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Composition and Antimicrobial Activity of the Essential Oil of *Salvia lanigera*

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Abstract: The aerial parts of *Salvia lanigera* (Labiatae) afforded an essential oil on steam distillation, which was analyzed by gas chromatography-mass spectrometry (GC/MS) using direct injection. Out of forty-eight peaks (representing 99.4% of the oil), forty-two components were identified representing 98.8% of the total oil composition. The major components were 1,8-cineole (36.2%), α-pinene (10.7%), terpin-4-ol (7.5%), β-pinene (6.5%), limonene (5.6%) and bornyl acetate (4.5%). Furthermore, the oil exhibited a very interesting antimicrobial profile after it was tested against seven gram-positive and –negative bacteria and two pathogenic fungi.

Key words: *Salvia lanigera*, Labiatae, essential oil, antimicrobial activity

Introduction

Salvia, the largest genus of the labiatae family, includes about 900 species widespread all over the world. In this genus, section *Salvia* contains the *Salvia* species richest in essential oils. Some members of this section, like *S. officinalis* and *S. fruticosa*, are of economical importance as flavouring agents in perfumery and cosmetology. Sage has been credited with a long list of medicinal uses: spasmolytic, antiseptic and astringent (Newall *et al*., 1996). In our continuing research on the essential oils of Greek plants, we have investigated the essential oil of *Salvia lanigera* and its biological activity. *Salvia lanigera* belongs to section *Salvia*. It is a perennial plant growing wild in the Mediterranean area (Hedge *et al*., 1976). The leaves of *S. lanigera* are used as an aromatic tea for a variety of abdominal troubles (AL-Hazimi *et al*., 1984). Previous reports on the plant have shown the presence of four diterpene-quinones of the royleanone type (Saleh *et al*., 1978). In addition, three diterpenes, namely isocarnosol, 12-hydroxyisocarnosol and methyl carnosoate were isolated from the petrol extract of the plant (Al-Hazimi *et al*., 1986). As no phytochemical and pharmacological studies on the essential oil of *S. lanigera* are reported. It was thought worthwhile to explore out its chemical composition as well as its antimicrobial activities to correlate the results with the traditional use of this plant for abdominal troubles.

Materials and Methods

Plant material: The aerial parts of *Salvia lanigera* were collected in March 2002 from Wadi Sabha near Zabirah, 200 Km north of Buraydah, Najd Province, Saudi Arabia. Dr. Atiqur-Rehman, Taxonmist identified the plant and a voucher specimen has been deposited at the herbarium, College of Pharmacy, King Saud University, for future reference.

Analysis of the essential oil: Fresh aerial parts (450 g) were crushed to coarse powder and steam distilled in a Clevenger apparatus for about 5 h to obtain the yellow coloured oil (2.4 ml, 0.53% v/w) which 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^2 x 1.0 μ Rtx- 5 sil MS (Restek); oven temperature: 40°C (hold 3 min) to 200°C at 8°C/ min then to 320°C at 6°C/min (hold 4 min); injector temperature: 320°C; sample size: 0.2 μ L, split 1: 100; mass range: 35- 500 amu, 3.17 scans/sec; carrier gas: He; ionization energy: 70 eV. The qualitative identification of different constituents was performed by comparison of their retention times and mass spectra with those of the library.

Pharmacological 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 (Ferraro *et al*., 2000) 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 h incubation periode.

Results and Discussion

The results of the analysis of the essential oil was qualitative and semi-quantitative. The fresh aerial parts of

Table 1: Identified chemical constituents in the essential oil of *Salvia lanigera*

Peak	Compound	Retention time (min)	% Area
1.	α-Thujene	6.56	0.2
2.	α-Pinene	6.77	10.7
3.	Camphene	7.23	0.8
4.	β-Pinene	7.97	6.5
5.	β-Myrcene	8.49	0.2
6.	P-Cymene	9.40	0.5
7.	Limonene	9.53	5.6
8.	1,8-Cineole	9.88	36.2
9.	γ-Terpinene	10.29	0.8
10.	Cis-Sabinene hydrate	10.66	0.4
11.	p -Cymenene	10.98	1.0
12.	Trans-Sabinene hydrate	11.12	0.7
13.	Linalool	11.46	1.1
14.	Trans-Sabinol	12.08	1.3
15.	Camphor	12.63	0.7
16.	Borneol	13.22	0.6
17.	Terpin-4-ol	13.44	7.5
18.	α-Terpineol	13.81	1.0
19.	Bornyl acetate	15.96	4.5
20.	Thymol	16.30	1.2
21.	Carvacrol	17.03	1.2
22.	α-Copaene	17.93	0.2
23.	β-Caryophyllene	18.82	2.5
24.	Trans- α -Bergamotene	19.12	0.2
25.	α-Humulene	19.63	0.2
26.	β-Acoradiene	19.71	0.2
27.	γ-Curcumene	20.10	0.3
28.	β-Bisabolene	20.37	0.4
29.	γ-Cadinene	20.80	0.1
30.	δ-Cadinene	20.95	2.0
31.	Trans-Sesquisabinene hydrate	21.60	0.3
32.	Caryophyllene Oxide	22.22	0.2
33.	Guaiol	21.73	5.4
34.	Humulene epoxide II	22.75	0.3
35.	α-Acorenol	22.91	0.4
36.	Epi- α -Cadinol	23.23	0.8
37.	β-Eudesmol	23.60	0.9
38.	α -Eudesmol	23.71	0.6
39.	α -Cadinol	23.87	0.6
40.	β-Bisabolol	23.98	0.6
41.	Cadalene	24.11	0.1
42.	α -Bisabolol	24.18	0.2

Table 2: Antimicrobial activity of the essential oil of *Salvia lanigera*

Microorganism	Inhibition Zone (mm) with 50 μl	MIC (μg ml^{-1})
Bacillus subtilis	8	600
Staphylococcus aureus	9	450
Staphylococcus epidermidis	9	450
Escherichia coli	NA	NT
Proteus mirabilis	7.5	550
Pseudomonas aeruginosa	NA	NT
Mycobacterium smegmatis	8	550
Candida albicans	12	450
Candida vaginalis	12	450

NT: Not tested NA: Not active

Salvia lanigera yielded 0.53% v/w of essential oil. This means that the plant was relatively rich in oil and fat constituents. The constituents identified by GC/MS analysis, their retention times and area percentage are summarized in Table 1. Out of forty-eight peaks (representing 99.4% of the oil), forty-two components were identified representing 98.8% of the total oil composition. Monoterpenic alcohols were the major constituents in the oil. In addition to 1,8-cineole (36.2%), that was the major monoterpenic alcohol, α-pinene (10.7%), terpin-4-ol (7.5%), β-pinene (6.5%), limonene (5.6%) and bornyl acetate (4.5%) were present in fairly good amount.

On the other hand, guaiol (5.4%) was the major oxygenated sesquiterpene present in the oil. Other sesquiterpene hydrocarbons such as β-caryophyllene (2.5%) and δ–cadinene (2.0%) were also detected in appreciable amounts. Moreover, some minor components were also detected of which trans-sabinol (1.3%), thymol (1.2%), carvacrol (1.2%), linalool (1.1%) and α-terpineol (1.0%) were identified.

Table 2 summarises the antimicrobial properties of the *S. lanigera* essential oil. The oil showed significant antibacterial and antifungal activity with 50 μl on various microorganisms tested. The minimum inhibitory concentration (MIC) of the oil was 600 μg ml^{-1} against *Bacillus subtilis*. Also, the oil exhibited the same MIC (450 μg ml^{-1}) against the two species of gram positive bacteria, *Staphyllococcus 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 (-) bacteria, *Proteus mirabilis* and *Mycobacterium smegmatis* was 550 μg ml^{-1}. Other microorganisms that were tested such as *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to the oil.

The above results strongly support using this plant traditionally as an aromatic tea for abdominal disorders caused by microorganisms tested. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

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