Analysis of active biochemical compounds of *Pstroselinum crispum* and *Apium graveolens* alcoholic extracts by Gas chromatography-Mass Spectrometry (GC-MS)and their antibacterial activity

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Abstract

The present study aimed to evaluate the antibacterial activity of plant extracts and their effect on isolated human pathogen bacteria and comparison of the effect of the active compounds of *Petroselinum crispim* extract and *Apium graveolens* extract, The results of GC-Mass of hot alcoholic extract of *Petroselinum crispim* showed many active compounds through 10 peaks, but the highest area (8.47) of active compounds found in peak No.7 as (3,7-dimethyl-6-octenyl ester, Butanoic acid, Bicyclo[3.1.1]heptane and 2,6,6-trimethyl). While the results of hot alcoholic extract of *Apium graveolens* showed the highest area (8.79) of active compounds found in peak No.6 as (Fumaric acid, Propanoic acid, 2-methyl-4-methylphenyl ester and Benzene).

The study was conducted on 100 samples were collected between March 2022 and May 2022, where the number of positive samples reached 83, while the number of negative samples reached 17, by taking urine samples from various patients in Ibn Al-baladi Hospital in Baghdad. Two types of bacteria were isolated: Escherichia coli, which appeared in 46 samples, 37 samples from Staphylococcus bacteria, all bacterial isolates were identified.

The results showed that the hot alcoholic extract of *Petroselinum crispum* give better inhibition zone in concentration No.1 (9.66mm) than in concentration No.2 (8.33mm) on *staphylococcus aureus*. While the hot alcoholic extract of *Petroselinum crispum* give better inhibition zone in concentration No.2 (13.33mm) than in concentration No.1 (12mm) on *Escherichia coli*.

The hot alcoholic extract of *Apium graveolens* give better inhibition zone in concentration No.2 (10mm) than in concentration No.1 (9.66mm) on *staphylococcus aureus*. The hot alcoholic extract of *Apium graveolens* give better inhibition zone in concentration No.2 (13mm) than in concentration No.1 (11.66mm) on *Escherichia coli*.

Keywords: - (*Petroselinum crispum*, *Apium graveolens*, antibacterial, antimicrobial, phytochemical, active compound, *Escherichia coli*, *Staphylococcus aureus* , Gas chromatography-mass spectrometry, GC-MS).

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Introduction

Parsley is regarded as an aromatic, culinary, and medicinal plant and is used in the cosmetic, food, and pharmaceutical industries. However, few studies with conflicting results have been conducted on the antimicrobial activity of parsley essential oil (1, 2 , 3).

Herbal plants have long been used in medicinal applications by human civilization until now, various herbal plants are known to have pharmacological effect in the teatment of a variety of disorders. Celery is one of the herbal plants that can be used as a therapy (4 , 5 , 6).

Material and Methods

Plant Sample collection -

From October 2021 to December 2022, samples of *Apium graveolens* (celery) and *Petroselinum crispum* (parsley) were taken from the local market. The plant leaves dried and grinded in to powder from by mechanical grinder, it kept at 4°C until further investigation.

- Hot Alcoholic Plant Extract preparation

To extraction more active principle compounds from the *Apium graveolens* (Celery) and *Petroselinum crispum* (Parsley), soxhlet extraction had used. Known amount (25g) powder of both plants separately was filled into the Soxhlet apparatus. A cotton plug was used at the place of thimble to stop the entry of the crude material into the Siphoning tube. The required solvent chloroform was filled up ten times more than total amount of the sample material into the flask of the apparatus. The apparatus was then connected with the water supply to the condenser.

The temperature of the heating mantle was maintained at boiling point of solvents. The process was carried out for 5 to 6 hours for each sample. The extract was transferred to Petri plates and solvent was allowed to evaporate. The evaporated material was weighed to calculate yield percentage and then, stored in the refrigerator for further use (7).

- Gas Chromatography- Mass Spectrometry analysis

The Industrial Research and Development Authority's Ibn Al-Bitar Research Center used the Shimadzu GC-2010 Plus and Shimadzu GCMS-Q2010 Ultra to perform a GC-MS analysis. Capillary column from Gl Sciences in Japan (Inert Cap 1MS, 0.25mm, 30m, 0.25m). Helium is

the carrier gas, and the auto injector is a Shimadzu AOC-20i, with a constant flow rate of 1 ml/min. 5 l of injection volume.

Pillar oven 100 °C is the temperature. Oven temperature was adjusted to 100 °C for three minutes, 240 °C for nine minutes, 280 °C for five minutes, and 300 °C for two minutes. It stood at 15.

- Bacterial sample collection

One hundred samples were collected from patients of Ibn Al-baladi Hospital in Baghdad. The samples include female with ages ranging between 18-35years, were collected during study period from March 2022 to May 2022.

The samples were collected in sterile condition and labeled with the information of patients, time of collection, and date. The samples collected by using sterile urine cups.

- Preparation of culture media

All media were prepared according to their manufacturer's instructions, autoclaved at 121°C, 15 pounds/Inch2 for15 minutes and distributed into sterile Petri dishes or tubes, and left to solidify at room temperature. The Petri- dishes were incubated at 37°C for 24 hours, to ensure sterilized. Some of these media need special additives. After that they were ready to use for isolation of bacteria or kept at 4°C in refrigerator (9).

Antimicrobial activity of plant extracts using well diffusion method

Agar well-diffusion method was used to determine the antimicrobial activity of plant extracts. Mueller-Hinton agar plates are swabbed by bacterial colonies (sterile cotton swabs). Wells (9mm diameter) were made in each of these plates using a sterile blue micropipette tips.

Different concentrations of plant extracts are then placed and controlled. Controlled use in this experiment (chloroform for alcoholic extract, distle water for water and nano extract), were added by sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs .Then the plates were incubated at 37°C for 18-24 h for bacterial pathogens.

The diameter of the inhibition zone (mm) was measured. The experiment was repeated three times, readings were taken in three different fixed directions for each replicate and the average values were recorded (10).

Results

Active compounds profiling using gas chromatography-mass spectrometry

1. Hot alcoholic extract of *Petroselinum crispum*

Table 1: Active compounds of hot alcoholic *Petroselinum crispum* extract.

Peak	Active compounds	R.T. min	Area %
1	Trichloromethane	7.974	0.32
2	Ethane Methane	13.638	0.24
3	3-Methyl-2-butenoic acid Phenyl ester	14.820	0.23
4	Phenol	15.756	0.43
5	Oxalic acid Isobutyl tetradecyl ester Dichloroacetic acid Trichloroacetic acid Tridecyl ester	16.370	0.22
6	Dichloroacetic acid Heptadecyl ester 2- Chloropropionic acid Pentadecyl ester Carbonic acid Hexadecyl 2,2,2-tri chloroethyl ester	19.547	0.69
7	3,7-dimethyl-6-octenyl ester Butanoic acid Bicyclo[3.1.1]heptane 2,6,6-trimethyl	20.181	8.47
8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol 9-Octadecyne	20.540	1.11
9	Butanoic acid 3,7-dimethyl-6-octenyl ester	20.843	1.24
10	Benzene 1-ethenyl-4-methoxy Benzofuran 2,3-dihydro-2-methyl	22.488	1.55

	Trichloroacetic acid Pentadecyl ester		

2. Hot alcoholic extract of *Apium graveolens*

Table 2: Active compounds of hot alcoholic *Apium graveolens* extract.

Peak	Active compounds	R.T. min	Area %
1	Dichloroacetic anhydride Spiro[bicyclo[3.3.0]octan-6-one-3-cyclopropane] Oxalic acid cyclohexyl nonyl ester	13.496	0.68
2	Naphthalene Decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-[4aR-(4a.alpha.,7.alpha.,8a.beta.)] 1,2,3,4,4a,5,6,8a-octahydro-4a, 8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)] .alpha.-Guaiene	14.933	2.76
3	Phenol 2,4-bis(1,1-dimethylethyl)	15.765	0.26
4	Cyclopropane 1-ethenyl-2-hexenyl[1.alpha.,2.beta.(E)] 5-Undecen-3-yne	18.366	1.28
5	2-Butenoic acid 4-phenoxy-methyl ester 3-Butenoic acid 3-Methyl-2,3- dihydrobenzofuran-3-carboxylic acid methyl ester	18.867	1.57

6	Fumaric acid Propanoic acid 2-methyl-4-methylphenyl ester Benzene	19.727	8.79
7	8-Hexadecyne 3- Octadecyne 9- Octadecyne	20.153	8.54
8	2-Pyridinamine N-methyl-meso-Hydrobenzoin Phenol	20.521	1.02
9	Methylphenyl ester Chloroacetic acid Tricyclo[3.3.0.0(2,8)]octan-3-one	20.796	0.80
10	2- Chloropropionic acid hexadecyl ester 1-Heneicosyl formate Heptafluorobutyric acid Pentadecyl ester	22.375	0.30

Macroscopic examination

All samples cultured by blood ager and MacConky ager, under aseptic technique, then incubated under aerobic conditions at 37 °C/ 24-48 hrs. Morphological and biochemical characterized a pure colony after growth are important for distinguishing various types of bacteria. Inspection of pure colony growth, morphology, size, and hemolysis ability on blood ager and lactose fermentation on MacConkey (11).

- Microscopic examination

A single colony from the agar was taken by loope and drop of water spread on clean slid with fixing by heat, and then staining with gram stains and then examined the bacteria cell under oil immersion by using light microscope to detect their response to the stain, sizes, shapes, and arrangement of the cells (9).

Antimicrobial activity of *Petroselinum crispum* and *Apium graveolens* extracts on *Staphylococcus aureus*

-Effect of alcoholic extracts on *Staphylococcus aureus*

Table3: Inhibition zone of alcoholic extract Concentration No. 1 and No.2 on *Staphylococcus aureus*

Extract	Mean ± SE		LSD value
	Concentration-1 50mg/ml	Concentration-2 25mg/ml	
Ext. 1	9.66 ±0.33 A a	8.33 ±0.33 B b	1.308 *
Ext. 2	9.66 ±0.33 A a	10.00 ±0.57 A a	1.851 NS
LSD value	1.331 NS	1.332 *	---

Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).

Ext.1=Hot *Petroselinum crispum* alcoholic extract.

Ext.2=Hot *Apium graveolens* alcoholic extract.

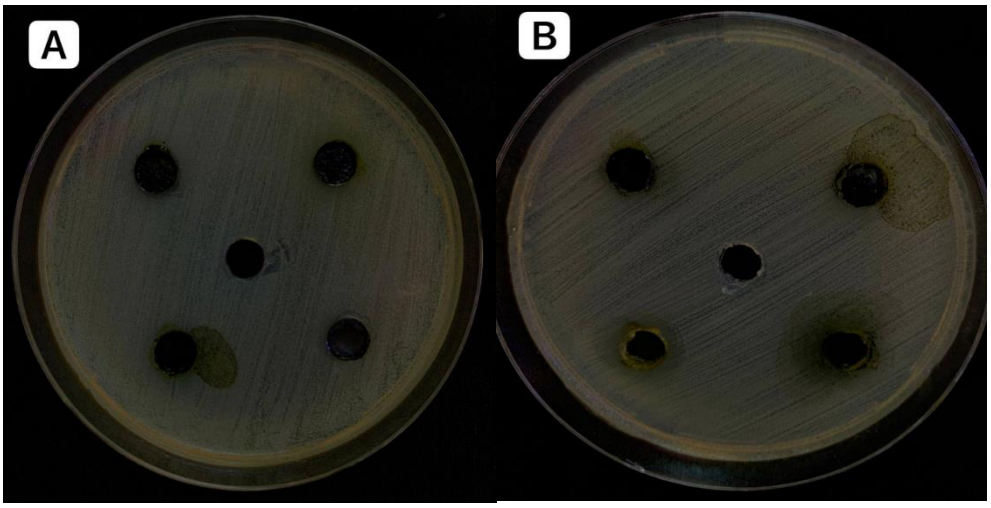

Figure1: (A) Effect of alcoholic extracts (Con.1) on *Staphylococcus aureus.* (B) Effect of alcoholic extracts (Con.2) on *Staphylococcus aureus.*

Antimicrobial activity of *Petroselinum crispum* and *Apium graveolens* extracts on *Escherichia coli*

- Effect of alcoholic extracts on *Escherichia coli*

Table4: Inhibition zone of alcoholic extract Concentration No. 1 and No.2 on *Escherichia coli*

Extract	Mean ± SE		LSD value
	Concentration-1	Concentration-2	

	50mg/ml	25mg/ml	
Ext. 1	12.00 ±0.57 B a	13.33 ±0.66 A a	2.448 NS
Ext. 2	11.66 ±0.88 B a	13.00 ±0.57 A a	2.926 NS
LSD value	1.718 *	2.033 NS	---

Means with different big letters in the same column and small letters in the same row are significantly different. * (P≤0.05).

Ext.1=Hot *Petroselinum crispum* alcoholic extract.

Ext.2=Hot *Apium graveolens* alcoholic extract.

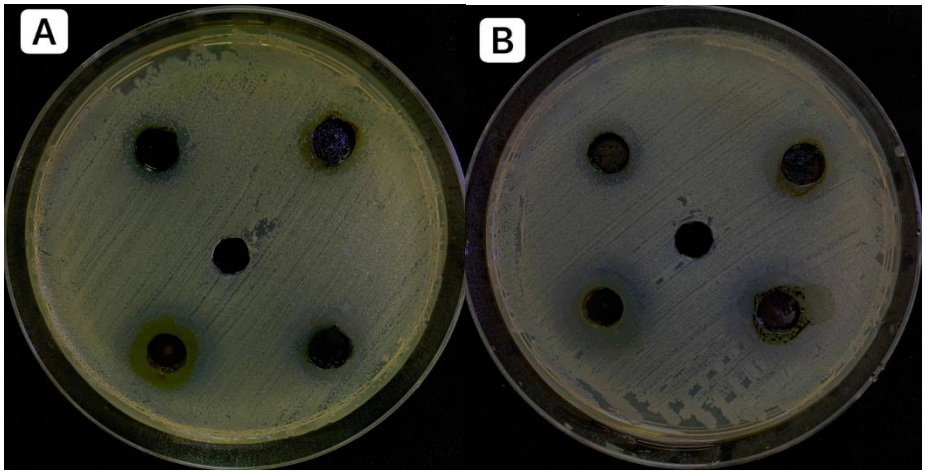

Figure2: (A) Effect of alcoholic extracts (Con.1) on *Escherichia coli*.

(B) Effect of alcoholic extracts (Con.2) on *Escherichia coli.*

References

1. Gutierrez J, Rodriguez G, Barry-Ryan C and Bourke P (2008). Efficacy of plant essential oils against foodborne pathogens and spoilage bacteria associated with ready-to-eat vegetables: antimicrobial and sensory screening. J. Food Prot. 71: 1846-1854.
2. Viuda-Martos M, Mohamady MA, Fernández-López J, Abd ElRazik KA, et al. (2011). In vitro antioxidant and antibacterial activities of essentials oils obtained from Egyptian aromatic plants. Food Contr. 22: 1715-1722.
3. Teixeira B, Marques A, Ramos C, Neng NR, et al. (2013). Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind. Crops Prod. 43: 587-595.

4. Khairullah AR, Solikhah TI, Ansori ANM, Fadholly A, Ramandinianto SC, Ansharieta R, Widodo A, Riwu KHP, Putri N, Proboningrat A, Kusala MKJ, Rendragraha BW, Putra ARS, and Anshori A. A review of an important medicinal plant: Alpinia galanga (L.) Willd. Systematic Reviews in Pharmacy. 2020; 11(10): 387-395.
5. Solikhah, T. I., Setiawan, B., & Ismukada, D. R. Antidiabetic activity of papaya leaf extract (Carica Papaya L.) isolated with maceration method in alloxan-induces diabetic mice. Systematic Reviews in Pharmacy. 2020: 11(9): 774–778.
6. Kumar S, Paul S, Walia YK, Kumar A, Singhal P. Therapeutic potential of medicinal plants: A review. Journal of Biological and Chemical Chronicles. 2015; 1(1): 46-54.
7. Salhi N.; Saghir S. A.M.; Terzi V.; Brahmi I.; Ghedairi N. and Bissati S. (2017). Antifungal Activity of Aqueous Extracts of Some Dominant Algerian Medicinal Plants. BioMed Research International Volume 2017; Article ID 7526291; 6 pages.
8. Harbone J. B. (1984). Phytochemical methods. A guide to modern techniques of plant analysis. 2nd ed.; Chapman and Hall. London. pp: 288.
9. Markey, B.K., Leonard, F.C., Archambault, M. Cullinane, A. and Maguire, D. (2014). Clinical Veterinary Microbiology. 2nd ed. Mosby Elsevier. Pp.161.
10. Sia, C. and Yim, H. A.(2010). Commercial virgin coconut oil:assessment of antimicrobial potential. Asian Journal of Food and Agro-Industry, Volume 3, pp. 567- 579.
11. Khan, F., Rizvi, M., Shukla, I. and Malik, A. (2011) A novel approach for identification of members of Enterobacteriaceae isolated from clinical sample. Biology and Medicine Journal. 3 (2): 313 – 319.