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Research Paper

Chemical composition of essential oil extracted from *Micromeria Barbata* growing in Lebanon and their antimicrobial and antioxidant properties

Youssef Bakkour[1, 2], Saer Alwan[1], Hiam Soufi[1], Nassim El-Ashi[2], Mohammad Tabcheh[2], Fawaz El Omar[2*]

[1]Laboratory of Pharmacognosy, Department of Pharmacy, Faculty of Public Health, University Of Jinan, P. O. Box 818 , Tripoli, Lebanon

[2]Laboratory of Applied Chemistry, Faculty of Science III, Lebanese University, P. O. Box 826, Tripoli, Lebanon

* Corresponding Author

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ABSTRACT

The chemical composition of essential oil obtained by hydrodistillation from the dried leaves of *Micromeria Barbata* was analyzed by GC/MS. The antimicrobial activity of the oil was evaluated against six microorganisms (considered as a major pathogens causing nosocomial infection) using screening test and broth micro dilution methods. The essential oil was found to show a broad spectrum of antimicrobial activity against the tested microbial strains. The antioxidant activity was employed by the 2.2-diphenyl-1-picrylhaydrazyl (DPPH) radical scavenging activity resulting in appreciable effect compared to the positive control Butylated hydroxyltoulene (BHT).

Keywords: *Micromeria Barbata;* Essential oil; GC/MS; Antioxidant; Antimicrobial.

INTRODUCTION

The genus *Micromeria* is a member of *Labiatae* family. The species of *Micromeria* are well known as aromatic species because they contain considerable quantities of essential oil (Al-Hamwui, et al., 2011). *Micromeria* genus was grown naturally in Lebanon and in the eastern Mediterranean region (Dudai, et al., 2001; Marinkovic, et al., 2002,). In general, several micromeria species are used against heart disorders, headache, wounds and skin infections and the most usage of *Micromeria* species are in colds (Baser, et al., 1996; Ali-Shtayeh., 1998; Dudai, et al., 2001; Duru, et al., 2004). The extracts of the majority of *Micromeria* species exhibit biological activities (öztürk, et al., 2011). The aim of this study is to identify the essential oil composition of *Micromeria barbata* and to evaluate its antimicrobial and antioxidant activities in order to try to find a plausible explanation for the numerous medical applications of the plant.

MATERIALS AND METHODS

Sample collection: *Micromeria Barbata* was coolected from Dennieh, North Lebanon in July 2011 and authenticated by Pr. George Thome, Botanist, (National Council for Scientific Research, Lebanon), Lebanon.

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Essential Oil Extraction: The samples were air dried, crushed. The essential oil was extracted by hydrodistillation (4 hours) using a Clevenger–type apparatus yielding yellowish oil. Its percent composition was calculated on moisture free basis to be 2%. The oil was collected in dark glass and stored at temperature below 4°C till analysis.

Gas chromatography/mass spectrometry: A Schimadzu QP 2010 plus gas chromatography system interfaced to a 2010 mass spectrometer was used for analysis of the samples. The separation was performed on a 30mx0.25mm internal diameter fused silica capillary column coated with 0.25µm film Rtx-5MS. The injector and the detector temperatures were respectively 250 and 280°C. The oven temperature was held at 40°C for 5 min, and programmed from 40 to 100°C at 4°C/min then to 280°C at 19°C/min and finally maintained at 280°C for 5 min. Split injection was conducted with a split ratio of 5:10. Helium was used as carrier gas, and flow-rate was 1.62 ml/min. The mass spectra were recorded over a range of 30-1000 atomic mass units at 0.5s/scan. Solvent cut time was 3 min. Ionization energy was 70 eV. The inlet and ionization source temperature were 280°C. The chemical composition of the oil was identified by comparing their spectra with those of a NIST library and confirmed by comparing their retention indices with data published in various literatures.

Antimicrobial Activity: Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC): The essential oil was tested against six clinical strains Pseudomonas aeruginosa (Imipenemase), *Staphylococcus aureus* (Methicillin resistant), *Staphylococcus aureus* (Methicillin sensitive), *Escherichia coli* (wild type), *Enterococcus sp* and *Candida albicans*. The screening diffusion method was employed to determine the antimicrobial activity of the essential oil (NCLLS, 2003). A suspension of the tested microorganisms was spread on the solid media plates (Mueller Hinton agar).A well on the solid agar was filled with 100µl of the essential oil. The plates were incubated at 37°C for 24 hours. Then the diameter of the inhibition zones were measured and expressed in millimeters. Each test was performed in three replicates and repeated twice. Levofloxacin serves as positive control. A broth micro dilution method was used to determine the MIC and MBC. All tests were performed in nutrient agar broth supplemented with Tween 80 at final concentration of 0.5% (v/v) for all microbes. Serial doubling dilutions of the oil were prepared in a series of 11 tubes ranged from 1 to 1/2048.The final concentration of each strain was adjusted to 10^6 CFU/ml. Tubes were incubated at 37°C for 24h. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth. The microorganism growth was indicated by the turbidity. To determine MBC, broth was taken from each well and incubated in Mueller Hinton for all bacteria except for Enterococcus sp that was incubated in Columbia agar with 5 % of human blood and in sabouraud dextrose agar for Candida albicans at a temperature of 37°C for 24h. The MBC was defined as the lowest concentration of the essential oil at which incubated microorganism was completely killed. Each test was performed in three replicates and repeated twice. Levofloxacin serves as positive control.

Antioxidant activity (DPPH assay): The antioxidant activities of essential oil and the BHT were assessed on the basis of radical scavenging effect of the stable DPPH free radical (Blois, 1958). 200µl of a 0.26mM solution of DPPH radical in methanol were mixed with a range of (15µl-180µl) oil and the total volume was adjusted to 4 ml by methanol. The concentrations of oil were 3.08-36.95mg/ml. The reaction mixture was shaken and then incubated for 60 minutes in dark at room temperature. The DPPH radical inhibition was measured at 517nm by using a Shimadzu UV spectrophotometer.

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RESULTS AND DISCUSSION

The main constituents of the essential oil of *Micromeria Barbata* used in present study are shown in Table 1. *M. Barbata* essential oil was characterized by the presence of 17 components. Pulegone (20.19%), Limonene (16.59%), Neomenthol (12.37%0, Menthol (6.19%), β-pinene (3.29%), and Piperitone (4.22%) were the major constituents. The content of pulegone relatively lower than those indicated by other studies (Ravid, et al., 1994; Marinković, et al., 2002; Telci, et al., 2007; Stojanović, et al., 2008; Hilan, et al., 2011) makes plants growin in Lebanon less Toxic.

Antimicrobial Activity: The disc diameters of zone of inhibition (DDs); minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of essential oil for the microorganisms tested are shown in table 2. The essential oil of *Micromeria Barbata* showed inhibition zones against all microorganisms tested. Generally, large DDs correlated with lower MICs. The data obtained from the disc diffusion method showed that *Peudomonas aeruginosa* had a zone of inhibition of (27 mm), the inhibition zone of *Escherichia coli* was (22mm), the inhibition zone of *Staphylococcus aureus* (MTS) was (13mm), the inhibition zone of *Staphyphylococcus aureus* (MTR) was (14mm), the inhibition zone of *Enteroccoccus sp* was (17mm) and those for Candida albicans was (30mm). The results of MIC indicated *S.aureus* and *C.albicans* had the lowest MICs (1/256), the highest MIC was (1/2) for *Pseudomonas aeruginosa*. The lowest MBC was for *Enterococcus sp* (1/512), *Pseudomonas aeruginosa* had the highest MBC of (1/2). The results of MBC/MIC ratio of all tested microorganisms ranged from 1 to 3.3. Overall, the essential oil displayed a bactericidal activity on the tested microorganisms and exerted a much stronger antimicrobial effect against gram positive bacteria than gram negative bacteria. Although, all gram positive bacteria tested had the smallest zone of inhibition and this may be due to the poor diffusion of gram positive bacteria in Muller Hinton agar.

Antimicrobial assays results justified and supported partly the popular usage of the flowers as traditional remedies for some infections. It was of interest to note that the broad antimicrobial activity of the oil against clinical strains especially *pseudomonas aeruginosa*, *Escherichia coli*, Methicillin resistant *staphylococci*, *Enterococcus sp*, *Candida albicans* which were established as major nosocomial pathogens responsible for a wide variety of infections, thus suggested this essential oil could be new medicinal resource for multi-resistant microbes.

Antioxidant Activity: The degree of inhibition was calculated as a percentage using the following formula:

% DDPH radical Scavenging = (Absorbance of blank – Absorbance of Sample/ Absorbance of blank) x 100

The essential oil of the *Micromeria Barbata* transformed the DPPH radical into its reduced form. A dose response relationship was observed for the essential oil (table 3). The E.O.'s IC_{50} is 4.9mg/ml whereas the control BHT has an IC_{50} of 0.4mg/ml .

The radical scavenging activity of the oil can possibly be due to the presence of Limonene, which is one of the major components of the oil. Previous studies indicate that Limonene has a high radical scavenging activity (Wei, et al., 2007; Yang, et al., 2010).

CONCLUSION

The essential oil of *M. Barbata is* mainly composed of pulegone and limonene. The oil possesses a stronger antimicrobial effect against gram positive bacteria than gram

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negative bacteria and also significant free radical scavenging activity. Antimicrobial and antioxidant effects may help to use essential oil as a natural additive in food, cosmetics and pharmaceutical industries.

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Table- 1: Chemical composition of the essential oil of the *Micromeria Barbata.*

name	RT	Ret Index	%area
α-Pinene	7.967	933	1.76
Camphene	8.183	953	0.1
β-Pinene	8.617	978	3.29
b-Myrcene	8.808	991	1.56
3-octanol	8.883	999	1.16
Limonene	9.525	1030	16.59
Ocimene	9.792	1046	0.17
terpinolene	10.558	1052	0.1
Menthone	11.467	1158	0.16
Neomenthol	11.642	1170	12.37
menthol	11.833	1184	6.19
Pulegone	12.442	1241	20.19
Piperitone	12.542	1267	4.22
menthyl acetate	12.808	1290	0.35
Isomenthol acetate	12.933	1305	0.89
Cadinene	14.150	1512	1.06
Cedrol	14.858	1610	0.49
Total			70.65

Table- 2: Antimicrobial activity of Micromeria barbata essential oil.

Microorganisms Clinically isolated strains	Essential Oil			Levofloxacin		
	DD[a]	MIC[b]	MBC[b]	DD[c]	MIC[d]	MBC[d]
Pseudomonas aeruginosa (semi penemase)	27	0.5	0.5	1	NA	NA
Staphylococcus aureus (MTS)	13	0.00391	0.0078	26	NT	NT
Staphylococcus aureus (MTR)	14	0.00391	0.00391	24	NT	NT
Escherichia coli (wild type)	22	0.015625	0.015625	31	2.44	2.44
Enterococcus sp	17	0.00098	0.001953	16	9.77	9.77
Candida albicans	30	0.00098	0.00391	NT	NT	NT

- DD: diameter of zone of inhibition (mm) including disc diameter of 66mm.
- NT, not tested, NA, not active.
- [a]Tested at a concentration of 100µl/well. [b]Values given as $\log^2$. [c]Tested at a concentration of 5µg/disc.
- [d]Values given as µg/ml.

Table- 3: radical scavenging activity of Micromeria Barbata essential oil

Oil mg/ml	% Scavenging activity
3.08	41.11
6.15	59.4
9.24	61.66
15.4	63.88
18.48	65.5
21.56	66.11
27.72	67.78
30.79	68.88
36.95	71.66

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