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Gas Chromatography-Mass Analysis and Evaluation of Antibacterial Activity of Mint and Basil Essential Oil

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Abstract: Essential oils have grown in popularity in recent years due to their antimicrobial qualities. The aim of study to analyses the chemical component evaluate the antibacterial activities *Mentha arvensis*, Mint essential oil (MEO) and *Ocimum basilicum*, Basil essential oil (BEO), and compares the essential oil's effects in inhibition of *Staphylococcus. aureus* bacteria that responsible for enterotoxin production. Water distillation method used for MEO and BEO extraction, then analyzed secondary metabolites using Gas Chromatography-Mass Spectroscopy (GC-MS) techniques sixty-four compounds were identified from MEO (11.52%) 2-ispropyl-5-methylcyclohexanone(menthol), (11.33%) D-limonene, (9.92%) 2-cyclohexen-1-methylethylidene, (6.28%) Cyclohexanone and (3.71%) Cyclohexanol also fifty-three compounds were identified in BEO estragole (55.44%), linalool (18.41%), propanal,2-methyl-3phenyl (4.67%),(cyclopropylmethy) 4methyloxy benzene(1.99%), and 2(10) pinene (1.89%). This Investigation used an antibacterial disk diffusion assay and the MEO, BEO have antibacterial activities against *S.aureus* but MEO exceeds BEO as it more antibcterial activity against *S. aureus*. It was concluded that the diameter of the inhibition zones of MEO was greater than BEO.

Keywords, *Ocimum basilicum*, *Mentha arvensis, Staphylococcus aureus*, GC-MS, antibacterial activities

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Introduction

Phytochemicals compound, depending on how they contribute to plant metabolism, are categorized as primary and secondary metabolites. Carbohydrates, amino acids, proteins, and lipids are examples of primary metabolites which are essential for plant existence. The remaining plant chemicals that cells generate through metabolic pathways descended from the primary metabolic pathways are known as secondary metabolites. The phytochemical constituents of medicinal plants have antibacterial activities, proving their therapeutic value (1). They confirmed that the antibacterial activities of medicinal plants are very significant. *M. arvensi*s a genus of the plant family *(Lamiaceae)*. It is widely distributed across the world's temperate regions; cultivation is crucial for this plant for a variety of uses as a medicine or as fragrant. The genus *basilicum,* belonging to same family called sweet basil, and its EO is used as food flavorings, commercial fragrances, and food preservatives (2). *Staphylococcus. aureus* can infect humans and induce food poisoning. considered as an opportunistic pathogen worldwide (3) Staphylococcal food poisoning outbreaks are often caused by Staphylococcul Enterotoxin (SE). The likelihood of causing human infection and food poisoning would rise if there was a high prevalence of foodborne *S. aureus* (4).

Essential oils have been shown to have positive biological benefits, including antioxidant, antiviral, and antibacterial properties (5).

The mechanisms of action work by inhibiting metabolic processes and protein synthesis, interfering with the synthesis of cell walls, and ultimately lysing the bacterial membrane (6).

The aims of the present study are to determine the active chemical compounds by gas chromatography (GC MS) and evaluated the antbacterial effect of *Mentha arvensis* and *Ocimum basilicum* essential oil against *S.aureus*.

Materials and methods

Mentha arvensis and *Ocimum basilicum* essential oils extract

Mentha arvensis and *Ocimum basilicum* leaves were bought in bulk from a neighborhood market in Baghdad and were classified in the College of Science, Department of Life Biology, University of Baghdad. The leaves were cleaned twice: once with running tap water and once with sterilized distilled water.

In a Clevenger device, the plant material was subjected to steam distillation. In a 1000 ml round flask with lower and upper portions linked to the flask and a condenser, 500 ml of distilled water was added to 50 g of fresh Mint or Basil leaves, and then the distillation process was carried out at 80-90 °C process lasted for 2 hours (7).

Gas chromatography–mass spectrometry (GC–MS) analysis.

The GC–MS analysis for EO composition was carried out using Shimadzu QP-2010 plus gas chromatography using a flame ionization detection (FID) detector fitted with a 60 m × 0.25 mm × 0.25 µm WCOT column coated with diethylene glycol (AB-Innowax 7031428, Japan). The carrier gas was helium, with a 3 ml/min flow rate. The temperature of both the injector and detector was maintained at 260°C, while oven temperature was programmed from 60 to 240°C with a ramp of 3°C/ min and then held at 260°C for 10 min. Essential oil (0.2 µl) was injected into the column with a split ratio of 80:1, 80 parts were injected, and 1 part went into a column. The relative percentage composition was calculated using area normalization, followed by MS analysis: ionization voltage (EI) 70 eV, peak width 2 s, mass range 40–850 m/z, and detector voltage 1.5 V. The mass spectral data were matched with the National Institute of Standards and Technology (NIST12 or NIST62) and Wiley 229 mass spectrometry libraries to identify the individual components (8).

Isolates and identification of *Staphylococcus aureus*

Twenty-five samples of different sources of meat were included in this study, isolated in the Central Health Laboratory Food Microbiology Department, then purified by subculture on brain-heart infusion (BHI) agar, and then re-cultured on mannitol salt agar, blood agar, nutrient agar, tryptic soy agar, and Baird barker agar *S.aureus* was identified depending on the morphological features on culture media and biochemical tests according to Bergey's manual (9).

Determination of the inhibitory activity of *Mentha Arvensis* and *Ocimum basilicum* oils

The agar well diffusion method was used in the current study to determine the antibacterial. The various MEO and BEO concentrations were created by dissolving them in (DMSO) dimethyl sulfoxide, 1:9 DMSO, to EO.

Bacterial cultures were used to test the antibacterial activity of different concentrations of MEO and BEO (0.6%, 1.25%, 2.5%, 5%, and 10%).

Muller-Hinton agar media were separately put onto a Petri plate. The active cell suspension (1 mL) was evenly dispersed across the agar surface that contained 1.5×10^8 CFU/ml utilizing a sterile swap, then six wells with a diameter of 6 mm each were created Utilizing a sterile cork borer (10).

Twenty µL from MEO and BEO were added to the wells. Only DMSO was used to fill the well control. The EOs were allowed to diffuse in the well for one hour, and then the plates were incubated at (37°C) for 24 hours. After the incubation time, the diameter of the inhibition zone was measured.

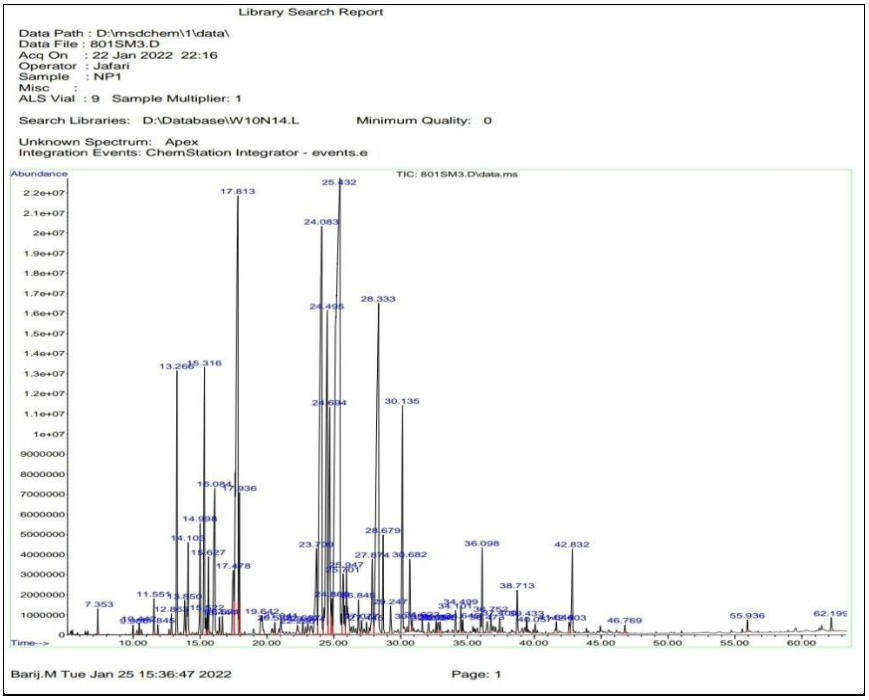
Results and discussion

Essential oils extraction

The Clevenger device was used to extract the EOs from the fresh leaves of the *M.arvensis* and *O.basilicum*. and was then kept in a refrigerator at 4 °C before use (12).

Gas chromatography–mass spectrometry (GC–MS) Analysis.

Figure (1) and Table (1) show the chemical compositions of the MEO using gas chromatography and mass spectroscopy. Sixty-four compounds were identified from, MEO was (11.52%) 2- isopropyl-5-methyl cyclohexanone (menthol), (11.33%)D-limonene, (9.92%)2-cyclohexene-1-methyl ethylidene and (6.28%) Cyclohexanone and Cyclohexanol (3.71%).



Figue (1): Gas Chromatogram (GC-MS) of *Mentha arivensis*

Table (1): Chemical Compound of *Mentha arivensis*.

Peak	Compound	R.time	Area%
1	2- ispropyl-5-methylcyclohexanone	24.0	11.52
2	D-limonene	17.8	11.33
3	2-cyclohexen-1-methylethylidene	38.3	9.92
4	Cyclohexanone	24.4	6.28
5	Cyclohexanol	24.6	3.71
6	2(10)-pinene	15.3	3.01
7	Bicyclo 4,1,0 heptane	30.1	2.86
8	2-pinene	13.2	2.42
9	Ethyl amyl carbinol	16.0	2.36
10	5 methyl-2-(propl-en-yl)cyclohexanol	23.7	1.81
11	5 methyl 2	27.8	1.18
12	Cymene	17.4	1.17
13	3 cyclohexene-lethanol alpha	25.9	1.15
14	Neoisomenthol	25.7	1.11
15	Caryophyllene	36.0	0.97
16	3 methyl-4- isopropylphenol	30.6	0.96
17	2 Cyclohexen lono 3methyl	28.6	0.91
18	2,4 diispropropylphend	38.7	0.47
19	Oxatricyclol		1.09
20	Cyclohexanone	14.0	1.06
21	Other compounds	--------	34.71

Fifty-three compounds were identified from the *O. basilicum* compound. The percentage of abundant constituents of the chemical compounds of BEO was estragole (55.44%), linalool (18.41%), (4.67%) propanal,2-methyl-3phenyl, 1(cyclopro-pylmethyl) 4 methyloxy benzene (1.99%), and 2 (10) pinene (1.89%), as showen in (Figure 2 and Table 2).

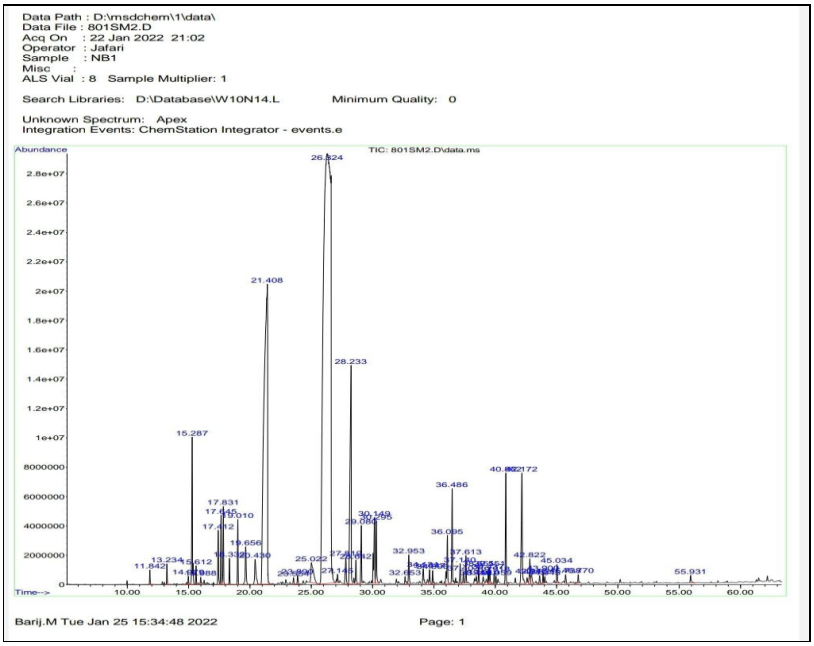


Figure (2): Gas Chromatogram (GC-MS) of *Ocimum basilicum*.

Table (2): Chemical Compound of *Ocimum basilicum*.

Peak	Compound	R.time	Area%
1	Estragole	26.31	55.44
2	Linalool	21.4	18.41
3	Propanal,2-methyl-3phenyl	28.2	4.67
4	1(cyclopropylmethyl)4methyloxy benzene	42.1	1.99
5	2(10)pinene	15.2	1.87
6	Cyclohexene	40.8	1.36
7	Trans-alpha-bergamatene	36.4	1.17
8	4 isopropylcyclohexa 1,3 dienecarb	30.1	1.16
9	Cyclohexand	17.0	0.92
10	Benzenemethanol 17.8	30.2	0.86
11	2-oxabicyclorimethyl	12.8	0.84
12	Caryphyllene	36.0	0.77
13	D-limonene	17.6	0.72
14	Citral	29.0	0.70
15	1,4-Cyclohexadiene	19.0	0.66
16	Ethyl-2(5methyl-5 vinyttetrahydr furan-2-yl propane 2-ylcarbonate	19.6	0.63
17	2(5 methyl 5viny tetrattydro 2-fu	20.4	0.61
18	o-cymene	17.4	0.60
19	2,6 Octadiend,3,7 dimethyl	27.8	0.47
20	5 oxatricyclo	42.8	0.36
21	Another compound	------	5.79

Isolates and identification of *Staphylococcus aureus*

All twenty-five isolates were identified depending on conventional cultural and properties.The Gram positive bacteria which appeared purple in color with coccus spheroid shaped, mainly grouped in clusters according to Berger-Bächi, and Rohrer (9), On blood agar, *S. aureus* usually displays a light to golden yellow pigment. was considered as *S.aureus* (13), on Baird-Parker medium that the positive result black and shiny, with a thin white border, surrounded by a light area around the colony (14). and ability to ferment mannitol aerobically on mannitol salt agar and appears in yellow colonies (15). with golden colonies shown on nutrient agar and tryptic soy agar (16). finally, *S. aureus* is positive for catalase (17), and coagulase (18).

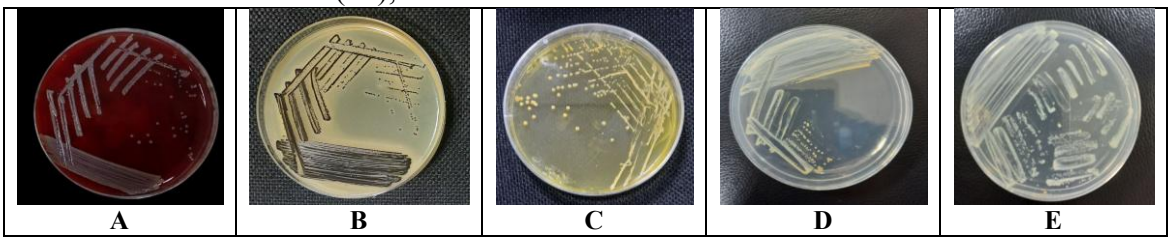
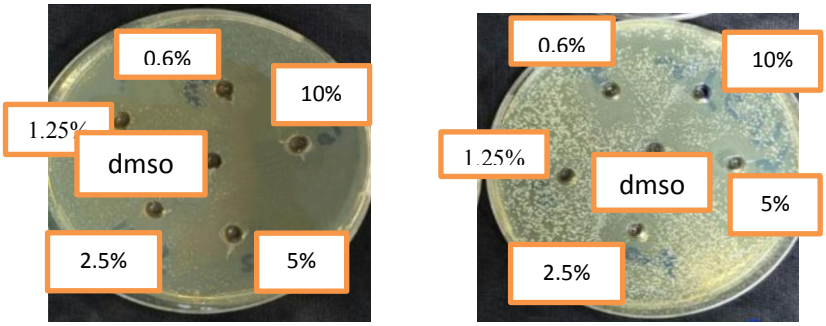


Figure (3): Colonies of *Staphylococcus aureus* growth on blood agar(A) , Baird-Parker agar(B), mannitol salt agar(C), nutrient agar(D), and tryptic soy agar(E) in 37 °C for 24 hr.

Antibacterial activity of mint essential oil and basil essential oil used agar well diffusion method (ADM).

The Agar well diffusion method was used for the antibacterial activity test of MEO *and* BEO. As the results of this test indicate that the antibacterial activity of MEO is stronger than of BEO. The diameter of the inhibition zone strong inhibitory effect reach at 24 um (19).



(A) **(B)**

Figure (4):antibacterial acitivity of Mint essential (A) and Basil essential oil (B) used Agar well Diffusion Method against *Staphylococcus aureus*.

Table (3): Comparing the antimicrobial efficacy of Mint essential oil and Basil essential oil against *Staphylococcus aureus*.

Mint essential oil		**Basil essential oil**	
Concentration %	Zone inhibition	**Concentration %**	Zone inhibition
10%	24 mm	**10%**	13 mm
5%	24 mm	**5%**	12mm
2.5%	18 mm	**2.5%**	8mm
1.25%	11 mm	**1.25%**	9mm
0.6%	7 mm	**0.6%**	8mm
LSD value	4.392 *	**LSD value**	3.405 *
*** (P≤0.05).**			

The antibacterial activity of MEO and BEO was found to be effective against tested bacterial strains. MEO and BEO had antibacterial activity and was shown to have the ability to suppress the development of *S.aureus* isolates. The diameter of the inhibition zone of MEO reached a maximum of 24 mm at a concentration of 10% and 5%. In comparision, the minor diameter of the inhibition zone was 7 mm at a concentration of 0.6%. The other diameter inhibition zone ranged from 18 to 11 mm at concentrations of 2.5% and 1.25%. The diameter inhibition zone of Basil reached a maximum of 13 mm at a concentration of 10 %. In contrast, the minor diameter of the inhibition zone was 8 mm at a concentration of 0.6% and 2.5%. The other diameter inhibition zone ranged from 12 to 9 mm at concentrations 5% and 1.25%. The antibacterial activity was different significantly throughout concentrations. Figure (4) showed that MEO is more effective than BEO in inhibiting *S.aureus* bacteria., the plant is rich in a wide variety of secondary metabolites such as tannins, phenols, steroids, flavonoids and volatile oils, which were found.

The reason may be that MEO possess menthol as fundemental compound the antimicrobial activity of EO might be correlated to its chemical composition due to the hydrophobic nature which allows them to interact with microbial membranes causing cell lysis, and inhibiting protein synthesis (20).

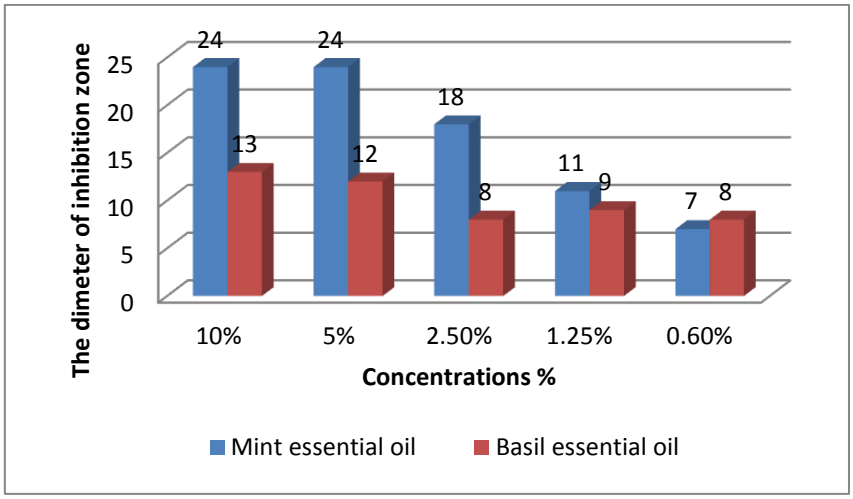


Figure (5): Comparing the antimicrobial efficacy of Mint essential oil and Basil essential oil against *Staphylococcus aureus*.

Conclusion

Mentha arevnsis and *Ocimum basilum* plants contain phytochemical compounds such as menthol, limonene, methylethylidene and other compound for the MEO and Estragole, linolol, Propanal,2-methyl-3phenyl and other compound for BEO that had bacterial activity due of increase the oxidative stress in microbial cells, causing damages of intracellular macromolecules leading cell death.

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