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CHEMICAL COMPOSITION AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *PETROSELINUM CRISPUM* (MILL) NYM ESSENTIAL OIL ISOLATED FROM MAYSAN CITY IRAQ

Dr. Rashid Rahim Hateet Alattwani
Ph.D. Bioscience, Dean Assistant,
College of Pharmacy, University of Maysan, Maysan,
Iraq
Masterr78@ymail.com

Dr. Rasha Khalil Abudaljalil
Alsaad Lecturer, M.Sc. Parasitology,
College of Pharmacy & College of Medicine,
University of Maysan, Maysan,
Iraq
Ahmeds201258@yahoo.com

Dr. Redha Alwan Hasan Alhashimi
M.B. Ch.B., F.I.B.M.S,
Department of Medicine, College of Medicine,
University of Maysan, Maysan,
Iraq
Ahmedsalihdr2008@yahoo.com

ABSTRACT
Antimicrobial activities of Petroselinum crispum essential oil was studied by disc agar well diffusion technique against tested organisms (Esherichia coli, Staphylococcus aureus, Psedomonas aeruginosa, Streptococcus pyogenes, Bacillus cerrus and Proteus vulgaris), The essential oil of Petroselinum crispum showed activity against all bacterial The inhibition zones exhibited by essential oil ranged between 12-40 mm diameter except P. aeruginosa was inactive. MIC test revealed that the essential oil of Petroselinum crispum exhibited a minimal inhibition values ranged between 200 -550 ug/L. The MBC test revealed that the essential oil of Petroselinum crispum exhibited a minimal inhibition values ranged between 250-600 ug/L. A verification of non-toxicity of the fungal extract against human blood revealed a negative test. The results were also showed that the essential oil of Petroselinum crispum could be an alternative source of antimicrobial agents against clinical pathogens. The DPPH free radical scavenging assay. Results showed that the parsley oil Petroselinum crispum possessed a certain degree of antioxidant activities.

Keywords: Essential oil, antibacterial activity, Petroselinum crispum.

1. INTRODUCTION
Medicinal plants form a sizeable component of traditional medicine and are mainstay for about 80% of the people in developing nations. Biologically active compounds found in plants appear to be more adaptable, acceptable and safer than synthetic compounds and display a wealthy source of potential pathogens control agents. Medical plants have used for years in daily life to treat diseases all over the world and all the researchers are looking for them. Essential oils are volatile organic compounds found in various plant tissues such as fruits, leaves, flowers, bark, stem, seeds, wood and roots. The quality of essential oils depends on the several factors including

the part of the plant used, the plant variety and its country of origin, the method of extraction and the refining process. Essential oils have a long history of use as natural microbial agents. Essential oils have recently been used in a number of pharmaceutical, food, and cosmetic products because these oils effectively inhibit the growth of a wide range of microorganisms, and they cause fewer side effects than synthetic antimicrobial agents in humans. Despite the widespread use of essential oils by humans, little is known about the exact mechanism of their antimicrobial action. The development of resistance to the antibiotics by the pathogens increases mortality and severe effects. The increased usage of specific antimicrobials correlates with the increased levels of bacterial resistance to those agents. Thus, many researchers have recently attempted to identify the antimicrobial properties of essential oils. Free radicals cause the oxidation of bio molecules which leads to cell injury and death. This has raised the possibility that antioxidants could act as prophylactic agents. It has long been recognized that naturally occurring substances in higher plants have antioxidant activities. Antioxidants are also important to the food industry. Many foods are subject to many factors that lead to the quality deterioration. Among these undesirable factors, lipid auto oxidation is one of the most concerned. Although there are some synthetic antioxidant compounds such as butylated hydroxyl toluene (BHT), this compound is associated with some side effects. Many studies have been shown that the presence of natural antioxidants from various aromatic and medicinal plants is closely related to the reduction of chronic diseases such as DNA damage, mutagenesis, and carcinogenesis.

2. MATERIALS AND METHODS

2.1 Plant material

Fresh leaves were collected during the flowering stage of *Petroselinum crispum* leaves came from the Maysan city at south of Iraq.

2.2 Essential oil extraction

The plant was chopped into small pieces (100 g) and subjected to simple hydro distillation for 3 hours using a Clevenger type apparatus then obtained essential oil was dried with anhydrous Na2SO4, and stored in a sealed dark glass under refrigeration conditions. The average yield of essential oil (ratio final volume of essential oil / weight of fresh vegetal material) from four ended distillations (200 g each) was 0.89%.

2.3 The test microorganism

Esherichia coli from urine, Staphylococcus aureus from, Streptococcus pyogenes from Throat, Pseudomonas aeruginosa and Klebsiella pneumoniae from burns were isolated from clinical cases in Al-Sadder Hospital and identified in their laboratories. All tests were performed in triplicate with two growth controls negative hexane (negative control). The organisms were cultured on maintenance media until use.

2.4 Antimicrobial assay

The antibacterial properties of the essential oils were done using the agar disk diffusion method. Bacteria were grown in 20 ml nutrient broth at 37°C overnight. The cultures were then diluted to the McFarland No.5 standard (1.0×10^8 CFU/ml). Standard Petri dishes containing nutrient agar were then inoculated with the bacteria suspension (1.0×10^8 CFU/ml). Sterile paper disks (6 mm) were placed on the inoculated plates and 10 µl of 10 mg/ml of the essential oils in 1% DMSO were added to the paper disk. The plates were then incubated at 37°C for 24 hours and the zone of inhibition measured. Tests were performed in triplicate and the mean values reported [9].

2.5 Minimum inhibitory concentration and the minimum bactericidal concentration

Minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were evaluated at concentrations between 20 µg/mL and 800 µg/mL. To this end, micro titer plates were capped and incubated at 37º C, for 24 hours following this incubation period, 15 mL of a resazurin (Sigma) solution at 0.02 % in sterile distilled water was added to each well, for analysis of the results. This system facilitates microbial growth detection; the blue color indicates the absence of microbial growth, while the red color indicates growth of viable cells. For determination of the Minimal Bactericidal Concentration (MBC), before the addition of resazurin an aliquot of the inoculum was aseptically removed from each well presenting no apparent growth, and then plated on to agar Mueller-Hinton supplemented with sheep blood (5%); the plates were incubated as previously described.

2.6 Cytotoxicity Studies by Haemolytic Activity

Hemolytic activity was done as described previously [10]. The hemolytic activities of the essential oil were determined on human red blood cells. Human erythrocytes from healthy individuals were collected in tubes containing EDTA as anticoagulant. The erythrocytes were harvested by centrifugation for 10m at 3000 rpm and washed three times in PBS. To the pellet, PBS was added to yield a 10% (v/v) erythrocytes/PBS suspension. The 10% suspension was then diluted 1:10 in PBS. From each suspension, 100 µL was added in triplicate to 100 µL of a different dilution series of essential oil in the same buffer in eppendorf tubes. Fluoride was used as positive control. Total hemolysis was achieved with 1% Triton X-100. The tubes were incubated for 1 h at 37◦C and then centrifuged for 10m at 2000 rpm at 20º C. From the supernatant fluid, 150 µL was transferred to a flat-bottomed micro titer plate, and the absorbance was measured spectrophotometrically at 450 nm. The hemolysis percentage was calculated by following equation:

Hemolysis % =((A450 of test compound treated sample - A450 of buffer treated sample)/(A450 of 1% TritonX-100 treated sample - A450 of buffer treated sample))×100

2.7 Gas chromatography–mass spectrometry (Gc-mass)

The distilled Plant oil was subsequently analyzed using a Gas chromatography-mass spectrometer (GC-Ms) to evaluate of branches oil quality. The GC-Ms analysis of the essential oil samples was carried out using gas chromatography mass spectrometry instrument stands at the lions Ins in India.

2.8 Antioxidant activity

The antioxidant capacity of the crude essential oil sample was initially determined by the DPPH (2,2-diphenyl-1-1-picrylhydrazyl radical) free radical scavenging assay to determine the free radical scavenging activity of the crude essential oil sample [11]. The reaction mixture containing 0.1 ML of sample dissolved in methanol, 0.3 mL of methanol, and 0.4 mL of 0.3 µM DPPH reagent dissolved in methanol was shaken and incubated in the darkness at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured spectrophotometrically at 517 nm. The scavenging effect of DPPH free radical was calculated by using the following equation:

Scavenging effect % = (1-(absorbance of sample)/(absorbance of control))×100

2.9 Statistical analysis

Statistical analysis of data. Analysis of variance (ANOVA) was used to determine the significance ($p \leq 0.05$) of the data obtained in all experiments.

3. RESULTS AND DISCUSSION

The essential oils are natural plant products and their formation and accumulation in plants have been reviewed by [12]. The essential oils from aromatic plants are for the most volatile part and thus lead themselves to several methods of extraction such as solvent extraction and water and steam distillation [13].The results of the antibacterial susceptibility test of the *P. crispum* essential oil are shown in tables 1, by the inhibition zone diameter (IZD) varied according to bacteria strains . The maximum zone of inhibition was found to be 40.0 mm in diameter against S. aureus followed by E. coli with inhibition zone diameter (IZD) 29.0 mm while the minimum zone of inhibition was found to be 12.0 mm in diameter against *B. mirailis* and inactive against *P. aergunosa* , the antibacterial activity of P. crispum essential oil perhaps because contain on some compounds as alkaloids, anthraquinones, glycosides, flavonoids, tannins, steroids, phlobatanins and triterpenoids. Other studies refer to the antibacterial activity of P. crispum essential for example [14]. The lack of antibacterial activity in *P. crispum* essential oil may also be due to absence or denaturation of some active components of essential oil which are responsible for the bacteriostatic or bactericidal activities [15].

The essential oil obtained from the leaves of the plant species *P. crispum* furnished MIC 550µg/mL and MBC 600 µg/mL for all the tested bacterial strains, except for *S. aureus*, which yielded MIC 200µg/mL and MBC 250µg/mL, which resulted in a bacteriostatic effect. Therefore, the essential oil obtained from *P. crispum* did not furnish promising results against the selected bacteria. [16] described that the essential oil of a plant can only be considered promising when MIC less than 100 µg/mL is achieved table 2. The oil antimicrobial activity could be attributed to the presence of compounds like 1,3-benzodioxole, 4-methoxy-6-(2-propenyl), α and β pinene and other compounds which have been reported [15] to have antibacterial properties.

Haemolytic activity on human erythrocytes was studied. Human red blood cells provide a handy tool for toxicity studies of the essential oil, because they are readily available, their membrane properties are well known, and their lysis is easily monitored by measuring the release of hemoglobin [16]. A verification of non-toxicity of the essential oil against human blood revealed a negative test. A result of GC/MS analyses 7 compounds have been identified in essential oil of *P. crispum* shown in Figure 2, table 3.

The DPPH radical scavenging method was used to evaluate the antioxidant property of essential oil *P. crispum.* in comparison with natural antioxidant, BHT. The concentrations of the essential oil required to scavenge DPPH showed a dose dependent response. Free radical scavenging capacity of the extract was found to be increased with increasing concentrations. It was observed that the scavenging activity of essential oil *P. crispum* at all concentrations from 1.95 to1000 µg/ml is rather strong (18 -80 %). The essential oil improved 80% inhibition at higher concentrations, indicating lesser antioxidant capacity than positive control Figure (3).

Table 1. Growth inhibition zones (mm diameter) exhibited by the essential oil of *P. crispum* against six isolates of bacteria

Bacterial strains	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. pyogenes*	*B. cerrus*	*P. vulgaris*
Essential oil	29.0	40.0	0	28.0	12.0	22.0

Table 2. The minimal inhibitory concentrations (MIC and MBC) of essential oil *P. crispum* against six isolates of bacteria

Bacterial isolates	MIC (µg/mL)	MBC (µg/mL)
E. coli	500	550
S. aureus	200	250
P. aeruginosa	-	-
S. pyogenes	550	600
B. cerrus	550	600
P. vulgaris	550	600

Table 2. Chemical composition of crude essential oil of *P. crispum*

Tension time (min)	Compounds
2.75	1R-α-pinene
3. 27	β- pinene
3.68	β- phellandrene
4.15	Styrene, p, α-dimethyl
5.76	2-caren-4-ol
7.40	1,3-benzodioxole,4-methoxy-6-(2-propenyl)
8.42	1,3-benzodioxole,4,7-dimethoxy-5-(2-propenyl)

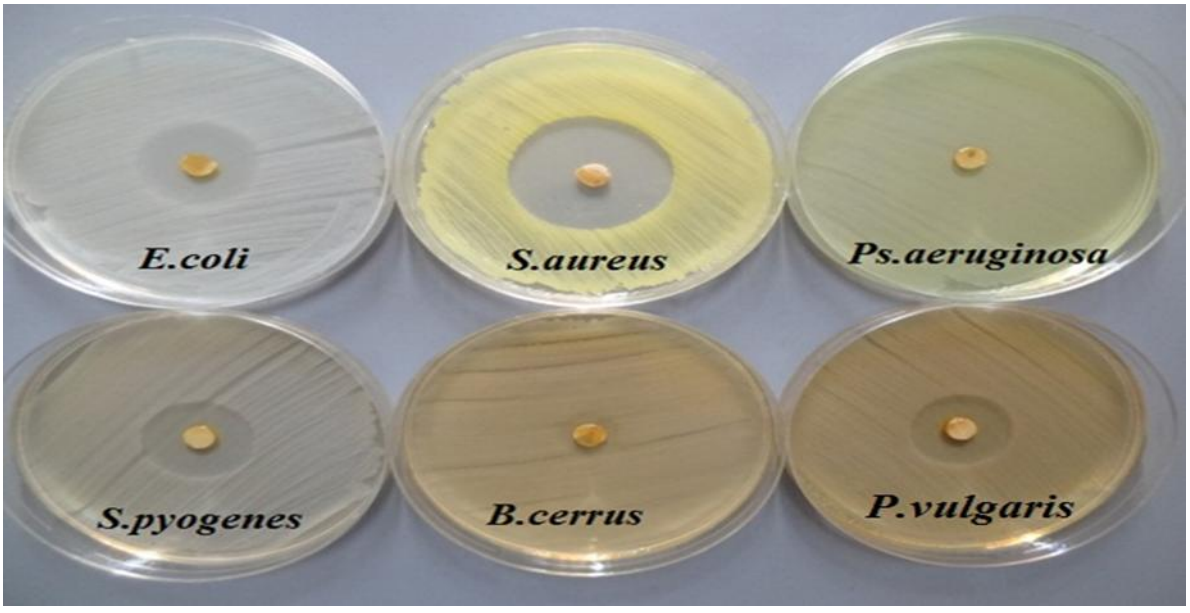

Fig 1: Inhibition zone diameter (IZD) by *P. crispum* essential oil

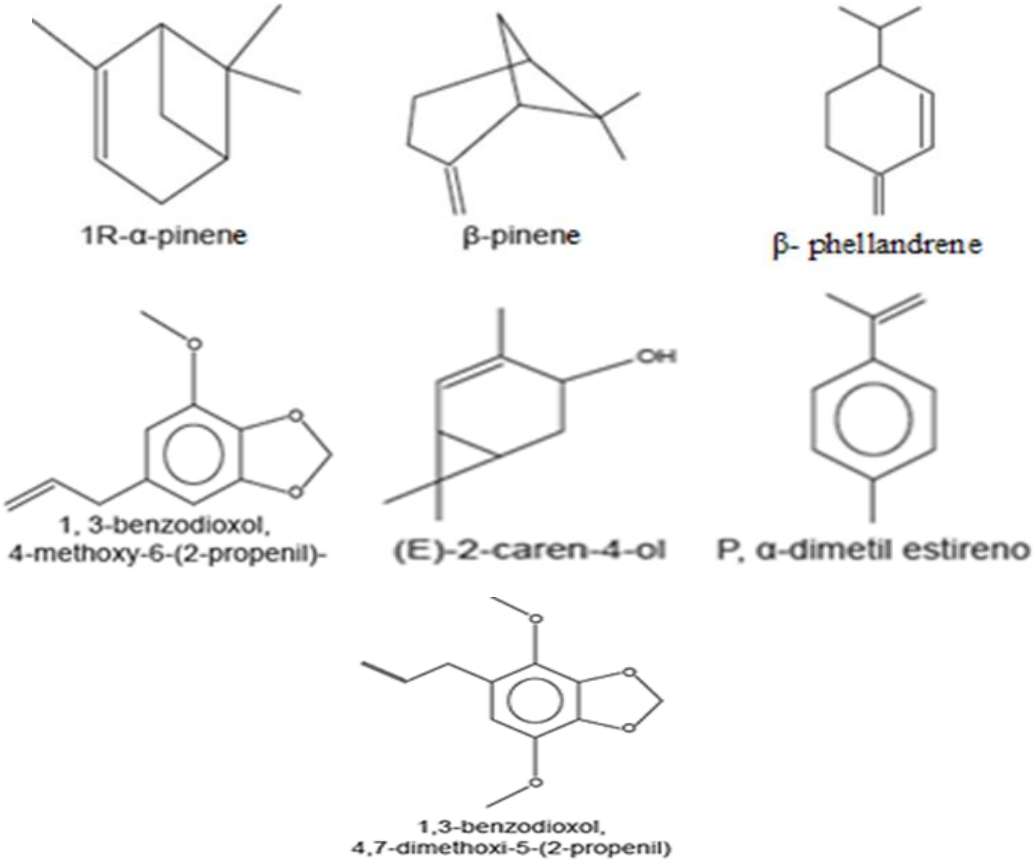

Fig 2: Chemical compounds of the crude essential oil *P. crispum*

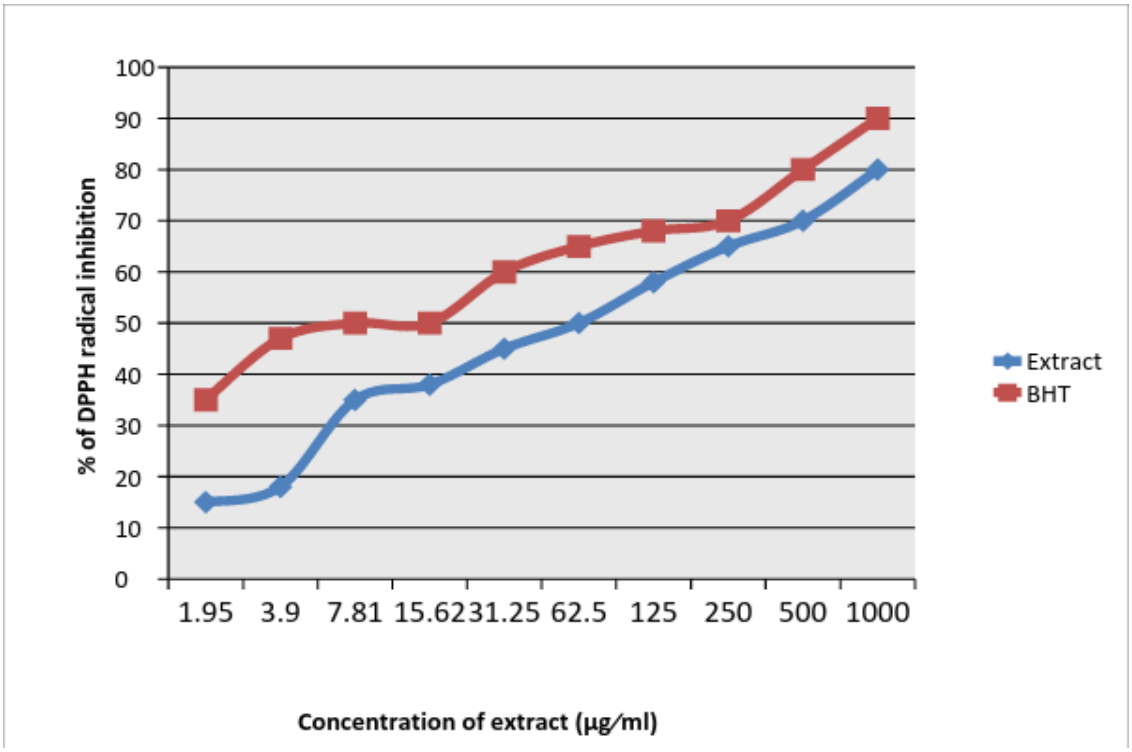

Fig 3: Free radical scavenging activity of *P. crispum* essential oil and positive controls (BHT)

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