# Antimicrobial Activity of Some *Salvia* Species Essential Oils from Iran

Morteza Yousefzadi[a,*], Ali Sonboli[b], Farah Karimi[c], Samad Nejad Ebrahimi[d], Behvar Asghari[d], and Amineh Zeinali[a]

[a] Department of Ecology and Systematic, Research Institute of Applied Science, ACECR, Evin, P. O. Box 19835-169, Tehran, Iran. Fax: (+9821) 29903038.
E-mail: morteza110110@gmail.com
[b] Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran
[c] Department of Biology, Faculty of Science, Shahed University, Tehran, Iran
[d] Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran

* Author for correspondence and reprint requests

Z. Naturforsch. **62c**, 514–518 (2007); received January 23, 2007

The aerial parts of *Salvia multicaulis*, *S. sclarea* and *S. verticillata* were collected at full flowering stage. The essential oils were isolated by hydrodistillation and analyzed by combination of capillary GC and GC-MS. The *in vitro* antimicrobial activity of the essential oils were studied against eight Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Bacillus pumulis*, *Enterococcus faecalis*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*). The results of antibacterial activity tests of the essential oils according to the disc diffusion method and MIC values indicated that all the samples have moderate to high inhibitory activity against the tested bacteria except for *P. aeruginosa* which was totally resistant. In contrast to antibacterial activity, the oils exhibited no or slight antifungal property, in which only the oil of *S. multicaulis* showed weak activity against two tested yeasts, *C. albicans* and *S. cerevisiae.*

*Key words:* Antimicrobial Activity, Essential Oil Compositions, *Salvia*

## Introduction

One of the largest genera of the family Lamiaceae, *Salvia* L., is represented by over 900 species and is widely distributed all over the world. Fifty-eight species of this genus are documented in the Flora of Iran, 17 of them are endemic (Mozaffarian, 1996). *Salvia* species have been used since ancient times for different ailments ranging from aches to epilepsy, and mainly to treat colds, bronchitis, tuberculosis, hemorrhage, and menstrual disorders (Topcu, 2006). Based on their well-characterized antioxidant, aromatic and antimicrobial properties, it is not surprising that members of this genus have been used in the cosmetic industry, popular medicine and as food flavouring and preservation products (Vallejo *et al.*, 2006).

In the present study antimicrobial activity of the essential oils of three *Salvia* species named *S. verticillata*, *S. sclarea* and *S. multicaulis* were investigated. The literature survey revealed that only chemical composition of the oils of the aforementioned species has previously been published but their antimicrobial properties were not studied up to now. Twenty seven components were characterized for *S. verticillata* with $\beta$-caryophyllene (24.7%), $\gamma$-muurolene (22.8%), limonene (8.9%) and $\alpha$-humulene (7.8%) as the major constituents (Sefidkon and Khajavi, 1999). In the oil of *S. multicaulis*, camphor (11.0%), 1,8-cineole (10.7%), borneol (8.9%) and $\alpha$-pinene (7.5%) have been characterized as main compounds from fifty identified components (Morteza-Semnani *et al.*, 2005). The chemical composition of the essential oils of *S. sclarea* from different countries has already been published and linalool, linalyl acetate, terpineol, geraniol, geranyl acetate, germacrene D, $\beta$-caryophyllene and sclareol were identified (Soules and Argyriadou, 1997; Moretti *et al.*, 1997; Peana *et al.*, 1999; Pitarokili *et al.*, 2002; Lorenzo *et al.*, 2004).

## Material and Methods

### *Plant material*

The aerial parts of three *Salvia* species were collected from the province Tehran, Iran, at an alti-

Table I. Information on *Saliva* spp. and their essential oil yields and compositions.

| Species | Locality | Voucher number | Oil yields (%) | Major components | Percentage |
|---|---|---|---|---|---|
| *S. multicaulis* Vahl. | Teharn: Tehran-Ghazvin road, Alamot May 10, 2006 | As 85011 | 0.5 | 1,8-Cineole<br>$\alpha$-Pinene<br>$\beta$-Caryophyllene<br>Borneol<br>Camphene | 21.0<br>16.5<br>8.9<br>8.3<br>7.5 |
| *S. verticillata* L. | Teharn: Jajrood, Latian dam, May 21, 2006 | As 85095 | 0.4 | $\beta$-Caryophyllene<br>Germacrene D<br>Limonene<br>$\alpha$-Pinene<br>$\alpha$-Humulene | 31.5<br>16.2<br>15.5<br>10.4<br>9.4 |
| *S. sclarea* L. | Teharn: Taleghan July 11, 2006 | As 85105 | 0.6 | Sclareol<br>Germacrene D<br>Linalool<br>$\alpha$-Terpineol<br>Geraniol | 11<br>9.8<br>9.0<br>7.4<br>4.8 |

tude of 1800–2500 m around May–July 2006. Voucher specimens are deposited at the Herbarium of Ecology and Systematic Department, Research Institute of Applied Science, Shahid Beheshti University, Tehran, Iran.

*Essential oil isolation*

The powdered plant parts (250 g) were hydrodistilled using a Clevenger type apparatus for 3 h. The resulting essential oils were dried over anhydrous sodium sulfate and stored at 4 °C until analyzed and tested.

*Essential oil analysis and identification procedure*

GC-FID analyses of the oil were conducted using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i. d., film thickness 0.25 $\mu$m). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The split ratio was 1/50. The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively. GC-MS analysis was carried out on a Thermoquest-Finnigan Trace GC-MS instrument equipped with the same column and temperature programming as mentioned for GC. Transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio equal to 1/50.

The constituents of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkanes ($C_6$–$C_{24}$) and the oil on a DB-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7.0) or with those of authentic compounds and confirmed by comparison of their retention indices with those of authentic compounds or with those reported in the literature (Adams, 2001). Semi-quantitative data was obtained from FID area percentages without the use of correction factors.

*Bioassay procedure*

Eleven microbial strains were used which included; *Bacillus subtilis* (ATCC 465), *B. pumulis* (PTCC 1274), *Enterococcus faecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 85327), *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763).

The antimicrobial activity of the essential oils and their main components was determined by the disc diffusion method (NCCLS, 1997). Briefly, 0.1 ml of a suspension of the test microorganism ($10^8$ cells/ml) was spread on Mueller-Hinton agar plates for bacteria and Sabouraud dextrose agar for the fungi. Sterile 6 mm discs, each containing 10 $\mu$l of essential oils were placed on the microbial lawns. Discs containing 10 $\mu$l of limonene, ocimene, 1,8-cineole, $\alpha$-pinene and sclareol (10 $\mu$l of

0.01 mg/ml) were used to study the antimicrobial activity of the major oil components. The plates were incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for fungi. The diameters of the zones of inhibition were measured and reported in mm. Triplicate tests were carried out for each oil.

MIC values were determined by the broth microdilution assay recommended by NCCLS (1999). Serial two-fold dilutions of the essential oils were made in Mueller-Hinton broth containing 0.5% Tween 80 for bacteria and Sabouraud dextrose broth with 0.5% Tween 80 for fungi in 96-well microtiter plates. Fresh microbial suspensions prepared from overnight grown cultures in the same media were added to give a final concentration of $5 \times 10^5$ organisms/ml. Controls of medium with microorganisms or the essential oil alone were included. The microplates were incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for fungi. The first dilution with no microbial growth was recorded as MIC.

## Results and Discussion

Hydrodistilled essential oils of three examined *Salvia* species yielded 0.5, 0.4 and 0.6%, respectively (Table I). Monoterpenes, 1,8-cineole (21.0%) and $\alpha$-pinene (16.5%), were the main constituents of the oil of *S. multicaulis.* In the oil of *S. verticillata* sesquiterpenoids were the major compound group including $\beta$-caryophyllene (31.5%) and germacrene D (16.2%). Sclareol (11.0%) and germacrene D (9.8%) have been determined as main constituents of *S. sclarea* essential oil.

The results of the antibacterial activity test of the essential oils according to the disc diffusion method and MIC values indicated that all the samples have moderate to high inhibitory activity against the tested bacteria except for *P. aeruginosa* which was totally resistant (Table II). From Gram-positive bacteria, *S. epidermidis*, *B. pumulis, B. subtilis* and *S. aureus* were more sensitive to the oils with MIC values of 3.75–7.5 mg/ml for *S. multicaulis* and 7.5 mg/ml and 15 mg/ml for *S. sclarea* and *S. verticillata,* respectively, compared to *E. faecalis* which was more or less resistant. From Gram-negative bacteria*, E. coli* and *K. pneumoniae* showed moderate sensitivity with inhibition zones ranging from 12–14 mm and 9–10 mm and also MIC values of 15 and > 15 mg/ml, respectively. In contrast to antibacterial activity, the oils exhibited

Table II. Antimicrobial activity of essential oils from three species of *Salvia*.

| Microorganism | *S. multicaulis* | | *S. sclarea* | | *S. verticillata* | | Standard antibiotics | |
|---|---|---|---|---|---|---|---|---|
| | IZ[a] | MIC[b] | IZ | MIC | IZ | MIC | Ampicillin[c] | Nystatine[d] |
| *B. subtilis* | 17 ± 0.3 | 7.5 | 17 ± 0.5 | 7.5 | 14 ± 0.2 | 15 | 14 ± 0.4 | – |
| *B. pumulis* | 18 ± 0.2 | 7.5 | 16 ± 0.3 | 7.5 | 14 ± 0.4 | 15 | 15 ± 0.3 | – |
| *E. faecalis* | 9 ± 0.4 | > 15 | 9 ± 0.2 | > 15 | 10 ± 0.1 | >15 | 11 ± 0.3 | – |
| *S. aureus* | 15 ± 0.3 | 7.5 | 15 ± 0.2 | 7.5 | 13 ± 0.3 | 15 | 13 ± 0.3 | – |
| *S. epidermidis* | 19 ± 0.5 | 3.75 | 15 ± 0.4 | 7.5 | 13 ± 0.4 | 15 | 19 ± 0.5 | – |
| *E. coli* | 13 ± 0.6 | 15 | 14 ± 0.5 | 15 | 12 ± 0.5 | 15 | 12 ± 0.2 | – |
| *K. pneumoniae* | 10 ± 0.4 | 15 | 10 ± 0.6 | > 15 | 9 ± 0.2 | >15 | – | – |
| *P. aeruginosa* | – | nt | – | nt | – | nt | 10 ± 0.3 | – |
| *A. niger* | – | nt | – | nt | – | nt | – | 16 ± 0.4 |
| *C. albicans* | 9 ± 0.5 | > 10 | – | nt | – | nt | – | 18 ± 0.5 |
| *S. cerevisiae* | 11 ± 0.3 | 10 | – | nt | – | nt | – | 18 ± 0.2 |

Values are given as mean ± standard deviation.
[a] Inhibition zone includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml.
[c] Tested at 10 $\mu$g/disc.
[d] Tested at 30 $\mu$g/disc.
–, Inactive; 7–14, moderately active; > 14, highly active; nt, not tested.

Table III. Antimicrobial activity of the main compounds of the essential oils from *Salvia* species.

| Microorganism | α-Pinene | | Ocimene | | 1,8-Cineole | | Sclareol | | Limonene | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IZ[a] | MIC[b] | IZ | MIC | IZ | MIC | IZ | MIC | IZ | MIC |
| *B. subtilis* | 15 ± 0.2 | 3.7 (27.6) | 14 ± 0.4 | 7.5 (55.1) | 31 ± 0.3 | 0.9 (6.0) | 30 ± 0.5 | 0.9 (3.0) | 16 ± 0.4 | 3.7 (27.6) |
| *B. pumulis* | 14 ± 0.3 | 3.7 (27.6) | 15 ± 0.3 | 7.5 (55.1) | 34 ± 0.2 | 0.9 (6.0) | 28 ± 0.2 | 0.9 (3.0) | 15 ± 0.3 | 7.5 (55.1) |
| *E. faecalis* | – | nt | – | nt | 10 ± 0.4 | 7.5 (48.7) | 15 ± 0.6 | 7 (24.4) | – | – |
| *S. aureus* | 13 ± 0.4 | 7.5 (55.1) | 9 ± 0.2 | 15 (110.3) | 23 ± 0.5 | 1.9 (12.1) | 24 ± 0.6 | 1.9 (6.1) | 14 ± 0.5 | 7 (55.1) |
| *S. epidermidis* | 14 ± 0.2 | 7.5 (55.1) | 12 ± 0.3 | 7.5 (55.1) | 27 ± 0.3 | 0.9 (6.0) | 29 ± 0.2 | 0.9 (3.0) | 17 ± 0.4 | 3.7 (27.6) |
| *E. coli* | 10 ± 0.1 | 15 (110.3) | 9 ± 0.1 | > 15 (110.3) | 22 ± 0.4 | 0.9 (6.0) | 15 ± 0.4 | 3.7 (12.2) | 10 ± 0.3 | 15 (110.3) |
| *K. pneumoniae* | – | nt | – | nt | 12 ± 0.4 | 7.5 (48.7) | 13 ± 0.2 | 15 (48.7) | – | – |
| *P. aeruginosa* | – | nt | – | nt | – | nt | 10 ± 0.2 | > 15 (48.7) | – | – |
| *A. niger* | – | nt | – | nt | – | nt | – | nt | – | – |
| *C. albicans* | – | nt | – | nt | 10 ± 0.4 | > 10 (64.9) | 11 ± 0.2 | > 10 (32.5) | – | – |
| *S. cerevisiae* | – | nt | – | nt | 13 ± 0.2 | 10 (64.9) | 14 ± 0.51 | 10 (32.5) | – | – |

Values are given as mean ± standard deviation.
[a] Inhibition zone includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml (mM).
Main compounds tested at 10 μl/disc. Sclareol tested at 10 μl/disc of 0.01 mg/ml.
–, Inactive; 7–14, moderately active; > 14, highly active; nt, not tested.

no or slight antifungal property, in which only the oil of *S. multicaulis* showed weak activity against two tested yeasts, *C. albicans* and *S. cerevisiae* with MIC values of >10 and 10 mg/ml, respectively.

Table III shows the antimicrobial activity of five major components of the oils tested. Among them, 1,8-cineole and sclareol exhibited high to moderate antibacterial activity against the test bacteria, while their antifungal properties were moderate. α-Pinene and ocimene showed moderate antibacterial activity except for *K. pneumoniae* and *P. aeruginosa* with inhibition zones ranging from 10–15 and 9–15 mm, respectively. No antifungal activity was determined against these two components. Antifungal property of limonene was noticeable with MIC values of 2.5–5 mg/ml compared to its antibacterial activity which was moderate with MIC values of 3.75–15 mg/ml. Two other main components of the tested oils, borneol and β-caryophyllene, were evaluated and no antimicrobial activity was characterized.

*Acknowledgements*

Academic Center for Education, Culture and Research (ACECR) is acknowledged for financial support of this work (grant number: 1184–11).

Adams R. P. (2001), Identification of Essential Oils Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured Publishing Co., Illinois, USA.

Lorenzo D., Paz D., Davies P., Villamil J., Vila R., Canigueral S., and Dellacassa E. (2004), Characterization and enantiomeric distribution of some terpenes in the essential oil of a Uruguayan biotype of *Salvia sclarea* L. Flavour Fragr. J. **19**, 303–307.

Moretti M. D. L., Peana A. T., and Satta M. (1997), A study on anti-inflammatory and peripheral analgesic action of *Salvia sclarea* oil and its main components. J. Essent. Oil Res. **9**, 199–204.

Morteza-Semnani K., Goodarzi A., and Azadbakht M. (2005), The essential oil of *Salvia aethiopis* L. J. Essent. Oil Res. **17**, 274–275.

Mozaffarian V. (1996), A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran.

National Committee for Clinical Laboratory Standards (NCCLS) (1997), Performance Standards for Antimicrobial Disk Susceptibility Test, 6th ed. Approved Standard. M100-A6. Wayne, Pennsylvania, USA.

National Committee for Clinical Laboratory Standard (NCCLS) (1999), Performance Standards for Antimicrobial Susceptibility Testing, 9th International Supplement. M100-S9. Wayne, Pennsylvania, USA.

Peana A. T., Moretti M. D. L., and Juliano C. (1999), Chemical composition and antimicrobial action of the essential oils of *Salvia desoleana* and *S. sclarea*. Planta Med. **65**, 752–754.

Pitarokili D., Couladis M., Petsikos-Panayotarou N., and Tzakou O. (2002), Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. J. Agric. Food Chem. **50**, 6688–6691.

Sefidkon F. and Khajavi M. S. (1999), Chemical composition of the essential oils of two *Salvia* species from Iran: *Salvia verticillata* L. and *Salvia santolinifolia* Boiss. Flavour Fragr. J. **14**, 77–78.

Souleles C. and Argyriadou N. (1997), Constituents of the essential oil of *Salvia sclarea* growing wild in Greece. Pharm. Biol. **35**, 218–220.

Topcu G. (2006), Bioactive triterpenoids from *Salvia* species. J. Nat. Prod. **69**, 482–487.

Vallejo M. C., Moujir L., Burillo J., Guerra L., Gonzalez M., Pentae R. D., Andres L., Luis J. G., Blanco F. L., and Galarreta M. R. (2006), Chemical composition and biological activities of the essential oil of *Salvia canariensis*. Flavour Fragr. J. **21**, 277–281.