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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 crispum* extract and *Apium graveolens* extract, The results of GC-Mass of hot aqueous extract of *Petroselinum crispum* showed many active compounds through 10 peaks, but the highest area (7.37) of active compounds found in peak No.5 as (Trolamine and Benzofuran). While the results of hot aqueous extract of *Apium graveolens* showed the highest area (3.83) of active compounds found in peak No.1 as (Pentanoic acid).

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 and 37 samples from *Staphylococcus* bacteria, all bacterial isolates were identified.

The results showed that the hot aqueous extract of *Petroselinum crispum* give better inhibition zone in concentration No.1 (10mm) than in concentration No.2 (7mm) on *staphylococcus aureus*. The hot aqueous extract of *Petroselinum crispum* give better inhibition zone in concentration No.1 (7.33mm) than in concentration No.2 (7mm) on *Escherichia coli*.

The hot aqueous extract of *Apium graveolens* give better inhibition zone in concentration No.1 (9.66mm) than in concentration No.2 (7.66mm) on *staphylococcus aureus*. The hot aqueous extract of *Apium graveolens* give the same inhibition zone in concentration No.1 and concentration No.2 (8.33mm) on *Escherichia coli*.

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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 treatment 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 Water Plant Extract preparation

For preparing the hot water extract 50 g of both plants powder was taken and placed in a beaker, add 500 ml of boiling distilled water and then placed in the shaking incubator at 28° C for 24 hours.

The extracts were then separated from the sample residue by filtration through Whatman No.1 filter paper, the extracts were poured into glass plates to allow the water to evaporate for drying and then placed in a sealed tube and kept in the refrigerator at 4°C until use (7,8).

- Gas Chromatography- Mass Spectrum 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.

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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 for 15 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 water extract of *Petroselinum crispum*

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

Peak	Active compounds	R.T. min	Area%
1	6H-Purin-6-one	3.767	2.17
	2-amino-1,7-dihydro-1-methyl		
	1,2,3-Oxadiazolium		
	3-[2-(2,4-dimethoxyphenyl)ethyl]-5-hydroxy-hydroxide		
	Inner salt 3-(4-Nitrophenyl)propan-1,2-diamin		
2	3,4-Dihydro-6-nitrocoumarin	4.012	1.10
	Benzyl alcohol		
	Benzyldimethylsilyl ether		
	Dimethyl(isopropyl)silyloxymethyl Benzene		
3	N-Allyl-N-ethylformamide	4.268	2.03
	Piracetam		
	Cyclohexane		
4	Cyclooctanone	4.400	3.31
	Piperidine		
	2,3-dimethyl-6-Amino-hex-2-en-1-ol		
5	Trolamine	5.629	7.37
	Benzofuran		
6	Benzeneacetamide	5.941	5.59
	N-(phenylacetyl)		
	Glutarimide		
	N-(2-phenylpropyl)-4-trimethylsilylmethylbenzamide		
7	3,3-Diisopropoxy-1,1,1,5,5,5-hexa	6.168	4.61
	Methyltrisiloxane		
	1,2-Benzisothiazol-3-amine		
	1,4-Bis(trimethylsilyl)benzene		
8	3-Ethylheptanoic acid	6.386	1.84
	D-Gluco-heptulosan N-Cyclododecylacetamide		

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9	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane Cyclotetrasiloxane	9.355	2.78
10	Pentanoic acid 1- methylethyl ester Butanoic acid propyl ester 5-Hexyl-1,3-oxathiolan-2-one	11.038	0.79

2. Hot water extract of *Apium graveolens*

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

Peak	Active compound	R.T. min	Area%
1	Pentanoic acid	6.168	3.83
2	Pentanoic acid	6.802	1.08
	Silver butanoate		
3	Pentanoic acid	7.766	0.93
	Silver butanoate		
4	Ethyl .alpha.-d-glucopyranoside	9.336	3.09
5	Heptanoic acid	9.534	0.63
	Hexanoic acid		
	.beta.-D-Glucopyranoside		
6	Pentanoic acid	10.858	0.94
	.beta.-Methyl xyloside		
	Ethyl .beta.-d-riboside		
7	.alpha.-d-Ribopyranoside	11.028	1.17
	.beta.-D-Ribopyranoside		
8	Octanoic acid	12.522	0.90
	Acetylcysteine		
	Methyl isopropylidene-.beta.-d-arabinoside		
9	Octanoic acid	14.716	1.42
	N-Acetyl-d-serine		
	2-Acetylamino-3-hydroxy-propionic acid		
10	Octanoic acid	15.444	1.18
	Tridecanoic acid		
	Acetamide		

- 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*

1. Effect of aqueous extracts on *Staphylococcus aureus*

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

Extract	Mean ± SE		LSD value
	Concentration-1	Concentration-2	
Ext. 1	10.00 ±0.57 A a	7.00 ±0.04 A b	1.603 *
Ext. 2	9.66 ±0.33 AB a	7.66 ±0.67 A a	2.069 NS
LSD value	1.631 *	1.331 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* water extract.

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

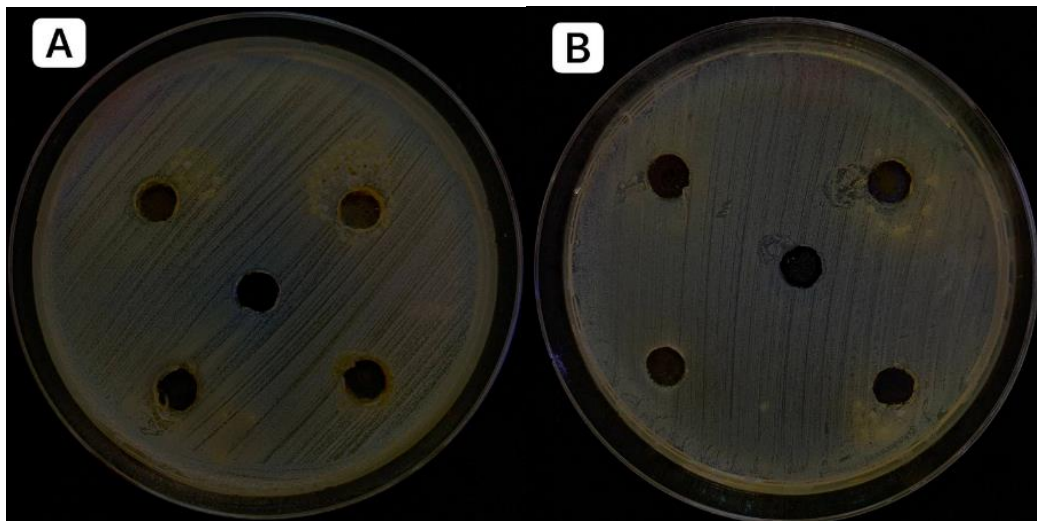


Figure1: (A) Effect of aqueous extracts (Con.1) on *Staphylococcus aureus*. (B) Effect of aqueous extracts (Con.2) on *Staphylococcus aureus*.

Effect of aqueous extracts on *Escherichia coli*

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

Extract	Mean \pm SE		LSD value
	Concentration-1	Concentration-2	
Ext. 1	7.33 \pm 0.33 B a	7.00 \pm 0.05 B a	0.925 NS
Ext. 2	8.33 \pm 0.33 AB a	8.33 \pm 0.33 A a	1.308 NS
LSD value	1.087 *	1.087 *	---

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

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

Ext.3=Hot *Apium graveolens* water extract.

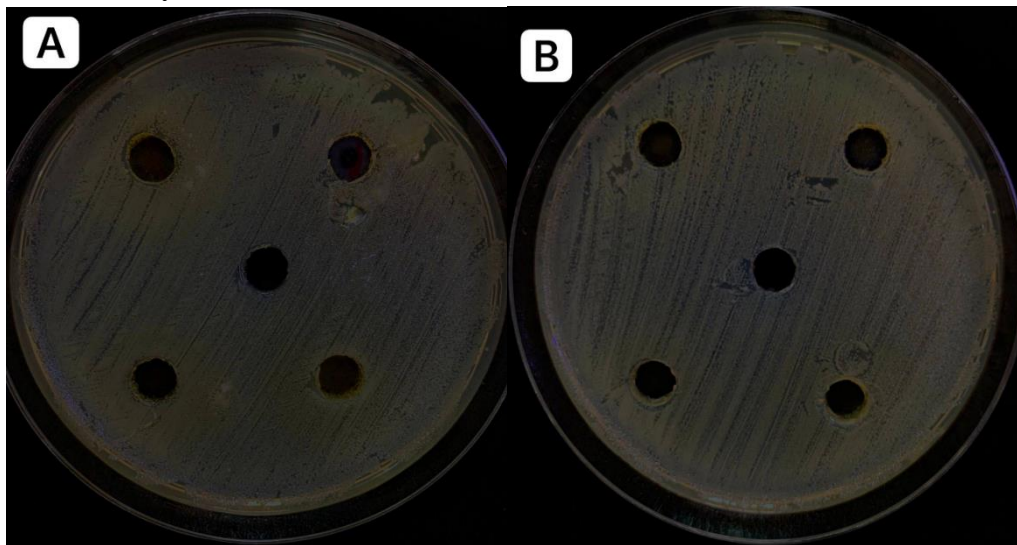


Figure 2: (A) Effect of aqueous extracts (Con.1) on *Escherichia coli*. (B) Effect of aqueous extracts (Con.2) on *Escherichia coli*.

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