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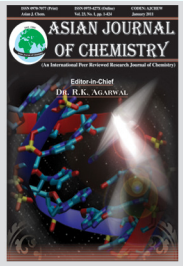

Antibacterial Activity and Chemical Constitutions of *Melissa officinalis* L.

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Two different isolation techniques, conventional hydrodistillation (HD) and solvent free microwave extraction (SFME) have been used to analyze the volatile constituents from the aerial parts of *M. officinalis* by gas chromatography coupled to mass spectrometry. The means of relative peak area (%) for every compound calculated with statistical analysis. Hydrodistillation and solvent free microwave extraction techniques produced quantitatively (yield, 0.5 % and 0.4 %) and qualitatively (aromatic profile) almost similar essential oil. E-citral (41.01-33.81 %), Z-citral (35.4-23.06 %), β-caryophyllene (8.38-8.09 %) and caryophyllene oxide (4.24-20.8 %) were identified as major constituents of this Iranian endemic specie. Besides, the hydrodistilled oil of *M. officinalis* was evaluated for antibacterial activity against gram-positive and gram-negative bacteria using agar dilution method and minimum inhibitory concentration (MIC) were determined. The hydrodistillation oil of *M. officinalis* showed antibacterial activity against *Bacillus subtilis* (gram-positive) growth with a MIC value of 25 and 150 µg/mL for *Escherichia coli* (gram-negative) as efficiently inhibition.

Key Words: Antibacterial activity, *Melissa officinalis* L., GC/MS, Essential oil.

INTRODUCTION

Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest. Some synthetic chemicals have been made to control microbial growth and reduce the incidence of food poisoning and spoilage. Although these synthetic preservatives are effective, they might be detrimental to human health. Consumers are concerned about the safety of foods containing artificial preservatives[1].

From ancient times, indigenous plants have been used in herbal medicine for curing various diseases[2]. Recently, notice to traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics have led authors to investigate the antimicrobial activity of medicinal plants[3,4].

The genus *Melissa* known as 'Varangbo' in Persian, have one specie in Iran. *Melissa* specie is well known as medicinal plants because of their biological and pharmacological properties[5]. The essential oil of *M. officinalis* has shown *in vivo* growth inhibitory effects in a number of cancers. The effect of the essential oil was compared with the anticancerogenic effects of the methotrexate (MTX) and vepesid[6]. Also, reported the chemical composition, antibacterial, antioxidative and radical-scavenging properties of the essential oils of *M. officinalis* obtained by conventional distillation methods and solvent-free microwave extraction method[7-9]. An alternative method for extracting natural products by using microwave energy has been developed[10]. Solvent free microwave extraction (SFME) is based on the combination of microwave heating and distillation is performed at atmospheric pressure[11]. Some of the advantages of this method over hydrodistillation includes, rapidity in attaining the extraction temperature of 100 °C for the first essential oil droplet, high yield of essential oil, lower energy requirement and high purity of the oil extracted using solvent free microwave extraction[12]. There are only a few articles in literature that have reported the acceleration of essential oil extraction by microwave irradiation[13-15].

The aim of the present study was to investigate an applicability of solvent free microwave extraction technique as an alternative to conventional hydrodistillation for isolation of *M. officinalis* volatiles.

Also, within scope of the present work, the hydrodistilled oil of *M. officinalis* was further evaluated for antibacterial properties.The present work reports about the composition and biological activity of *M. officinalis* essential oil growing wild in Iran.

EXPERIMENTAL

The aerial parts of *M. officinalis* were collected from East of Iran, located in height of Binaloud Mountain, Province of northern khorasan in June 2009. Voucher specimens have been deposited at the herbarium of the Research Institute of Forest and Rangelands (TARI), Tehran, Iran.

Extraction of the essential oil: For hydrodistillation, 100 g the air-dried aerial parts in 2 L flask were subjected to hydrodistillation for 3.5 h using a Clevenger type according to the standard procedure described in the European pharmacopeia[16].

In the SFME procedure, 50 g of spice was moistened prior to extraction by soaking in water for 1 h and then draining off excess water[11]. Moistened spices were placed in reactor and heated by microwave irradiation with 200 W power for 40 min. The essential oil was collected, dried under anhydrous sodium sulphate and stored at 4°C until analyzed.

For irradiation used a millestone 'Microsynth' microwave oven that had a multimode microwave reactor 2.45 GHz with a maximum delivered power of 1500 W variable in 1 W increments.

Temperature was monitored by a shielded thermocouple (ATC-FO) which inserted directly into the sample container and temperature was controlled by a feedback to the microwave power regulator without using correction factors.

A cooling system outside the microwave oven condenses the distillate continuously. The dimensions of PTFE coated cavity are 55 cm × 55 cm × 55 cm. During experiments, time, temperature, pressure and power were controlled with 'easy-control' software package.

Gas chromatographic analysis was carried out on a Hewlett-Packard 6890 gas chromatograph equipped with a split (20:1) injector (250 °C) and a flame ionization detector (250 °C). Nitrogen was used carrier gas (1 mL/min).The capillary column used was DB-5 (30 m × 0.25 mm, 0.32 μm film thickness). The oven temperature was held at 60 °C for 3 min, then heated to 220 °C with a 5°C rate and kept constant at 220 °C for 5 min. Quantitative data were obtained from GC (FID) area percent without using correction factor.

GC/MS analysis was performed using a Hewlett-Packard 6890/5973 GC/MS that equipted with a 30 m × 0.25 mm, film thickness 0.32 μm HP-5MS column. Helium (99.999 %) was used as carrier gas (1.0 mL/min). The temperature program was as like as gas chromatography. The injection temperature and ion source temperature were 250 and 230 °C, respectively. Mass spectra were taken at 70 eV. All data were obtained by collecting the full scan mass spectra within the scan range 40-450 amu. The GC/MS was equipped with chemstation software and Wiley 275 library. Identification of constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples[17].

Antimicrobial activity: Minimum inhibitory concentrations (MICs) of the essential oil was determined by agar dilution method (EUCAST, 2000) with respect to different test microorganisms including Gram-positive (*Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* PTCC 1330) bacteria.

A series of eight dilutions of the oil was prepared in ethanol (1 mL). Each dilute was added to molten Muller Hinton (MH) agar at 50 °C to give the final concentrations of 0.0125, 0.05, 0.1,0.15, 0.25, 0.5, 1, 1.5 mg/mL.

The bacteria inocula were prepared by suspending overnight colonies from MH agar media in sterile saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to *ca.* 10^7 cfu/mL.

Statistical analysis: All extractions with HD and SFME were performed in duplicate and a general linear model (GLM) procedure from SAS (Statistical Analysis Software, version 9.1, SAS Institute Inc., Cary, NC, USA) was used to compare among the means.

RESULTS AND DISCUSSION

The chemical composition of *M. officinalis* essential oil was identified by comparision of mass spectra library and retention indices with authentic sample. Eight components in the HD method and nine components in the SFME method were identified. The identified compounds and their percentages were listed in Table-1, in order of their elution on the HP-5 column. The hydrodistillation of the dried aerial parts of *M. officinalis* performed for 210 min gave the light yellowish oil with a pleasant smell in 0.5 % yield. Compounds were characterized representing 95.88 % of the oil obtained by hydrodistillation. Solvent free microwave extraction technique allowed the isolation of the oil in 0.4 % yield for 40 min. In this application, microwave irradiation highly accelerated the extraction process, but without causing considerable changes in the volatile oil composition. The major components included citral, β-caryophyllene and caryo-phyllene oxide were common in both methods (Table-1).

TABLE-1
CHEMICAL COMPOSITION OF ESSENTIAL OILS OBTAINED BY HYDRODISTILLATION (HD) AND SOLVENT FREE MICROWAVE EXTRACTION (SFME) OF *Melissa officinalis L.* AERIAL PARTS

No.	Compound[a]	RI[b]	Relative peak area (%)[c]	
			HD(%)	SFME(%)
1	Linalool	1100	0.74± 0.2	1.04±0.0
2	Z-Citral	1252	35.4±0.1	23.06±0.2
3	Geraniol	1259	1.09±0.1	0.92±0.1
4	E-Citral	1281	41.09±0.4	33.81±0.1
5	Thymol	1298	–	2.1±0.2
6	Geranyl acetate	1388	3.81±0.2	5.16±0.1
7	β-Caryophyllen	1456	8.38±0.3	8.09±0.0
8	Muurola-4(14),5-diene(*trans*)	1517	1.13±1.0	1.15±2.8
9	Caryophyllen oxide	1627	4.24±0.6	20.8±0.6
Total oxygenated fraction (%)			86.37	86.89
Total peak area (%)			95.88	96.13
Total extraction time (min)			210	40
Yield (%)			0.5	0.4

[a]Compounds listed in order of elution.
[b]RI, Kovat's indices as determined on a DB-5 column using the homologous series of *n*-hydrocarbons (C9-C19).
[c]Mean ± SD (n = 2), In each component identified, the means are not significantly different ($p > 0.05$) for each component identified.

In comparision, SFME yielded a higher amount (30.04 %) of heavier compounds (*i.e.* sesquiterpenes), while with HD method only 13.57 % of these compounds were isolated (Table-1).

The antibacterial activity of the oil was evaluated only for HD method and is summarized in Table-2. The essential oil inhibited bacterial growth within a narrow concentration range of 25-150 μg/mL. Both Gram-positive and Gram-negative bacteria were highly sensitive to this potent essential oil,

TABLE-2
MINIMUM INHIBITORY CONCENTRATIONS (MICs) OF ESSENTIAL OILS OF IRANIAN *Melissa officinalis* L. COLLECTED FROM BINALOUD, DETERMINED BY AGAR DILUTION METHOD

Bacteria	MIC (µg/mL)
Bacillus subtilis	25
Escherichia coli	150

especially *Bacillus subtilis* with the MIC of 25 µg/mL and *E. coli* with the MIC of 150 µg/mL.

The antibacterial activity showed the essential oil can be attributed to the presence of terpenes in major compounds. Also complicated mixture of monoterpenes and sesquiterpenes in the whole oil can represent a stronger barrier to bacterial infections[18].

Conclusion

The results on the application of SFME technique to *M. officinalis* volatiles provided a scientific support for the use this technique in the oil productions. Also, the data presented confirm the antibacterial potential of *M. officinalis* essential oil. The oils tested represent an inexpensive source of natural antibacterial substances for use in pathogenic systems to prevent the growth of bacteria and extend the shelf life of the processed food.

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