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Research Article

Essential Oil Composition and Antimicrobial Activities of *Lavandula pubescens, Marrubium vulgare* and *Meriandra bengalensis* from Yemen

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Abstract

In Yemen, family Lamiaceae is one of the most important families due to its use in folk medicine and for the commercial production of essential oils. In this study, three Lamiaceae plants; *Lavandula pubescens* Decne*, Marrubium vulgare* L. and *Meriandra bengalensis* (Roxb.) Benth*,* were chemically investigated by GC/MS as well as their antimicrobial activity was assessed using agar-well diffusion method. The major compounds in *Lavandula pubescens* oil were carvacrol (54.98%), thymol methyl ether (12.15%) and *Z*-caryophyllene (4.47%), while those of *Marrubium vulgare* were *Z*-caryophyllene (10.95%), octadecanol (10.44%) and *α*-bisabolene (9.72%). *Meriandra bengalensis* contained camphor (64.87%) as a major compound with 1,8 cineole (12.16%) and camphene (11.96%). The strongest antimicrobial activity was observed in *Lavandula pubescens* against *Klebsielle pneumoniae (*1.95 µL/mL) which is higher than that of gentamycin, *Enterobacter cloacae (*0.98 µL/mL), and *Bacillis subtilis* (0.49 µL/mL) that are similar in activity to that of standards (gentamycin and ampicillin). Oils of *Marrubium vulgare* L. and *Meriandra bengalensis* exhibited a range of activities between weak and none. Our result concludes that *Lavandula pubescens* possess pronounced antimicrobial activity against *Klebsielle pneumoniae, Enterobacter cloacae* and *Bacillis subtilis* giving it a highly medicinal impact value to be further studied *in vivo* against different types of microbial infectious diseases

Keywords: Lamiaceae; Volatile Oil; Antimicrobial Activity

Introduction

Lamiaceae, formerly called Labiatae, the mint family of flowering plants, with 252 genera and 6700 taxa, the largest family of the order Lamiales. Lamiaceae is distributed nearly worldwide, and their species are cultivated for their fragrant leaves and attractive flowers. The family is of high importance for flavor, fragrance, or medicinal activities [1,2].

In Yemen, Lamiaceae family is represented by 23 genus with 23 species are endemic to Yemen. Lamiaceae EOs showed the highest antimicrobial efficiency against many microorganisms as *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus fumigatus*, *Candida albicans...etc*) [3,4]. These properties could be attributed to the main constituents of Lamiaceae essential oils such as carvacrol, thymol, *p*-cymene, 1,8-cineole, caryophyllene [5-7].

Food borne diseases have become one of the public health problems as microbial resistance against lots of antibiotics has increased worldwide. Foodborne pathogens are the causes of illness and death in developing countries [8]. The spectrum of foodborne pathogens includes a variety of enteric bacteria, aerobes and anaerobes, viral pathogens and parasites [9]. Salmonella, *Escherichia coli*, Shigella and *Staphylococcus aureus* are considered as most

common foodborne pathogens [10]. With the renaissance of "back to nature", increasing concerns over food safety and a high demand for eco-friendly natural products has led to a growing interest in natural antimicrobial compounds.

Essential oils exhibit a wide range of antimicrobial activities, and different plant extracts for food preservation [11]. The chemical composition and properties of the essential oils of plants growing in Bani Matar area (Yemen) have never been investigated before. So, in this study, the chemical composition of essential oils isolated from *Lavandula pubescens* Decne*, Marrubium vulgare* L. and *Meriandra bengalensis* (Roxb.) Benth and their antimicrobial activities have been assessed.

Materials and Methods

Plant material

Three medicinal Lamiaceae taxa were collected; *Lavandula pubescens, Marrubium vulgare* and *Meriandra bengalensis*, during the rainy season in 2-22/8/2015 from different locations in Bani Matar District, Sanaa governorate, Yemen (Table 1). The identification of the specimens was done by utilizing the available taxonomic and floristic literatures [8-11]. Voucher specimens have been deposited at the Herbarium of Faculty of Pharmay, Ain Shams University (PHG-LP-303, PHG-MV-304, PHG-MB-305) and a duplicate of each herbarium specimen was kept at the Herbarium of Biology Department, Faculty of science Sanaa University.

No.	Medicinal plant	Local name	% yield*	Date
1	*Lavandula pubescens* Decne.[a)]	Vaheia	1.00	14/9/2015
2	*Marrubium vulgare* L.[b)]	Hei wjazp	0.05	20/5/2016
3	*Meriandra bengalensis* (Roxb.)Benth.[c)]	ḍarū	5.00	10/9/2015
Plants were collected at altitude 2402-3353 m nearby following villages: [a)] Kusher; [b)] Al-saih (Jabal An-Nabi Shuayb); [c)] Kusher. *% v/w: volume of oil in mL obtained from 100 g of fresh plant material.				

Table 1: Data of collection and yield of essential oil obtained from ***Lavandula*** *pubescens*, *Marrubium vulgare* and *Meriandra bengalensis.*

Isolation of the essential oil

The fresh leaves and green branches of the three plants were chopped into small pieces. The essential oil was isolated from each part by hydro distillation for 5 h using a Clevenger-type all glass apparatus. Each oil was transferred to a screw-capped glass vial, dried (Na_2SO_4) and stored at 4 °C in the dark until analysis.

Analysis of essential oils by GC and GC-MS

GC analysis was carried out using a GC HP 5890 Hewlett Packard equipped with FID and HP-5 fused silica capillary column 30 m × 0.25 mm i.d., film thickness 0.25 µm, using a sample volume of 0.03 µL. Oven temperature was programmed from 60 ºC to 240 ºC at 3 ºC/min; injector temperature 250ºC; detector temperature 280 ºC; carrier gas was helium, flow rate was 1.0 mL/min; automatic sample injection, 0.02 µL of the oil; split was 1/70. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization. GC-MS analysis was performed on a Perkin-Elmer quadrupole MS system (Model 5) coupled with the GC HP 5972, equipped with a HP-5 capillary column. Oven temperature was programmed from 45 ºC to 240 ºC at 3 ºC/min; injector temperature 250 ºC; carrier gas was helium, flow rate was 0.5 mL/min; automatic sample injection, 0.02 µL of the oil; split: 1/70. The MS operating parameters were interface temperature: 300 ºC, ion source temperature: 200 ºC, EI mode: 70 eV, scan range was 41-400 amu.

Identification of the components

Mass spectra of the individual GC peaks were identified by a computer search of the commercial libraries (WILEY, NIST), as well as matching with published mass spectra [12-14]. The identification was further confirmed by the calculation of the retention indices (RI) relative to (C6-C22) *n*-alkanes [15].

Antimicrobial screening

A series of bacterial and fungal strains available in stock culture of the Regional Center for Mycology and Biotechnology Antimicrobial Unite (RCMB), Cairo, Egypt, were used for antibiotic sensitivity testing comprising: Gram-positive Bacteria [Staphylococcus aureus (RCMB 010067) *Enterococcus faecalis* (RCMB 010028); *Bacillus subtilis* (RCMB 010063)], Gram-negative Bacteria [*Enterobacter cloacae* (RCMB 010072); *Klebsiella pneumoniae* (RCMB 010052); *Escherichia coli* (RCMB 010093], and Fungi [*Aspergillus fumigatus* (RCMB 02568), *Candida albicans* (RCMB 05036), *Saccharomyces cerevisiae* (RCMB 05177);]. The previously prepared essential oil

was diluted 1/3 v/v in dimethyl sulphoxide (E-Merck), 30 µl (containing 10 µl of pure oil) were used in the test. Dimethyl sulphoxide (50 µl) was used as a negative control. The agar diffusion method [16] was applied using trypticase soy agar (Difco) medium inoculated with the bacterial or fungal suspension of the test organisms. Discs 5 mm in diameter were impregnated with the oils or the control. Then the discs were placed onto the surface of the culture medium. Discs of ampicillin, gentamycin and amphotericin B were used as standard antibacterial and antifungal agent, respectively. The plates were incubated at 35- 37°C for 24-48 hours in case of bacteria, 25°C for 48 hours in case of filamentous fungi, while yeasts were incubated at 30°C for 24-48 hours. After incubation, the diameters of inhibition zones were recorded in millimeters and the results were compiled in table 4. The minimum inhibitory concentrations (MIC) of S. sempervirens oil against the tested microorganisms were also determined by micro dilution method [17].

Results and Discussion

Many Lamiaceae plants induced antimicrobial activities against wide range of bacteria and fungi. For example, the antimicrobial properties of *Dracocephalum kotschyi Boiss*. were determined against 12 strains of microorganism where the highest activities were against *Aspergillus brasiliensis*, *Bacillus subtilis* and *Candida albicans* [18]. Meanwhile, the essential oils obtained from both leaves and flowers of *Salvia hydrangea* may have potential application as bactericidal agents against *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Klebsiella pneumoniae* [19].

Yield of oils and data of collection are listed in Table 1. The higher yield aws to *Marrubium vulgare* 5.00 % v/w and the lowest one was 0.05 % v/w for *Meriandra bengalensis.* Moderate yield was observed in *Lavandula pubescens* (1.00 % v/w). Fourteen peaks were detected in *Lavandula pubescens* oil (90%), (Table 2). Carvacrol represented the major peak (54.9%), followed by thymol methyl ether (12.2%). The most abundant constituents of the *Marrubium vulgare* chromatogram (88.9%) were *Z*-caryophyllene (10.9%), octadecanol (10.4%), and *α*-bisabolene (9.7%), (Table 3). Seventeen peaks were recorded (99.6%) in *Meriandra bengalensis* oil. The major constituents were camphor (64.9%), 1,8-cineole (12.2%) and camphene (12.0%), (Table 4).

No.	Formula	Compounds	KI	Oil Area [%][a]
1	$C_{10}H_{16}$	*α*-Pinene	936	0.23
2	$C_{10}H_{16}$	*β*-Myrcene	990	2.59
3	$C_{10}H_{16}$	*δ*-3 Carene	1009	1.25
4	$C_{10}H_{14}$	*p*-Cymene	1028	0.79
5	$C_{10}H_{16}$	Limonene	1031	0.42
6	$C_{10}H_{16}$	Terpinolene	1087	4.87
7	$C_{10}H_{16}O$	*trans*-epoxy Ocimene	1140	1.01
8	$C_{10}H_{12}O_2$	Phenyl ethyl acetate	1194	1.64
9	$C_{11}H_{16}O$	Thymol methyl ether	1231	12.15
10	$C_{10}H_{14}O$	Carvacrol	1296	54.98
11	$C_{15}H_{24}$	*Z*-Caryophyllene	1404	4.47
12	$C_{15}H_{24}$	*α*-Bisabolene	1507	1.30
13	$C_{15}H_{24}O$	Caryophyllene oxide	1580	3.78
14	$C_{20}H_{32}$	Cembrene	1939	0.55
		Total Identified		90.03
Functional group		Total peak (%)		
Monoterpene hydrocarbons		10.15		
Sesquiterpene hydrocarbons		5.77		
Diterpene hydrocarbons		0.55		
Oxygenated monoterpene		69.78		
Oxygenated sesquiterpene		3.78		
Total hydrocarbons compounds		16.47		
Total oxygenated compounds		73.56		

[a] Values are expressed as relative area percentage; [b]Kovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).

Table 2: Essential oil composition of *Lavandula pubescens.*

No.	Formula	Compounds	KI	Oil Area [%][a]
1	$C_{10}H_{16}$	*α*-Pinene	936	2.27
2	$C_8H_{16}O$	Octadienal	1105	2.23
3	$C_{10}H_{14}O$	Carvacrol	1296	1.11
4	$C_{15}H_{24}$	*α*-Copaene	1374	2.71
5	$C_{15}H_{24}$	*Z*-Caryophyllene	1404	10.95
6	$C_{15}H_{24}$	*Z*-*β*-Farnesane	1444	6.91
7	$C_{15}H_{24}$	*trans* Muuroladiene	1457	3.97
8	$C_{14}H_{24}O$	*α*-Ionol isomethyl	1466	4.18
9	$C_{15}H_{24}$	*α*-Bisabolene	1507	9.72
10	$C_{15}H_{24}$	-Cadinene	1515	4.35
11	$C_{15}H_{26}O$	*Z*-Nerolidol	1534	1.06
12	$C_{15}H_{24}O$	Caryophyllene oxide	1583	3.72
13	$C_{14}H_{24}O_3$	Isoborneol	1632	1.76
14	$C_{15}H_{30}O$	Pentadecanone	1697	1.46
15	$C_{12}H_{14}O_2$	*E*-Ligustilide	1796	1.82
16	$C_{20}H_{40}O$	Phytol	1943	2.54
18	$C_{20}H_{34}O$	*Z, Z*-Geranyl linalol	1961	5.86
19	$C_{20}H_{36}O$	Pseudo phytol	1988	1.95
20	$C_{11}H_{10}O_3$	7-Hydroxy-4,8-dimethyl Coumarin	2013	1.35
21	$C_{20}H_{34}O$	Manool	2060	1.98
22	$C_{18}H_{38}O$	Octadecanol	2077	10.44
23	$C_{18}H_{34}O_2$	Oleic acid	2142	1.21
24	$C_{20}H_{40}O_2$	Octadecenol acetate	2209	5.36
		Total Identified		88.91

Functional group	Total peak (%)
Monoterpene hydrocarbons	2.27
Sesquiterpene hydrocarbons	38.61
Oxygenated monoterpene	3.34
Oxygenated sesquiterpene	14
Oxygenated diterpene	30.69
Total Hydrocarbons compound	40.88
Total oxygenated compound	48.03

[a] Values are expressed as relative area percentage; [b]Kovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).

Table 3: Essential oil composition of *Marrubium vulgare.*

No.	Formula	Compounds	KI	Oil Area [%][a)]
1	$C_{10}H_{16}$	Tricyclene	924	0.57
2	$C_{10}H_{16}$	α-Pinene	936	3.45
3	$C_{10}H_{16}$	Camphene	953	11.96
4	$C_{10}H_{16}$	Sabinene	975	0.22
5	$C_{10}H_{16}$	β-Pinene	981	1.24
6	$C_{10}H_{16}$	δ-3Carene	1009	0.97
7	$C_{10}H_{18}O$	1,8 cineole	1035	12.16
8	$C_9H_{14}O$	Camphenilone	1085	0.23
9	$C_{10}H_{16}O$	trans-Pinocarveol	1143	0.36
10	$C_{10}H_{16}O$	Camphor	1147	64.87
11	$C_{10}H_{18}O$	Borneol	1175	1.33
12	$C10H_{16}O$	cis-Pinocarveol	1185	0.16
13	$C_{10}H_{14}O$	Myrtenal	1198	0.21
14	$C_{10}H_{14}O$	Verbenone	1208	0.35
15	$C_{15}H_{24}O$	Caryophyllene Oxide	1580	0.24
16	$C_{15}H_{26}O$	Eudesmol-epi-	1620	0.16
17	$C_{15}H_{26}O$	Eudesmol	1630	1.11
		Total all		99.59

Functional group	Total peak (%)
Monoterpene hydrocarbons	18.41
Oxygenated monoterpene	79.67
Oxygenated sesquiterpene	1.51
Total hydrocarbon compounds	18.41
Total oxygenated compounds	81.18

[a] Values are expressed as relative area percentage; [b]Kovats retention index calculated on DB-5 column; The major components are highlighted in bold. (Values expressed as relative area percentages to the total identified components).

Table 4: Essential oil composition of *Meriandra bengalensis.*

Reported literatures concerning *Lavandula pubescens* oil revealed that carvacrol was the major compound in the oil (60.9-70%) [20]. It is the major peak in the leaf oil of eight wild *Lavandula pubescens* plants from different locations in Yemen [20].

Different compositional patterns appear obviously upon comparing our data with previous studies regarding EO of *Marrubium vulgare.* Iranian oil contained *β*-caryophyllene (32.2%), (E)-*β*-farnesene (11.4%) and 1,8-cineole (8.2%) [21]. Essential oil isolated from Egyptian species composed of carvacrol (36.3%) and *β*-phellandrene (15.5%) [22]. Turkeian oil composed of *α*-pinene (28.9%), *β*-pinene (18.3%) and *β*-phellandrene (17.4%) [23]. Carvacrol content is quite high which explains the antimicrobial effect on both Gram negative and Gram positive bacteria as well as fungi. The antimicrobial effect of carvacrol on bacterial cells has been widely explained [24,25].

The content of camphor (64.9%) in our sample of *Meriandra bengalensis* EO was close with those previously reported in Yemen (43.6%). The major component from Indian oil [26] was *l*-linalool

(68.4%) followed by 1,8-cineole (17.4%) that agrees with our result. These differences in components might be attributed to climatic and environmental conditions [27].

Tables 5 and 6 displayed zone of inhibition and minimum inhibitory concentration (MIC) of the three oils. According to literatures which pointed to the potency of a drug according to its MIC: MIC of < 0.5 uL/mL are considered potent; 0.6-1.5 uL/mL are considered moderate; > 1.5 uL/mL are considered weak [28,29]. *Lavandula pubescens* oil induced potent antibacterial activities against *Enterobacter cloacae* (0.98 µL/mL), *Klebsielle pneumoniae (*1.95 µL/mL), which is higher than that of gentamycin and *Bacillus subtilis* (0.49 µL/mL) that is of similar to ampicillin activity. It showed moderate activity against *Escherichia coli* and *Saccharomyces cerevisiae* (1.95 and 0.98 µL/mL respectively). A weak activity was induced against *Enterococcus faecalis* and *Aspergillus fumigatus* (7.81 and 1.95 µL/mL). No activity was observed against *Candida albicans.*

Meriandra bengalensis exhibited moderate activitiy against *Saccharomyces cerevisiae* (3.9 µL/mL) and weak or even no activity against the rest of tested microorganisms.

Also, the EO of *Lavandula pubescens* induced weak activities against *Escherichia coli, Staphylococcus aureus*, *Aspergillus fumigatus* and *Candida albicans,* and no activity against the rest tested organisms.

This diversity of activity of the tested oils could be attributed mainly to the presence of carvacrol thymol methyl ether, *Z*-caryophyllene. It was reported that carvacrol induces antimicrobial activity [30] on some bacteria as *E. coli* and *Salmonella typhimurium*; (MIC: 1 and 3 mM) [31]. Thymol methyl ether was the main constituent of *Satureja sp,* and *Thymus fallax* oils that exhibited antimicrobial activity (MIC values of the oils vary with the bacterial strain tested, ranging from 15.63 to

Plant	Diameter of inhibition zone (mm)[a)b)c)d)]								
	Gram-negative bacteria			Gram-positive bacteria			Fungal species		
	EC	KP	Eco	SA	EF	BS	AF	CA	SC
LP	22.9 ± 0.37	21.4 ± 0.58	20.7 ± 0.63	22.3 ± 0.63	18.1 ± 0.72	24.2 ± 0.25	21.2 ± 0.58	NA	22.4 ± 0.58
MV	NA	11.6 ± 0.63	16.3 ± 0.63	NA	15.2 ± 1.2	NA	12.3 ± 1.2	15.2 ± 0.72	NA
MB	16.2 ± 0.44	15.8 ± 0.12	18.2 ± 0.44	16.2 ± 0.37	NA	18.3 ± 0.37	18.2 ± 0.25	NA	18.9 ± 1.2
Standard	Gentamicin			Ampicillin			Amphotericin B		
	23.8 ± 0.63	20.2 ± 0.12	27.3 ± 0.44	28.9 ± 0.14	25.3 ± 0.58	26.4 ± 0.34	23.7 ± 0.10	21.9 ± 0.12	27.8 ± 0.58

Table 5: Antimicrobial activity of the volatile oils of *Lavandula pubescens*, *Marrubium vulgare* and *Meriandra bengalensis* as inhibition zone diameter.

[a)] Mean zone of inhibition in mm _ standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. The concentration used for the standard antibiotic was 30 mg/mL.

[b)] The test was done using the diffusion agar technique. Well diameter: 6.0 mm. RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms.

[c)] Results are Mean ± SD of triplicate values.

[d)] 6-9 mm: no activity; 12-15 mm: low activity; 15-18 mm: good activity; above 18 mm: significant activity.

LP: *Lavandula pubescens* Decne, MV: *Marrubium vulgare* L. and MB: *Meriandra bengalensis*

AF: *Aspergillus fumigatus* (RCMB 02568), CA: *Candida albicans* (RCMB 05036), SC: *Saccharomyces cerevisiae* (RCMB 05177); SA: *Staphylococcus aureus* (RCMB 010067); EF: *Enterococcus faecalis* (RCMB 010028); BS: *Bacillus subtilis* (RCMB 010063); EC: *Enterobacter cloacae* (RCMB 010072); KP: *Klebsiella pneumoniae* (RCMB 010052); ECo: *Escherichia coli* (RCMB 010093)

NA: no activity.

Plant	Gram-negative bacteria			Gram-positive bacteria					
	EC	KP	ECo	SA	EF	BS	AF	CA	SC
LP	0.98	1.95	1.95	0.98	7.81	0.49	1.95	NA	0.98
MV	NA	NA	125	31.25	NA	NA	125	31.25	NA
MB	31.25	31.25	7.81	15.63	NA	7.81	7.81	NA	3.9
Standard	Gentamicin			Ampicillin			Amphotericin B		
	0.98	3.9	0.98	0.49	0.98	0.49	0.49	0.98	0.49

Table 6: Antimicrobial activities (MIC, uL/mL) of the volatile oils of *Lavandula pubescens*, *Marrubium vulgare* and *Meriandra bengalensis*

[a] Mean zone of inhibition in mm _ standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms. The concentration used for the standard antibiotic was 30 mg/mL.

[b] The test was done using the diffusion agar technique. Well diameter: 6.0 mm. RCMB: Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms.

[c] Results are Mean ± SD of triplicate values.

[d] 6-9 mm: no activity; 12-15 mm: low activity; 15-18 mm: good activity; above 18 mm: significant activity.

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NA: no activity.

125.0 μLmL−1) [32]. One of the major components identified in *Nectandra megapotamica* oil is *Z*-caryophyllene which exhibited strong activity against *Streptococcus mutans* (MIC: 50 μg/mL), *Streptococcus sobrinus* (MIC: 20 μg/mL), *Prevotella nigrescens* (MIC: 50 μg/mL) and *Bacteroides fragilis* (MIC: 31.25 μ/mL) [33]. *β*-Caryophyllene demonstrated selective antibacterial activity against *S. aureus* (MIC 3 ± 1.0 μM) and more pronounced antifungal activity than kanamycin [34]. Some researchers have demonstrated that whole EOs usually induce higher antimicrobial activity than the mixtures of their major compounds, suggesting that the minor compounds are critical to the synergistic activity, though antagonistic and additive effects have also been observed.

Conclusions

It could be concluded that the oil of *Lavandula pubescens,* is more effective as antimicrobial agent than *Marrubium vulgare* and *Meriandra bengalensis*. It showed strong antimicrobial activities against most tested pathogenic microbial and could be considered as potential natural antibiotic for many infections. Its essential oils showed a great importance to be clinically used in the treatment of various *Klebsielle pneumoniae, Enterobacter cloacae* and *Bacillis subtilis Klebsielle pneumoniae, Enterobacter cloacae* and *Bacillis subtilis* infections. However, its activity should be more deeply investigated before proposing it as a natural antibiotic for many infections. Moreover, its toxicity should be tested before any potential application on animal and human beings.

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Volume 5 Issue 10 October 2021