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Chemical composition and antimicrobial activities of the volatile oil of *Mentha- pulegium* (*Labiatae*)

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ABSTRACT

This study was aimed to evaluate the chemical coposition and antimicrobial activities of the volatile oil of *Mentha pulegium* (*Labiatae*). Twenty one volatile compounds were identified by using gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The results showed that the volatile oils mainly contained about 31.66 % 1-Menthol and 27.76 % 1-Menthone. Antimicrobial activities of volatile oil was studied against three bacterial strains (*E. coli., Staphyllococcus aureus* and *Salmonella typhi*) and four fungal species (*Fusarium, Aspergillus sp., penicillium sp. and Trichoderma sp*) at concentration 0.1 ml and 0.3 ml (10 mg / 1 ml)). The volatile oil had strong inhibitory effect for all bacterial and fungal species at concentration 0.3 ml (10 mg / 1 ml) of the volatile oil.

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KEYWORDS

Mentha pulegium;
Volatile oil;
Chemical composition;
GC-MS;
Antibacterial and antifungal activities.

INTRODUCTION

Mentha; the genus of Labiatae family, includes 20 species that spread all over the world. *Mentha pulegium L*. is one of the Mentha species commonly known as pennyroyal. It is native species of Europe, North Africa and in Asia Minor and near East[2]. One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals[4]. The Mentha genus is a member of this family and represents by about 6 species in the flora of Iran[7]. Mentha species are generally known under the name ''na'na'' and ''pooneh'' in Iran and commonly used as herbal tea, flavoring agent, and medicinal plant[8]. Analysis of the essential oil revealed the presence of piperitone (38.0%), piperitenone (33.0%), terpineol (4.7%), and pulegone (2.3%) as the major components. The results showed a significant activity against microorganisms especially Gram-positive bacteria with inhibition zones and minimal inhibitory concentration values in the range of 8 to 21 mm and 0.25 to 4 µl/ml, respectively, whereas the least susceptible were Gram-negative bacteria especially Escherichia coli[5]. The dependence of *Mentha pulegium L*. (pennyroyal) essential oil composition, obtained by supercritical carbon dioxide (SC-CO2), with the following parameters: pressure, tem-

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perature, extraction time (dynamic), and modifier (methanol) was studied. The results were also compared with those obtained by conventional hydro distillation method in laboratory conditions. Regarding the percentages of menthone (30.3%) and pulegone (52.0%). The evaluation of the composition of each extract was performed by gas chromatography–mass spectrometry[1]. The chemical composition of European pennyroyal (*Mentha-pulegium*) essential oil and to characterize the in vitro antioxidant and antimicrobial activities of its water (hot and cold) and ethanolic extracts and of the essential oil. The essential oil revealed menthone, pulegone and neo-menthol as the main constituents, comprising 35.9, 23.2 and 9.2% of the essential oil, respectively[9]. The aim of this study was to identify the chemical composition and evaluate the antimicrobial activity of Egyptian *Mentha pulegium L*. oil against different microorganisms in order to validate its traditionally used.

MATERIALS AND METHODS

Isolation of the volatile oils

Air-dried aerial parts were cut in small pieces and subjected to steam distillation for three hours using the method described by Marcus and Lichtenstein[6], and Weaver[11]. The volatile oils were dried over anhydrous sodium sulfate and stored under N2 atmosphere in amber vials at 4 °C until they were analyzed.

GC-MS analysis conditions

For qualification, the essential oil was analyzed on Gas Chromatography Mass Spectrometry HP 6890 Series A (Agilent) by using A Thermo Scientific (TR-5MS), (5% Phenyl Polysil Phenylene Siloxane) capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness). Helium (He), having a flow rate of 1.00 ml/min, was used as carrier gas. The GC oven temperature was kept at 50 ºC for 5 minutes and programmed to 250 ºC. The injector temperature was 250 °C. The amount of injection was 1µL.

Identification of components

Retention indices for all compounds were determined according to the Van Den Dool method[3]. While identification of the components was based on comparison of their mass spectra with those of internal (computer) library, Wiley7n.1 and PMW_Tox3.1 libraries and some reference compounds.

Antimicrobial activities of volatile oils

1. Microbial strains

The bacterial and fungal strains were obtained from the microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were *E. coli., Staphyllococcus aureus* and *Salmonella typhi* and fungal species were *Fusarium, Aspergillus sp., penicillium sp. and Trichoderma sp*. The oil was dissolved in dimethylforamide (DMF) for antimicrobial investigation at the final concentration of (10 mg / 1 ml).

2. Antibacterial activity

Antibacterial activities of volatile oil were tested using pour plate technique at two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml). Culturing and incubated of different bacterial species were carried out at 37 ºC for 24 hours. After the elapse of incubation periods, the diameter of inhibition zones was measured. The inhibition zone formed by the volatile oil against the particular test bacterial strain determined as the antibacterial activities[10].

3. Antifungal activity

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup pores (15 mm) diameter. Two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml) of the volatile oil were transferred into the well. Dimethyl foramide (DMF) was used only as a control. The plates were incubated for 7 days at 30 ºC. The inhibition zone formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

RESULTS AND DISCUSSION

Chemical composition of the volatile oils

The results obtained from the gas liquid chromatogram were reported in TABLE 1, we can see from these results that we have twenty one compounds were characterized. The major components are as follows: 30.85 % 1-Menthol and 27.76 % 1-Menthone.

TABLE 1 : The volatile compounds identified from the volatile oil of *Mentha pulegium* by using GC-MS

Number	Compound	Retention time in min.	%	Molecular weight	Molecular formula
1.	Alpha-Pinene	7.06	0.37	136	$C_{10}H_{16}$
2.	Beta-Pinene	8.49	0.59	136	$C_{10}H_{16}$
3.	Limonene	10.13	2.75	136	$C_{10}H_{16}$
4.	1.8-Cineole	10.28	4.68	154	$C_{10}H_{18}O$
5.	1-Menthone	14.42	27.76	154	$C_{10}H_{18}O$
6.	Mentho-furan	14.53	2.36	150	$C_{10}H_{14}O$
7.	L-Menthone	14.76	7.16	154	$C_{10}H_{18}O$
8.	Neo-menthol	14.83	5.29	156	$C_{10}H_{20}O$
9.	1-Menthol	15.19	31.66	156	$C_{10}H_{20}O$
10.	Alpha -Terpinol	15.69	0.53	154	$C_{10}H_{18}O$
11.	D-Pulegone	16.87	1.53	152	$C_{10}H_{16}O$
12.	L-Carvone	17.15	1.41	150	$C_{10}H_{14}O$
13.	Piperitone	17.44	1.14	152	$C_{10}H_{16}O$
14.	(+)-Menthyl Acetate	17.97	5.27	198	$C_{12}H_{22}O_2$
15.	Iso-Menthylacetate	18.41	0.21	198	$C_{12}H_{22}O_2$
16.	Alpha-Bourbonene	20.36	0.40	204	$C_{15}H_{24}$
17.	Beta-Caryophyllene	21.35	2.81	204	$C_{15}H_{24}$
18.	Delta-Cadinene	23.81	0.27	204	$C_{15}H_{24}$
19.	Caryophylleneoxide	25.62	0.66	220	$C_{15}H_{24}O$
20.	gamma-selinene	25.95	0.74	204	$C_{15}H_{24}$
21.	Thujopsene	26.58	0.86	204	$C_{15}H_{24}$

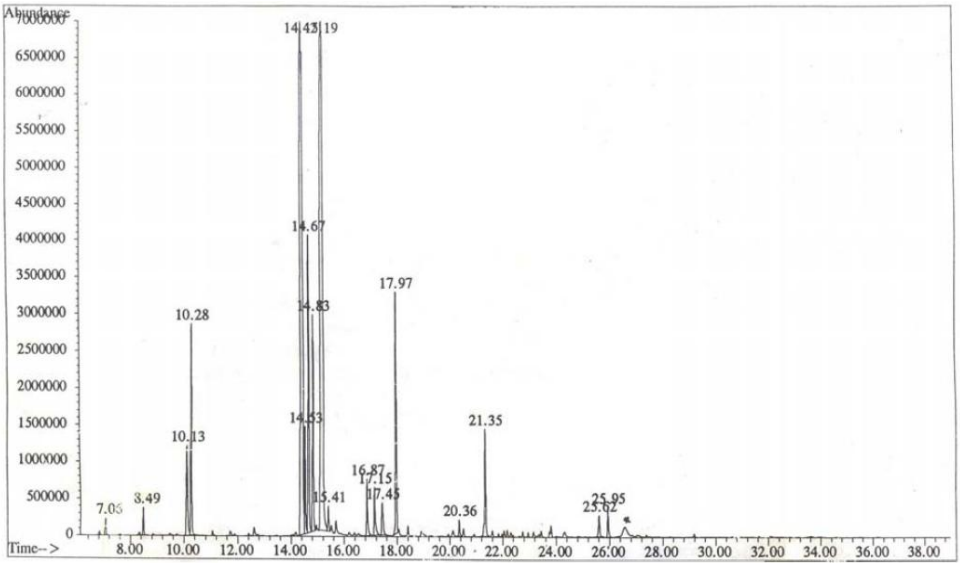

Figure 1 : Gas chromatography-mass spectroscopy (GC-MS) of the volatile oil of *Mentha-pulegium*

Antimicrobial screening

The antimicrobial activities of *Mentha-pulegium* volatile oil were evaluated by a pour plate technique method against bacterial species (*E. coli*. *Staphyllococcus aureus* and *Salmonella typhi*) and fungal species (*Fusarium, Aspergillus sp., penicillium sp.* and *Trichoderma sp.*). Volatile oils strongly exhibited antimicrobial activity against the tested strains at all concentration.

Antibacterial activity

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TABLE 2 : Antibacterial activity of volatile oil of *Mentha- pulegium*

Bacterial species	Inhibition zone diameter in mm	
	0.1 ml	0.3 ml
Escherichia-coli	7	10.0
Staphyllococcus aureus	-	15.0
Salmonella typhi	13	20.0

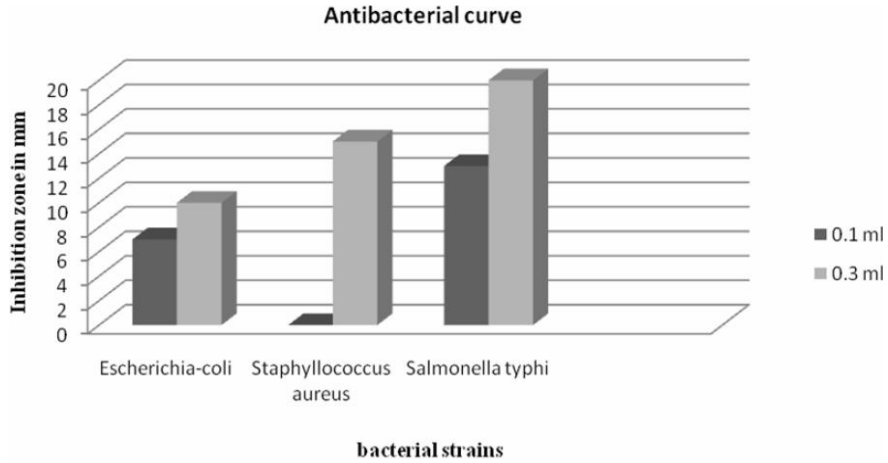

Figure 2 : Antibacterial activity of the volatile oil of *Mentha-pulegium*

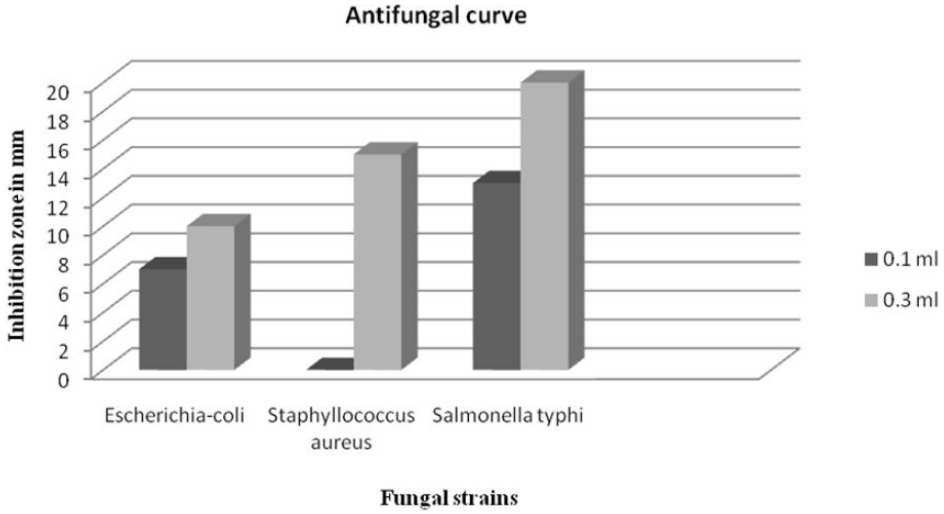

Figure 3 : Antifungal activity of the volatile oil of *Mentha-pulegium*

Data in TABLE 2 and in Figure 2 evaluate that The maximum inhibitory responses are indicated after the treatment of *E. coli.*, *Staphyllococcus aureus* and *Salmonella typhi* with highest concentration of the oil (0.3 ml), while the moderating inhibitory response after the treatment of *E. coli.*, and *Salmonella typhi* with normal concentration of the oil (0.1 ml). The result was showed that *Staphyllococcus aureus* had is the highest resistance species to the oil at 0.1 ml concentration.

Antifungal activity

TABLE 3 : Antifungal activity of the volatile oil of *Mentha - pulegium*

Fungal strains	Inhibition zone diameter in mm	
	0.1 ml	0.3 ml
Fusarium oxysporum	35.0	45.0
Aspergillus sp.	28.0	40.0
Penicillium sp.	38.0	58.0
Trichoderma sp.	12.0	30.0

Antifungal activities of the volatile oil of *Mentha-pulegium* were evaluated against the tested fungal

strains. The oil showed strongly inhibitory activity against all species at 0.1 and 0.3 ml concentrations as shown in TABLE 3 and Figure 3.

CONCLUSIONS

The chemical composition of Egyptian *Mentha-pulegium* oil was investigated. Volatile oil from *Mentha pulegium*, was obtained by steam-distillation method, and its chemical composition was determined by GC-MS. The results indicated that the volatile oils mainly had about 30.85 % 1-Menthol and 27.76 % 1-Menthone.

The results showed that volatile oils of *Mentha-pulegium* have strong antibacterial activities against (*E. coli*. and *Salmonella typhi* at concentration 0.1 ml and 0.3 ml (10 mg / 1 ml)) and antifungal activities against (*Fusarium, Aspergillus sp., penicillium sp.* and *Trichoderma sp.* at the same concentrations.

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