on overall. As indicated in Fig.2, the highest percentage of Rn -222 was $137.129 \text{ Bq}/\text{m}^3$, while the lowest concentration was $25.4092 \text{ Bq}/\text{m}^3$ in the Ahmed scented tea specimen. The highest CEEC level was $54.8515 \text{ Bq}/\text{m}^3$ in the Ahmed scented tea specimen while the lowest was $10.1637 \text{ Bq}/\text{m}^3$ in the Prairie tea specimen according to the estimated CEEC values. The mean CEEC for ^{222}Rn was $29.074 \text{ Bq}/\text{m}^3$, indicating that the amount of radon released from the specimens is not solely dependent on the amount of Ra-226 present. For the entire tea

specimen collection, the total average result of the indicative (WLM) of ^{222}Rn quantities was estimated to be 0.407. In the Ahmed scented tea sample, the maximum result was 0.76792, while the lowest result was 0.14229 in the Prairie tea specimen.

The mean yearly active dose E for tea specimens was 1.831 mSv/y, with a highest of 3.45565 mSv/y in the Ahmed scented tea specimen and a lowest of 0.64031 mSv/y in the the Prairie tea specimen

The association among Eff in (mSv/y) and radon level activity (Bq/kg) is depicted in Fig. 4. The correlations are linearly rising, and effective matching equations could be obtained by Fig.4

$$\text{Eff.} = 0.0252 * C_{\text{Rn}} (\text{Bq/m}^3) \quad 7$$

For the entire collection of tea specimens, the mean result of Excess Lung Cancer per Million Persons per Year (ELC) was 1100.26. Ahmed scented tea specimens had the greatest average score of 2075.72, while Prairie tea specimens had the lowest average value of 384.619. This discrepancy in readings is also related to changes in the type of the tea specimens The association among ECL and radon level Bq/m^3 is seen in Figure 5. The continuity formula, based on this figure, is a good fit.

$$ELC = 15.137 C_{\text{Rn}} (\text{Bq/m}^3)$$

For entire tea data sample, the expected mean radon emission rate in (mBq/m².h) was 8.359. Ahmed scented tea specimen had the greatest rating of 15.76981, while Prairie tea specimen had the lowest value of 2.922053. Furthermore, the variances in the readings are related to variations in the quality of the tea specimens.

Conclusions

A closed can technological technique was used to detect radon concentrations and breathing rate. According to the findings, radon concentrations for tea specimens ranged from (25.4092 to 137.129) Bq/m^3 . Furthermore, the highest percentage ratio was observed in Ahmeed Perfume tea, while the lowest concentration ratio was identified in Prairie tea. It also indicates that certain tea specimens have the same mean radon content of 72.687 Bq/cm^3 . Surface exhalation rate and radon levels have a positive connection ($R^2 = 0.893$). In terms of health hazard impacts, the findings showed that specimens are acceptable. These values were below the ICRP's suggested action levels of 200-600 Bq/m^3 . The specimens examined did not present any health risks, depending to the findings.

Table and figures

Table 1: Summary of radon concentration examined in nine tea brands being sold in Iraqi markets

Serial No.	Tea Brand / Samples	Density /Track/cm2	CRn Bq/m ³	F	EEC=C*F (Bq/m ³)	WL*10 ⁻³	WLMY	Eff	ELC	Ex Rate*10 ⁻³
T1	Prairie tea	126	25.4092	0.4	10.1637	2.74694	0.14229	0.64031	384.619	2.922053
T2	Ahmad tea with cardamom	150	30.249	0.4	12.0996	3.27016	0.16939	0.76228	457.879	3.478635
T3	Apple tea	290	58.4814	0.4	23.3926	6.32231	0.3275	1.47373	885.233	6.725361
T4	Mahmoud tea	336	67.7578	0.4	27.1031	7.32516	0.37944	1.7075	1025.65	7.792142
T5	Lepton tea	390	78.6474	0.4	31.459	8.50242	0.44043	1.98191	1190.49	9.044451
T6	Ceylon tea with cardamom	397	80.059	0.4	32.0236	8.65503	0.44833	2.01749	1211.85	9.206787
T7	Deer tea	430	86.7138	0.4	34.6855	9.37447	0.4856	2.18519	1312.59	9.972087
T8	Ahmed English Tea	445	89.7387	0.4	35.8955	9.70148	0.50254	2.26142	1358.38	10.31995
T9	Ahmed Perfume tea	680	137.129	0.4	54.8515	14.8247	0.76792	3.45565	2075.72	15.76981
	Average	360.44	72.687	0.4	29.074	7.8580	0.407	1.831	1100.267	8.359

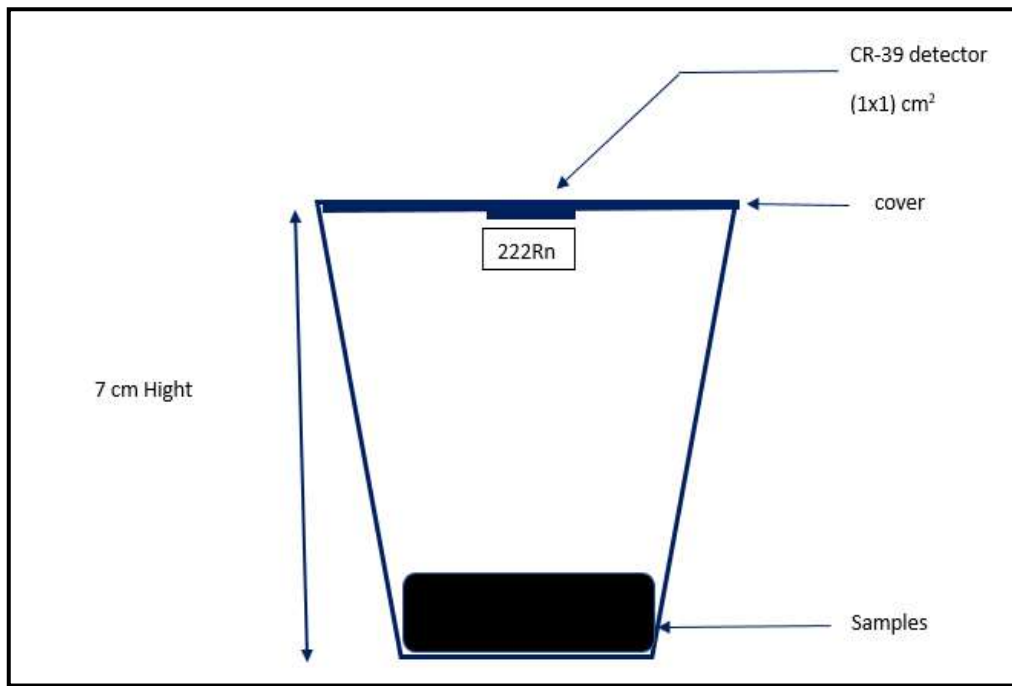


Figure 1: Schematic diagram showing the closed can technique for CR-39 with tea samples

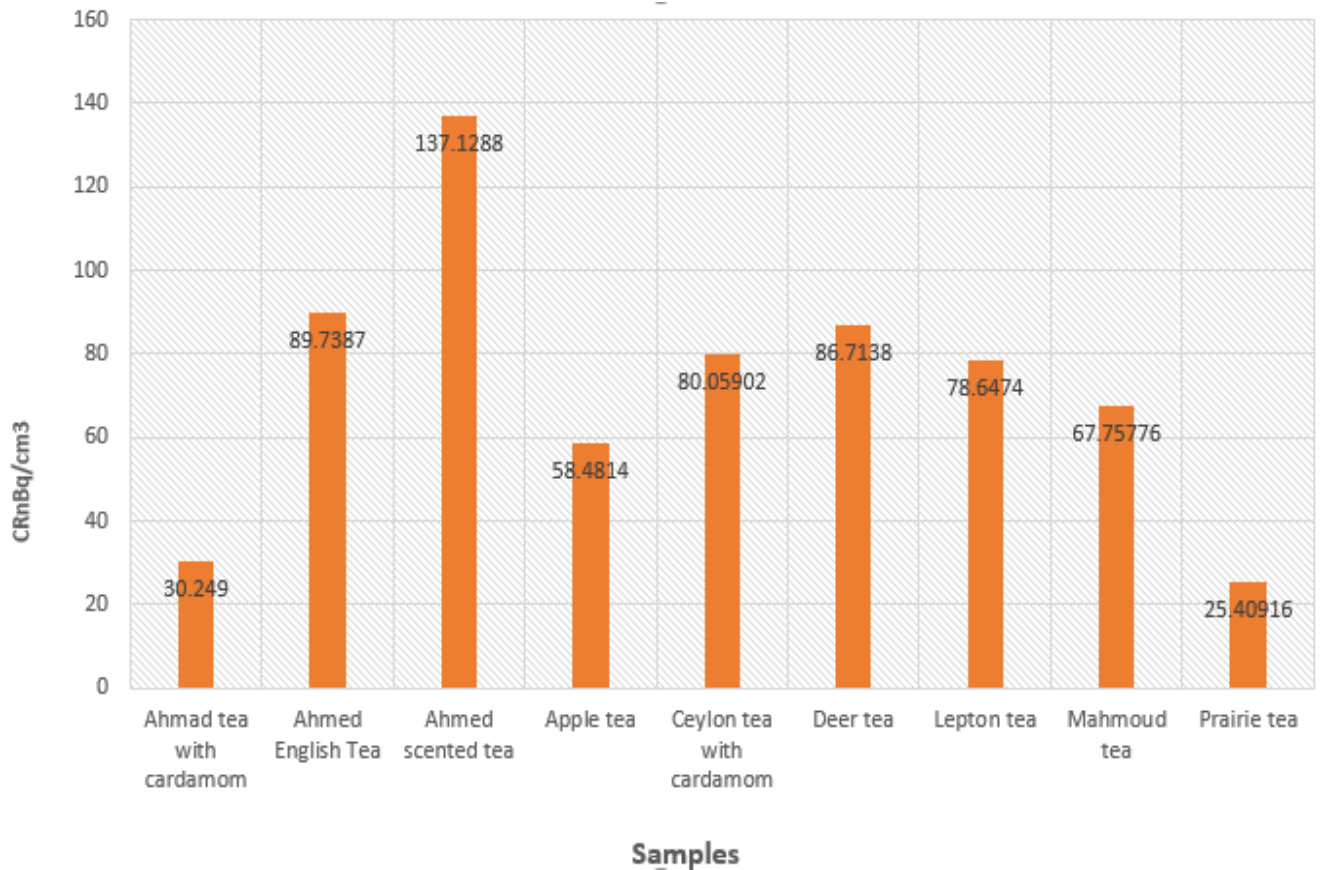


Figure 2. Radon 222- concentration in all tea samples.

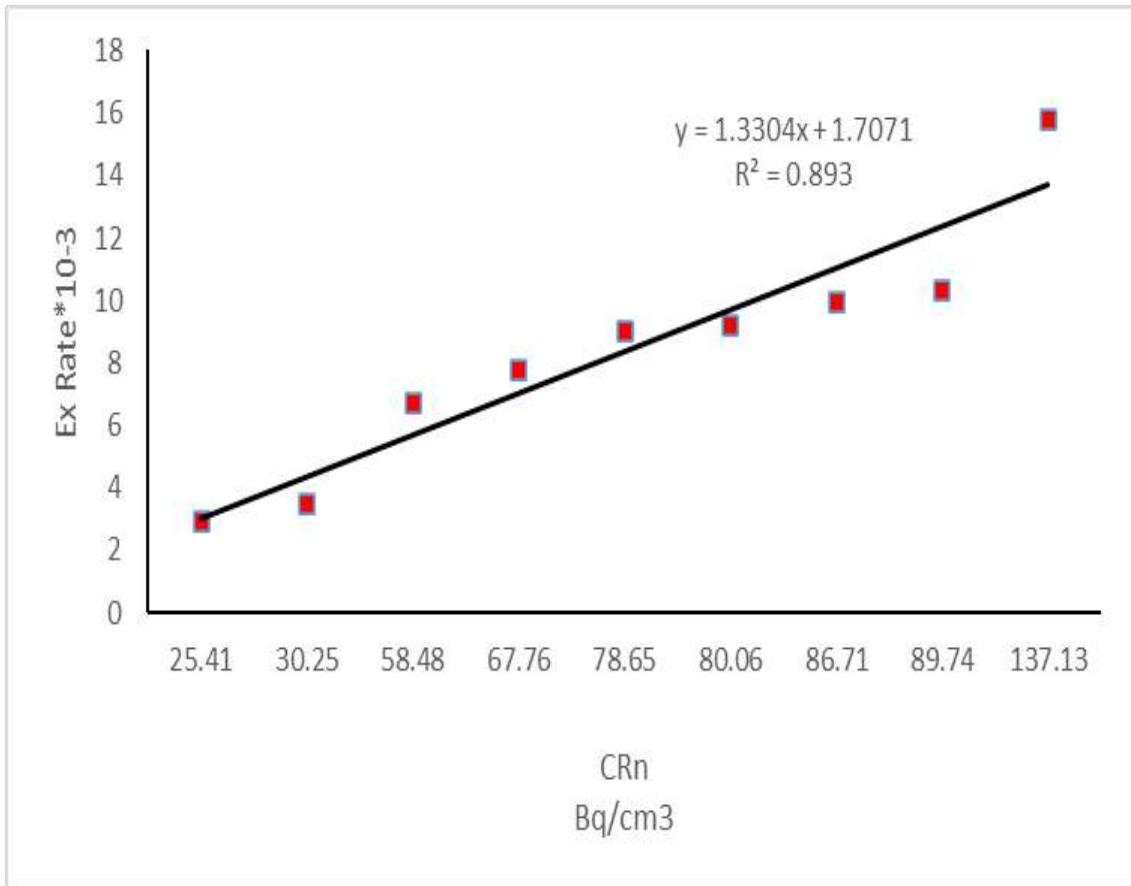


Figure 3: Relationship between (Ex Rate) and CRn Bq/m³

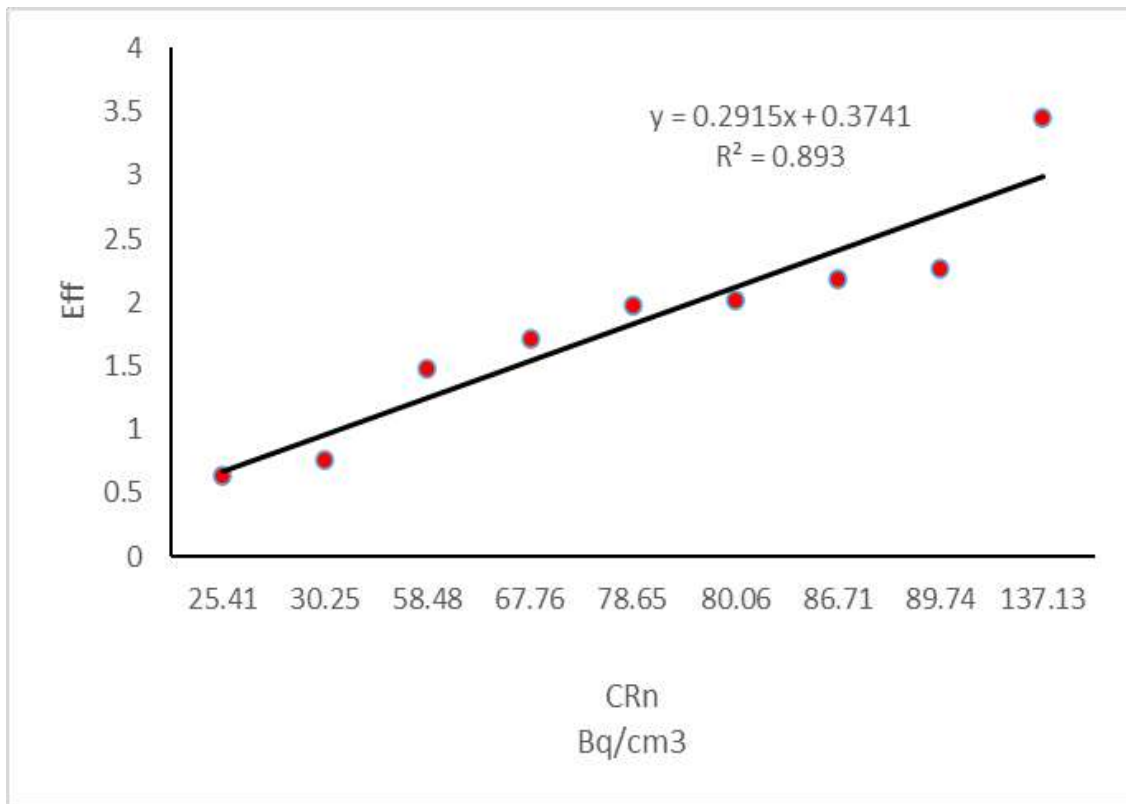


Figure 4: Relationship between (Eff) and CRn Bq/m³

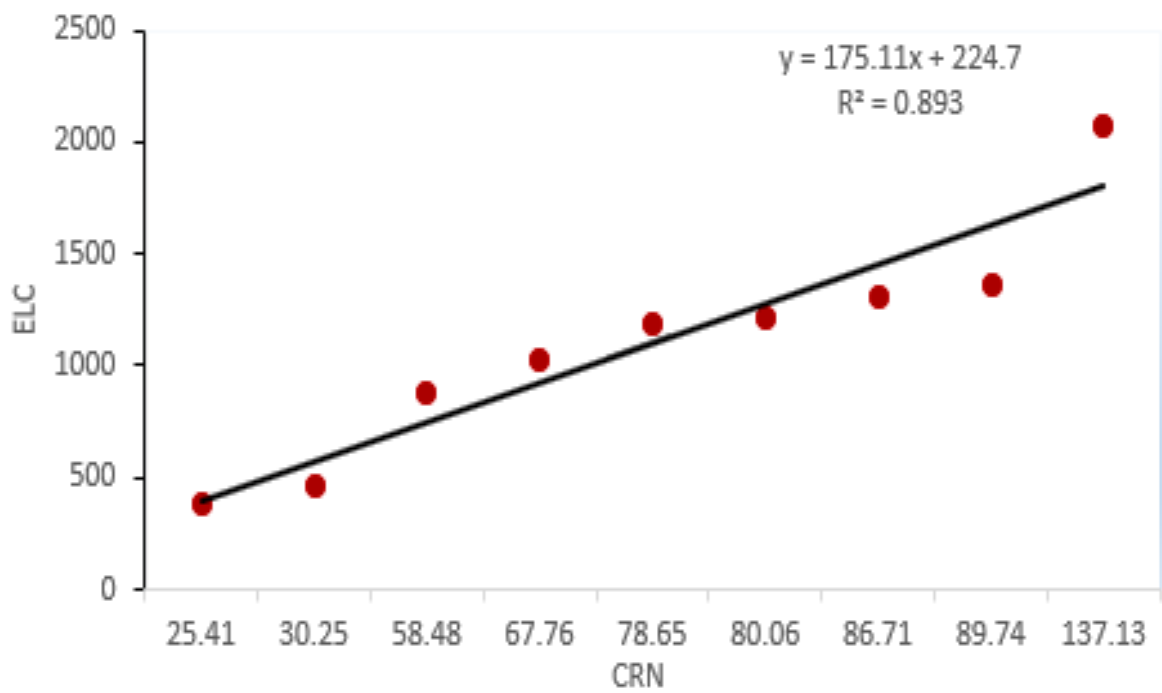


Figure 5: Relationship between (ELC) and CRn Bq/m³

References:

1. Mittal, S., S. S. Bhatti, A. S. Jodha, S. Saravanan, D. Gopalani, S. Kumar and P. C. Kalsi (1998). Proc. XI National Symp "On SSNTDs and applications", ed. Surinder Singh, Amritsar, 307.
2. Iyer, R. H. and N. K. Chaudhuri (1997). Development of the Track Registration Technique in Solid State Nuclear Track Detector from Solution Media and Its Application. *J. Rad. Meas*, 27(4) : 529-.548.
3. United Nations Scientific Committee on the Effect of Atomic Radiation Sources, Effects and Risks of Ionizing Radiation. Report to the General Assembly, with Scientific Annexes, United Nations, New York, 2000.
4. United Nations Scientific Committee on the Effects of Atomic Radiation Sources, Effects and Risks of Ionizing Radiation. Report to the General Assembly, with Scientific Annexes. United Nations, New York. 1988.
5. Aswood M S, Jaafar M S, Salih N. Estimation of annual effective dose due to natural radioactivity in ingestion of vegetables from Cameron Highlands, Malaysia. *Environmental Technology & Innovation*. 2017; 8: 96-102.
6. Adrović F, Jakupi B, Vasić P, Mitić D. Measurements of radon concentration. *Radiation measurements*. 1995; 25(1-4): 643-4.
7. United Nations Scientific Committee on the Effects of Atomic Radiation Sources, Effects and Risks of Ionizing Radiation. Report to the General Assembly, with Scientific Annexes. United Nations, New York (2000).
8. 6. P. Li, R. Zhang, M. Gu and G. Zheng, *Science of The Total Environment* 612, 436-441 (2018).
9. M. Poschl and L. M. Nollet, *Radionuclide concentrations in food and the environment*. (CRC Press, 2006).
10. Aswood M S, Jaafar M S, Bauk S. Measuring Radon Concentration Levels in Fertilizers Using CR-39 Detector. In *Advanced Materials Research*. 2014; 925: 610-3.
11. BEIR (Committee on Biological Effects of Ionizing Radiation) (1999). *Health Effects of Exposure to Radon*. BEIR VI. National Academy Press, Washington, DC.
12. Younis, M., Atia, Khetam Abd Alade, Mahood, B.H. and Hssoni, A.M. 2010. *Journal of Basra Searches*, 6: 336.
13. F. Abu-Jarad, F., Fremlin, J.h. and R. Bull, R. 1980. *Phys. Med. Biol.* 25: 683- 694 (1980).
14. Nada F. Tawfiq, Hussein M. Nasir and Rafaat Khalid, 2012. "Determination of Radon oncentrations in AL-NAJAF Governorate by Using Nuclear Track Detector CR-39", *Journal of Al-Nahrain University*, 15(1): 83-87.
15. Khan, A.J., Varhney, A.K., Prasad, R., Tyagi, R.K. and Ramachandran, T.V. 1990. *Nucl. Tracks. Radiat. Meas.*, 17(1990): 497-502.
16. UNSCEAR (United National Scientific Committee on the Effects of Atomic Radiation), Report to the General Assembly, United Nations, New York, (2010).
17. Qureshi, A.A., Kakar, D.M., Akram, M., Khattak, N.U., Tufail, M. and. Mehmood, K. 2000. *Journal of Environmental Radioactivity*, 48(2): 203-209.

**RELATIONSHIP OF ESSENTIALLY SEMISMALL QUASI-DEDEKIND
MODULES WITH SCALAR AND MULTIPLICATION MODULES**

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RELATIONSHIP OF ESSENTIALLY SEMISMALL QUASI-DEDEKIND MODULES WITH SCALAR AND MULTIPLICATION MODULES

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Abstract:

Let R be a ring with 1 and W is a left Module over R . A Submodule D of an R -Module W is small in W ($D \ll W$) if whenever a Submodule V of W s.t $W = D + V$ then $V = W$. A proper Submodule Y of an R -Module W is semismall in W ($Y \ll_S W$) if $Y = 0$ or $Y/F \ll W/F \forall$ nonzero Submodules F of Y . A Submodule U of an R -Module E is essentially semismall ($U \ll_{es} E$), if for every non zero semismall Submodule V of E , $V \cap U \neq 0$. An R -Module E is essentially semismall quasi-Dedekind (ESSQD) if $\text{Hom}(E/W, E) = 0 \forall W \ll_{es} E$. A ring R is ESSQD if R is an ESSQD R -Module. An R -Module E is a scalar R -Module if, \forall, \exists s.t $V(e) = ze \forall$. In this paper, we study the relationship between ESSQD Modules with scalar and multiplication Modules. We show that if E is scalar semismall quasi-prime R -Module. Then E is an ESSQD R -Module, we show that if E is faithful multiplication R -Module, thus E is an essentially semismall prime R -Module iff R is an ESSQD ring.

Key words: Essentially Semismall Quasi-Dedekind Modules, Scalar Modules, Multiplication Modules.



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Introduction:

A Submodule D of an \mathbb{R} -Module W is small in $W(D \ll W)$ if whenever a Submodule V of W s.t $W = D + V$ then $V = W$ [1].

A proper Submodule Y of an R -Module W is semismall in $W(Y \ll_S W)$ if $Y = 0$ or $Y/F \ll W/F \forall$ nonzero Submodules F of Y [2].

A Submodule U of an \mathbb{R} -Module E is essentially semismall($U \ll_{es} E$), if for every non zero semismall Submodule V of E , $V \cap U \neq 0$ [3].

An \mathbb{R} -Module E is essentially semismall quasi-Dedekind(ESSQD) if $\text{Hom}(E/W, E) = 0 \forall W \ll_{es} E$ [3].

A ring \mathbb{R} is ESSQD if \mathbb{R} is an ESSQD \mathbb{R} -Module[3].

An \mathbb{R} -Module E is a scalar \mathbb{R} -Module if, $\forall V \in \text{End}_R(E)$, $\exists z \in R$ s.t $V(e) = ze \forall e \in E$ [4].

An \mathbb{R} -Module E semismall quasi-invertible if $\text{Hom}(E/V, E) = 0, \forall 0 \neq V \ll_S E$ [5].

Proposition1: If W is a semismall quasi-invertible R -Submodule of E , then $\text{ann}(E) = \text{ann}(W)$.

Proof: Clearly $\text{ann}(E) \subseteq \text{ann}(W)$. let $w \in \text{ann}(W)$. Define $u : E/W \rightarrow E$ by $u(e+W) = re, \forall e \in E$. Clearly u is well-defined homomorphism. Then $u = 0$, therefore $w \in \text{ann}(E)$.

An \mathbb{R} -Module W is essentially semismall prime (ESSP) if, $\text{ann}_R(W) = \text{ann}_R(V)$ for all $V \ll_{es} W$.

Proposition2: Let E be an ESSQD R -Module, then E is an ESSP R -Module.

Proof: From Prop.1.

Proposition3: Let R be a ring. If R is a semiprime ring then R is an ESSQD ring.

Proof: Let $g \in \text{End}_R(E)$ s.t $\text{Ker } g \ll_{es} R$. Suppose $g \neq 0, \exists 0 \neq w \in R$ s.t $g(a) = wa \forall a \in R$. Since $\text{Ker } g \ll_{es} R$ and $0 \neq w \in R$, thus $\exists 0 \neq x \in R$ s.t $0 \neq wx \in \text{ker } g$, hence $0 = g(wx) = wg(x) = w^2x$. This implies $(wx)^2 = 0$. Since R is semiprime, $wx = 0$ which is a contradiction. Therefore $g = 0$, thus R is ESSQD ring.

Proposition4: Let E be an ESSP faithful R -Module. Then R is an ESSQD ring.

Proof: Let I be an ideal of R s.t $I^2 = (0)$. Suppose $I \neq (0)$. On the other hand, $IE \neq (0)$, if $IE = (0)$ implies $I \subseteq \text{ann}_R(E) = (0)$; that is $I = (0)$, a contradiction. But $IE \ll_{es} E$, If $IE \not\ll_{es} E$, since E is an ESSP faithful R -Module thus $\text{ann}_R(IE) = \text{ann}_R(E) = (0)$. Clearly $I \subseteq \text{ann}_R(IE) = (0)$, then $I = (0)$ which is contradiction, thus $IE \not\ll_{es} E$. Let W be a relative complement for IE , thus $IE \oplus W \ll_{es} E$. Then $\text{ann}_R(IE \oplus W) = \text{ann}_R(E) = (0)$, but $IW \subseteq IE$ and $IW \subseteq W$, thus $IW \subseteq IE \cap W = (0)$ and hence $I(IE \oplus W) = I^2E + IW = (0)$. Therefore $I \subseteq \text{ann}_R(IE \oplus W) = (0)$, hence $I = (0)$, a contradiction. Then our assumption is false. Thus $I = (0)$ and R is a semiprime ring. Thus, by Prop.3, R is an ESSQD ring.

Lemma5: Let E be an R -Module. Then E is an ESSP R -Module if and only if E is an ESSP \bar{R} -Module, where $\bar{R} = R/\text{ann}_R(E)$.

Proof: \Rightarrow) Let U be an essentially semismall \overline{R} -Submodule of E . Clearly U is an R -Submodule of E . Let V be a nonzero R -Submodule of E , thus V is an \overline{R} -Submodule of E and hence $U \cap V \neq (0)$. Then U is an essentially semismall R -Submodule of E , thus $ann_R(E) = ann_R(U)$. It follows that $ann_{\overline{R}}(U) = ann_{\overline{R}}(E)$, since

$r + ann_R(E) \in ann_{\overline{R}}(U)$, thus $(r + ann_R(E))U = 0$; that is $rU = 0$ and $r \in ann_R(U) = ann_R(E)$. Then $r + ann_R(E) \in ann_{\overline{R}}(E)$.

\Leftarrow) The proof of the converse is similarly.

Corollary6: If E is an ESSP R -Module, then $\overline{R} = R/ann_R(E)$ is an ESSQD ring.

Proof: It follows by [3, Cor. 18] and Prop 4.

In the following proposition If E is ESSQD \mathbb{R} -Module, then $End_R(E)$ will be ESSQD ring.

Proposition7: Assume that E is a scalar \mathbb{R} -Module with $ann_R(E)$ is a semiprime ideal of \mathbb{R} , thus $End_R(E)$ is ESSQD ring.

Proof: Since E is a scalar \mathbb{R} -Module, thus by [6, Lemma6.2, p.80], $End_R(E) \cong R/ann_R(E)$, since $ann_R(E)$ is semiprime ideal of \mathbb{R} , thus $\overline{R} = R/ann_R(E)$ is a semiprime ring. Then $End_R(E)$ is a semiprime ring. Therefore by [3, Prop`7], $End_R(E)$ is an ESSQD ring.

Corollary8: Let E be a scalar \mathbb{R} -Module. Then (1) \Rightarrow (2) \Rightarrow (3),(3) $\not\Rightarrow$ (1) and(3) $\not\Rightarrow$ (2).

- 1) E is ESSQD \mathbb{R} -Module.
- 2) E is ESSP \mathbb{R} -Module.
- 3) $End_R(E)$ is ESSQD ring.

Proof: (1) \Rightarrow (2): Follows by Prop.2.

(2) \Rightarrow (3): Since E is an ESSP, then by Coro 6, $\overline{R} = R/ann_R(E)$ is an ESSQD ring. But E is a scalar \mathbb{R} -Module, thus by [6, Lemma 6.2, p.80], $End_R(E) \cong R/ann_R(E)$, Then $End_R(E)$ is an ESSQD ring.

In the following example explains that (3) $\not\Rightarrow$ (1) and (3) $\not\Rightarrow$ (2).

Example9: Z_p^∞ as Z -Module is not ESSQD. But $End_Z(Z_p^\infty)$ is an integral domain, Clearly it is an ESSQD ring. But Z_p^∞ as Z -Module is not ESSP, since if $H = (\frac{1}{p} + Z)_{\leq e} Z_p^\infty$, then $ann_Z(H) = pZ \neq ann_Z(E) = (0)$, where P is prime number.

The following corollary shows that $End_R(E)$ is ESSQD ring iff \mathbb{R} is ESSQD ring under the class of faithful scalar Modules.

Corollary10: Assume that E is a faithful scalar \mathbb{R} -Module. Then $End_R(E)$ is ESSQD ring iff \mathbb{R} is ESSQD ring.

Proof: Since E is a scalar \mathbb{R} -Module, then by [6 Lemma 6.2, p.80], $End_R(E) \cong R/ann_R(E) \cong R$. Hence we get the result.

An \mathbb{R} -Module E is a semismall quasi-prime(SSQP) if, $ann_R(H)$ is a prime ideal of \mathbb{R} for each non zero semismall Submodule H of E . In addition, a proper semismall Submodule H of E is SSQP if $[H:d]$ be semismall prime ideal of $\mathbb{R} \forall d \in E, d \notin H$.

Theorem11: Assume that E is an \mathbb{R} -module. Then (1) \Rightarrow (2) \Rightarrow (3) \Rightarrow (4)

1. E is SSQP \mathbb{R} -module.
2. $ann_{\mathbb{R}}H = ann_{\mathbb{R}}rH$ for each semismall submodule H of E such that $rH = (0), r \in \mathbb{R}$.
3. $ann_{\mathbb{R}}(d) = ann_{\mathbb{R}}(rd)$ for each $d \in E$ such that $rd \neq 0, r \in \mathbb{R}$.
4. $ann_{\mathbb{R}}(d)$ be semismall prime ideal of \mathbb{R} for each $d \in E$.

Proof: (1) \Rightarrow (2) Since $rH \subseteq H$ then $ann_{\mathbb{R}}H \subseteq ann_{\mathbb{R}}rH$. Let $a \in ann_{\mathbb{R}}rH$ so $arH = 0$ implies $ar \in ann_{\mathbb{R}}H$ is a prime ideal. Then either $a \in ann_{\mathbb{R}}H$ or $r \in ann_{\mathbb{R}}H$. If $r \in ann_{\mathbb{R}}H$, thus $rH = 0$ which is a contradiction. Then, $a \in ann_{\mathbb{R}}H$.

(2) \Rightarrow (3) Clear.

(3) \Rightarrow (4) Let $ab \in ann_{\mathbb{R}}(d)$ and suppose that $b \in ann_{\mathbb{R}}(d)$. Thus $abd = 0$ and $bd = 0$, which implies that $a \in ann_{\mathbb{R}}(bd)$. But by (3), $a \in ann_{\mathbb{R}}(d)$.

Proposition12: Let D be proper Submodule of an \mathbb{R} -Module E . The following are equivalent:

1. D is SSQP Submodule of E .
2. $[D:_{\mathbb{R}} W]$ is semismall prime ideal of \mathbb{R} for each Submodule W of E where $[D:_{\mathbb{R}} W] = \{e \in D, eW \subseteq D\}$.
3. $[D:_{\mathbb{R}}(e)] = [D:_{\mathbb{R}} W]$ for each $e \in E, r \in \mathbb{R}, [D:_{\mathbb{R}}(e)]$

Proof: (1) \Rightarrow (2) Let D be a SSQP Submodule of E . Then $[D:_{\mathbb{R}}(d)]$ is semismall prime ideal of \mathbb{R} , for each $e \in E$. Thus $[D:_{\mathbb{R}}(e)]$ is a semismall prime ideal for each $e \in W$ and $[D:_{\mathbb{R}} W]$ is a semismall prime ideal of \mathbb{R} .

(2) \Rightarrow (3) Clearly $[D:_{\mathbb{R}}(e)] \subseteq [D:_{\mathbb{R}}(we)]$. Let $x \in [D:_{\mathbb{R}}(we)]$ for each $w \in [D:_{\mathbb{R}}(e)]$ and $e \in D$. Hence $x(we) \subseteq D$. It follows that $xw \in [D:_{\mathbb{R}}(e)]$ which is a semismall prime ideal by (2). But $w \in [D:_{\mathbb{R}}(e)]$ thus $x \in [D:_{\mathbb{R}}(e)]$. Then, $[D:_{\mathbb{R}}(we)] \subseteq [D:_{\mathbb{R}}(e)]$. Therefore, $[D:_{\mathbb{R}}(e)] = [D:_{\mathbb{R}}(we)]$.

(3) \Rightarrow (1) Let $e \in E$ and $x, y \in \mathbb{R}$ s.t $xy \in [D:_{\mathbb{R}}(e)]$, suppose $y \in [D:_{\mathbb{R}}(e)]$, thus by (3), $[D:_{\mathbb{R}}(ye)] = [D:_{\mathbb{R}}(e)]$. But $x \in [D:_{\mathbb{R}}(ye)]$, then $x \in [D:_{\mathbb{R}}(e)]$. Thus, D is a SSQP Submodule.

Proposition13: An \mathbb{R} -Module E is SSQP iff (0) is a SSQP Submodule of E .

Proof: Since E is SSQP \mathbb{R} -Module, then by Th. 11, $\text{ann}_{\mathbb{R}}(d)$ is semismall prime ideal of \mathbb{R} for every $d \in E$. But $\text{ann}_{\mathbb{R}}(d) = [0:_{\mathbb{R}}(d)] \forall d \in E$, thus by prop.12, (0) is a SSQP Submodule of E .

Proposition14: Assume that E is a scalar SSQP \mathbb{R} -Module. Thus E is ESSQD \mathbb{R} -Module, and \mathbb{R} is ESSQD ring.

Proof: First: Assume $h \in \text{End}_R(E)$, $h \neq 0$. To prove that $\text{Ker } h \not\ll_{\text{es}} E$. Since E is a scalar \mathbb{R} -Module, then $\exists 0 \neq n \in R$ s.t $h(e) = ne, \forall e \in E$. Suppose that $\text{Ker } h \ll_{\text{es}} E$, then for any $0 \neq m \in E, \exists 0 \neq z \in R$ s.t $0 \neq zm \in \text{Ker } h$, thus $h(zm) = 0$; that is $zm = 0$, so $nz \in \text{ann}_R(m)$. Since E is a SSQP \mathbb{R} -Module, thus $\text{ann}_R(m)$ is a prime ideal of \mathbb{R} , then either $n \in \text{ann}_R(m)$ or $z \in \text{ann}_R(m)$; that is either $nm = 0$ or $zm = 0$, but $zm \neq 0$, thus $nm = 0$ for any $m \in E$. Then $h = 0$ which is a contradiction. Therefore $\text{Ker } h \not\ll_{\text{es}} E$ and hence E is an ESSQD \mathbb{R} -Module.

Second: Since E is a SSQP \mathbb{R} -Module, then by Prop.13, (0) is a SSQP Submodule of E and hence (0) is a semiprime ideal of \mathbb{R} . Thus \mathbb{R} is a semiprime ring. Then by [3, Prop. 7], \mathbb{R} is ESSQD ring.

A Submodule U of an \mathbb{R} -Module E is semismall invertible if $U^{-1}U = E$, where $U^{-1} = \{r \in \mathbb{R}_T: rU \ll E\}$ and \mathbb{R}_T is the localization of \mathbb{R} at T in the usual sence, $T = \{g \in G: gd = 0 \text{ for some } d \in E, \text{ then } d = 0\}$, where G is the set of all nonzero divisors of \mathbb{R} [3].

An \mathbb{R} -Module E is semismall quasi-Dedekind(SSQD) if every non zero Submodule H of E is semismall quasi-invertible[5].

A ring \mathbb{R} is SSQD if \mathbb{R} is SSQD \mathbb{R} -Module[5].

Theorem15: Assume E is a faithful multiplication \mathbb{R} -Module. Then E is ESSP \mathbb{R} - Module iff \mathbb{R} is ESSQD ring.

Proof: (\Leftarrow) Let $Y \ll_{\text{es}} E$. Since E is a faithful multiplication \mathbb{R} -Module, then by[7], $\exists D \ll_e \mathbb{R}$ s.t $Y = DE$. Clearly $\text{ann}_R(Y) = \text{ann}_R(D)$. Since \mathbb{R} is an ESSQD ring, thus D is a semismall quasi-invertible ideal of \mathbb{R} , then $\text{ann}_R(D) = 0$. It follows that $\text{ann}_R(Y) = 0 = \text{ann}_R(E)$. Thus E is ESSP \mathbb{R} -Module.

\Rightarrow) Follows by Prop.4.

Proposition16: Assume that E is a multiplication \mathbb{R} -Module. If $\text{End}_R(E)$ is integral domain then E is a SSQD \mathbb{R} -Module.

Proof: Let $h \in \text{End}_R(E)$, $h \neq 0$. Since $\text{End}_R(E)$ is an integral domain, h is nonzero divisor. But E is a multiplication \mathbb{R} -Module, so h is monomorphism, by [8, Lemma 2.2]. Thus E is a SSQD \mathbb{R} -Module.

proposition17: Assume that E is an ESSP \mathbb{R} -Module with $\text{ann}_R(E) = \text{ann}_R(\bar{E})$, thus \bar{E} is an ESSP \mathbb{R} -Module.

Proof: Let $H \ll_{\text{es}} \bar{E}$. Since $E \ll_{\text{es}} \bar{E}$ then $0 \neq H \cap E \ll_{\text{es}} \bar{E}$. Let $U \leq E$, $U \neq 0$, thus $U \leq \bar{E}$. Then $(H \cap E) \cap U \neq 0$, thus $(H \cap E) \ll_{\text{es}} E$. Since E is an ESSP \mathbb{R} -Module, then $\text{ann}_R(H \cap E) = \text{ann}_R(E)$, but $\text{ann}_R(H) + \text{ann}_R(E) \subseteq \text{ann}_R(H \cap E)$, hence $\text{ann}_R(H) + \text{ann}_R(E) \subseteq \text{ann}_R(E)$, then $\text{ann}_R(H) + \text{ann}_R(\bar{E}) \subseteq \text{ann}_R(\bar{E})$, so $\text{ann}_R(H) \subseteq \text{ann}_R(\bar{E})$. Since $\text{ann}_R(\bar{E}) \subseteq \text{ann}_R(H)$ then $\text{ann}_R(H) = \text{ann}_R(\bar{E})$. Thus \bar{E} is an ESSP \mathbb{R} -Module.

References

- [1] F.Kasch, Modules and rings, Academic Press., London, 1982.
- [2] Ali Inaam.M, Muhmood Layla.S. "semi small submodules and semi-lifting Modules", 3rd scientific conference of the college of science, university of Baghdad, 2009, 385-393.
- [3] Hussain Mukdad Q., AbdulKareem Darya j. Essentially Semismall Quasi Dedekind modules", 1st scientific conference of the college of Education, Duhook university, 2019,. p. 21-28.
- [4] Shihab B.N., Scalar Reflexive Modules, Ph .D.Thesis, College of Education, Ibn AL-Haitham, University of Baghdad, 2004.
- [5] Saleh M. A. Hussain M. Q.,Semismall Quasi-Dedekind modules", 1st scientific conference of the college of Education, karbala university, 2017,. p. 386-396.

- [6] Mohamed–Ali E. A., On Ikeda-Nakayama Modules, Ph.D.Thesis, College of Education, Ibn AL-Haitham, University of Baghdad, 2006.
- [7] EL-Bast Z. A., Smith P.F., Multiplication Modules, Comm In Algebra,16, 1988, 755 – 779 .
- [8] Naoum A .G., AL- Aubaidy W.K.H., A note on multiplication modules and their Rings of endomorphisms, Kyungpook Math, J.Vo 1.35 , No.2, 1995,223 – 228.

**INFLUENCE OF AQUEOUS BARLEY SPROUTS EXTRACT , TREHALOSE ,
AND CALCIUM ON CARROT FLORAL BIOLOGY**

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INFLUENCE OF AQUEOUS BARLEY SPROUTS EXTRACT, TREHALOSE, AND CALCIUM ON CARROT FLORAL BIOLOGY

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Abstract:

This research was implemented at vegetable field of the College of Agricultural Engineering Sciences - University of Baghdad during spring season 2019. The experiment was conducted using factorial within Randomized Complete Block Design arrangement with three factors and replicates (3X3X2). The aqueous barley sprouts extract (B0, B1) (0, 100 g.L-1) represented the first factor. Trehalose (T0, T1, T2) (0, 50, 75 mmole.L-1) represented the second factor. Calcium (C0, C1, C2) (0, 1, 2 ml.L-1) represented the third factor. The research objectives are assessing the impact of the mentioned factors and their interaction on carrot floral biology traits. Results showed the effectiveness of three ways interaction treatment B1T2C1 in producing significant increases in umbel diameter (17.33, 11, 7.934 cm) and setting percentage (100, 100, 87.67%) for the first, second, third umbels respectively. While B1T2C2 produced the highest number of umbel.umbel-1 (193, 123, 111.6) for the first, second, third umbels respectively. And the highest number of flowers. umbellet -1 for the first, second, third umbels found in B0T1C0 (167.3, 113, 48.67).

Key words: Carrot Umbel, Umbel Order, Stecklings, Sugars, Sprouted Grains.



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Introduction:

Floral biology of carrot plant is considered very complicated since it requires special care. The biennial carrot plant needs to be vernalized in order to flower, in addition to the existence of a protandry state, and then the maturity of its seeds happened in several stages with different quality according to the umbel starting with the primary umbel, followed by the second and tertiary umbels, which entails several things, including the quantity and quality of the resulting seeds (Rubatzky et al, 1999; Simon et al., 2019).

Sprouted seeds have been widely used in the last decade in many fields, as they are characterized by their richness in simple and easy to absorb form. They are rich in gibberellin and low in abscisic acid, as these sprouts are characterized by being a rich source of natural growth-stimulating hormones (Bewley et al., 2013; Taiz et al., 2014). They also abundant in nutrients, vitamins, amino acids and sugars in an easily absorbed form (Marton and Mandoki, 2010; Fazaeli et al., 2012). As a result; their aqueous extracts can be sprayed directly on the plant.

Trehalose is one of the non-reducing disaccharides, which made from the combining of two glucose molecules. Trehalose has multiple functions in the plant, as it affects the formation of embryos and flowering as well as the regulation of carbon metabolism in the plant (Iturriaga et al., 2009; Burek et al., 2015).

Calcium is classified as one of the elements that have a pivotal role in the fertilization process in plants due to its role in the growth and polarity of the pollen tube. Cells need it for the activity and effectiveness of their membranes. Calcium is a second messenger of many responses to environmental and hormonal signals (Taiz et al., 2014). As for what mentioned above; our study aimed to assess the impact of stated factors on the floral biology of carrot plant.

Materials And Methods

This experiment was conducted during spring season (2019) to study carrot floral biology at researches station (A) College of Agricultural Engineering Sciences, University of Baghdad (Al-Jadiryah). Table 1 shows the chemical and physical characteristics of the soil for both seasons. The stecklings of carrot that produced from fall season 2018 were sowed on two lines on terraces in 5/February. The field was under drip irrigation system. Mineral fertilizer was added as recommended for carrot plants (120 kg.ha⁻¹, 120 K₂O kg. ha⁻¹, 40 K₂O kg. ha⁻¹) to all plots before planting (ali etal, 2015). The spacing between one plant and another was 0.3 m. The stecklings were planted in a plant density 147620 plants.ha⁻¹.

The experiment was implemented as factorial arrangement (2X3X3) within randomized complete block design with three replicates. Spraying barley aqueous sprouted grains extract was represented the first factor with two levels (0, 100g.L⁻¹ DW) which symbolized (B₀, B₁). The second factor is spraying with three levels of trehalose (0, 50, 75 mmol.L⁻¹) which symbolized (T₀, T₁, T₂). The third factor is spraying with three levels of calcium (0, 1, 2 ml.L⁻¹) (as chelated calcium 30% Ca), which symbolized (C₀, C₁, C₂). The first spraying was after 10 days from thinning. The second spraying was after 15 days from the first spraying. The third spraying was after 15 days from the second spraying.

The Study traits were Determination of the following; umbel diameter (cm), number of umbellets.umbel-1, number of flowers.umbel-1, setting percentage (%) in umbels. The collected data analyzed using analyses of variance and the means were compared according to L.S.D. test under 5% probability.

Table 1. Physical and chemical characteristics of the soil.

Character	Values
pH	7.44
EC1:1 (ds.m-1)	392.
Total N (mg kg-1)	55.0
P (mg kg-1)	13.7
K (mg kg-1)	170
Ca (mg kg-1)	187
Fe (mg kg-1)	2.60
Na (Meq L-1)	61.0
Cl- (Meq L-1)	51.0
SO4-2 (Meq L-1)	207
HCO3- (Meq L-1)	477
O.M. (%)	10.3
Gypsum (%)	320
Sand (%)	12.0
Silt (%)	40.0
Clay (%)	48.0
Texture	Clay Loam

Results And Discussion

Umbel diameter and Setting percentage

We note from the results of Tables 2A, 2B and 2C that the three ways interaction treatment C1T2B1 showed significant superiority in compare to all interaction treatments, including the control treatment by producing it the highest umbel diameter of the for the primary, secondary, tertiary umbels, and this superiority continued for the same concentrations in Its the two ways interaction represented by the treatments C1T2, B1 T2 and C1 B1 (except the interaction of calcium with the sprouted barley aqueous extract for the tertiary umbels, which did not reach the level of significance) and its singular factors (B1, C1 and T2) compared to the control treatment for each of them.

The results of the same tables showed that the primary and secondary umbels reached the percentage of full setting (100%) for several treatments in the three and two ways interaction, as the three ways interaction of trehalose sugar were achieved with both concentrations (T1 and T2) alone or with the first concentration of calcium only (C1) or with the aqueous extract of sprouted barley (B1) or both, the percentage of complete setting of the primary and secondary umbels (100%), while the lowest setting of the primary and secondary umbels were found in the three ways interaction treatment C2T2B1, the setting percentage of the tertiary umbels for the three ways interaction didn't reach the level of significance.

Table 2 A. umbel diameter (cm) and setting percentage (%) for the primary (p), secondary (s), tertiary (t) umbels after treatment for three ways interaction

traits treatments	Umbel diameter (p)	umbel diameter (s)	Umbel diameter (t)	Setting percentage (p)	Setting percentage (s)	Setting percentage (t)
B0T0C0	11.90	7.00	5.033	91.33	89.67	71.00
B0T0C1	12.86	7.87	5.732	100.00	97.33	77.33
B0T0C2	12.56	7.57	5.400	88.67	84.67	69.00
B0T1C0	13.40	8.40	6.167	100.00	100.00	81.67
B0T1C1	14.30	9.30	6.533	100.00	100.00	82.00
B0T1C2	13.73	8.73	6.133	87.00	84.33	64.33
B0T2C0	13.74	8.73	6.400	100.00	100.00	81.00
B0T2C1	14.53	9.53	7.333	100.00	100.00	84.67
B0T2C2	13.26	8.27	5.833	85.67	86.00	66.33
B1T0C0	12.73	7.73	5.567	92.67	92.00	71.00
B1T0C1	12.40	7.40	5.400	94.00	93.33	71.00
B1T0C2	12.16	7.17	5.167	89.00	88.67	69.33
B1T1C0	13.26	8.27	6.267	100.00	100.00	77.67
B1T1C1	14.96	9.97	7.384	100.00	100.00	86.33
B1T1C2	13.16	8.17	6.067	86.67	85.67	68.00
B1T2C0	14.60	9.60	7.380	100.00	100.00	80.67
B1T2C1	17.33	11.00	7.934	100.00	100.00	87.67
B1T2C2	13.66	8.67	6.333	85.33	83.33	66.33
LSD 0.05	0.93	0.64	0.547	2.58	2.97	N.S.

Table 2 B. umbel diameter (cm) and setting percentage (%) for the primary (p), secondary (s), tertiary (t) umbels after treatment for two ways interaction

Traits treatments	Umbel diameter (p)	umbel diameter (s)	Umbel diameter (t)	Setting percentage (p)	Setting percentage (s)	Setting percentage (t)
BXT						
BOT0	12.44	7.48	5.389	93.33	90.56	72.44
BOT1	13.81	8.811	6.278	95.67	94.78	76.00
BOT2	13.84	8.84	6.522	95.22	95.33	77.33
B1T0	12.43	7.43	5.378	91.89	91.33	70.44
B1T1	13.80	8.80	6.589	95.56	95.22	77.33
B1T2	15.20	9.76	7.222	95.11	94.44	78.56
LSD 0.05	0.54	0.37	0.32	N.S.	N.S.	N.S.
BXC						
BOC0	13.01	8.04	5.867	97.11	96.56	77.89
BOC1	13.90	8.90	6.533	100.0	99.11	81.33
BOC2	13.19	8.19	5.789	87.11	85.00	66.56
B1C0	13.53	8.53	6.411	97.56	97.33	76.44
B1C1	14.90	9.46	6.922	98.00	97.78	81.67
B1C2	13.00	8.00	5.856	87.00	85.89	68.22
LSD 0.05	0.54	0.37	N.S.	N.S.	N.S.	N.S.
TXC						
TOC0	12.31	7.37	5.300	92.00	90.83	71.00
TOC1	12.63	7.63	5.567	97.00	95.33	74.17
TOC2	12.36	7.37	5.283	88.83	86.67	69.17
T1C0	13.33	8.33	6.217	100.00	100.00	79.67
T1C1	14.63	9.63	6.983	100.0	100.00	84.17
T1C2	13.45	8.45	6.100	86.83	85.00	66.83
T2C0	14.16	9.17	6.900	100.00	100.00	80.83
T2C1	15.93	10.26	7.633	100.00	100.00	86.17
T2C2	13.46	8.47	6.083	85.50	84.67	66.17
LSD 0.05	0.66	0.45	0.387	1.82	2.10	3.75

Table 2C. umbel diameter (cm) and setting percentage (%) for the primary (p), secondary (s), tertiary (t) umbels after treatment individual factors

traits treatments	Umbel diameter (p)	umbel diameter (s)	Umbel diameter (t)	Setting percentage (p)	Setting percentage (s)	Setting percentage (t)
B						
B0	13.36	8.38	6.063	94.74	93.56	75.26
B1	13.81	8.66	6.396	94.19	93.67	75.44
LSD 0.05	0.31	0.21	0.182	N.S.	N.S.	N.S.
T						
T0	12.43	7.46	5.383	92.61	90.94	71.44
T1	13.80	8.81	6.433	95.61	95.00	76.67
T2	14.52	9.30	6.872	95.17	94.89	77.94
LSD 0.05	0.38	0.26	0.223	1.05	1.21	2.17
C						
C0	13.27	8.29	6.139	97.33	96.94	77.17
C1	14.40	9.18	6.728	99.00	98.44	81.50
C2	13.09	8.10	5.822	87.06	85.44	67.39
LSD 0.05	0.38	0.26	0.233	1.05	1.21	2.17

2. Number of umbellet.umbel-1 and number of flowers.umbellet-1

The results of tables 3A,3B, 3C showed significant differences in Number of umbellet.umbel-1 of carrot plants in different directions according to the physiological effect of the concentrations of the added treatments, as the highest number of umbellet.umbel-1 for the primary, secondary, tertiary umbels was found in B1C2T2 and the lowest number of umbellet. umbel-1 for the primary, secondary, tertiary umbels was found C0T1B0 for all orders (Table 3A) The two ways interaction between trehalose and the extract did not reach the level of significance for the mentioned trait and for all umbels orders, two ways interaction C2B0 and C2T2 produced the highest number of umbellet. umbel-1 compared to the lowest number of umbellet.umbel-1, which was found at C1B0 and C0T1 treatments for the primary, secondary, tertiary umbels (Table 3B).

As for the individual factors (Table 3C), the C2 and B1 treatments were superior for the primary, secondary, tertiary umbels, while the control treatment outperformed both trehalose concentrations in producing the highest number of umbellet. umbel-1 for all umbels ranks.

The results of the same tables indicated the significant superiority of the C0T1B0 treatment in increasing the number of flowers. umbellet -1 for the for the primary, secondary, tertiary umbels compared to the control treatment and all other treatments. Also, the two ways interaction treatments T1B0 and C0T1 outperformed the control treatment and all the other treatments in the aforementioned trait and for all umbels ranks. As for the two ways interaction between the aqueous extract of barley sprouts and calcium, the control treatment C0B0 produced the highest number of flowers. umbellet -1 for the primary umbels and treatment C1B0 for the secondary and tertiary umbels. Table 4C indicates the superiority of

individual treatments B0 over treatment B1, T1 and T2 over T0 and C0 and C1 over C2 by producing the highest number of flowers in umbellet -1 for all orders.

Table 3A. Number of umbellet.umbel-1 and number of flowers.umbellet-1 (%) for the primary (p), secondary (s), tertiary (t) umbels after treatment for three ways interaction

Traits treatments	Number of umbellet. umbel-1 (p)	Number of umbellet. umbel-1 (s)	Number of umbellet. umbel-1 (t)	Number of flowers. umbellet-1 (p)	Number of flowers. umbellet-1(s)	Number of flowers. umbellet-1 (t)
B0T0C0	139.0	88.3	73.67	56.70	33.67	29.33
B0T0C1	83.0	53.0	47.67	114.70	92.68	43.33
B0T0C2	171.7	104.0	92.33	55.30	33.33	24.00
B0T1C0	57.0	39.0	35.67	167.3	113.00	48.67
B0T1C1	96.0	63.0	54.00	103.00	63.33	37.67
B0T1C2	183.0	117.0	104.00	88.00	57.00	36.67
B0T2C0	92.0	59.3	52.00	90.70	55.33	36.67
B0T2C1	94.0	62.0	50.67	99.00	66.33	41.00
B0T2C2	191.0	119.6	107.00	79.00	50.00	31.67
B1T0C0	130.0	79.3	66.00	53.70	31.33	21.00
B1T0C1	134.0	80.3	71.67	65.00	47.67	35.67
B1T0C2	171.0	101.0	99.33	50.70	31.67	22.66
B1T1C0	104.7	65.7	54.33	74.70	48.33	37.67
B1T1C1	90.3	60.7	51.34	94.00	61.67	37.00
B1T1C2	173.7	108.6	101.20	60.70	36.67	27.67
B1T2C0	98.3	65.3	54.67	88.00	54.33	36.00
B1T2C1	106.7	69.0	58.00	77.00	44.33	35.67
B1T2C2	193.0	123.0	111.60	60.70	36.34	30.66
LSD 0.05	17.1	9.4	6.65	14.00	10.62	2.61

Table 3 B. Number of umbellet.umbel-1 and number of flowers.umbellet-1 for the primary (p), secondary (s), tertiary (t) umbels after treatment for two ways interaction

Traits treatments	Number of umbellet. umbel-1 (p)	Number of umbellet. umbel-1 (s)	Number of umbellet. umbel-1 (t)	Number of flowers. umbellet-1 (p)	Number of flowers. umbellet-1(s)	Number of flowers. umbellet-1 (t)
BXT						
B0T0	131.2	8.81	71.22	750.6	53.22	32.22
B0T1	112.0	73.0	64.56	118.80	77.78	41.00
B0T2	125.8	80.3	69.89	89.6	59.22	36.44
B1T0	145.0	86.9	79.00	55.4	36.89	26.44
B1T1	122.9	78.3	69.00	76.4	48.89	34.11
B1T2	132.7	85.8	74.78	75.2	43.00	34.10
LSD 0.05	N.S.	N.S.	N.S.	8.09	6.13	1.51
BXC						
B0C0	96.1	62.2	53.78	104.90	67.33	38.22
B0C1	91.0	59.3	50.78	103.90	74.11	40.67
B0C2	181.9	113.5	101.10	75.10	46.78	30.78
B1C0	111.0	70.1	58.33	72.10	44.67	31.56
B1C1	110.3	70.0	60.34	71.30	49.22	36.11
B1C2	179.2	110.9	104.10	63.70	34.89	27.00
LSD 0.05	9.9	5.4	3.8	8.09	6.13	1.51
TXC						
T0C0	134.5	83.8	69.83	55.20	32.50	25.17
T0C1	108.5	66.7	59.67	87.30	70.17	39.50
T0C2	171.3	102.5	95.83	54.00	32.50	23.33
T1C0	80.8	52.3	45.00	121.00	80.67	43.17
T1C1	93.2	61.8	52.67	96.00	62.50	37.33
T1C2	178.3	112.8	102.60	75.80	46.83	32.17
T2C0	95.3	62.3	53.33	89.30	54.82	36.33
T2C1	100.3	65.5	54.33	79.50	52.33	38.34
T2C2	192.0	121.3	109.30	78.30	43.17	31.17
LSD 0.05	12.11	6.62	4.71	9.90	7.51	1.85

Table 3C. Number of umbellet. umbel-1 and number of flowers.umbellet-1(%) for the primary (p), secondary (s), tertiary (t) umbels after treatment individual factors

traits treatments	Number of umbellet. umbel-1 (p)	Number of umbellet. umbel-1 (s)	Number of umbellet. umbel-1 (t)	Number of flowers. umbellet-1 (p)	Number of flowers. umbellet-1 (s)	Number of flowers. umbellet-1 (t)
B						
B0	123.0	78.37	68.56	94.60	62.74	36.56
B1	135.5	83.67	74.26	69.00	42.93	31.55
LSD 0.05	5.71	3.120	2.218	4.67	3.54	0.87
T						
T0	138.1	84.33	75.11	65.50	45.06	29.33
T1	117.4	75.67	66.78	97.60	63.33	37.56
T2	129.2	83.06	72.33	82.40	50.11	35.28
LSD 0.05	6.99	3.821	2.716	5.72	4.33	1.07
C						
C0	103.6	66.17	56.06	88.5	56.00	34.89
C1	100.7	64.67	55.56	87.6	61.67	38.39
C2	180.6	112.2	102.6	69.4	40.83	28.89
LSD 0.05	6.99	3.821	2.716	5.72	4.33	1.07

It was clear from field observations that the best umbel architecture for carrot plants is represented by their large diameter, giving them a relatively low number of umbellets, and a large number of flowers within their umbellet, , as this type of umbels is characterized by being not compact and permeated by air, which facilitates the movement of pollinators within them, and thus increase The setting percent as well as their strength due to the strength and thickness of the petioles of their flower stalks, which makes them not subject to breakage or scattering, This distinct architecture was obtained in a number of tree ways interactions (except for the interactions containing C2. C1T2B1 and its binary and singular factors was characterized by producing the best architecture for the flowering inflorescences of carrot plant, as the reason may be attributed to the contribution of trehalose sugar in the formation of this form of umbels due to Its effect on the integrity of flower structures, as one of the studies on corn plant showed a distortion in the flower structures of the mentioned plant due to a mutation that caused the absence of trehalose from flowering meristems (Satoh-Nagasawa et al., 2006).

As well as that; The first concentration of calcium (C1) and barley extract (B1) (in the tree and two ways interactions and their individual factors) contributed in producing good floral indicators to their physiological action that supported the action of trehalose, as the action of calcium is known in the fertilization process by facilitating the germination of pollen on the stigma and the beginning of the formation of the pollen tube (calcium is directional cue of its growth through the so-called chemotropic or chemotactic tactic, meaning that the growth of the pollen tube is attracted to the higher concentrations of calcium. Calcium also establishes the polarity of the pollen tube and stimulates its growth and elongation (Ge et al., 2007, Taiz et al., 2014 and Bhatla and Lal, 2019). As for the action of the aqueous extract of barley sprouts, it is represented by providing formulas of important nutrients (Table 2) in the development of floral structures and then in fruit, as phosphorous, which is important in

promoting flowering and proper seed development, as well as its action in the phosphorylation of trehalose (Bhatla and Lal, 2019).

In contrast to what was mentioned above, the umbel architecture of the C2T2B1 and C2T0B1 treatments was characterized by the abundance of flowering umbellets, the small number of flowers inside the umbellets, and the somewhat small diameter of the floral umbels, which made them compact to a large extent and hindered the movement of air and pollinators inside them, which affected their setting percent as well as the fragility of their petioles which make them more susceptible to breakage and scattering.

References

- Ali, N. S., H. S. Rahi, and A. A. Shackir, 2014. Soil Fertility. Arabic Community publisher for Publication and Distribution 1st ed , pp: 307.
- Bewley, J. D., K.J. Bradford, H. W. M. Hilhorst, and H. Nonogaki. 2013. Seeds: Physiology of Development, Germination and Dormancy (3rd Ed.) Springer, New York – Heidelberg – Dordrecht – London, pp: 392.
- Bhatla, S. C., and M. A.Lal. 2019. Plant Physiology, Development and Metabolism. Springer Nature, Library of Congress, pp: 1237.
- Burek, M, S. Waskiewicz, L. Wandzik, and K. Kaminska. 2015. Trehalose – properties, biosynthesis and applications. CHEMIK, 69(8): 469-476.
- Fazaeli, H., H. A. Golmohammadi, S.N. Tabatabayee and M. Asghari-Tabrizi. 2012. Productivity and nutritive value of barley green fodder yield in hydroponic system. World Applied Sciences Journal 16 (4): 531-539.
- Ge, L., H. Tian, and S. Russell. 2007. Calcium function and distribution during fertilization in angiosperms. American journal of botany. 94(6): 1046–1060.
- Iturriaga, G., R. Suárez, and B. N. Franco. 2009. Trehalose metabolism: from osmoprotection to signaling. Int. J. Mol. Sci., 10, 3793-3810.
- Marton, M., and Z. Mandoki. 2010. The role of sprouts in human nutrition a review. Acta Univ. Sapientiae, Alimentaria, (3) 81-117.
- Rubatzky, V. E., C. F. Quiros and P. W. Simon. 1999. Carrots and Related Vegetables Umbelliferae. CABI Publishing. pp:294.
- Satoh-Nagasawa N, N. Nagasawa, S. Malcomber, H. Sakai, and D. Jackson. 2006. A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature 441: 227–230.
- Simon, P., M. Lorizzo, D. Grzebelus, and R. Baranski. 2019. The Carrot Genome. Springer Nature Switzerland AG 1st ed. pp: 372.
- Taiz, L. , E. Zeiger, I. M. Moller, and A. Murphy. 2014. Plant Physiology and Development. 6th ed, Sinauer Associates, Inc., Publishers Sunderland, Massachusetts. pp, 761.



**EVALUATION OF ELECTROMAGNETIC POLLUTION OF CELLULAR
MOBILE NETWORK**

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EVALUATION OF ELECTROMAGNETIC POLLUTION OF CELLULAR MOBILE NETWORK

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Abstract:

Wireless communications are characterized by their fastest growth in history, as they used ever-evolving and renewed technologies, which have allowed them to spread widely. Every day, communication technology introduces a new invention with features that differ from its predecessor. Bell Laboratories first suggested mobile wireless communication services to the general population in the late 1940s. Still, it wasn't easy at that time to use on a large scale due to its high costs. This paper aims to describe the state of cellular mobile networks; by comparing the sources of electromagnetic pollution caused by these networks, measure the level of power density in some residential areas, and compare them with international standards adopted in determining the level of power density by calculating the effect of the transmitted power and the angle of transmission of the antenna from the station. The importance of the paper lies in the fact that it investigates the levels of non-ionizing radiation produced by cellular mobile networks and the identification of other types of pollution caused by these networks.

Key words: Cell Phone, Electromagnetic Pollution, Electromagnetic Radiation, Locomotion.



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Introduction:

Communication was and still of great importance to humanity. The human voice is the simplest and oldest means of communication used to communicate between people within close distances. However, when the distance between the two parties of communication increases, other tools such as stimuli (horns) and drums are used. When communication was developed, the available optical technologies such as flags, smokes were, fire or fireworks were used for communication in farther distances. Thus the visible part (light) of the electromagnetic spectrum was used. The electromagnetic spectrum outside the visible area has been used as a more recent means of communication through radio (radio communications)[1-3].

In 1946, the Federal Communications Commission (FCC) granted a license to operate a wireless mobile phone system for commercial purposes in 25 cities in the United States of America. With the development of electronics, cellular communication systems as wireless communications have become widely used because they allow many people to use them efficiently. Cellular communication systems have gone through several generations and attained the present state[4].

1.2 Sources of electromagnetic radiation in mobile phone networks

Three sources emit electromagnetic radiation in mobile phone networks that effected the human body and may be exposed to them at different levels and frequencies. Radiations are fired either from the base stations (the towers) or from the microwave antennas connecting the stations or the telephone device that the subscriber uses to make calls[5,6].

1.2.1. Base stations

The radiation source is the antennas installed on the towers at high altitudes, such as the roofs of houses and high buildings, and perhaps above water tanks. By looking around, one can notice how dense these towers are, and their locations are selected to obtain the required signal coverages. People are generally exposed to the radiation emanating from those antennas of the base stations of the mobile phone networks, as shown in Figure (1). Whether they are mobile phone users or other applications, they are close to their residence and work. The extent of its impact depends on several factors, the most prominent of which are[7,8,9]:

- The station's transmitting power: the more significant the transmitter power (P_t), the greater its effect on the objects exposed to radiation.
- It was transmitting antenna gain: The greater the antenna's gain towards the exposed object, the greater its effect on the exposed object.
- Transmitting antenna height: One of the important ways to reduce the impact of the base station antenna radiation is to increase its height, although placing the antennas at higher altitudes is mainly intended to obtain better coverage.
- The distance between the radiation centre in the antenna and the object exposed to the radiation should be considered.
- The duration of radiation exposure: an increase in the duration of exposure leads to an increase in the amount of electromagnetic energy absorbed by the body.

- The radiation pattern: the antenna radiates more energy in one direction than it does in other directions. The object's location exposed to the main beam of the antenna radiation is more exposed to the radiation than the object being within the influence of the back lobes or side lobes.
- The presence of reflective buffers in radiation such as walls of the houses and others bodies can reflect the wave emanating from the antenna, increasing the intensity of the radiation power that reaches the exposed object.
- Factors related to the body exposed to radiation include the body's orientation exposed in a whole or part and the length of the object exposed. Often the human body is represented by a monopole antenna that stands above the ground and captures electromagnetic waves.
- Factors related to the environment, such as air movement, humidity, and temperature, may affect the extent of exposure of living organisms to radiation.
- Factors related to the number of channels used for transmission at the station, the operating frequencies, and the communication density at the time of exposure increased the influences of radiations.
- Factors related to the tilt angle of the antenna towards the ground, as the antennas are directed towards the ground to obtain the required coverage, may have specific effects.

The level of exposure to radiation from the station's antenna is expressed in power density, representing the rate of energy passing through the unit area in the far-field area of the antenna and is measured in watts per square meter.

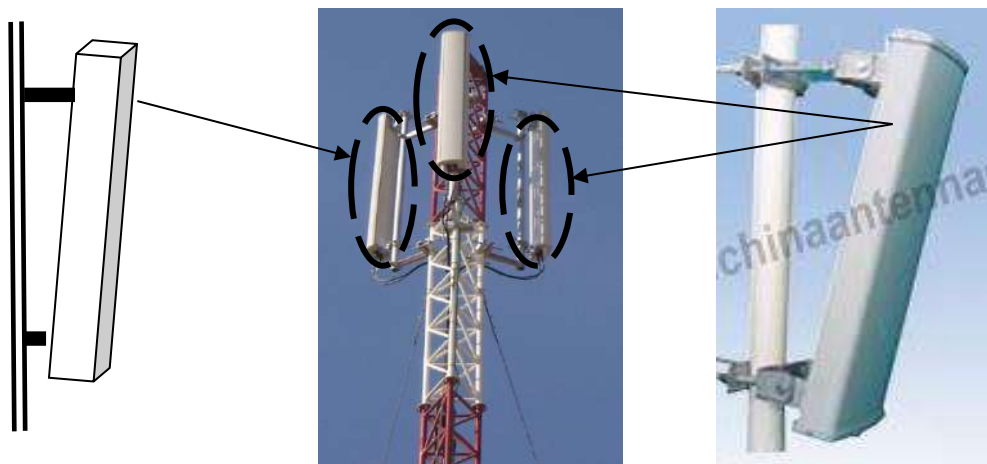


Figure 1: Base station antennas in mobile phone networks

The equation can find the power density:

$$S = \frac{P_t G_t}{4 \pi r^2} \quad \text{.....} \quad 1$$

Where (S) represents the power density in watts per square meter (W / m²) and (P_t) is the power transmitted through the transmitting antenna in the station, (G_t) the transmitting antenna gain and (r) is the distance travelled by the radial component. The power sent down to the point at which the power density is to be calculated.

As shown in Figure (2), it is possible to calculate the power density at any point located in the far-field of the antenna, where the radial distance (r) can be calculated between the centre of radiation in the antenna and any point on the ground. It is possible to note that the distance (r_1) is the distance between the antenna and the foot of the human body exposed to radiation, while (r_2) is equal to the distance between the antenna and its head.

$$r_1 = \sqrt{H^2 + d^2}$$

$$r_2 = \sqrt{(H-h)^2 + d^2}$$

Where (H) is the height of the antenna in meters, (h) represents the height of the exposed object, (d) the horizontal distance between the base of the tower and the exposed object, (r) represents the distance between the radiation centre of the station antenna and the point at which the power density of the exposed object is to be calculated[10].

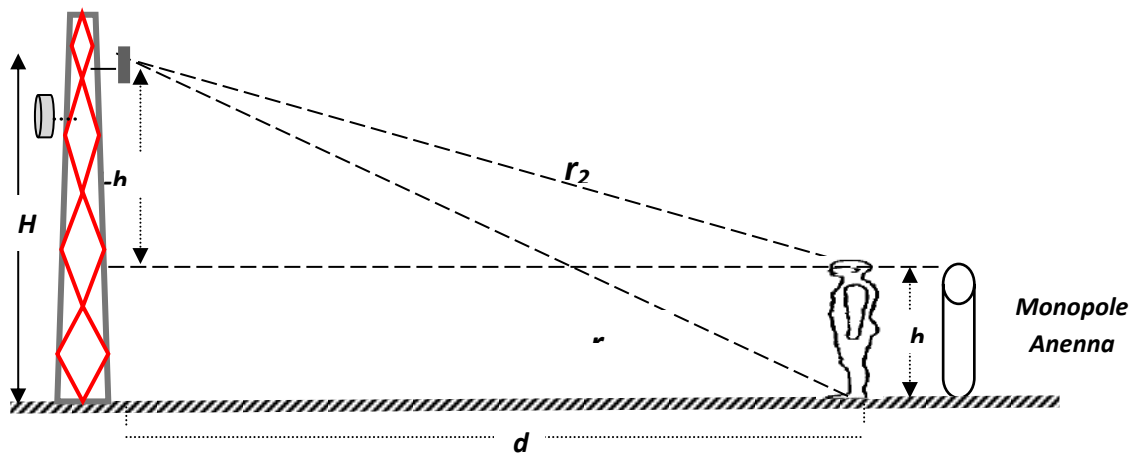


Figure 2: Sketch for the calculation of the power density resulting from the transmitting antennas of the stations

The strength of the electric and magnetic field can be calculated as the components of the electromagnetic wave. The equation linking the power density with the electric and magnetic field strength is:

$$S = \frac{P_t G_t}{4 \pi r^2} = \frac{E^2}{120 \pi} = 120 \pi H^2 \quad \dots\dots\dots 2$$

Where (S) is the power density in watts per square meter (W / m^2), (E) the electric field strength in volts per meter (V / m), and (H) is the magnetic field strength in amperes per meter (A / m).

Therefore, power density, electric field strength, or magnetic field strength can be estimated by the level of exposure.

1.2.2 Microwave antennas in base stations

These are dish antennas that are installed at high altitudes in the base of the station towers. The purpose of these antennas is to achieve a link between a station and the nearby stations and then to the central control centre (MSC), as shown in Figure (3). It may be possible to connect these terminals to optical fibres, but using a microwave connection is

easier technically and economically. These antennas operate on the principle of connection between two points, and in the mobile phone systems, it connects between two base stations and operates at high frequencies up to (18GHz).

Given that, these antennas are installed at high altitudes to achieve the best connection between one point and another point (point-to-point), that is, between one cell and another. It is necessary to obtain a clear path free of obstacles and barriers between the two microwave antennas. Thus it is difficult for a person to be exposed to the radiation from these antennas. Except for engineers and technicians, they are likely to be exposed to microwave radiation when they adjust the pointing of these antennas together to obtain a clear line-of-sight path and when these antennas are in working order. Figure (3) shows the microwave antennas used to connect the stations and then to the control center[11].

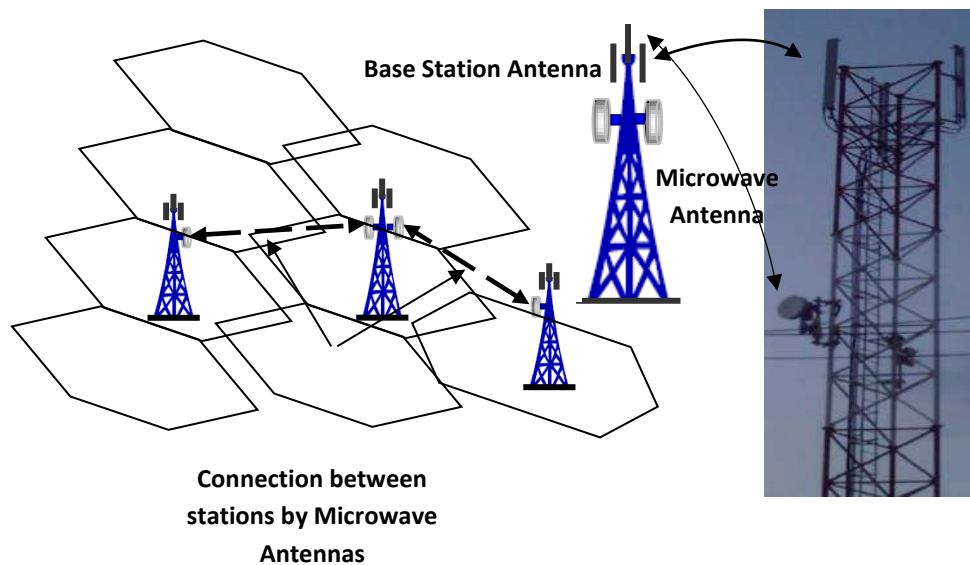


Figure 3: Exhibited the microwave antennas to connect the base stations in mobile phone networks

When the mobile phone is transmitting, the speaker's voice is transformed by the microphone in the device into an electrical signal that turns into electromagnetic waves in the antenna installed in the device. Thus, the user of the mobile phone is surrounded by electromagnetic radiation when making a phone call. The danger of the radiation that emits from the transmitter antenna in the mobile phone lies in the fact that it is close to the head area, which contains tissues that can absorb the energy of electromagnetic waves after the wave penetrates the living tissues of the head.

The normal state of using a mobile phone is holding it close or touching the ear, which makes it close to the brain, which means the head tissues will be in the near field from the transmitting antenna to the phone. It is necessary to mention that the redness of the ear after a long call is not due to the absorbed electromagnetic energy, but due to the dissipation of the device's battery energy in a heated manner when using the device or pressure on the phone device when holding it towards the head. Nevertheless, keeping the phone device away from the head by a few centimetres reduces the effect of electromagnetic radiation significantly. It is worth noting that the mobile phone emits these radiations when it is on (ON) even if it is

not used in a phone call, and the radiation will be less than when it is used in phone calls. The emission stops when it is in the OFF state.

The use of an external phoneme (headphone) reduces the potential effects of radiation from the phone. However, these simulators are intended to make calls without holding the device by hand (hand free) and not for protection purposes.

Electromagnetic waves penetrate the living tissues of the speaker's head at an amount that depends on the frequency, power and properties of the incident wave. The amount of energy absorbed during a given period of exposure depends on the electric field induced in the living tissue and the electrical and thermal properties of the tissue.

The effect of electromagnetic radiation on living tissues of the human body is expressed at the Specific Absorption Rate or SAR. The time rate of power absorption per unit mass of biological tissue when tissue is exposed to electromagnetic radiation, measured in units of watts per kilogram (W / kg). The Specific Absorption Rate (SAR) is defined as the time rate of increase (gain) or decrease (loss) of energy per unit mass that occupies a specific volume and has a specific density.

The acquisition by living tissues of the electromagnetic energy emitted from the mobile device during the transmission leads to an increase in the temperature due to the increase in the kinetic energy of the molecules of living tissue. The amount of heat gained depends on:

a) The power transmitted by the telephone device or the value of the electric field penetrating the living tissue. The transmitting power depends on [12]:

- adequate radiated power, which depends on the transmitted power and the gain (gain) of the antenna of the telephone equipment through which the transmitter is transmitted in a particular direction.
- The amount of power or field induced in the tissue that the electromagnetic wave penetrates shows that this power is less than the power falling on the head area (the skin) before it penetrates. The power excited in the living tissue is equivalent to (13.53%) of the power falling on the head at the penetrating distance. The induced field equals its value (36.78%) of the field falling outside the field head before penetration.

Table 1. The effects on the received signal level (coverage area) were presented by changing the transmitted power from 20 dBm to 38 dBm according to the following equation. Figure (4) illustrates the effect of the transmitted power on the signal strength of the coverage area.

$$Pr = Pt + Gt + Gr - PL - A \quad \dots\dots (3)$$

Where Pr is the received power, Pt the transmitted power, Gt is the gain of the transmitting antenna, Gr the receiving antenna gain

Table 1: The effects on the received signal strength by changing the transmitted power for different pathloss models (Closed-In model, A Global Partnership 3GPP model and Stanford University model);

Txd power (dBm)	CI Rxd (dBm)	3GPP Rxd (dBm)	SUI Rxd (dBm)
20	-119.9	-119	-192.8
22	-117	-116.2	-190.7
24	-114.2	-113.4	-188.6
26	-111.5	-110.7	-186.5
28	-108.9	-108	-184.4
30	-106.3	-105.4	-182.4
32	-103.7	-120.9	-180.3
34	-101.2	-100.3	-178.2
36	-98.65	-97.81	-176.2
38	-96.17	-95.34	-174.1

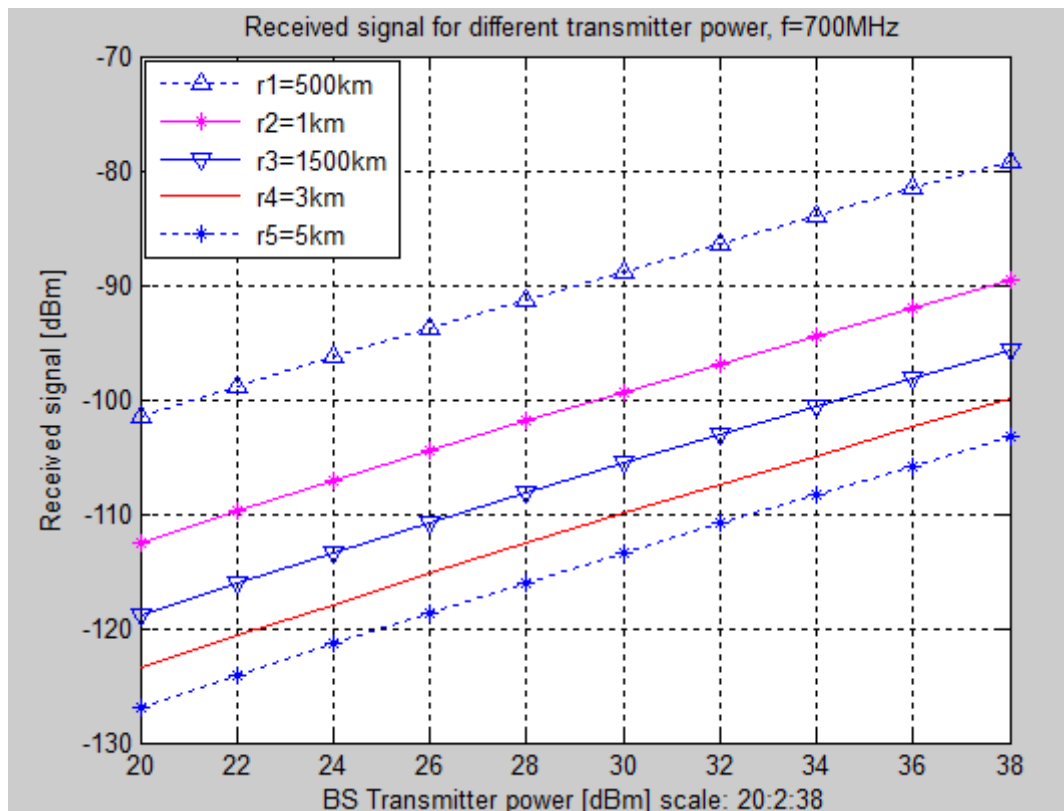


Figure 4: Cover forecast diagram, showing the effect of the transmitted power on the (receiving) signal strength.

1.2.3. The effect of tilting the antenna on coverage area

The total effect of the antenna tilt is the sum of both the electrical and the mechanical tilt. The electrical tilt is constant at 2 degrees because it is manufacturer-specific, while the mechanical tilt varies from 0 degrees to 5 degrees. So, the overall slope usually varies from 0 ° to approximately 7 °, but this can be used in practice. The tilt angles can be estimated by a simple calculation of the vertical angle between the antenna and the area of interest. In other words, the choice of the inclination angle is according to the desired coverage areas, and it is in the direction of the vertical diagram.

Using the basic formula of Pythagoras; tangent $\Theta = \text{opposite} / \text{adjacent}$ (3)

angle = cosine (height / distance)

Where; opposite = height

adjacent = distance

Note: height and distance must be the same units of measurement.

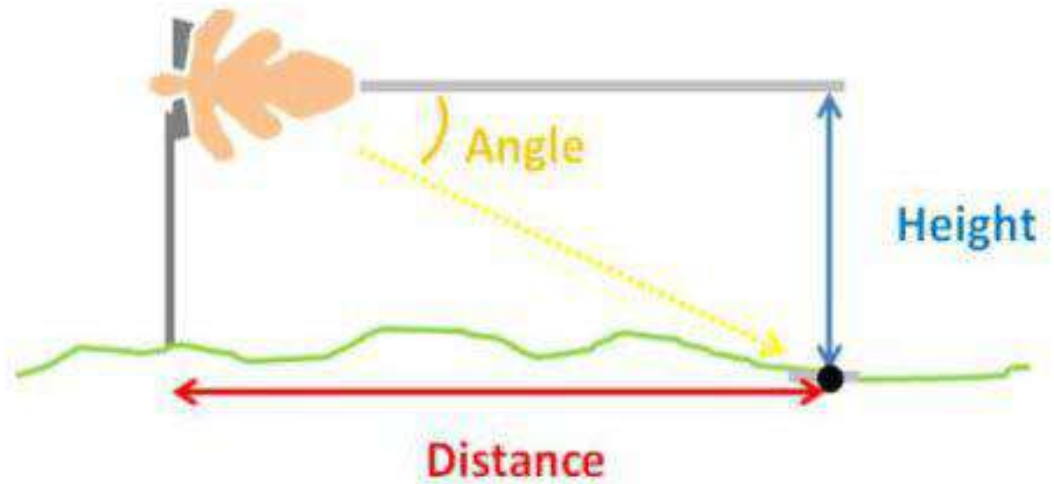


Figure 5: The relationship between antenna height, tilt and T-R distance[12]

Using the analogy, one can always obtain the value of the antenna angle, Transmitter - Receiver distance (T-R), or the height of the antenna.

Therefore, at $\Theta = 0^\circ - 5^\circ$, respectively, the values for the antenna height are obtained, which is used with equations (3). The obtained values are presented in Table 2. Table 2 summarizes the effect on the received signal level (coverage area) by changing the inclination of the antenna and illustrated in Fig. (6).

Table 2: The effect on the received signal strength by changing the tilt of the base station antenna

Tilt angle (degree)	0	1	2	3	4	5
CI- Rxd (dBm)	-248.1	-249	-225.1	-301.13	-205	-228
3GPP- Rxd (dBm)	-249	-249.2	-225.2	-302.03	-250.5	-229
SUI- Rxd (dBm)	-350.7	-340.3	-302	-441.15	-350	-290

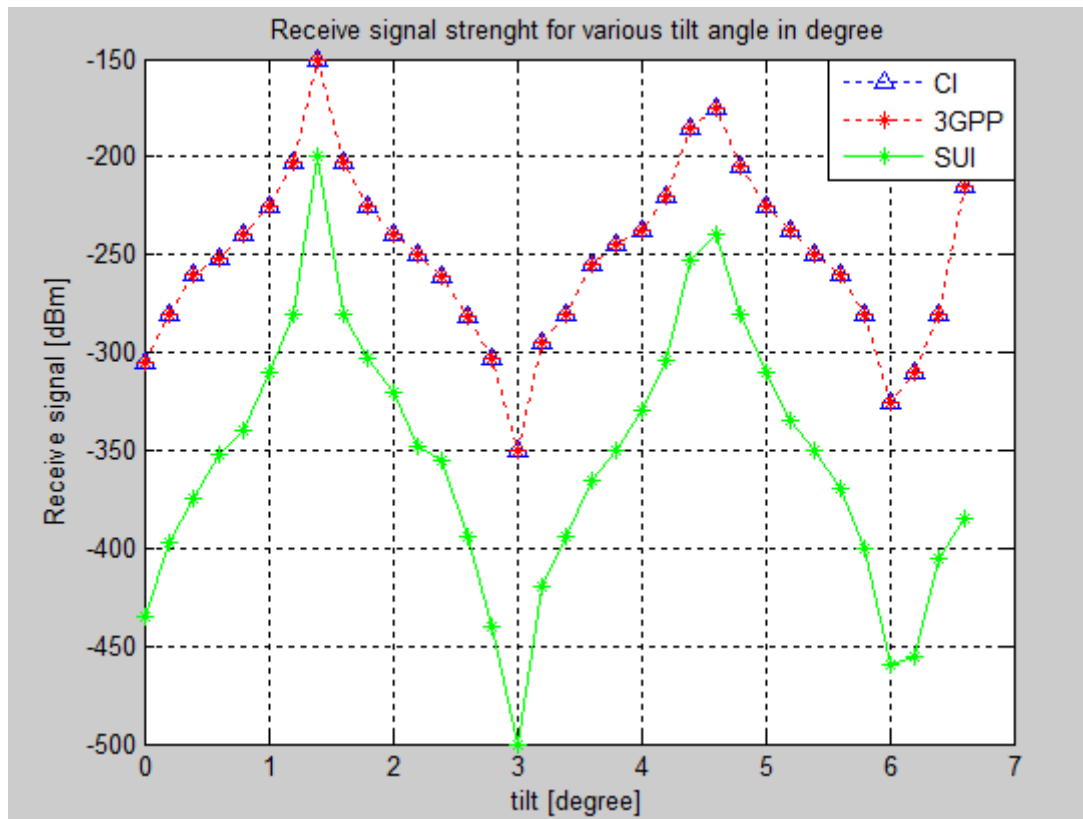


Figure 6: The effect of slope on the received signal level

1.3 Anxiety about having mobile network towers

Our bodies received electromagnetic waves from radio and television broadcasting towers and mobile phone towers. They are monopole receiving antennas that absorb or receive the energy of electromagnetic radiations.

To answer the cause of the increasing concern of mobile phone stations compared to broadcasting stations. The following points should be mentioned:

1. The number of mobile phone stations is large compared to the number of radio broadcasting towers. The radio broadcasting stations transmit with high capacities, not less than 1 kilowatt, to obtain a more comprehensive coverage area for their programs. While the mobile phone stations transmit with fewer capacities to cover a small geographical area, the cell area, especially in urban areas (cities), is small, although one station broadcast on more than one channel (more than one frequency).
2. The frequencies on which the mobile phone operates are higher than the regular radio and television transmission frequencies. They are close to the frequencies of microwave ovens or Wi-Fi devices in the fourth generation (4G).
3. The concern about mobile phone towers in residential areas is not accompanied by concern about radio broadcasting towers. Even though they transmit with high transmission capacities that reach ten times the capacity of mobile phones at the very least, and some of them are located within the municipal boundaries of cities. The reasons behind the absence of concern about the presence of broadcasting towers in cities compared to the increased concern about the presence of mobile phone networks are due to:

- The numbers required of radio broadcasting towers to obtain the required coverage are very few compared to the number of mobile phone towers needed to achieve the required coverage.
- Radio broadcasting towers are erected at the highest point in the area, while mobile phone towers are erected in different places of varying heights.
- A forbidden area often surrounds radio broadcasting towers, and fences prevent the general public from entering it, which keeps them from exposure to high radiation levels.
- Building of the house transmitters and signal transmission lines are surrounded by materials that prevent the signal from leaking out and preventing them

1.4 Conclusions

The mobile network service providers choose the towers installing sites according to specific criteria and principles related to the objective of the operating company or to provide the best coverage of the geographical area to get high-quality services. It comes through installing base stations in places close to the areas where the subscribers are located, whether residential or commercial gathering areas or areas close to the workshops.

The primary purpose of having large numbers of towers is to increase the network capacity to accommodate the most significant number of subscribers. It is essential to know that increasing the capacity means more cells where each cell contains a base station, meaning that a more significant number of subscribers means more stations installed.

The general questions are the reasons for setting up coverage station towers in certain places and not in others. In this regard, you are within the range of geographic coverage by the network service whenever you are near the base station. You also need the least possible amount of signal capacity that you send when making phone calls. Thus, you are exposed to the potential risk less than when you are away from the station

To achieve a balance between the engineering requirements that must be met to ensure the quality of coverage and service on the one hand and to achieve the environmental conditions, and the following conditions should be observed when installing mobile phone stations or towers:

- Towers are installed over tall buildings, whether they are natural (elevated terrain) or on the roofs of the tall buildings.
- Avoid placing the antennas near reflective surfaces, as this increases the intensity of the radiated power.
- The antenna height should be higher than the height of the neighbouring buildings to ensure that the radio signal is not blocked. The company engineers conduct a field survey of the tall buildings in the area where the tower is to be erected to discover how the signal will arrive between the station and the subscribers. Ensure that the station is linked to other stations through the appropriate connection method such as microwave dishes.
- The roof of the building on which the tower that carries the antennas is installed must be constructed of reinforced concrete to bear the tower's weight.

- When resorting to erect more than one tower for two different equipment on the roof of one building, it must be considered that there is a distance between the two towers to ensure that interference does not occur.

References

- [1] International Commission on Non-Ionizing Radiation Protection. ICNIRP guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). *Health Phys* 1998; 74: 494–522.
- [2] Institute of Electrical and Electronics Engineers. IEEE C95.7-2014—IEEE recommended practice for radio frequency safety programs, 3 kHz to 300 GHz. IEEE Standards Association, 2014. https://standards.ieee.org/standard/C95_7-2014.html (accessed Nov 6, 2018).
- [3] Volkow ND, Tomasi D, Wang GJ, et al. Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. *JAMA* 2019; 305: 808–13.
- [4] Schmid MR, Loughran SP, Regel SJ, et al. Sleep EEG alterations: effects of different pulse-modulated radio frequency electromagnetic fields. *J Sleep Res* 2020; 21: 50–58.
- [5] Kimata H. Microwave radiation from cellular phones increases allergen-specific IgE production. *Allergy* 2005; 60: 838–39.
- [6] Kim, J.H., Lee, J.K., Kim, H.G., Kim, K.B. and Kim, H.R. Possible effects of radiofrequency electromagnetic field exposure on central nerve system. *Biomol. Ther.*, 27(3): (2019) 265-275.
- [7] Ouadah, N.S., Lecomte, A., Robidel, F., Olsson, A., Deltour, I., Schüz, J., Blazy, K. and Villegier, A.S. Possible effects of radiofrequency electromagnetic fields on in vivo C6 brain tumors in Wistar rats. *J. Neurooncol.*, 140(3): 539-546. (2018)
- [8] Xu, F., Bai, Q., Zhou, K., Ma, L., Duan, J., Zhuang, F., Xie, C., Li, W., Zou, P. and Zhu, C. Age-dependent acute interference with stem and progenitor cell proliferation in the hippocampus after exposure to 1800 MHz electromagnetic radiation. *Electromagn. Biol. Med.*, 36(2): (2017) 158-166.
- [9] International Agency for Research on Cancer, World Health Organization. Classifies Radiofrequency Electromagnetic Fields as Possibly Carcinogenic to Humans. Vol. 208. International Agency for Research on Cancer, World Health Organization, Geneva. (2011).
- [10] H.-S. Kim, B.-J. Park, H.-J. Jang et al., “Continuous exposure to 60Hz magnetic fields induces duration- and dose-dependent apoptosis of testicular germ cells,” *Bioelectromagnetics*, vol. 35, no. 2, pp. 100–107, 2014.
- [11] W. Duan, C. Liu, H. Wu et al., “Effects of exposure to extremely low frequency magnetic fields on spermatogenesis in adult rats,” *Bioelectromagnetics*, vol. 35, no. 1, pp. 58–69, 2014.
- [12] R. Rago, P. Salacone, L. Caponecchia et al., “Te semen quality of the mobile phone users,” *Journal of endocrinological investigation*, vol. 36, pp. 970–974, 2013.

A petri dish held by a hand, showing various fungal colonies. A large green colony is in the center, surrounded by several smaller white and yellow colonies. The background is a blurred laboratory setting.

**INHIBITORY EFFECT OF SOME COMMERCIAL DETERGENTS ON FUNGI
ISOLATED FROM INDOOR AIR**

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SABA ABDULAMEER KADHIM AL - ZIADI
AHMED ABDULAMEER KADHIM**

INHIBITORY EFFECT OF SOME COMMERCIAL DETERGENTS ON FUNGI ISOLATED FROM INDOOR AIR

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Ahmed Abdulameer KADHIM³

Abstract:

The present study was aimed to investigate about the inhibitory effect of some detergents spread in the Iraqi markets contain a group of fungi that pollute the air of homes for this purpose a group of indoor air pollutant fungi of the following species have been isolated such as (*Aspergillus niger*, *Alternaria alternata*, *Fusarium solani*, *F.oxysporum*, *Mucore sp.*, *Rhizopus stolanifer* and *Penicillium*). The species (*Penicillium*, *Fusarium solani*, and *Aspergillus niger*) isolated from six houses chosen randomly in variant residential neighborhoods were selected to test the ability of some commercial detergent handwashing liquid Ays trade mark, white bleach (sodium hypochlorate) and Al Emlaq super jel (multi uses cleaning jell), which were randomly tested from the local markets of Diwaniyah city in inhibiting their growth, the results showed that Ays brand hand wash did not affect the fungus *Penicillium* at any concentration of the concentrations used in the study, while it had an inhibitory effect on the other two air pollutant fungi (*Fusarium solani*, and *Aspergillus niger*) while hypochlorite sodium and Al Emlaq super jel were effective on all fungi used in the study.

Key words: Indoor Air Pollutant Fungi, Inhibitory Effects, Commercial Detergents.



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Introduction:

The issue of environmental pollution is one of the important topics that arouse interest in the current era, as this phenomenon is classified among environmental crimes and its seriousness is embodied in many indicators that directly or indirectly affect human life and endanger him [1]. The air is considered one of the important components of the environment and is subject to contamination with many materials such as dust, fumes, fungi, bacteria, pollen, and others. Air pollution is one of the main causes of disease and death that can be avoided worldwide, with 4.3 million deaths recorded every year, most of them in developing countries, from exposure to household air pollution (indoor air). An additional 3.7 million deaths a year are attributable to air pollution. Fungi as indoor air pollutants are widely distributed and pose a severe hazard to human health in enclosed spaces [1]. Detergents are one of the most important substances used to reduce the number of pathogens [3]. To get rid of all germs, including spores, whether bacteria, fungi, or viruses [4]. Air fungi are spread by air currents in the form of spores and pieces of fungal threads. Microorganisms, including fungi, can enter the buildings by entering the outside air, during heating, ventilation, the air conditioning system, and through doors and windows. For a sufficient period [5], air pollutant fungi have become increasingly important as they have harmful effects on health, meaning that exposure to fungi causes irritations, allergies, and toxic effects, and toxic fungi cause many health problems for humans. The information obtained from samples of air fungi can help in the medical evaluation, determining treatment methods, and estimating health risks, and it is also useful in stimulating the follow-up of measuring indoor air quality to avoid the harmful causes. [5,6, 7]. A group of household cleaners has spread, whether they are used for personal hygiene for individuals or to clean the house and all its facilities, the principle of its work is as follows: - The detergent molecule consists of two parts, namely the tail (a hydrophobic carbon chain) and the head (a hydrophilic ionic group).

- When adding detergent to water, it reduces the surface tension of the water. - The detergent particles arrange themselves so that the tail is pointing towards the spots and the head towards the water. When mechanical friction, similar charges repel each other.

This study was aimed to investigate the inhibitory effect of many detergents which are commercially available on some common air pollutant fungi.

Work methods:

1. Preparation of the culture media

Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA) were used, and the preparation was done according to the instructions mentioned on the package by the manufacturer.

2. Isolation and diagnosis of fungi from homes

6 houses were randomly identified for 6 areas of the city of Diwaniyah city, which are (Al-Jamaa neighborhood, Al-Jazira district, Al-Furat neighborhood, Umm Al- Khail, Al-Jalabiyah and Al-Orouba neighborhood). The plate containing the cultural media prepared for the purpose of isolation was opened inside The house for a period of 5 minutes, then the plate

was closed and transferred to the incubator for incubation for a period of 5 to 7 days at a temperature of 25 ° C. After the end of the incubation period, the isolated fungi were diagnosed based on the outward appearance of the fungal colony, as well as microscopic examination to confirm the diagnosis of the fungus at the species level, depending on [8 ,9 10].

3. Commercial detergents tests

3 types of detergent have been tested (liquid hand soap Ays trade mark, Bleachand Al Emlaq jel for cleaning floors)

4. Fungi sensitivity test

Fungi sensitivity test to detergents minimum inhibitory concentration of the detergent used to test their sensitivity to fungi was determined by following the Serial Dilution Method to find the lowest inhibitory concentration.

5. The percentage of inhibition estimation

was calculated by measuring the colony diameter for the treatments relative to the control treatment, which was represented by a culture medium without any addition and according to the following equation: Inhibition percentage = (control colony diameter - treatment colony diameter) x 100.

Results and discussion:

A group of fungal species polluting indoor air have been isolated from the following genera (*A. niger*, *A. alternata*, *F. solani*, *F.oxysporum*, *Mucore spp.*, *R. stolanifer* and *Penicillium spp*).

The species (*Penicillium spp*, *F. solani*, and *A. niger*) were selected to test the ability of some commercial detergents (Ays hand washing liquid, sodium hypochlorite and Elemlaq jel) which were randomly tested from the local markets of Diwaniyah city to inhibit their growth.

The results showed high effectiveness of commercial detergent in inhibiting the tested fungi, as three concentrations were prepared for each treatment (50, 75, and 100%), and all concentrations showed an inhibitory effect on the growth of fungi, but with varying proportions, as the best concentration was 100%. the table 1 show the inhibitory effect of detergent used in the study according to their concentration , the figure (1) refer to use of (Ays) to inhibit the growth of *F.solani*, and the concentration gave 100% the best result in inhibiting the growth of the fungus as well for *A niger* Figure (2) and (3) for the fungus *Penicillium*

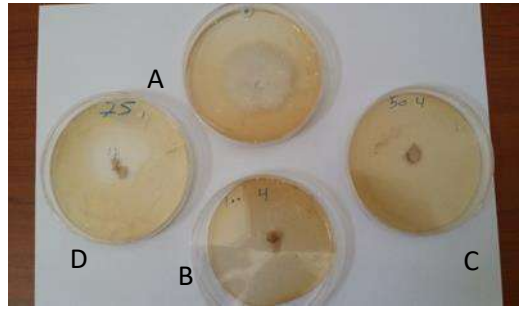


Figure (1) shows the growth of *F. solani* on the medium (PDA), in addition to concentrations of Ays solution, A: plate represents the comparison treatment, B: plate represents a concentration of 100%, C: plate is a concentration of 50% and D: leftplate is a concentration of 75%.

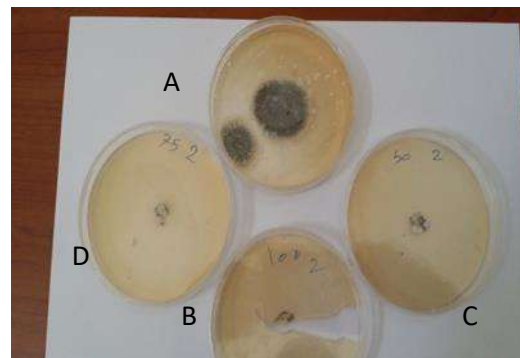


Figure (2) shows the growth of the fungus *A. niger* on the middle PDA, plus concentrations of Ays hand washing , A: represents the comparison treatment, the B: plate represents a concentration of 100%, C: plate a concentration of 50% and D: plate a concentration of 75%

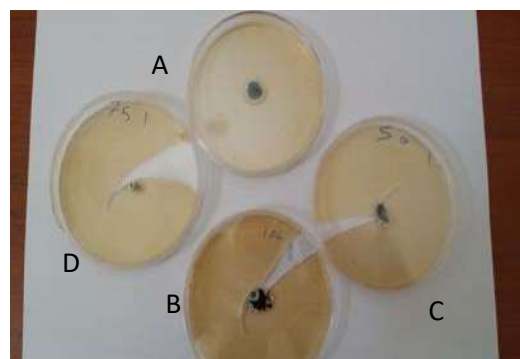


Figure (3) shows the growth of the fungus *Penicillium spp* on the medium (PDA), adding concentrations of Ays hand washing, A: plate represents the comparison treatment, B:plate represents a concentration of 100%, C: plate is a concentration of 50% and D: plate is a concentration of 75%

The same is the case for the commercial (El-emlaq) treatment in inhibiting the growth of *F. solani*, Figure. (4), as well as for *A. niger*, Figure. (5) and Figure. (6) for *Penicillium*, as the concentration gave 100% better result of inhibition of the growth of the tested fungi, as is the case for the treatment of Al- Emlaq jell.

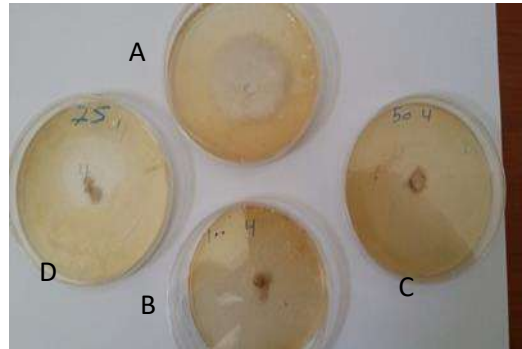


Figure (4) shows the growth of *F. solani* on the medium (PDA), with added concentrations of the commercial sodium hypochlorate, A: plate represents the comparison treatment, B: plate represents a concentration of 100%, C: plate a concentration of 50% and D: plate a concentration of 75%

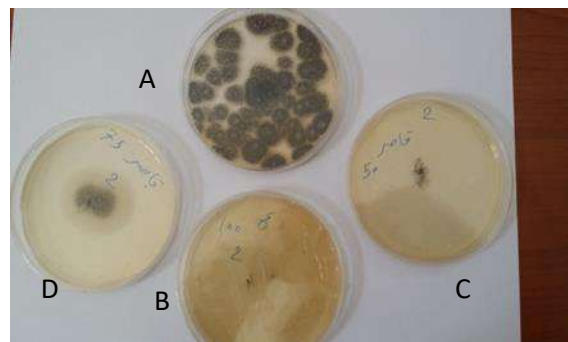


Figure (5) shows the growth of the fungus *A. niger* on the medium (PDA), in addition to concentrations of the commercial sodium hypochlorate, as the upper plate represents the comparison treatment, the lower plate represents a concentration of 100%, the right plate a concentration of 50% and the left plate a concentration of 75%

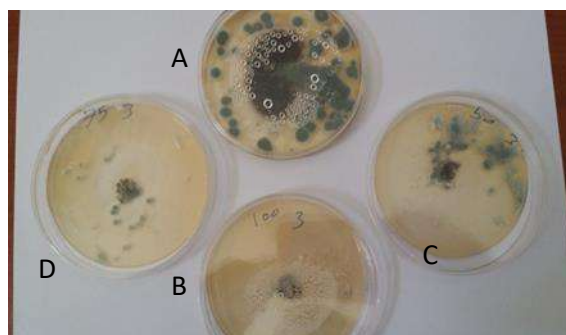


Figure (6) shows the growth of the *Penicillium* fungus on the medium (PDA), in addition to concentrations of the commercial sodium hypochlorate, as the upper plate represents the comparison treatment, the lower plate represents a concentration of 100%, the right plate is a concentration of 50% and the left plate is a concentration of 75%.

The inhibitory effect of detergents comes from the biosynthesis of free organic acids, sodium tri poly phosphate and ethoxyl-oleyl-cetyl alcohol which had a strong stimulating effect to increase liquid wetting (which is also a property of surface active substances) can result in severe destructive changes in the fungal cell wall, decomposition of cytoplasmic membrane lipids, protein denaturation, disruption of the cell division process, and an increase in the oxidoreduction potential, eventually leading to microorganism cell death.

Conclusion

Household commercial detergents have a clear inhibitory effect on some fungi that pollute the air of homes, which makes them gain great importance in sterilizing homes and thus the possibility of reducing diseases that these fungi may cause.

Table 1 the ratio of growth inhibition as detergent dilution rate

Detergent	<i>Penicillium</i>			<i>Fusarium solani</i>			<i>Aspergillus niger</i>		
	50%	75%	100%	50%	75%	100%	50%	75%	100%
Ays (handwashing)	0.00	0.00	0.00	12.00	11.00	0.00	22.00	13.00	0.00
El –emlaq superjell	20.00	0.00	0.00	11.00	10.50	0.00	35.00	10.00	0.00
Bleach	0.00	0.00	0.00	13.00	12.00	0.00	0.00	0.00	0.00
Control	25.00			55.00			85.5		

References

- [1] Khan, A. H., & Karuppayil, S. M. (2012). Fungal pollution of indoor environments and its management. *Saudi journal of biological sciences*, 19(4), 405-426.
- [2] WHO. The burden of disease from ambient air pollution for 2012, http://www.who.int/phe/health_topics/out.
- [3] Boyano, A., Kaps, R., Medyna, G., & Wolf, O. (2017). Revision of six EU Ecolabel Criteria for detergents and cleaning products.
- [4] Rutala, W. A., & Weber, D. J. (2008). *Guideline for disinfection and sterilization in healthcare facilities*, 2008.
- [5] Shelton, B. G. , K. H. Kirkland, W. D. Flanders, and G. K. Morris. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology*, 68(4): 1743 – 1753.
- [6] Hedayati, M. T., Mayahi, S., Aghili, R., & Goharimoghadam, K. (2005). Airborne fungi in indoor and outdoor of asthmatic patients' home, living
- [7] in the city of Sari. *Iranian Journal of Allergy, Asthma and Immunology*, 189-191.
- [8] Basilico, J. C. (2007). Influence of environmental factors on airborne fungi in houses of Santa Fe City, Argentina. *Science of the Total Environment*, 376(1-3), 143-150.
- [9] Tille, P. (2015). *Bailey & Scott's diagnostic microbiology-E-Book*. Elsevier Health Sciences.
- [10] K Hussain, K., Malavia, D., M Johnson, E., Littlechild, J., Winlove, C. P., Vollmer, F., & Gow, N. A. (2020). Biosensors and diagnostics for fungal detection. *Journal of Fungi*, 6(4), 349.
- [11] Wagner, K., Springer, B., Pires, V. P., & Keller, P. M. (2018). Molecular detection of fungal pathogens in clinical specimens by 18S rDNA high- throughput screening in comparison to ITS PCR and culture. *Scientific reports*, 8(1), 1-7.



**THE ANTIBACTERIAL EFFECTS OF GREEN TEA EXTRACT ON
RESISTANT BACTERIA ISOLATED FROM HUMAN EYE INFECTIONS**

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THE ANTIBACTERIAL EFFECTS OF GREEN TEA EXTRACT ON RESISTANT BACTERIA ISOLATED FROM HUMAN EYE INFECTIONS

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Abstract:

Ocular infection is a world wide issue especially for public health field which could be a result to its own normal flora due to subjection to external factors (e.g. stress, getting older, hits, surgical operations, systemic diseases and losing commensal flora). Ocular pathogens could be healed by a group of topical antibiotics, but with time, drug resistance had been developed, which more magnified by wrong diagnosis and random use of antibiotics leading to unexpected complications e.g. visual problems, leading to blindness at last .

Alternative therapy had been used to treat such infections including plant extracts like Green Tea (*Camellia sinensis*) .

Eye swabs about (50) samples were gathered from people had ocular infections, then biochemical tests diagnosed (30) bacterial isolates. There were (17) isolates (6 isolates of *Staphylococcus* spp. and 11 isolates were *Enterococcus*) out of the (30) isolates showed multiple antibiotic resistance to nine antibiotics by disc-diffusion method, there were high complete resistance to Moxifloxacin and Bacitracin, in contrast to Ciprofloxacin and Chloramphenicol.

The antibacterial effects of hot water, cold water, Acetone, Ethanol and Methanol Green tea extracts was examined against the (17) multiple antibiotic resistant isolates by agar-well diffusion method using. Only the Ethanol and Methanol green tea extract showed promising results, without any effect of the remaining green tea extracts. Green tea extracts were equal to Ciprofloxacin and Sulphamethoxazole in effectiveness against antibiotic resistant isolates . The (17) isolates were tested for production of biofilm and protease. (12) isolates were biofilm-producer but after subjection to Ethanol Green tea extract changed into non biofilm-former. (13) isolate were protease-producer but after subjection to Ethanol Green tea extract changed into non protease-former.

Key words: Eye Swabs, Antibiotic Resistance, Alternative Therapy, Green Tea Extracts, Biofilm and Protease.



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Introduction:

Eyes are the most precious and sensitive sense organs (1). One of the Eyes diseases causes was Bacteria especially when they armed with virulence factors supported by immunity deficiency with different severe complications depending on socio– economical levels, health of person, age, conditions of life, quality of food, genes and physiological conditions (2).

Eye infections could be resulted from blood infections or external invasion from surrounding environment, which were so localized to eyes, but may spread to other organs or tissues (3).

Staphylococcus spp., *Streptococcus spp.* and *Pseudomonas aeruginosa* were the most frequent microbes of eyes due to their opportunistic nature (4).

Ocular infections need specific treatment by antibiotics, which in turn had their own pharmacological activity depending on definite diagnosis of the disease. The failure of antibiotics therapy plus multiple antibiotic resistant bacteria led to substitute them by plant and insects natural products e.g. honey and tea as an old-new therapy (5).

Tea is a rich plant of polyphenols by different types and levels depending on the preparation method (e.g. green, black and oolong). Green tea is so rich source of monomeric polyphenols (e.g. epicatechin, epicatechingallate, and epigallocatechin). Black tea rich by simple catechins which oxidized to much higher molecular weight molecules and their fermented forms (6).

catechins present at green tea had its antibacterial effects, which was mentioned by many researchers on different Gram +re and Gram -re aerobic bacteria, anaerobic bacteria, viruses, fungus, and parasites (7).

Presence of biofilm had its significance in medical infections, because it is a sign of reoccurrence or causing even more chronic infections (8), especially its role in real pathogenicity and opportunistic ones (9).

The study aimed to isolate and diagnose bacteria among persons who had ocular infections, then test its sensitivity patterns to antibiotics. Antibacterial effects of Green tea extracts prepared in different solvents were tested against antibiotic resistant isolates, plus Biofilm and protease production before and after subjection to the most effective Green tea extract.

Materials and Methods

Eye swab samples collection

Fifty eye swabs were collected from patients of ocular infections from Al-Kindy Hospital in Baghdad, during Dec. 2018. Sterile moistened swabs were used to get the eyes discharges gently, then put in a sterile saline transport test tube in order to reach the lab within less than 1 hr. Each eye swab was cultured on plates of Brain Heart Infusion Agar, then incubated at 37°C for 24 hr.

Bacterial isolates Identification

The isolates were re-cultured several times on Nutrient Agar, incubated at 37°C for 24 hrs to get pure bacterial isolates, then were cultured on Chrom agar and subjected to different biochemical tests for more identification.

Antibiotic sensitivity test

The sensitivity of isolates to antibiotics was done by Kirby-Bauer disc diffusion method by using the following antibiotic discs: Sulphamethoxazole (SMZ, 50 µg), Moxifloxacin (MXF, 5 µg), Tobramycin (.TOB, 10 µg), Erythromycin (E, 15 µg), Ciprofloxacin (Cip, 5 µg), Neomycin (N, 30 µg), Bacitracin (B), Nitrofurantion (F, 300 µg) and Chloramphenicol (C, 30 µg). Isolates were recorded either as sensitive (S), moderate sensitive (I), and resistant (R) depending on (10).

Green tea extracts preparation

10 g of silani green tea (*Camellia sinensis*) dried leaves granules were taken from local market, then put in one hundred ml of (hot boiling and cold distilled water), Ethanol, Methanol and Acetone for 24 hr, then filtered by using the whatman filter paper (No.1), after that, centrifuged at 3000 rpm /15 min to get filtrated extracts, then were stored at -20°C until further use (11).

Antibacterial activity of Green tea extracts

A 24-hr old culture of bacterial isolates were transferred into 2 ml of normal saline to make a suspension for each bacterial isolate (about 1.5×10^8 CFU/ml), then Muller-Hinton Agar plates were cultured by the bacterial isolate suspension, left at room temperature for 15 minutes until dry.

Wells were made into Muller-Hinton Agar plates, then 50 µl of each Green tea extracts was added. A negative control was sterilized distilled water. Plates incubated at 37 °C for 24 hr. The zones of inhibition diameters was recorded (12).

Biofilm production assay

Biofilm production was detected as mentioned at (13):

- 1- Bacterial culture by 24 hr age was cultured in Tryptic Soy Broth +1% sucrose for 20 hr at 37° C in duplicate for each bacterial isolate.
- 2- Tubes were emptied and washed with sterile Normal Saline for 3 times, then strongly removed the non-attached bacteria by shaking the tube.
- 3- Fixation of the attached bacteria was done by adding ethanol (96%) for each tube for 15 min.
- 4- Tubes were emptied and dried, then stained by crystal violet for 5 min and excess stain was removed by washing the tubes by tap water.
- 5- After tubes drying, biofilms were appeared as purple spots on the tubes sides .

Protease production detection

Protease production was detected on skim milk Columbia medium by stabbing with 24hr bacterial colonies, then plates were incubated in 37°C for 24 hrs .Presence of halo areas around the colonies represents a positive result (14).

Results

Fifty eye swabs were collected from ocular infections patients, then biochemical tests revealed (30) bacterial isolate, (17) isolate out of the (30) isolates were identified (6 isolates of *Staphylococcus* spp. and 11 isolate were *Enterococcus*) based on the biochemical tests and its characters on Chrom agar

Antibiotic susceptibility test of the (17) isolates showed high complete resistance to Moxifloxacin and Bacitracin, there were (14) isolate out of the (17) isolate resistant to Tobramycin and Neomycin, (10) out of (17) isolates were resistant to Nitrofurantion,(9) out of (17) isolates were resistant to Erythromycin followed by (7) out of (17) isolates were resistant to Ciprofloxacin and Chloramphenicol as showed at (Figure 1 and Table 1).



Figure 1: Antibiotic sensitivity test of bacterial isolates

Table 1 : Antibiotic sensitivity test of bacterial isolates

Isolate Name & No.	Sulphamethoxazole	Moxifloxacin	Tobramycin	Erythromycin	Ciprofloxacin	Neomycin	Bacitracin	Nitrofurantion	Chloramphenicol
Staph. 1	I	R	R	R	R	R	R	R	R
Staph. 2	R	R	R	R	R	S	R	R	R
Staph. 3	I	R	R	R	R	R	R	R	R
Staph. 4	R	R	R	R	R	R	R	R	R
Staph. 5	R	R	R	R	R	S	R	R	R
Staph. 6	R	R	R	R	R	S	R	R	R
Entero. 1	I	R	S	I	S	R	R	R	S
Entero. 2	S	R	I	I	S	R	R	S	S
Entero. 3	S	R	R	R	S	R	R	S	S
Entero. 4	S	R	R	I	R	R	R	R	R
Entero. 5	S	R	R	I	S	R	R	I	S
Entero. 6	R	R	R	I	S	R	R	R	I
Entero. 7	S	R	R	I	S	R	R	I	I
Entero. 8	S	R	R	I	S	R	R	R	I
Entero. 9	S	R	S	R	S	R	R	I	I
Entero. 10	S	R	R	I	S	R	R	I	I
Entero. 11	S	R	R	R	S	R	R	S	S

These 17 isolates were used for the agar well diffusion method of Green tea extracts which showed significant results especially for ethanol and methanol extracts, while there were no effect of hot,cold green tea water extracts and Acetone green tea extract. Ethanol green tea extract caused inhibition zones ranged between (10 to 15) mm, compared with Methanol green tea extract caused inhibition zones ranged between (8 to 12) mm as shown in (Figure 2 and Table 2).



Figure 2 : Antibacterial activity of Green tea extract

1. Hot water green tea extract
2. Cold water green tea extract
3. Ethanol green tea extract
4. Methanol green extract
5. Acetone green tea extract

Table 2 : Antibacterial activity of green tea extract by mm

Isolate Name & No.	Hot water green tea extract	Cold water green tea extract	Acetone green tea extract	Ethanol green tea extract	Methanol green tea extract
Staph. 1	-	-	-	12	9
Staph. 2	-	-	-	15	12
Staph. 3	-	-	-	13	9
Staph. 4	-	-	-	13	11
Staph. 5	-	-	-	12	-
Staph. 6	-	-	-	15	-
Enter. 1	-	-	-	13	10
Enter. 2	-	-	-	15	10
Enter. 3	-	-	-	13	8
Enter. 4	-	-	-	13	10
Enter. 5	-	-	-	12	10
Enter. 6	-	-	-	13	10
Enter. 7	-	-	-	15	10
Enter. 8	-	-	-	12	-
Enter. 9	-	-	-	10	10
Enter. 10	-	-	-	15	-
Enter. 11	-	-	-	12	10

Different plant extracts were used medically because their antimicrobial activity. Antibiotic resistance is the main obstacle on antibiotics use field, especially random use in developing countries which cause absolute failure. Wide use of the first-line antibiotics now days led to lose the effectiveness against common microbes and re-treatment by the same resistable antibiotics led us to second-line and third-line antibiotics which became in turn more costing and cause different side effects (15).

Green tea showed an extraordinary anti_oxidant, anti_bacterial, anti_inflammatory, anti_cancer, anti androgen and immunomodulatory properties (16). One of the components of green tea which stand behind the anti oxidant properties, is catechins (17), which had inhibitory effect on bacterial growth (18), by triggering stress genes (19). *P. aeruginosa* had two important virulence factors : biofilm and antibiotic resistance (20). Plant extracts had been used extensively against biofilm-former bacteria (21), in addition to (22) who used green tea extracts against resistant antibiotic bacteria.

Standard Strains of *Staph. aureus*, *E.coli*, *P. aeruginosa* and clinical isolates of Methicillin Resistant *Staphylococcus aureus* and Multidrug Resistant *P. aeruginosa* were effectively treated by green tea aqueous extract (23).

A Researcher (24) found that green tea extract caused inhibition of bacterial causes of eye infection, the most common bacteria inhibited by extraction is *Staph. aureus* followed by *Ps. aeruginosa* and *Streptococcus pneumoniae*.

The antibacterial activity of catechin was related to its antioxidant character on a phospholipid membrane model (25). The anti oxidant and anti bacterial activity of green tea resulted from epigallocatechin gallate (26).

Green tea extracts act directly on bacteria by cell membrane damage, bacterial fatty acids synthesis inhibition, important bacterial membrane integrated-enzymes inhibition as DNA gyrase, protein tyrosine kinase, ATP synthase, cysteine proteinases, and inhibition of the membrane efflux pump (27).

The massive increase in antibiotic resistance (e.g. penicillin-G, ciprofloxacin and erythromycin) which represented the first line therapy led to therapy problems. So, the alternative natural therapy of multiple antibiotic resistant bacteria had appeared to the scene, by the use of green tea in the daily use that could control or even slow the growth of these resistable microbes.

Researchers proposed that the green tea extract as anti-adhesive agent, it could stop the adhesion of bacteria on cell membrane of the host cell (28). Hot water extract of green tea was efficient against mouth bacterial isolates, and can be consumed by pregnant women as a safe mouth wash for periodontitis pathogenic bacteria (29).

Also a researcher (30) found high resistance to chloramphenicol, erythromycin, cefixime between eyes bacterial isolates, in addition to, a significant antibacterial activity of black and green tea extracts. The latter extract scored higher inhibition zones ranged between (11 to 25) mm, Perhaps had been related to the presence of tannic acid which represented a significant inhibitor of microbial growth, besides tea contents of phenolic compounds (catechins and flavonoids) which had the ability of controlling the bacterial infections.

These (17) isolates (multiple antibiotic resistant) were tested for biofilm and protease production due to the significance of these virulence factors in eyes infection. There were (12) isolate out of the (17) isolate were biofilm producer but after subjection to Ethanol green tea extract changed into non-biofilm former. From other side, (13) isolate out of the (17) isolate were protease producer but after subjection to Ethanol green tea extract changed into non-protease former as shown in (Figure 3 and Table 3).

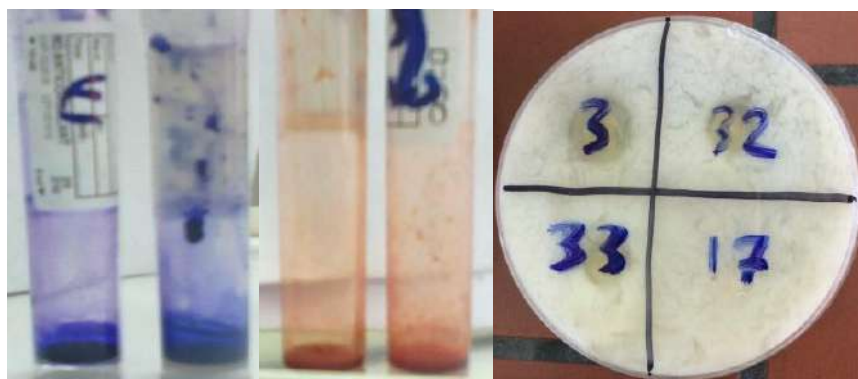


Figure 3 :Biofilm and protease production of bacterial isolates

Biofilm of Enterococci makes the bacterial colonies more resistable to antibiotics. *Enterococcus faecalis* are biofilm-former on materials of ocular lens (e.g. silicone, acrylic and polymethymethacrylate) (31).

Biofilm improves bacterial existence on abiotic surfaces, especially the nosocomial isolates (32), due to the importance of biofilm in the first step of attachments, penetration and finally persistence (33).

The use of contact lens or intraocular lenses for vision correction consider a tool to build a biofilm on them by microbes leading to lenses failure. Even these infections could be evolved into secondary persistent infection resulting in vision impairment and finally blindness, like what happened when acute bacterial endophthalmitis or corneal ulceration occurred (34).

Table 3 : Production of Biofilm and protease from bacterial isolates before and after subjection to Ethanol Green tea extract

Isolate Name & No.	Biofilm production		Protease production	
	Before	After	Before	After
Staph. 1	+	-	-	Not tested
Staph. 2	+	-	-	Not tested
Staph. 3	+	-	+	-
Staph. 4	+	-	-	Not tested
Staph. 5	-	Not tested	+	-
Staph. 6	-	Not tested	-	Not tested
Enteroc. 1	+	-	+	-
Enteroc. 2	+	-	+	-
Enteroc. 3	+	-	+	-
Enteroc. 4	+	-	+	-
Enteroc. 5	+	-	+	-
Enteroc. 6	-	Not tested	+	-
Enteroc. 7	-	Not tested	+	-
Enteroc. 8	+	-	+	-
Enteroc. 9	+	-	+	-
Enteroc. 10	+	-	+	-
Enteroc. 11	-	Not tested	+	-

α B-crystallin, a heat shock protein produced in eyes retina continuously depending on stress conditions (35), A researcher (36) found that *S. aureus* protease split α B-crystallin, leading to lose its protectively, which leads to magnify the apoptosis in the eyes retina leading to huge tissue damage, and finally failure of retinal function.

Results of (37) was compatible with this study especially about protease inhibition, because they found that black tea extract caused inhibition of *Pseudomonas* on blood agar, besides inhibition the activity of protease enzyme and adhesion capability which in fundamental factors in Bacterial infection of human eyes.

Recommendations

Alternative therapy represented by plant extracts could be used to prepare an active component which could be used in pharmacological uses as ointments or solutions by reasonable cost, safe use and active therapy.

Biofilm prevention is the termination key of different bacterial infections . So, the creation of an old-new therapy under medical supervision may lead to effective therapy for biofilm-former bacteria.

Conclusions

Ethanol green tea extract showed high antibacterial activity against multiple antibiotic resistant bacteria isolate from human ocular infections. Besides its good effect on two of the most important virulence factors of bacteria (Biofilm and Protease). Future studied are needed to find active ingredients in order to use it in pharmacy as effective alternative therapy, especially biofilm prevention in order to stop or even reduce ocular infection .

References

- (1). Dagnachew, M. ; Yitayih, W. ; Feleke, M. ; Tesfaye, N. and Getachew, F. (2014) Types and drug susceptibility patterns of bacterial isolates from eye discharge samples at Gondar University Hospital, Northwest Ethiopia. BMC Res. Notes, 7(292): 1-5.
- (2). Ubani, U. (2009) Common bacterial isolates from infected eyes, JNOA, 15: 40-47.
- (3). Bharathi, M.J. ; Ramakrishnan, R. ; Shivakumar, C. ; Meenakshi, R. and Lionalraj, D. (2015) Etiology and antibacterial susceptibility pattern of community-acquired bacterial ocular infections in a tertiary eye care hospital in south India. Indian J. Ophthalmol., 58(6): 497-507.
- (4). Kwapong, P.K. ; Ilechie, A. and Kusi, R. (2013) Comparative antibacterial activity of stingless bee honey and standard antibiotics against common eye pathogens. J. Microbial. Biotech. Res., 3(1): 162-168.
- (5). Omoya, F.O. and Akharaiyi, F.C. (2012) Mixture of honey and ginger extract for antibacterial assessment on some clinical isolates. Int. J. Pharma. Biomed. Res., 2(1): 39-47.
- (6). Astill, C. ; Birch, M.R. ; Dacombe, C. ; Humphrey, P.G. and Martin, P.T. (2001) Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J Agric Food Chem., 49(11):5340–5347.
- (7). Reygaert, W.C. (2017) An Update on the Health Benefits of Green Tea. Review Beverages, 3(6):1-14.
- (8). Murugan, S. ; Devi, P. and John, P.N. (2011) Antimicrobial susceptibility pattern of biofilm Producing *E. coli* of urinary tract infections. Curr. Res. Bacteriol., 4: 73-80.
- (9). Lassaro, M.A.; Salinger, N.; Zhang, J.; Wang, Y. and Zhong, Z. (2009) F1C Fimbriae play an important role in biofilm formation and intestinal colonization by the *E. coli* commensal strain Nissle. App. Environ. Microbiol., 75: 246-251.
- (10). Clinical and Laboratory Standards Institute. (2013) Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement m100-s23, Clsi, Wayne, Pa, Usa.
- (11). Basam, B.M. ; Enas, I.J. ; Enaam, H.B. ; Zainab, H.S. and Khitam, A.O. (2016) Antimicrobial Effects of *Rubus idaeus*, *Origanum vulgare*, *Petroselinum crispum* and Bacteria Infecting Urinary Tract. IJOPILS, 4(2): 1-17.
- (12). Kumar, S. and Narain, S. (2010) Herbal remedies of wetlands macrophytes in India. Int. J. Pharm. Biosci., 1(2): 1-12.
- (13). Tahmourespour, A. and Kermanshahi, R.K. (2011) The effect of a probiotic strain (*Lactobacillus acidophilus*). Bosnian J. Basic Med. Sci., 11(1): 37-40.
- (14). Johnson, J.R. ; Moseley, S.L. ; Roberts, P.L. ; Stamm W.E. (1988) Aerobactin and other virulence factor genes among strains of *E. coli* causing urosepsis: Association with patient characteristics. Infect Immun, 56:405-12.
- (15). Vidal, C.A. ; Sousa, E.O. ; Fabiola, F.G. ; Rodrigues, R.C, Lacerda, S.R. and Costa, J.G. (2012) Phytochemical screening and synergistic interactions between aminoglycosides, selected antibiotics and extracts from the bryophyte *Octoblepharum albidum* Hedw (calymperaceae). Arch. Biol. Sci. Belgrade., 64 (2): 465-470.

- (16). Siddiqui, M. W. ; Sharangi, A. B. ; Singh, J. P. ; Thakur, P. K., ; Ayala-Zavala, J. F. ; Singh, A. and Dhua, R. S. (2016). Antimicrobial properties of teas and their extracts *in vitro*. Critical Reviews in Food Science and Nutrition, 56(9), 1428–1439.
- (17). Ojo, O. O. ; Ladeji, O. and Nadro, M. S. (2007) Studies of the antioxidative effects of green and black tea (*Camellia sinensis*) extracts in rats. Journal of Medicinal Food, 10(2), 345–9.
- (18). Bai, L. ; Takagi, S. ; Ando, T. ; Yoneyama, H. ; Ito, K. ; Mizugai, H. and Isogai, E.(2016). Antimicrobial activity of tea catechin against canine oral bacteria and the functional mechanisms. J. Vet. Med. Sci. Advanced Publication, Article ID: 16–0198.
- (19). Liu, X. ; Li, J. ; Wang, Y. ; Li, T. ; Zhao, J. and Zhang, C. (2013) Green tea polyphenols function as prooxidants to inhibit *P. aeruginosa* and induce the expression of oxidative stress-related genes. Folia Microbiologica, 58(3):211–7.
- (20). Mah, T. C. ; Pitts, B. ; Pellock, B. ; Walker, G. C. ; Stewart, P. S. and O’Toole, G. A. (2003) A genetic basis for *P. aeruginosa* biofilm antibiotic resistance. Nature, 426(6964):306–310.
- (21). Abidi, S. H. ; Ahmed, K. ; Sherwani, S. K. and Kazmi, S. U. (2014) Reduction
- (22). and removal of *P. aeruginosa* biofilm by natural agents. Int. J. Chem. Pharma. Sci., 5: 28–34.
- (23). Radji, M. ; Agustama, R. A. ; Elya, B. and Tjampakasari, C. R. (2013) Antimicrobial activity of green tea extract against isolates of methicillin–resistant *Staph. aureus* and multi–drug resistant *P. aeruginosa*. Asian Pacific J. Trop. Biomed., 3(8): 663–667.
- (24). Dubey, N. and Mehta A. (2016) *In vitro* study of the antimicrobial property of Green tea extract against standard (ATCC) bacterial strains and clinical isolates of methicillin resistant *Staph. aureus* & multidrug resistant *P. aeruginosa*. Indian J Microbiol. Res.,3(3): 230-235.
- (25). Hassan, A. A. (2018) Assessment of antibacterial activity of green tea against bacterial eye infection in Shendi Town. MSc. Discretion in Medical Laboratory Science (Microbiology), Shendi University,Sudan.
- (26). Caturla, N. ; Vera-Samper, E. ; Villalaín, J. ; Mateo, C. R. and Micol, V. (2003) The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radical Biology and Medicine, 34(6): 648–662.
- (27). Vidigal, P. G. ; Müsken, M. ; Becker, K. A. ; Häussler, S. ; Wingender, J. ; Steinmann, E. ; Kehrmann, J. ; Gulbins, E. ; Buer, J. ; Rath, P.M. and Steinmann, J. (2014) Effects of green tea compound epigallocatechin-3-gallate against *Stenotrophomonas maltophilia* infection and biofilm. PloS One, 9(4): e92876.
- (28). Reygaert, W.C.(2014) The antimicrobial possibilities of green tea. Front. Microbiol., 5: 434.
- (29). Maksum, R. ; Rafael, A.A. ; Berna, E. and Conny, R.T. (2013) Antimicrobial activity of green tea extract against isolates of methicillin resistant *Staph.aureus* and multi-drug resistant *P.aeruginosa*. Asian Pacific J. Trop. Biomed., 3(8): 663-667.
- (30). Enas, Y.S.(2014) Antibacterial effect of black and green tea on oral bacteria in pregnant. Int. J. Enhanced Res. Sci. Technol. Engi., 3(4): 1-14.

- (31). Jasim,E. I. ; Al-Fraji, B.B. and Shawket, D.S.(2017) *Lactobacillus casei* isolated from Human Milk and Three Natural Agents act as Antibacterial against Gram Negative Pathogenic Bacteria isolated from Infected Eye .Int. J. Curr. Microbiol. App. Sci., 6(4): 2568-2580
- (32). Kobayakawa, S. ; Jett, B. D. and Gilmore, M. S. (2005) Biofilm formation by *E. faecalis* on intraocular lens material. Curr. Eye Res., 30:741–745.
- (33). 32-Srinivasa, R. ; Karthika, R. ; Singh, P. ; Shashikala, P.; Kanungo, R.; Jayachandran, S. and Prashanth, K.(2008) Correlation on between biofilm production on and multiple drug resistance in imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian J. Med. Microbiol., 26(4): 333-337.
- (34). 33-Ghanbarpour, R. and Salehi, M. (2010) Determination of adhesion encoding genes in *E. coli* isolates from Omphalitis of chicks. American J. Anim. Vet. Sci., 5: 91-96.
- (35). 34-Paulo, J. M. ; Haas,B.W. and Gilmore, M.S. (2015) Biofilms in Infections of the Eye. Review.Pathogens, 4: 111-136.
- (36). 35- Yaung, J. ; Jin,M. ; Barron, E. ; Spee, C. ; Wawrousek,E. ; Kannan, R. and
- (37). Hinton, D. (2007) Alpha-crystallin distribution in retinal pigment epithelium and effect of gene knockouts on sensitivity to oxidative stress. Mol. Vis.,13:566–577.
- (38). 36- Whiston,E. ; Sugi,N. ; Kamradt,M. ; Sack,C.; Heimer,R. ; Engelbert, M. ; Wawrousek,E.; Gilmore,M. ; Ksander,R. and Gregory, M.(2008) α B-Crystallin Protects Retinal Tissue during *Staph.aureus*-Induced Endophthalmitis. Infect. Immun.,76(4) : 1781–1790 .
- (39). 37- Flayyih,M. T. ; Yousif, H.S. and Subhi,I.M. (2013) Antimicrobial effects of Black Tea (*Camellia sinensis*) on *Pseudomonas aeruginosa* Isolated from eye infection. Iraqi J. Science., 54 (2): 255-265.

USING INCRETIN IN TREATMENT OF DIABETES MELLITUS DISEASE

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USING INCRETIN IN TREATMENT OF DIABETES MELLITUS DISEASE

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Abstract:

Incretin hormones are gut peptides secreted in response to nutrient ingestion, which play a key role in the regulation of islet function and blood glucose levels. In humans, the major incretin hormones are glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP), and together they fully account for the incretin effect which is defined as the phenomenon whereby orally ingested glucose elicits a much greater insulin response than that obtained when glucose is infused intravenously to give identical blood glucose levels.

there is evidence to suggest that impairments in secretion and/or action of incretin hormones arise secondarily to the development of insulin resistance, glucose intolerance, and/or increases in body weight rather than being causative factors. In separate studies, insulin sensitivity, glucose tolerance, and body mass index (BMI) have all been identified as independent factors associated with reductions in GLP-1 secretion and an impaired incretin effect.

In patients with type 2 diabetes, the incretin effect is clearly reduced, which results in an inappropriately low insulin response to the ingestion of nutrients. Several early studies indicated that the reduced incretin effect could, at least in part, be related to impaired secretion of GLP-1 (whereas secretion of GIP is generally found to be unaltered).

Impaired meal-stimulated GLP-1 levels have been reported in some studies of patients with type 2 diabetes.

- incretins exert antidiabetic actions in a glucose-dependent manner
- Glucagon-like peptide 1 receptor (GLP-1r) agonists, but not dipeptidyl peptidase-4 (DPP-4) inhibitors, inhibit gastric emptying and might cause weight loss
- DPP-4 inhibitors can be administered orally and are well tolerated
- GLP-1r agonists must be administered by subcutaneous injection and commonly cause nausea.

Key words: Incretin, Diabetes Mellitus..



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Introduction:

Diabetes is a heterogeneous metabolic disorder that is characterized by elevated blood glucose due to either insufficient amount of insulin or the body's inability to use the produced insulin efficiently, or both [1]. Diabetes is a chronic condition affecting 8% (24 million) of the US population [2] and associated with an increased risk for mortality as well as morbidity. The prevalence of diabetes (type 1 and type 2) is expected to increase by 54% to >54.9 million persons between 2015 and 2030 just in the United States [3]. By 1930, the total number of annual deaths from 2015 to 2030 is expected to increase to 385,800 in the United States, with associated annual medical and societal costs expected to reach \$622 billion by 2030.

In type 1 of diabetes mellitus (T1DM), autoreactive T cells attack the insulin secreting pancreatic β cells, resulting in no insulin being synthesized in the β -cells. However, in type 2 diabetes Mellitus (T2DM), which accounts for about 90–95% of the total diabetes-affected population, there is not only impaired insulin production by the β -cells of the pancreas but also impaired insulin release in response to high blood glucose levels (a condition termed hyperglycemia). This increased glucose level causes insulin resistance in the tissues. The complex interplay of both genetic and life style factors are involved in the pathophysiology of T2D. Both T1DM and T2DM, if not controlled, can result in long-term complications, like defective leptin signalling and damage to the central nervous system. These complications could be the result of a pathogenic process at the mitochondrial level [4]. Studies have shown that mitochondrial dysfunction is linked with T2DM and age-related insulin resistance. Besides diabetes, several other factors also can also affect mitochondrial function, such as genetic factors, oxidative stress, mitochondrial biogenesis, and aging [5].

Types of Diabetes Mellitus

1- Type 1 diabetes mellitus

Only 5%–10% of patients are affected with T1DM, of which 80–90% are children in their adolescence [7,8]. T1DM, sometimes called juvenile DM, involves Autoreactive T-cells and circulating antibodies of the immune system destroy insulin-secreting pancreatic β - cells, leading to lifelong dependency on an external source of insulin. Insulin, isoforms of glutamic acid decarboxylase (GAD), protein tyrosine phosphatase (IA2 and IA2 β) and zinc transporter protein (ZnT8A) are the major autoantigens of T1DM [10]. The human leukocyte complex (HLA), especially the DR and the DQ genes, plays a major role in the manifestation of T1DM [6]. The HLA-DQA1, HLA-DQB1, and HLA-DRB1 genes belong to HLA family. The HLA complex helps the immune system distinguish the body's own proteins from those proteins produced by foreign invaders, such as viruses and bacteria.

2- Type 2 diabetes mellitus

Type 2 diabetes mellitus also known as adult –onset diabetes, occurs during the later stages of life and comprises 95% of the diabetes-affected population [10,11]. T2DM is characterized by declining insulin production and eventual pancreatic β -cell failure. This leads to a decrease in glucose transport into the liver, muscle cells, and fat cells [12,13]. T2DM goes undiagnosed for several years resulting in long-term effects because

hyperglycaemia develops gradually over the years. The precise causal factors for T2DM is not completely understood.

T2DM is a polygenic disorder that develops due to a complex interaction between multiple genes and environmental factors. T2DM is largely associated with aging. Lifestyle conditions, including physical inactivity, sedentary lifestyle, cigarette smoking and generous consumption of alcohol, are reported to play an important role in the development of T2DM.

3- Type 3 diabetes mellitus

Recent studies have established links between systemic metabolic dysfunction, such as diabetes, and neurocognitive impairment, including dementia, obesity, insulin resistance, diabetes, and metabolic syndrome [14]. Brains of patients with Alzheimer's disease (AD) showed reduced expression of insulin and neuronal insulin receptors, as compared with those of age-matched controls. This event gradually leads to a breakdown of the entire insulin-signalling pathway, which manifests insulin resistance [15]. Studies have shown the links between derangements of carbohydrates, proteins, lipids, proteins and brain dysfunction, and cognitive impairment [14].

T3D can be defined as neuroendocrine disorder that represents the progression of T2DM to AD [16]. These include insulin growth factor signalling, acetylcholine esterase activity, inflammatory responses, ApoE4A allele on synaptic plasticity, and vascular dysregulation of brain capillaries.

4- Gestational diabetes mellitus

During pregnancy, gestational diabetes in the mothers is linked to hyperglycemia in the fetus. Hyperglycaemia in pregnant women increases the risk of adverse maternal, fetal, and neonatal outcomes. Gestational diabetes is characterized by carbohydrate intolerance during pregnancy [1]. A glucose challenge test is done between 22 and 24 weeks of pregnancy by giving an oral glucose load of 50 g of glucose. If the 2-h post-glucose value is >140 mg/dL, the test is positive.

Incretin Hormone

In 1930, La Barre et al. claimed that crude secretin can further be purified into two fractions—one which stimulates the exocrine pancreas and another which stimulates insulin secretion. They postulated that a part of glucose lowering effect was due to an additional independent effect of the preparation per se, because the fraction lowered the blood glucose even in pancreatectomized dogs.[16] In 1932, La Barre coined the term incretine (incretin) to denote a factor extracted from the upper gut mucosa, which produced hypoglycemia by stimulating insulin secretion without having any effect on pancreatic exocrine secretion and also asserted that incretin could lower the blood glucose level only in pancreas-intact animals. They also predicted the use of incretin in the treatment of human diabetes.[17] In the next few years, Loew et al., along with other research workers, concluded from their experiments that the existence of incretins was “questionable.”[18-19] This led to premature condemnation of the incretin concept and for the next 3 decades no further research was done in this area.

Incretin hormones are gut peptides secreted in response to nutrient ingestion, which

play a key role in the regulation of islet function and blood glucose levels. In humans, the major incretin hormones are glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP), and together they fully account for the incretin effect [20]. The incretin effect is defined as the phenomenon whereby orally ingested glucose elicits a much greater insulin response than that obtained when glucose is infused intravenously to give identical blood glucose levels (the so-called isoglycemic glucose infusion). Depending on the size of the stimulus, the incretin effect can account for up to 70% of glucose-induced insulin secretion in healthy humans [21]. It is explained by the fact that oral, but not intravenous, glucose stimulates the release of the incretin hormones which then enhance glucose-stimulated insulin secretion.

Interestingly, a similar phenomenon has recently been reported for lipids, with oral ingestion being associated with increased insulin (and incretin hormone) responses compared with matched intravenous lipid infusions [22]. GIP is produced in the K-cells, located predominantly in the more proximal parts of the small intestine, with highest density in the duodenum. In contrast, GLP-1 is produced by the more distally situated L-cells, primarily in the ileum, and is also found in high density in the colon [23]. However, both cell types can be found throughout the whole intestine, and there is also evidence that a population of cells exists in which both GLP-1 and GIP are co-localized [24].

The discovery that DPP-4 has a key role in the degradation of GLP-1 has led to the development of DPP-4 inhibitors as therapeutic agents in the management of type 2 diabetes [25-26].

Incretin Hormone Biosynthesis and Metabolism

GLP-1 is derived from the post-translational processing of the proglucagon gene product by prohormone convertase 1/3 (PC-1/3) in the intestinal L-cells. This process also results in production of the related peptide GLP-2. In the pancreatic α -cell, the action of PC-2 gives rise to glucagon [29]. However, recent studies suggest that, under some circumstances, small amounts of GLP-1 may also be produced in the α -cell, with isolated rat and human islets being shown to contain GLP-1 following exposure to high glucose concentrations [30-31]. In the case of GIP, PC-1/3 processing of pro-GIP within the K-cells results in the formation of a single bioactive 42-residue peptide [32].

As for GLP-1, there is some evidence to suggest that α -cells may also be able to produce small amounts of GIP, which undergoes processing by PC-2 to form a C-terminally truncated peptide, GIP(1-30) [33].

In the intestinal enteroendocrine cells, both GLP-1 and GIP are stored as intact peptides within secretory granules until they are secreted. Once released, N-terminal degradation by DPP-4 forms the metabolites, GLP-1(937)/GLP-1(9-36)NH₂ and GIP(3-42), which account for the majority of the circulating forms of the two incretins [34]. This degradation is a rapid process, giving the intact peptides short half-lives of only 1-2 minutes (GLP-1) [31-35] and 2-3 minutes (GIP) [36-37].

Incretin Hormones in Disease States

Interpretation of the precise relationship between disease and incretin hormone secretion and the incretin effect is complicated. However, there is evidence to suggest that impairments in secretion and/or action of incretin hormones arise secondarily to the development of insulin resistance, glucose intolerance, and/or increases in body weight rather than being causative factors. In separate studies, insulin sensitivity, glucose tolerance, and body mass index (BMI) have all been identified as independent factors associated with reductions in GLP-1 secretion and an impaired incretin effect [38-39].

Incretin Hormones in Obesity

Interest in the role of incretin hormones in obese subjects originates from findings indicating roles for GIP as a potential mediator of increased triglyceride storage in adipose tissue and, thus, of weight gain and obesity. Conversely, the appetite-reducing activity of GLP-1 suggests a role in inhibiting food intake and weight gain.[40]

Incretin Hormones in Type 2 Diabetes Mellitus

The incretin hormones Although there are probably many postprandially released hormones with an effect on insulin secretion[41], the available experimental evidence suggests that the two most important ones are glucose- dependent insulinotropic polypeptide (GIP) and glucagon- like peptide-1 [42].

Type 2 diabetes In patients with type 2 diabetes, the incretin effect is clearly reduced [43-44], which results in an inappropriately low insulin response to the ingestion of nutrients .Several early studies indicated that the reduced incretin effect could, at least in part, be related to impaired secretion of GLP- 1 (whereas secretion of GIP is generally found to be unaltered).

Impaired meal-stimulated GLP-1 levels have been reported in some studies of patients with type 2 diabetes [45-46]. However, in other studies, no such impairment was observed [47-48].

Incretin Function in Type 2 Dm .

Given that GIP and GLP-1 together are responsible for the incretin effect in healthy subjects, it is now possible to analyse the incretin defect in patients with Type 2 diabetes. Theoretically, the defect could be due to impaired secretion or accelerated metabolism of the incretin hormones; alternatively, the effect of the hormones could be compromised. There are many publications on the secretion of GIP in Type 2 diabetes, and both increased, normal and decreased secretion have been reported [49]. Impression is that GIP secretion is normal or slightly impaired. A recent study in patients with Type 2 diabetes, covering a very wide clinical spectrum of the disease [50], near normal fasting concentrations and meal responses were found, with no correlations between metabolic parameters and GIP responses. In the same study, a very significant impairment of the secretion of GLP-1 was observed. By multiple regression analysis, the impairment was found to be related to impaired cell function. In a previous study in a small group of identical twins discordant for Type 2

diabetes, the GLP-1 response was lower in the diabetic twins [51]. Furthermore, in first degree relatives of diabetic patients, 24-h GLP-1 profiles were normal [52]. The lower GLP-1 concentrations could also be caused by an increased elimination of GLP-1 in diabetic subjects compared to healthy subjects. An impaired secretion of GLP-1, therefore, seems to contribute to the impaired incretin effect in Type 2 diabetes.

But what about the effect of the hormones.?

Here a dramatic difference emerges. The effects of iv infusions of GIP and GLP-1 were studied [53] in moderate Type 2 diabetic patients and matched control subjects, and it was found that the insulinotropic effect of GIP was almost lost in the patients, whereas the insulin response to GLP-1 was similar to that observed in the control subjects. Similar results were obtained by another study [54]. Based

on chromatography, the structure of the two hormones seems to be normal in diabetic patients, and recent studies have shown that mutations in the genes that encode GLP-1 and GIP are unlikely to be common causes for the impaired incretin effect [55-56]. This points to an impaired effect of GIP on the beta cell as an important contributor to the incretin defect in Type 2 diabetes mellitus and raises the question of the function of GIP receptors in these patients. Loss of function mutations or impaired expression of GIP receptors could explain the impaired effect [57]. Several groups have reported polymorphisms in the coding region of the GIP receptor gene, but these were neither associated with diabetes [58] nor with defective signalling of the receptor [59]. In contrast, a defective expression of the GIP receptor has been observed in animals with experimental diabetes [60]. Studies in glucose-tolerant first-degree relatives of diabetic patients show a reduced insulinotropic effectiveness of GIP in 50% of the subjects compared to the control subjects without a family history of diabetes, indicating that the GIP defect could be a genetically determined and possibly primary defect [61]. In an attempt to pursue this finding in more detail, there designed a test consisting of bolus injections of GIP and GLP-1 (with or without simultaneous glucose administration) [62]. By comparing the response to GIP with that obtained with GLP-1, was the result if responses to GLP-1 were low (as in poorly controlled diabetic subjects), the responses to GIP were equally impaired and vice versa. conducted studies and the results was the GLP-1 the subjects showed a completely normal insulin response to glucose. A similar observation (that GLP-1 can restore beta-cell responsiveness glucose in patients with Type 2 diabetes) was made by a study using an entirely different approach [63]. Moreover, the normal glucose induced inhibition of glucagon secretion was completely restored.

GIP, however, regardless of dose, had neither effect on insulin secretion nor on glucose turnover. It was concluded that the GIP defect in Type 2 diabetes is very severe indeed, but restricted to "late phase" insulin secretion, which could be particularly relevant for postprandial insulin secretion. In further studies using the same technique, was investigated groups of diabetic patients known or suspected to have diabetic etiologies different from those of the classic obese elderly Type 2 diabetic patients [64]. The groups included patients with Type 1 diabetes, diabetes secondary to pancreatitis, and patients with LADA. It was the underlying hypothesis that these patients would not exhibit a comparable GIP defect,

based on the assumption that the GIP defect was a genetic defect contributing to the phenotype of the typical Type 2 diabetic patient. Again, however, the results were unexpected. The different groups had clearly lower relative responses to GIP than the non-diabetic control

group, and for example, the patients with secondary diabetes had virtually absent late response to GIP and no effect on glucose turnover. It was concluded that the observed GIP defect is a consequence of the diabetic state, and although a genetic component might also be involved in Type 2 diabetic patients, as shown in the study of the first degree relatives [65], the defect induced by diabetes is very severe. The differential responsiveness of the cells to GIP and GLP-1 is surprising because of the many similarities between the two hormones, their receptors and their signal transduction mechanisms [66-67]. Apparently, however, the interaction between glucose stimulation of insulin secretion and the potentiating actions of the two hormones differ in spite of the fact that both seem to depend on an initial accumulation of cAMP.

summarize the state-of-the-art science on incretin hormones including their role in physiology and in the pathophysiology of obesity and type 2 diabetes, and the therapeutic perspective that can be derived from these findings.

Incretin-Based Therapies For Type 2 Diabetes Mellitus

As we have said previously, the incretin hormone glucagon-like peptide 1 (GLP-1) stimulates insulin release in response to an enteric glucose load in humans [65] was followed by major advances in our understanding of how GLP-1 regulates glucose metabolism. [70-71] In addition, GLP-1—unlike the other incretin hormone, glucose-dependent insulinotropic polypeptide (GIP)—retains its glucose-regulatory actions in patients with diabetes mellitus. These findings led to the discovery and generation of structurally distinct GLP-1 receptor (GLP-1R) agonists, which mimic the actions of GLP-1 in vivo in humans [72-73] Furthermore, characterization of the essential role of dipeptidyl peptidase 4 (DPP-4) in the inactivation of bioactive GLP-1 and GIP [74-75] promoted the development of orally available DPP-4 inhibitors, administration of which stabilizes both incretin hormones at physiologically active levels.

1 - DPP-4 inhibitors

2 - GLP-1R agonists

Conclusions

Incretin hormone secretion is regulated by a variety of different signaling pathways, which allow a coordinated response to a physiological stimulus. Many factors (nutrients, nerves, hormones, and even drugs) can affect K- and L-cell responses via direct and indirect mechanisms. However in humans, direct stimulation by nutrients in contact with the apical membrane of the endocrine cells appears to be the principal factor determining incretin hormone secretion. Alterations in GLP-1 and GIP responses are reported to be related to changes in body weight, insulin resistance, and glucose

tolerance, but the precise ways in which these factors impact on the enteroendocrine cells remains unclear. Nevertheless, changes in incretin hormone levels have been reported in obese glucose-tolerant subjects, with impairments in incretin action becoming evident as glycemic control deteriorates. These impairments probably contribute to the ongoing reduction in β -cell function and developing hyperglycemia observed when overt diabetes becomes established. Emerging data are suggesting that incretin hormones also have other actions in addition to their accepted roles in the regulation of normal islet function and glucose homeostasis. These include potentially beneficial cardiovascular and neuroprotective actions. However, while the glucoregulatory actions of the incretins have been convincingly demonstrated to be impaired in subjects with diabetes, it is currently unknown whether this also applies to any of their non-glucose effects. Given the increasing use of incretin-based therapies as part of the treatment paradigm for type 2 diabetes, further investigation of the pleiotropic effects of the incretin hormones in disease states is clearly warranted.

References

1. A.T. Kharroubi, H.M. Darwish Diabetes mellitus: the epidemic of the century *World J. Diabetes*, 6 (2015), pp. 850-867
2. W.I. Sivitz, M.A. Yorek Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities *Antioxid. Redox Signal.*, 12 (2010), pp. 537-577
3. W.R. Rowley, C. Bezold, Y. Arikian, E. Byrne, S. Krohe Diabetes 2030: insights from yesterday, today, and future trends *Popul. Health Manag.*, 20 (2017), pp. 6-12
4. K. Akter, E.A. Lanza, S.A. Martin, N. Myronyuk, M. Rua, R.B. Raffa Diabetes mellitus and Alzheimer's disease: shared pathology and treatment? *Br. J. Clin. Pharmacol.*, 71 (2011), pp. 365-376
5. J.A. Kim, Y. Wei, J.R. Sowers Role of mitochondrial dysfunction in insulin resistance *Circ. Res.*, 102 (2008), pp. 401-414
6. A. American Diabetes Diagnosis and classification of diabetes mellitus *Diabetes Care*, 37 (Suppl. 1) (2014), pp. S81-S90
7. D. Dabelea, E.J. Mayer-Davis, S. Saydah, G. Imperatore, B. Linder, J. Divers, R. Bell, A. Badaru, J.W. Talton, T. Crume, A.D. Liese, A.T. Merchant, J.M. Lawrence, K. Reynolds, L. Dolan, L.L. Liu, R.F. Hamman, S.f.D.i.Y. Study, Prevalence of type 1 and type 2 diabetes among children and adolescents from 2001 to 2009, *JAMA*, 311 (2014) 1778–1786.
8. D.M. Maahs, N.A. West, J.M. Lawrence, E.J. Mayer-Davis Epidemiology of type 1 diabetes *Endocrinol. Metab. Clin. N. Am.*, 39 (2010), pp. 481-497
9. I. Vermeulen, I. Weets, M. Asanghanwa, J. Ruige, L. Van Gaal, C. Mathieu, B. Keymeulen, V. Lampasona, J.M. Wenzlau, J.C. Hutton, D.G. Pipeleers, F.K. Gorus, R. Belgian Diabetes Contribution of antibodies against IA-2beta and zinc transporter 8 to classification of diabetes diagnosed under 40 years of age *Diabetes Care*, 34 (2011), pp. 1760-1765
10. J.E. Fradkin Confronting the urgent challenge of diabetes: an overview *Health Aff (Millwood)*, 31 (2012), pp. 12-19
11. J.S. Bhatti, G.K. Bhatti, P.H. Reddy Mitochondrial dysfunction and oxidative stress in metabolic disorders - a step towards mitochondria based therapeutic strategies *Biochim. Biophys. Acta*, 1863 (2017), pp. 1066-1077
12. C.R. Kahn, Banting Lecture Insulin action, diabetogenes, and the cause of type II diabetes *Diabetes*, 43 (1994), pp. 1066-1084
13. A.B. Olokoba, O.A. Obateru, L.B. Olokoba Type 2 diabetes mellitus: a review of current trends *Oman Med. J.*, 27 (2012), pp. 269-273
14. L.E. Stoeckel, Z. Arvanitakis, S. Gandy, D. Small, C.R. Kahn, A. Pascual-Leone, A.Pawlyk, R. Sherwin, P. Smith Complex mechanisms linking neurocognitive dysfunction to insulin resistance and other metabolic dysfunction *F1000Res*, 5 (2016), p. 353

15. S. Supale, N. Li, T. Brun, P. Maechler Mitochondrial dysfunction in pancreatic beta cells *Trends Endocrinol. Metab.*, 23 (2012), pp. 477-487
16. P. Maechler, N. Li, M. Casimir, L. Vetterli, F. Frigerio, T. Brun Role of mitochondria in beta-cell function and dysfunction *Adv. Exp. Med. Biol.*, 654 (2010), pp. 193-216
17. La Barre J, Still EU. Studies on the physiology of secretin. *Am J Physiol.* 1930;91:649-53.
18. La Barre J. Sur les possibilités d'un traitement du diabète par L'incretine. *Bull Acad R Med Belg.* 1932;12:62034.
19. Loew ER, Gray JS, Ivy AC. The effect of duodenal instillation of hydrochloric acid upon the fasting blood sugar of dogs. *Am J Physiol.* 1939;126:270-6.
20. Loew ER, Gray JS, Ivy AC. The effect of acid stimulation of the duodenum upon experimental hyperglycemia and utilization of glucose. *Am J Physiol.* 1940;128:298-308.
21. Creutzfeldt W. The incretin concept today. *Diabetologia.* 1979;16:75–85. [PubMed] [Google Scholar]
22. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab.* 1986;63:492–498. [PubMed] [Google Scholar]
23. Lindgren O, Carr RD, Deacon CF, Holst JJ, Pacini G, Mari A, Ahren B. Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. *J Clin Endocrinol Metab.* 2011;96:2519–2524. [PubMed] [Google Scholar]
24. Eissele R, Göke R, Willemer S, Harthus HP, Vermeer H, Arnold R, Göke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest.* 1992;22:283–291. [PubMed] [Google Scholar]
25. Mortensen K, Christensen LL, Holst JJ, Orskov C. GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul Pept.* 2003;114:189–196. [PubMed] [Google Scholar]
26. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J Clin Endocrinol Metab.* 1995;80:952–957. [PubMed] [Google Scholar]
27. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab.* 2000;85:3575–3581. [PubMed] [Google Scholar]
28. Amori RE, Lau J, Pittas AG (July 2007). "Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis". *JAMA.* 298 (2): 194–206. doi:10.1001/jama.298.2.194 . PMID 17622601 .
29. Nauck MA, Homberger E, Siegel EG, Allen RC, Eaton RP, Ebert R, Creutzfeldt W. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J Clin Endocrinol Metab* 1986. 63:492-498.
30. Holst JJ. The physiology of glucagon-like peptide 1. *Physiol Rev.* 2007;87:1409–1439. [PubMed] [Google Scholar]
31. Heller RS, Aponte GW. Intra-islet regulation of hormone secretion by glucagon-like peptide-1-(7-36) amide. *Am J Physiol.* 1995;269:G852–G860. [PubMed] [Google Scholar]
32. Whalley N, Pritchard L, Smith DM, White A. Processing of proglucagon to GLP-1 in

- pancreatic alpha cells: is this a paracrine mechanism enabling GLP-1 to act on beta cells? *J Endocrinol.* 2011 In press. [PubMed] [Google Scholar]
33. Ugleholdt R, Poulsen ML, Holst PJ, Irminger JC, Orskov C, Pedersen J, Rosenkilde MM, Zhu X, Steiner DF, Holst JJ. Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor. *J Biol Chem.* 2006;281:11050–11057. [PubMed] [Google Scholar]
34. Fujita Y, Wideman RD, Asadi A, Yang GK, Baker R, Webber T, Zhang T, Wang R, Ao Z, Warnock GL, Kwok YN, Kieffer TJ. Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alphacells and promotes insulin secretion. *Gastroenterology.* 2010;138:1966–1975. [PubMed] [Google Scholar]
35. Deacon CF, Pridal L, Klarskov L, Olesen M, Holst JJ. Glucagon-like peptide 1 undergoes differential tissue-specific metabolism in the anesthetized pig. *Am J Physiol.* 1996;271:E458–E464. [PubMed] [Google Scholar]
36. Vilsboll T, Agerso H, Krarup T, Holst JJ. Similar elimination rates of glucagon-like peptide-1 in obese type 2 diabetic patients and healthy subjects. *J Clin Endocrinol Metab.* 2003;88:220–224. [PubMed] [Google Scholar]
37. Vilsboll T, Agerso H, Lauritsen T, Deacon CF, Aaboe K, Madsbad S, Krarup T, Holst JJ. The elimination rates of intact GIP as well as its primary metabolite, GIP 3-42, are similar in type 2 diabetic patients and healthy subjects. *Regul Pept.* 2006;137:168–172. [PubMed] [Google Scholar]
38. Deacon CF, Nauck MA, Meier J, Hucking K, Holst JJ. Degradation of endogenous and exogenous gastric inhibitory polypeptide in healthy and in type 2 diabetic subjects as revealed using a new assay for the intact peptide. *J Clin Endocrinol Metab.* 2000;85:3575–3581. [PubMed] [Google Scholar]
39. Rask E, Olsson T, Söderberg S, Johnson O, Seckl J, Holst JJ, Ahren B. Impaired incretin response after a mixed meal is associated with insulin resistance in nondiabetic men. *Diabetes Care.* 2001;24:1640–1645. [PubMed] [Google Scholar]
40. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes.* 2008;57:1340–1348. [PubMed] [Google Scholar]
41. Creutzfeldt W, Ebert R, Willms B, Frerichs H, Brown JC. Gastric inhibitory polypeptide (GIP) and insulin in obesity: increased response to stimulation and defective feedback control of serum levels. *Diabetologia.* 1978;14:15-24.
42. Holst JJ, Orskov C. Incretin hormones—an update (2001) *Scand J Clin Lab Invest* 234 [Suppl]:75–85
43. Fehmann HC, Goke R, Goke B (1995) Cell and molecular biology of the incretin hormones glucagon-like peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 16:390–410
44. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulindependent) diabetes. *Diabetologia.* 1986;29:46–52. [PubMed] [Google Scholar]
45. Bagger JI, Knop FK, Lund A, Vestergaard H, Holst JJ, Vilsboll T. Impaired regulation of the incretin effect in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2011;96:737–345. [PubMed] [Google Scholar]

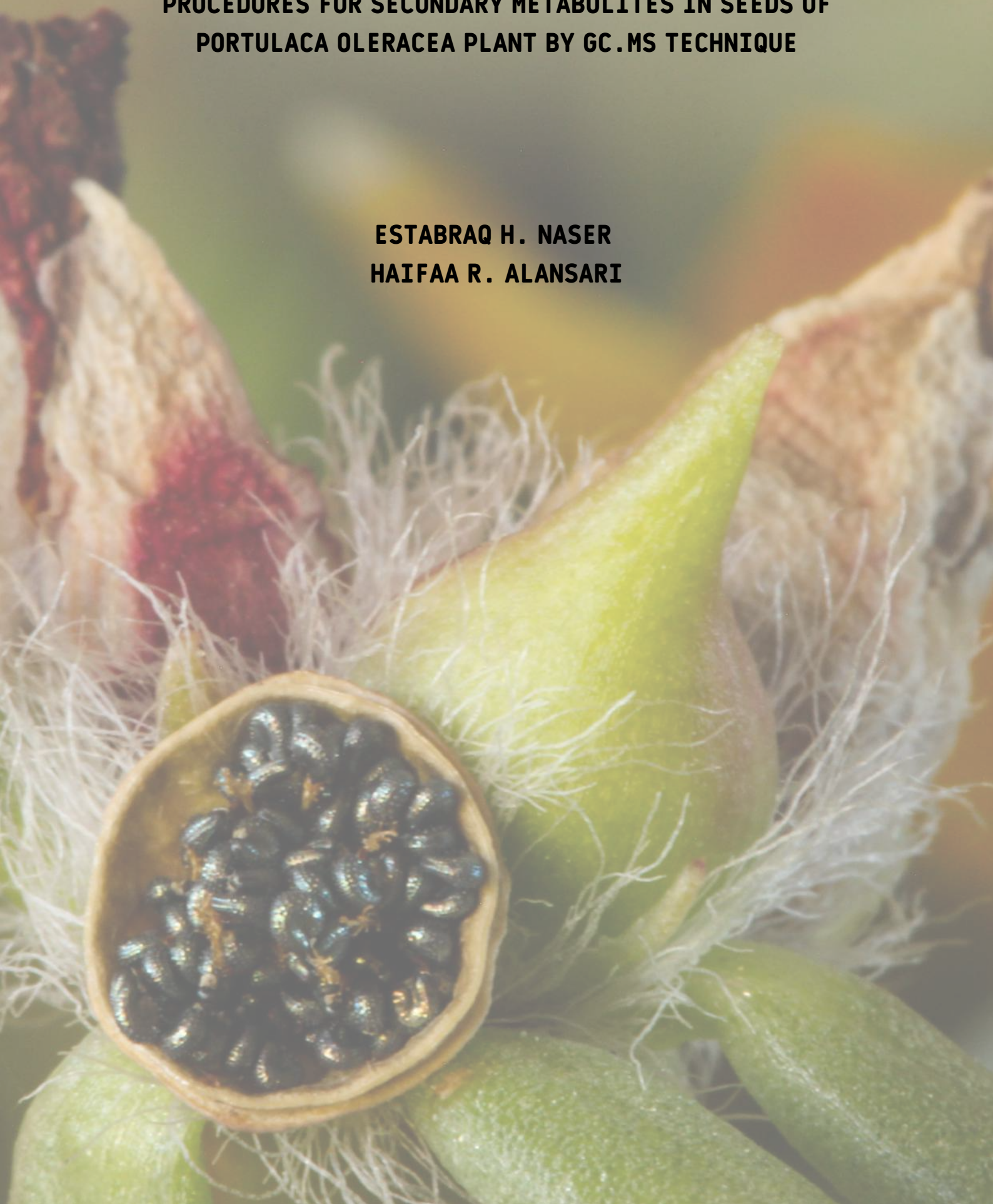
- 46.. Muscelli E, Mari A, Casolaro A, Camastra S, Seghieri G, Gastaldelli A, Holst JJ, Ferrannini E. Separate impact of obesity and glucose tolerance on the incretin effect in normal subjects and type 2 diabetic patients. *Diabetes*. 2008;57:1340–1348. [PubMed] [Google Scholar]
47. . Knop FK, Aaboe K, Vilsboll T, Madsbad S, Holst JJ, Krarup T. Reduced incretin effect in obese subjects with normal glucose tolerance as compared to lean control subjects. *Diabetes*. 2008;57(Suppl 1):A410. [Google Scholar]
48. Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes*. 2001;50:609–613. [PubMed] [Google Scholar]
49. Ryskjaer J, Deacon CF, Carr RD, Krarup T, Madsbad S, Holst J, Vilsboll T. Plasma dipeptidyl peptidase-IV activity in patients with type- 2 diabetes mellitus correlates positively with HbA1c levels, but is not acutely affected by food intake. *Eur J Endocrinol*. 2006;155:485–493. [PubMed] [Google Scholar]
50. Krarup T (1988) Immunoreactive gastric inhibitory polypeptide. *Endocr Rev* 9:122–134
51. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK et al. (2001) Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 86:3717–3723
52. Vaag AA, Holst JJ, Volund A, Beck-Nielsen HB (1996) Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)—evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. *Eur J Endocrinol* 135:425–432
53. Nyholm B, Walker M, Gravholt CH, Shearing PA, Sturis J, Alberti KG et al. (1999) Twenty-four-hour insulin secretion rates, circulating concentrations of fuel substrates and gut incretin hormones in healthy offspring of Type II (non-insulin-dependent) diabetic parents: evidence of several aberrations. *Diabetologia* 4:1314–1323
54. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W (1993) Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 91:301–307
55. Elahi D, McAloon Dyke M, Fukagawa NK, Meneilly GS, Sclater AL, Minaker KL et al. (1994) The insulinotropic actions of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (7–37) in normal and diabetic subjects. *Regul Pept* 51:63–74
56. Nauck M, Hahn S, Sauerwald A, Schmeigel W (2000) Lack of germline mutations in the proglucagon gene region coding for glucagon-like peptide 1 in type 2 diabetic (NIDDM) patients. *Exp Clin Endocrinol Diabetes* 108:72–75
- 57.. Orskov C, Vilsboll T, Krarup T, Inagaki N, Hjorth SA (1999) Lack of germ-line mutations in the GIP coding region of the pro-GIP in type II diabetic patients. *Diabetes* 48 [Suppl 1]:A427 (abstract)
58. Holst JJ, Gromada J, Nauck MA (1997) The pathogenesis of NIDDM involves a defective expression of the GIP receptor. *Diabetologia* 40:984–986
59. Kubota A, Yamada Y, Hayami T, Yasuda K, Someya Y, Ihara Y et al. (1996) Identification of two missense mutations in the GIP receptor gene: a functional study and

association analysis with NIDDM: no evidence of association with Japanese NIDDM subjects. *Diabetes* 45:1701–1705

60. Almind K, Ambye L, Urhammer SA, Hansen T, Echwald SM, Holst JJ et al. (1998) Discovery of amino acid variants in the human glucose- dependent insulinotropic polypeptide (GIP) receptor: the impact on thepancreatic beta cell responses and functional expression studies in Chinese hamster fibroblast cells. *Diabetologia* 41:1194–1198
61. Lynn FC, Pamir N, Ng EH, McIntosh CH, Kieffer TJ, Pederson RA (2001) Defective glucosedependent insulinotropic polypeptide receptor expression in diabetic fatty Zucker rats. *Diabetes* 50:1004–1011
62. Meier JJ, Huckling K, Holst JJ, Deacon CF, Schmiegel WH, Nauck MA (2001) Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* 50:2497–2504
63. Vilsboll T, Toft-Nielsen MB, Krarup T, Madsbad S, Dinesen B, Holst JJ (2000) Evaluation of beta-cell secretory capacity using glucagon-like peptide 1. *Diabetes Care* 23:807–812
64. Kjems LL, Holst JJ, Volund A, Madsbad S (2003) The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes* 52:380–386
65. Fehmann HC, Goke R, Goke B (1995) Cell and molecular biology of the incretin hormones glucagonlike peptide-I and glucose-dependent insulin releasing polypeptide. *Endocr Rev* 16:390–410
66. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B et al. (2003) International Union of Pharmacology. XXXV. The glucagon receptor family. *Pharmacol Rev* 55: 167–194
67. Gromada J, Holst JJ, Rorsman P (1998) Cellular regulation of islet hormone secretion by the incretin hormone glucagon-like peptide 1. *Pflugers Arch* 435:583–594
68. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet*. 2006;368:1696-1705.
69. Nauck M. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. *Diabetes Obes Metab*. 2016;18:203-216.
70. Kreymann, B., Williams, G., Ghatei, M. A. & Bloom, S. R. Glucagon- like peptide-17–36 a physiological incretin in man. *Lancet* 2, 1300–1304 (1987).
71. Deacon, C. F. Therapeutic strategies based on glucagon-like peptide 1. *Diabetes* 53, 2181–2189 (2004).
72. Drucker, D. J. The biology of incretin hormones. *Cell. Metab*. 3, 153–165 (2006).
73. Drucker, D. J. & Nauck, M. A. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 368, 1696–1705 (2006).
74. Deacon, C. F., Johnsen, A. H. & Holst, J. J. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J. Clin. Endocrinol. Metab*. 80, 952–957 (1995).
75. Kieffer, T. J., McIntosh, C. H. & Pederson, R. A. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon- like peptide 1 in vitro and in vivo by dipeptidyl peptidase iv. *Endocrinology* 136, 3585–3596 (1995).

**COMPARATIVE STUDY OF THREE SEQUENTIAL EXTRACTION
PROCEDURES FOR SECONDARY METABOLITES IN SEEDS OF
PORTULACA OLERACEA PLANT BY GC.MS TECHNIQUE**

**ESTABRAQ H. NASER
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PLANT BY GC.MS TECHNIQUE**

Estabraq H. NASER¹
Haifaa R. ALANSARI²

Abstract:

Secondary metabolites referred to as phytochemical compounds that have importance in the recovery of many ailments. In order to identify these compounds and get their benefits, they must be identified and isolated from their parent plant; one of them is *Portulaca oleracea* that belongs to portulacaceae family. The seeds are extracted by three different sequential extraction methods including petroleum ether, Ethylacetate, and methanol in way based on gradual increasing solvent polarity. The quantification and identification of the compounds were carried out by gas chromatography mass detection apparatus. The result shown that these seeds contain many secondary metabolites like flavonoids, alkaloids, saponins, steroids and phenolic compounds. The GC-MS analysis of the seeds extracts showed the presence of many important compounds such as, trans-geranylgeraniol, tetracosanoic acid, trans-farnesol, digitoxin, vitamin E, estrol and cis-6-octadecenoic acid in high percent in addition to little percent distributed between β -sitosterol, gitoxigenin, (+)- α -tocopherol, camphene, hexadecane, δ -elemene, 3- methoxybenzyl alcohol, ecgonine, (-)-myrtenol, cholecalciferol, 9-cis-retinal, limonene-6-ol,pivalate, p-cymene, γ -terpinene, humulene, patulin, 1,12-tridecadiene, isopropyl linoleate, pentadecane, elaidic acid, ascorbic acid,permethyl-, dodecanedioic acid, carvedilol, heptadecanoic acid, linolenic acid, kampferol-3,4'-dimethyl ether, and scopoletin, it is important to mention that these compounds can be utilized in the treatment of many diseases as single drug.

Key words: Digitoxin, Vitamin E, Portulacaceae, GC.MS, Phytochemical Screening.



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Introduction:

The pharmacological effects of medicinal plants are generally based on their phytochemical compositions, which classified into primary and secondary metabolites. Generally, Primary metabolites are implicated in development, reproduction, and growth of the organism. Secondary metabolites are organic compounds that produced through primary metabolite modification. They have many biological effects, like antiviral, antibiotic, antifungal and are able to protect the plants from several pathogens. [1]

A long time ago medicinal plants have been used in diseases treatment, *portulaca oleracea* was one of the medicinal plants that listed in the World Health Organization and it has been called ‘Global Panacea’ [2]. *P. oleracea* plant in Arabic called ‘Rejlah’ belongs to Portulacaceae family, and commonly known as Purslane, it is an herbaceous plant spread around Mediterranean and tropical Asian countries. *Portulaca oleracea* has different therapeutic effects such as cardiovascular diseases in the prevention of infections, preservation of the immune system in addition to strong antioxidant effect [3]. According to the health benefit, *P. oleracea* called “vegetable for long life” in Chinese medicinal plants culture, and it’s used in traditional and folk medicine as a remedy for many diseases, including headache, dermatitis, inflammation, intestinal worms, abdominal pain, urinary tract infections, high fever, and etc., due to its have a large spectrum of pharmaceutical effects (antioxidant, analgesic, bactericide, anti-inflammatory, hypoglycemic, and hypocholesterolemic). [4] *P. oleracea* seeds contain fatty acids, beta-carotene, ascorbic acid, omega-3, coumarins, apigenin, luteolin, kaempferol, genistein, glutathione, alpha tocopherols, *s*-carotene, melatonin, minerals (calcium, magnesium, copper, zinc, manganese, iron selenium and phosphorus), dopamine, quercetin, α -linolenic acid, aspartic acid, vitamins(A,C, and B), glutamic acid, and oxalate [5] and other alkaloids, flavonoids, anthocyanin, and saponins.

The lipid-soluble compounds and essential oil are known to have greatest effect in prevention of obesity as aromatherapy for obese women with middle-age. [6]

Portulaca oleracea plant is an edible wild plant that widely distributed throughout the world, it’s traditionally used either in raw as salad or in cooked form. [7] It’s distributed largely in subtropical and tropical regions of the world, in different parts of the United States and is added to salad and soup around tropical Asian and Mediterranean countries. *Portulaca oleracea* might be originated in Asia now ever present in the Mediterranean region and Africa. [8]

Stems and leaves are the main edible parts of the plant, which are characterize by their chemical compositions and nutritional values in different reports. [9] Aerial parts of the plant had a huge nutritional usefulness due to the presence of large quantities of alpha linolenic acid, however large quantity of oleic acid and nitrates hinder the wide plant uses and acceptance in human diet. *P. oleracea* seeds according to ethnobotanical studies, possess pharmacological properties similar to the aerial parts so they can be utilized by human beings and animals. [10] Seed extracts of *P. oleracea* have antibacterial activities against *Staphylococcus aureus*, [11] antioxidant properties and antihyperlipidemic. Seed oils are so nourishing as they are wealthy in polyunsaturated fatty acids be composed of linoleic acid, alpha linolenic acid and oleic acid, also they formed of phenolic lipids (alkylresorcinols) and phenolic compounds (protocatechuic and p-hydroxybenzoic acids).[12,13]

However, the aim of this study was to evaluate chemical compositions in *P. oleracea* seeds to propose them as possible complementary/alternative matrices for food industry, also for pharmaceuticals industries. In addition the effects of seeds oil yield by extraction methods and its chemical compositions were discussed.

Materials and methods:

In order to investigate *Portulaca oleracea* seeds were identified at department of Pharmacognosy and medicinal plants, Pharmacy college, Kerbala university. Two hundred and fifty grams of seeds of *P. oleracea* plant were cleaned and dried carefully, grind by mechanical grinder to coarse powder, then extracted by sequential extraction procedure.

First by reflux apparatus with petroleum ether for one hour then the crude extract cooled at room temperature and left macerated in the same solvent for one week, the petroleum ether extract filtered, the clear filtrate was evaporate by rotatory evaporator under reduced pressure at temperature didn't exceed 40°C.

Second the remaining marc re-extracted by maceration with Ethylacetate for one week, the Ethylacetate extract filtered, the clear filtrate was evaporate by rotatory evaporator under reduced pressure at temperature didn't exceed 40°C.

Third the remaining marc re-extracted again by maceration with methanol for one week, the methanolic extract filtered, the clear filtrate was evaporate by rotatory evaporator under reduced pressure at temperature didn't exceed 40°C.

Phytochemical screening

Preliminary phytochemical screening were accomplished by the following experiments in order to identify the presence of phytochemical compounds.

Saponins test

Foam or Frothing test

Boil 2g of the powdered plant with twenty milliliter of D.W in the water bath then filter. Take 10ml of filtrate and mix with 5ml of D.W.[14]

Test for flavonoid

Alkaline reagent test

Ten ml of ethyl acetate were added to 0.2 g of the plant powder, heated for 3 minutes on a water bath, then cooled, filter, 1ml of diluted ammonia was added to the filtrate.[15]

Alkaloids test

In a test tube Put 5 g of the plant powder then pour 20 ml methanol into the test tube. Allow the mixture to boil in the water bath for 2 min., then cooled and filtered. Add two drops of Dragendoff's reagent (potassium bismuth iodide solution) to 2 ml of filtrate; add two drops of Wagner's reagent (potassium iodide and iodine solution) to 5 ml of filtrate; To 2 ml of filtrate add two drops of Meyer's reagent (solution of potassium mercuric iodide). Add two drops of Hager's reagent (solution of saturated picric acid) to 5 ml of the filtrate.[16]

Tannins test

Plant powder about 0.5 grams put with 20ml of distilled water in a test tube then boiled, and filtered, 0.1% FeCl₃ was added to the filtrate, a blue black or brownish green color indicate presence of tannins.[17]

Test for steroid

Salkowski test

Dissolve 0.2 g of extract in chloroform and add few drops concentrated sulphuric acid were to the solution. The presence of a reddish color upper chloroform layer indicate the presence of steroids.[8]

Phenolic compounds test

Dissolve 0.5g of the extract in 5 mL of distilled water, add few drops of 5% FeCl₃ solution. The presence of phenolic compounds were indicated by the presence of dark green color.[17]

Chemical investigation by GC-MS apparatus:

Petroleum ether, Ethylacetate, and Methanol extracts were analyzed using a GC (Agilent Technologies 7890A) equipped with a polar Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (0.25 μm film thickness and 30 m × 0.25 mm i. d.) and interfaced with a Mass-selective detector (MSD, Agilent 7000). Helium is the carrier gas and its linear velocity is 1 ml/min. The temperature of injector and detector was 200 °C and 250 °C, respectively. The injected sample volume was 1 μl. The parameters for operating MS were: acquisition mass range 50-800, interface temperature 250 °C, and ionization potential 70 eV.

Different components were investigated depend on comparing their retention time and mass spectra with standard one and by computerize matching with compounds in library of WILEY and NIST also by comparing the fragmentation patterns of their Mass Spectral data with that recorded in the literature.

Results

The results displayed that the percentage yield of *P. oleracea* crude extracts from seeds extraction by method no.1 was 3.248% which is 8.12 grams higher than that get by method no. 2 and no. 3 which produce percentage yields of 2.012% and 0.732% which are 5.03 and 1.83 grams respectively, as shown in **Table (1)**. The phytochemical investigations exhibit the existence of terpenoid, flavonoids, alkaloids, saponins, and steroids in addition to the absence of flavonoids, and tannins in plant seeds, but in dissimilar concentrations, as appear in **Table (2)**.

Table (1) percentage yields of *Portulaca oleracea* seeds extraction

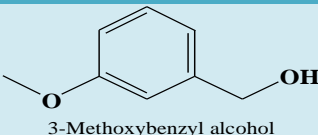
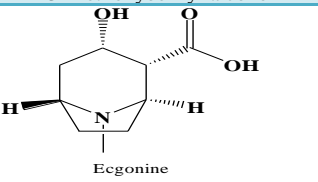
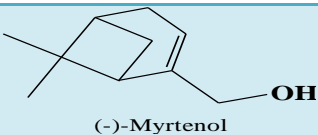
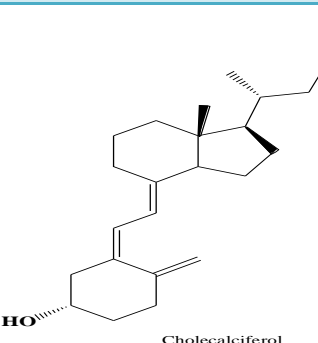
Extraction procedure	Percentage yield
Petroleum ether extract	3.248%
Ethylacetate extract	2.012%
Methanol extract	0.732%

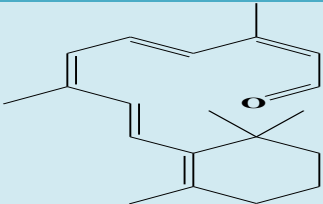
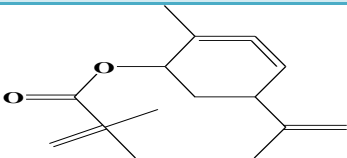



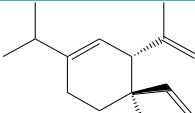
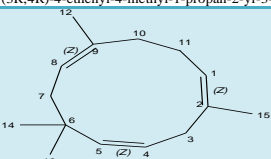

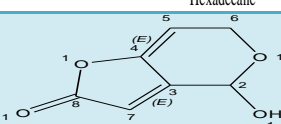

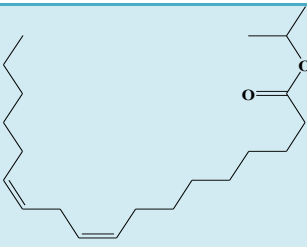


Table(2) Preliminary phytochemical screening of *Portulaca oleracea* seeds

Test name	Result
Saponin	+
Flavonoid	+
Alkaloid	+
Tannin	-
Steroid	+
Phenolic compounds	+

The examinations of these three methods carried out by using GC-MS apparatus, exhibit around thirty-four different components of *P. oleracea* seeds crude extract rely on method of extraction as appear in Fig. 1,2 and 3 and Table 3,4, and 5

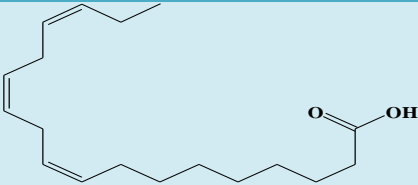
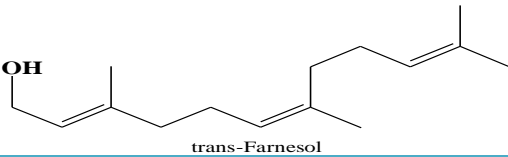
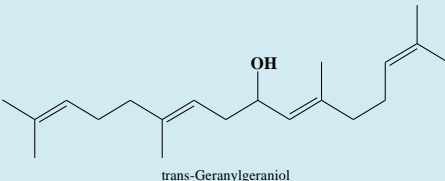
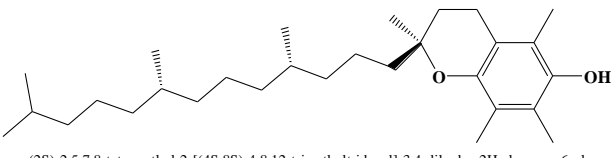
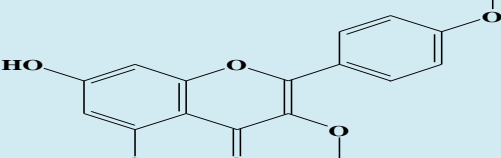
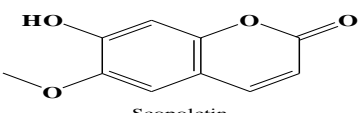
Table(3) GC-MS analysis of Petroleum ether extract

No.	RT (min)	Compound name	Structure	Area sum %
1	4.94	3-Methoxybenzyl alcohol	 3-Methoxybenzyl alcohol	0.9
2	5.34	Egonine	 Egonine	0.9
3	5.98	(-)-Myrtenol	 (-)-Myrtenol	0.12
4	6.47	Cholecalciferol	 Cholecalciferol	0.11

5	6.5	9-cis-Retinal	 <p>9-cis-Retinal</p>	0.1
6	6.75	Limonen-6-ol, pivalate	 <p>5-Isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate</p>	0.27
7	6.91	p-Cymene	 <p>p-Cymene</p>	0.22
8	7.07	Camphene	 <p>Camphene</p>	2.44
9	7.36	γ-Terpinene	 <p>p-Mentha-1,4-diene</p>	0.54
10	7.69	δ-Elemene	 <p>(3R,4R)-4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene</p>	1.18
11	8.705	Humulene		0.72
12	8.85	Hexadecane	 <p>Hexadecane</p>	1.44
13	9.07	Patulin		0.54
14	9.6	1,12-Tridecadiene	 <p>1,12-Tridecadiene</p>	0.58
15	9.8	Isopropyl linoleate	 <p>Isopropyl linoleate</p>	0.42
16	10.64	Pentadecane	 <p>Pentadecane</p>	0.37
17	11.46	Elaidic acid	 <p>Elaidic acid</p>	0.55

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18	11.87	Ascorbic acid, permethyl-	<p>Ascorbic acid, 2,3,5,6-tetramethyl-</p>	0.4
19	11.99	Dodecanedioic acid	<p>Dodecanedioic acid</p>	0.39
20	12.6	Cis-6-Octadecenoic acid	<p>Cis-6-Octadecenoic acid</p>	0.42
21	12.713	Carvedilol	<p>1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol</p>	0.38
22	14.4	Vitamin E	<p>Vitamin E</p>	0.54
23	14.85	Tetracosanoic acid	<p>Tetracosanoic acid</p>	24
24	15.25	Heptadecanoic acid	<p>Heptadecanoic acid</p>	0.78
25	15.27	Digitoxin		0.69
26	15.4	Estrol	<p>Estrol</p>	1.07
27	15.57	Linolenic acid		0.9

			 <p>Linolenic acid</p>	
28	16.15	trans-Farnesol	 <p>trans-Farnesol</p>	21.4 4
29	16.48	trans-Geranylgeraniol	 <p>trans-Geranylgeraniol</p>	34.6 2
30	17.8	(+)- α -Tocopherol	 <p>(2S)-2,5,7,8-tetramethyl-2-[(4S,8S)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-chromen-6-ol</p>	3.13
31	18.189	Kampferol-3,4'-dimethyl ether	 <p>3,4'-Dimethoxy-5,7-dihydroxyflavone</p>	0.89
32	21.14	Scopoletin	 <p>Scopoletin</p>	0.61

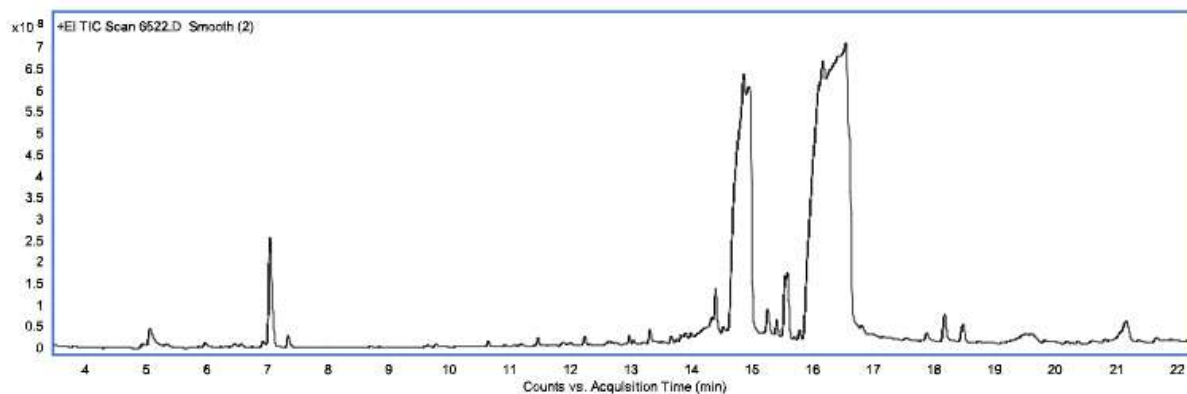
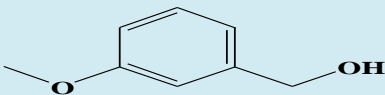
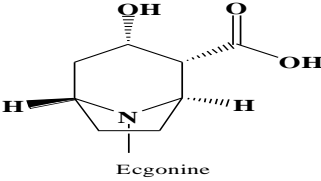
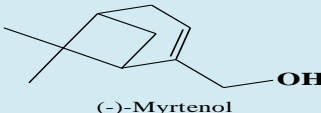
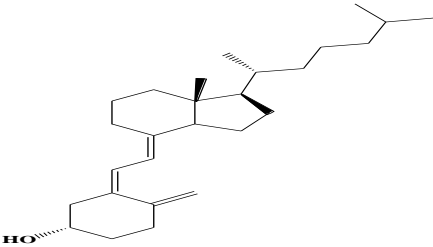
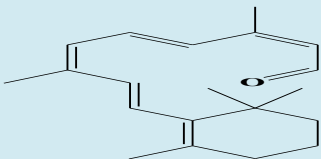
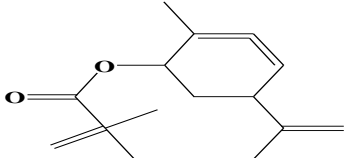
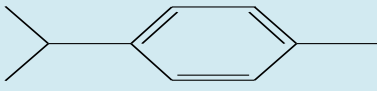

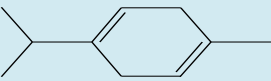
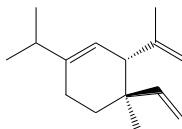
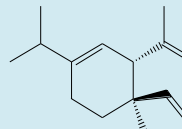
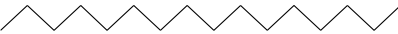
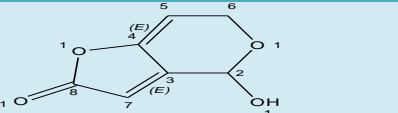

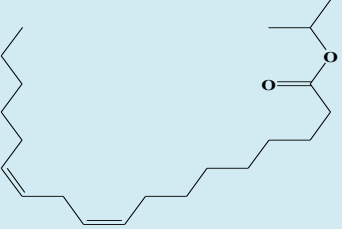
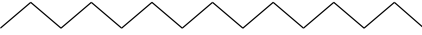
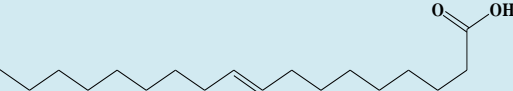
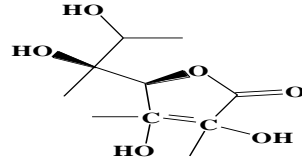
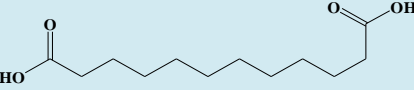



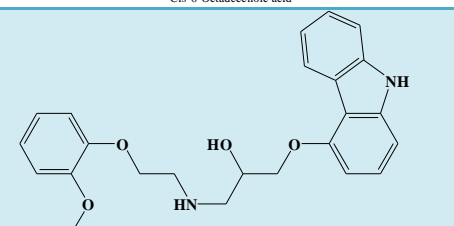
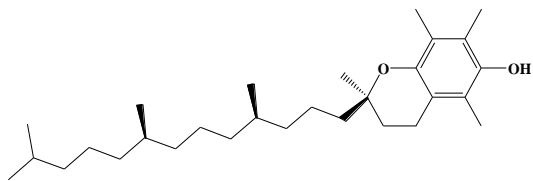

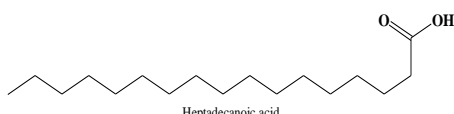
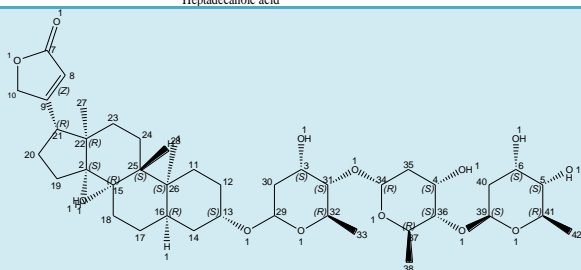
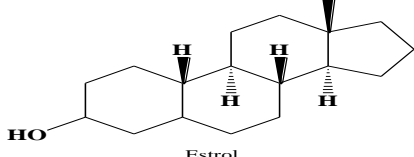
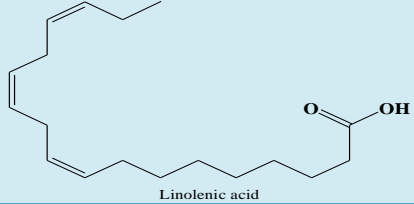
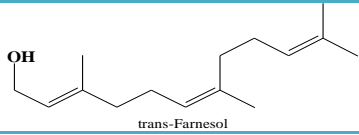
Figure (1) GC-MS chromatogram of phytochemical compounds of *P. oleracea* petroleum ether extract

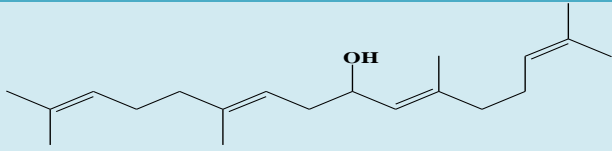
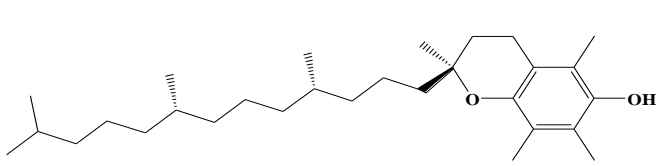
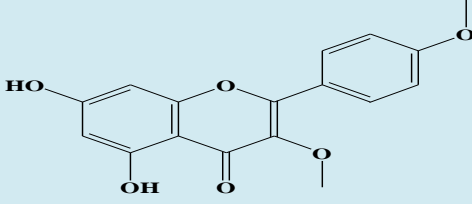
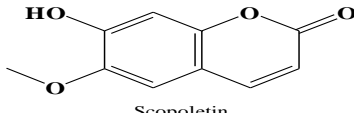

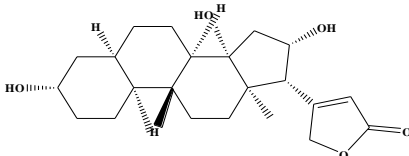
Table(4) GC-MS analysis of Ethylacetate extract

No.	RT (min)	Compound name	Structure	Area sum %
1	4.94	3-Methoxybenzyl alcohol	 <p>3-Methoxybenzyl alcohol</p>	0.6
2	5.34	Ecgonine	 <p>Ecgonine</p>	0.5
3	5.98	(-)-Myrtenol	 <p>(-)-Myrtenol</p>	0.48
4	6.47	Cholecalciferol	 <p>Cholecalciferol</p>	0.18
5	6.5	9-cis-Retinal	 <p>9-cis-Retinal</p>	0.2
6	6.75	Limonen-6-ol, pivalate	 <p>5-Isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate</p>	0.2
7	6.91	p-Cymene	 <p>p-Cymene</p>	0.6

8	7.07	Camphene	 Camphene	0.13
9	7.36	γ -Terpinene	 p-Mentha-1,4-diene	0.11
10	7.69	δ -Elemene	 (3R,4R)-4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene	0.15
11	8.705	Humulene	 (3R,4R)-4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene	0.23
12	8.85	Hexadecane	 Hexadecane	0.24
13	9.07	Patulin		0.93
14	9.6	1,12-Tridecadiene	 1,12-Tridecadiene	0.54
15	9.8	Isopropyl linoleate	 Isopropyl linoleate	0.68
16	10.64	Pentadecane	 Pentadecane	0.55
17	11.46	Elaidic acid	 Elaidic acid	0.82
18	11.87	Ascorbic acid, permethyl-	 Ascorbic acid, 2,3,5,6-tetramethyl-	0.56
19	11.99	Dodecanedioic acid	 Dodecanedioic acid	0.52

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20	12.6	Cis-6-Octadecenoic acid	 <p>Cis-6-Octadecenoic acid</p>	0.54
21	12.713	Carvedilol	 <p>1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol</p>	0.62
22	14.4	Vitamin E	 <p>Vitamin E</p>	23.93
23	14.85	Tetracosanoic acid	 <p>Tetracosanoic acid</p>	0.85
24	15.25	Heptadecanoic acid	 <p>Heptadecanoic acid</p>	1.09
25	15.27	Digitoxin		46
26	15.4	Estrol	 <p>Estrol</p>	4.5
27	15.57	Linolenic acid	 <p>Linolenic acid</p>	0.79
28	16.15	trans-Farnesol	 <p>trans-Farnesol</p>	0.76

29	16.48	trans-Geranylgeraniol	 <p>trans-Geranylgeraniol</p>	0.61
30	17.8	(+)- α -Tocopherol	 <p>(2S)-2,5,7,8-tetramethyl-2-[(4S,8S)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-chromen-6-ol</p>	2.5
31	18.189	Kampferol-3,4'-dimethyl ether	 <p>3,4'-Dimethoxy-5,7-dihydroxyflavone</p>	1.9
32	21.14	Scopoletin	 <p>Scopoletin</p>	2.02
33	21.95	β -Sitosterol		1.46
34	22.6	Gitoxigenin		4

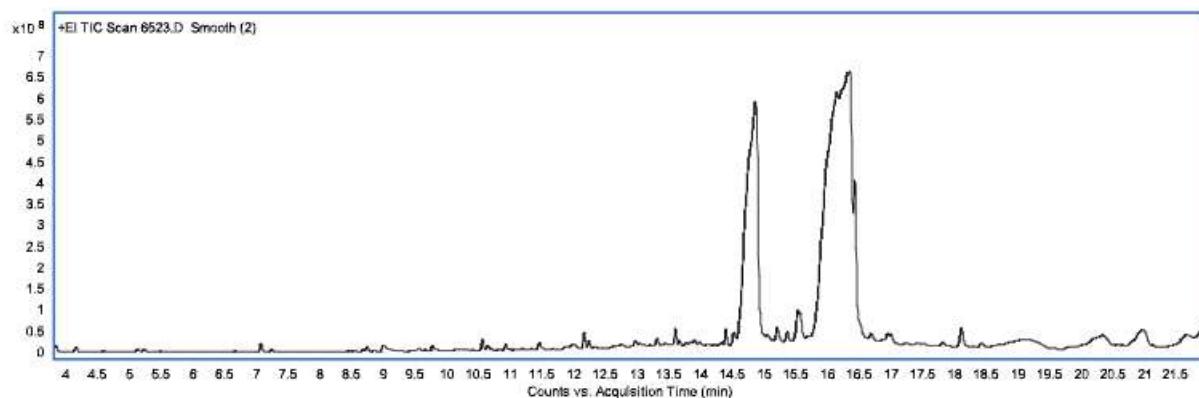
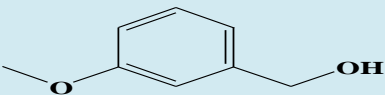
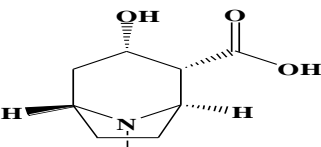
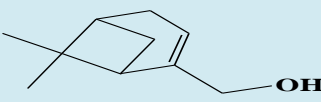
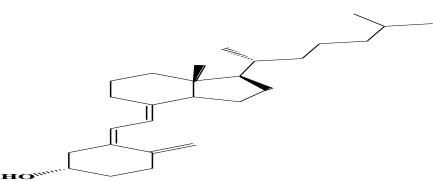
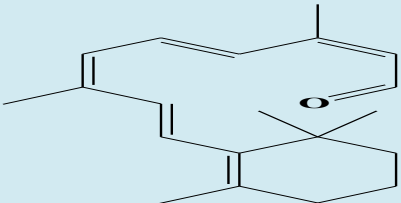
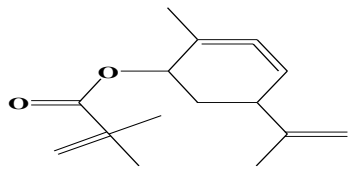
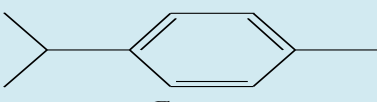
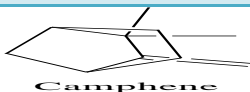
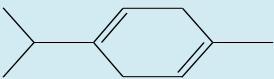
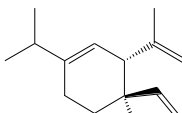
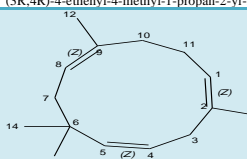
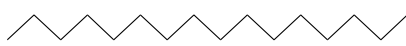
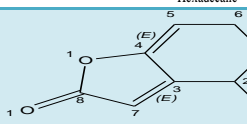

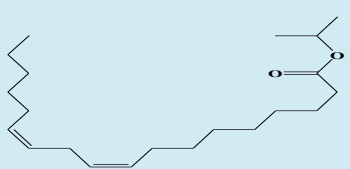

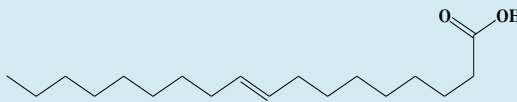
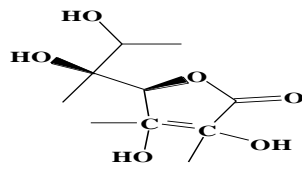
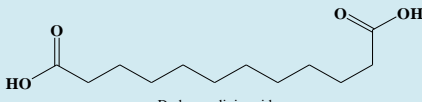
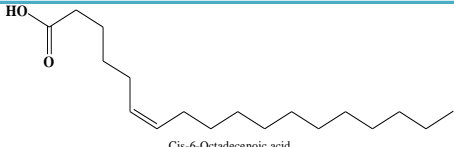
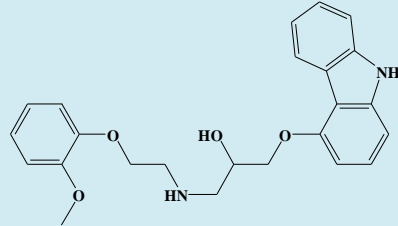


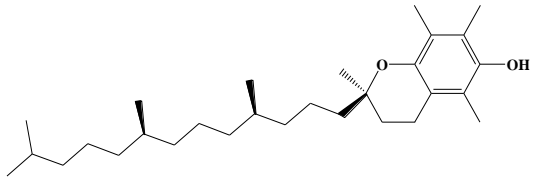

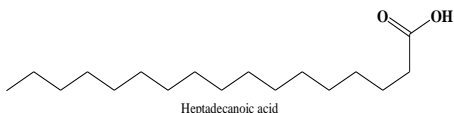
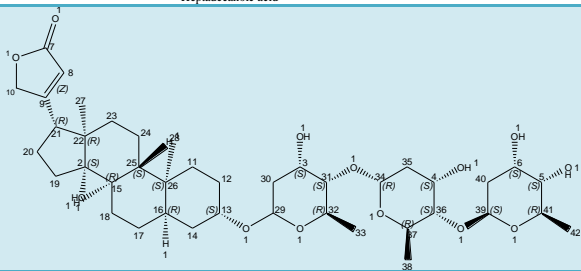
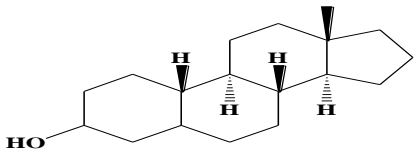
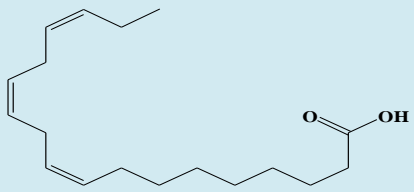
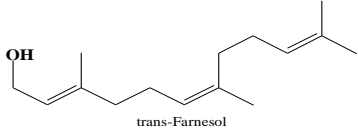
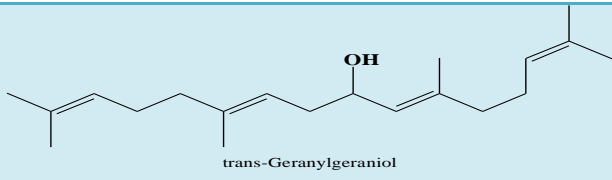
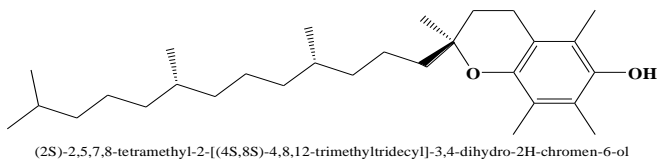
Figure (2) GC-MS chromatogram of phytochemical compounds of *P. oleracea* ethylacetate extract

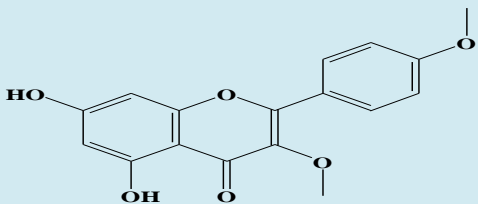
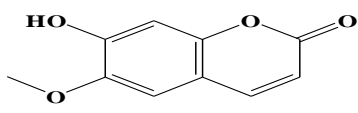
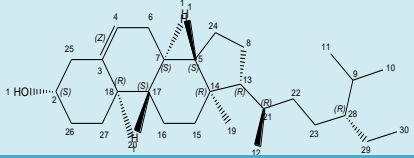
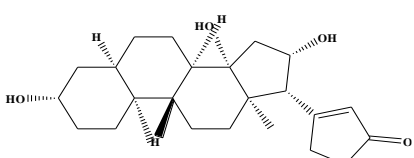
Table(5) GC-MS analysis of Methanol extract

No.	RT (min)	Compound name	Structure	Area sum %
1	4.94	3-Methoxybenzyl alcohol	 3-Methoxybenzyl alcohol	0.55
2	5.34	Ecgonine	 Ecgonine	0.55
3	5.98	(-)-Myrtenol	 (-)-Myrtenol	0.86
4	6.47	Cholecalciferol	 Cholecalciferol	0.53
5	6.5	9-cis-Retinal	 9-cis-Retinal	0.76
6	6.75	Limonen-6-ol, pivalate	 5-Isopropenyl-2-methyl-2-cyclohexen-1-yl pivalate	0.56
7	6.91	p-Cymene	 p-Cymene	0.66
8	7.07	Camphene	 Camphene	0.54
9	7.36	γ-Terpinene	 p-Mentha-1,4-diene	0.55

10	7.69	δ -Elemene	 <small>(3R,4R)-4-ethenyl-4-methyl-1-propan-2-yl-3-prop-1-en-2-ylcyclohexene</small>	1.08
11	8.705	Humulene		0.55
12	8.85	Hexadecane	 Hexadecane	0.44
13	9.07	Patulin	 Patulin	1.09
14	9.6	1,12-Tridecadiene	 1,12-Tridecadiene	1.6
15	9.8	Isopropyl linoleate	 Isopropyl linoleate	0.63
16	10.64	Pentadecane	 Pentadecane	0.62
17	11.46	Elaidic acid	 Elaidic acid	2.94
18	11.87	Ascorbic acid, permethyl-	 Ascorbic acid, 2,3,5,6-tetramethyl-	0.52
19	11.99	Dodecanedioic acid	 Dodecanedioic acid	2.08
20	12.6	Cis-6-Octadecenoic acid	 Cis-6-Octadecenoic acid	17.41
21	12.713	Carvedilol	 1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol	0.92

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22	14.4	Vitamin E	 <p>Vitamin E</p>	0.55
23	14.85	Tetracosanoic acid	 <p>Tetracosanoic acid</p>	0.96
24	15.25	Heptadecanoic acid	 <p>Heptadecanoic acid</p>	0.43
25	15.27	Digitoxin		6.49
26	15.4	Estrol	 <p>Estrol</p>	39.38
27	15.57	Linolenic acid	 <p>Linolenic acid</p>	2.66
28	16.15	trans-Farnesol	 <p>trans-Farnesol</p>	0.53
29	16.48	trans-Geranylgeraniol	 <p>trans-Geranylgeraniol</p>	0.54
30	17.8	(+)- α -Tocopherol	 <p>(2S)-2,5,7,8-tetramethyl-2-[(4S,8S)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-chromen-6-ol</p>	0.46

31	18.189	Kampferol-3,4'-dimethyl ether	 3,4'-Dimethoxy-5,7-dihydroxyflavone	4.32
32	21.14	Scopoletin	 Scopoletin	1.85
33	21.95	β -Sitosterol		4.32
34	22.6	Gitoxigenin		2.08

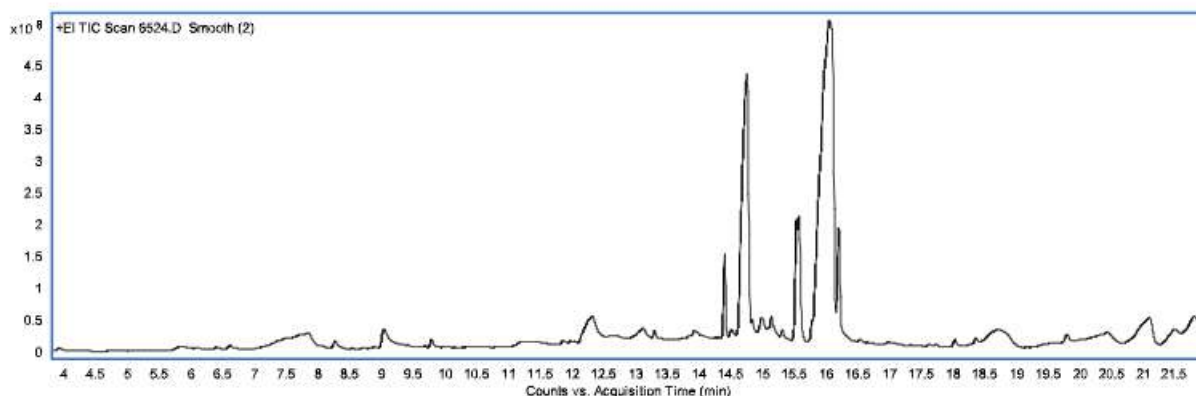


Figure (3) GC-MS chromatogram of phytochemical compounds of *P. oleracea* methanol extract

Discussion

The outcomes exhibit that the better method for extraction of *P. oleracea* seeds was method no.1 that produced percentage yield about 3.248% of crude extract, higher than that obtained by method no. 2 and method no. 3. These differences belong to extraction methods, and to the polarity of extracting solvents petroleum ether, Ethylacetate and methanol. The preliminary phytochemical screenings by employing many chemical reagents showed *P. oleracea* seeds include different active components like terpenoids, alkaloid, steroids saponin, flavonoids, hydroxyl coumarins, fatty acids and essential oils and that agree with GC-MS analysis result.[18]

Chemical components of petroleum ether extract of the seeds of *P. oleracea* that recognized by GC-MS are arranged in **Table 3**. The phytochemical compounds in the three extracts composed mostly from thirty-four compounds vary from fatty acids, steroids,

terpenes, coumarins, essential oils, vitamins, phenolic compounds, alkaloids, and others. The predominant constituents were trans-geranylgeraniol, tetracosanoic acid, trans-farnesol, digitoxin, vitamin E, estrol and cis-6-octadecenoic acid in addition to little percent distributed between β -sitosterol, gitoxigenin, (+)- α -tocopherol, camphene, hexadecane, δ -elemene, 3-methoxybenzyl alcohol, ecgonine, (-)-myrtenol, cholecalciferol, 9-cis-retinal, limonene-6-ol, pivalate, p-cymene, γ -terpinene, humulene, patulin, 1,12-tridecadiene, isopropyl linoleate, pentadecane, elaidic acid, ascorbic acid, permethyl-, dodecanedioic acid, carvedilol, heptadecanoic acid, linolenic acid, kampferol-3,4'-dimethyl ether, and scopoletin. Most of the compounds are terpenes and fatty acids depended on the structures and what present in their phytochemical screenings as appear in **figure(1)**. High level percent in petroleum ether seeds extract belongs to trans-geranylgeraniol 34.62%, tetracosanoic acid 24%, trans-farnesol 21.44%, (+)- α -tocopherol 3.13%, camphene 2.44%, hexadecane 1.44%, δ -elemene 1.18%, and estrol 1.07% while high level percent in Ethylacetate seeds extract due to digitoxin 46%, vitamin E 23.93%, estrol 4.5%, gitoxigenin 4%, (+)- α -tocopherol 2.5%, scopoletin 2.02%, kampferol-3,4'-dimethyl ether 1.9%, β -sitosterol 1.46%, and heptadecanoic acid 1.09%, the highest percent in methanol seeds extract belongs to estrol 39.38%, cis-6-octadecenoic acid 17.41%, digitoxin 6.49%, kampferol-3,4'-dimethyl ether 4.32%, β -sitosterol 4.32%, elaidic acid 2.94%, linolenic acid 2.66%, gitoxigenin 2.08%, dodecanoic acid 2.08%, and scopoletin 1.85%. The disagreements in these percent described by 'like dissolve like' rule, more polar component visible in methanol and vice versa.[19]

Conclusions

In this work, we have reported that most abundant compounds in the three extracts were trans-geranylgeraniol, tetracosanoic acid, trans-farnesol, digitoxin, vitamin E, estrol and cis-6-octadecenoic acid. However, it is important to mention that these compounds can be utilized in the treatment of many diseases as a single drug after isolation of them as they are present in large quantities in each extract.

References

- [1] Hussein R. A. and El-Anssary A. A.(2018). Plants Secondary Metabolites: The Key Drivers of the Pharmacological Actions of Medicinal Plants. *Intechopen*. 76139.
- [2] Zhou YX, Xin HL, Rahman K, Wang SJ, Peng C, Zhang H.(2015). *Portulaca oleracea* L.: A review of phytochemistry and pharmacological effects. *Biomed Res Int*. 2015:1-11.
- [3] Dreny EGE.(2020). Antidiabetic Activity of Aerial Parts and Seeds of Purslane (*Portulaca oleracea*) on Diabetic Rats. *Eur J Nutr Food Saf*. 12(7):13–23.
- [4] Fernández-Poyatos MDP, Llorent-Martínez EJ, Ruiz-Medina A.(2021). Phytochemical composition and antioxidant activity of *Portulaca oleracea*: Influence of the steaming cooking process. *Foods*.10(1).
- [5] Desta M, Molla A, Yusuf Z.(2020). Characterization of physico-chemical properties and antioxidant activity of oil from seed, leaf and stem of purslane (*Portulaca oleracea* L.). *Biotechnol Reports*. 27:e00512.
- [6] Konstantinidi M, Koutelidakis AE.(2019). Functional Foods and Bioactive Compounds : A Review of Its Possible Role on Weight Management and Obesity's Metabolic Consequences. *Medicines*.6(94):1-24
- [7] Petropoulos, S. A., Karkanis, A., Martins, N., & Ferreira, I. C. F. R.(2016). Phytochemical composition and bioactive compounds of common purslane (*Portulaca oleracea* L.) as affected by crop management practices. *Trends in Food Science & Technology*.55:1–10.
- [8] Ojah EO, Oladele EO, Chukwuem P.(2021). Phytochemical and antibacterial properties of rootextracts from *Portulaca oleracea* Linn. (Purslane) in the management of diseases in Nigeria. *Journal of medicinal plants for economic developement*.5(1):1–13.
- [9] Petropoulos, S., Karkanis, A., Fernandes, Â., Barros, L., Ferreira, I. C. F. R., Ntatsi, G., et al.(2015). Chemical composition and yield of six genotypes of common purslane (*Portulaca oleracea* L.): An alternative source of omega-3 fatty acids. *Plant Foods for Human Nutrition*.70(4):420–426.
- [10] Petropoulos SA, Fernandes Â, Arampatzis DA, Tsiropoulos NG, Petrović J, Soković M, et al.(2020). Seed oil and seed oil byproducts of common purslane (*Portulaca oleracea* L.): A new insight to plant-based sources rich in omega-3 fatty acids. *Lwt*.123.
- [11] Tayel, A. A., Shaban, S. M., Moussa, S. H., Elguindy, N. M., Diab, A. M., Mazrou, K. E., et al.(2018). Bioactivity and application of plant seeds' extracts to fight resistant strains of *Staphylococcus aureus*. *Annals of Agricultural Science*.63(1):47–53.
- [12] Nazeam, J. A., El-Hefnawy, H. M., Omran, G., & Singab, A. N.(2018). Chemical profile and antihyperlipidemic effect of *Portulaca oleracea* L. seeds in streptozotocin-induced diabetic rats. *Natural Product Research*. 32(12):1484–1488.
- [13] Jalali Mousavi, S. R., Niazmand, R., & Shahidi Noghahi, M.(2015). Antioxidant activity of purslane (*Portulaca oleracea* L.) seed hydro-alcoholic extract on the stability of soybean oil. *Journal of Agricultural Science and Technology*. 17(6):1473–1480.
- [14] Victor, B.K.; Al. Shaikh Hamed, W.M.A.(2014). Preliminary Phytochemical Screening and Evaluation of in vitro antioxidant activity of Iraqi species of *Hypericum perforatum* aerial part. *International research Journal of Pharmacy*. 5(5):369-373.
- [15] Onwukaeme, D.N.; Ikuegbrweha, T.B; Asonye, C.C.(2007). Evaluation of phytochemical constituents, Antibacterial activities and Effect exudate of *pycanthus*

- Angolensis weld wrab (Myristicaceae) on corneal ulcers in rabbits. *Tropical Journal of Pharmaceutical Research*. 6(2):725-730.
- [16] Kashamar AM, Naser EH and Almaali HAA.(2018). Clinical study of three medicinal plants (*F. vulgare*, *Zea mays* and *Petroselinum sativum*) against urinary tract infection and stones. *J Pharm Sci & Res*. 10(4): 755-758.
- [17] Mir MA, Sawhney SS, Jassal MMS.(2017). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Biology*. 2(1):001–005.
- [18] Vaidya SK, Golwala DK, Patel DS, Sahoo S. (2020). Evaluation of Antioxidant and Anti-Parkinson Activity of *Portulaca oleracea* Seed Methanolic Extract. *European J Med Plants*.31(2):10–7.
- [19] Durgawale TP, Khanwelkar CC, Durgawale PP.(2018). Phytochemical analysis of *portulaca oleracea* and *portulaca quadrifida* extracts using gas chromatography–mass spectrometry. *Asian J Pharm Clin Res*.11(9):204–7.



**STRUCTURAL AND OPTICAL PROPERTIES OF FE₂O₃: "CDTE THIN
FILMS GROWN BY PULSE LASER DEPOSITION AT DIFFERENT X
CONTENT**

**ASMAA N. MOHAMMED ALI
ABOTHER GHANIM ALMOHANA
JINAN MOHAMMED ABBAS**

STRUCTURAL AND OPTICAL PROPERTIES OF Fe_2O_3 : $CdTe$ THIN FILMS GROWN BY PULSE LASER DEPOSITION AT DIFFERENT X CONTENT

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Abstract:

Iron oxide (Fe_2O_3) doped Cadmium telluride ($CdTe$) thin films with various x substance (0.05, 0.08 and 0.1) % have been arranged by pulsed laser deposition procedure (PLD) at room temperatures (RT). The morphological construction of these films has been inspected utilizing AFM investigation. Atomic Force Measurement (AFM) estimations observed that the normal worth of grain size for all movies at RT decline with expanding of $CdTe$ content (341.31, 188.58, 106.26) separately. While a normal roughness values increment with expanding x substance (0.89, 18.4, 26.32) individually separately. The optical properties which are incorporate transmission retention coefficient, The energy band hole of arranged films movies decline as x substance increments (2.00, 1.70, 1.40) individually, Also, the optical constants have been still up in the air.

Key words: (Fe_2O_3): $CdTe$, Structural and Optical Properties, PLD Technique".



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Introduction:

One of the main metal oxide is Iron Oxides in light of the fact that these materials are valuable for some applications [1] like resistive warmers, electrochemical, significant sensors, impetuses and photocells[2]. Iron Oxide (Fe_2O_3) is a minimal expense semiconductor having hexagonal construction [3], a moderately low band hole of (2.2) eV [4], subsequently it can assimilate the vast majority of the apparent light and can be utilized for possible application as photograph anodes in photograph electrochemical sun oriented cells [5]. Cadmium telluride (CdTe) semiconductor having a place with II-VI family has been read up for a long time. CdTe has been for the most part examined as a polycrystalline dainty film and as a quantum speck [6]. As a meager film, it has been arranged by close space fume transport (CSVVT), laser removal, electro testimony and splash pyrolysis, and faltering, and it has been for the most part utilized as the safeguard material of slim film sun oriented cells. Later testimony strategies of CdTe depend on taking movies by utilizing extremely basic and modest statement procedures, for example, plunge covering or twist covering and a toughening interaction [7].

2. Experimental

Iron oxide (Fe_2O_3) and cadmium telluride powders with a perfection of 99.999% were used as starting materials to plan thin films (Fe_2O_3) by laser-struck affidavit technique with a thickness of 250 nm . various x substance (0 .05, 0.08 and 0.1)%. These materials were mixed in the inlet mortar for 60 minutes. From there the mixture was pressed to 5 tons to trace a target 2.5 cm wide and 4 cm thick. Finally, the lenses were sintered at a temperature of 873 K to ensure the homogeneity of the materials. The lens in general should be essentially as thick and homogeneous as possible to ensure the ideal texture of the shop slides. Pulsed laser claim study is performed in a vacuum chamber observed under vacuum conditions (10^{-3} Torr) at low voltage of a base gas for specific oxide and nitride events. The Nd:YAG laser focused on the spot surface was completed using a raised round quartz for mixing with a center length of 10 cm and the mapping attribute was set inside the vacuum chamber. This laser centering aims to overcome the trailing edge energy thickness when the laser hits the outer layer of the target material. The shades of laser are the Nd:YAG laser with a heartbeat energy of 800 mJ and a repetition of 6 Hz and a repetition of 1064 nm, and the number of shots is close to 500 lbs. The properties of the (Fe_2O_3) structure are not completely permanent. The surface morphology of(Fe_2O_3) -doped CdTe films was examined by atomic force microscopy evaluation using a (AA3000 test microscope for type testing). The optical properties of the stored films were characterized at a repeat level of 190-1100 nm using a UV-VIS spectrophotometer (Optima-3000).

3. Results and Discussion

Fig. (1) Shows 3D AFM pictures for slight films at different x substance (0.05, 0.08, and 0.1) % at RT. When in doubt, AFM assessment shows that the grain size values for all motion pictures decline with extending CdTe content. While the ordinary Roughness values increase with extending CdTe content as shown in table 1. Our results were a respectable simultaneousness with P. Elttayef [8].

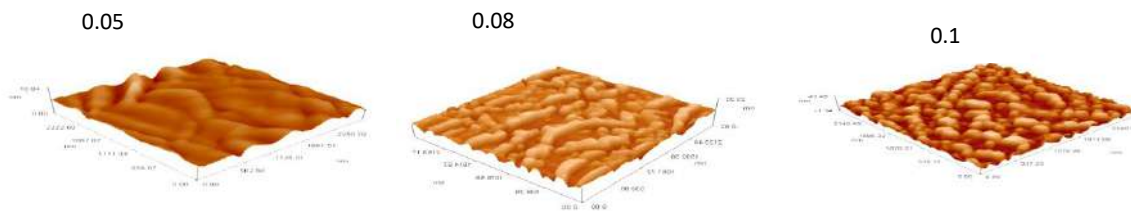


Fig. 1: AFM images for films with” different x content at RT.”

Table 1: Average grain size, average roughness and Peak- Peak for films with different x content at RT.

x	Ave. grain size (nm)	Ave. Roughness (nm)	Peak-Peak (nm)
0.05	341.31	0.89	9.15
0.08	188.58	18.4	22.86
0.1	106.26	26.32	13.4

The optical image of Fe₂O₃:CdTe samples was observed at (λ) range from (190 – 1100nm). Figure 2 shows the progress for (Fe₂O₃): CdTe films have an orderly arrangement on the glass at RT (293K) at different degrees X. It should be apparent from this figure that the vehicle decreases with the X degree relaxation, indicating an improvement in reflex and support. The beginning of the lead for films was less sharp, a rapid consequence of how more definite glass-like dimensions are being handled. Progression progression reaches longer frequencies (lower energies) showing a decrease in E_g with the creation of x-substance [9].

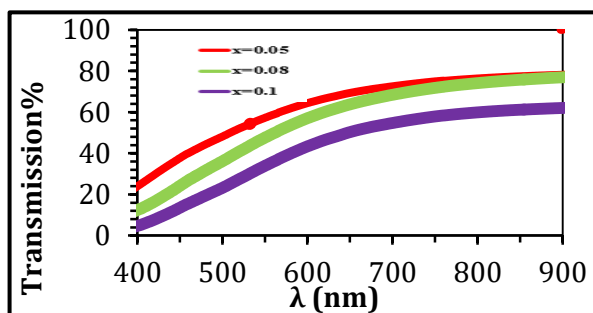


Fig. 2: Transmittance spectrum as a function of wavelength for Fe₂O₃: CdTe films at different x content at RT.

The absorbance range for Fe₂O₃: CdTe films with different x substance was recorded in the recurrent reach 190-1100 nm. Figure 3 shows that the ingestion range inside the indisputable recurrent locale in range (400-600) nm for (Fe₂O₃): CdTe slight film range. .it will generally speaking be seen from Fig.3 that the absorbance loosened up with creating of x

substance. This is relied on to make the confined states in the energy opening lead to lessen in the transmission and a short period of time later decrease in the energy opening.

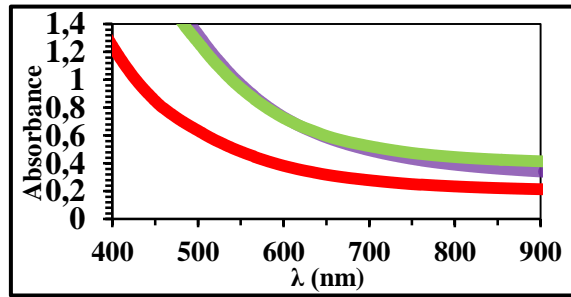


Fig. 3: Absorbance spectrum as a function of wavelength for Fe₂O₃: CdTe films at different x content at RT.

(α) was assessed from the area of high ingestion at the fundamental absorption edge of the film from condition [10]:

$$\alpha = 2.303 A / t \dots \dots \dots (1)$$

Where (A) is the absorbance and t is the thickness.

The optical energy opening qualities (E_g) for Fe₂O₃: CdTe films with different x (x= 0.05, 0.08 1.0) not totally firmly established by using Tauc condition [11]:

$$\alpha (h\nu) = B (h\nu - E_g)^r \dots \dots \dots (2)$$

The optical energy aperture decreases with the magnification of substance x as indicated in Table 2. Just as substance x increases from 0.05 to 0.1. This is because the progress from CdTe to Fe₂O₃ made the material weaker (higher absorption). The reduction of E_g after the addition of x to Fe₂O₃ [12].

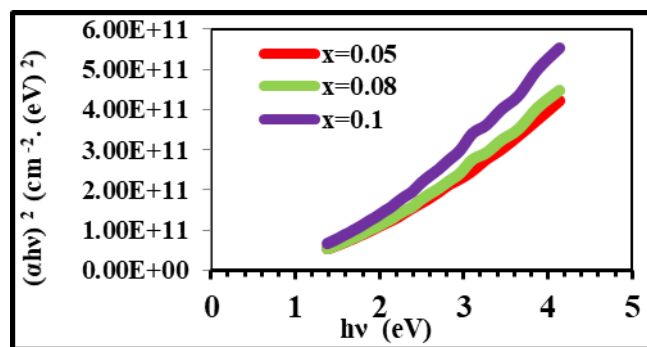


Fig.4: (hν)² as a function of hν for (Fe₂O₃) : CdTe films at different x content at RT.

The refractive logarithmic mixture as part of Refrashi appears at 190–1100 nm.

Another element x is shown in Figure 5. One more pause for (Fe₂O₃): CdTe x films are not completely resolved by the state at infinity [11]:

$$n = \{4R K2\}^{1/2} (R + 1) \dots \dots \dots (3)$$

$$(R-1)^2 (R-1)$$

R is the chronicle of reflection and n is the chronicle of refraction. From Figure 5 and Table 3 it can be seen that the refractive log increases from 0.05 to 0.1 as substance x relaxes. This adjustment can be a quick consequence of the difference in the length of the bond with the improvement of the reduction of the thickness of the distortion, that is, of the reduction of the reflection on which the diffraction bar is fixed. This result is similar to that of Cheng et al. [13] close.

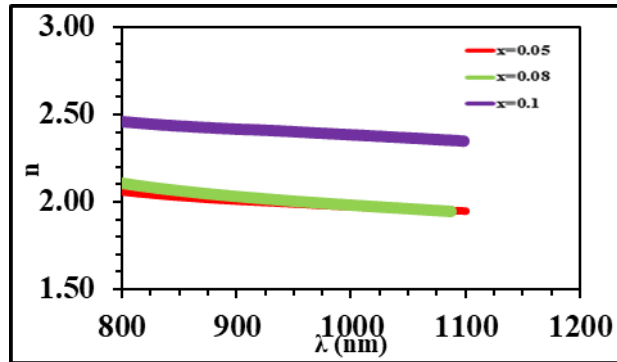


Fig.5: Variation of (n) as a function of wavelength for (Fe₂O₃) CdTe films at different x content at RT.

The potential gains of demolition not altogether settled forever by using condition [14]:

$$k = \alpha\lambda/4\pi \dots \dots \dots (4)$$

The demolition coefficient increases (0.086, 0.105, 0.118) with extending of x substance at RT, due to more grain limits with extension of CdTe to Fe₂O₃ and decreased the energy opening in this manner to growing the absorption with growing x substance [15].

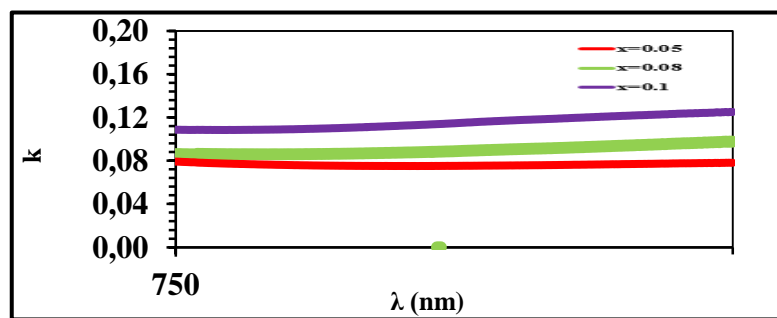


Fig.6: Variation of (k) as a function of wavelength for(Fe₂O₃) : CdTe films at different x content at RT.

The plots of (ϵ_r and ϵ_i) parts of the dielectric constant with wavelength in the range of 750-1100nm for (Fe_2O_3) : CdTe slim films put away at different x substance are shown in Figures 7 and 8. Authentic and nonexistent dielectric constants were resolved using conditions:

[16, 17]:

$$\epsilon_r = n^2 - k^2 \dots \dots \dots (5)$$

$$\epsilon_i = 2nk \dots \dots \dots (6)$$

It is seen that ϵ_r and ϵ_i relaxed with making of x substance as displayed in Table 2. The strategy for overseeing acting of ϵ_r is like that of refractive record considering the way that the more direct worth of k^2 separated and n^2 , While ϵ_i is predominantly relies upon the k attributes, which are associated with the mix of help coefficient. . This direct is in synchronization with M. Powalla and D. Cap [18].

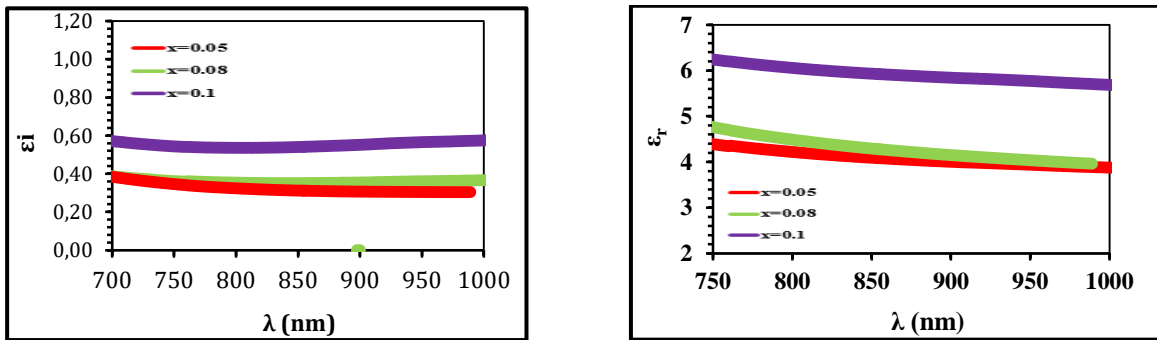


Fig.7: Imaginary part and Real part of dielectric constant as a function of wavelength for wavelength for Fe2O3: CdTe films at different x content at RT.

Table 2: Optical parameters of Fe2O3: CdTe films at different x content at RT.

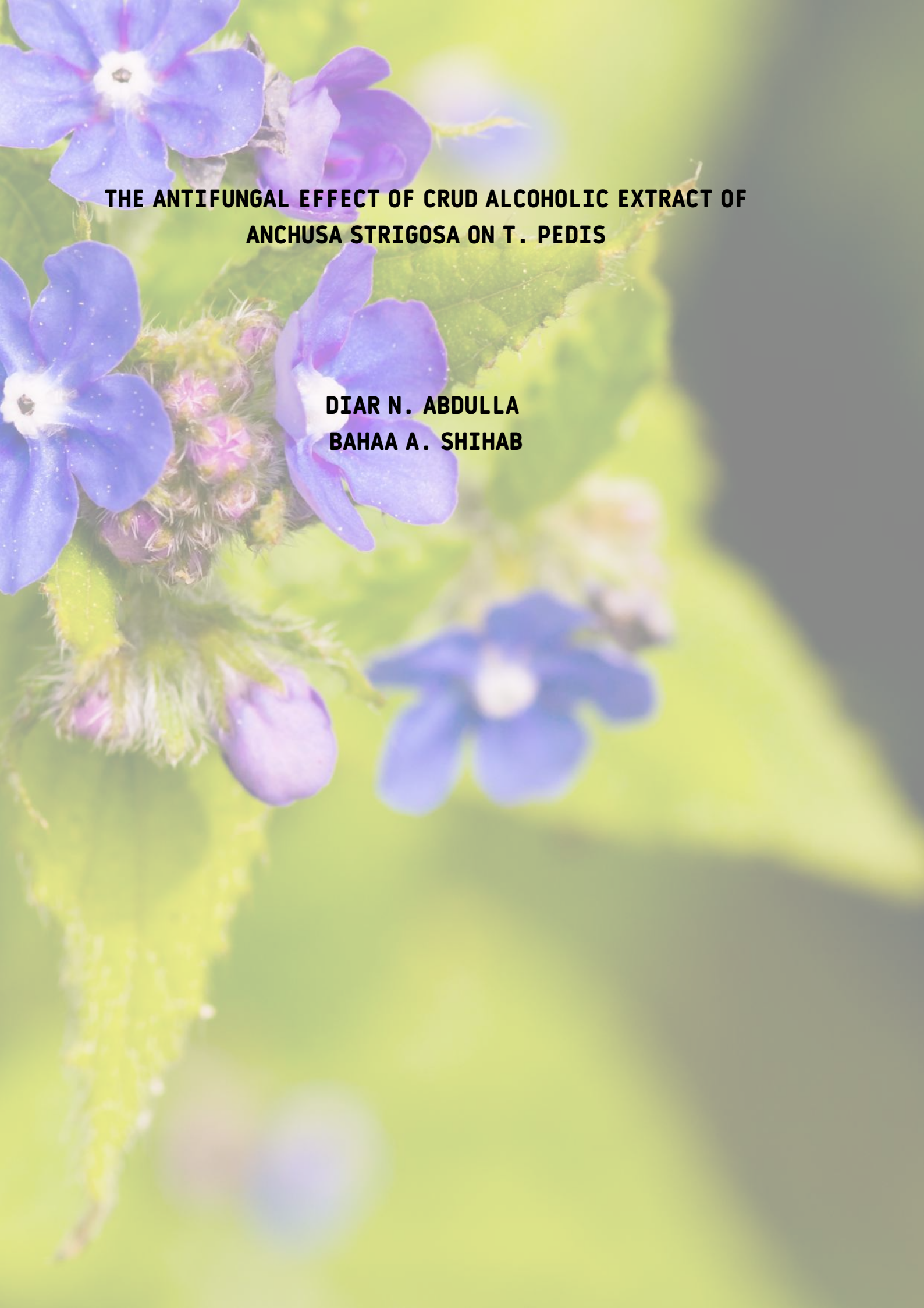
t(nm)	T%	α (cm ⁻¹)	K	n	ϵ_r	ϵ_i	Eg (eV)
0.05	76.020	13709	0.086	2.065	4.257	0.355	2.00
0.08	63.170	14373	0.105	2.395	5.723	0.512	1.70
0.1	59.464	17326	0.118	2.471	6.095	0.587	1.40

4. Conclusions

A polycrystalline advancement for the Fe₂O₃: CdTe films coordinated effectively by beat laser promise at various x substance (0.05, 0.08 and 0.1) %. AFM evaluations showed that that the run of the mill grain size values for films at RT decline with broadening x substance. While the run of the mill Roughness values increment with broadening x substance. The optical advances in Fe₂O₃: CdTe film is speedy progression and the worth of optical energy opening expansion with stretching out of x substance. Along these lines the property of Fe₂O₃: CdTe thin movies coordinated by beat laser verbalization rely unequivocally on the set up condition like x substance.

References

- [1] O. Lupan et al., "Localized synthesis of iron oxide nanowires and fabrication of high performance nanosensors based on a single Fe₂O₃ nanowire," *Small*, vol. 13, no. 16, p. 1602868, 2017.
- [2] Hasany S, Abdurahman N, Sunarti A, Jose R. Magnetic Iron Oxide Nanoparticles: Chemical Synthesis and Applications Review. *CNANO*. 9(5):561-575, 2013.
- [3] 3-Laurent, Sophie; Forge, Delphine; Port, Marc; Roch, Alain; Robic, Caroline; Vander Elst, Luce; Muller, Robert N. (2008).
- [4] 4-Y. Fu, J. Chen, and H. Zhang, "Synthesis of Fe₂O₃ nanowires by oxidation of iron," *Chem. Phys. Lett.*, vol.350, no. 5- 6, pp. 491 – 494, 2001.
- [5] 5-Bushra K.H.Al- Maiyaly, "The Effect of Thickness on Electrical Conductivity and Optical Constant of Fe₂O₃ Thin Films," *Ibn Al -Haitham Jour. Pure Appl. Sci*, vol. 27, no. 3, pp. 237 – 246, 2014.
- [6] A. Bosio, N. Romeo, S. Mazzamuto, and V. Canevari, "Polycrystalline CdTe thin films for photovoltaic applications," *Progress in Crystal Growth and Characterization of Materials*, vol. 52, no. 4, pp. 247–279, 2006.
- [7] X. Wu, "High-efficiency polycrystalline CdTe thin-film solar cells," *Solar Energy*, vol. 77, no. 6, pp. 803–814, 2004.
- [8] 8-P. A. K. Elttayef, A. Prof, M. H. Jaduaa, and M. A. Muhammed, "Study the structural and optical properties of Fe₂O₃ thin films prepared by RF magnetron," *Int. J. Appl. or Innov. Eng. Manag*, vol. 4, no. September, pp. 99 – 105, 2015.
- [9] A. Tanushevski and D. Sokolovski, "Structural and optical properties of CdTe thin films obtained by electrodeposition," *RAD Conf. Proc.*, vol. 2, pp. 149– 154, 2017.
- [10] Ahmed M N, Nadia A A, Nariman S and Farid El-Tantawy *Journal of Applied Physics*, 2014.
- [11] Burns G *Solid State Physics Academic Press Inc, Harcourt Brace Jovanovich, New York*, 1985.
- [12] T.S.Moss, "Optical Properties of Semiconductors", London Butter Worthes Scientific Publications (1959).
- [13] W.X.Cheng, A. L.Ding, X. S. Zheng, P. S. Qiu and X. Y. He, *Journal of Physics: Conference Series*, vol.152, P.P: 12-36, (2009).
- [14] Taus J *Amorphous and Liquid Semiconductor Plenums Press New York and London*, 1974.
- [15] S. M. Sze, "Physics of Semiconductor Devices", 2nd ed., John Wiley and Sons, Inc., New York, (1981).
- [16] Pankove J I *Optical Process in Semiconductors Dover Publications New York*, 1971
- [17] C.Chang, A. Segmüller, L.L.Chang, and L.Esaki, *Appl. Phys.Lett.*, Vol.38, No.11, pp.912-914 (1981).
- [18] M. Powalla and D. Bonnet, "Thin-film solar cells based on the polycrystalline compound semiconductors CIS and CdTe," *Advances in Opto Electronics*, vol. 2007, Article ID 97545, 2007.



**THE ANTIFUNGAL EFFECT OF CRUD ALCOHOLIC EXTRACT OF
ANCHUSA STRIGOSA ON T. PEDIS**

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THE ANTIFUNGAL EFFECT OF CRUD ALCOHOLIC EXTRACT OF ANCHUSA STRIGOSA ON T. PEDIS

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Abstract:

Tinea pedis is the most common type of fungal infection in human. The samples have been taken from fifty patients (26 female, 24 male) their ages ranged from 11 to over 60 years diagnosed as having Tinea pedis in Baghdad Teaching Hospital. All samples were examined directly after the addition of a drop of 10% KOH solution and then cultured on selective fungal culture media. The results showed that the rate of infection was more in females, and more in patients with age ranging from (30-39) years old, followed by those between (40-49) years old, and the causative fungi agents of fungal infection of T. pedis were mainly Trichophyton rubrum (72%) followed by Trichophyton mentagrophyte (32%) then Epidermophyton floccosum (12%) respectively.

The inhibitory effect of alcoholic extract of Anchusa strigosa was tested in vitro on some causative fungi of Tinea pedis and the result were compared with that of Antifungal Tolnaftate, and the result of it showed that Trichophyton mentagrophyte was affected by the extract more than Trichophyton rubrum and the percentage of growth inhibition by the extract for Trichophyton mentagrophyte at a concentration of (500) and (1000) P.P.M. were (65.11% and 100%) respectively, while that for Trichophyton rubrum were (53.68%, 87.36% and 100%) at a concentration of (500, 1000 and 1500) P.P.M. respectively. This extract was shown to be a promising antifungal agent in the future.

Key words: Anchusa Strigosa, Tinea Pedis, Alcoholic Extract, Dermatophytes, Tolnaftate.



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Introduction:

Tinea pedis is the most common type of fungal infection in human, most cases are caused by one of three organisms *Trichophyton rubrum* (the most common and stubborn), *Trichophyton mentagrophytes var interdigitale* and *Epidermophyton floccosum* (Ilkit, M., 2014). The sharing of wash places (e.g. in showers) and of swimming pools, predisposes to infection and occlusive foot wears encourages relapses (Gupta, A et al 2003) Also other types of dermatophytes including zoophilic one can do it. There are three common clinical patterns:

1. Soggy interdigital scaling, particularly in the fourth and fifth interspaces.
2. A diffuse dry scaling of the soles.
3. Recurrent episodes of vesication. (Richard Weller et al. 2015)

The diagnosis of Tinea pedis and other cutaneous mycosis depends on clinical features in addition to laboratory tests which include the microscopic examination of skin scraping from the scaly margin of the lesion after clearing in KOH solution to see the branching hyphae, and the spores. Exact identification of causative fungus is generally determined by culture on Sabouraud dextrose agar. Many topical antifungal agents are effective in treatments of tinea pedis and other cutaneous mycosis like clotrimazole, miconazole, naftifine and others (William D. James et al. 2020). Tolnaftate is one of the topical antifungal drugs which belong to Thiocarbamates compounds which is found in the form of solution or powder that can be fungistatic or fungicidal. (Hay and Moore, 1998; Yamaguchi et al., 2001).

Anchusa strigosa belong to the Boraginaceae family were distributed in the temperate, especially in Mediterranean and tropical regions (Ketabchis, et al. 2011; Abbas M. et al. 2009), and it is found on road side of the Northern regions of Iraq. The active materials of this plant include pyrrolizidine alkaloids, tannins, lycopsamine, mucilage and viridiflorate (Al-Zubaidi et al., 1996). Chemical studies also showed that the active compounds in *Anchusa strigosa* include aliphatic hydrocarbons, oil, proteins, pyrrolizidine alkaloids and polyphenols (Abbas M. et al. 2009; Al Ali. Et al 2007; Kohli K.& Ali M. 2003; Siciliano T. et al 2005; Braca A. et al. 2003).

Anchusa strigosa possessed many pharmacological effects which include gastric protective effect, antimicrobial, hypotensive and antidiabetic effects (Ali et al .2014). The aqueous extract of *Anchusa strigosa* also exerted antifungal activity. (Ali-Shtayeh MS and Abu Ghdeib SI., 1999).

Materials And Methods:

Specimens collection:

Fifty specimens were collected from patients (26 female, 24 male) their ages ranged from 11 to over 60 years diagnosed as having Tinea pedis in Baghdad Teaching Hospital. The specimens were taken from the active scaly border of Tinea pedis by using a blunt scalpel. Blade.

Microscopic Examination:

The specimens were put on sterile glass slide and a drop of 10% KOH solution was run under the coverslip then the specimen heated by Bunsen burner and examined under the microscope after (10-15) minutes to see the fungal hyphae and sometimes the spores.

Culture of The Specimens:

Part of the specimen was taken and distributed on the surface of the Sabourud Dextrose agar (SDA) which contain chloramphenicol and Cycloheximide to prevent growth of bacteria and other saprophytic fungi and then incubated for three weeks at (30C°). The growth was diagnosed according to taxonomic keys of medical fungi (Koneman and Robert, 1985; Koneman et al., 1997; Midgley et al., 1997). The specimens were also cultured on potato Dextrose agar (PDA) and cornmeal agar (CA). and the Lactophenol-Cotton blue stain was used to identify the fungal infection isolates of T.pedis microscopically (Fingold & Paron , 1986) In addition to biochemical test for urea enzyme production (Tilton, 1992; Baron et al. 1994).

Collection of The Plant:

Anchusa strigosa plant was collected in the period of flowering from Tikrit city, and the plant was identified depending on (Al-Musawi, 1982; Al-Zubaidi, 1989). The leaves and flower of the plant were cleaned and dried in air and in a shadow place then divided into small species and grind to become in a powder form.

Crud Extraction:

A (250gm) were taken from the plant powder and put in (800ml) of 95% ethanol alcohol for 48 hours, then filtered by (Whatman No.1) filter paper, then evaporated by Rotary Vacuum evaporator at 40 C° until it became thick liquid and kept in the fridge depending on (Twaij and others, 1985).

Estimation of MIC and MFC on Solid Media:

A volume of (100, 200, 300) microliter was taken from the extract and was added to (200ml) of (SDA) in the Petri dishes so as the concentrations of the plant extract were ranged from 500 P.P.M. to 1500 parts per million. Then the dishes were inoculated by the fungal growth and incubated at (30C°) for different periods according to the type of fungus and for three times for each type of fungi. After few days the diameter of colonies were measured and compared with controls (Mishura et al., 1994; Singh and Singh, 1997).

Statistical Analysis:

Carried out by using chi-square test and the results were tested at ($P < 0.05$). Duncan test was used to show the inhibitory effect of plant extract and the drug Tolnaftate on the growth of the fungi in comparison to the control and in comparison, between the plant extract and the Tolnaftat drug, according to. (Bruning and Kintz, 1977).

Result & Discussion:

Isolation of Fungal Infection of *T. pedis*:

In this study fifty patients with Tinea pedis infection were included and diagnosed clinically and by laboratory test and culture. The results in table (1) showed that the rate of infection was more in females, and more in patients with age ranging from (30-39) years old, followed by those between (40-49) years and this disagree with (Terragni et al., 1991), Who said that *T. pedis* is similar in incidence between male and females before puberty but it becomes more in males at and after puberty.

This may be because most of these females are house wives and are in direct contact with water and detergent due to home duties and this will increase fungal growth due to wet environment.

Also we can notice that there is an increase in infection rates in the age periods between (40-49) and (50-59) years old which is consistent with (page and others, 1991) who find that the infection is increase with age.

Table (1) Number of *T. pedis* patients according to gender and age:

Range of age in years	Number		Total
	Male	Female	
11 – 19	2	2	4
20 – 29	2	8	10
30 – 39	3	10	13
40 – 49	6	5	11
50 – 59	8	1	9
Above 60 years	3	-	3
Total	24	26	50

The results showed that there is a significant difference in the percentage of isolated dermatophytes ($p < 0.0001$) as in table (2) which show that the isolated dermatophyte which cause most of *T. pedis* infections were mainly *T. rubrum*, *T. mentagrophytes* and *E. floccosum* and this agree with (Hay & Moore, 1998). *T. rubrum* was the most commonly isolated fungus in *T. pedis* and this agree with (Geary and Lucky, 1999).

Table (2) Type of isolated dermatophytes from T. pedis patients:

Name	Frequency	Male	Female	%
1 <i>T. rubrum</i>	36	16	20	72
2 <i>T. mentagrophyte</i> <i>var. interdigitale</i> <i>var. mentagrophyte</i>	16 (14) (2)	6	10	32
3 <i>E. floccosum</i>	6	4	2	12
4 <i>T. tonsurans</i>	2	0	2	4
Total	60	26	34	

Significant difference between percentages of isolated fungi ($\chi^2 = 46.1$, $p < 0.0001$).

No significant difference between males and females in the type of isolated fungi ($\chi^2 = 3.1$, $p > 0.05$).

The differentiation of isolated dermatophytes was possible from the characters possessed by each one, as the following:-

Trichophyton Rubrum:

It had a slow growth on culture media as it reaches a diameter of (2.5cm) after incubation for (14) days at (30C°). The colonies were whitish in color and cottony with an elevated center and had grooves that extends from center to its periphery. On microscopic examination there was a production of tear drops or peg-shaped micro conidia laterally on fertile hyphae. *T. rubrum* was giving a red pigment on Cornmeal agar. It was differentiated from *T. mentagrophytes* as *T. rubrum* give negative Hair perforation test and negative urea’s test in addition to production of red pigment in cornmeal agar and most of isolates were similar to Downy variants depending on (Frey et al., 1979).

Trichophyton Mentagrophytes:

The colonies were grown rapidly on SDA, it was (6cm) in diameter after two weeks incubation at (30C°) and had white color and flattened. On microscopic examination it produces septate hyphae, the macroconidia were abundant which had cylindrical shape with smooth walls. The microconidia were sawlajan in shape. *T. mentagrophyte* showed positive Hair perforation test and positive Ureas test but not produce red pigment on corn meal agar (Basak P.et al (2019); Carmen V. Sciortino Jr. ,2017)

Epidermophyton Floccosum:

The diameter of the colony was about (2.3cm) after (10) days incubation at (30C°) on (SDA). The color of the colonies was white but with the progress of time it takes a light yellow color, and had a cotton texture. This fungus was differentiated by the presence of smooth, thin walled, club-shaped macroconidia and absence of microconidia. The macroconidia are born singly or in clusters of 2 or 3, and consisting of 1 to 9 septa. (Carmen V. Sciortino Jr. ,2017).

The Antifungal Effect Of Crud Alcoholic Extract Of Anchusa Strigosa On Tinea Pedis:

It has been found that this extract had a noticeable effect at a concentration of 500 P.P.M. table (3) and *T. mentagrophytes* was affected by the extract more than *T. rubrum*. The percentage of growth inhibition by the extract for *T. mentagrophytes* at a concentration of (500) and (1000) P.P.M. were (65.11% and 100%) respectively as in table (3) picture no.(1),

while that for *T. rubrum* were (53.68% , 87.36% and 100%) at a concentrations of (500 , 1000 and 1500) P.P.M respectively as shown in table (3) and picture (2).

Table (3) The effect of alcoholic extract of *Anchusa strigosa* on colony diameter grown on culture media:

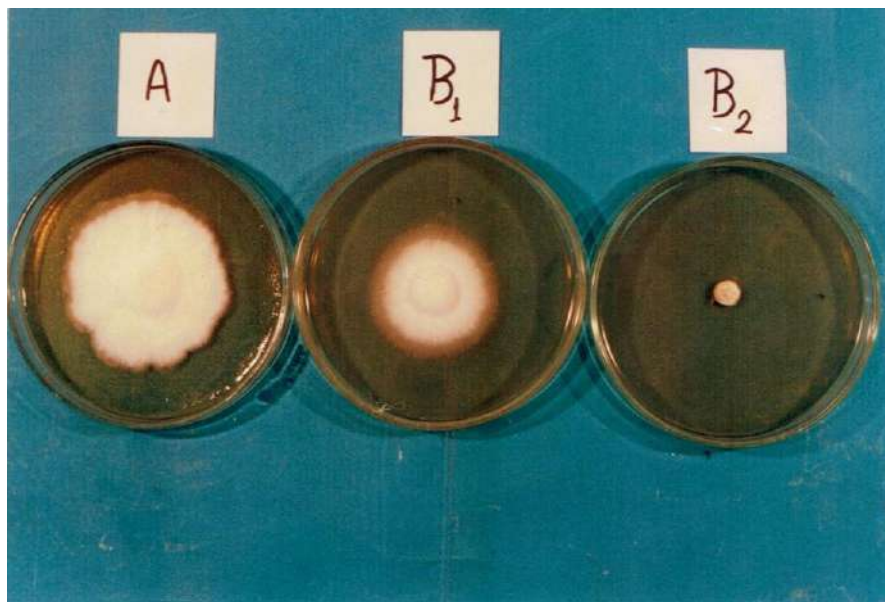
Name Concentration PPM	T. rubrum		T. mentagrophytes	
	Diameter of colony (mm)	Growth inhibition %	Diameter of colony (mm)	Growth inhibition %
Control	47.5 A	0	90 A	0
500	22.0 B	53.68	31.3 B	65.5
1000	6 C	87.36	0 C	100
1500	0 D	100	0 C	100

The different letters vertically means there is significant difference at p 0.05 according to Duncan test.



Picture (1) The effect of alcoholic extract of *Anchusa strigosa* on *T. mentagrophytes*

A: control B1: Minimum inhibitory concentration (M.I.C) B2: Minimum Fatal concentration (M.F.C)



Picture (2) The effect of alcoholic extract of *Anchusa strigosa* on *T. rubrum*

A: control B1: M.I.C B2: M.F.C

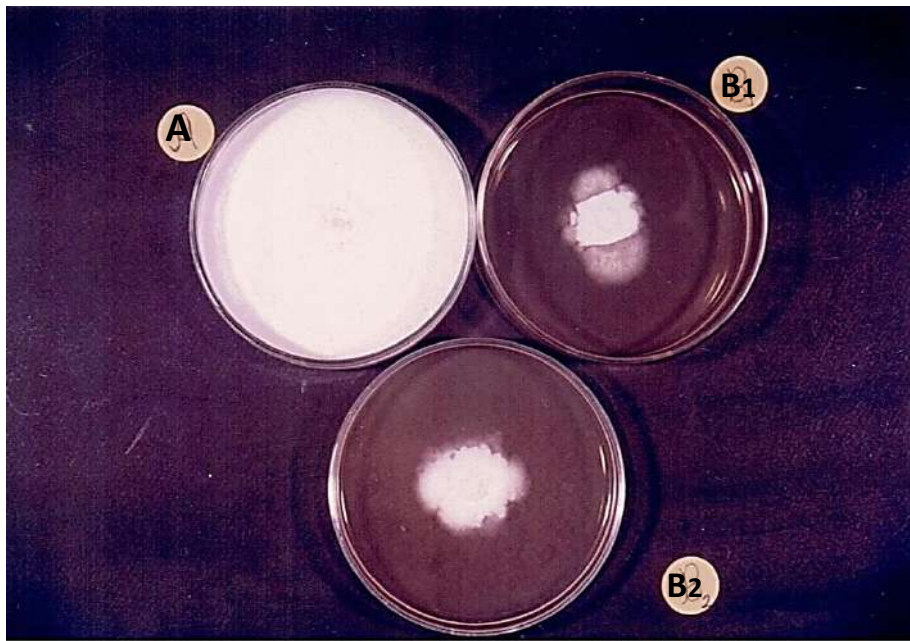
The Inhibitory Effect Of Tolnaftate Drug On Colony Growth Diameter (mm) In Culture Media:

The percentage of growth inhibition by Tolnaftate for *T. mentagrophytes* were (64.88 , 64.88 and 63.88%) at concentration of (500 , 1000 and 1500) P.P.M respectively as in table (4) picture no. (3). While that for *T. rubrum*, they were (56.59, 62.51 and 62.51%) at (500, 1000 and 1500) P.P.M. as in table (4) picture no. (4).

Table (4) The effect of Tolnaftate drug on the diameter of Fungal colony (mm) that grown on culture media:

Fungus Name Concentration PPM	T. rubrum		T. mentagrophytes	
	Colony diameter (mm)	Percentage of growth inhibition	Colony diameter(mm)	Percentage of growth inhibition
Control	67.5 A	0.0	90.0 A	0.0
500	29.3 B	56.59	31.6 B	64.88
1000	25.3 B	62.51	31.6 B	64.88
1500	25.3 B	62.51	32.5 B	63.88

The different letters vertically mean the presence of significant difference at probability level 0.05 according to Duncan test.

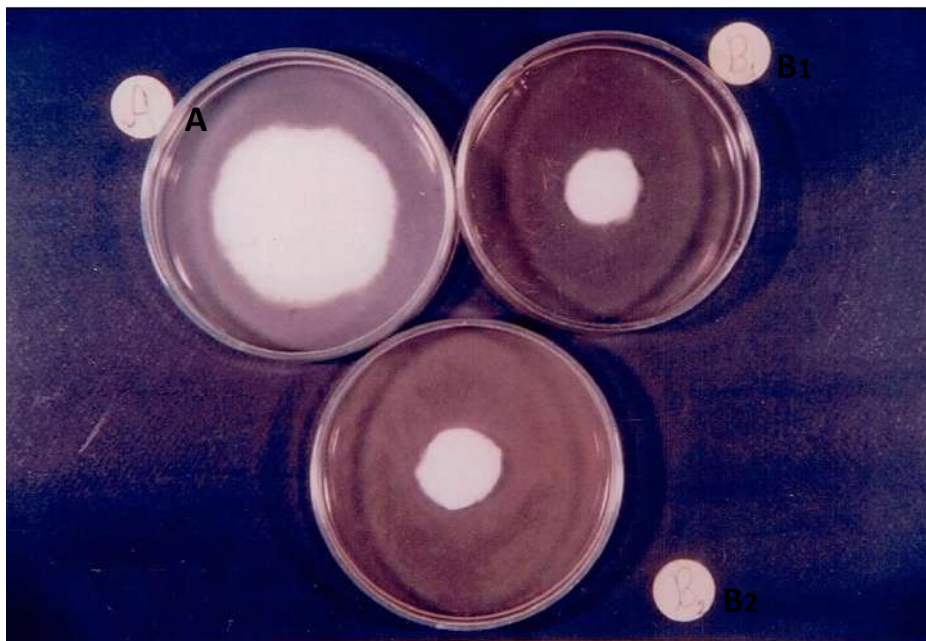


Picture (3) The effect of Tolnaftat drug on *T. mentagraphyte*

A: control

B1: M.I.C

B2: M.F.C



Picture (4) The effect of Tolnaftat drug on *T. rubrum*

A: control

B1: M.I.C

B2: M.F.C

In this study we found that the crud alcoholic extract of *Anchusa strigosa* was effective antifungal agent as it show a noticeable inhibitory effect at the minimal concentration (500) P.P.M. and it was more effective on *T. mentagrophyte* than *T. rubrum*, and when we compare the effect of plant extract with that of Tolnaftate drug, table (5), we notice that the plant extract at (1500) P.P.M was the best for *T. rubrum* and show a significant difference ($P \leq 0.05$) followed by a concentration of (1000) P.P.M then Tolnaftate drug ; while for *T. mentagrophyte*, the plant extract had the best growth inhibitory effect at (1000 and 1500) P.P.M., and the percentage of growth inhibition was (100%) and show a significant difference ($P \leq 0.05$) from others, As shown in table (5).

Table (5): Comparison between the effects of crud alcoholic extract of *Anchusa strigosa* and Tolnaftate drug on fungal colony diameter.

	Fungal Name	T. rubrum Growth inhibitory %	T. mentagrophyte Growth inhibitory %
	Conc. P.P.M.		
Alcoholic extract of <i>Anchusa strigosa</i>	500	53.68 D	65.18 B
	1000	87.37 B	100.00 A
	1500	100.00 A	100.00 A
Tolnaftate drug	500	56.54 CD	64.78 B
	1000	62.47 C	64.78 B
	1500	62.47 C	63.89 B

The different vertical letters mean there is a significant difference at probability level 0.05 according to Duncan test.

Many topical antifungal drugs have been used for treatment of *T. pedis* and other dermatophyte infection but they have many side effects and used for a long period of time, so, its important to find anew effective antifungal agent that may give a better result in a short period of time to avoid the undesirable side effects of treatment.

In this study we conclude that the crud alcoholic extract of *Anchusa strigosa* is a promising antifungal agent and can compete the used antifungal drugs, and this may be due to its content of alkaloids which has the ability to combine with DNA which lead to growth inhibition (Cowan, 1999).

References:

- Abbas M, Disi A, Al-Khalil S. Isolation and identification of anti-ulcer components from *Anchusa strigosa* root. *Jordan Journal of Pharmaceutical Sciences* 2009; 2(2):131-139.
- Alali F, Tawaha K, El-Elimat T, Syouf M, El-Fayad M, Abulaila K. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. *Natural Product Research* 2007; 21:1121-1131.
- Ali E. et al., (2014), The Pharmacology Of *Anchusa Italica* And *Anchusa Strigosa*. A Review, *International Journal of Pharmacy and Pharmaceutical Sciences*, Vol 6, Issue 4.
- Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plants extracts, against dermatophytes. *Mycoses* 1999; 42: 665-672.
- Al-Mussawy, A. Hussein (1982). The key for plant groups that present in Iraq with some of it's characteristic, Al-Hathara office for pressing and engineering drawing. Baghdad.
- Al-Zubaidi, A. Mohan (1989). Classification of genus *Anchusa* L. Boraginaceae in Iraq. Master thesis, collage of science, Baghdad university.
- Al-Zubaidi, Z. Najeeb, Baban H. Abdulkareem and Fleih F. Kazum(1996) Treatment by Iraqi Medical Herbs , Mosul University.
- Baron, E.J.; Peterson, L.R. and Finegdo, S.M. (1994). *Diagnostic Microbiology*. 9th edition, Mosby, pp.754-760.
- Basak P. et al (2019). Prevalence of dermatophytic infections including antifungal susceptibility pattern of dermatophytes in a tertiary care hospital, *Int J Res Med Sci*. 2019 Mar;7(3).
- Braca A, Bader A, Siciliano T, Moreelli I, Tommasi N D. New pyrrolizidine alkaloids and glycosides from *Anchusa strigosa*. *Planta Med* 2003; 69(9): 835-841.
- Braca A, Bader A, Siciliano T, Moreelli I, Tommasi ND, new pyrrolizidine alkaloids and glycosides from *anchusa strigosa*. *Planta Med* 2003; 69 (9): 835-841.
- Bruning, J. L. and Kintz, B. L. (1977) *computational Handbook of statistics*. 2nd edition, scott Foresman and co., pp.116-119, 230-237.
- Carmen V. Sciortino Jr. (2017), *Atlas of Clinically Important Fungi*, John Wiley & Sons, Inc
- Cowan, M. M. (1999). Plant products as Antimicrobial agent. *12 (4): 564-574*.
- Fingold ,S.M. and Baron, E.T.(1986). *Bailey and Scott`s Diagnostic Microbiology*. 7th ed , Mosby , p914.
- Frey, D., Oldfield, R. J. and Bridger, R. C. 1979. *A color atlas of pathogenic fungi*. Wolfe Medical publications Ltd., Smeets Weert, Holland.
- Geary, R.J. and Lucky, A.M. (1999). Tinea pedis in children presenting as unilateral inflammatory leasions of the sole. *Pediatr. Dermatol.*, 16(4): 255-258.
- Gupta, A. K., Chow, M., Daniel, C. R., & Aly, R. (2003). Treatments of tinea pedis. *Dermatologic Clinics*, 21(3), 431–462.
- Hay, R. J. and Moore, M. (1998). *Mycology*. In: *textbook of Dermatology by champion, R.H., Burton, J. L.; Burns, D.A. and breanhnach, S.M.*, 6th edition, vol.2, Black well science, pp.1277-1345.
- Ilkit, M., & Durdu, M. (2014). Tinea pedis: The etiology and global epidemiology of a common fungal infection. *Critical Reviews in Microbiology*, 41(3), 374–388.

- Ketabchi S, Moatari A, Shadram M, Rostami Y. The anti influenza virus activity of *Anchusa italic*-Asian J. Exp Biol Sci 2011;2(4):558-561
- Kohli K and Ali M. Phytochemical studies of Iranian *Anchusa strigosa* Linn. Recent Progress in Medicinal Plants: Phytochemistry and Pharmacology, J.N.Govil and V.K.Singh (eds.), Studium Press, LLC, Houston, Texas, USA 2003, V2, pp 247-251.
- Kohlik and Ali M. phytochemical studies of Iranian *Anchusa strigos* Linn. Recent progress in Medicinal plants: phytochemistry and pharmacology, J. N. Govil and V.K. singh (eds), stadium press, LLC, Houston, Texas, USA 2003, V2, pp246-251.
- Koneman, E.W. and Robert, G.D. (1985) practical laboratory Mycology. 3rd edition. The Williams and Wilkins company Baltimore, USA..
- Koneman, E.W.; Aiiien, E.D.; Janda, I.M.; Schreckenberger, U.C. and Winn, A.C. (1997). Color Atlas and textbook of diagnostic microbiology. Lippinott- Rave publishers.
- Midgley, G.; Clayton, Y. M. and Hay, P.R.J. (1997). Diagnosis in color medical mycology. 1st edition, Mosby-wolf.
- Mishura, A. K. and Dubey, N.K. (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities Appl. Env. Micro., 60(4): 1101-1105. (Abs.)
- Page, JC. : Abramson, C.; Lee, W. L.; Mc Carthy, D. J. and Williams, D. (1991). Diagnosis and treatment of tinea pedis. A review and update. J. Am. Pediatr. Med. Assoc., 81 (6): 304-316.
- RICHARD W., Hamish H. and Margaret M. Clinical Dermatology, fifth edition, T. pedis, 2015, pp.239-240.
- Siciliano T, Leo MD, Bader A, Tommasi ND, Vrieling K, Braca A and Morelli I. Pyrrolizidine alkaloids from *Anchusa strigosa* and their antifeedant activity. Phytochemistry 2005; 66(13): 1593-1600.
- Singh, C.J. and Singh, B. G. (1997). Antifungal activity of some plant extracts against dermatophytes, and related keratinophilic fungi.
- Terragni, L.; Buzzetti, I.; Lasagni, A. and Orianig A. (1991). Tinea pedic in children. Mycoses, 34 (5): 273-276.
- Tilton, R.C. (1992). Fungi In: Clinical Laboratory medicin by Tilton, R.C.; Balows, A.; Hohnadel, D.C. and Reiss, R.F. Mosby, pp.727-762.
- Twaij, H. A.; Elisha, E.E. and Al-Jeboory, A. A. (1985). Screening of Iraqi medical plants for diuretic activity. Indian J. pharmacy., 73-76.
- William D. James; Dirk M. Elston; James R. treat; Mish A. Rosenbach; Isaac M. Neuhaus. Andrews textbook of dermatology 13th ed, 2020, p 293.
- Yamaguchi, H.; Uchida, K.; Tanaka, T. and Yamaguchi, T.(2001)therapeutic efficacy of a topical tolnaftate preparation in guinea pig model of tina pedis Japan J. Antibot., 54(6):323-330.

**NATURAL LIGHTING IN RESIDENTIAL BUILDINGS
HANANO NEIGHBORHOOD - ALEPPO CITY AS A CASE STUDY**

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MAAN SAHAB MOHAMMED
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**NATURAL LIGHTING IN RESIDENTIAL BUILDINGS
HANANO NEIGHBORHOOD - ALEPPO CITY AS A CASE STUDY**

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Abstract:

The good natural lighting is considered one of the principal aims that man and architect to use it in the buildings. This side acquired his attention through the ages, so he used different architecture types suited the available techniques in each place and time. Many of the architectural engineers contributed in utilizing this factor in their buildings. This developed by the development of sustainable concepts and focusing on utilizing the natural energy, including solar energy as main source for natural lighting. So, this search determines the designing and planning parameters which achieve the maximum possible natural illumination to serve the sustainable architectural goals from social, economic and environmental viewpoints. Then applying the theoretical frame on a collection of building in Aleppo to reach parameters meeting the principal determinations of natural lighting design in building to realize clear imaginations in knowledge and application which assist in decreasing power consumption average and to be the base in developing the designing operation within the architectural concepts and sustainable buildings in Aleppo. The research problem is crystallized in the lack of knowledge and application of the designing parameters of the natural lighting in selected samples from Aleppo buildings and the effects on the visual and hygiene environment within the architectural space, as the using of the artificial illumination is considered a crescent burden for the energy economies of that buildings, the research concluded by the recommendations and the result.

Key words: Natural Lighting, Sustainable Architecture, Passive Solar Energy.



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الإضاءة الطبيعية في المباني السكنية حي هنانو - مدينة حلب كحالة دراسية

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معن سحاب محمد⁵

ربيع خليل ذنون⁶

الملخص:

تعتبر الإضاءة الطبيعية الناجمة واحدة من الأهداف الأساسية التي سعى الإنسان والمعماري لتوظيفها في مبانيه، ونال هذا الجانب اهتمامه عبر العصور فاعتمد أساليب وأنماط معمارية مختلفة تناسب والتقنيات المتوفرة لكل زمان ومكان . وقد ساهم العديد من المعماريين منذ مطلع القرن العشرين بتوظيف هذا العامل في مبانيهم، وتطور هذا الأمر بتطور الفكر المعماري بمفاهيم الاستدامة والتركيز على توظيف الطاقات الطبيعية ومنها الطاقة الشمسية كمصدر رئيسي للإضاءة الطبيعية. يحاول البحث تحديد المعايير التصميمية والتخطيطية التي تحقق أقصى إضاءة طبيعية ممكنة تخدم أهداف العمارة المستدامة من الجوانب الاجتماعية والاقتصادية والبيئية ومن ثم تطبيق الإطار النظري على مجموعة من المباني في مدينة حلب للوصول إلى معايير تراعي المحددات الأساسية لتصميم الإضاءة الطبيعية في المباني لتحقيق تصورات معرفية وتطبيقية واضحة تساعد على الحد من ارتفاع معدلات استهلاك الطاقة وتكون أساساً في تطوير العملية التصميمية ضمن مفاهيم العمارة والأبنية المستدامة في مدينة حلب. وتتبلور المشكلة البحثية في القصور المعرفي والتطبيقي للمعايير التصميمية للإضاءة الطبيعية في نماذج منتخبة لمباني في مدينة حلب وتأثيراتها على البيئة الصحية والبصرية داخل الفراغ المعماري، لاسيما أن استخدام الإضاءة الصناعية يعتبر عبئاً متزايداً على اقتصاديات الطاقة لتلك المباني، واختتم البحث بالنتائج والتوصيات.

الكلمات المفتاحية: إضاءة طبيعية، عمارة مستدامة، طاقة شمسية سلبية.

المقدمة:

الإضاءة lighting هي إسقاط ضوء على سطوح الأشياء يمكن من رؤيتها بالعين المجردة أو من تبين شكلها وتسجيل وجودها بوسائل أخرى تتحسس بالضوء، وتكمن أهمية الإضاءة في أن البشر يلتمسون المعرفة ويحصلون على القسم الأعظم من معلوماتهم عن العالم المحيط بهم بطريق الرؤية أو الإبصار، كما أن الإضاءة تساهم في تحقيق الاستقرار النفسي للإنسان في عمله وفي أوقات راحته إلى جانب إسهامها في المحافظة على صحة الإنسان وسلامته. فعندما تكون الإضاءة حسنة والرؤية جيدة يزداد مردود العمل ويتحسن نوعه وتتناقص إصابات العمل وأخطاؤه، وتنخفض حوادث الطرق وتتحسن أحوال المعيشة. ولا شك في أن الإنفاق على تحسين شروط الإضاءة كبير الجدوى وسريع التعويض اقتصادياً. والإضاءة النموذجية rational هي الإضاءة التي تستجيب لمتطلبات الصحة والاقتصاد معاً، أما المقصود بالإضاءة الطبيعية natural lighting فهي الإضاءة الناتجة عن ضوء النهار والذي بدوره ناتج من إضاءة أشعة الشمس المباشرة وغير مباشرة والتي تنعكس عن قبة السماء والغيوم والمحيط الخارجي للمباني وتنعكس عن الأرض والمباني المجاورة، وتمثل الشمس المصدر الأساسي للضوء الطبيعي على الكرة الأرضية، وللأشعة الشمسية تطبيقات عديدة في كثير من المجالات ولكن أهم هذه التطبيقات هي التطبيقات المنزلية ولها مميزات عديدة منها أنها منتشرة بكثرة بتراكيب بسيطة وأيضاً تكاليفها قليلة ، وحتى أن تخزين الحرارة الآن أصبح في الإمكان بسهولة وذلك للاستفادة منها أثناء الليل وذلك لأن الطاقة الحرارية لها الاستخدام الأكبر من بين العديد من الطاقات^[1]. وفي الوقت الذي يتوقف فيه مدى توفر مصادر الطاقة الحالية على أماكنها، وبالتالي تأثير تكلفة الاستخراج على قيمة المنتج من

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هذه الطاقة وسعر الاستهلاك، أو زيادة التكلفة نتيجة لنقلها أو استيرادها، فإن الطاقة الشمسية تتميز بالتوفر في كل مكان تقريباً، إذ أن نسبة سطوع الشمس في بعض المناطق وخصوصاً البلاد العربية تصل إلى ٧٠ % شتاء وتزداد هذه النسبة إلى ٩٠ % صيفاً كما أن ساعات سطوع الشمس في مصر - على سبيل المثال تصل إلى حوالي ٣٠٠٠ - ٤٠٠٠ ساعة في العام، كما أنه وفي الوقت الذي تتعرض فيه مصادر الطاقة الحفرية للنضوب نتيجة لزيادة الاستهلاك، فإن الطاقة الشمسية تتميز بأنها مستديمة طالما أن هناك حياة وأن الشمس مازالت تشرق، ولا تحتاج إلى تكلفة استخراج أو نقل من مكان إلى آخر [2].

مشكلة البحث

على الرغم من الإمكانيات الهائلة التي تتمتع بها الدول العربية من كميات السطوع الشمسي سنوياً، إلا أنه لم يتم توظيفها بشكل فعال كمصدر بديل للطاقة المستخدمة في إضاءتها نتيجة لقصور ملحوظ في تطبيق المعايير التصميمية للإضاءة الطبيعية في المباني، الأمر الذي أدى إلى عدم توفر بيئة صحية بصرية مريحة داخل الفراغ المعماري، كما أن استخدام الإضاءة الصناعية يشكل عبئاً متزايداً على اقتصاديات الطاقة الحفرية (بترو - فحم - غاز طبيعي) والتي قاربت مصادرها على النضوب.

هدف البحث

- تحديد المعايير التصميمية للإضاءة الطبيعية وصولاً لتحقيق بيئة صحية وبصرية جيدة لمستخدمي المباني.
- دراسة أثر معايير الإضاءة الطبيعية على المباني بهدف تقييم فعالية الإضاءة الطبيعية.

1- الأشعة الشمسية كمصدر للطاقة والإضاءة:

إن الطاقة التي يحتاجها الإنسان في ممارسة أنشطته حياته المختلفة لها أكثر من صورة، فهي إما طاقة حرارية يستخدمها في تحقيق الدفء والحرارة اللازمة للأغراض المختلفة: التسخين أو التبريد، أو طاقة ضوئية، أو طاقة ميكانيكية كصورة متحركة عن صور الطاقة الحرارية أو الضوئية، وهذا ما استطاع الإنسان الحصول عليه من خلال العمليات المختلفة لتحويلات الطاقة. ولا تختلف الطاقة الشمسية عن المصادر الأخرى للطاقة في هذا النطاق، فقد اتجهت الأبحاث عقب أزمة البترول العالمية في عام ١٩٧٣ إلى البحث عن مصادر أخرى للطاقة البديلة والمتجددة، وكان من الطبيعي أن تتجه هذه الأبحاث نحو الطاقة الشمسية، ومن خلال عملية البحث أمكن التوصل إلى كل الصور التي يحتاجها الإنسان من هذه الطاقة، وإن كانت بعض هذه الصور مازالت تتوقف على الجدوى الاقتصادية والمقارنة بين اقتصاديات الطاقة الشمسية ومصادر الطاقة الأخرى [3]، ومن هذه الاستخدامات ما يرتبط بتقنيات الطاقة الشمسية السالبة (Passive Solar Energy Techniques) وهي لا تمثل أية مشكلة تصميمية أو اقتصادية، فقد نفذت فيما سبق العديد من المعالجات المعمارية والتخطيطية التي ساعدت في تحقيق الراحة الحرارية قبل ابتكار الأجهزة الميكانيكية، ويبقى على الممارسين والمخططين مراعاة هذه الجوانب وتطويرها في التصميم والتخطيط بقصد ترشيد الطاقة، وإن لم تتمكن هذه الحلول من الاستغناء بشكل نهائي عن الطاقة التقليدية فعلى الأقل يمكن أن تقلل من الاعتماد عليها بشكل كامل. فعلى سبيل المثال تمثل مشكلة تبريد الهواء في البلاد العربية ذات المناخ الحار حين أن هذا الأمر يمكن توفيره أو الإقلال منه بالاعتماد على تقنيات الطاقة الشمسية السالبة في عمارة وتخطيط المدن الجديدة، وذلك بتصميم المباني على أفنية داخلية واستعمال ملاقف الهواء (أبراج الهواء)، ومواد البناء ذات العزل الحراري الكبير، كما أن تظليل الفتحات يحقق عزلاً قدره ٥٠ % من كمية الحرارة التي يكتسبها الفراغ الداخلي إذا لم تكن الفتحات مظلمة هذا بجانب إتباع أسس التخطيط العمراني المتضام عن طريق تصميم المجاورات السكنية بشكل يقلل الفراغات الخارجية ويقلل مسافات السير بين الخدمات المختلفة بحيث يقل الاعتماد على السيارة والترشيد في استهلاك الطاقة، مع الإكثار من النباتات والأشجار في الفراغات المختلفة سواء داخل المباني أو خارجها أما تقنيات الاستخدام الموجب للطاقة الشمسية (Active Solar Energy Techniques) وهي تعنى استخدام وسائل ميكانيكية أجهزة، أو خلايا شمسية خاصة (لأداء كل العمليات، بداية من تجميع الطاقة وتحويلها إلى أية صورة أخرى، وحتى أداء الغرض المطلوب، سواء تدفئة أو تبريد أو تسخين مياه أو إنتاج طاقة كهربائية أو ميكانيكية لكافة الاستخدامات الأخرى، وتعد فكرة استخدام الإضاءة الشمسية في توليد الحرارة اللازمة للتسخين (الماء - الهواء) باستخدام السخان الشمسي من

التطبيقات البسيطة والمتوفرة في كثير من بلاد العالم مثل الأردن ومصر وأمريكا وأستراليا والهند واليابان) تمتلك طوكيو وحدها حوالي ٢,٥ مليون وحدة)، تمتاز هذه الوحدات بسهولة استخدامها وتوزيعها حيث يمكن وضع هذه المجمعات الشمسية في أماكن مختلفة على الأسطح أو على الواجهات بحيث يمكن استغلالها في عملية التشكيل المعماري سواء للمباني المنخفضة الارتفاع أو العالية، صغيرة أو كبيرة الحجم، السكنية أو مباني الفنادق والمستشفيات والمراكز التجارية والأندية الرياضية^[4].

2- الإضاءة والإنسان:

الإضاءة أهمية كبيرة في حياة الإنسان حيث يؤكد الباحثون على أن عملية الرؤية تستهلك ربع الطاقة الكلية اللازمة للجسم في حالة الإضاءة الصحية و النظر السليم , وأن أي نقص في هذه الإضاءة معناه استنزاف الطاقة من الجسم لتعويض هذا النقص^[5]، وتختلف حاجة الإنسان للإضاءة في كل مرحلة عمرية عن الأخرى، فقد توصل مورتنتسون Mortenson ، وريتشارد بلاكويل Blackwell إلى أن الناس بين الأعمار من ٣٠ إلى ٤٠ عامًا تحتاج إلى كمية إضاءة مقدارها ١٧,١ مرة قدر ما يحتاجه من هم في سن من ٢٠ إلى ٣٠ عامًا لكي يحصلوا على نفس درجة الوضوح في الإضاءة، وأن كبار السن ما بين ٦٠ إلى ٧٠ عامًا يحتاجون إلى كمية إضاءة مقدارها ٥١,٢ مرة قدر الإضاءة اللازمة للشباب بين ٢٠ إلى ٣٠ عام^[4]. أن الإنسان يحتاج إلى التغيير المستمر في المرئيات حتى يمكنه المحافظة على مستوى ذكائه، كما أن الحرمان من هذه التغييرات إن طال فإنه يصيب الإنسان بهلوسة في الرؤية وكذلك في حاسة السمع علاوة على انخفاض مستوى ذكائه. وفي المركز الطبي لجامعة "ديك" بأمريكا قام كل من الأستاذة "مورفي" Murphy عام ١٩٥٤ م وكذلك "سيلفر مان Silverman وسلبورن Celborn عام ١٩٦١ م (بدراسة أثر تعرض الإنسان لمرئيات لا يطرأ عليها تغييرًا فوجدوا أن مثل هذا الثبات له تأثير سئ بالنسبة لمعدلات إفراز الهرمونات ونشاط مركز الأعصاب والجهاز التنفسي وحيوية الأوعية الدموية القريبة من الجلد، وكذلك مقدرة الإنسان على الإحساس، لأن الإضاءة الطبيعية توفر التغييرات المطلوبة في الأشكال المحيطة به وتساعد على استمتاعه بالحياة والصحة الجيدة وتعد الإضاءة الطبيعية أفضل أنواع الإضاءة بالنسبة لتمييز الألوان، لأن غالبية مصادر الضوء الصناعي إذا ما سقطت على الألوان المتشابهة ظهرت مختلفة حتى بالنسبة لمصادر الإضاءة الواحدة كالفلوريسنت (مثلا) بينما تبهت الألوان تحت تأثير مصادر ضوء أخرى، وتختفي خاصية الألوان في مصادر ضوء اصطناعية ثالثة وتظهر بلون مصفر (مثلا) بدرجات مختلفة ومتباينة حيث إن وجود الشمس في الفراغات المعمارية يضيف تغييرًا وتنوعًا في هذه الفراغات وذلك بسبب تغير الألوان والإضاءة، وهذا التباين يضيف ديناميكية في هذا الفراغ يصعب تحقيقها في حالة استخدام الإضاءة الصناعية^[6]. كما ثبت أن للإضاءة تأثيرًا على الجهاز العصبي للإنسان فقد قدم الدكتور "راندوت" Randot عام 1963 إلى معهد C.I.E. للإضاءة بفرنسا جاء فيه أن للإضاءة تأثيرًا منشطًا للأعصاب، وكذلك على حيوية الإنسان ونشاط أعضائه، وأنه في الأيام التي تقل فيها الإضاءة أو تحت ظروف إضاءة صناعية فإن الإنسان يصاب بالخمول والكسل، ولقد درس هذا التأثير من خلال ملاحظة التغييرات التي تطرأ على كرات الدم البيضاء عند تعرض الإنسان للإضاءة كذلك ترتبط الإضاءة بالأشعة فوق البنفسجية، ويفيد التعرض لهذه الأشعة من قبل الكبار إلى امتصاص الكالسيوم، كما تفيد في علاج الأطفال من أمراض لين العظام، وكما لاحظ الدكتور دانتسيج Dantsig وزملائه انخفاضًا ملحوظًا في أمراض الجهاز التنفسي بين خمس الأطفال من تلاميذ المدارس الذين تم تعريضهم لهذه الأشعة بصفة يومية^[7]، بيد أن الإضاءة لا تقتصر فقط على وضوح الرؤية أو تنظيم وظائف أعضاء الجسم بل إن المطلوب من الإضاءة أيضًا هو التخفيف من الصراع النفسي الذي يعاني منه الإنسان نتيجة للعالم الصناعي الذي جاء من صنعه، والذي لم يثبت نجاحه في التوفيق بين غرائزه التي تدفعه ليعيش طبيعيًا وبين أسلوب الحياة المصطنع الذي يفرض على الإنسان أن يتعايش فيه.

3- الإضاءة الطبيعية والفراغ المعماري للأبنية السكنية في الدراسات المعمارية السابقة

أوضحت الدراسات المعمارية أن الفراغات المعمارية المضاءة بضوء النهار تبدو متسعة ورحبة وأكثر عندما يستخدم ضوء النهار من قبل شاغلي المبنى أو مستخدمو المبنى لتحقيق راحتهم الحياتية طوال الفترة التي يقضونها بالمبنى. فهم كبشر، يكوون بتكليف راحتهم البصرية الفسيولوجية من خلال التعرض إلى ضوء النهار الطبيعي^[8]، وبالتالي نحتاج إليه كعنصر أساسي في بنايتنا خلال ساعات النهار. وان تغيره الديناميكي هو حافز لدورتنا النهارية والليلية ويمكنه التحكم في مزاجنا وصحتنا على التوالي.

تشجيعاً على الإحساس بالسعادة والسرور عنها في حالة الفراغات المضاءة بالإضاءة الصناعية نظراً لارتباطها بالخارج من خلال الفتحات والنوافذ كما أن فتحات الإضاءة وخصوصاً الجانبية تعد بمثابة قنوات اتصال بصرية بالمحيط الخارجي، بما يمثله من مناظر طبيعية وظروف مناخية متغيرة واختلاف حالة السماء، ويصعب على الإنسان العيش وممارسة مهام حياته بدون هذا الاتصال، كما أن هناك دراسات متعددة كتبت في الإضاءة أكدت على أن النوافذ والاتصال الخارجي ضروري من الناحية النفسية بالنسبة لمستخدمي هذه الفراغات، وأن الاتصال الخارجي للسكان بالمحيط ضرورة سيكولوجية هامة، تؤثر على الأداء والاستمتاع والحضور الذهني في الفراغات التي تمت هذه البحوث فيها^[9]. وأشارت بعض الدراسات أيضاً إلى الجوانب المتعلقة بالطاقة فيمكن للتأثير الحراري للضوء الطبيعي أن يوفر حرارة مجانية للمبنى، مما يقلل من استهلاك الطاقة للتدفئة في الشتاء^[10]. كما تشير الدراسات، وقبل وجود الإضاءة الاصطناعية كان هذا العامل مهم جداً. فبسبب نقص ضوء النهار في شمال أوروبا، وخاصة في فصل الشتاء، تم استخدام النوافذ الكبيرة والسقوف العالية لتوفير إضاءة طبيعية كافية في المساحة الداخلية. تم العثور على تصميمات مختلفة للنوافذ في البلدان الجنوبية بسبب الحاجة إلى تقليل اكتساب الحرارة أثناء الصيف إلى جانب توفير إضاءة طبيعية مناسبة. لقد وجدت بعض الدراسات مثلاً أن دمج الفناء في تصميم المبنى يوفر حلاً مقبولاً لتوفير ضوء النهار في المساحات الداخلية^[11]

3-1 مصادر الإضاءة الطبيعية في المباني^[12]:

- الضوء المباشر الصادر من الشمس أو السماء، وضوء السماء يكون أكثر راحة من ضوء الشمس الذي يحتاج إلى أساليب تحكم مخصصة من أجل الاكتساب الحراري، اللعان، والأشعة المضرة.
- الضوء الصادر عن عناصر عاكسة من الخارج مثل سطح الأرض، الأبنية، جلسات النوافذ العريضة، البروزات العاكسة.

- الضوء الصادر عن عناصر عاكسة من الداخل مثل الجدران المحيطة الداخلية، السقف، الأرضية وهذه الأسطح هي عناصر عاكسة مهمة من أجل اكتساب كمية إضاءة جيدة داخل الغرفة، وإهم سطح هو سقف الغرفة لذلك يجب أن يكون أملس بطلاء فاتح عالي الانعكاس.

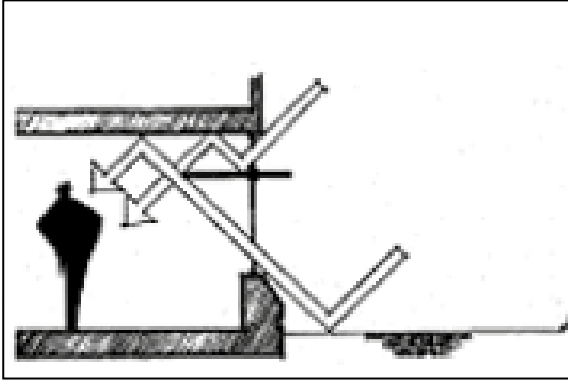
4- الشروط التصميمية المرتبطة بالإضاءة الطبيعية:

تكمّن فعالية التصميم المعماري الجيد في تحقيق شروط الراحة البصرية للمستخدم داخل الفراغات المعمارية، وهي ما يمكن ترجمته في شكل مجموعة من الشروط هي^[13]:

- إضاءة عناصر المبنى بالإضاءة الطبيعية.
- تخصيص أماكن بالمبنى تمكن الإنسان من الاستفادة من الأشعة فوق البنفسجية Ultra-violet مع تحقيق الخصوصية البصرية
- زيادة الإضاءة سواء كانت طبيعية أو صناعية داخل المبنى قدر الإمكان ليقارب مستوى الإضاءة الطبيعية بالخارج.
- السماح لأشعة الشمس بالنفاذ إلى المبنى لمدة لا تقل عن ساعة يوميًا
- التحكم في توزيع الشبابيك بحيث تحقق عامل الخصوصية، حتى لا يضطر الناس إلى قفل الشيش (الابجور- ضلف الشمسية-) أو استخدام الستائر المعتمدة طوال النهار.
- الإقلال من الألوان الماصة للضوء داخل المبنى وخصوصاً الألوان القاتمة، ويكون اللون الأبيض والألوان الفاتحة هي الغالبة.
- توفير شدة الإضاءة المناسبة والموزعة بانتظام في الحيز المعماري، وهي حوالي 45-65 شمعة / قدم مربع للأنشطة العادية، وقد تزيد: وتبلغ هذه الشدة في حالات خاصة لتصل إلى 100 شمعة / قدم مربع
- منع السطوع، والذي يحدث بسبب:
 1. وجود فرق كبير في شدة الاستضاءة بين الأجزاء المضيئة والأخرى المظلمة.
 2. سقوط الضوء على سطح عاكس.
- وقد يحدث السطوع في مستوى إضاءة عالية أو خافتة وهو عملية نسبية.

1-4 المعالجات المعمارية للإضاءة الطبيعية [14].

تتوقف طرق معالجة الإضاءة على وظيفة وحجم الحيز ومستوى شدة الاستضاءة المطلوب داخل الحيز، وبشكل عام يمكن تحديد أربعة طرق لمعالجة الإضاءة في المباني هي:



شكل 1 زيادة نسبة غلاف المبني [14]

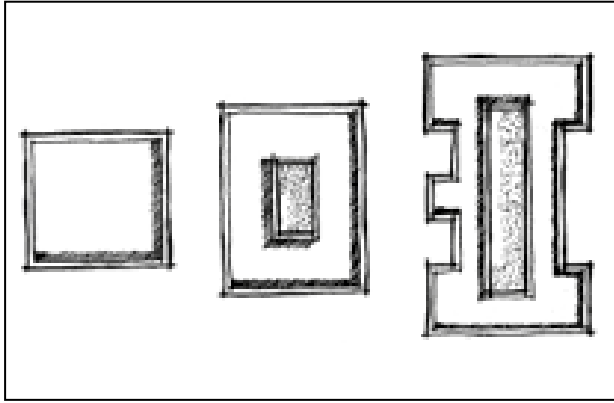
- إضاءة مباشرة: Direct Lighting وفيها يكون مصدر الضوء (سواء طبيعي أو صناعي) مكشوف للمستخدم.
- إضاءة موزعة Diffused Lighting: وفيها يتم توزيع الإضاءة بواسطة وسائل خاصة
- إضاءة نصف مباشرة Semi-direct Lighting: ويتم الحصول عليها بوضع حواجز خاصة أمام مصدر الإضاءة

- إضاءة غير مباشرة: Indirect Lighting وفيها تنعكس الإضاءة على سطح آخر ليصبح هو بمثابة مصدر للضوء.

5- المؤشرات التصميمية المؤثرة في حصد الإضاءة الطبيعية في المباني:

1-5 تصميم المبني وغلافه الخارجي

يشترط في المباني التي تصمم لتحقيق أفضل حصد شمسي لتوفير الإضاءة الطبيعية أن تراعي المعايير الآتية وحسب ما قدمته الدراسات:



شكل 2 الرفوف الضوئية [16]

- زيادة النسبة المئوية لمناطق الإضاءة الطبيعية المحيطة عن طريق زيادة طول محيط البناء أو زيادة النسبة غلاف المبني/ حجم المبني (شكل 1).
- تفضل المساقط الأفقية الضيقة والطويلة بالنسبة للإضاءة الطبيعية
- توجيه المحور الطولي باتجاه شرق- غرب لتحقيق الاستفادة قصوى من الشمس في الاتجاه الجنوبي .
- يمكن اعتماد (القاعدة الذهبية) في حساب أبعاد الغرفة من أجل إضاءة طبيعية كافية

- $D=1,5d$ عمق الغرفة = D ، ارتفاع النافذة = d [15].

- يصمم مخطط المبني بحيث لا يزيد عرضه عن 60 قدم (الأمام إلى الخلف) أو بحدود 33 قدم باتجاه النوافذ من أي موقع عمل أو وظيفة.

- استخدام مناور مصممة بطريقة فعالة لإدخال الضوء الطبيعي للفضاءات الاشغالية والخدمية.

- استخدام الفتحات السقفية (skylight)

- استخدام الرفوف الضوئية في الواجهات (light shelf) وهي عبارة عن بروزات أفقية مزودة بسطح عالي الانعكاس (شكل 2)، تعمل على زيادة مستوى الإضاءة الطبيعية وانتشارها والتخفيف من اللعان.

- استخدام تقنيات متطورة للتحكم الآلي بحصد الإضاءة الطبيعية مثل الكاسرات المتحركة [16].

- توفير منافذ شمسية كافية لإدخال أقصى كمية من الإشعاع الشمسي لغرض الإضاءة الطبيعية [17].

- ضرورة تحقيق تكامل تصميم واجهات المبني مع الأنظمة الشمسية السالبة لتوفير أقصى إضاءة طبيعية [18].

- استخدام الشرفات في الواجهات بمثابة سطوح عاكسة للإشعاع الشمسي الساقط كأداة لحصد أكبر كمية ممكنة من الإضاءة الطبيعية^[19].
- اختيار أشكال وكتل معمارية لتحقيق استجابة ملائمة للمناخ وطبيعة توجيه الإشعاع الشمسي مع السطوح والفتحات والشرفات^[20].
- يمكن تصميم السقف الثانوي بزاوية مائلة إلى الداخل من أجل تحقيق انسيابية لدخول إضاءة طبيعية أكبر إلى الفضاء^[21].

2-5-2 تصميم النافذة

يراعى عند تصميم النوافذ أن تؤدي الأدوار الرئيسية المطلوبة منها وهي:

- زيادة كمية الإضاءة الطبيعية: عادة ما يكون ذلك بزيادة الإشعاع الشمسي المشتت من القبة السماوية أو المنعكس على الأسطح المحيطة، وذلك بهدف تقليل الطاقة المستهلكة في الإضاءة الصناعية وتقليل التشوه اللوني الذي تسببه، وتحقيق الحد الأدنى من الإضاءة عند انقطاع التيار الكهربائي.
 - حسن التوزيع: عادة ما تكون المناطق القريبة من النوافذ اشد في درجة إضاءتها عن باقي مناطق الفراغ، مما يعني إن جزءاً من الغرفة قد يكون جيد الإضاءة بينما تتسم أجزاء أخرى بنقصها، فيجبر المستخدمين على استخدام الإضاءة الصناعية، ويساهم في ارتفاع النافذة وحسن تصميمها واستخدام معالجات مناسبة في تحسين توزيع الإضاءة داخل الفراغ.
 - منع الوهج: السطوح Glare هو وجود سطح أو جزء من الفراغ ذو شدة إضاءة أعلى بكثير من الأجزاء الأخرى مما يرهق العين. وعادة ما تكون النافذة هي أكثر الأسطح استضاءة، ويمثل دخول الإشعاع الشمسي المباشر سبباً للوهج، حيث ترتفع شدة استضاءة الأسطح المعرضة له بقدر كبير^[22].
 - تصميم نوافذ عالية لإدخال الضوء الطبيعي عميقاً إلى المبنى.
 - اعتماد النوافذ المتصلة والمستمرة لحصد أقصى إضاءة طبيعية منتظمة مع ملاحظة أن النوافذ المنفصلة مقبولة، ولكن الفصل بينها يمكن أن يشكل تضادات في الضوء وخلق مساحات معتمة^[23].
 - توفير نوافذ متحركة (قابلة للفتح) لضمان إدخال أقصى إضاءة طبيعية مباشرة لكل فضاء مشغول^[24].
 - اختيار مواقع ملائمة لتوقيع النوافذ بقرب القواطع الداخلية فضلاً عن مركز الفضاء لتوفير أقصى إضاءة مباشرة ومنعكسة^[25].
 - تصميم جوانب النافذة بأسطح مائلة (reveals) لحصد أكبر كمية من الإضاءة الطبيعية^[26].
- أما أهم العوامل التي يجب مراعاتها عند تصميم النوافذ فهي:

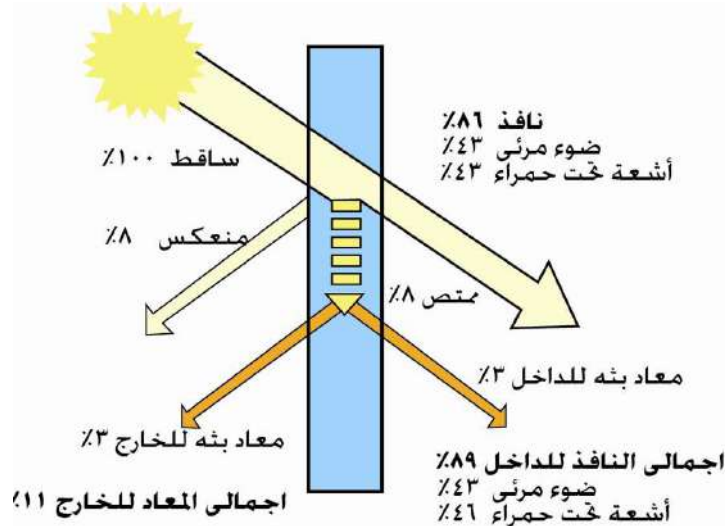
1-2-5 مساحة النافذة (نسبة مساحة النافذة إلى مساحة ارض الغرفة):

من أهم الوسائل التي تساعد في فهم اثر التصميم على الإضاءة الداخلية الناتجة عن الضوء الطبيعي هو العلاقة بين مساحة النافذة ومساحة أرضية الغرفة، ولقد حرصت العديد من الدول والهيئات الدولية على تبني معايير ومواصفات لاستخدام الإضاءة الطبيعية، وتوصي المواصفات البريطانية بكميات متفاوتة من الإضاءة الطبيعية الداخلية، بحيث لا تقل عن 5% من الضوء الطبيعي الخارجي في حال عدم استخدام الإنارة الكهربائية لمعظم أيام السنة، وبنسبة لا تقل عن 2% من الضوء الطبيعي الخارجي إذا كانت الإنارة الكهربائية ستستخدم خلال ساعات النهار، ولقد استخدمت نسبة مساحة النافذة إلى مساحة ارض الغرفة كمييار للإضاءة الطبيعية. ففي اليابان - كمثال - يفضل ألا تقل نسبة مساحة زجاج النافذة إلى مساحة ارض الغرفة عن 7:1 في المنازل وان تتراوح ما بين 5:1 و10:1 في المباني الأخرى. هناك أيضاً من يطرح نسبة 16:1 مساحة النافذة إلى مساحة أرضية الغرفة كضمان لإضاءة طبيعية جيدة للغرفة في المناطق المشمسة، وان نسبة 25:1 يمكن أن توفر إحساساً بان الغرفة مضاءة طبيعياً بصورة جيدة للمناطق الملبدة بالغيوم^[27].

2-2-5 نوعية الزجاج المستخدم في النوافذ:

يعتبر الزجاج هو السطح الناقل للطاقة الشمسية (ضوء - حرارة) إلى داخل الفضاءات المعمارية لهذا تعتبر عملية السيطرة على هذا السطح هي الوسيلة الرئيسية للسيطرة على هذا الانتقال، ويحدد نوع الزجاج المستخدم وسمكه كمية الأشعة الشمسية الداخلة إلى الفضاء، وكانت دائماً عملية تظليل زجاج الفتحة عملية غاية في البساطة

تمكن من السيطرة على هذا الإشعاع، فيمكن إسدال ستارة أو إغلاق ضلفة خشبية مصممة أمام النافذة في أي وقت تسقط فيه أشعة الشمس المباشر على الفتحة فيتم إزلالها، وتنتهي المشكلة وهذا الحل رغم كفاءته في تقليل نفاذ الإشعاع الحراري، إلا أن له عدة عيوب فهو يمنع وظائف هامة للنافذة، ويحولها إلى حائط، وتعتمد جودة تصميم النافذة على السماح للأشعة المشتتة بالدخول بقدر يكفي للإضاءة، ومنع الأشعة المباشرة والمنعكسة التي تحمل قدرًا أكبر من الحرارة.



شكل 3 مرور الإشعاع الشمسي من الزجاج العادي [28]

لهذا ومنذ زمن طويل كانت هناك تجارب وأنواع عديدة من الزجاج تمت معالجته حتى يمكنها السيطرة على هذا الإشعاع وتغيير النفاذية تبعاً للحاجة، وهي ثلاث فئات رئيسية (جدول 1)، أولها الزجاج ذو النفاذية الانتقائية للطول الموجي، الذي ينفذ الضوء المرئي أكثر من الأشعة تحت الحمراء، وأفضل أنواعه الزجاج الأخضر. وتشمل الزجاج منخفض الانبعاثية، ويتميز بتقليل الحرارة المعاد بثها للفضاء نتيجة قلة انبعاثيته للأشعة تحت الحمراء [29].

وثانيهما الزجاج المتغير النفاذية، كالزجاج الكهربائي الذي يتم فتحه أو غلقه بضغطة زر أو التحكم الإلكتروني، وتتركز حوله الأبحاث حالياً.

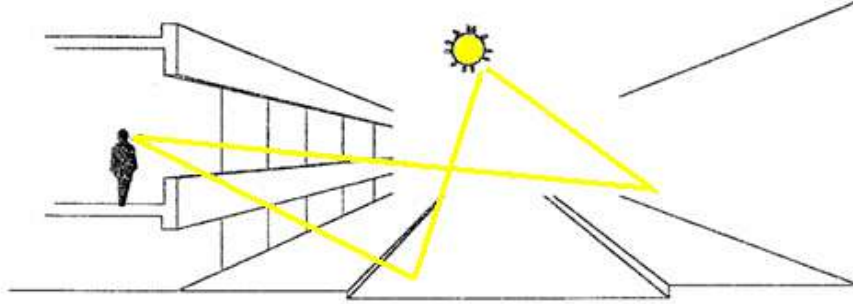
ج الزجاج ذو الانتقائية الزاوية السقوط Angle Selective	ب- الزجاج متغير النفاذية Switchable Glass	أ- الزجاج ذو الانتقائية للطول الموجي Spectrum Selective
الزجاج المتجه غير المتجانس anisotropic • الشوائب المترامية مغناطيسياً • التبخير المائل • الحفر الضوئي لطبقة عاكسة الزجاج المعتمد على الانعكاس • الزجاج المنشوري • الزجاج المقطع بالليزر • الزجاج المجمع كاسرات الشمس المدمجة • التقطيع المتتابع • الشرائح المخدوشة طولياً	1- الزجاج متغير النفاذية ضوئياً Photo-Chromic 2- الزجاج متغير النفاذية حرارياً Thermo-Chromic 3- الزجاج متغير النفاذية كهربياً (الزجاج الذكي) Electro-Chromic • البلورات السائلة • الحبيبات المعلقة • الهيدريد العاكس الزجاج متغير اللون بحقن الأيونات	1- الزجاج الماص • الزجاج المدخن (البنى) • الزجاج الأخضر • الزجاج الأزرق 2- الزجاج العاكس • المطلي بالذهب • المطلي بمواد أخرى • العاكس الماص 3- الزجاج منخفض الانبعاثية • الطبقة المخفضة من الداخل • الطبقة المخفضة من الخارج

جدول 1 التقنيات المستخدمة في الزجاج ذو النفاذية الاختيارية [30]

وثالثهما الزجاج ذو الانتقائية لزاوية السقوط الذي يحمل فرصة عالية للنجاح في توفير الطاقة تماثل تلك التي لكاسرات الشمس مع التخلص من عيوبها.

3-5 خصائص الإقليم الجغرافي:

تختلف شدة الإضاءة الطبيعية تبعا للمنطقة الجغرافية حيث تتباين هذه المناطق في شدة وكمية



شكل 4 انعكاس الوهج من الأسطح الخارجية [30]

الإشعاع الشمسي الساقط عليها إضافة إلى زاوية سقوطه وطول اليوم الشمسي فيها وتقسم هذه المناطق بشكل عام إلى [30].

1. المناطق المدارية: في هذه المناطق يصاحب الإشعاع الشمسي الضوء الطبيعي، أي أن أية زيادة في الضوء تعني زيادة في الإشعاع الحراري والذي يسبب إجهادا أكثر من الإجهاد الذي يسببه قلة الضوء، فمن ناحية نفسية، كلما قلت شدة الضوء كلما شعر الإنسان بالارتياح، ذلك لارتباط الضوء بالحرارة الشديدة. ويحتاج هذا الأمر إلى المعالجة الماهرة بحيث يتم توفير الضوء المناسب من جهة ، والتقليل من الشعور الذاتي بالظلام ، من جهة أخرى، والذي ينشأ من التباين القوي بين البيئة الخارجية الباهرة الضوء والإضاءة الداخلية الخافتة.

وتتلخص ايجابيات ومشاكل الإضاءة الطبيعية في المناطق المدارية في الآتي:

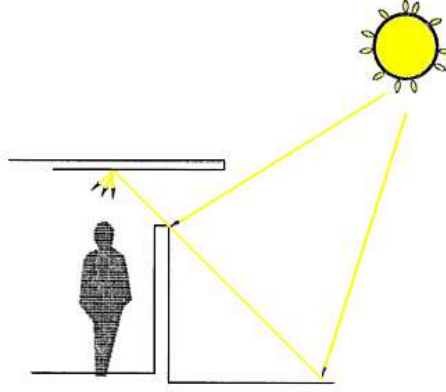
- توفير القدر الكافي من الضوء حتى في وجود العواض والشبكات والمظلات المانعة للحرارة.

- إقصاء الأسطح المضادة بواسطة الشمس، تلك التي تسبب الوهج، من المجال البصري.

2. المناطق الحارة الجافة: في هذه المناطق ، يجب إقصاء ضوء الشمس عن المباني للأسباب الحرارية أولا، ولتسببه بالوهج ثانيا. وعادة ما تكون النوافذ هنا صغيرة الحجم بحيث لا يمكن إلا رؤية جزء صغير من السماء من أي نقطة داخل المبنى.

ولأن السماء تكون ذات سطوع منخفض فإن المركبة السماوية للضوء الطبيعي تكون منخفضة، ومن جهة أخرى فإن سطوع السماء عند الأفق يكون كبيرا وربما أدى إلى إحداث الانبهار إذا لم يتم استعمال الظلان المانعة لصدده. كذلك، فإن أسطح المباني الأخرى وسطح الأرض عادة ما تكون ذات ألوان فاتحة وتشكل الأشعة الضوئية المنعكسة منها مصدرا رئيسيا للوهج أيضا، لذا يجب أن يتم معالجتها بعناية لمنع هذا الوهج .

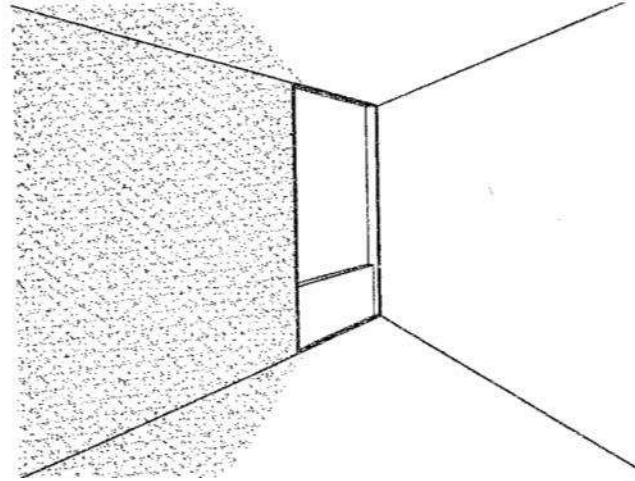
وأكثر الإشكال ملائمة للإضاءة الطبيعية في هذه المنطقة هو الضوء المنعكس داخليا ويمكن توفي ذلك باستعمال النوافذ المرتفعة High Level Windows والتي تكون فيها جلية النافذة أعلى من مستوى النظر بحيث تسمح هذه النافذة بمرور الضوء المنعكس إلى السقف الداخلي. وإذا كان هذا السقف ابيضا فان ذلك يؤمن ضوءا مشتتا كافيا للإضاءة الداخلية من نافذة صغيرة نسبيا (شكل 5).



شكل 5 تشتيت الضوء المنعكس بواسطة السقف الداخلي [27]

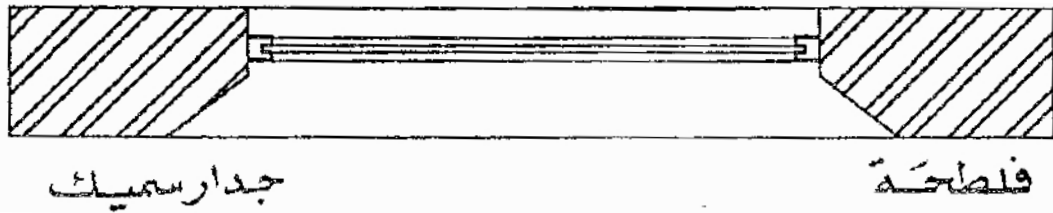
في حالة استعمال المظلات أو الكواسر الشمسية، يجب وضع هذه المظلات بحيث لا تكون ظاهرة من الداخل مع مراعاة عدم استعمال مادة عاكسة فيها، لان ذلك يشكل مصدرا للوهج. ويمكن استعمال النوافذ العادية إذا كانت تفتح على فناء Courtyard ظليل ومزروع. أما إذا لم يكن هناك بد من أن تكون النافذة مطلة على جهة مضاءة بواسطة الشمس فيمكن معالجة التباين بين السطوح القوي في الخارج والجهة المجاورة للنافذة في الداخل كما يلي:

- طلاء الجدار المجاور للفتحة بلون فاتح.
- دهان إطار النافذة الداخلي بالأبيض.
- فلطحة (إمالة) Splay جانب النافذة في الجدار السميك وخلافه بلون فاتح (شكل 6).



شكل 6 معالجة جوانب النوافذ [27]

- عمل فتحات أخرى في الجهة المقابلة لكي تلقي ضوءا على الجدار المحيط بالنافذة. واحد الحلول المتاحة لهذه المشكلة هي عمل نافذة راسية ضيقة في ركن الدار أو الحجرة بحيث تلقي ضوءا على سطح الجدار وبالتالي تكون مصدرا ظاهرا لسطوع اقل (شكل 7)



شكل 7 معالجة جوانب النوافذ [27]

3. المناطق الحارة الرطبة: تكون المباني في هذه المناطق ذات هيكل بنائي خفيف وبفتحات واسعة لتأمين التهوية والحركة الكافية للهواء ومظلات كبيرة. يتم إقصاء ضوء الشمس المباشر للأسباب الحرارية، وتكون السماء ناصعة بحيث تسمح بالقدر الكافي من الضوء ولكن نسبة للسطوع العالي فان ذلك يسبب الوهج، ويمكن حماية المباني من الوهج باستعمال المظلات والأشجار.

4-5 التكامل التصميمي بين الإضاءة الطبيعية والإنارة الصناعية

تسمح النوافذ ذات المساحات الكبيرة بإنارة طبيعية أكثر ولكنها في نفس الوقت تؤدي إلى زيادة في الاكتساب أو الفقدان الحراري مما يؤدي إلى زيادة في أحمال التدفئة أو التبريد و نتيجة لذلك زيادة في استهلاك الطاقة الكهربائية. و يمكن الحل التصميمي الأمثل بتصميم نوافذ بحيث يكون هناك أوازن بين متطلبات الإنارة الطبيعية و الأحمال الحرارية مما يؤدي إلى استهلاك أقل للطاقة الكهربائية في المبنى.

أظهرت الدراسات انخفاض واضح في الطاقة المستهلكة بواسطة الإنارة الصناعية بحوالي 35% وكذلك انخفض إجمالي استهلاك الطاقة الكهربائية بحوالي 13% عند دمج الإنارة الطبيعية مع الصناعية في المباني. هذا بالإضافة أن أحمال التبريد في المبنى انخفضت مما يؤدي إلى استخدام نظام تكييف للهواء بحجم أصغر. وخلال هذه البحوث أجريت دراسة تحليلية لعناصر تصميم النافذة المختلفة لغرض التعرف على تأثير هذه العناصر على الأداء الحراري و استهلاك الطاقة في المباني الإدارية عندما يكون هناك دمج و استغلال للإنارة الطبيعية. و شملت الدراسات التحليلية نسب متعددة لمساحة النافذة إلى الجدار و ارتفاعات متعددة للنافذة و أنواع مختلفة للزجاج. و كنتيجة تم تطوير أداة تصميمية مساعدة الهدف منها مساعدة المصممين على اختيار و تصميم نوافذ متوافقة مع متطلبات الإنارة الطبيعية و بأقل استهلاك للطاقة الكهربائية في المبنى أو الفراغ^[31].

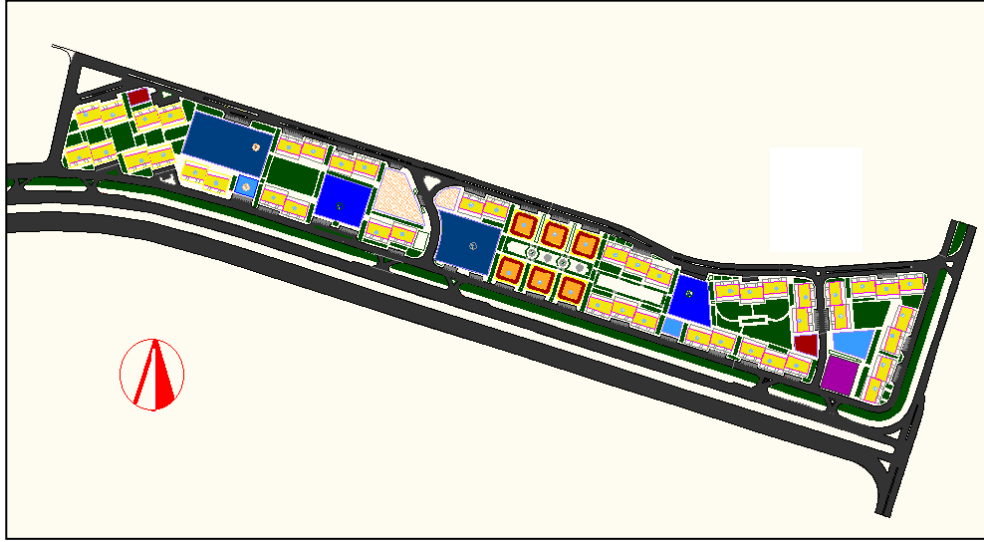
5-5 اللون:

تعتبر الألوان من العوامل المساعد على تحقيق فضاءات معمارية مضاءة بصورة مريحة ومبهجة حيث انه يمكن اختيار الألوان الفاتحة للسقوف من أجل زيادة قيمة الضوء المنعكس وتحسين الضوء المنتشر بشكل متساوي^[32] ، ويعد لون الضوء ذاته من المتغيرات المهمة في القرار الضوئي، إذ لألوان الإضاءة الدافئة والباردة تأثير في ردود أفعال معينة كالانشراح أو خلق مزاج أو جو خاص بالفضاء الداخلي. ويمكن أن تعمل المرشحات على طرح أجزاء مختارة من الطيف الضوئي بعيدا عن مصادر الضوء الطبيعي^[33]، ويعتمد مؤشر القيمة للون (Value) كمقياس لعلاقة اللون مع الإضاءة فهو يمثل مقياس السطوع للون ويتعلق بالشدة الضوئية للون أي أن كمية اللون الأبيض يعكس الشعاع الضوئي 100% تقريبا أو اللون الأسود الذي لا يعكس من الشعاع الضوئي شيئا لذلك كلما كان اللون ساطعا أكثر كلما كانت قيمته أكبر ويصدر ضوءا أكثر، كما أن أقسام الجدار الموجودة بين النوافذ تبدو في الغالب معتمة بالنسبة للنوافذ المضئية لذلك يتوجب طلاء الجدران الحاوية على نوافذ بألوان فاتحة أكثر من بقية الجدران في الغرفة، كما إن اختيار الألوان المستخدمة في طلاء الفضاء يجب لها أن تتناسب مع وظيفة كل فراغ، بحيث تؤدي دورا في تحقيق التأثير المطلوب في سلوك ومزاج الناس الذين يوجدون في تلك الفراغات، ويمكن أن نلاحظ بعض التأثيرات النفسية للألوان في الفراغات الداخلية حيث يحتفظ اللون الأحمر بخاصية انه يحاكي الرغبة، لذا من المفيد استعماله في غرف الطعام، أما لونا الأزرق والأخضر، فأنهما يمدان الإنسان بالشعور بالهدوء والراحة النسبية وحب الحياة، فهما ألوان الطبيعة لذلك من المناسب استعمالهما في غرف النوم. ومن جهة أخرى فاللونان الأصفر والبرتقالي، هما لونا الطاقة لذا من المفيد توظيفهما في

الفراغات الأكثر حيوية، مثل غرف المعيشة، ومكان تحضير الطعام ، كما يقوم اللون البرتقالي الفاتح لدور الحافز الايجابي ،وبذلك يمكن أن يكون اللون المسيطر في غرفة المكتب^[34].

6-5 وظيفة الفضاء المعماري: تختلف حاجة الفضاءات المعمارية إلى الإضاءة الطبيعية وكميتها حسب طبيعة فعالية الفضاء ففي المتاحف مثلاً يجب الاستفادة من الإضاءة الطبيعية قدر الإمكان والتي لا تسبب وهج داخل المتحف بالاعتماد على تشكيل فتحات السقف والفتحات العالية في الحوائط التي يجب ألا تقل زاوية ميل أشعة الضوء لها عن 45°، ويتم عكس الضوء بواسطة مرايا في الأركان. ويفضل استخدام الكاسرات الزجاجية والستائر والأبواب للتحكم في الضوء، في حين تعتمد الفضاءات التعليمية نوافذ مباشرة تسمح بتوزيع الإضاءة الطبيعية توزيعاً متجانساً يمنع الوهج والضوء المنعكس، أما بالنسبة للفعاليات السكنية فيمكن ان تتسلسل أهمية الإضاءة الطبيعية وكميتها بالنسبة للفعاليات داخل الفضاءات بالترتيب كما يأتي: (الفضاءات الاشغالية) فضاءات المعيشة، المكتب، النوم، المطبخ، الحمام ودورات المياه، (الفضاءات الانتقالية) ، المدخل، الممرات، الدرج.

6- عينات الدراسة العملية:



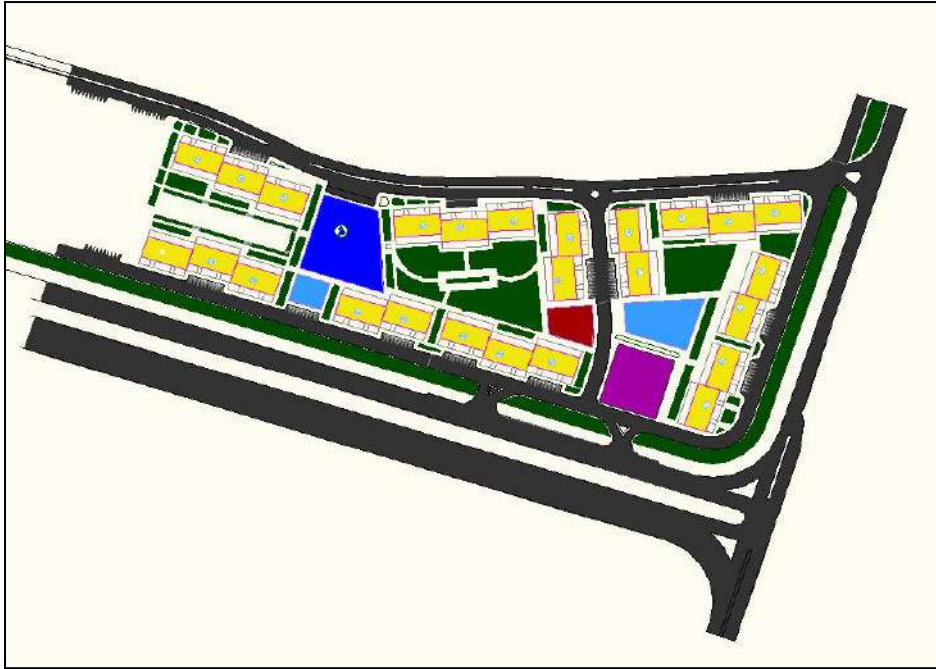
مخطط 1 الموقع العام للمجمع السكني - شمال هنانو [28]

لمحة عن المناخ في مدينة حلب: تقع مدينة حلب في شمال سوريا على خط طول 37 درجة وخط

عرض 36 درجة وتحصل المدينة على حوالي 75% ساعات إضاءة طبيعية على مدار العام وتعتبر مدينة حلب من المناطق الحارة الجافة، يعاني مناخ المدينة من الفروقات المناخية الكبيرة بين الليل والنهار وعلى مدار السنة ويؤدي تكدس البلوكات السكنية وتلاصقها مع بعضها البعض في مدينة حلب وزيادة المساحات الزفتية على رفع درجة الحرارة صيفاً بالمقارنة مع المحيط المجاور بسبب الإشعاع الشمسي المنعكس والتمشيت الذي يساعد في عملية الإضاءة لكنه في نفس الوقت ،وهكذا نجد بأن درجة الحرارة في أجواء مدينة حلب تزيد صيفاً/3 درجات مئوية عن حرارة الأجواء الخارجية .

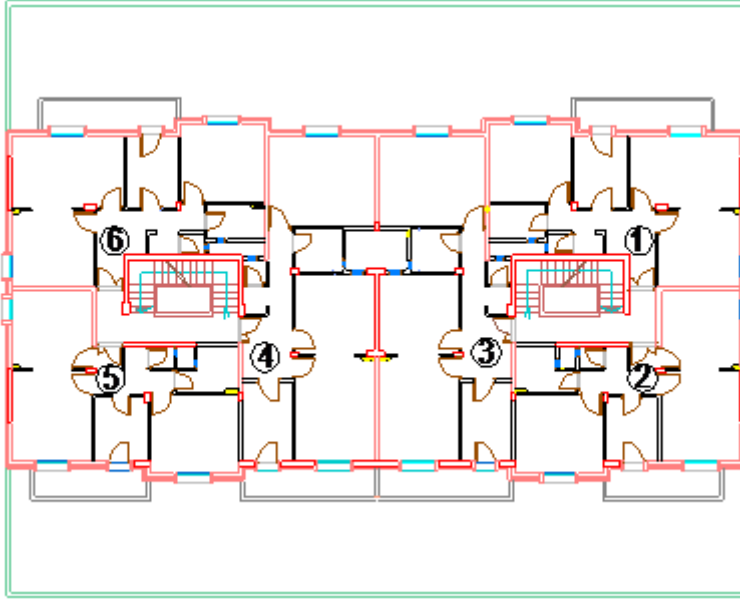
لمتطلبات أهداف البحث تم انتخاب مجموعة من المباني السكنية في مدينة حلب لغرض اختبار ووصف فعالية وكفاءة أداء الإضاءة الطبيعية فيها وشملت الأتي:

- مجمع المباني السكنية في منطقة شمال هنانو - مدينة حلب
- مجمع المباني السكنية في منطقة حلب الجديدة جمعية المهندسين- المشروع الحادي عشر



النموذج الأول: مجمع المباني السكنية في منطقة شمال هنانو التابعة لمدينة حلب
مخطط 2 تجمع لعدد من البلوكات السكنية حول ساحات داخلية [28]
النظام العمراني المعتمد هو سكن جماعي متصل: ويبلغ عدد مقاسمه 45 مقسم بوجائب من الجهة الأمامية
والخلفية 5م وجيبة أمامية (على الواجهة الرئيسية) و5م وجيبة خلفية





مخطط 1 مسقط الطابق المتكرر للبلوكة السكنية

صورة رقم 2 واجهة احد
الأبنية في الحالة الدراسية 1

مساحة المقسم 2م815
والمساحة المبنية 2م505، تتوضع
البلوكات بشكل يحاول أن يخلق
ساحات داخلية، من حيث لا تتوضع
بلوكة أمام أخرى، إلا في بعض الحالات،
ويبقى الوصول إليها من المحيط وليس
من الساحة الداخلية.

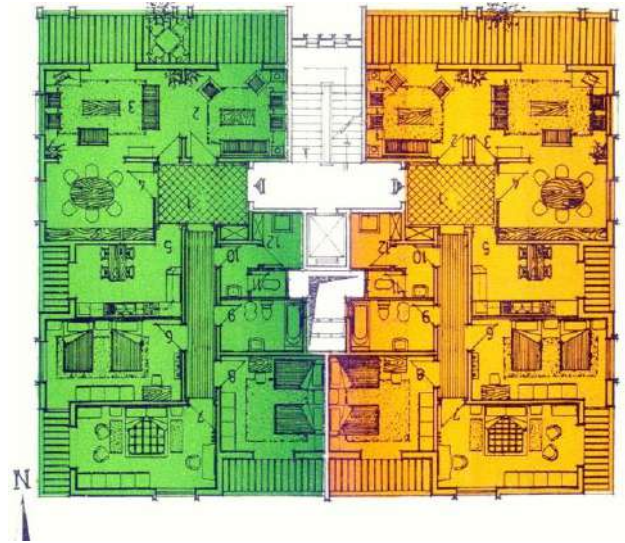
النموذج الثاني: مجمع المباني السكنية في منطقة حلب الجديدة - جمعية المهندسين النظام العمراني المعتمد
هوسكن جماعي منفصل: ويبلغ عدد مقاسمه 136 مقسم بجنايب من الجهة الأمامية والخلفية وجيبة أمامية (على
الواجهة الرئيسية) ووجيبة خلفية، تتوزع المقاسم باتجاهين شمال - جنوب وشرق - غرب، المسافات البينية تحددها
الوجائب والساحات المخصصة للخدمات فقط.



مخطط 3 الموقع العام للمشروع الحادي عشر - جمعية المهندسين [35]



صورة رقم 3 منظور ل احد الأبنية في الحالة الدراسية 2[35]



مخطط 2 مسقط الطابق المتكرر[35]

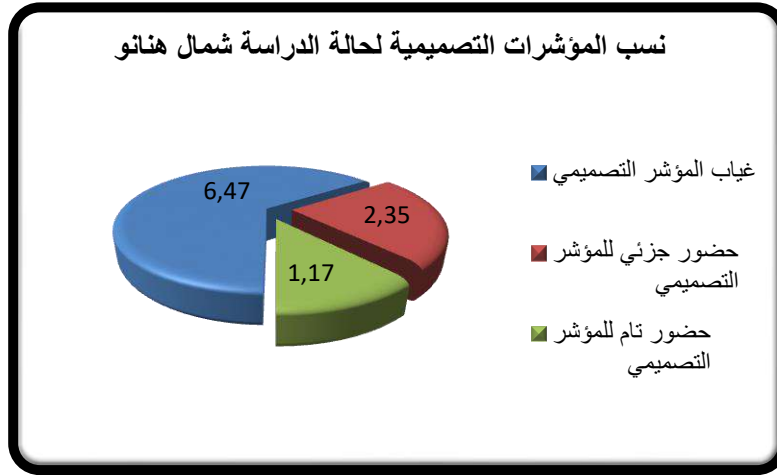
الحالة التطبيقية		المؤشرات التصميمية	
الموقع 2 حلب الجديدة	الموقع 1 شمال هنانو		
○	○	1 توجيه المبنى	
×	×	2 استخدام المناور للإغراض الإضاءة الطبيعية	
×	×	3 استخدام الفتحات السقفية	
×	×	4 استخدام الرفوف الضوئية	
×	×	5 توفير منافذ شمسية	
×	×	6 تصميم السقف الثانوي	
×	×	7 تصميم نوافذ عالية	
○	×	8 اعتماد نوافذ متصلة ومستمرة	
✓	✓	9 توفير نوافذ متحركة	
○	○	10 توقيع النوافذ بقرب القواطع الداخلية	
×	×	11 تصميم جوانب مائلة للنوافذ	
○	×	12 نوعية الزجاج	
✓	✓	13 استخدام الألوان الفاتحة في الفضاء المعماري	
✓	○	14 وظيفة الفضاءات الاشغالية بالعلاقة مع الإضاءة الطبيعية	
○	×	15 وظيفة الفضاءات الانتقالية بالعلاقة مع الإضاءة الطبيعية	
○	×	16 كفاية المسافات البينية (المسافات المتروكة بين الأبنية)	
✓	○	17 استخدام الشرفات	
✓ حضور تام للمؤشر التصميمي في المباني قيد الدراسة ○ حضور جزئي للمؤشر التصميمي في المباني قيد الدراسة × غياب المؤشر التصميمي في المباني قيد الدراسة			

جدول 2 تحليل المؤشرات التصميمية المنتخبة في الحالات الدراسية

تم اعتماد عدد من المؤشرات التصميمية والتي اختيرت اعتمادا على المعايير العمرانية المعتمدة في تصميم المجمعات السكنية في المدينة بعد مقارنتها مع آراء الساكنين والملاحظات التي قام الباحثين برصدها اثناء الزيارات الميدانية لمواقع الحالات التطبيقية موضوع الدراسة واعتماد الأكثر تكرارا فيها ثم عمل مقارنة لتطبيقها في تلك المواقع وحسب الجدول الموضح.

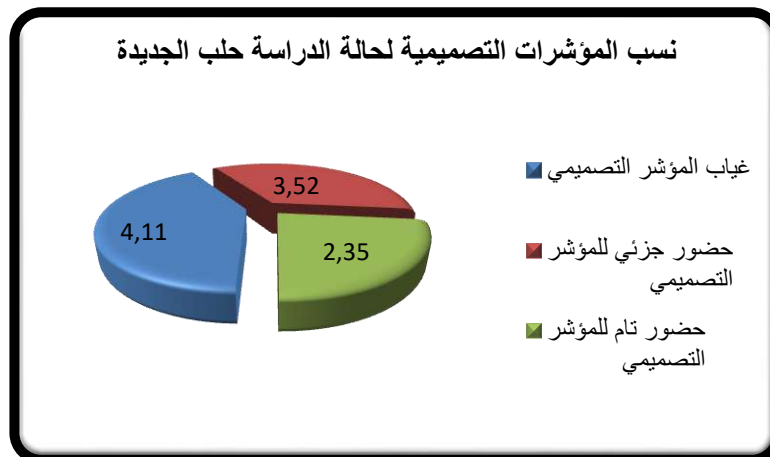
7- نتائج التطبيق: أظهرت نتائج التطبيق الآتي:

1- حضور تام لمؤشرات تصميمية تقليدية وشكلت نسبتها 11.7% في موقع هنانو و 23.5% في موقع حلب الجديدة: وجود النوافذ المتحركة واستخدام الألوان الفاتحة في طلاء الفضاء المعماري بالنسبة لنموذجي الدراسة وحصلت الفضاءات الاشغالية والانتقالية في النموذج الدراسي رقم 2- حلب الجديدة على إضاءة مناسبة ولوحظ فيها أيضا حضور تام للشرفات.



2- حضور جزئي وشكلت نسبتها 23.5% في موقع هنانو و 35.2% في موقع حلب الجديدة: مؤشرات التوجيه، توقيع النوافذ بالقرب من القواطع الداخلية بالنسبة لنموذجي الدراسة، كما وحصلت الفضاءات الاشغالية في النموذج الدراسي رقم 1 –شمال هنانو على حضور جزئي للإضاءة الطبيعية واستخدام الشرفات. واستخدمت بعض النوعيات غير التقليدية من الزجاج بشكل جزئي في بعض مباني النموذج الدراسي رقم 2 – حلب الجديدة

3- غياب المؤشرات التصميمية: استخدام الفتحات السقفية، استخدام الرفوف الضوئية، توفير منافذ شمسية، تصميم السقف الثانوي، تصميم نوافذ عالية، تصميم جوانب مائلة لحافات النوافذ بالنسبة لنموذجي الدراسة، ولم يلاحظ في النموذج الدراسي رقم 1 – شمال هنانو أي استخدام لنوعية زجاج مغايرة، ولا اعتماد نوافذ متصلة ومستمرة.



8- الاستنتاجات النهائية

- التصميم المعماري:

- 1- لم يراعي التصميم المعماري بشكل كافي تطبيق المؤشرات التصميمية الخاصة بمصدر الإضاءة الطبيعية التي رصدها البحث: استخدام الفتحات السقفية، استخدام الرفوف الضوئية، توفير منافذ شمسية، تصميم السقف الثانوي، تصميم نوافذ عالية، تصميم جوانب مائلة لحافات النوافذ.
- 2- النماذج قيد الدراسة أخذت بالاعتبار بعض المؤشرات التصميمية ومنها التوجيه، توقيت النوافذ بالقرب من القواطع الداخلية، وحصول بعض الفضاءات الاشغالية على الإضاءة الطبيعية واستخدام الشرفات. واستخدام بعض النوعيات غير التقليدية من الزجاج بشكل جزئي

- التخطيط العمراني:

- 1- اظهر المخطط العمراني ضعف في توزيع الكتل البنائية للعمارات السكنية وخلل في تقدير المسافات البنائية بالعلاقة مع متطلبات الإضاءة الطبيعية.
- 2- أظهرت الدراسة ضعف وقصور في ضوابط البناء والتنظيم العمراني لمعطيات الاستدامة فيما

9- التوصيات

- 1- إن الطاقة الشمسية كمصدر رئيسي للإضاءة يمكن لها إن تشكل قاعدة مهمة من القواعد الاقتصادية في عملية التنمية العمرانية المستدامة، لما توفره من وفر في الطاقة المستخدمة في حال تم توظيفها تصميميا وعمرانيا بصورة صحيحة ، الأمر الذي يستلزم تطوير تقنيات توظيفها المختلفة، والبدء في الإحلال التدريجي لهذه التقنيات بدلا من الأساليب التقليدية الحالية.
- 2- اعتماد معايير تصميمية للأبنية المستدامة لحصد الطاقة الشمسية لغرض توفير أقصى إضاءة طبيعية.
- 3- إعداد دراسات تطويرية للأبنية قيد الدراسة للإغراض معالجة الأخطاء التصميمية التي رصدها البحث، قدر الإمكان.
- 4- التأكيد على تخطيط عمراني متكامل يأخذ بالاعتبار عوامل الإضاءة الطبيعية والتوجيه الشمسي وإيجاد مناوور ومداخل شمسية ومسافات بينية كافية لتوفير أقصى إضاءة.
- 5- إن التقنيات السالبة للطاقة الشمسية لا تمثل أية مشكلة في الاستخدام سواء على مستوى العمارة أو التخطيط، فقد ثبت نجاحها في تحقيق الراحة الحرارية للإنسان قبل التوصل إلى الوسائل الميكانيكية، وبأني الدور على المعماريين والمخططين لاستغلال هذه الإمكانيات الطبيعية واستنباط الحلول الملائمة للعصر ولطبيعة المجتمع والموقع، حتى يتحقق الترشيح في استخدام الطاقة الكهربائية والميكانيكية تمهيدا للاستغناء تماما عن الأجهزة التي تعمل بأنواع الطاقة التقليدية وخصوصا في التبريد والتدفئة
- 6- عدم استخدام النوافذ الملونة التي تعطي جوا ضبابيا داخليا والتي هي غالبا الألوان البرونزية.
- 7- المشاركة مع نظام تحكم فعال من أجل الحصول على تخفيض في كلف تشغيل الإنارة الاصطناعية.
- 8- إعادة النظر في ضوابط البناء والتشريعات العمرانية ذات العلاقة بمسألة الإضاءة الطبيعية وتطبيق مؤشرات التصميمية.

المصادر

1. فكري أحمد وأحمد وآخرون، 2007، **العلاقة التكاملية بين المباني والخلايا الفوتوفولتية**، مؤتمر الهندسة البيئية، كلية الهندسة، جامعة عين شمس، القاهرة 10-12 نيسان، ص 20
2. حسن نوبي محمد والإكيابي محمود عبد الهادي، 1999، **استخدام تقنيات الطاقة الشمسية في المدن الجديدة قاعدة اقتصادية في عملية التنمية العمرانية المستدامة**، -بحث منشور في ندوة المدن الجديدة ودورها في التنمية المستدامة 24-27 نوفمبر، أغادير، المملكة المغربية، ص3، 4،
3. حسن، مصدر سابق ص4
4. شعبان، رزق نمر، 1996، **الإضاءة النهارية والصناعية في العمارة**، المركز العربي للخدمات الطلابية، عمان، الأردن، ص32
5. وزير، يحيى، 2003، **التصميم المعماري الصديقة للبيئة**، نحو عمارة خضراء، مكتبة مدبولي، القاهرة، ص118.
6. يوسف وجيه فوزي، 1982، **العمارة وحياة الإنسان**، مجلة المهندسين، العدد 324، القاهرة، ص3
7. يوسف، مصدر سابق ص36.
8. Kime, Bukar Ali . 2018. "**Comparative Analysis of Day lighting and Artificial Lighting in Library Building; Analyzing the Energy Usage of the Library.**" International Journal of Engineering and Technology 5 (12). P. 7665
9. الزعفراني، مصدر سابق ص 24
10. Kaheneko, Omary. 2021. "**Research on Application of Natural Light in Modern Architecture Design.**" The International Journal of Science & Technoledge 9(2) ,p.44
11. Al-Ashwal, Najib T. , and Ahmad Sanusi Hassan. 2018. "**The Impact Of Daylighting-Artificial Lighting Integration On Building Occupants' Health And Performance.**" International Transaction Journal of Engineering, Management, & Applied Sciences & Technologies .P.98
12. عثمان، لينا، 2008، **اثر مبادئ العمارة المستدامة وهندسة القيمة في تخفيض كلفة تشغيل المبني**، رسالة ماجستير، كلية الهندسة المعمارية، جامعة حلب، ص65
13. يوسف، مصدر سابق ص39.
14. وزير، مصدر سابق ص 126
15. عثمان، مصدر سابق ص66
16. Yudelson, Jerry, 2007, **Green Building A to Z**, New Society Publisher, p62,112.
17. Smith, Carl, 2008, **Residential Landscape Sustainability**, Blackwell Publishing Ltd., London, p37.
18. Birkeland, Janis, 2003, **Design for Sustainability**, Earth Scan Publications Ltd., London, p93.
19. Scott, Andrew, 2005, **Dimensions of Sustainability**, E&Spon, London, p180.
20. Crowther, 1992, Richard, **Ecologic Architecture**, Butterworth architecture, USA, p195.
21. عثمان، مصدر سابق ص 68
22. الزعفراني د. عباس محمد وفكري أحمد أحمد، ٢٠٠٦، **الزجاج ذو النفاذية الاختيارية للإشعاع الشمسي مدخل للتصميم البيئي للفتحات الخارجية في المباني** مؤتمر قسم الهندسة المعمارية، كلية الهندسة – جامعة القاهرة، ص ص 3-2
23. عثمان، مصدر سابق ص 67
24. Yudelson, ibid p112.
25. Crowther, ibid p195.
26. Scott, ibid p180.

27. الشيباني، خالد عسكر، 2002، العلاقة الهندسية بيل النافذة ومساحة الغرفة، المجلة العلمية لجامعة الملك فيصل، العدد الأول مارس 2003
28. الزعفراني، مصدر سابق ص 24
29. الزعفراني، مصدر سابق ص 26
30. إبراهيم، د. حاتم جلال عبد العظيم، (2006) محاضرات الإضاءة، كلية الهندسة ، جامعة القاهرة
31. Yudelson, ibid p116
32. عثمان, مصدر سابق ص 68
33. العكام اكرم جاسم محمد و الهاشمي شيماء نبيل نور ، ٢٠٠٧ ، التشكيلات الفنية للإضاءة الداخلية لبهو الفنادق، مجلة الهندسة والتكنولوجيا ، المجلد ٢٥ ، العدد ٣، ص 116.
34. عثمان, مصدر سابق ص 69
35. دبس وزيت حسام وعبد الرزاق معاد ، البعد الوظيفي والجمالي للألوان في التصميم الداخلي المعاصر، 2008، مجلة جامعة دمشق للعلوم الهندسية مجلد 24 عدد 2، ص 353



**POTENTIAL BENEFITS OF SUSTAINABLE MANAGEMENT IN
ENGINEERING PROJECTS**

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POTENTIAL BENEFITS OF SUSTAINABLE MANAGEMENT IN ENGINEERING PROJECTS

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Abstract:

Sustainability in project management is one of the most prominent issues of the current era that must be paid attention to. In this research, the concept of sustainable project management and its importance in managing realistic projects is addressed, and it deals with the meaning of sustainability and sustainable management. This research discusses the potential benefits of implementing sustainability in sustainable project management during a lifespan. Project and management's role in construction practice, management processes, and comparison between the traditional method of construction management and the sustainable management of construction, particularly in the level of detail

This research aims to identify the common challenges faced during the management of sustainable projects and their impact on project performance, such as the high costs of sustainable building materials, technical difficulties during work and communication between team members. etc., and this research focuses on some aspects of sustainable practices in the construction phase management Which includes factors such as: site selection through feasibility assessment and sustainable on-site practices, materials selection through life cycle assessment, time, cost and resource controls, quality management, machinery and transportation management, waste management, durability and stability, correct occupancy and based designs. Innovative ideas and the role of Building Information Modeling (BIM) as a design and visualization tool in project management and the possibility of improving performance and thus building efficiency significantly to achieve maximum benefit. The research indicates that the task of constructing a sustainable building is not fully accomplished until the concept of sustainability is integrated into each step of the life cycle of the project in general and in all the practices of the implementation phase in particular, and the process is not limited to the construction of a building with sustainable materials only.

The lack of investment in sustainable project management is the most important and impactful barrier. To overcome these barriers, a project management framework must be developed, and the adoption of sustainable project management approaches to the future must be promoted.

Key words: Sustainability, Project Management, Sustainable Project Management, Building Information Modeling (BIM).



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الفوائد المحتملة في إرادة المشاريع المستدامة

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الملخص:

تعد الاستدامة في إرادة المشاريع من أبرز قضايا العصر الحالي التي يجب الألتفات إليها، في هذا البحث يتم تناول مفهوم الإرادة المستدامة للمشاريع وأهميتها في إرادة المشاريع الواقعية، ويتناول معنى الاستدامة والإرادة المستدامة و يناقش هذا البحث الفوائد المحتملة لتنفيذ الاستدامة في إرادة المشاريع المستدامة خلال فترة حياة المشروع و دور الإرادة في ممارسة البناء، وعمليات الإرادة، والمقارنة بين الطريقة التقليدية لإرادة البناء والإرادة المستدامة للبناء لا سيما في مستوى التفاصيل.

يهدف هذا البحث إلى تحديد التحديات المشتركة التي يواجهها أثناء إرادة المشاريع المستدامة وتأثيرها على أداء المشروع مثل التكاليف المرتفعة لمواد البناء المستدامة، الصعوبات الفنية أثناء العمل و التواصل بين اعضاء الفريق .. الخ، و يركز هذا البحث على بعض جوانب الممارسات المستدامة في إرادة مرحلة البناء والتي تشمل عوامل مثل: اختيار الموقع من خلال تقييم الجدوى و الممارسات المستدامة في الموقع، اختيار المواد من خلال تقييم دورة الحياة، الوقت والتكلفة وضوابط الموارد، إرادة الجودة، إرادة الآليات و النقل، إرادة النفايات، المتانة والاستقرار، صحة الإشغال و التصميم القائم على الأفكار المبتكرة و دور نمذجة معلومات البناء BIM كأداة تصميم و تصور في إرادة المشاريع وعن إمكانية تحسين الأداء وبالتالي كفاءة المبنى بشكل كبير لتحقيق أقصى فائدة. و يشير البحث إلى أن مهمة تشييد مبنى مستدام لا يتم إنجازها بالكامل إلا عندما يتم دمج مفهوم الاستدامة في كل خطوة من دورة حياة المشروع ب شكل عام و في كل ممارسات مرحلة التنفيذ بشكل خاص ولا تقتصر العملية على تشييد مبنى بمواد مستدامة فقط . و يعد نقص الاستثمار في إرادة المشاريع المستدامة هو الحاجز الأكثر أهمية وتأثير. وللتغلب على هذه الحواجز يجب تطوير إطار عمل لإرادة المشروع، و تعزيز اعتماد نهج الإرادة المستدامة للمشاريع في المستقبل.

الكلمات المفتاحية: الاستدامة، إرادة المشاريع، الإرادة المستدامة للمشاريع، نمذجة معلومات البناء BIM.

المقدمة:

أصبحت الاستدامة مهمة لأنجاز مشاريع البناء وفقاً للأهتمام المتزايد من قبل الحكومات والمؤسسات غير الحكومية وكذلك من عامة الناس و ذلك لضرورتها القصوى لان العالم يتغير بسرعة، وإذا لم نبدأ في اتخاذ الإجراءات، فقد ينتهي بنا الأمر إلى التأثير ليس فقط على حياة أجيالنا المستقبلية ولكن أيضاً على نظام الكوكب بأكمله، فأصبحت الحكومات تتخذ تدابير قانونية للسيطرة على التلوث والحفاظ على الموارد وحماية النظم البيئية الطبيعية.

و ركزت الابحاث بشكل أساسي على جوانب التصميم للمبنى من ناحية قدرته على استخدام الموارد الطبيعية بكفاءة مثل ضوء الشمس و الماء و استخدام مواد بناء صديقة للبيئة غير ضارة و لها قابلية على اعادة التدوير .. الخ و مع ذلك لا يشير البناء المستدام إلى تصميم المبنى فحسب بل يشير أيضاً إلى مراحل البناء و الاستخدام و الصيانة و الهدم و من هنا يمكن ان يعرف المبنى المستدام على أنه منشأ مصمم تم بناؤه وتشغيله و هدمه بطريقة موفرة للموارد باستخدام نهج بيئي سليم و هو استجابة إلى التغيرات السلبية السريعة في بيئة الأرض وأنظمتها البيئية. هناك أدلة متزايدة على التدمير المتسارع للنظم البيئية و الزيادةات الهائلة في عدد السكان والاستهلاك. إضافة إلى المشاكل التي تسبب بها الإنسان مثل تغير المناخ وإزالة الغابات والتصحر.

في هذا البحث، تم تسليط الضوء على الاستدامة في إرادة المشاريع، وتناقش سياسات الاستدامة في إرادة المشاريع، الطرق المحددة والمستقبلية الممكنة للاستدامة في المشاريع والتحديات المشتركة التي يواجهها مديرو

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المشاريع أثناء إرادة مشاريع تشييد المستدامة و اقتراح بعض الحلول المعقولة لتحسين إرادة مشروع البناء المستدام .

مشكلة البحث:

تكمن مشكلة البحث في التساؤل الآتي

هل للأستدامة في إرادة المشاريع دور في المحافظة على النظم البيئية، و هل بإمكانها مراعاة متطلبات المستقبل

فرضية البحث:

تكمن فرضية البحث في ان تحقيق الاستدامة في إرادة المشاريع بما يتناسب مع متطلبات البيئة المستقبلية .

أهداف البحث:

- 1 - الهدف الرئيسي من الاستدامة هو مراعاة المتطلبات المستقبلية و تلبية احتياجات السكان الحالية وحماية البيئة والموارد للمستقبل .
- 2- الدافع الرئيسي وراء حركة المباني المستدامة عالية الاداء هو نموذج التنمية المستدامة و الذي لا يغير الهياكل المادية فحسب بل يغير أيضاً طريقة عمل الشركات و المؤسسات التي تعمل على تنفيذ تلك المباني .
- 3- اختيار التدابير اللازمة لإرادة المشاريع المستدامة.

المبحث الأول: التأصيل المفاهيمي و الإطار النظري

أولاً: مفهوم الاستدامة

مصطلح "الاستدامة" ليس جديداً في الواقع، إن الاستدامة بدأت مع بداية الحضارة الإنسانية. يذكر الصندوق العالمي للطبيعة أن الاهتمام الكبير بالاستدامة والاستخدام الواسع للمصطلح لم يبدأ في الانتشار حتى منتصف القرن العشرين حيث بدأت الحركة البيئية و أوضح أن هناك تكاليف بيئية مرتبطة بإنتاج واستخدام المواد التي يتمتع بها الناس في هذه الأثناء، يتم تعريف مفهوم التنمية المستدامة التي تعتبر المظلة الشاملة المرتبطة باستدامة الإنسان على كوكب الأرض على أنها "التنمية التي تلبى احتياجات الحاضر دون المساس بقدرة الأجيال القادمة على تلبية احتياجاتهم الخاصة" (الجمعية العامة للأمم المتحدة، 1987) و سلط الضوء على بعض القضايا لمحاولة معالجتها من خلال دمج التنمية المستدامة في مختلف القطاعات الحكومية والمستويات التنظيمية والعامه. (Kahachi, 2017, p. 88)

ثانياً: الاستدامة و صناعة البناء

تركز الاستدامة على الرؤية طويلة المدى و تسعى إلى دمج الجوانب الاجتماعية والبيئية والاقتصادية للمشروع رغم ذلك فإن تكامل الجوانب الاقتصادية والبيئية والاجتماعية في إرادة المشاريع لا يمثل نهجاً شاملاً للاستدامة في إرادة المشروع، أن ملائمة نهج المحصلة الثلاثية يرتبط بشكل أساسي باستراتيجية ومنظور المشروع، والتي قد تختلف من مشروع إلى آخر لأن كل مشروع هو حدث فردي غير قابل للتكرار

يمكن وصف مفهوم الاستدامة على انه محاولة لضمان تحقيق النمو الاقتصادي مع الحد من الاستغلال غير المعقول للموارد الطبيعية و الاخلال بالأنظمة البيئية القائمة .

و يشمل مفهوم الاستدامة تقييم جميع التكاليف و الفوائد المتعلقة بالأنشطة الاقتصادية بما في ذلك التخصيص الفعال للموارد و تحسين نوعية الحياة، الاستدامة مهمة لاستمرارية دائرة الحياة في بيئتنا الطبيعية و مهمة للحفاظ على مصادر المياه و الموارد الطبيعية فلا يمكن ان نتجاهل المعايير البيئية و الاقتصادية و الاجتماعية الاستدامة عند ممارستنا لعمليات البناء و التشييد و التي هي من اكبر مسببات الأضرار على البيئة مثل التغير المناخي الناتج عن الغلاف الجوي' الاحتباس الحراري و انبعاثات CO2 و غيرها من المشاكل البيئية

تلعب صناعة البناء دوراً رئيسياً في وضع مفهوم الاستدامة موضع التنفيذ كونها المستهلك الرئيسي للموارد الطبيعية و ملوثة للبيئة و يمكن ارجاع ما يقارب (Kibert, 2016, p. 46)

14% من الاستهلاك العالمي للمياه الصالحة للشرب

30% من المخلفات و هدر النفايات

38% من انبعاثات ال CO 2

40% من استهلاك المواد الخام

37% من استخدام الطاقة

72% من استهلاك الكهرباء

فإن تأثير مشاريع البناء على البيئة ينشأ من مواد البناء و طبيعة التصميم و طرق البناء و الموقع و التخطيط و كذلك من استهلاك الطاقة و من الصناعات المتعلقة بالبناء مثل:

- إنتاج مواد مثل الاسمنت و الالمنيوم و البلاستيك
- حركة المعدات و المواد بين المواقع
- تشغيل المعدات في الموقع أثناء العمل و بعد اكتمال المبني

يمكن تصنيف الاثار السلبية لمشاريع البناء على النحو التالي:

- تدهور الموارد و استنزاف موارد الغابات عن طريق استخدام الخشب و استخراج الرمل و الطين و الرواسب الأخرى مثل الحجر الجيري، استخدام الطاقة في إنتاج و نقل هذه المواد إلى موقع العمل

- الاضطراب المادي للنظم البيئية و التغيرات المناخية الطويلة المدى، تحويل المجاري المائية الطبيعية بسبب السدود و فقدان النباتات و الحيوانات و اضطراب التوازن البيئي
- التلوث الضوضائي الناتج عن المباني في المناطق الحضرية .

ثالثاً: دور إرادة المشروع في تحقيق الاستدامة

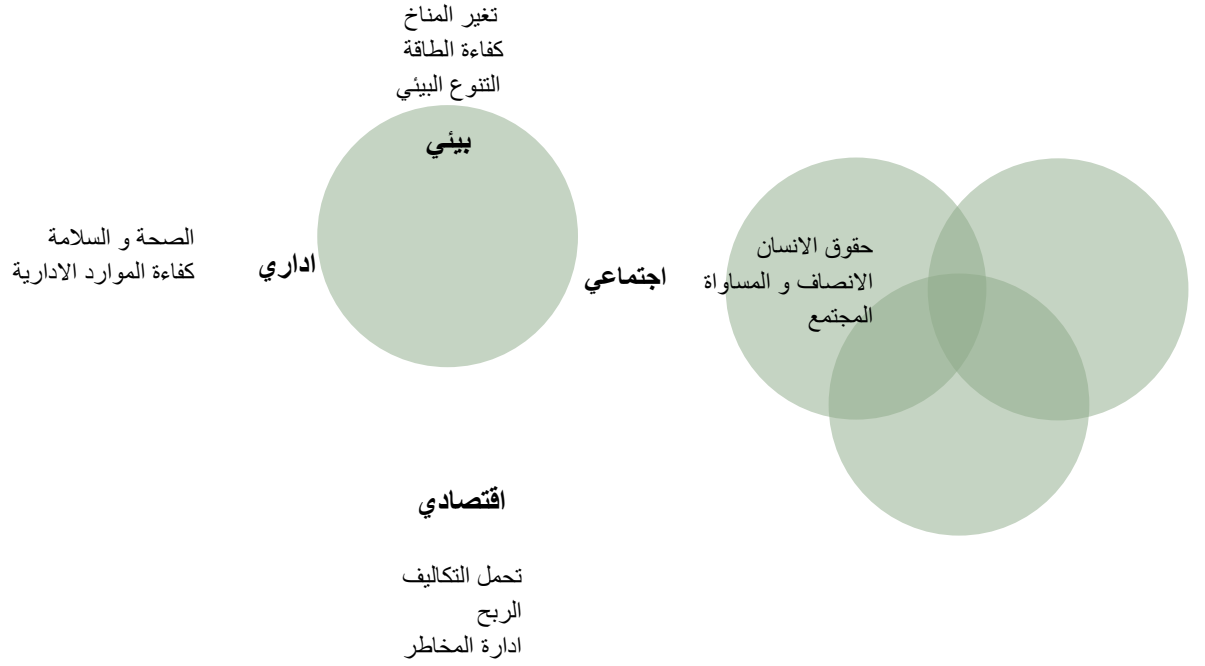
تطورت ممارسة إرادة المشاريع بسرعة على مر السنين منذ أن تم تقديمها رسمياً في عام 1957. وقد تطورت لتصبح عنصرًا حيويًا في أدوات تطوير المشروع . تعتبر ممارسات إرادة المشاريع الصحيحة ذات أهمية كبيرة لنجاح المشروع . (Abdul Aziz Abdullah, 2021) يظهر دورالشركات والمؤسسات في اتخاذ بعض الخطوات الجادة نحو تحقيق التنمية المستدامة، أن الشركات مسؤولة من خلال ممارساتها عن غالبية انبعاثات ثاني أكسيد الكربون و الاستهلاك المفرط لمصادر الموارد الطبيعية والطاقة، و من خلال تعزيز أداء هذه الشركات من حيث الاستدامة، من الممكن تحقيق خفض كبير في انبعاثات ثاني أكسيد الكربون، وآثار تغير المناخ واتخاذ خطوات كبيرة نحو التنمية المستدامة ومع ذلك، فإن تحقيق الاستدامة على مستوى الشركات ليس بالمهمة السهلة لأن معظم هذه الشركات والشركات موجهة نحو الربح، لذلك بدأ مفهوم تحقيق الاستدامة من خلال إرادة المشاريع في الانتشار في العقدين الماضيين مع تسليط الضوء على عدد من الفوائد للشركات من خلال "إرادة المشاريع المستدامة . (Armenia, 2019, p. 9)

1- فوائد تطبيق الاستدامة في إرادة المشاريع:

تشمل فوائد دمج الاستدامة في إرادة المشروع بعدة طرق، يمكن أن يساعد على زيادة قيمة المنظمة، وخلق الفرص، والقضاء على أو تقليل المخاطر، وزيادة الربح وتقليل التكلفة. يمكن للاستدامة أن تخلق فرصًا جديدة وتزيد من جودة الأداء وتوفر وصولاً أفضل إلى أسواق معينة وتميز منتجات الشركة عن المنافسين الآخرين مما يمنحها مزايا تنافسية وتزيد من البيع. الناس سوف تجذبهم العلامة التجارية للشركة في السوق وممارسات الاستدامة الخاصة بها. وإعادة تقدير التكلفة لأنها تساعد في تعزيز العلاقة داخل سلسلة التوريد. سيكون الموردون والهيئات التنظيمية وأصحاب المصلحة الخارجيون داعمين للشركة، بالإضافة إلى الحد من استخدام الطاقة والمواد بسبب الاستخدام الأكثر ابتكارًا واستدامة لها، مما يقلل من التكلفة الإجمالية، ينطبق هذا أيضاً على تكلفة العمالة ورأس المال الذي يمكن أيضاً تخفيضه، حيث تتكامل الشركة مع الاستدامة في ممارسات إرادة المشاريع الخاصة بها حيث تقل الحاجة إلى العمال ورأس المال

2-دوافع الاستدامة في إرادة المشاريع:

يعد مصطلح البناء المستدام في الأصل لتوضيح مسؤولية صناعة البناء لتحقيق الاستدامة ويُعرف للبناء المستدام على أنه " .. إنشاء بيئة مبنية صحية باستخدام مبادئ بيئية مستدامة تتسم بالكفاءة، قسم إلى أربع ركائز: اجتماعية، اقتصادية، بيئية و ادارية تسلط الاستدامة الاجتماعية الضوء على التحسينات في جودة حياة الإنسان، الانصاف والمساواة والمجتمع . تشمل استدامة الاقتصاد السلع والخدمات وتحقق مجرد استخدام مستدام. تخفيض تكاليف تشغيل المبنى، بعد تطبيق الحلول الحديثة سواء في مجال التصميم، المواد التطبيقية، الاستغلال، تخفيض تكاليف الهدم . تكشف الاستدامة البيئية عن الدافع الذي يحتاجه البناء المستدام لحماية البيئة الطبيعية بدلاً من تلويثها، ويشجع على استخدام متجدد مستدام ويقلل من استخدام المياه والطاقة والأراضي في كل مرحلة من مراحل المشروع، اي الحفاظ على الطبيعة بصورة رئيسة وسليمة من خلال خفض صرف الطاقة والمياه والمواد الخام وانبعاثات غازات الاحتباس الحراري وإنتاج النفايات تتطلب الاستدامة الفنية الأداء العالي والمتانة والجودة والاستخدام المختلط للمبنى . (Kahachi, 2017, p. 89)



الشكل (1) دوافع الاستدامة في إرادة المشاريع
(APM body of knowledge, 2019)

3- أهداف الاستدامة في إرادة المشاريع:

يعالج البناء المستدام بشكل شامل القضايا البيئية والاجتماعية والاقتصادية للمبنى في سياق مجتمعه.

جدول (1)

أهداف الاستدامة في إرادة المشاريع

موضوع الهدف	الفوائد الاقتصادية	الفوائد البيئية	الفوائد الاجتماعية
كفاءة استخدام الطاقة	انخفاض استهلاك الوقود و الكهرباء و انخفاض الطلب على الطاقة و بالتالي انخفاض التكاليف	انخفاض استهلاك الكهرباء، الوقود الأحفوري، تلوث للهواء و انبعاثات ثاني أكسيد الكربون	توفير البيئة المريحة للشاغلين مستقبلا
كفاءة استخدام المياه	خفض تكاليف المياه	الحفاظ على الموارد للأجيال القادمة و الاستخدامات الزراعية و الترفيهيه، تقليل عدد محطات معالجة المياه	انخفاض استخدام المياه الصالحة للشرب و تقليل التصريف في المجاري المائية و اجهاد اقل على النظم البيئية المائية من اجل الحياة البرية و الزراعية
المواد و الموارد	انخفاض التكلفة من خلال اعادة تدوير المواد و انخفاض تكاليف التخلص من النفايات	قلة مدافن النفايات، زيادة الاسواق المنتجة للمواد الصديقة للبيئة، انخفاض النقل و الطاقة بسبب استخدام مواد محلية و اقليمية	تقليل الضغط على مدافن النفايات، تقليل استخدام المواد، انخفاض التلوث

(الباحث)

- عوامل دمج الاستدامة في إرادة المشاريع:

هناك مجموعة من العوامل تساعد في دمج الاستدامة في إرادة المشاريع وهي كالآتي:

- دورة حياة المشروع: جميع المشاريع لها دورة حياة بغض النظر عن عدد المراحل التي يمكن تحديدها يرتبط توجيه الاستدامة بدورة حياة المشروع وله بعض القيود تجاه المشاريع قصيرة المدة، تمر دورة حياة المشروع بمراحلها بدرجة متغيرة من الشدة، في بداية المشروع تكون مستويات الموارد والتكاليف منخفضة نوعًا ما في المشروع، ثم يزداد الجهد تدريجياً حتى أقصى نقطة في مرحلة التنفيذ، في مرحلة الإنهاء يحدث انخفاض سريع لذلك يجب أن يتم تخطيط الموارد في وقت مبكر من دورة حياة المشروع وعبر مراحل المشروع تتكامل دورة حياة المشروع المستدام من خلال تطبيق مبادئ الاستدامة في كل مرحلة من دورة حياة . (Gareis, 2013, p. 3)
- الأخلاقيات في المشاريع: يجب إكمال المشروع مع مراعاة الحفاظ على القيم الأخلاقية والاجتماعية مثل النزاهة، والصدق، والمسؤولية، والاحترام.
- سياسات وإجراءات واضحة للمشاريع: تُظهر السياسات والإجراءات الواضحة للمشاريع في الشركة مدى انفتاح الشركة في صياغة سياساتها وإجراءاتها واتخاذ القرارات والإجراءات بشأن مختلف القضايا اليومية. علاوة على ذلك، فإنه يشير أيضاً إلى كيفية تأثير سياسات وقرارات الشركة على البيئة وتعزز تأثيرها على أصحاب المصلحة والطبيعة والمجتمع. يمكن دمج الاستدامة في إرادة المشروع من قبل الشركات مع سياساتها وإجراءاتها ومسؤوليتها الواضحة لتحقيق الاستدامة في إرادة المشاريع . (V.K. Chawla*, 2017, p. 158)
- مراعاة مصالح أصحاب المصلحة: تعتبر مشاركة أصحاب المصلحة في المشاريع أمراً مهماً من وجهة نظر الاستدامة والتواصل في إرادة المشروع ويؤدي كذلك إلى الإرادة الشاملة لأصحاب المصلحة يمكن لأصحاب المصلحة كشركاء تحديد المشاكل في المشروع ويمكنهم تطوير حلول للمشكلات المحددة، تنفيذ الحلول ومواصلة قياس التقدم المحرز في المشاريع . (Silvius & Schipper, 2014, p. 70)
- إرادة المخاطر بالنسبة للمشاريع المستدامة: إن المخاطر شائعة جداً في إرادة المشروع. يمكن أن تؤثر بعض الأحداث غير المؤكدة أو مجموعة الأحداث غير المؤكدة على العائد الإجمالي وأهداف أي مشروع لتجنب الأحداث غير المؤكدة أثناء تنفيذ المشروع يجب تحديد المخاطر ومعالجتها بشكل مناسب لتحقيق الإرادة المستدامة للمشروع كما أنه يساعد في الحد من استثمار الموارد، والحفاظ على المعايير في المشاريع وما إلى ذلك .
- التخلص من النفايات في المشاريع: من أجل الحصول على الموارد الكافية، فإن التخلص من النفايات أمر ضروري للغاية في كل عملية وأنشطة للإرادة المستدامة للمشروع. في مجال إرادة المشروع، يمكن تحديد النفايات في العمليات والأنشطة المختلفة على أنها تغيير غير ضروري في الخطط والمتطلبات، والموارد الزائدة وغير المستخدمة، والإفراط في تقدير الموارد وما إلى ذلك يمكن التخلص من أنشطة النفايات في مواقع مشاريع البناء مثلاً عن طريق التصنيع المسبق خارج الموقع، يتم تنفيذ الكتل الأسمنتية، وقضبان الحديد الملحومة، وخللائط الأسمنت بدلاً من القيام بالتصنيع في موقع البناء وغيرها من الممارسات التي تساعد على خلق فرص للاستدامة في إرادة المشروع من خلال تقليل الهدر والجودة العالية والسعر الأقل والاستخدام المناسب للموارد وتطبيق التكنولوجيا والمهارات المتقدمة والنهوض العام للاقتصاد والعديد من المزايا التي يمكن تحقيقها. (KHALFAN, 2006, p. 43)

المبحث الثاني: تحديات و سياسات و ممارسات الاستدامة في إرادة المشاريع

أولاً: تحديات الاستدامة في إرادة المشاريع

1 - التكاليف المرتفعة لممارسات و مواد البناء المستدام بالمقارنة مع المشاريع التقليدية:

حيث تتراوح تكاليف الرأسمالية للمشاريع المستدامة من 1 - 25 % اعلى من المشاريع التقليدية و ترجع هذه الزيادة إلى التعقيد في التصميم و تكاليف النمذجة اللازمة لدمج الممارسات المستدامة في المشاريع و ترتبط أيضاً هذه التكاليف المرتفعة بأسعار المواد و تقنيات البناء المستدام حيث ان تكاليف المواد المستدامة تكون اعلى بنسبة 3 % إلى 4 % من تكاليف المواد التقليدية و تؤثر هذه الزيادة في التكاليف بشكل مباشر على إرادة المشروع لانهم مسؤولون عن إرادة و تسليم المشروع ضمن ميزانية مخصصة . (Zhang, 2011, p. 160)

2- الصعوبة الفنية أثناء عملية البناء:

يقوم مدير المشروع بتنفيذ خطة المشروع من خلال التصريح بتنفيذ الانشطة لغرض تسليم المشروع بالوقت و الجودة و التكلفة المطلوبة و في كثير من الاحيان يتطلب المشروع المستدام تقنيات و عمليات بناء معقدة فأذا لم تتم معالجة التعقيدات بشكل جيد قد تؤثر على اداء مدير المشروع . (Hwang, 2012, p. 341)

3- المخاطر انواع العقود المختلفة لتسليم المشروع:

ان نجاح تطوير و تنفيذ اي تصميم مستدام يعتمد بشكل كبير على نوع العقد المختار لتسليم المشروع . يجب ان يتضمن العقد المستخدم في المشاريع المستدامة جميع التفاصيل الخاصة بالتصميم المتكامل و هذا قد يحث مشكلة اذا تمت إضافة تفاصيل تتعلق بالتصميم المستدام فيما بعد و مما يؤدي تكلفة اجمالية اعلى للمشروع . (Hwang, 2012, p. 337)

4 - عملية الموافقة على التقنيات المستدامة الجديدة و المواد المعاد تدويرها:

تقترح بيئة السوق ان عملية التخطيط قد تطول لان عملية الموافقة على استخدام التقنيات المستدامة و المواد المعاد تدويرها يمكن ان تكون طويلة و هذا يسبب مشكلة لإرادة المشروع حيث يجب عليهم تغير الجدول الزمني للمشروع .

5- عدم التطابق مع التقنيات المستدامة:

لقد أثبتت العديد من الدراسات أن التقنيات الخضراء تشكل تحديات معينة للمطورين والعملاء والمقاولين. قلة المعرفة أو الخبرة الفنية وعدم الإلمام بالمنتجات أو المواد أو النظام أو التصميم. يتمثل التحدي الرئيسي حيث ان التقنيات المستدامة عادة ما تكون أكثر تعقيداً وتختلف عن التقنيات التقليدية، يتعين على مدير المشروع تسليم المشروع بالأداء المطلوب المحدد من قبل العميل وقد يؤثر عدم الإلمام بأداء التقنيات المستدامة على نتائج الأداء. (Pettersen, 1991, p. 100)

6 - التواصل بين أعضاء فريق المشروع:

لتحقيق النجاح، يجب على مدير المشروع إرادة عدد كبير من الموردين والمقاولين والمقاولين الثانويين وأعضاء الفريق. يعد الاتصال أمراً بالغ الأهمية بشكل خاص للمشروع المستدام من أجل نقل الممارسات المستدامة المطلوبة من أعضاء الفريق. (Hwang, 2012, p. 337)

7 - الوقت و التنفيذ المستدام في الموقع:

يتطلب عادة إجراء فحوصات عشوائية وزيارات ميدانية من قبل مديري المشاريع لضمان تنفيذ الممارسات المستدامة في الموقع). هذا ضروري لأن العمال قد يميلون إلى التخلي عن بعض الممارسات المستدامة التي تستغرق وقتاً طويلاً عندما تكون هناك ضغوط زمنية لإكمال المشروع. (Hwang, 2012, p. 338)

ثانياً: اختيار التدابير في إرادة المشاريع المستدامة

ان معايير الاستدامة أثناء اختيار التدابير في إرادة المشروع. يجب أن تتناول القرارات في سياق المشاريع الاستدامة في جميع مراحل تخطيط المشروع والجدولة والتنفيذ والإنجاز، علاوة على ذلك، يجب على صانعي القرار مراعاة فوائد العملاء والمجتمع والطبيعة، من أجل اتخاذ القرارات الصحيحة واختيار التدابير المناسبة، يجب على صانعي القرار تحسين وتقوية معلوماتهم ومهاراتهم في المجالات الرئيسية والفرعية لضمان مشاريع ناجحة ومستدامة. يصبح من الواضح أن عمليات اختيار التدابير المناسبة أو النظر في القرار الصحيح ذات أهمية كبيرة لإرادة المشروع المستدامة. من المفهوم أيضاً أن اتخاذ القرار الصحيح هو سمة فريدة لأي صانع قرار والتي يجب تحسينها باستمرار لتحقيق كفاءة عالية ومعدل نجاح في الإجراءات المتخذة للإرادة المستدامة للمشروع في ضوء القرارات المتخذة. (Kibert, 2016, p. 196)

ثالثاً: سياسات إرادة المشاريع المستدامة

يعتبر تحقيق الاستدامة في إرادة المشروع امراً مهماً يضمن القيمة والفوائد في العمليات الإجمالية. يمكن قياس الاستدامة بشكل عام من خلال ثلاثة عوامل أساسية وهي العامل البيئي والعامل الاقتصادي والعامل الاجتماعي. في المشاريع، تعتبر استدامة المنتج وعمليات تطويره ذات أهمية كبيرة في ضوء آثارها الكبيرة على العوامل البيئية والاجتماعية. تصبح إرادة المشروع المستدامة أكثر حيوية وحاسمة للمشاريع التي تواجه تغييرات في المجتمع يضمن التحكم في المشروع تحقيق الأهداف المحددة للمشروع، ولكن لتحقيق الاستدامة في المشاريع، يجب مراعاة ممارسات مراقبة أكثر تنوعاً للاستدامة يجب أن يكون هناك اعتبار مناسب لتطبيق الاستدامة أثناء تنفيذ المشروع، وإجراءات مراقبة المشروع والاتفاق المتبادل بين أصحاب المصلحة في المشروع، بشكل عام، يمكن تصنيف مفهوم الاستدامة إلى ثلاث فئات رئيسية مرتبطة ببعضها البعض ولها نفس الأهمية من وجهة نظر عمليات وإرادة المشروع المستدامة. الفئات الثلاث هي الاستدامة البيئية، والاستدامة الاقتصادية، والاستدامة الاجتماعية. في وقت سابق، كان التركيز الرئيسي لعمليات المشروع هو الحفاظ على معدل نمو مرتفع واستدامة اقتصادية عالية مع أقل الاعتبارات المتعلقة بالاستدامة البيئية والاستدامة الاجتماعية، ولكن من المقبول عالمياً الآن أن الاستدامة البيئية والاستدامة الاجتماعية والاستدامة المالية لها نفس الأهمية بالنسبة لتحقيق هدف إرادة المشاريع المستدامة .

رابعاً: الممارسات المستدامة في إرادة المشروع

يمكن تصنيف إرادة أي مشروع على أساس النظام والحجم والموقع. ولكن بشكل أساسي لبناء مبنى مستدام، يجب مراعاة الآتي:

1- **الموقع** : بعد عملية اختيار الموقع المناسب للمشروع طبقاً لمعايير الاستدامة في المراحل الأولية من المشروع حيث يجب ان يقع الموقع في مكان به وسائل راحة أساسية كافية مثل المدارس، والملعب، والسوق، والمكتبة، والمواصلات، ومرافق الاتصالات، والجامعة، والمركز الطبي، والمستشفى، وما إلى ذلك و تشمل الاستدامة في الموقع تحليل للموقع وتقييم احتياجات المشروع و وضع خطة عمل مستدامة لضمان تنفيذ المشروع المستدام مع مراعاة النقاط الآتية: (Pulaski, 2004, p. 34)

- وضع خطط وصول مفصلة إلى الموقع في جميع مراحل العمل الرئيسية
- وضع تدابير مفصلة للتحكم في ترسيب التربة / التعرية من المقاول
- استخدام المواد المعاد تدويرها في الموقع للردم لتقليل استهلاك الوقود .
- وضع خطة لحماية الغطاء النباتي الموجود في الموقع خلال جميع أنشطة البناء
- تقليل اهدار العمالة و الموارد المادية .
- تحديد اقرب محطات الوقود و ذلك لتزويد معدات العمل .
- السيطرة على الضوضاء
- منع تلوث المياه الجوفية
- الامتثال للقوانين و اللوائح البيئية
- توفير فرص تدريب للمقاولين لفهم مسؤولياته في تشييد المباني المستدامة .

- ان انشاء اي مبنى مستدام مرتبط بتحقيق الثلاثية البيئية و الاقتصادية و الاجتماعية و هنا يتم فهم العامل الاجتماعي بتماشي المبنى مع ثقافة المجتمع و متطلبات الزبائن و هو ما ينقص من مفهوم الاستدامة بالشكل العام حيث انه يتناسى اهمية إجراءات السلامة الخاصة بالموقع فالسلامة تعتبر من المفاهيم الأساسية في الاستدامة البشرية في إرادة المشاريع.

2- إرادة الجودة:

تعتبر إرادة الجودة عملية مهمة مرافقة لكافة عمليات الإنتاج و التصميم هدفها تقليل الهدر و الأخطاء إلى اقل حد ممكن او اعادة العمليات الناتجة عن سوء التنفيذ، ان إرادة الجودة خلال مرحلة التنفيذ من اهم العمليات لارتباطها بالاعمال و الهدر في المواد الناتج عن تعديل الاخطاء و الذي بالتالي سيزيد من كلفة المشروع و يزيد من هدر المواد و بالتالي يؤثر على محصلة الانبعاثات و من ناحية أخرى ان إرادة الجودة تساعد في عدم تأخير العمل و بالتالي استخدام المواد ضمن الفترة المخططة لها مما يقلل حدوث مخاطر نتيجة عدم الالتزام بالخطة الزمنية للمشروع. (Pica, 2015, p. 7)

3- إرادة الآليات و خطط النقل:

يتطلب تطبيق مفهوم الاستدامة فيما يتعلق بآليات التشييد تغير بالتفكير النمطي حول شراء الآليات و تشغيلها و الذي يعتمد على النظرة الاقتصادية و هذه بعض القضايا التي يجب الانتباه لها:

- اختيار الآليات: تعتبر الآليات هي المصدر الرئيسي للتلوث في الموقع، فانه عند العمل باستراتيجية الاستدامة يصبح اختيار الآليات احد اهم الامور التي يجب مراعاتها للحد من التلوث. مثلا عند اختيار الآليات المخصصة لمشروع معين النقل مثلا فان تحقيق الاستدامة يتم من خلال المقارنة بين عدد و انتاجية الآليات و في إطار هذه المقارنة قد نصل إلى نتيجة ان الشاحنات الكبيرة الحجم هي الافضل لكن في الحقيقة هذه المقارنة لا تأخذ بعين الاعتبار انبعاثات الكربون و بالتالي نحصل على ضرر بيئي أكثر مقابل كلفة اقل.

- الصيانة الدورية: ان الصيانة الدورية للآليات التي تملكها الشركة يعتبر جوهر الاستدامة بالنسبة للآليات فان تاخير صيانة إحدى الآليات يعتبر مفيد اقتصاديا في الوقت الحالي لكن عند صيانتها مستقبلا ستكون الكلفة أكثر فضلا عن تاثير هذا التأخير على العمر الاقتصادي للآلية.

- كمية الوقود المستهلك: يجب إدخال معايير بيئية للمقارنة و لاختيار الآليات بحيث يضمن استخدام الآلية بكفاءة في استخدام الوقود و كما يجب اتخاذ إجراءات قانونية تجبر الشركات على شراء الآليات التي تحقق المعايير البيئية التي توضع من قبل خبراء

- اجهاد الآليات: عند استخدام الآلية معينة في الموقع و في سبيل الحصول على إنتاجية أكبر يتم تشغيل الآلية بكامل طاقتها خلال اليوم و هذا الامر يجهد الآلية و يؤثر على انتاجيتها على المدى الطويل و على عمرها و بالتالي يصبح هذا الاستخدام غير مستدام.

- اعادة تدوير زيوت الآليات:

عندما ترتفع نسبة الشوائب في زيوت الآليات تصبح غير قادرة على أداء الوظيفة المطلوبة و بالتالي يتم تبديلها و لذلك يعتبر اعادة تدوير هذه الزيوت تقنية مفيدة جدا من الناحية البيئية و الاقتصادية.

4- إرادة نفايات البناء:

نفايات البناء هي جميع المواد التي يتم توليدها نتيجة البناء و يتم التخلي عنها بغض النظر عما يتم معالجته.

تتضمن نفايات البناء الخشب، الواح السمنت، البلوك، الطابوق، الانابيب المعدنية، حديد التسليح، مواد العزل، الأسلاك الكهربائية، الورق و الزجاج... الخ و يعد التخلص من هذه النفايات مشكلة كبيرة نظرا لكمياتها و نفاذ الأماكن المخصصة لذلك.

فتعرف إرادة النفايات انها عملية للتخلص من هذه المواد بطريقة بيئية عن طريق تقليل النفايات و اعادة تدويرها و استخدامها مرة أخرى

كما يمكن التقليل من هذه النفايات عن طريق زيادة الدقة في الشراء و التصميم و إرادة المواد الخام بشكل أكثر فاعلية. (KHALFAN, 2006, p. 55)

المبحث الثالث: نمذجة معلومات البناء BIM وإدارة المشاريع

أصبحت نمذجة معلومات البناء BIM عملية تعاونية شاملة في صناعة البناء والتشييد، فقد شهدت BIM نموًا متزايدًا خلال العقد الماضي. يحدث هذا بشكل رئيسي بسبب قدراتها في مشاريع البناء. يمكن لـ BIM إنشاء لغة مشتركة بين جميع أقسام النظام في المشروع وجعلهم فريقًا متكاملًا. يتطابق نهجها بشدة مع أنظمة تسليم المشروع المتكاملة PPD. دورها كمنسق لنظام المشروع يشبه إلى حد بعيد واجبات مدير المشروع. يدمج BIM التخصصات المختلفة ويحلل أنظمة المشروع من أجل البناء، ويقدر تكلفة ووقت المشاريع ويرسم صورة كبيرة للمشاريع باستخدام التصور. كل هذا هو ما يفعله مدير المشروع على نطاق مختلف خلال دورة حياة المشروع. يحتوي BIM على بعض الميزات التي يمكن استخدامها بشكل فعال في إدارة المشاريع.

يمكن تلخيصها على النحو التالي (Rokooei, 2015, p. 10) :

- مساعدة مديري المشاريع والمصممين والمهندسين في إجراء المزيد من التحليلات، صنع القرار هو جانب آخر من جوانب BIM من خلال ربط نماذج معلومات البناء بالأدوات المناسبة سيكون من الممكن تحليل استهلاك الطاقة لمشروع بناء ومن ثم إيجاد حلول أفضل مثل تغيير المواد والاتجاه والكتلة ... إلخ.

- تقدير الوقت والتكلفة من ميزات BIM التي تمكن مديري المشاريع من تقدير وقت المشروع بشكل صحيح مع فهم واضح لمراحل المشروع. تسهيل عملية صنع القرار بأقل تكلفة ووقت مطلوب. علاوة على ذلك، و لل BIM القدرة على محاكاة البدائل المختلفة للمشروع وبالتالي تساعد مديري المشروع والمديرين التنفيذيين للتنبؤ بشكل موثوق بعواقب قراراتهم.

- التكامل، يمكن لفريق المشروع التعامل والتفاعل مع نموذج واحد للتخصصات المختلفة في المراحل المختلفة من المشروع يمكن لـ BIM تنسيق أنشطة التصميم والتحليل والبناء في المشروع و لذلك، يؤدي إلى سلامة المشاريع.

- تعزيز التعاون في المشروع والتحكم فيه بين أصحاب المصلحة

- إنتاجية محسنة (إعادة عمل أقل وتعاضات وتغييرات)

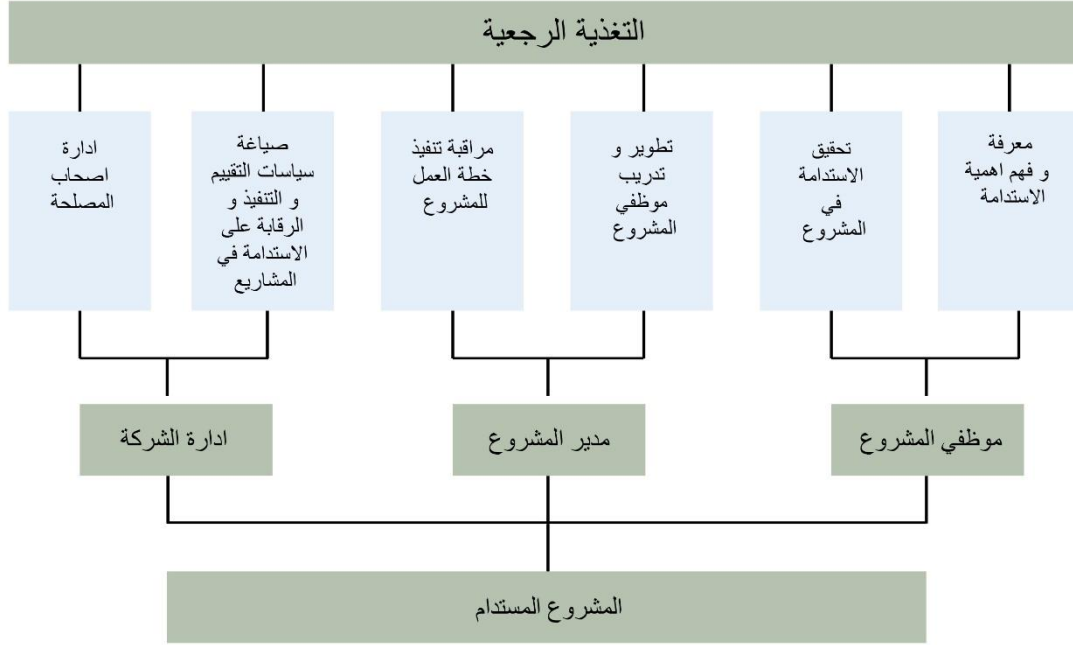
- جودة وأداء أفضل للمشروع

- تسليم المشروع أسرع

- انخفاض تكاليف البناء

الإطار المتكامل لإدارة المشاريع المستدامة:

تعتمد الإرادة المستدامة للمشروع بشكل كبير على صانعي القرار وواضعي السياسات وتنفيذ القرارات والسياسات نحو الاستدامة في المشاريع. يتم تنفيذ القرارات المتعلقة بسياسات الشركة وتنفيذها من قبل الموارد البشرية للشركة. توجد بشكل عام ثلاثة مستويات رئيسية للموارد البشرية في أي شركة هي، موظفوا المشروع (المستوى الأدنى يشمل الموظفين والمشرف والمديرين المبتدئين)، ومدير المشروع (المستوى الأوسط من الإرادة يشمل المدير، والمدير الأول، والمدير العام)، وإرادة المشروع (المستوى العالي من يشمل صانعو السياسات المديرين والمدير التنفيذي ونائب الرئيس والرئيس) من أجل الإرادة والعمليات التجارية السلسة، يجب أن يكون هناك تنسيق مناسب واتصال وتفويض مناسب للسلطة والمسؤوليات بين المستويات الثلاثة المذكورة للموارد البشرية ويجب أن يكون واضحًا بين الجميع. من أجل تحقيق الاستدامة في المشاريع، يجب إضافة عنصر التغذية الراجعة في كل مستوى من مستويات منظمة إرادة المشروع ويمكن أخذ التعليقات لكل قرار وإجراء وعملية، علاوة على ذلك، يجب تقييم التعليقات ومراجعتها قضائيًا من قبل المنظمة وأصحاب المصلحة والعملاء لاتخاذ الإجراءات التصحيحية من أجل تحقيق الاستدامة في المشاريع. كما تم توضيح إطار العمل المتكامل المقترح للاستدامة في المشاريع .



الشكل (2) الإطار المتكامل لتحقيق الاستدامة في المشاريع

المصدر: (2017، V.K. Chawlaa*)

الاستنتاجات:

1- أن مفهوم الإرادة المستدامة للمشاريع لا يزال في مهده لكن يجب التوجه نحو تكامل الاستدامة في عمليات إدارة المشاريع . كونها هي جوهر الأعمال التجارية للعديد من الشركات و المؤسسات ، لذلك لا يمكنهم تجاهل هذا المسار يجب عليهم تحديث عمليات إدارة المشاريع الخاصة بهم لتشمل مبادئ الاستدامة.

2- تعتبر مشاريع البناء / التشييد بطبيعتها أكثر اهتمامًا بتكامل النهج المستدامة، لأنها تساهم بقوة لانبعثات الكربون العالمية والمطالبة بكمية هائلة من الطاقة. في هذا البحث نهج في ممارسات إدارة المشروع التي تعتبر مواقع العوامل الرئيسية للتكامل الفعال و هي سياسات وممارسات الشركة، إدارة الموارد وتوجيه دورة الحياة ومشاركة أصحاب المصلحة

مع مراعاة الجدول الزمني الضيق لمشاريع البناء، قد لا يستغرق أعضاء فريق المشروع وقتًا كافيًا لفهم متطلبات الاستدامة ، مما يؤثر سلبيًا على اهتماماتهم فيها وبالمثل، قد يكون تنفيذ الممارسات الخضراء مكلفًا. عند إدخال تكنولوجيا وأنظمة جديدة، يحتاج أعضاء فريق المشروع إلى التدريب وحتى يظهروا مستوى معينًا من الأداء على المفهوم والممارسات المعتمدة حديثًا، نتيجة لذلك، ليس من المستغرب أن يصبح عدم الاهتمام المعلن من العملاء أحد التحديات الحاسمة في إدارة مشاريع البناء المستدام.

3- دمج الاستدامة إدارة المشروع و ذلك لفوائدها الواضحة على الرغم من ان الأدوات والأساليب المستخدمة لدمج الاستدامة لا تزال قيد التطوير وستحتاج إلى مزيد من الوقت للتطوير لتطبيقه على جميع المشاريع .

التوصيات:

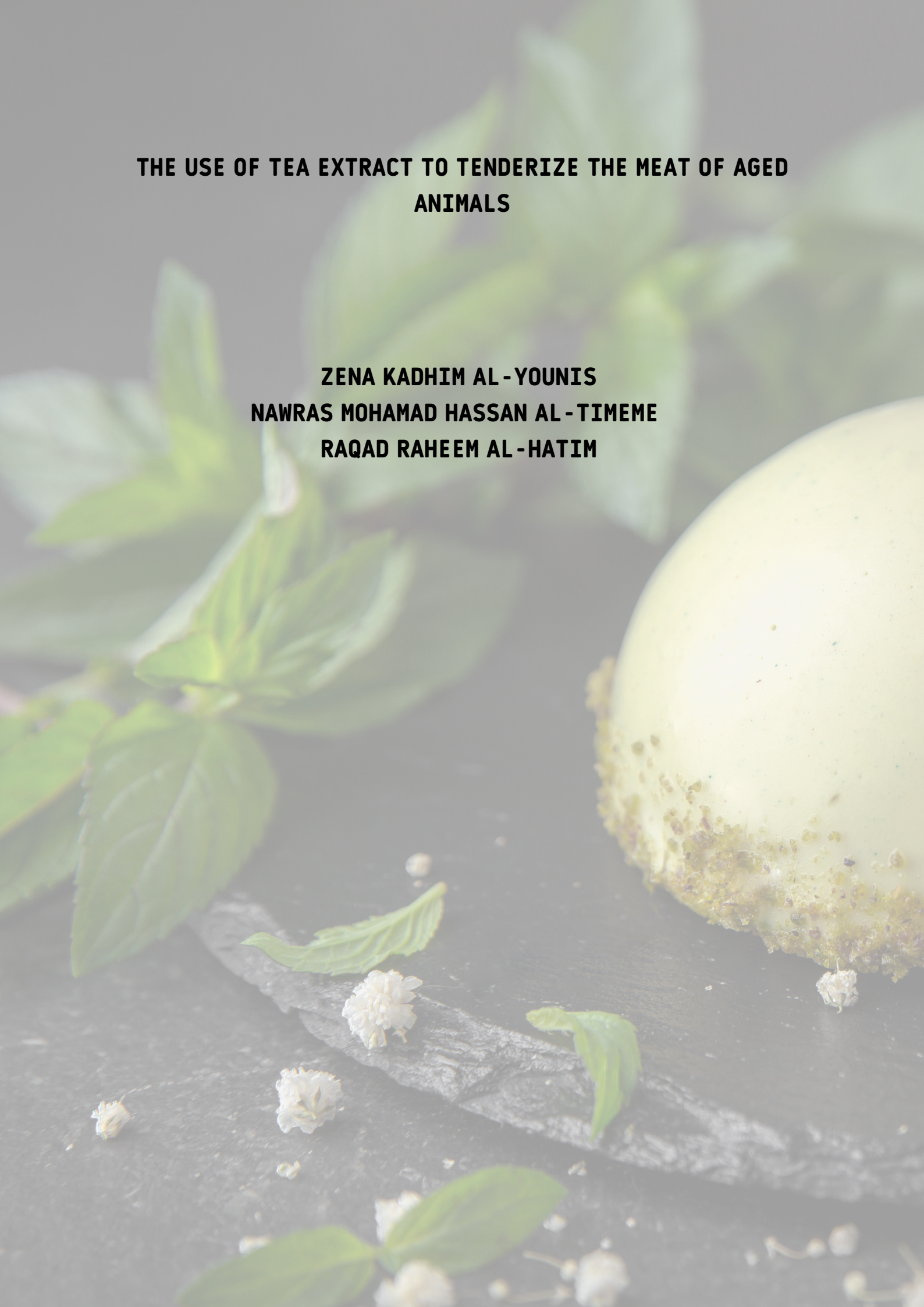
- 1- إطار عمل إرادة المشاريع المستدامة بالمقارنة مع إطار عمل إرادة مشاريع البناء التقليدي، يجب أن يكون إطار عمل إرادة المشاريع المستدامة أكثر تفصيلاً وأن يسمح بتواصل أكبر بين جميع الموظفين المعنيين. بالنظر إلى أن مثل هذا الإطار لا يزال غير متوفر في جميع البلدان ولا سيما البلدان النامية ويُنظر إليه على أنه غير مفيد .
- 2- يجب تنفيذ الإرادة المتكاملة للبيئة لتقليل الآثار السلبية وفي نفس الوقت، تعزيز فوائد المشاريع المستدامة على البيئة المحيطة. يجب إجراء التقييم البيئي لتحديد تأثيرات البناء المحتملة على البيئة ؛ البحث عن بدائل للتخفيف من الآثار وإنشاء خطط تصحيح وبرامج مراقبة إذا كان لا يمكن تخفيف الآثار أو إزالتها . يجب إجراء تقييم للتكاليف البيئية لدورة الحياة للموارد والمنتجات المستخدمة في مرحلة التخطيط حتى تتمكن فرق المشروع من تحديد واختيار المنتجات المناسبة لاستخدامها في المستدامة .
- 3- طوال مشروع البناء بأكمله، يجب أن يلتزم فريق المشروع بنظام إرادة البيئة، يجب عليهم النظر في معايير تقييم المشاريع المستدامة لتحديد كيفية بناء المبنى من أجل الامتثال للمتطلبات التشريعية أو للحصول على مستوى معين من الجوائز. تتمثل الخطوة الأخيرة في مرحلة التخطيط لمشروع البناء الأخضر في تطوير هيكل تنظيمي يحدد العلاقة بين الموظفين المهمين المشاركين في المشاريع. بعض الأمثلة على هؤلاء الموظفين هم المقاولون والاستشاريون والهندسيون وفريق الإرادة البيئية والعميل والأطراف المتضررة
- 4- يجب إنشاء طرق اتصال بين كل عضو في الفريق لتسهيل التواصل الجيد داخل فريق المشروع. يجب أيضاً إدراج مسؤوليات وسلطات جميع الموظفين وتحديد بوضوح لمنع أي توجيه أصابع الاتهام والارتباك أثناء عملية البناء. أثناء إنشاء المباني المستدامة ، يجب اعتماد برنامج إرادة البيئة على أساس متطلبات العلامة الخضراء في البناء المستدام. تتضمن ممارسات البناء المستدامة هذه الاستخدام الفعال للخرسانة لعناصر البناء، والحفاظ على هياكل المباني الحالية واستخدام المواد والمنتجات المستدامة .
- 5- يمكن تنفيذ خطط العقوبات والحوافز خلال مرحلة البناء لضمان الامتثال للمعايير البيئية المحلية. يجب مراقبة المشروع وتوثيقه لتعزيز إرادة عملية البناء بأكملها. أخيراً وليس آخراً، يجب تعزيز معرفة وخبرة المهنيين في تشييد المباني الخضراء والارتقاء بها من خلال دورات ترقية منتظمة لإبقائهم محدثين بالمعلومات المتطورة حول التقنيات والمنتجات والمواد ذات الصلة ببناء المباني الخضراء.

المصادر:

- 1- Abdullah, A. A., Abdul-Samad, Z., Abdul-Rahman, H., & Salleh, H. (2021). PROJECT MANAGEMENT METHODS, GUIDES AND STANDARDS: A CRITICAL OVERVIEW. *Journal Of Project Management Practice (JPMP)*, 1(1), 35-51.
- 2- Armenia, S., Dangelico, R. M., Nonino, F., & Pompei, A. (2019). Sustainable project management: A conceptualization-oriented review and a framework proposal for future studies. *Sustainability*, 11(9), 2664.
- 3- Gareis, R., Huemann, M., Martinuzzi, A., Weninger, C., & Sedlacko, M. (2013, April). Project management and sustainable development principles. Project Management Institute
- 4- Hwang, B. G., & Tan, J. S. (2012). Green building project management: obstacles and solutions for sustainable development. *Sustainable development*, 20(5), 335-349.
- 5- Kahachi, H. A. H. (2017). Sustainability and Project Management–The Drivers and Benefits. *Wasit Journal of Engineering Sciences*, 5(1), 87-103.
- 6- Khalfan, M. M. (2006). Managing sustainability within construction projects. *Journal of Environmental Assessment Policy and Management*, 8(01), 41-60.
- 7- Kibert, C. J. (2016). *Sustainable construction: green building design and delivery*. John Wiley & Sons.
- 8- Pettersen, N. (1991). What do we know about the effective project manager? *International Journal of Project Management*, 9(2), 99-104.
- 9- Pica, M. M. (2015). *Project life cycle economics: Cost estimation, management and effectiveness in construction projects*. Ashgate Publishing, Ltd..
- 10- Pulaski, M. H., Horman, M., Riley, D., Dahl, P., Hickey, A., Lapinski, A., ... & Vittori, G. (2004). Field guide for sustainable construction. *Pentagon Renovation and Construction Program Office Safety Sustainability and Environment IPT and Washington Headquarters Services Defense Facilities Directorate, USA*.
- 11- Rokooei, S. (2015). Building information modeling in project management: necessities, challenges and outcomes. *Procedia-Social and Behavioral Sciences*, 210, 87-95.
- 12- Silvius, A. J., & Schipper, R. P. (2014). Sustainability in project management: A literature review and impact analysis. *Social Business*, 4(1), 63-96.
- 13- Zhang, X., Shen, L., & Wu, Y. (2011). Green strategy for gaining competitive advantage in housing development: a China study. *Journal of cleaner production*, 19(2-3), 157-167.

**THE USE OF TEA EXTRACT TO TENDERIZE THE MEAT OF AGED
ANIMALS**

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NAWRAS MOHAMAD HASSAN AL -TIMEME
RAQAD RAHEEM AL -HATIM**



THE USE OF TEA EXTRACT TO TENDERIZE THE MEAT OF AGED ANIMALS

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Nawras Mohamad Hassan AL-TIMEME²

Raqad Raheem AL-HATIM³

Abstract:

The study included the preparation of the black tea extract with a 10% concentration and immerse the cow meat in this extract for 60, 90 minutes with a sample of control without immersion, then studied the qualitative characteristics for meat, which included moisture content, pH, water holding capacity and loss While weighing during cooking and Tyrusen / Teretovan coefficient (total, protein and non-protein). The results of meat treatment showed that the meat samples treated with the tea were significantly superior ($p < 0.05$) in humidity compared to the control treatment. Sample treatment that treated for a period of 90 minutes was the highest in moisture content (76.86%), The value of the pH amounted to the tea treatment for 90 minutes 5.76, the presence of a significant increase in the water holding capacity of meat samples treated with tea compared to the control sample, a significant decrease in weight loss during cooking for treated-samples compared to the control sample, all treatments in the study were significant ($p < 0.05$) in the values of the tyrosine/total, protein and non-protein tryptophan factor compared to the control treatment, and the 90-minute treatment sample recorded the highest rates which reached at 5.285, 1.325 and 3.933, respectively. It was clear that the black tea affected positively on the studied properties of treated-meat.

Key words: Black Tea, Tea Extract, Tenderize, Aged Animals, Cow Meat



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استعمال مستخلص الشاي في تطرية لحوم الحيوانات المسنة

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الملخص:

تضمنت الدراسة تحضير مستخلص الشاي الأسود بتركيز 10% وغمر قطع اللحم البقري في هذا المستخلص لفترة 30، 60 دقيقة مع ترك عينة السيطرة بدون غمر، بعد ذلك درست الصفات النوعية لقطع اللحم المغمورة والتي تضمنت المحتوى الرطوبي، الرقم الهيدروجيني، قابلية حمل الماء، نسبة الفقد بالوزن أثناء الطبخ ومعامل التايروسين/تربتوفان (الكلي والبروتيني وغير البروتيني).

وقد بينت نتائج معاملة اللحوم تفوق عينات اللحم المعاملة بالشاي معنوياً ($p < 0.05$) مقارنة بعينة السيطرة في المحتوى الرطوبي، إذ سجلت العينة المعاملة لفترة 60 دقيقة أعلى نسبة والتي بلغت 76.86%، وبلغت قيمة الرقم الهيدروجيني للعينة المعاملة بالشاي لفترة 60 دقيقة 5.76، وجود ارتفاع معنوي ($p < 0.05$) في قابلية حمل الماء لعينة اللحم المعاملة بالشاي لفترة 60 دقيقة مقارنة بعينة السيطرة، وكان هناك انخفاض معنوي ($p < 0.05$) في النسبة المئوية للفقد بالوزن أثناء الطبخ لعينات اللحم المعاملة بالشاي مقارنة بعينة السيطرة، حققت كافة المعاملات المستخدمة في الدراسة ارتفاعاً معنوياً ($p < 0.05$) في قيم معامل (التايروسين/تربتوفان) الكلي ومعامل (التايروسين/تربتوفان) البروتيني ومعامل (التايروسين/تربتوفان) غير البروتيني مقارنة بمعاملة السيطرة وسجلت العينة المعاملة لفترة 90 دقيقة أعلى نسب والتي بلغت 5.271، 1.323 و3.948 على التوالي.

الكلمات المفتاحية: الشاي الأسود، مستخلص الشاي، التطرية، لحم الأبقار، الحيوانات المسنة.

المقدمة:

يعتبر اللحم ذا قيمة غذائية عالية لكونه مصدراً رئيسياً للأحماض الأمينية الأساسية والعناصر المعدنية والأحماض الدهنية الأساسية والفيتامينات (Hocquette et al., 2010)

ان الصفات النوعية للحوم هي التي تحدد مدى قبول المستهلكين للحوم ومنتجاتها كذلك تحدد قابلية المستهلكين على شراء هذه اللحوم لأن معظم المستهلكين يرغبون في الحصول على لحوم ذات نوعية واستساغة عالية ومن أهم هذه الصفات اللون، النكهة، الطراوة، العصيرية وقابلية اللحم على مسك الماء والفقدان عند الطبخ (Lawrie, 2002).

الطراوة من الأمور الأكثر أهمية في علوم الأغذية وخاصة عمليات تحضير لحوم الحيوانات الزراعية وإعداد لحم ذي طراوة مقبولة، وتمثل الطراوة أكثر عوامل الاستساغة أهمية بالنسبة للمستهلك وهي أهم انطباع يشعر به الإنسان عند أكل اللحوم وتقطيعها بالفم إلى قطع صغيرة (Wahlgern et al., 2002)، وتتأثر بالعديد من العوامل منها العوامل الوراثية والتغيرات التي تحدث نتيجة الخزن أو قد تعزى هذه الاختلافات في الطراوة إلى جملة العوامل المشتركة (Pospiech et al., 1998 ; Koohmaria, 1996).

تتوجه الأنظار الآن إلى استخدام النباتات والأعشاب الطبية في تطرية اللحوم المسنة وذلك لاحتوائها على مواد فعالة تساهم في عملية التطرية ومواد نكهة ومضادات أكسدة ومواد أخرى لم تتطرق الدراسات إليها، كما أن هذه النباتات متوفرة محلياً ورخيصة الثمن ومنها الشاي.

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اكتشف الشاي صدفة من قبل Zhen hung إبان الأمبراطورية الصينية 2737 ق.م (Wheeler & Weeler, 2004) ويعد الان من أكثر المشروبات استهلاكاً في بلدان اسيا (Zeveri et al., 2006). يزرع الشاي في أكثر من 30 بلداً وتعد الصين، اليابان، تايوان، الهند، بنكلادش، سريلانكا وكينيا من أكثر البلدان زراعة لهذا المحصول (Shaheen et al., 2006). هناك أنواع عديدة منه منها الشاي الأخضر والأسود والشاي الأسود الصيني (الاولنج) ويتميز بأنه يمتلك تأثيرات صحية متنوعة في حاله استهلاكه منها كونه مضاد للالتهابات (Dona et al., 2003).

اتجهت الدراسات والبحوث نحو الاستفادة من لحوم الحيوانات المسنة والمستبعدة من التربية نتيجة انخفاض قابليتها الإنتاجية واستدعى الأمر الاهتمام بتحسين صفاتها النوعية والبحث عن مطريات رخيصة الثمن ومتوفرة محلياً، لذلك هدفت الدراسة الحالية إلى استعمال الشاي الأسود المتوفر والرخيص الثمن كمادة مطرية للحوم بدلا من الأنزيمات عالية الثمن.

المواد وطرائق العمل

- اللحم: تم الحصول على لحوم الأبقار من المجزرة التابعة لمحافظة البصرة بعمر 3 سنوات .
- الشاي: تم الحصول على الشاي (شاي الوزه) من الاسواق المحلية.

تحضير عينات اللحوم

بعد تقطيع اللحوم إلى قطع صغيرة (3سم × 3سم) تمت معاملتها بمستخلص الشاي 10% الذي حضر بإضافة 10 غم من الشاي إلى ماء ساخن واكمل الحجم إلى 100 مل حسب طريقة القزاز (2013)، ثم اضيفت اليه قطع اللحم وتركت لمدة 30 و60 دقيقة بدرجة حرارة المختبر فيما تركت معاملة السيطرة بدون إضافة. بعدها غسلت العينات بالماء المقطر ووضعت في الثلاجة على درجة حرارة 4م لحين إجراء الاختبارات الفيزيائية والكيميائية والحسية.

الاختبارات الفيزيائية والكيميائية للحوم المعاملة

1- نسبة الرطوبة Moistnrecontent

قدرت النسبة المئوية للرطوبة وفق (A.O.A.C (1980).

2- الرقم الهيدروجيني pH value

تم قياس الرقم الهيدروجيني حسب طريقة (Attken et al.(1962)

3- قابلية حمل الماء Water Holdig Capaci(W.H.C.)

اتبعت طريقة (Dolatowski & Stasiak(1999) في قياس قابلية حمل الماء وذلك باخذ 50 غم من اللحم المعامل بمحلول الانزيم وحسب الفترات 30 و60 دقيقة وجنست مع 50 مل ماء مقطر لمدة دقيقة واحدة، نبذ المزيج المجنس بالطرد المركزي بسرعة 10/5000 xg دقائق وبدرجة 4 م، حسبت نسبة قابلية حمل الماء كالتالي:-

وزن الماء المضاف للحوم - وزن الماء بعد النبذ المركزي

$$\text{قابلية حمل الماء (\%)} = \frac{\text{وزن الماء المضاف للحوم} - \text{وزن الماء بعد النبذ المركزي}}{100 \times \text{وزن النموذج}}$$

وزن النموذج

4- نسبة الفقد بالوزن أثناء الطبخ Cookig Test

قيست نسبة الفقد بالوزن أثناء الطبخ استنادا إلى (Purchas & Barton(1976)، اخذت كمية من اللحم المعامل بالانزيم وحسب الفترات الزمنية 30 و60 دقيقة ووضعت في أكياس من البولي اثيلين واغلقت باحكام وطبخت في حمام مائي بدرجة حرارة 70 م لمدة 90 دقيقة، سحب السائل من الكيس وخرنت في الثلاجة لمدة 24 ساعة، وزنت النماذج بعد ازالة السائل الموجود على سطح العينات وحسبت نسبة الفقد

$$\text{الفقد بالوزن أثناء الطبخ (\%)} = \frac{\text{وزن النموذج قبل الطبخ} - \text{وزن النموذج بعد الطبخ}}{\text{وزن النموذج قبل الطبخ}} \times 100$$

5-معامل التايروسين/تربتوفان Tyrosin/Tryptophan Index

استخدمت الطريقة التي ذكرها (El-Badawi *et al.*, 1964) في تقدير معامل التايروسين/تربتوفان في عينات اللحم وذلك بإضافة 50 مل من الماء المقطر إلى 10 غم من اللحم المفروم ثم خلط المزيج بصورة جيدة ورشح باستعمال ورق ترشيح (Whatt man No.1) وخفف الراشح أربع مرات بقدر حجمه بالماء المقطر وقدر الامتصاص الضوئي بواسطة جهاز المطياف الضوئي على طول موجي قدره 280 نانوميتر وبعد اخذ مقدار التخفيف في الاعتبار. اعتمدت النتيجة معامل التايروسين/تربتوفان غير البروتيني فقد تم تقديره بأخذ 20 مل من الراشح وأضيف إليه حجم مساوٍ من 15% Trichloroacetic acid ثم رشح واستعمل الراشح في تقدير معامل التايروسين/تربتوفان غير البروتيني على نفس الطول الموجي وبطرحة من معامل التايروسين/تربتوفان الكلي نحصل على معامل التايروسين/تربتوفان البروتيني.

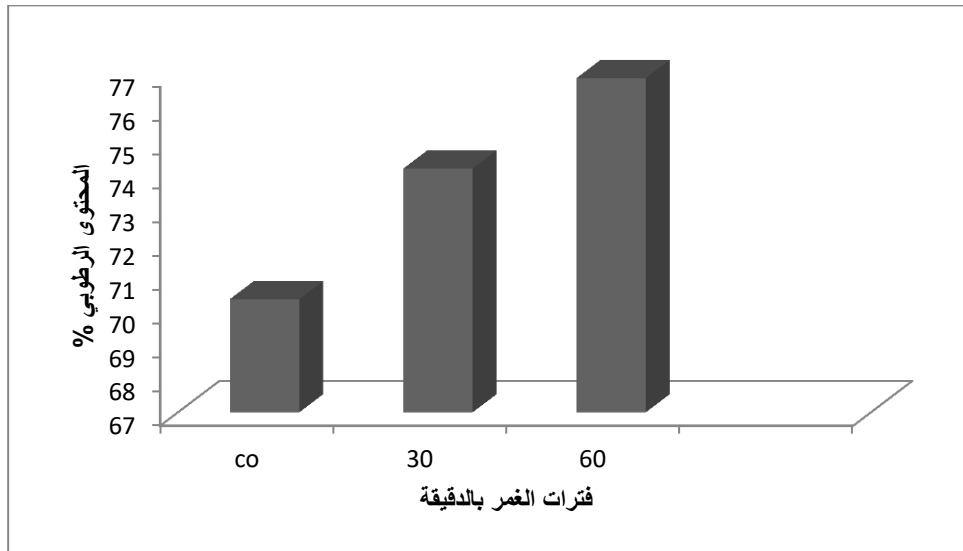
التحليل الإحصائي Statistical analysis

تم تحليل النتائج إحصائياً باستخدام التصميم العشوائي الكامل Complete Randomized Design وحللت البيانات إحصائياً باستخدام البرنامج الإحصائي الجاهز SPSS (2006, SPSS) وقورنت النتائج باستخدام اقل فرق معنوي R.L.S.D عند مستوى ($p < 0.05$) (الراوي وخلف الله، 2000).

النتائج والمناقشة

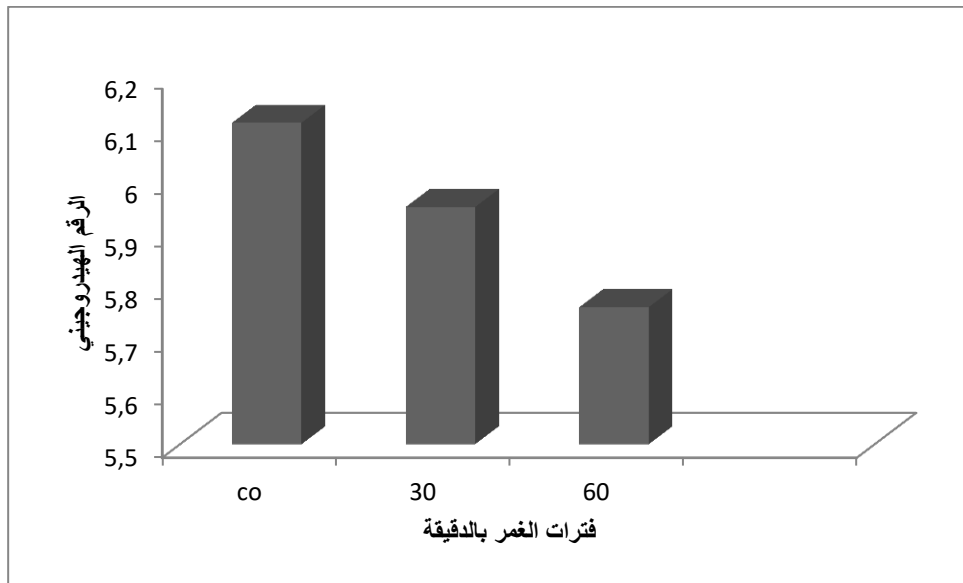
توضح النتائج في الشكل (1) الى ارتفاع نسبة الرطوبة في العينات المعاملة بالشاي لفترة ساعة ولفترة نصف ساعة والتي بلغت 76.86 % و 74.19% على التوالي مقارنة بالعينة غير المعاملة والتي بلغت 70.34% ويعزى سبب ارتفاع الرطوبة بالعينات المعاملة بالشاي إلى وجود حامض التانك الذي أدى إلى انتفاخ بروتينات المايوفبيرل كما انه خفض الرقم الهيدروجيني مما أدى إلى امتصاص الرطوبة أكثر خلال عملية الغمر وبالتالي زيادة قابلية اللحم على الارتباط بالماء (Hamm, 1960)، وتتفق نتائج هذه الدراسة مع النتائج التي حصل عليها محسن (1999) وزيادة (2005) والذين أشاروا إلى ارتفاع نسبة الرطوبة في عينات اللحم المعاملة بالأعشاب النباتية.

تبين النتائج في الشكل (2) ان قيم الرقم الهيدروجيني انخفض من 6.11 في اللحم غير المعامل الى 5.95، 5.76 في اللحم المعامل بالشاي لفترة نصف ساعة ولفترة ساعة على التوالي ان سبب هذا الانخفاض يعود إلى وجود حامض التانك الذي تكون عند تحضير المستخلص المائي لأوراق الشاي (توفيق واخرون، 2012)، إذ تزداد قابلية اللحم على حمل الماء كلما ابتعد الرقم الهيدروجيني عن نقطة التعادل الكهربائي 5.2-5.3 (Hopkins and Rowe *et al.*, 2001) (Thompson, 2002)



L.S.D 0.1998

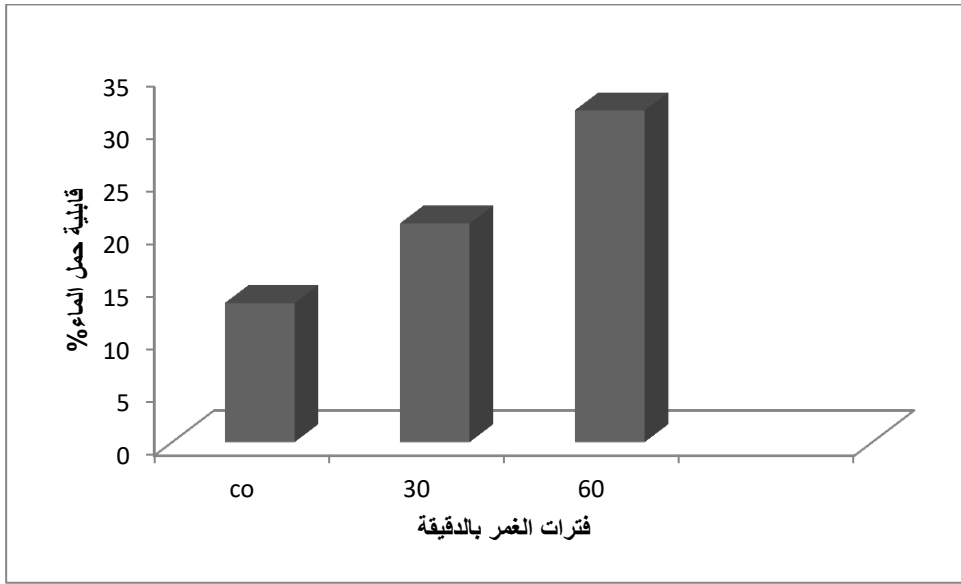
شكل (1) النسبة المئوية للمحتوى الرطوبي للحم البقر



L.S.D 0.1165

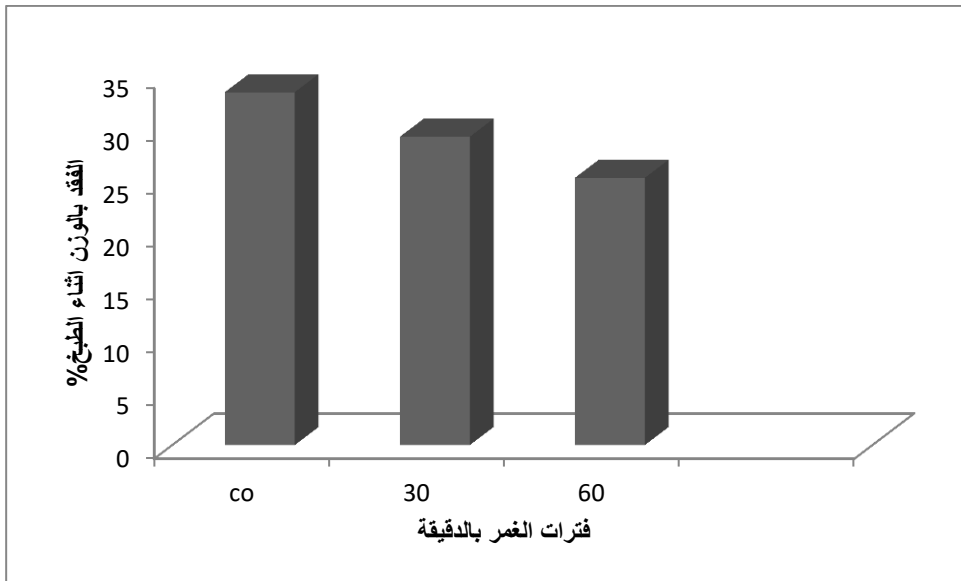
شكل (2) الدالة الحامضية للحم البقر

تبين النتائج في الشكل (3) إلى ان قابلية حمل الماء للحم المعامل بالشاي كانت 31.5% وهي اعلى من اللحم المعامل بالشاي لفترة نصف ساعة والتي بلغت 20.75% وان اللحم غير المعامل كان اقل قابلية لحمل الماء حيث بلغ 13.2%، يعزى سبب زيادة قابلية اللحم على حمل الماء إلى دور الشاي في المحافظة على ثباتية الغشاء الخلوي للحم وتثبيت الجذور الحرة بواسطة منع انتقال ذرة الهيدروجين إلى الجذر الحر فتصبح هذه الجذور ثابتة وانعكس ذلك في قلة فقدان خلال الطبخ وتحسين قابلية اللحم على ربط الماء إذ ان سلامة وحماية هذه الاغشية والحد من تمزقها تساهم في المحافظة على المكونات الخلوية للحم الامر الذي ادى إلى تحسين قابلية اللحم على مسك الماء (Haluk & Ramazan, 2001) لذلك كانت نسبة الفقد بالوزن أثناء الطبخ للحم المعامل بالشاي لفترة ساعة اقل من المعامل لفترة نصف وهذا بدوره كان اقل من اللحم غير المعامل والتي بلغت 25.24%، و29.12% و33.33% على التوالي والموضحة في الشكل (4).



L.S.D 0.1165

شكل(3)النسبة المئوية لقابلية حمل الماء للحم البقر

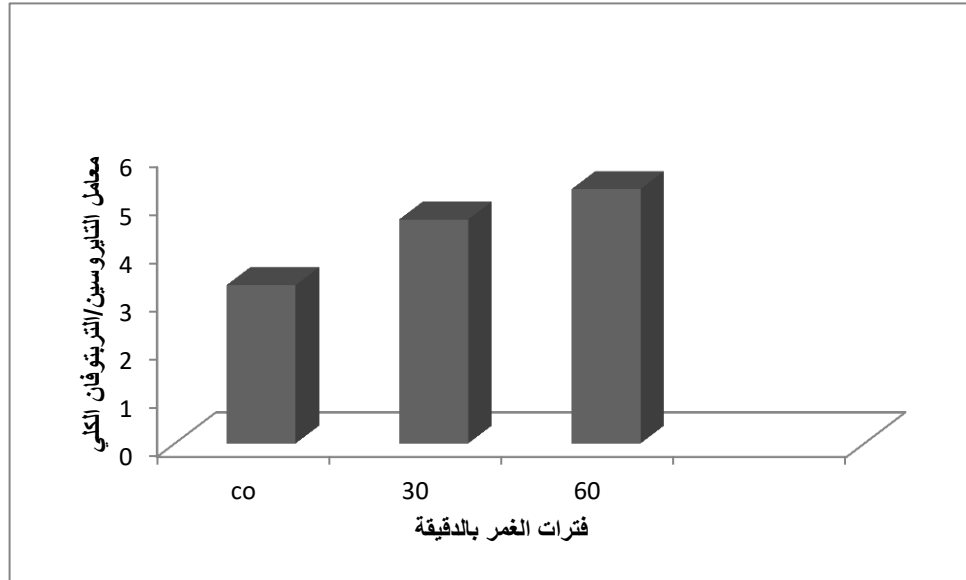


L.S.D 0.01998

شكل(4)النسبة المئوية للفقد بالوزن أثناء الطبخ للحم البقر

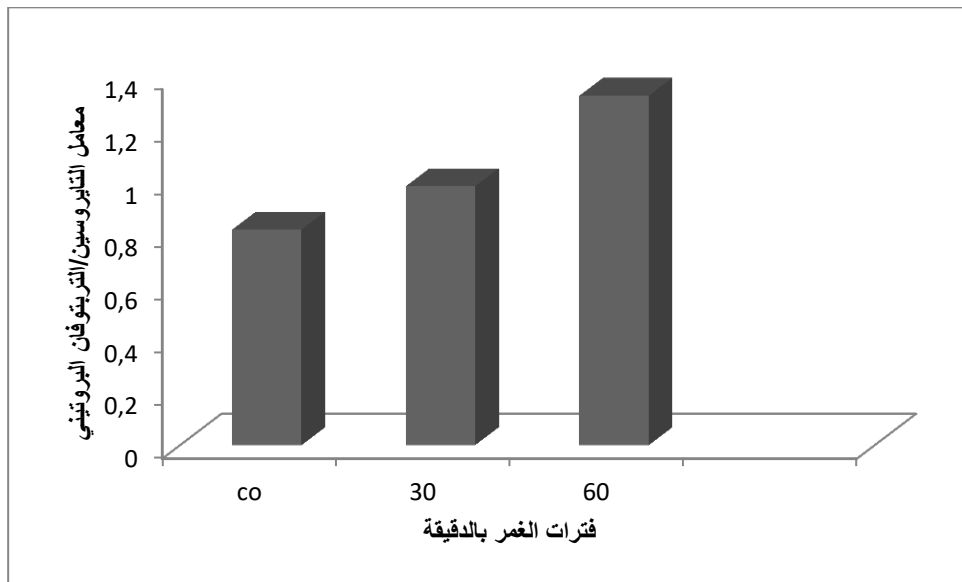
تشير الاشكال (5)، (6)و(7) إلى وجود فروق معنوية ($P < 0.05$) بين قيم معامل التايروسين/تربتوفان الكلي والبروتيني وغير البروتيني مع تقادم فترات الغمر (30، 60) دقيقة، اذ تبين النتائج أن معاملة لحوم الأبقار بمستخلص الشاي أدت إلى ارتفاع في قيم معامل التايروسين/تربتوفان الكلي والبروتيني وغير البروتيني مقارنة بعينة السيطرة وكانت اعلى قيمة للحم المعامل بمستخلص الشاي لفترة 60 دقيقة اذ بلغت 5.258، 1.325 و 3.933 على التوالي وهذه النتيجة قد تعزى إلى دور حامض التانك في زيادة تحلل بروتينات العضلات مما يزيد من نسبة الأحماض الأمينية وقيم قياس درجة التحلل الحامضي الحلقي التايروسين/ تربتوفان، يعتبر معامل التايروسين/تربتوفان مقياس لتركيز الأحماض الأمينية الاروماتية (الحلقية) وان ارتفاعه يدل على حدوث تحلل لبروتينات اللحوم (El-Badawi et al., 1964)،

واتفقت هذه النتائج مع النجموي (1985) أذ وجد أن قيم معامل التايروسين/تربتوفان يعد مقياساً لتركيز الأحماض الأمينية الحلقية التيروسين والتربتوفان نتيجة حصول تكسر وتحلل في بروتينات اللييفات العضلية مما يكون له دور في زيادة طراوة اللحم.



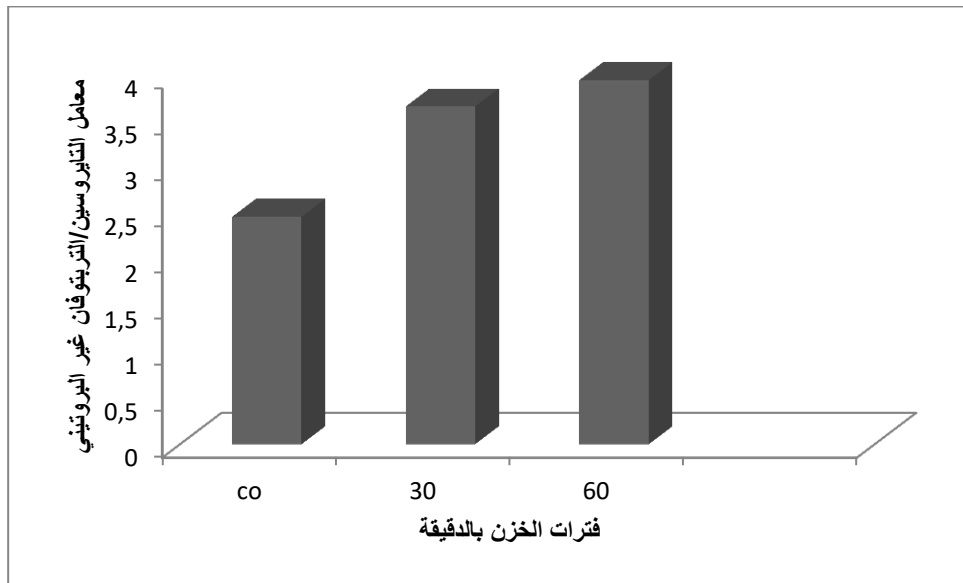
L.S.D 0.0546

شكل رقم (5) معامل التايروسين/تربتوفان الكلي للحم البقر



L.S.D 0.0518

شكل رقم (6) معامل التايروسين/تربتوفان البروتيني للحم البقر



L.S.D 0.0512

شكل رقم (7) معامل التايروسين/ترتوفان غير البروتيني للحم البقر

الاستنتاجات

نستنتج من النتائج اللاحقة امكانية استخدام مستخلص الشاي الأسود لتطرية لحوم الحيوانات المسنة لكون الشاي مادة طبيعية ومتوفرة ورخيصة الثمن بدلا من الأنزيمات باهضة الثمن او المواد الاخرى التي قد تؤثر سلبا على صحة الانسان.

المصادر

- الراوي، خاشع محمود وخلف الله، عبد العزيز محمد(2000). تصميم وتحليل التجارب الزراعية، ط2، دار الكتب للطباعة والنشر، جامعة الموصل، ص488.
- الربيعي، أميرة محمد صالح (2003). تحسين الصفات النوعية للحوم ذبائح إناث الماعز المسنة باستعمال التحفيز الكهربائي والمحاليل الملحية. أطروحة دكتوراه، كلية الزراعة، جامعة بغداد، العراق.
- الطائي، منير عبود جاسم (1986). تكنولوجيا اللحوم والأسماك. دار الكتب للطباعة والنشر، جامعة البصرة، العراق.
- القرزاز، محمد فاروق عبد الحميد(2013). تأثير غمر قطع لحم الصدر بالمستخلصات المائية للشاي الأخضر والبصل الاحمر وخل التمر على الصفات النوعية لفروج اللحم. مجلة علوم الدواجن العراقية، 7(1):1-13.
- النجماوي، معتصم حسين محمد (1985). تأثير التعتيق والمعاملة بالبوابين على بروتينات المايوفبيرل وبعض الصفات الاخرى للحوم الاغنام المسنة. رسالة ماجستير، كمية الزراعة، جامعة الموصل.
- توفيق، نبيل عارف، ايمان حسام محمد و ثابت نعمان جاسم (2013). تأثير زمن الغليان على كمية التانينات المستخلصة من أوراق الشاي. مجلة جامعة الانبار للعلوم الصرفة، 3(7):121-152.
- زيادة، حميد محمد سعيد (2005). استخدام بعض الأعشاب النباتية في تطرية لحوم الأغنام المسنة ودراسة صفاتها الفيزيائية والكيميائية والحسية. رسالة ماجستير، كلية الزراعة، جامعة البصرة، العراق.
- عباس، حميد رزاق وصالح، حاتم حسون (2012). استخدام المستخلص الخام لأنزيم بروتين Actinidin لفاكهة الكيوي في تحسين بعض الصفات النوعية للحوم الكباش العواسية. مجلة الانبار للعلوم البيطرية. 5(1): 10-20.
- محسن، افتخار حسن، منير عبود و محارب عبد الحميد (1999). تأثير نوع التوابل ونسبة الملح وفترات الخزن التجميدي على المحتوى والصفات الكيميائية لبيركر لحم البقر. مجلة أبحاث البصرة، العدد 22: 73-87.
- موسى، علاء كاظم (2007). تأثير استخدام بعض المستخلصات النباتية على الصفات النوعية للحوم الأبقار المسنة . رسالة ماجستير، كلية الزراعة، جامعة البصرة.
- ناصر، أميره كاظم، سمير اسطيفان حنا ورغدان هاشم محسن (2007). استخدام الأعشاب النباتية في تطرية لحوم الدواجن المسنة. مجلة البصرة للعلوم الزراعية، المجلد 20، العدد (2) .
- A. O. A. C. (1980). Official methods of Analysis of Association of Official Analytical Chemists, Washington, U.S.A.
- Attken, A., Cacey, C. J., Penny, I. F. and Volye, C. A. (1962). Effect of drying temperature in the accelerated freeze dried pork. J. Sci. and Food Agric., 13: 93 439 – 7.
- El-Badawi, A. A., Angiemor, A. F. and Gain, R. E. (1964). Effect of soaking in water thermal enzyme in activation in vadiation on the textural.
- Dolatowski, J. Z. and Stasiak, D. M. (1999). The effect of low frequency and intensity ultrasound on pre-rigor meat on structure and functional parameters of freezing and thawed beef semimembranosus muscle . Proc. 44th . Inc. Cong. Meat Sci. tech. Barcelona , Spain .
- Dona M, Dell'Aica I, Calabrese F, Benelli R, Morini M, Albini A, Garbisa S. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. J Immunol 2003; 170:4335-4341.
- Gerelf, B. Ikeuchi, Y. and Suzuki, A. (2000). Meat tenderization by proteolytic enzymes after osmotic dehydration. Meat Sci., 56: 311-318. Elsevier Science Ltd.

- Haluk, E. and G. Ramazan . 2011. Comparison of Marinating with Two Different Types of Marinade on Some Quality and Sensory Characteristics of Turkey Breast Meat. *Journal of Animal and Veterinary Advances*. Volume: 10 | Issue: 1 | Page No.: 60-67.
- Hocquette JF, Gondret F, Baéza E, Médale F, Jurie C, Pethick DW. Intramuscular fat content in meat-producing animals: Development, genetic and nutritional control, and identification of putative markers. *Animal*. 2010 Feb;4(2):303-319.
- Hopkins and Thompson, J. M. (2002). The relation ship between postmortem calcium concentration or pH and indicator of proteolysis in bovine muscle. *Meat. Sci.*, 61: 411-414.
- Kline, R .W. and Stewart, G. F. (1949). Glucose protein reaction dried egg albumin. *Ind. Eng Chem.*, 40 : 919 – 922 .
- Koohmaraie, M. (1996). Biochemical factors regulating the toughening and tenderization processes of meat. *Meat Sci*. 43: S193-S201.
- Lawrie, R. A. (2002). The eating quality of meat. In: *Meat Science*, 5th ed., pergarmann press, PP. 173-176, 184-188.
- Mendiratta, S. K., Ansaneyulu, A. S. and Bisht, G. S. (2003). Teuclerization and Autioxidant effect of ginger meat. *S. Food Sci. Tech. Mays*. 37: 651-655.
- Mendiratta, S. K., Ansaneyulu, A. S. and Bisht, G. S. (2003). Teuclerization and Autioxidant effect of ginger meat. *S. Food Sci. Tech. Mays*. 37: 651-655.
- Mendiratta, S. K., Nrganeyulu, A. S. R., Lakshmanan, V. and Devetlkal, S. (2004). Comparative efficacy of different tenderizing agents for tenderization of spent hen meat. *Indian . J. Poult. Sci.*, 39: 37– 42.
- Naveena, B. and Mendiratta, S. (2004). The tenderization of Buffalo meat using ginger extract. *Indian Vet. Res. Insitiute*, 4: 235-239.
- Pearson, D . (1970). *The chemical analysis of food*. Chemical publishing company. I.N.C. New York . 376 .
- Pospiech, E., Lyezynski, A., Urbaniak, M., Pietrzak, M., Rzosinska, E., Mikolajczak., B, Firlej, Z. and Iwanska, E. (1998). Meat quality depending on various feeding of parks in polish. *Symposium Krakow*, 132.
- Purchas, R. W. and Barton , R. A. (1976). The tenderness of meat several breeds of cattle raised under new Zealand pastoral conditions. *New Zealand J. Agric. Res*. 19: 421 – 428 .
- Shaheen, S.M., M.N. Hossen, M. Ahmed and M.S. Amran, 2006. Green Tea in Health Care: A natural medicine, a natural drink. *J. Applied Sci. Res.*, 2: 306-309.
- Wahlgern, N. M.; Paulsoud, K. & Gloemmi, J. K. (2002). The effect of suspension and chilling regime on pork tenderness. *Proceeding of the 48th ICOMST, Rome*, 1: 240-242.
- Wheeler,D.S. and Wheeler,W.J.(2004).The medicinal chemistry of tea.*Drug Dev. Res.*,61:45-65.
- Zaveri,N.T.(2006).Green tea and its polyphenolic catechis: medicinal use in cancer and noncancer applications.*Life Sci.*,78:2080.

ESSENTIALLY SEMIMALL QUASI-DEDEKIND MODULES AND ANTI-HOPFIAN MODULES

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ESSENTIALLY SEMIMALL QUASI-DEDEKIND MODULES AND ANTI-HOPFIAN MODULES

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Abstract:

Let V be a ring with identity and S be a unitary left Module over V . An \mathbf{R} -Module S is essentially semismall quasi-Dedekind (ESSQD) whether $\text{Hom}(S/H, S) = 0 \forall H \ll_{\text{es}} S$. A ring V is ESSQD if V is an ESSQD V -Module. An V -Module S is anti-hopfian if S is nonsimple and all nonzero factor Modules of S are isomorphic to S ; that is for all $Y \leq S$, $S/Y \cong S$. In this paper we study the relationship between ESSQD with anti-hopfian Modules and continuous Modules. We also give some examples to illustrate these relationships.

Key words: Essentially Semismall Quasi-Dedekind Modules, Anti-Hopfian Modules.



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Introduction:

Let V be a ring with identity and S be a unitary left Module over V . A Submodule Y of an V -Module S is small in S ($Y \ll S$) whether whenever a Submodule T of S together $S = Y + T$ lead $T = S$ [1]. A proper Submodule U of an V -Module S is semismall of S ($U \ll_s S$) if $U = 0$ or $U/E \ll S/E$ for all nonzero Submodule E of U [2]. An Submodule Y of an V -Module S is essentially semismall ($Y \ll_{se} S$), if for every nonzero semismall Submodule U of S , $U \cap Y \neq 0$ [3]. An V -Module S is Essentially Semismall Quasi-Dedekind (ESSQD) if $\text{Hom}(S/Q, S) = 0 \forall Q \ll_{es} S$ [3]. A ring V is ESSQD if V is an ESSQD V -Module [3]. An V -Module S is anti-hopfian if S is nonsimple and all nonzero factor Modules of S are isomorphic to S ; that is for all $D \leq S$, $S/D \cong S$ [4].

Proposition1: Assume S be an anti-hopfian V -Module. Then $F = \text{End}_v(S)$ is an ESSQD ring.

Proof: Since S is an anti-hopfian V -Module, then $F = \text{End}_v(S)$ is an integral domain [4], so $F = \text{End}_v(S)$ is an ESSQD ring [3].

Remark2: Every anti-hopfian Module is not ESSQD Module.

Proof: Since S be an anti-hopfian Module, thus $\forall D \leq S, S/D \cong S$. Since $\text{Hom}(S/D, S) \cong \text{Hom}(S, S) \neq 0$. Thus every semismall Submodule D of S is not semismall quasi-invertible. Then S is not ESSQD Module.

An V -Module S has C_1 property if, $\forall W \leq S, \exists D \leq^\oplus S$ with $W \leq_e D$. An V -Module S has C_2 property if, for all $W \leq^\oplus S$ and $D \leq S$ with $W \cong D$, then $D \leq^\oplus S$. An V -Module S has C_3 property if, for all $W_1, W_2 \leq^\oplus S$ with $W_1 \cap W_2 = 0$, then $W_1 \oplus W_2 \leq^\oplus S$. A Module is called extending (CS) if it satisfy C_1 property. A Module is called quasi-continou (π -injective) if it satisfy C_1 and C_3 properties. A Module is called continuous if it satisfy C_1 and C_2 properties [5].

Proposition3: If S is continuous, then S/Δ is a (von Neumann) regular ring and Δ equal J , the

Jacobson radical of S

Proof: Assume $x \in S$ and W be a complement of $F = \text{Ker}x$. By $C_1, W \leq^\oplus S$. Since $x|_W$ is a monomorphism, $xW \leq^\oplus S$ by C_2 . Then $\exists y \in S$ so $yx = 1_W$. Hence $(x - xyx)(F \oplus W) = (x - xyx)W = 0$, and $F \oplus W \leq \text{Ker}(x - xyx)$. Since $F \oplus W \leq_e S, x - xyx \in \Delta$. Subsequently S/Δ is a regular ring. Thus $J \leq \Delta$.

Assume $x \in \Delta$. Since $\text{Ker } x \cap \text{Ker}(1 - x) = 0$ and $\text{Ker } x \leq_e S, \text{Ker}(1 - x) = 0$. Hence $(1 - x)S \leq^\oplus S$ by C_2 . However $(1 - x)S \leq_e S$, since

$\text{Ker } x \leq (1 - x)S$. Then $(1 - x)S = S$, then $1 - x$ is a unit in S , thus $x \in J$ and hence $\Delta \leq J$. Then, $\Delta = J$.

An V -Module S is semismall K -nonsingular if, for each $h \in \text{End}_V(S)$, $\text{Ker } h \ll_{es} S$ then $h = 0$ [3].

Proposition4: If S is a semismall K -nonsingular and continuous Module, then $\text{End}_V(S)$ is regular and right continuous and $J(\text{End}_V(S)) = 0$

Proof: Since S is continuous then by Prop.3, $J(Q) = \{\varphi | \text{Ker } \varphi \ll_{es} S\}$ and $Q/J(Q)$ is Von Neumann regular and right continuous, By semismall K -nonsingularity, $J(Q) = 0$.

An V -Module S is semismall self-injective (semismall quasi-injective), if it is semismall S -injective.

Proposition5: Let S be a continuous V -Module. If S is an ESSQD V -Module, then $\text{End}_V(S)$ is regular and $J(\text{End}_V(S)) = 0$.

Proof: by Prop.4.

Corollary6: If S is a semismall quasi-injective and ESSQD V -Module, then $\text{End}_V(S)$ is regular and $J(\text{End}_V(S)) = 0$.

Proof: Since S is a semismall quasi-injective V -Module, thus S is a continuous V -Module. Thus by prop.5 $\text{End}_V(S)$ is regular and $J(\text{End}_V(S)) = 0$.

An P -homomorphism $f : S \rightarrow V$ is semismall homomorphism if $\text{ker } f \ll_{es} S$.

Assume S and W be V -Modules, W is semismall S -injective, if for each V -semismall monomorphism $f : A \rightarrow S$ (where A is V -Module) and for each V -homomorphism $r : A \rightarrow W$, \exists an V -homomorphism $k : S \rightarrow W$ such that $k \circ f = r$.

Theorem7: Assume A be a semismall quasi-injective right V -Module then $J(\text{End}_V(A)) = \{f \in \text{End}_V(A) | \text{Ker } f \ll_{es} A\}$.

Proof: Let $K = \{u \in \text{End}_V(A) | \text{Ker } u \ll_{es} A\}$, consider any $u, z \in K$. Since $(\text{Ker } u) \cap (\text{Ker } z) \leq \text{Ker}(u - z)$, we infer that $\text{Ker}(u - z) \ll_{es} A$, whence $u - z \in K$. Given any $h \in \text{End}_V(A)$, we have $\text{Ker}(uh) = h^{-1}(\text{Ker } u) \ll_{es} A$, whence $uh \in K$. Also, since $\text{Ker } u \leq \text{Ker}(uh)$, we see that $uh \in K$ as well. Therefore K is a two-sided ideal of $\text{End}_V(A)$.

Given any $u \in K$, we have $\text{Ker } u \ll_{es} A$ and $[\text{Ker } (1 - u)] \cap [\text{Ker } u] = 0$; hence $\text{Ker } (1 - u) = 0$. Thus $1 - u$ provides an isomorphism of A onto $(1 - u)A$, and the inverse isomorphism $(1 - u) : A \rightarrow A$ extends to a map $g \in \text{End}_v(A)$ s.t $g(1 - u) = 1$. Then u is a left quasi-regular element of $\text{End}_v(A)$.

Now K is a left quasi-regular ideal of $\text{End}_v(A)$, and $K \subseteq J(\text{End}_v(A))$. To show $K = J(\text{End}_v(A))$, we first prove that $\text{End}_v(A)/K$ is regular ring, follows $K = J(\text{End}_v(A))$. Consider any $f \in \text{End}_v(A)$, and B be a relative complement for $\text{Ker } f$ in A . Note that f restricts to isomorphism $fB \rightarrow B$ to some $g \in \text{End}_v(A)$. Now $(gf)|_B$ is the identity on B ; hence $(fgf - f)B = 0$, and consequently $B \oplus (\text{Ker } f) \subseteq \text{Ker } (fgf - f)$. In as much as $B \oplus (\text{Ker } f) \ll_{es} A$, we thus obtain $fgf - f \in K$, whence $\overline{fgf} = \bar{f}$ in $\text{End}_v(A)/K$ is regular ring. Now regular rings have zero radical, thus $J(\text{End}_v(A)/K) = 0$. On the other hand, since $K \subseteq J(\text{End}_v(A))$ we have $J(\text{End}_v(A)/K) = J(\text{End}_v(A))/K$, whence $J(\text{End}_v(A)) = K$.

Theorem8: Let S be a semismall quasi-injective V -Module such that $J(\text{End}_v(S)) = 0$. If W is an essential semismall V -Submodule of S then W is a semismall quasi-invertible V -Submodule of S .

Proof: Suppose W is an essential semismall V -Submodule of S and $f \in \text{Hom}(S/W, S)$, $f \neq 0$. Define $g = f \circ \pi$, where $\pi : S \rightarrow S/W$ is the natural homomorphism. Clearly $g \in \text{End}_v(S)$, $g \neq 0$ and $W \subseteq \text{Ker } g$. Since W is essential semismall, thus $\text{Ker } g$ is essential semismall by Th.7, $g \in J(\text{End}(S))$ and hence $g = 0$. Then $f = 0$. This is a contradiction. Therefore $\text{Hom}(S/W, S) = 0$, thus W is a semismall quasi-invertible V -Submodule.

Proposition9: Let S be a semismall quasi-injective V -Module with $J(\text{End}_v(S)) = 0$. Then S is an ESSQD V -Module.

Proof: Let $W \ll_{es} S$. Thus by Th.8, W is a semismall quasi-invertible Submodule of S . Then S is an ESSQD V -Module.

By Prop.9 and Coro 6, we have

Corollary10: If S is a semismall quasi-injective V -Module. Then S is an ESSQD V -Module if and only if. $J(\text{End}_v(S)) = 0$

Remark11: The condition $J(\text{End}_v(S)) = 0$ is necessarily in prop.9,. For example: Z_4 as Z -module is semismall quasi-injective which is not ESSQD. In fact $J(\text{End}_Z(Z_4)) \cong Z_2 \neq 0$

Proposition12: If S is an V -Module such that $End_v(S)$ is a regular ring, then S is an ESSQD V -Module.

Proof: Suppose $u \in End_v(S), u \neq 0$. To prove that $Ker u \not\ll_{es} S$. Since $End_v(S)$ is a regular ring, $\exists 0 \neq z \in End_v(S)$ s.t $u = uozou$ and so $zou = (zou)^2$, and $gof \neq 0$. Thus zou is an idempotent element in $End_v(S)$, thus $S = Ker(zou) \oplus Im(zou)$, that is $Ker(zou) \leq^{\oplus} S$, then $Ker(zou) \not\ll_{es} S$, since $Im(zou) \neq 0$ and $Ker(zou) \cap Im(zou) = 0$. But $keru \subseteq ker(zou)$, thus $Kerz \not\ll_{es} S$. Then S is an ESSQD V -Module.

Corollary13: Assume S be a semismall quasi-injective V -Module. Thus S is an ESSQD V -Module if and only if $End_v(S)$ is a regular ring.

Proof: From Coro 6 and Prop 12.

Corollary14: Let S be a multiplication V -Module. If each cyclic Submodule of S is injective, then S is an ESSQD V -Module.

Proof: Since S is a multiplication V -Module with any cyclic Submodule of S is injective, thus by[6], $End_v(S)$ is a regular ring. Then by Prop 12, S is an ESSQD V -Module.

The converse of Prop.12 is not true in general, as the following example shows:

Example15: Z as a Z -Module is ESSQD, but $End_Z(Z) \cong Z$ which is not a regular ring.

Proposition16: Let S an V -Module, and $W = End_v(S)$. If S is an ESSQD and quasi-continuous Module, then W_s satisfies C_3 . Conversely, if W_s has C_3 , then S has C_3 , for an arbitrary Module S .

Proof: Since S is ESSQD, so $\Delta = \{\phi \in W : Ker \phi \ll_{es} S\} = 0$, where $W = End_v(S)$, and by[5], W_s has C_3 .

To prove the converse. Assume $S_1, S \leq^{\oplus} S$ with $S_1 \cap S_2 = 0$, then $S = S_1 \oplus W_1, S = S_2 \oplus W_2$ for some $W_1, W_2 \leq S$. Consider the following : $S \xrightarrow{\rho_1} S_1 \xrightarrow{i_1} S$ and $S \xrightarrow{\rho_2} S_2 \xrightarrow{i_2} S$, where ρ_1, ρ_2 are the natural projection mappings, and i_1, i_2 are the inclusion mappings. Thus $i_1 \rho_1 \in D = End_v(S)$, $i_2 \rho_2 \in D = End_v(S)$. So $i_1 \rho_1 = (i_1 \rho_1)^2$, thus $i_1 \rho_1$ is an idempotent element in W_s , then $W = (i_1 \rho_1)W \oplus (I - i_1 \rho_1)W$; that is $(i_1 \rho_1)W \leq^{\oplus} W$, similarly $(i_2 \rho_2)W \leq^{\oplus} W$. But $(i_1 \rho_1)W \cap (i_2 \rho_2)W = 0$, since if, $\exists z \in (i_1 \rho_1)S$, $z \in (i_2 \rho_2)W$ then $z = (i_1 \rho_1)of_1$ and $z = (i_2 \rho_2)of_2$ for some $f_1, f_2 \in W$. Hence

$z(x) = (i_1 o \rho_1) o f_1(x) = (i_1 o \rho_1)(x_1 + k_1) = x_1$ and
 $z(x) = (i_2 o \rho_2) o f_2(x) = (i_2 o \rho_2)(x_2 + k_2) = x_2$, thus $z(x) = x_1 = x_2$, so
 $z(x) \in S_1 \cap S_2 = 0$, then $z = 0$. Since S_s satisfies C_3 , we get
 $(i_1 o \rho_1)W \oplus (i_2 o \rho_2)W \leq^\oplus W$. Then $[(i_1 o \rho_1)D \oplus (i_2 o \rho_2)D] \oplus E = D$, for some
 $E \leq W$. Since $I \in W$, (where $I =$ identity map on S), thus
 $I = [(i_1 o \rho_1) o g_1 + (i_2 o \rho_2) o g_2] + \psi$, where $\psi \in E$, $g_1, g_2 \in W$
 Thus $S = I(S) = [(i_1 o \rho_1) o g_1(S) + (i_2 o \rho_2) o g_2(S)] + \psi(S) \subseteq$
 $[(i_1 o \rho_1)(S) + (i_2 o \rho_2)(S)] + \psi(S) = (S_1 \oplus S_2) \oplus \psi(S)$. Hence $S_1 \oplus S_2 \leq^\oplus S$. Thus
 S has C_3 .

An V-Module is called to have (strong) summand intersection property(SSIP)
 SIP, if for an (infinite) finite index set I and $\forall (S_i)_{i \in I}$ with $S_i \leq^\oplus S, i \in I$, then
 $\bigcap_{i \in I} S_i \leq^\oplus S$. And a Module S has the summand sum property (SSP), if $\forall X, Y \leq^\oplus S$,
 then $X + Y \leq^\oplus S$ [7].

The left annihilator of $V \subseteq S$ in $S = \text{End}_V(S)$ [i.e all elements $\phi \in S$ such that
 $\phi V = 0$] is denoted by $L_S(V)$, the right annihilator of $T \subseteq S$ in S [i.e all elements $u \in S$
 such that $Tu = 0$] is denoted by $r_U(T)$ [8].

An V-Module S is Baer if, for all $W \leq S, L_S(W) = Ee$, with
 $e^2 = e \in E = \text{End}(S)$. Equivalently, S is Baer if, for all ideal $I \leq_S E, r_U(I) = eS$
 with $e^2 = e \in E = \text{End}_V(S)$ [8].

Proposition17: An ESSQD and quasi-continuous V-Module S has SSIP and SSP.

Proof: Since S has C_1 property; that is S is an extending V-Module. But S be an ESSQD
 extending V-Module, then S is a Baer V-Module, by[8]. Thus, by [7], S has SSIP. Assume
 $S_1, S_2 \leq^\oplus S$. Then by SSIP, $S_1 \cap S_2 \leq^\oplus S$. Let $E = S_1 \cap S_2$, implies $\exists Q \leq S$ s.t
 $E \oplus Q = S$. But $S_1 \leq^\oplus S$, implies $S_1 \oplus X = S$, for some $X \leq S$, then
 $E \oplus Q = S = S_1 \oplus X$. Similarly, $S_2 \leq^\oplus S$, thus $S_2 \oplus Y = S$, for some
 $Y \leq S$, then $E \oplus Q = S = S_2 \oplus Y$. We claim that $E \oplus (Q \cap S_1) = S_1$
 ... (1), $E \oplus (Q \cap S_2) = S_2$... (2). To prove (1). Since
 $E \oplus (Q \cap S_1) = (S_1 \cap S_2) \oplus (Q \cap S_1) \subseteq S_1$. Conversely, let $x \in S_1$, then
 $x = x + 0 \in S_1 \oplus X = S = E \oplus Q$, thus $x = a + b$, where $a \in E = (S_1 \cap S_2)$ and

$b \in Q$, implies $b = x - a \in Q \cap S_1$, then $x = a + b \in E \oplus (Q \cap S_1)$, similarly we can get(2). Assume that $Y_1 = Q \cap S_1$, $Y_2 = Q \cap S_2$, $S_1 + S_2 = (E \oplus Y_1) + (E \oplus Y_2) = (S \oplus Y_1) \oplus Y_2$, then $S_1 + S_2 = (E \oplus Y_1) \oplus Y_2$, but $E \oplus Y_1 = S_1 \leq^{\oplus} S$, also $Y_2 \leq^{\oplus} S$, since $E \oplus Y_2 = S_2$ and $S_2 \oplus Y = S$, thus $E \oplus Y_2 \oplus Y = S_2 \oplus Y = S$, then $Y_2 \leq^{\oplus} S$. To prove $(E \oplus Y_1) \cap Y_2 = 0$. Let $x \in (E \oplus Y_1)$ and $x \in Y_2 = Q \cap S_2$, hence $x = h + w_1$, $x \in Q$, $x \in S_2$, thus $w_1 \in Y_1 = Q \cap S_1$, then $w_1 \in S_1$, also $h \in E = S_1 \cap S_2$, so $h \in S_1$ and $h \in S_2$, thus $x = h + w_1 \in S_1$, but $x \in S_2$, thus $x \in S_1 \cap S_2 = E$, but $x \in Q$, then $x \in Q \cap E = 0$. Thus by property C_3 , $(E \oplus Y_1) \oplus Y_2 \leq^{\oplus} S$. Therefore $S_1 + S_2 \leq^{\oplus} S$. Thus, S has SSP.

References

- [1] F.Kasch, Modules and rings, Academic Press., London, 1982.
- [2] Ali I.M, Muhmood L.S. "semi small submodules and semi-lifting Modules", 3rd scientific conference of the college of science, university of Baghdad, 2009, 385-393.
- [3] Hussain M. Q., AbdulKareem D. j. Essentially Semismall Quasi-Dedekind modules", 1st scientific conference of the college of Education, Duhook university, 2019,. p. 21-28.
- [4] Hiraro Y., Mogami I., " On restricted anti-hopfian Modules ", Math .J. Okayama University, 28 (1986) , p. 119 - 131 .
- [5] Mohamed S . H., Muller B .J., " Continuous and discrete Modules ", London Math, Soc, LNC, 147 Cambridge univ . press, Cambridge , 1990 .
- [6] Naoum A . G ., " Regular multiplication Modules ", Periodica Math , Hungarica , Vo 1. 31(2), 1995, p. 155 – 162 .
- [7] Rizvi S.T.,Roman C.S., " On K- nonsingular Modules and applications ", Comm . In Algebra , 35 (2007), p. 2960 – 2982 .
- [8] Roman C. S ., " Baer and Quasi-Baer Modules ", Ph . D .Thesis, M.S., Graduate, School of Ohio, State University, 2004 .

