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Composition of the Essential Oil of *Micromeria biflora* ssp. *arabica* K. Walth

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Abstract: The essential oil of *Micromeria biflora* ssp. *arabica* K. Walth was obtained by hydrodistillation and analyzed by GC/MS. Out of 39 peaks (representing 99.0% of the oil); 30 components were identified, representing 98.2% of the total oil. The major constituents were trans-caryophyllene (43.7%), caryophyllene-oxide (18.0%), spathulenol (8.5%), α -humulene (4.6%), α -myrcene (3.1%) and germacrene-D (3.1%). The antimicrobial activity of the oil was also evaluated.

Key words: Labiatae, trans-caryophyllene, caryophyllene-oxide, spathulenol

INTRODUCTION

Micromeria biflora ssp. *arabica* K. Walth (Labiatae) is woody based twiggy herb, distributed in Southern Saudi Arabia (Collenette, 1999). Several species of the genus *Micromeria* have been used as remedies for a number of medical problems (Pedro *et al.*, 1995; Baser *et al.*, 1997; Vladimir-Knezevic *et al.*, 2000) and as herbal tea (Baser *et al.*, 1997). In addition, the essential oil composition of many *Micromeria* species such as *M. sinaica* (El-Hawary *et al.*, 1991), *M. myrtifolia* (Özek *et al.*, 1992), *M. graeca* (Tzakou and Couladis, 2001), *M. dolichodontha* (Baser *et al.*, 1997), *M. cilicica* (Duru *et al.*, 2004) and *M. libanotica* (Diab *et al.*, 2005) has been studied. In most of these species, pulegone, isomenthone, *p*-menthone, limonene, linalool, γ -pinene, α -pinene, *p*-cymene, α -terpinene, α -terpinene, α -terpineol, camphene, β -bourbonene and borneol are reported (Duru *et al.*, 2004). This study reports on the results of GC/MS analysis of the essential oil of *Micromeria biflora* ssp. *arabica*.

MATERIALS AND METHODS

Plant material: The aerial parts were collected in Al-Baha, Saudi Arabia in April 2004. The plant was identified by Dr. M. Atiqur Rahman, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 14885) was deposited at the herbarium of the College of Pharmacy, KS.

Oil isolation: Fresh aerial parts (700 g) were hydrodistilled for 4 h using a Clevenger-type apparatus. The oil was extracted from the distillate with peroxide-free diethyl

ether, followed by drying over anhydrous sodium sulphate and removal of the solvent yielded 0.07% (w/w) of the oil.

GC/MS analysis: The oil was analyzed using a GC/MS Finnigan mat SSQ7000 system operating at 70 eV ionization energy, equipped with a DB-5 FSC column (30 m \times 0.25 mm, film thickness 0.25 μ m). Helium (0.8 mL min⁻¹) was used as carrier gas. GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C min⁻¹ and then kept constant at 220°C for 10 min to 240°C at rate of 1°C min⁻¹. Mass range was recorded from m/z 38 to 400. The oil (0.2 μ L) was injected in split mode (split ratio 100:1). Injection port temperature was at 250°C. The qualitative identification of different components was performed by comparison of their retention times and mass spectra with those of the library.

Antimicrobial activity testing: Microorganisms used: The microorganisms used for the agar dilution assay were: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 90028 and *Mycobacterium smegmatis* ATCC 35797.

Agar dilution assay: (Mitscher *et al.*, 1972): The oil was dissolved in a small volume of DMSO and diluted with 10 mL warm nutrient agar (40°C) for most of organisms and Sabouraud agar for *Candida*, to give a concentration of 1000 and 2000 μ g mL⁻¹ and swirled carefully before congealing. The microorganisms were streaked in radial patterns on the agar plates. The plates were incubated at 37°C in the dark and examined after 24 and 48 h. Complete

inhibition of growth was required to declare bioactivity. The controls consisted of both negative (DMSO) and positive controls. Chloramphenicol was used as a positive control for Gram positive and Gram negative organisms, nystatin for *Candida albicans* and isonicotinic acid hydrazide for *Mycobacterium smegmatis*.

RESULTS AND DISCUSSION

The essential oil of *M. biflora* ssp. *arabica* was examined by GC/MS. The yield was 0.07% (w/w) of pale yellow oil. The constituents of the oil are given in Table 1. These constituents are listed in order of their elution from the DB-5 FSC column. Out of 39 peaks (representing 99.0% of the oil), thirty components were identified, representing 98.2% of the total oil. The oil is mainly composed of sesquiterpene (85.8%) and monoterpene (12.3%) compounds. The major sesquiterpene components were trans-caryophyllene (43.7%), (-)-caryophyllene-oxide (18.0%), spathulenol (8.5%), α -humulene (4.6%), germacrene-D (3.1%) and farnesene (2.0%). On the other hand, the major monoterpene compounds were α -myrcene (3.1%), R(+)-limonene (2.8%), α -pinene (2.0%) and cis-sabinene

hydrate (1.9%). Other monoterpenes, sesquiterpenes as well as non-terpenoid compounds were also present in smaller amounts.

The results of the present investigation indicated that *M. biflora* ssp. *arabica* is very rich in sesquiterpene constituents. It is very similar in composition to the Turkish *M. myrtifolia* (Özek *et al.*, 1992). In addition, the oil of this plant according to the composition of its monoterpene components can be grouped with the *Micromeria* species that have pinane type monoterpenes (Perez-Alonso *et al.*, 1996). The oil was also tested against a range of microorganisms (materials and methods part) and showed no activity at 1000 μ g mL.

From this study and owing to the high sesquiterpene contents of the oil of this plant, it can be concluded that *M. biflora* ssp. *arabica* can be used as an aroma fixation.

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Table 1: Identified constituents in the essential oil of *M. biflora* ssp. *arabica*

Peak No.	Retention time (min)	Compound	Area (%)
1	4.34	4-Methyloctane	0.01
2	6.70	3-Methyl-1-propylcyclopentene	0.02
3	8.50	Cyclofenchene	0.08
4	10.88	α -Pinene	2.00
5	11.74	α -Myrcene	3.10
6	13.79	R(+)-Limonene	2.81
7	14.40	Linalyl acetate	0.44
8	14.74	Neryl acetate	0.12
9	15.09	α -Terpinyl acetate	0.09
10	15.76	α -Phellandrene	0.02
11	16.45	δ -3-Carene	0.02
12	17.50	Linalool	0.81
13	19.05	L-linalool	0.06
14	22.22	Cis-sabinene hydrate	1.91
15	25.08	Cis-caran-trans-2-ol	0.85
16	36.77	Trans-caryophyllene	43.74
17	37.54	α -Humulene	4.58
18	38.08	Germacrene-D	3.09
19	38.39	Farnesene	2.02
20	38.63	Cadinene	0.29
21	42.24	(-)-Caryophyllene-oxide	18.01
22	42.80	Spathulenol	8.48
23	43.23	Diepicedren-1-oxide	0.81
24	43.77	Calarepoxide	0.82
25	44.24	Bulnesol	1.38
26	44.61	Isoaromadrenepoxide	1.25
27	45.13	Aromadrenepoxide isomer	0.20
28	45.39	Aromadrenepoxide isomer	0.09
29	46.25	7,7-Dichlorobicyclo[3.2.0]hept-2-en-6-one	0.21
30	46.60	α -Santalol	0.86
		Total	98.20

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