African Journal of Biotechnology Vol. 10(42), pp. 8397-8402, 8 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2615
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Antimicrobial activity of essential oil of *Salvia officinalis* L. collected in Syria

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Accepted 23 May, 2011

The essential oils of *Salvia officinalis* L. collected at two different altitudes in Syrian coastline were analyzed by gas chromatography. Plant's development stage and the ecological factors had impact on the qualitative composition of *S. officinalis* essential oil. Although, the major components of the essential oils extracted from plants grown at both altitudes were 1,8-cineol, camphor, borneol, α-pinene, β-pinene, camphene, β-myrcene and caryophyllene, their percentage changed according to the altitude. *S. officinalis* essential oil was for its antibacterial activities by using Gram- positive and negative bacteria. Both *Staphylococcus aureus* and *Streptococcus* group D were efficiently inhibited after 10 min of contact at oil concentration of 20 µl/ml. The inhibitory effect of the essential oil on *Candida albicans* was total and definitive within a minimum of contact time and oil concentration. But the essential oil showed a temporary bacteriostatic effect on *Escherichia coli*, *Salmonella typhi*, as well as *Pseudomonas aeruoginosa.* In comparison with most known antibiotics, the efficiency of *S. officinalis* essential oil was much better, especially against bacteria resistant to antibiotic.

Key words: Essential oil, *Salvia officinalis* L., antibacterial activities, inhibitory effect, bacteriostatic effect.

INTRODUCTION

Salvia officinalis L. from the family *Lamiaceae*, is a worldwide cultivated aromatic herb that is endemic with Syria where it is known as Kasiin or Mariamia. It grows along the Syrian coastline at different altitudes up to 900 m above the sea level (Mouterde, 1983). As a result of the favorable conditions, 19 species of genus *Salvia* are found in the Syrian flora (Tohme and Tohme, 2002). Although *S. officinalis* has many different uses, essentially, it has been used as herbal remedy for a wide range of disorders and illnesses by applying it either internally or externally. It is employed as diuretic, tonic, menstruation's promoter, local styptic, antiseptic, anti-inflammatory, antifungal and spasmodic pain relief (Ioannides, 2002). It is also used as treatment for dysentery, coughing, indigestion, ulcer, varicose veins, insect bites (Dweck, 2000; Izzo, 2005). On the other hand, it is used for treating nervous conditions, trembling, should be used carefully since large doses can be toxic (Jellin et al., 2000). The properties of *S. officinalis* were depression and to mitigate aging symptoms (Scholey et al., 2008). Moreover, it has a lot of cosmetic uses such as skin and hair care (Barnes and Phillipson, 2007). But in spite of its efficiency in herbal therapy, *S. officinalis* believed to be due to its volatile essential oil, whereas *S. officinalis* is considered to have the highest amount of this oil when compared to the other species within the genus *Salvia* (Giannouli and Kintzios, 2000).

The essential oil of *S. officinalis* has low viscosity and sharp herbal smell; the olfactive qualities of this oil are due to existence of caryophyllene (Chalcat et al., 1998). The content and composition of *S. officinalis* essential oil changes according to the surrounding ecological factors as well as to the plant's development stages (Perry et al., 1999; Santos-Gomes and Fernandes-Ferreira, 2001; Maksimovic et al., 2007).

This research aimed to investigate the composition of the essential oil extracted from *S. officinalis* grown in

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Syria; to evaluate the anti-microbial activities of *S. officinalis* oil against some Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Study locations and preparation of plant materials

Samples of *S. officinalis* were collected from two sites in the Syrian coastline; Al-bahlolia (100 m) and Al-Hafa (500 m). Collecting process took place before and after plant's blossoming in June, July, August and September.

Fresh green leaves were collected in clean polythene bags and codified as follows: 'S1' leaves collected from Al-bahlolia, 'S2' leaves collected from Al-Hafa. Each set, consisting of six leaves mixed together, were washed with distilled water, dried at room temperature until the water content dropped to 4.8%. The weight of each sample was 40 g.

Extraction of essential oil

The air-dried samples were crushed, and then subjected to hydrodistillation for three hours by using Clevenger-type apparatus according to the standard procedure described in the European Pharmacopoeia (1997). In order to eliminate all water, the extracted oils were treated with anhydrous sodium sulphate (Na_2SO_4), then filtered and kept in the dark at 4°C until tested and analyzed.

Gas chromatography (GC) analysis conditions

Separating and analyzing the essential oil of *S. officinalis* samples was done by a Shimadzu GC-17A system equipped with fused silica capillary column (30 m × 0.25 mm), coated with 0.25 µm film Rtx-5MS. The injector and detector temperatures were set at 250 and 280°C, respectively. The applied oven temperature program was: 40°C for 5 min, rising at 4°C/min to 100°C, rising at 19°C/min to 280°C and held for 5 min. The control mode is split and split ratio 5:10. Carrier gas was helium with flow-rate of 1.5 ml/min. The mass spectra were recorded over a range of 30 to 1000 atomic mass unit at 0.5 s/scan. Solvent cut time was 3 min. Ionization energy was 70 eV. The inlet and ionization source temperature were 280°C.

Antimicrobial activities

Microbial strains

A panel of six microorganisms was used to access the antimicrobial activities of *S. officinalis* essential oil. Testing for all microbial strains was performed first by pre-culturing inocula of *Escherichia coli* O157:H7, *Pseudomonas aeruoginosa* (ATCC 27853), *Salmonella typhi* O:9,12, Vi - H: d, *Staphylococcus aureus* (ATCC 25923), *Streptococcus* group D and *Candida albicans* (ATCC 10239) in the following selective media: Nutrient broth, Mannitol agar, Salmonella Shigella (SS) agar, Mac Conkey agar, nutrient agar 1.5%, Bile-Esculine agar Kligler iron agar and Mueller Hinton agar (Biolife, Milano, Italy). The dishes were incubated overnight at 37°C in the case of the bacteria, while *C. albicans* was incubated overnight at 30°C.

Disc diffusion method

Only one essential oil specimen was chosen for the antimicrobial test. This choice was based on the more total components and the higher percentage of certain components such as 1,8-cineole and borneol. As a result, the essential oil extracted from plants grown at 100 m above the sea level was used in this study.

In order to determine the antimicrobial activities of *S. officinalis* essential oil, the disc diffusion method was applied according to NCCLS (1997). Briefly, inocula were prepared by suspending colonies from the pre-cultured test microorganisms to have suspensions of *S. aureus* (47×10^6 cells/ml); *Streptococcus* group D (44×10^6 cells/ml); *E. coli* (1×10^8 cells/ml); *P. aeruginosa,* (18×10^6 cells/ml); *S. typhi* (3×10^8 cells/ml); *C. albicans* (18×10^6 cells/ml). The adjusted suspensions were swabbed onto the surfaces of Muller Hinton agar, then 6 mm filter paper discs were soaked with 5, 10 and 20 µl/ml of the essential oil which were placed on the inoculated dishes.

Petri dishes were kept for 2 h at 4°C, and then incubated for 24 h at 37°C in the case of bacteria, while the yeast was incubated at 30°C. Antimicrobial activity was assessed by measuring the diameter of the inhabitation area (including disc diameter of 6 mm) of the microorganism strain tested with the oil which was measured by a ruler and the number of germs inhibited by each antibiotic agent was calculated according to the following equation:

$$n = (a*N)/A$$

Where, n is the number of inhibited germs/ml; a is the area of the inhibition disc (πr^2) in cm^2; N is the number of germs per ml; A is the area of the Petri dish (πR^2) in cm^2. In order to have valid and reliable results, each test was repeated three times to eliminate any error.

Based on the same principle, disc diffusion method was used to perform the antibiogram to evaluate the antimicrobial activity of the essential oil versus the antibiotic agents (the concentrations of the antibiotic agents were equal to those used in the medicines sold in the drug stores): Disks of teicoplanine (10 µg), penicilline (10 µg), cloxacilline (5 µg), erythromycine (15 µg), lincomycine (2 µg), sulfonamide (300 µg), ampicillin (10 µg), tetracycline (30 µg), ciprofloxacine (5 µg), chloramphénicol (30 µg), gentamycine (10 µg), kanamycine (30 µg), gentamycine (10 µg), sulphamethoxazole and TRIMETHOPRIM (25 µg), kanamycine (30 µg), streptomycine (10 µg) and nalidixic acid (30 µg) were placed on Muller Hinton agar dishes that were inoculated with bacteria. After incubating the dishes at 37°C for 24 h, the diameter of the inhabitation area (including disc diameter of 6 mm) of the bacterial strain tested with antibiotics was measured by a ruler and the number of germs inhibited by each antibiotic agent was calculated according to previously mentioned equation.

RESULTS AND DISCUSSION

Analysis of *S. officinalis* essential oil

Perry et al. (1999), Santos-Gomes and Fernandes-Ferreira (2001) and Maksimovic et al. (2007) stated that many factors such as seasonal and geographical factors affect *S. officinalis* essential oil. Changes in quality and quantity of *S. officinalis* oil were also observed and confirmed in this study.

Altitude had an impact on the essential oil's yield as it was 0.52 and 0.14% at altitudes 100 and 500 m, respectively. Moreover, altitude had effects on the quantitative and qualitative composition of the essential oil. The total components of oil extracted from plants collected at 100 and 500 m were 45 and 30, respectively;

Table 1. Essential oil composition (% of major components) of *S. officinalis* collected in Syria.

Compound *	*S. officinalis* **	*S. officinalis* ***
(1R)-(+)-α- Pinene	3.70	4.50
(-)- Camphene	2.60	5.00
β – Pinene	6.00	5.20
Sabinene	-	0.30
β- Myrcene	3.00	3.50
α- Terpinene	-	0.40
(R)-(+) Limonene	-	-
1,8- Cineole	62.0	55.0
γ- Terpinene	0.30	0.50
p- Cymene	0.60	0.60
Terpinolene	-	0.20
(-)-α- Thujone	1.38	1.80
β- Thujone	0.72	1.50
Camphor	8.00	10.0
(-)- Linalool	0.80	0.80
Linalyl acetate	0.60	0.30
(-)-Trans- Caryophyllene	2.00	1.00
Monoterpene	1.26	1.10
(+)- Menthol	-	-
Borneol	5.00	4.50
α- Terpineol	0.20	-
Geranyl acetate	0.30	-
Geraniol	0.10	0.25
Phytol	0.18	-
Thymol	0.80	0.70
Carvacrol	0.20	0.40
Farnesol	0.20	-
Trans-trans- Farnesol	0.06	0.15
Totals Components	45	30

*Compounds of essential oil extracted from fresh green leaves and flowering top. ***S. officinalis* L.*:* collected at 100 m above the sea level; ****S. officinalis* L.*:* collected at 500 m above the sea level.

sabinene, α-terpinene and terpinolene only existed in essential oil extracted from plants collected at 500 m, while α-terpineol, geranyl acetate, phytol and farnesol existed only in essential oil extracted from plants collected at 100 m (Table 1). Table 1 revealed that the essential oils extracted from 100 and 500 m had the same major constituents, but their percentages differed due to the altitude. The major component in both oils was 1,8-cineol (62-55%), followed by camphor (8 to 10%), borneol (5 to 4.5%), α-pinene (3.7 to 4.5%), β-pinene (6 to 5.2%), camphene (2.6 to 5%), β-myrcene (3 to 3.5%), caryophyllene (2 to 1%); while α and β-thujone were in low concentration (0.72 to 1.5%). The concentrations of the major constituents in the essential oil of *S. officinalis* observed in this study were different from those obtained by Grella and Picci (1988), Marino et al. (2001) and Tucker et al. (1980). These differences can be attributed to the fact that essential oils are heterogeneous and their quality and quantity vary with the growth stages, ecological conditions and the extraction method (Kim et al., 1995; Ozcan and Erkmen, 2001).

Antimicrobial activities of *S. officinalis* essential oil

Many researches reported that the antimicrobial activities of the essential oils were due to the presence of some major and minor constituents. Ulubelen et al. (1994) observed strong antimicrobial activities of the essential oil of *S. sclarea* and attributed them to the existence of caryophyllene. Dorman and Deans (2000) stated that the minor components of essential oil such as a-pinene and borneol have antimicrobial activities. Also the antimicrobial effects of borneol were also reported by Vardar-Unlu et al. (2003). 1,8-cineole and camphor are well-known chemicals that possess antifungal as well as antibacterial activities (Jalsenjak et al., 1987; Sur et al., 1991; Tzakou et al., 2001).

The essential oil extracted from plants collected at 100 m was used in this study as it had more total component than that of 500 m. Moreover, it had higher percentage of 1,8-cineol and borneol. The essential oil showed antimicrobial activities against the test microorganisms used in this study. The results presented in Table 2 shows that the essential oil of *S. officinalis* inhibited the growth of Gram-positive bacteria (*S. aureus* and *Streptococcus* group D) completely at concentration of 20 µl/ml with the minimum time of contact. At the lowest concentration (5 µl/ml), the inhabitation started after 10 min of contact and increased with time and concentration.

The results presented in Table 3 revealed that gram-negative bacteria (*E. coli*, *S. typhi* and *P. aeruoginosa*) were temporary bacteriostatic in relation to contact time and oil concentration:

1. In spite of the inhibitory effect of the essential oil against *E. coli*, the bacteria resumed their growth after 24 h at concentrations of 5 and 10 µl/ml. But the inhabitation was definitive at high oil concentration.
2. Regarding *S. typhi*, the bacteria growth was inhibited after 1 h of contact with concentrations of 5 and 10 µl/ml, but the bacteria resumed their growth after 24 h even when the highest concentration was used.
3. In the case of *P. aeruginosa* which was proven to be resistant bacteria to *S. officinalis* essential oil, the number of the inhibited bacteria after 10 min and 1 h of contact at any concentration of the essential oil was limited, and the optimum growth was reached after 24 h.

It was observed from this study that the antimicrobial activity of the essential oil was more definite against Gram-positive than against Gram-negative bacteria, that was also verified by Nostro et al. (2000). The higher resistance among Gram-negative bacteria might be due

Table 2. Minimal and maximal inhibitory concentrations of *S. officinalis* essential oil against Gram-positive bacteria.

Bacteria G(+)	V. O.	5 µl			10 µl			20 µl		
	Time	10'	1 h	24 h	10'	1 h	24 h	10'	1 h	24 h
S. aureus, 47 x 10^6 cells/ml	10^{-3}	100	1	0	80	0	0	0	0	0
	10^{-4}	10	0	0	7	0	0	0	0	0
	10^{-5}	0	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0
Streptococcus groupe D, 44 x 10^6 cells/ml	10^{-3}	120	0	0	114	0	0	0	0	0
	10^{-4}	14	0	0	11	0	0	0	0	0
	10^{-5}	5	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0

Table 3. Minimal and maximal bacteriostatic concentraions of *S. officinalis* essential oil against Gram-negative bacteria.

Bacteria G(-)	V. O.	5 µl			10 µl			20 µl		
	Time	10'	1 h	24 h	10'	1 h	24 h	10'	1 h	24 h
E. coli, O:157,H7 1 x 10^8 cells/ml	10^{-3}	65	65	h.g.*	60	22	h.g.	1	0	0
	10^{-4}	6	0	h.g.	2	0	h.g.	0	0	0
	10^{-5}	1	0	h.g.	1	0	h.g.	0	0	0
	10^{-6}	0	0	h.g.	0	0	h.g.	0	0	0
P. aeruginosa, 18 x 10^6 cells/ml	10^{-3}	240	h.g.	h.g.	230	h.g.	h.g.	200	h.g.	h.g.
	10^{-4}	33	h.g.	h.g.	30	150	h.g.	21	37	h.g.
	10^{-5}	6	55	h.g.	1	25	h.g.	1	10	h.g.
	10^{-6}	0	0	h.g.	0	5	h.g.	0	2	h.g.
S. typhi, 0:9,12, Vi - H: d, 3 x 10^8 cells/ml	10^{-3}	88	0	h.g.	87	0	h.g.	27	0	h.g.
	10^{-4}	55	0	h.g.	54	0	h.g.	13	0	h.g.
	10^{-5}	14	0	h.g.	11	0	h.g.	7	0	0
	10^{-6}	6	0	h.g.	0	0	h.g.	0	0	0

*h.g. = High growth.

Table 4. Inhibitory concentrations of *S. officinalis* essential oil against yeast (*C. albicans*).

Yeast	V.O.	5 µl			10 µl			20 µl		
	Time	10'	1 h	24 h	10'	1 h	24 h	10'	1 h	24 h
Candida albicans 18 x 10^6 cells/ml	10^{-3}	0	0	0	0	0	0	0	0	0
	10^{-4}	0	0	0	0	0	0	0	0	0
	10^{-5}	0	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0

to the presence of phospholipidic membrane which limits the effect of oil on the cell membrane (Nikaido and Vaara, 1985).

Table 4 shows that *C. albicans*, was the most susceptible to the essential oil among all the test microorganisms. The inhibition was total and definitive within a minimum time of contact and a minimum concentration. Although the concentration of *C. albicans* was the highest, the remaining number of yeast after the inhabitation test was zero. This remarkable activity against *C. albicans* can be attributed to pinene (Dorman and Deans, 2000).

Results in Table 5 revealed that the numbers of bacteria inhibited by the essential oil were: *E. coli* (100), *S. typhi* (294), *P. aeruginosa* (18), *S. aureus* (470)

Table 5. Minimum inhibitory concentration (MIC) of *S. officinalis* essential oil and anti-microbial discs against Gram-negative and positive bacteria.

Test bacteria	Time of contact	Bacterial concentration	*S. officinalis* L. oil concentration (µl)	Antibiotic concentration/µg
E. coli	10'	100	5	Chloramphenicol (30 µg)
(1 x 10^8 cells/ml)	1h	10,000	5	
Salmonella typhi (3 x 10^8 cells/ml)	1h	300,000	5	Sulphamethoxazole (25 µg) Gentamicine (10 µg)
P. aeruginosa	10'	18	10	Resistant
(18 x 10^6 cells/ml)	1h	18	5	
Staphylocccus aureus	10'	470	5	Chloramphenicol (30 µg)
(47 x 10^6 cells/ml)	1h	4,700	10	(2+)
Streptococcus group D,	10'	44	5	Ampicillin (10 µg) Chloramphenicol (30 µg) Sulphamethoxazole (25 µg)
(44 x 10^6 cells/ml)	1h	44,000	10	

and *Streptococcus* group D (44). The efficiency of the anti-microbial against the used bacteria were as follows: The highest efficiency among the anti-bacterial discs used was by chloramphenicol (30 µg) for *E. coli*; sulphamethoxazole (25 µg) and gentamicine (10 µg) for *S. typhi*, chloramphenicol (30 µg) for *S. aureus*; ampiciline (10 µg), chloramphenicol (30 µg) and sulphamethoxazole (25 µg) for *Streptococcus* group D; while *P. aeruginosa* was resistant to all antibiotics, it was obvious that the anti-bacterial activity of *S. officinalis* had strongly exceeded that of the usual antibiotics which inhibited the same numbers of bacteria but with the higher concentrations mentioned earlier.

Conclusion

The essential oil profile of *S. officinalis* was proven to be affected by the ecological and seasonal factors. But despite all factors, the predominance components were 1,8-cineol, camphor, borneol, α-pinene, β-pinene, camphene and β-myrcene and caryophyllene. The essential oil of *S. officinalis* proved to have antibacterial activity against Gram- positive and negative bacteria. This activity was more obvious against Gram- positive than negative bacteria, which might be due the existence of the outer phospholipid membrane of the gram-negative bacteria. In comparison with the commercialized antibiotics, the essential oil exhibited a better efficiency, especially against resistant bacteria to antibiotics. This made this essential oil a good alternative to the traditional antibiotics as well as food preservatives.

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