Short Communications

COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *SALVIA PALAESTINA* BENTHAM GROWING IN SAUDI ARABIA

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تمت معالجة الأجزاء الهوائية للنبات العطري سالفيا باليستاينا الذي ينتمي للعائلة الشفوية الذي ينمو في المملكة العربية السعودية بالتقطير المائي لاستخراج الزيت العطري له والذي تم تحليله عن طريق استخدام جهاز كروماتوجرافيا الغاز مع جهاز مطياف الكتلة (GC/MS). أوضح التحليل الكروماتوجرافي وجود ثمانية وثلاثين مركباً (تمثل 99.2% من كمية الزيت العطري) الذي تم تحليله. كما تم التعرف على أربعة وثلاثين مركباً (تمثل 97.1% من مجموع الزيت العطري). وقد أوضح التحليل وجود مركبات بنسبة عالية في الزيت العطري للنبات سالفيا باليستاينا، من أهمها سكليرول (26.8%)، بيتاكار يوفيللين (16.9%)، لينالول (7.8%)، جوايول (5.4%)، وأخيراً - 8,1 سنيول (5.2%). في هذه الدراسة تم اختبار الفاعلية المضادة للميكروبات للزيت العطري ضد سبعة أنواع من البكتيريا الموجبة والسالبة الجرام بالإضافة إلى نوعين من الأنواع المسببة للأمراض من الفطريات، حيث أظهرت الدراسة نتائج إيجابية ومشجعة مضادة للميكروبات.

The aerial parts of *Salvia palaestina* Bentham (Lamiaceae) on steam distillation afforded an essential oil, which was analyzed by gas chromatography-mass spectrometry (GC/MS). Out of thirty-eight peaks (representing 99.2 % of the oil), thirty-four components representing 97.1 % of the total oil composition were identified. The major components were sclareol (26.8 %), β-caryophyllene (16.9 %), linalool (7.8 %), guaiol (5.4 %), and 1,8 -cineole (5.2 %). The oil was tested against seven Gram positive and negative bacteria and two pathogenic fungi, to exhibit an interesting antimicrobial profile.

Keywords: *Salvia palaestina*, Lamiaceae, essential oil, GC/MS, antimicrobial activity.

Introduction

Salvia, the largest genus of the Lamiacea family, includes about 900 species widespread all over the different flora of the world. In this genus, the *Salvia* species (commonly known as sage) are the richest in essential oils. Some members of this section, like *S. officinalis* L. and *S. fruticosa* Poir, are of economical importance as flavouring agents in perfumery and cosmetology. Sage has been credited with a long list of folk medicinal uses: spasmolytic, antiseptic, and astringent (1). In our continuing research on the essential oils of Greek plants growing in Saudi Arabia, we have investigated the essential oil of *Salvia palaestina* Bentham that belongs to genus *Salvia* in order to uncover its biological activity. It is a perennial herb growing wild in the Mediterranean area (2) characterized by a basal rosette of sparsely hairy slightly lobed crenate leaves; branched flower stems to 45 cm tall, blue flowers 2.5 cm long. Previous reports on the plant have shown the presence of three diterpenoids namely, salvipalolide **(1)** 2-oxocandesalvone A **(2)** and salvipalestinoic acid **(3)** In addition, a new methoxylupane derivative **(4)** and several known triterpenes and abietane diterpenes, and several flavonoids together with sitosterol and a mixture of fatty acid esters of tyrosol (3,4). Furthermore, the known terpenoids vergatic acid, ursolic acid, crataegolic acid, lupane-3β-,11α,20-triol and sclareol and sitosteryl 3β-glucoside have been isolated from the leaves of *S. palaestina* Bentham (5,6). The literature survey revealed that no

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phytochemical and pharmacological work has been done so far on the essential oil of the aerial parts of this Saudi plant. We report herein the composition and antimicrobial activity of the essential oil, wherein thirty-four components were identified by gas chromatography-mass spectrometry (GC/MS) using direct injection (Table-1). The antimicrobial activity was screened against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa*, *Mycobacterium smegmatis, Candida albicans, and Candida vaginalis* (Table-2).

Materials and Methods

Plant material: The aerial parts of *Salvia palaestina* Bentham in its flowering shape were collected in March 2005 from Shaeeb Muharaq, 140 km North East of Riyadh, Najd Province, Saudi Arabia. The taxonomist of the department, Dr. Atiqu-Rahman identified the plant, and a voucher specimen has been deposited at the herbarium (#1635), College of Pharmacy, King Saud University for future reference.

Analysis of the essential oil: Fresh aerial parts (330 g) were crushed to coarse powder and steam distilled in a Clevenger apparatus for about eight hours to obtain the yellow colored oil (2.2 ml, 0.66 % v/w), bp 250 -252 ^{o}C; $[\alpha]_D^{22}$ 37.7^{o} (c; 0.5 in CH_3OH) , with a specific gravity of 1.12 at 22 ^{o}C. The oil was subjected to analysis by GC/MS using direct injection in the split mode under the following conditions:

Hewlett-Packard 5973 MSD GC/MS equipped with a quartz capillary column: 30 x 0.32 mm x 1.0 µ Rtx- 5 sil MS (Restek); oven temperature: 40 ^{o}C (hold 3 min) to 200 ^{o}C at 8 ^{o}C/ min then to 320^{o} C at 6 ^{o}C/min (hold 4 min); Injector temperature: 320 ^{o}C; Sample size: 0.2 µ L, split 1: 100; mass range: 35-500 amu, 3.17 scans /sec; carrier gas: He with a flow rate of 1.0 ml/min; ionization energy: 70 eV. The qualitative identification of different constituents was performed by comparison of their retention times and mass spectra with those of Wiley 7th edition mass spectral library.

Antimicrobial screening: The antimicrobial activity was tested according to the National Committee of Clinical Laboratory Standards (NCCLS 2000) using American Type of Culture Collection (ATCC) standard (7) against various microorganisms namely: *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Mycobacterium smegmatis, Candida albicans,* and *Candida vaginalis.* The positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition after 24 hrs incubation period.

Results and Discussion

The results of the analysis of the essential oil were qualitative and quantitative. The fresh aerial parts of *Salvia palaestina* Bentham yielded 0.66 % v/w of essential oil (2.2 ml), bp 250 -252 ^{o}C; $[\alpha]_D^{22}$ 37.7^{o} (c; 0.5 in CH_3OH) , with a specific gravity of 1.12 at 22 ^{o}C. This means that the plant was relatively rich in oil compared with other species of the genus *Salvia*. The constituents identified by GC/MS analysis, their retention times and relative area percentage are summarized in Table-1. Out of thirty-eight peaks (representing 99.2 % of the oil), thirty-four components were identified representing 97.1 % of the total oil composition. Diterpenic alcohols (26.8 %) and sesquiterpene hydrocarbons (25.4 %) were the major constituents in the oil. In addition to sclareol (26.8 %), that was the major diterpenic alcohol, β-caryophyllene (16.9 %), linalool (7.8 %), guaiol (5.4 %), and 1,8- cineole (5.2 %) were present in fairly good amount.

On the other hand, linalool (7.8 %) and 1,8-cineol (5.2 %) were the major monoterpenes present in the oil. Other sesquiterpene hydrocarbons such as β-caryophellene (16.9 %), α–humulene (2.8 %), and δ-cadinene (2.4 %) were also detected in appreciable amounts. Moreover, some minor components were also detected of which bornyl acetate (2.0 %), terpin-4-ol (1.5 %), thymol (1.2 %), carvacrol (1.2 %), α-acorenol (1.1 %), and α-terpineol (1.0 %) were identified. Table 3 shows the previously reported oil percentage in other species of the genus *Salvia* growing in Saudi Arabia including *S. palaestina* Bentham (8,9).

Table 1: Identified chemical constituents in the essential oil of *Salvia palaestina* Bentham.

Peak No	Compound	Retention time (min)	M^+	Major fragments m/z	B. P. (°C)	% Area
1.	α-Thujene	6.57	136	105, 93, 77	152	0.2
2.	α-Pinene	6.77	136	121, 105, 93, 77	155	3.7
3.	Camphene	7.22	136	121, 107, 93	159	0.8
4.	β-Pinene	7.98	136	121, 107, 93	161	3.0
5.	β-Myrcene	8.50	136	93, 69, 53	165	0.2
6.	P-Cymene	9.42	134	119, 103, 91	177	0.5
7.	Limonene	9.53	136	121, 107, 93	176	3.0
8.	1,8-Cineole	9.88	154	139, 108, 93	177	5.2
9.	γ -Terpinene	10.30	136	121, 105, 93	183	0.6
10.	Cis-Sabinene hydrate	10.67	154	139, 121, 111, 93	164	0.4
11.	p -Cymenene	10.98	132	117, 91	198	1.0
12.	Trans-Sabinene hydrate	11.13	154	139, 121, 111, 93	199	0.7
13.	Linalool	11.45	154	121, 93	202	7.8
14.	Trans-Sabinol	12.10	152	134, 119, 93	202	1.3
15.	Camphor	12.65	152	108, 95, 77	204	0.7
16.	Borneol	13.21	154	139, 121, 95	Sublimes	0.6
17.	Terpinen-4-ol	13.45	154	136, 111, 93	211	1.5
18.	α-Terpineol	13.81	154	136, 121, 93	219	1.0
19.	Bornyl acetate	15.97	196	154, 136, 121	226	2.0
20.	Thymol	16.31	150	135, 115, 91	232	1.2
21.	Carvacrol	17.04	150	135, 115, 91	237	1.2
22.	β-Caryophyllene	18.82	204	189, 161, 133, 105	244	16.9
23.	α-Humulene	19.65	204	189, 147, 121, 107	248	2.8
24.	γ-Curcumene	20.11	204	161, 134, 119, 105	250	0.9
25.	β-Bisabolene	20.37	204	189, 161, 134, 119	252	0.4
26.	δ-Cadinene	20.96	204	189, 161, 134, 119	253	2.4
27.	Guaiol	21.74	222	204, 189, 161, 119	255	5.4
28.	Sclareol	22.82	308	290, 257, 204, 191	258	26.8
29.	α-Acorenol	22.91	222	204, 161, 119	258	1.1
30.	Epi- α -Cadinol	23.23	222	204, 189, 161	260	0.8
31.	β-Eudesmol	23.60	222	204, 189, 161	262	0.9
32.	α -Eudesmol	23.73	222	204, 189, 161, 149	263	0.6
33.	α -Cadinol	23.87	222	204, 189, 161	264	0.9
34.	β-Bisabolol	23.99	222	204, 119, 111, 93	266	0.6
35.	unknown	8.98	_	_	_	0.7
36.	unknown	14.61	_	_	_	0.3
37.	unknown	17.92	_	_	_	0.4
38.	unknown	25.22	_	_	_	0.7

Table 2: Antimicrobial activity of the essential oil of *Salvia palaestina*.

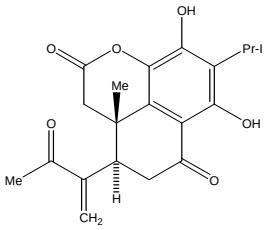
Microorganism	Inhibition Zone (mm) with 50 µl	MIC (µg/ml) of oil	Inhibition Zone (mm) (10 µg/ml) Gentamicin	MIC (µg/ml) Gentamicin	Inhibition Zone (mm) (10 µg/ml) Amphotericin B	MIC (µg/ml) Amphotericin B
Bacillus subtilis	8	500	12	4	NA	NT
Staphylococcus aureus	10	400	14	2	NA	NT
Staphylococcus epidermidis	10	400	12	2	NA	NT
Escherichia coli	NA	NT	16	1.5	NA	NT
Proteus mirabilis	8	550	16	2	NA	NT
Pseudomonas aeruginosa	NA	NT	7	4	NA	NT
Mycobacterium smegmatis	9	550	16	16	NA	NT
Candida albicans	13	400	NA	NT	16	25
Candida vaginalis	13	400	NA	NT	16	30

NA: Not active
NT: Not tested

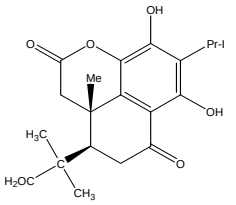
Table 3: Identified chemical constituents in the essential oil of some *Salvia* Spp. Growing in Saudi Arabia.

		% Area		
Peak No.	**Compound**	***S. lanigera***	***S. verbenaca***	***S. palaestina***
1.	α- Thujene	0.2	1.0	0.2
2.	α- Pinene	10.7	7.3	3.7
3.	Camphene	0.8	1.5	0.8
4.	Sabinene	_	16.0	_
5.	β- Pinene	6.5	6.3	3.0
6.	1-Octen-3-ol	_	0.3	_
7.	β- Myrcene	0.2	0.3	0.2
8.	α- Phellandrene	_	0.3	_
9.	δ-3- Carene	_	4.0	_
10.	α- Terpinene	_	1.1	_
11.	o- Cymene	_	0.3	_
12.	p- Cymene	0.5	1.7	0.5
13.	Limonene	5.6	6.7	3.0
14.	1,8- Cineole	36.2	_	5.2
15.	γ- Terpinene	0.8	2.7	0.6
16.	Sabinene hydrate	_	0.2	_
17.	Cis- Sabinene hydrate	0.4	_	0.4

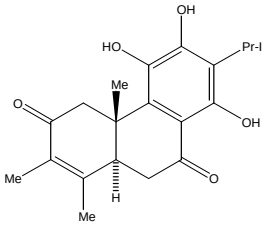
18.	Terpinolene	_	0.7	_
19.	p- Cymenene	1.0	_	1.0
20.	Trans- Sabinene hydrate	0.7	_	0.7
21.	Linalool	1.1	3.1	7.8
22.	Trans-1-methyl-4-isopropyl-2-cyclohexene-1-ol	_	0.4	_
23.	Trans-Pinocarveol	_	0.3	_
24.	Cis-1-methyl-4-isopropyl-2-cyclohexene-1-ol	_	0.4	_
25.	Trans- Sabinol	1.3	_	1.3
26.	Camphor	0.7	_	0.7
27.	Borneol	0.6	1.2	0.6
28.	4- Terpineol	_	7.4	_
29.	Terpin-4-ol	7.5	_	1.5
30.	α- Terpineol	1.0	1.2	1.0
31.	Bornyl acetate	4.5	0.3	2.0
32.	Thymol	1.2	_	1.2
33.	Carvacrol	1.2	_	1.2
34.	α- Copaene	0.2	3.0	_
35.	Bourbonene	_	0.8	_
36.	β- Elemene	_	3.4	_
37.	β- Caryophellene	2.5	5.0	16.9
38.	γ- Elemene	_	1.0	_
39.	δ-Guaiene	_	0.5	_
40.	Trans-α- Bergamotene	0.2	_	_
41.	α- Humulene	0.2	1.4	2.8
42.	α- Amorphene	_	0.7	_
43.	Germacrene-D	_	0.5	_
44.	β- Selinene	_	0.8	_
45.	β -Guaiene	_	1.2	_
46.	β- Acoradiene	0.2	_	_
47.	γ- Curcumene	0.3	_	0.9
48.	β- Bisabolene	0.4	_	0.4
49.	γ- Cadinene	0.1	_	_
50.	δ- Cadinene	2.0	_	2.4
51.	Trans- Sesquisabinene hydrate	0.3	_	_
52.	Caryophellene Oxide	0.2	_	_
53.	Guaiol	5.4	_	5.4
54.	Humulene epoxide II	0.3	_	_
55.	α- Acorenol	0.4	_	1.1
56.	Sclareol	_	_	26.8
57.	Epi-α- Cadinol	0.8	_	0.8
58.	β- Eudesmol	0.9	_	0.9
59.	α- Eudesmol	0.6	_	0.6
60.	α- Cadinol	0.6	_	0.9
61.	Cadelene	0.1	_	_
62.	α- Bisabolol	0.2	_	_
63.	β- Bisabolol	0.6	_	0.6



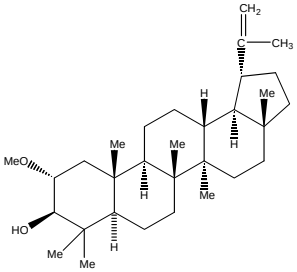
Salvipalolide (1)



Salvipalestinoic acid (3)



2-oxocandesalvone A (2)



Methoxylupane derivative (4)

Table 2 summarizes the antimicrobial properties of the *S. palaestina* Bentham essential oil. The oil showed significant antibacterial and antifungal activity with 50µl of pure oil on various microorganisms tested. The minimum inhibitory concentration (MIC) of the oil was 500 µg/ml against *Bacillus subtilis.* Also, the oil exhibited the same MIC (400 µg/ml) against the two species of Gram positive bacteria, *Staphylococcus aureus* and *S. epidermidis* as well as the two species of the pathogenic fungi, *Candida albicans* and *C. vaginalis*. In addition, the minimum inhibitory concentration against the two Gram negative bacteria, *Proteus mirabilis* and *Mycobacterium smegmatis* was 550 µg/ml. Other microorganisms that were tested such as *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to the oil. Standard antibiotics (Gentamicin and Amphotericin B) were used in order to control the sensitivity of the tested organisms.

Further toxicological and clinical studies are required to prove the safety of oil for medicinal use in order to recommend the oil in the treatment of skin diseases and diarrhea caused by the tested microorganisms.

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