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Volatile oil profile of some lamiaceous plants growing in Saudi Arabia and their biological activities

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Abstract: A comparative investigation of hydro-distilled essential oils from aerial parts of *Mentha longifolia* L. (ML), *Mentha microphylla* K.Koch (MM), *Mentha australis* R.Br. (MA), and *Teucrium polium* L. (TP) growing in Al Madinah Al Munawwarah, Saudi Arabia, was carried out. The total numbers of identified constituents were 22, 23, 14, and 20 in ML, MM, MA, and TP oils, representing 93.0, 99.3, 78.1, and 81.1% of the total oil composition, respectively. Pulegone (40.7%) and cineole (33.4%) were the major components in ML, whereas carvone (64.6%) was the major one in MM. Furthermore, β-linalool (22.9%) and α-terpineol (12%) were the major components in MA, whereas, (*E*)-3-caren-2-ol accounted for 12.1% in TP. The essential oils of TP and MA exhibited promising activities against *Leishmania donovani* promastigotes with IC_{50} values of 2.3 and 3.7 μg/mL, respectively. In contrast, MA essential oils exhibited antifungal activities towards *Candida krusei* and *C. glabrata* with IC_{50} values of 1 and 1.2 μg/mL, respectively.

Keywords: antimicrobial; antiprotozoal; *Mentha* species; *Teucrium polium*; volatile oils.

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1 Introduction

Aromatic plants are well recognized for their medicinal properties, their use in perfumes and cosmetics, and as flavoring agents in the food industry. These uses are mostly attributed to their volatile oil (essential oil) content [1]. Essential oils possess various bioactivities such as antibacterial [2], antiviral, antioxidant, antidiabetic [3], antimalarial [4], and antileishmanial [5]. They may be useful in the prevention and treatment of cancer and cardiovascular diseases such as atherosclerosis and thrombosis [3]. The Lamiaceae (mint family) is a very important volatile oil-containing plant family. *Mentha* species are native to Southern and Western Europe, extending northwards to the Netherlands; they are cultivated as pot-herbs and are naturalized in northern and central parts of Europe [6, 7]. Also, they are widely distributed in Central Asia and Southern Africa. In Saudi Arabia, they are grown in the Wadi Najran, Taif region, and Al Madinah Al Munawwarah (Abyar Al-Mashy) [8]. *Teucrium polium* L. occurs throughout the Mediterranean and Irano-Turanian regions [9]. In Saudi Arabia, it is widely distributed in Aljuf, Hail, and Al Madinah Al Munawwarah (Gabal Al-aquiq) [8].

The Saudi Arabian flora comprises about 2250 plant species that are distributed throughout the Kingdom [10]. Many of these have been used by the local communities for the treatment of a large number of ailments. The family Lamiaceae is represented in Saudi Arabia by 76 species [11]. Four lamiaceous species are widely growing in Al Madinah Al Munawwarah [12] and are commonly used in folk medicine by Saudi people. A *Mentha longifolia* L. (Naana, Habak) leaf extract is used as a remedy

for cough and breathing problems [13]. *Mentha microphylla* K. Koch (Niena'a barri-niena'a) is used as an analeptic, appetizer, and a carminative [11, 14]. *Teucrium polium* L. (Ja'adah) is used as stomachic and vermifuge, but also in steam baths to alleviate cold and fever [11]. The leaf, flower, and stem of *Mentha* sp. are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to impart a certain aroma and flavor [15, 16].

Based on a literature survey, there are no reports on detailed analyses of the essential oils of *M. longifolia*, *M. microphylla*, *M. australis*, and *T. polium* growing in Saudi Arabia. Only the essential oil of the aerial parts of *T. polium*, collected in the northern region of Saudi Arabia (Aljuf) has been analyzed and reported as being enriched in γ-muurolene (8.7%), α-cadinol (5.9%), and δ-cadinene (5.1%) [17].

The major goal of this study was to investigate the chemical composition of the essential oils isolated from the aerial parts of these plants, as well as assessing their antimicrobial, antimalarial, and antileishmanial activities.

2 Experimental

2.1 General

GC/MS analysis was performed with a Shimadzu Model GC-17A gas chromatograph interfaced with a Shimadzu model QP-5000 mass spectrometer (Kyoto, Japan). Volatiles were separated on a DB5-MS column (30 m length, 0.5 mm i.d., and 0.25 μm film) (J&W Scientific, Santa Clara, CA, USA). Injections were done with a split ratio of 1:10 for 30 s. Operating conditions were the following: injector 220 °C, column oven 38 °C for 3 min, then programmed at a rate of 12 °C min^{-1} to 220 °C and kept for 2 min, He carrier gas at 1 mL min^{-1}. The transfer line and ion-source temperatures were adjusted to 230 and 180 °C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at m/z 40–500. The volatile components were identified using the procedures described previously [18]. Peaks were first de-convoluted using AMDIS software (www.amid.net) and compounds subsequently identified by their retention indices (RI) relative to *n*-alkanes (C6–C20), and by matching their mass spectra to the NIST, WILEY library database (>90% match) as well as to those of authentic standards when available.

2.2 Plant material

The fresh flowering aerial parts of *M. longifolia*, *M. microphylla*, and *M. australis* were collected from Abyar Al-Mashy and those of *T. polium* from Gabal Al-aquiq, Al Madinah Al Munawwarah in March 2013. Plant samples were authenticated based on morphological characteristics by Dr. Emad A. Alsherif, Associate Professor of Plant Ecology, Department of Biology, Faculty of Science & Arts, Khulais, King Abdulaziz University, Saudi Arabia. A herbarium specimen of each collected plant was deposited at the herbarium of the Department of Natural Products and Alternative Medicine, Faculty of Pharmacy, King Abdulaziz University (ML-1-2013, MM-2-2013, MA-3-2013, and TP-4-2013, respectively).

2.3 Preparation of volatile oil

Fresh plant material (500 g) of each of the four species was subjected separately to hydro-distillation [19]. The volatile oils obtained were dried over anhydrous sodium sulfate to give 1.74, 1.41, 0.93, and 0.68 g for *M. longifolia*, *M. microphylla*, *M. australis*, and *T. polium*, respectively. They were kept in a refrigerator for GC/MS analysis and biological study. All samples were dissolved in DMSO prior to use.

2.4 Antimicrobial assay

All essential oils (conc. 1–20 μg/mL) were tested for antimicrobial activity against *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Aspergillus fumigatus* ATCC 90906, *Cryptococcus neoformans* ATCC 90113, methicillin-resistant *Staphylococcus aureus* ATCC 33591, *Staphylococcus aureus* ATCC 2921, *Escherichia coli* ATCC 35218, and *Pseudomonus aeruginosa* ATCC 27853. Susceptibility tests were performed for all organisms using modified versions of the methods recommended by the Clinical and Laboratory Standards Institute (CLSI). Samples were transferred in duplicate to 96-well flat bottom microplates. Concentration (CFU/mL) of the microbial inocula were determined by monitoring the absorbance of the cell suspensions at 630 nm. These suspensions were then diluted as needed in the assay broth to afford the desired CFU/mL recommended for the experiment. Drug controls [ciprofloxacin for bacteria and amphotericin B for fungi (both from ICN Biomedicals, Aurora, OH, USA)] were included in each assay. Optical densities of the cultures were read at either 630 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Winooski, VT,

USA) or by excitation at 544 nm and determining emission at 590 nm (*A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Offenburg, Germany) prior to and after incubation [20, 21]. Percent growth was plotted versus test concentration to afford the IC_{50}.

2.5 Antimalarial assay

The antimalarial activity of the oils at concentrations ranging from 1 to 20 μg/mL was tested on chloroquine-sensitive (D6, Sierra Leone) and -resistant (W2, Indochina) strains of *Plasmodium falciparum*. For the assay, which is based on the determination of plasmodial LDH activity, a suspension of red blood cells infected with the D6 strain of *Plasmodium falciparum* (200 μL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL amikacin) was added to the wells of a 96-well plate containing 10 μL of serially diluted test samples. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 °C for 72 h in a modular incubation chamber (Billups-Rothenberg, Del Mar, CA, USA). Briefly, 20 μL of the incubation mixture was mixed with 100 μL of the Malstat™ reagent (Flow Inc., Portland, OR, USA) and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma-Aldrich, St. Louis, MO, USA) was then added and the plate was further incubated in the dark for 1 h. The reaction was stopped by the addition of 100 μL of 5% acetic acid, and the plate was read at 650 nm. The experiment was carried out in triplicate. The standard antimalarial agents, chloroquine and artemisinin, were used as positive controls, while DMSO was used as the negative one. IC_{50} values were computed from the dose – response curves [21, 22].

2.6 Antileishmanial assay

The *in vitro* antileishmanial activity of the essential oils was tested at concentrations ranging from 1 to 20 μg/mL against *Leishmania donovani* promastigotes grown in RPMI 1640 medium supplemented with 10% GIBCO fetal calf serum at 26 °C. A 3-day-old culture was diluted to 5×10^5 promastigotes/mL. Samples were added directly to the cultures in a 96-well plate, followed by incubation for 48 h at 26 °C. Growth of the promastigotes was determined by the Alamar Blue assay (BioSource International, Camarillo, CA, USA). Standard fluorescence was measured by a Fluostar Galaxy plate reader (excitation at 544 nm; emission at 590 nm). The experiment was carried out in triplicate. Pentamidine was used as positive standard. Percent growth was calculated and plotted against the tested concentrations in order to determine the IC_{50} values [20, 21, 23].

3 Results and discussion

The chemical composition of the water-distilled essential oils from aerial parts of ML, MM, MA, and TP was characterized by GC/MS (Table 1 and Figures S1 and S2 in Supplementary Materials). In the essential oils of ML, MM, MA, and TP, total numbers of 22, 23, 14, and 20 compounds were identified, representing 93.0, 99.3, 78.1, and 81.1%, respectively, of the oils' components. Cineole (33.4%) and pulegone (40.7%) were the major compounds in the ML essential oil, differing from those present in the essential oil from *M. longifolia* L. ssp. *schimperi* grown in Sudan, which was rich in carvone (67.3%) and limonene (13.5%) [24]. Among the minor components were *cis*-pulegone oxide (2.5%), epoxy-*p*-mentha-1,8(10)-diene (2.4%), menthone (2.4%), isomenthone (1.9%), β-pinene (1.4%), limonene (1.3%), and isopulegone (1.3%).

The MM essential oil mainly consisted of carvone (64.6%) and limonene (19.5%). Previously, pulegone (34.1%), piperitenone oxide (32.9%), and piperitenone (11.3%) had been reported as the main constituents of the essential oil obtained by hydro-distillation of the fresh aerial parts of *M. microphylla* collected in the Gennargentu Mountains of Sardinia, Italy [25, 26]. MM essential oil reported herein did not contain any of these three components. This variation may be attributed to geographical and seasonal variation. Quantitative and qualitative variation both within and between populations/biotypes of the same species have been reported before [27].

The essential oil of MA was mostly enriched in β-linalool (22.9%) and α-terpineol (12%), while in the TP oil (*E*)-3-caren-2-ol (12.1%) was the major component, in addition to δ-cadinene (8.4%), spathulenol (7%), γ-muurolene (5.9%), 4-terpineol (5.8%), (*E*)-pinocarveol (5.4%), β-linalool (5.3%), and t-cadinene (5%). A previous report on TP essential oil collected from the northern region of Saudi Arabia (Aljuf) had revealed the presence of γ-muurolene (8.7%), α-cadinol (5.9%), δ-cadinene (5.1%), β-pinene (4.6%), β-gurjurene (4.4%), α-limonene (4.3%), α-pinene (3.8%), α-thujene (3.7%), spathulenol (3.4%), p-cymene (3.0%), γ-cadinene (2.8%), and sabinene (2.5%) [17].

It has been reported that biosynthesis and metabolism of essential oils are strongly influenced by environmental factors, such as temperature, nutrition, and salinity [28],

Table 1: Relative percentage of volatile compounds in the essential oils of *Mentha longifolia*, *M. microphylla*, *M. australis*, and *Teucrium polium* identified by GC-MS.

Peak no.	Kovats index	Compound	%			
			M. longifolia	*M. microphylla*	*M. australis*	*T. polium*
1	937	α-Pinene[a]	0.8	0.9	–	–
2	954	α-Fenchene	0.1	0.1	–	–
3	962	2-Heptenal	0.1	–	–	–
4	976	Sabinene	0.2	0.4	–	–
5	982	β-Pinene[a]	1.4	1.2	–	–
6	990	β-Myrcene[a]	0.4	0.3	–	–
7	999	3-Octanol	–	0.4	–	–
8	1028	*m*-Cymene	0.3	0.3	–	–
9	1034	Limonene[a]	1.3	19.5	–	–
10	1039	Cineole[a]	33.4	4.3	–	–
11	1057	3-Thujen-2-ol	–	–	–	2.2
12	1077	Unknown monoterpene	–	–	–	0.6
13	1101	β-Linalool[a]	–	–	22.9	5.3
14	1109	β-Terpinene	–	–	–	1.4
15	1120	Unknown monoterpene	–	–	–	1.8
16	1128	(*Z*)-allo-ocimene	–	–	–	1.8
17	1130	1,3,8-*p*-Menthatriene	–	0.1	–	–
18	1135	Unknown	–	–	–	2.2
19	1141	Limonene oxide	–	0.5	–	–
20	1143	(*E*)-Pinocarveol	–	–	–	5.4
21	1152	(*E*)-3-Caren-2-ol	–	–	–	12.1
22	1155	Menthone	2.4	–	–	–
23	1165	Isomenthone	1.9	0.4	–	–
24	1169	Unknown	–	–	–	0.1
25	1170	Ocimenol	0.4	0.1	–	–
26	1180	Dihydrocarvone	0.2	2.5	–	–
27	1184	Borneol	–	–	3.4	–
28	1185	Isopulegone	1.3	0.3	–	–
29	1191	4-Terpineol	–	0.2	–	5.8
30	1192	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	–	–	8.6	–
31	1194	*p*-Cymen-3-ol	–	–	–	4.3
32	1195	α-Terpineol	0.6	0.2	12	2.9
33	1218	(3*Z*,5*E*)-1,3,5-Undecatriene	0.2	–	–	–
34	1211	Verbenone	–	–	–	3.0
35	1229	(*E*)-Carveol	–	0.9	–	–
36	1238	Unknown monoterpene	1.0	–	1.7	–
37	1251	Pulegone	40.7	0.9	2.3	–
38	1253	Unknown	–	–	–	1.6
39	1256	Carvone[a]	0.6	64.6	1.8	–
40	1273	(*Z*)-Pulegone oxide	2.5	–	–	–
41	1276	2,6-Dimethyl-2,7-octadiene-1,6-diol	–	–	2.2	–
42	1278	Carvone oxide	–	0.4	–	–
43	1283	2,6-Octadien-1-ol, 3,7-dimethyl acetate	–	–	1.5	–
44	1294	Borneol acetate	–	–	1.4	1.6
45	1299	Epoxy-*p*-mentha-1,8(10)-diene	2.4	–	–	–
46	1301	Unknown monoterpene	–	–	3.2	–
47	1303	Unknown aromatic	–	–	–	1.4
48	1314	Unknown monoterpene	1.8	–	–	–
49	1321	Unknown	–	–	2.7	–
50	1334	Unknown monoterpene	–	–	2.5	–
51	1345	Eucarvone	1.1	–	–	–
52	1355	β-Terpinyl acetate	–	0.2	–	2.4
53	1359	Unknown monoterpene	–	–	1.9	–
54	1362	Mint furanone	0.7	–	–	–

Table 1 (continued)

Peak no.	Kovats index	Compound	%			
			M. longifolia	*M. microphylla*	*M. australis*	*T. polium*
55	1370	Unknown	0.6	–	–	–
56	1375	Epoxylinalool	–	–	3.5	–
57	1378	Unknown	0.4	–	–	–
58	1392	α-Cubebene	–	–	–	0.8
59	1395	1-Methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	–	–	2.2	–
60	1379	α-Bourbonene	–	0.5	–	–
61	1403	β-Bourbonene	–	–	–	2.1
62	1405	Unknown	0.3	–	–	–
63	1426	Unknown	–	0.5	–	–
64	1439	β-Farnesene[a]	–	–	–	2.6
65	1442	Unknown monoterpene	–	–	3.0	–
66	1453	Unknown	2.4	–	–	–
67	1482	τ-Gurjunene	–	–	–	3.5
68	1514	τ-Muurolene	–	–	–	5.9
69	1519	Unknown	0.5	–	–	–
70	1522	δ-Cadinene	–	–	–	8.4
71	1562	Chamigrene	–	–	7.3	–
72	1601	Spathulenol	–	–	–	7.0
73	1607	Unknown	–	–	3.3	5.3
74	1618	*t*-Gurjunene	–	–	8.1	–
75	1649	Epijunenyl acetate	–	–	0.9	–
76	1628	*t*-Cadinene	–	–	–	2.6
77	1672	Unknown sesquiterpene	–	–	–	5.0
78	1676	Unknown sesquiterpene	–	–	3.6	–
Non-oxygenated compounds		Monoterpenes and aliphatic hydrocarbons (%)	4.8	22.8	–	3.2
		Sesquiterpene hydrocarbons (%)	–	0.5	15.4	25.9
Oxygenated compounds		Alcohols (%)	3.4	2.3	51.3	45.0
			–	0.5	–	7.0
		Oxides (%)	33.4	4.8	–	–
		Epoxides (%)	2.4	–	3.5	–
		Ketones (%)	51.4	68.7	4.1	3
		Esters (%)	–	0.2	3.8	4.0
Total			100.0	99.8	100.0	99.1
Not identified			7.0	0.5	21.9	18.0
Identified			93.0	99.3	78.1	81.1

[a]Volatiles identified by comparison with standard.

as well as by factors related to genotype and agronomic conditions, such as harvesting time, plant age, and crop density [29].

The essential oils were evaluated for their antimicrobial activities against *Candida albicans*, *C. glabrata*, *C. krusei*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, methicillin-resistant *Staphylococcus aureus* (MRSA), *S. aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and for antileishmanial activities against *Leishmania donovani* promastigotes, as well as for antimalarial activities against chloroquine-sensitive and -resistant strains of *Plasmodium falciparum*. TP and MA essential oils exhibited antileishmanial activity with IC_{50} values of 2.3 and 3.7 μg/mL, respectively, compared to the IC_{50} of 2.1 μg/mL of the positive control, pentamidin. The MM essential oil exhibited weaker activity (IC_{50} 6.1 μg/mL). The antileishmanial activity of MA is likely due to its high linalool content (22.9%) [30], and that of TP may be due to

(*E*)-3-caren-2-ol (12.1%). Moreover, MA exhibited moderate antifungal activity against *C. krusei* and *C. glabrata* with IC_{50} values of 1.02 and 1.23 µg/mL, respectively, compared to amphotericin B (IC_{50} 0.34 µg/mL). Only weak antibacterial activities were observed towards all examined strains, and none of the samples inhibited the growth of the two *P. falciparum* strains.

4 Conclusion

The essential oils of *M. longifolia*, *M. microphylla*, *M. australis*, and *T. polium* were dominated by the large amounts of oxygenated compounds which were highly odoriferous and most valuable in terms of their contribution to the oil fragrance in addition to their antimicrobial effect. The essential oils of TP and MA showed promising antileishmanial activities towards *L. donovani*. In contrast, MA essential oil exhibited antifungal activities towards *Candida kursei* and *Candida glabrata*.

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