AL-KUFA UNIVERSITY JOURNAL FOR BIOLOGY-2023. VOL.15 NO.2 P:1-9
ISSN (Print): 2073-8854 ISSN **(online):** 2311-6544

Original Research Paper *Journal homepage: https://journal.uokufa.edu.iq/index.php/ajb/index*

Gc-MS Analysis and Anti-Bacterial Activity of *Lantana Camara* Extracts

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Article history
Received: 10 / 5 /2023
Revised: 25 / 7 /2023
Accepted: 1 / 8 /2023
DOI:
10.36320/ajb/v15.i2.11985
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Abstract: *Scientists from all over the world are currently investigating medicinal plants. Plants have long been used as a source of medicine by humans due to their low cost and long history of use. Due to technological limitations, most traditional medicines in the past were derived from plants. In this study, the anti-bacterial and phytochemical properties of Lantana camara extracts from Iraq were analyzed. The extract composition was analyzed using GC/MS. The agar well diffusion method and broth dilution assay to evaluate the antimicrobial activity of each extract. The aerial parts of the Lantana camara herb were extracted with n-hexane, ethanol, and methanol. The beneficial phytochemicals in L. camara include triterpenoids, flavonoids, alkaloids, saponins, steroids, and tannins. The anti-bacterial activity of extract's was evaluated., The n-hexane extract was found to have a 400 µg/mL minimum inhibitory concentration (MBC), against Proteus valguris, while the ethanolic and methanolic extracts showed similar anti-bacterial activity at 100 µg/mL., While the lowest inhibitory concentration of n-hexane and ethanolic extracts was 400 µg/mL, and methanolic extract was 200 µg/mL. against Bacillus subtilis. This means that the methanolic extract is more effective and inhibits bacterial growth at lower concentrations. This article will be useful for researchers studying the biological activity, phytochemical composition, and anti-bacterial activity of Lantana camara.*

***Keywords*: *Lantana camara, n-Hexane extract, Ethanolic extract, anti-bacterial activity.* GC-MS**

1.Introduction

There are many compelling reasons to pursue the discovery of anti-bacterial plants with potential medical applications. We urgently need new methods of treating bacterial infections as antibiotic resistance continues to rise. For hundreds of years, people have turned to medicinal plants as a source of new compounds that kill bacteria to treat everything from common colds to bacterial infections. Unlike antibiotics, medicinal plants are typically less expensive and more accessible. This is especially true in developing nations, where access to medical care and antibiotics may be limited [1,2]. Due to their low cost and wide availability, medicinal plants are rapidly gaining favour as a primary source of healthcare for underserved communities [3]. Medicinal plants are used more frequently because of their importance in medicine and the activity of the secondary metabolites they contain [4].

Lantana camara is a small shrub with triangular stems. Its maximum dimensions are 1–3 meters in height and 2.5 meters in width. The leaves are ovate in shape, crenate-serrate, rugose on top and scabrous on the underside, and pointed either acutely or sub-acutely. They are between 3 and 8 centimeters in length and 3 and 6 centimeters in width and are a bright green color. The plant's leaves and stems are covered in coarse hairs [5].

There is a wide range of possible shades of red, orange, and white because of the gradual color changes that occur as these colors age. Flowers with yellow throats bloom for a long time due to the axillary head's role in their nearly constant bloom and gradual color change. The axillary head is constantly covered in yellow-throated flowers. The calyx is tiny, the corolla tube is short, and the lobes on the 6-7 mm broad limb are all different sizes. The flower clusters, or inflorescences, are set up in pairs in the axils of the leaves on opposite sides of the plant. About 24–40 dead flowers were discovered inside. The plant's root system quickly recovers from repeated cuttings and produces new shoots [6].

Lantana camara is an essential plant that has been utilized in alternative medical practices for many years. The *Lantana camara* is a species that belongs to the genus Lantana, the division Magnoliophyte, the class Magnoliopsida, the order Lamiales, and the family Verbenaceae. It has been used as a treatment for a wide range of diseases in different countries all over the world. Medical professionals have successfully used the leaves to treat a wide variety of conditions, including but not limited to: wounds, rheumatism, ulcers, catarrhal infections, cancer, tetanus, malaria, asthma, ulcers, eczema, bilious fever, tumors, chicken pox, swelling, sores, Measles, ataxy of the abdominal viscera; fevers; the common cold; and hypertension. Patients in Ghana suffering from bronchitis frequently consume tea made from the boiled plant. A combination of powdered root and milk is given to children suffering from stomachaches or worms. When applied to the skin, lantana oil helps heal minor cuts and prevents wounds from becoming more widespread. Both leprosy and scabies were treated with decoctions used to the affected areas of the skin [7,12].

It has been reported that an aqueous extract of *L. camara* has anti-inflammatory properties in albino rats. In the carrageenan-induced paw oedema test with rats, extract treatment (500 mg/kg body weight) significantly reduced paw volume [13]. An antiulcerogenic effect of a methanol extract of *L. camara* leaves was observed in rats with ethanol, aspirin and cold-resistant stress-induced gastric lesions. When the affected rats were pretreated with the extract (200 and 400 mg/kg body weight), it significantly reduced the severity of ulcers caused by aspirin, ethanol, and cold restraint stress. The extract's antiulcerogenic activity wasdose-dependent across all models [14]. It has been demonstrated that calcium oxalate urolithiasis can be prevented in male albino rats exposed to ethylene glycol and ammonium chloride by administering an ethanolic extract of the leaves of *L. camara*. Following treatment with the extract, there was a notable reduction in the accumulation of calcium and oxalate andan increase in the amount of calcium, oxalate, and creatinine that was excreted in the urine [15]. Rats that had been given alloxan to make them diabetic found that a methanol extract of *L. camara* leaves lowered their blood sugar. When rats with diabetes caused by alloxan were given an oral dose of 400 milligrams per kilogram of body weight of a methanol extract of L. camara leaves, their blood sugar levels dropped to 121.94 milligrams per deciliter [16].

Several different cultivars of the *Lantana camara* plant have been found to have properties that stop bacteria from growing on their leaves and flowers. The anti-bacterial activity of three solvent extracts of leaves and flowers from four different kinds of *L. camara* was found to be very strong against *Escherichia coli, Bacillus subtilis, and Pseudomonas aeruginosa*, but not so strong against *Staphylococcus aureus* [17]. The leaves and roots of *Lantana camara* are said to kill bacteria when they are extracted with ethanol. The effectiveness of the anti-bacterial treatment was evaluated in vitro using Microdilution assays. The extracts successfully eliminated two multi resistant strains of Escherichia coli and Staphylococcus aureus and other bacteria, including *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholera*, and others [18].

Using the broth microdilution and agar well diffusion method, methanolic extracts of various *Lantana camara* parts were examined for antimicrobial action against ten bacteria and five fungi. Both Gram-positive *Bacillus cereus* and Gram-negative *Salmonella typhi* were inhibited by *L. camara* leaf extract [19]. A typical plant pathogen, Alternaria sp., was used to see if *Lantana camara* could stop plant diseases caused by it. The food poisoning plate method was used to compare the antifungal activity of extract concentrations of 10, 15, and 20 mg/mL. At a 20 mg/mL concentration, *L. camara* was very effective against the fungus Alternaria sp [20]. Both ethanol and hot water extracts of *L. camara* were looked at to see if they could stop white rot and brown rot in wood. Even at such a low concentration (0.01%), the ethanol extract still killed the white and brown rot fungi. The other extract worked just as well [21].

2. Methodology

Collection of plant

From August 1st, 2021, through December 30th, 2021, fresh aerial parts of *Lantana camara* were collected from Baghdad City. Was. Deionized water was then used to wash them once more. Finally, they were allowed to air-dry at room temperature. The dried plant was then broken up into smaller pieces and kept in a cool, dark place until an extract was needed.

Chemical solvents

Maceration is one of the simplest methods of extraction. It entails soaking coarse and powdered plant material in solvents such as n-hexane (99%), ethanol (99.99%), and methanol (99.8%). Extraction of bioactive compounds from plants using this method is standard and does not cost very much. Polar compounds can be extracted with methanol and ethanol, while nonpolar compounds are extracted with n-hexane and other nonpolar solvents [22,24].

Preparation of Extracts by Maceration method

Lantana camara's aerial parts were removed by maceration with solvents of different polarities. In a nutshell, 800 mL of n-Hexane, 800 mL of ethanol, and 500 mL of methanol were used to treat 100 g of powder, 95 g of powder, and 90 g of powder, respectively. The supernatant was filtered out in the dark at 25 degrees Celsius for 48 hours. To further purify the extracts and isolate the bioactive compounds, this procedure was repeated, and the solvent in the supernatant was evaporated using a vacuum rotary evaporator [25].

GC/MS analysis

The condition of GC/MS is as follows:

1. The type of detector used was a mass spectrometer (MS), the injection technique used was a split (80.1), the injector temperature was 260 degrees Celsius, the injector volume was one microliter, the carrier gas was helium, the flow rate was 1 milliliter per minute, and the mode used was scanned 50-550.

2. The capillary columns used were HP-5MS and measured 30 millimeters in length, 0.25 millimeters in diameter, and 0.25 micrometers in thickness .

3. temperature program: 60 degrees Celsius for four minutes, then three degrees Celsius per minute up to 100 degrees Celsius for two minutes, and then four degrees Celsius per minute up to 260 degrees Celsius for five minutes.

4 .Scan Range (50–500) and (EM) Detector for the Mass Spectrometer (70 V).

5. By comparing the average peak area of each component to the total measurement area, we were able to calculate the relative percentage amount of each element. Because of this, we were able to figure out how much each part made up of the whole. The MS Solution software was the system's operating system and data collection tool.

Preparation of bacteria

The pathogenic bacteria (*Proteus valguris and Bacillus subtilis*) were obtained from the BPC laboratory analysis center.

Preparation of Mueller-Hinton broth

Mueller-Hinton broth powder requires 21 grams per liter of water. Use a mixer to combine and dissolve them combine and dissolve them thoroughly. To ensure sterilization, add them to the final storage containers (like a conical flask) and autoclave at 121 degrees for 15 minutes [26].

Broth dilution assay

The broth dilution assay was utilized to perform the Minimum Inhibitory Concentration (MIC) test. The minimal inhibitory concentration (MIC) test determines the lowest concentration of antimicrobial agent that significantly slows growth. A suspension with a turbidity of 0.5 Mc Farland was used to prepare the bacteria. The dilutions of the aerial part extract were 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125 µg/ml (W/V). The extract was then given a bacterial suspension, and it was incubated for 24 hours at 37 degrees Celsius. The MIC was the lowest, clearest concentration. Three times of experiments were conducted on each tested specimen [27].

3.Results and Discussion

Maceration method

The solvents n-hexane, ethanol, and methanol were used to extract the 100 g of air-dried whole plants. A crude extract evaporated the organic solvent at 30–40 ^{o} C under low pressure.

During the maceration extraction method, the aerial parts of *Lantana camra* are broken down and put into a container. The container is then covered, and it stays that way for at least three days. The polarity of the solvents can influence the maceration method. *Lantana camara* aerial parts were extracted in the following order: n-hexane in 800 ml, ethanol in 800 ml, and methanol in 500 ml. The percentage yields of the extracts were 2% (light green), 3.6% (dark green), and 3.3% (dark green) for n-hexane, ethanol, and methanol, respectively. These findings demonstrated that the aerial parts of *Lantana camara* were more soluble in ethanol. The results of GC/MS analysis showed that the plant *Lantana camara* contains squalene, beta-amyrin, hexane dioic acid, eucalyptol, naphthalene, alpha Tocospiro A,trans-geranylgeraniol,caryophyllene, caryophyllene oxide, Octadecatrienoic acid, phytol, Neophytadiene, Alfa-Copaene, Vitamin E, alpha amyrin, iso spathulenol, Mequinol, Methoxy-4-vinylphenol, hexadecatrienoic acid, these compounds have many biological activities, as displayed in tables (1,2 and 3).

GC/MS analysis of n-Hexane extract

The chromatograms of the aerial part of the *Lantana camara* plant reveal that different compounds remain in the plant for varying amounts of time. The GC/MS chromatogram of the n-hexane extract (111) distinct compounds, as shown in Figure (1). The bioactive compound in the aerial part of *Lantana camara* contributes to the plant's medicinal value.

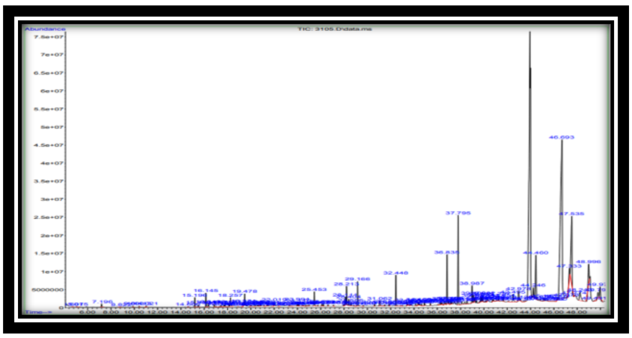

Figure (1): GC/MS of the n-Hexane extract of aerial part of *lantana camara.*

In *Lantana camara* n-Hexane extract, the most essential phytochemical compounds are Eucalyptol, Naphthalene1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis), Alpha Tocospiro A,trans-Geranylgeraniol Caryophyllene oxide, 9,12,15-Octadecatrienoic acid, methyl ester (ZZZ)-, Phytol, Neophytadiene, Alfa-Copaene, , Vitamin E, Alpha amyrin and Iso spathulenol. As Table (1) shows, this chemical is used for a wide range of biological purposes, including**: -**

Table (1): GC/MS of n-Hexane extract and biological activity of each compound.

No	Compounds	Area %	RT (min)	Biological activity
1	Eucalyptol	0.25	4.689	anti-inflammatory antioxidant [28)]
2	Alfa-Copaene	0.58	15.196	Anticancer antigenotoxic Antioxidant [29]
3	Naphthalene1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) (1S-cis)	0.78	18.257	Antimicrobial anti-inflammatory [29]
4	Caryophyllene oxide	0.71	19.476	Antitumor Antioxidant Analgesic anti-inflammatory [30,31]
5	Neophytadiene	0.23	23.996	anti-inflammatory antimicrobial [32]
6	9,12 Octadecatrienoic acid (Z, Z), methyl ester	0.42	28.11	Anti-inflammatory Immunomodulatory [33]
7	9,12,15-Octadecatrienoic acid, methyl ester, (ZZZ)	1.29	28.214	Anti-inflammatory immunomodulatory [33]
8	Phytol	0.50	28.406	Antioxidant anti-Inflammatory Analgesic [34]
9	Alpha Tocospiro A	0.34	38.114	anti-inflammatory anti-diabetic (35)
10	trans-Geranylgeraniol	0.35	39.712	Anticancer [36]
11	Vitamin E	0.39	41.258	Antioxidant anti-inflammatory [37]
12	Alpha amyrin	0.33	45.145	Antioxidant [38]
13	Iso spathulenol	0.25	20.348	Antioxidant anti-Inflammatory [39]

GC/MS analysis of an ethanolic extract

The chromatograms of the aerial part of the *Lantana camara* plant reveal that different compounds remain in the plant for varying amounts of time. The GC-MS chromatogram of the ethanolic extract shows [28] distinct compounds, as displayed in Figure (2). The bioactive compound in the aerial part of *Lantana camara* contributes to the plant's medicinal value.

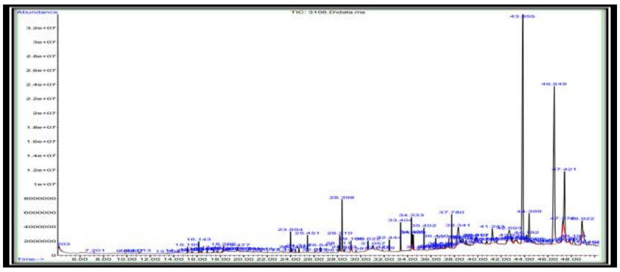

Figure (2): GC/MS of the ethanolic extract of the aerial part of *lantana camara*.

Alfa Copaene, Caryophyllene, Neophytadiene, and 9, 12, 15 Octadecatrienoic Acid, Methyl Ester (ZZZ), are the most essential phytochemical compounds found in *Lantana camara* ethanol extract. This chemical has a wide range of biological activities, as shown in Table (2).

Table (2): GC/MS of an ethanolic extract and biological activity of each compound.

No	Compounds	Area %	RT (min)	Biological activity
1	Alfa Copaene	0.32	15.196	Anticancer antigenotoxic Antioxidant [28]
2	Caryophyllene	0.61	16.145	antioxidant antimicrobial anticancer [30,31]
3	Neophytadiene	0.95	23.996	anti-inflammatory antimicrobial [32]
4	9,12,15 Octadecatrienoic Acid, Methyl Ester (ZZZ)	0.95	28.209	Anti-inflammatory immunomodulatory [33]

GC-MS analysis of a methanolic extract

The chromatograms of the aerial part of the *Lantana camara* plant reveal that different compounds remain in the plant for varying amounts of time. The methanolic extract's GC-MS chromatogram reveals 43 distinct compounds, as shown in Figure (3). The bioactive compound in the aerial part of *Lantana camara* contributes to the plant's medicinal value.

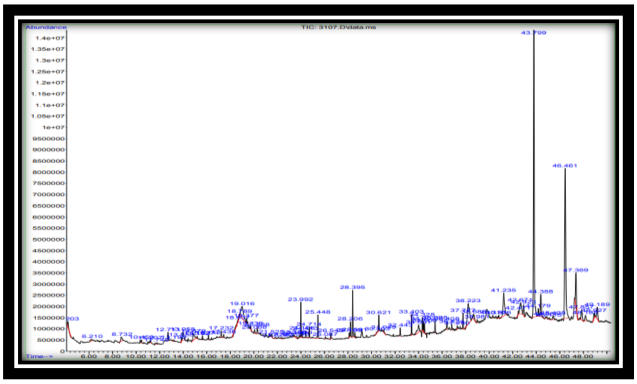

Figure (3): GC-MS of the methanolic extract of the aerial part of *lantana camara.*

The main phytochemical compounds found in *Lantana camara* methanol extract are Mequinol, Methoxy-4-vinyl phenol, 7, 10, 13-Hexadecatrienoic acid, methyl ester, Hexanedioic acid, dioctyl ester and Squalene. This chemical has a wide range of biological activities, as shown in Table (3).

Table (3): GC-MS of methanolic extract and biological activity of each compound.

No	Compounds	Area%	RT (min)	Biological activity
1	Mequinol	0.55	8.731	Topical treatment of melasma [40]
2	Methoxy-4-vinylphenol	1.11	13.956	Anti-bacterial [41]
3	7,10,13-Hexadecatrienoic acid, methyl ester	0.66	28.204	Antimicrobial Antioxidant Anti-inflammatory[42]
4	Hexanedioic acid, dioctyl ester	0.42	34.326	Antioxidant antiandrogenic [43]
5	Squalene	0.76	37.777	Anti-bacterial antioxidant Antitumor [43]

Broth microdilution assay

In the anti-bacterial analysis, minimum inhibitory concentration was used. The aerial part extract is prepared in three different solvents: n-hexane, ethanol, and methanol, and the bacteria analyzed are *Proteus valguris and Bacillus subtilis* in various concentrations of {3.125, 6.25, 12.5, 25, 50, 100, 200 and 400} micrograms per milliliter. A lower concentration was created by diluting the highest. There are remarkable positive results in the anti-bacterial analysis. To determine how well an anti-bacterial agent works, we need to know its minimum inhibitory concentration (MIC) value. A low MIC value could mean that the bioactive compound is very effective or that microorganisms will not likely become resistant to it. Figures 4 and 5 show what happens to *Proteus valguris and Bacillus subtilis* when the MIC value of each extract is changed by changing the concentration used. MIC values for *Proteus vulgaris and Bacillus subtilis* were between 100 and 400 [µg/ml].

The synthesis and accumulation of the natural plant products known as phytochemical in plants have been examined by [26] It is hypothesized that the extract's active components, including flavonoids, alkal -oids, and tannins, are what give it its antibacterial activity. The results of the study [27] which suggested that the active components in plant extracts are highly chemicals that prevent fungi from growing [28].

According to a study, the potently poisonous chemicals that make up plant extracts' active components prevent fungi from growing. Cellular mortality will result from non-specialized interactions between active chemicals in extracts and succinate dehydrogenase and NADH because they will impede the enzymes and cofactors necessary for vital metabolic processes. The active components of the extract, including flavones, alkaloids, and tannins, are thought to be the cause of its antibacterial capability. The results of [29].

Discuss the antibacterial properties of the phenolic component extraction and the therapeutic value of the M. sativa plant. Some molecules involved in secondary metabolism have phenolic rings with a single substitution and the highest possible degree of oxidation. The medicinal herbs include phenols, a powerful antibacterial compound [30]. The oxidized molecule may block enzymes, there may be a reaction with sulfhydryl groups, or there may be more general interactions with proteins that cause phenolic toxicity to microorganisms. [31] Carboxylic acids, which have been shown to be a potent antibacterial agent, were discovered to be associated to numerous antimicrobial and antifungal actions. These acids are known to exist in diverse plant metabolite molecular structures [32]. These results agree with [33], the phenolic extracts at concentration 500 mg/ml gave highest inhibition zone for leaves 25mm, fruits 19mm and barks 21mm against *S. aureus.*

The results showed the ability of ZnO nanop -articles to effect on *S.aureas* and *E.coli* when used in different concentrations and showed the ability of high concentration as compared to little concentration. ability of this Nano material to interact with organic compound of surface wall bacteria and destroy it. That led to destroy the cell wall and death of bacteria [34]. ZnO NPs antibacterial movement specifically relates with their focus as announced by a few examinations, in a similar manner, the action is estimating subordinate. In any case, this reliance is additionally impacted by convergence of NPs. Bigger surface region and higher fixation are responsible for ZnO NPs antibacterial action [35].

Conclusions

Phenol compound and Zno nanoparticles has height inhibition for Antimicrobial.

References

1. Atai Z, Atapour M and Mohseni M. (2009). Inhibitory effect of ginger extract on *Candida albicans*. American Journal of Applied Sciences, 6 (6), 1067-1069.
2. Sanata, D. Genc, G.E., Erturan, Z. (2010) The antifungal susceptibilities of oral candida spp. Isolation from HIV infected patient. African Journal of Microbiology Research, 4(6):466-470.
3. Taweechaisupapong S, Choopan T, Singha ra S, Chatrchaiwiwatana S, Wong kham S. (2005) In vitro inhibitory effect of Streblus asper leaf-extract on adhesion of Candida albicans to human buccal epithet -lial cells.

Journal Ethnopharma cology. Jan 4;96 (1-2):221-6.

4. Shepherd, M. G. (1986). The pathogenesis and host defence mechanisms of oral candidosis. New Zealand Dental Journal 82, 78–81.
5. Gadea, I., Cuenca, M., Gegúndez, M. I. et al. (1997). Effect of pH and buffer system on the in-vitro activity of five antifungals against yeasts.Journal of Antimicrobial Chemotherapy 39, 453–9.
6. Poulain, D.; Jouault, T. (2004). Candida albicanscell wall glycans, host receptors and responses: elements for a decisive crosstalk. *Current Op. Micro*. 7, 342-349.
7. KwonChung, K.J., Bennett, J.E. (1992) Medical Mycology. Lea and Febiger, Phila -delphia, London.
8. Mills, E., Jean-Jacques, D., Dan, P , Gede, K. (2006). Herbal medicines in preg -nancy and lactation . An evidences –based Approach, London and New York.
9. Bhat, S.; P. Maheshwari; S. Kumar & A. Kumar (2002). "Mentha species: In vitro Regeneration and genetic transformation". Molecular Biology Today. 3(1):
10. Livermore.D.M.(2003).Bacterialresistance:Origins, epidemiology and impact, Clin. Infect. Dis. 36 :11–23.
11. Fatima, A.; Modolo, L.V.; Conegero, L.S.; Pilli, R.A.; Ferreira, C.V.; Kohn, L.K. and Carvalho, J. E. (2006). Lactones and their derivatives : biological activities, mechan -isms of action and potential leads for drug design. Curr. Med.Chem. 13:3371-3384.
12. Dimitri, M. J. (1980) Encyclopedia Argen -tina of Agriculture and Gardener. Buenos Aires.
13. Tang, K. S., Konczak, I., and Zhao, J. (2016). Identification and quantification of phenolic in Australian native mint (*Mentha australis* R. Br.). Food Chem. 192, 698–705.
14. Leon, W. N.; Aly, S.; Jacques , S.; Dayeri, D. and Alfred S. Traore. (2012). In vitro Antimicrobial Activity of Some Phenolic Compounds (Coumarin and Quercetin) Against Gastroenteritis Bacterial Strains. International Journal of Microbiological Research .3 (3) . 2079-2093.
15. Kar, A. (2007). Pharmaocgnosy and Phar -macobiotechnology Revised- Expanded Se -cond Edition. New Age International Lim -ted Publishres New Delhi. pp 332-600.
16. Skalniy , A.V. and Rudakov, I. A. (2004) Bioelements in Medcine. M.: Onix Vol.21 : 272 pp. (in Russian).
17. Ramesh , P. ; Rajendran, A. ; Meena shisundarm (2014). Green Syntheis of zinc oxide Nanoparticles using flower Extract Cassia Auriculata . *Journal of Nano Scie -nce and NanoTechnology*. 41-45 pp.
18. Al-Ibrahemi .N ; AL-Yassiry, A. ; AL-Laith. Z. N. & Al-Musawi, B. H. (2022). Chemical Analysis Of Phytochemical For the *Anethum graveolens L*. Fresh And Commercial Dry By Gas Chromatography Mass- Spectrometer. IOP. Conference series: Earth and Environmental Science.
19. AL-Ibrahemi .N; Hasan. R. M. (2019). Identification of Artemisinin compound in Artemisia herba alba belong to the Aster -acea by HPLC and GC/MS. *Al-Kufa University Journal for Biology*. VOL.11 / NO.2.-2073 8854
20. AL-Ibrahemi .N; Hasan. R. M; Alslman. K. (2020). Efect of Zinc oxid nanop -articles on the oxidant stress (Malon aldehde MDA, lipid peroxidation level LPO) and antioxidant GSH glutation) Medico-Legal Update 20 (1), 882-888.
21. Nadia N.H.ALMasaoodi, Batool Shakir Abed Almjalawi , Dhuha Qasim ، Nibras Al-Ibrahemi (2022). Effect study volatile oil and phenol compound isolated from *Petroselinum sativum* L. on the *Tricho -phyton mentagrophytes and Micro -sporum canis* . IOP. Conference series: Earth and Environmental Science.
22. Wala almosawy , Roaa A. R. Al_Samak , Zaynab hayder alwtwt , Nibras Al-Ibrahemi (2022) . Evaluate the Antibac -terial of Zinc oxide nanoparticles and phenol extract from dried seeds of *Negilla Sativa* L. IOP. Conference series: Earth and Environmental Science.
23. Harborne, J. B. (1984). Phytochemical Methods.; A Guide to Modern Techniques of Plant Analysis, 2nd ed. Chapman and Hall, London.
24. Naser , N.K. ; ALMasoody , I.H. & Al-Ibrahemi, N. (2022) .Antibiotic and chemi -cal study for the petroselinum sativ -um L. that belong for Umbellifera family .*International Journal of Health Sciences* , (6) (S6),6066-6073.

25. Harley, J. H. and Prescott. (1996). Labora - tory exercises in microbiology. 3ed , USA. Pp :449.
26. Croteau, R. (1986). Biochemistry of mono - terpenes and sesquiterpenes of the essential oils. Herbs, spices and medicinal plants: recent advances in botany, horticulture and pharmacology. 1: 81-135. Oryx press, phoenix, Az.
27. Al-qertani, Y.M. (2012). Effect of plant extracts of some plants species on Afla toxin B1 produced by Aspergillus flavus. MSc Thesis, College of Education for Pure Sciences, Diyala University, Iraq.
28. Tegos, G., Stermilz, F. R., Lomovskaya , O., Lewis, K. (2002) .Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. Antimicrobial Agent Chemotherapy, 46(10):3133-3141.
29. Guenther, E. (1972). The production of essential oils: method of distillation, effenrage, maceration, and extraction with volatile solvents. In: Guenther, E.(ed.). the essential oils. History-origin in plants. Production Analysis. 1:85-188. Krieger publ. Co Malabar, FL.
30. Brantner, A. ; Males, Z. ; Papeljnjak, S. and Antolic, A, (1996). Antimicrobial acti -vity of paliurus spina – Christi mill. J. Eth - nopharmacol. 52:119-122.
31. Mason, T. L. and Wasserman, B. P. (1987). Inactivation of red beet beta-glucan synthase by native and oxidized phenolic compounds. Phytochemistry 26:2197-2202.
32. Sultana, T; Rashid, M.A; Ali, M.A, and Mahmood, S.F. (2010). Hepatoprotective and antibacterial activity of ursolic acid extracted from Hedyotis corymbosa L. Bangladesh J. Sci. Ind. Res. 4:27–34.
33. Al-Hadad, A A. S. (2017). Qualitative, quantitative and Antimicrobial activity study of some active compounds of Casuarina Cunninghamiana extracts. A Thesis Submitted to the Council of the Faculty of Science / University of Kufa.
34. Rizwan, W.; Young-Soon, K.; Amrita, M.; Soon, Y.; Hyung-Shik, S. (2010). Form -ation of ZnO micro-flowers prepared via solution process and their antibacterial activity, J. Nanoscale Res. Lett., 5(10) :1675–1681.
35. Peng, X.; Palma, S.; Fisher, N.S.; Wong, S.S. (2011). Effect of morphology of ZnO nanostructures on their toxicity to marine algae, Aquat. Toxicol., 102 (3):186-196.

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