Antimicrobial Activity of Six Constituents of Essential Oil from *Salvia*

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The antimicrobial activity of three *Salvia* species, *i.e. S. santolinifolia, S. hydrangea* and *S. mirzayanii,* essential oils were investigated. The essential oils were obtained from the aerial parts of plants and analyzed by GC-MS. The main constituents of aforementioned species were α-pinene (72.4%), β-pinene (6.6%) and limonene (5.3%); β-caryophyllene (25.1%), 1,8-cineol (15.2%) and caryophyllene oxide (11.5%); α-terpinenyl acetate (22.6%), 1,8-cineol (21.2%) and linalool (8.9%), respectively. Bioassays exhibited that the property of the oil of *S. myrzayanii* was superior to others. The antimicrobial activity of essential oil from *Salvia* species may well be due to the presence of synergy between six tested compounds (linalool, 1,8-cineol, α-pinene, β-pinene, β-caryophyllene and limonene) and other constituents of the oils with various degrees of antimicrobial activity. Among these, linalool and 1,8-cineol had the highest antimicrobial activity.

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

Introduction

The genus *Salvia* L. is one of the largest members of the Lamiaceae family and includes about 900 species distributed all over the world. In flora of Iran this genus is represented by 61 species, 17 of which are endemic (Rechinger, 1982). Members of the genus *Salvia* and especially the most well-known species *S. officinalis* have been shown to possess a significant array of biological and pharmacological properties such as spasmolytic, astringent and antiseptic. In folk medicine of Iran, decoction of the leaves of *S. mirzayanii* (local name: Moor Talkh) was used for stomach pain and infusion of the flowers of *S. hydrangea* (local name: Gol-e Arooneh) for treating colds (Ghannadi, 2002). Phytochemically, a sesterterpene (salvimirzacolide) of *S. mirzayanii* has already been isolated and identified (Moghaddam *et al.,* 1998). The literature survey revealed that antimicrobial activity of the essential oils of *S. mirzayanii, S. hydrangea* and *S. santolinifolia* has not previously been published, although there are several reports on the compositions of the essential oils of some Iranian *Salvia* species (Rustaiyan *et al.*, 1997a, b, 1999, 2000; Sefidkon and Mirza, 1999; Sefidkon and Khajavi, 1999; Mirza and Sefidkon, 1999; Ahmadi and Mirza, 1999; Mirza and Ahmadi, 2000; Javidnia *et al.*, 2002; Salehi *et al.*, 2005a, b).

Antimicrobial and antioxidant activities of the essential oils and various extracts of some *Salvia* species have recently been investigated (Tepe *et al.,* 2004, 2005; Tzakou *et al.,* 2001; Weng and Wang, 2000). In the framework of our investigation on the essential oils composition and biological activities of the Iranian Lamiaceae family (Sonboli *et al.,* 2004), we report here the antimicrobial activity of the essential oils of three species of *Salvia,* which have not been the subject of previous investigations.

Material and Methods

Plant materials

The aerial parts of plants were collected from wild populations as follows: *S. mirzayanii,* Fars: Darab, Kase-tarashan mountain, at an altitude of 1500 m, on May 8, 2004; *S. santolinifolia,* Hormozgan: Hajiabad, Golzar protected area, at an altitude of 950 m, on May 9, 2004; *S. hydrangea,* Fars: Abadeh, Dahaneh mountain, at an altitude of 2250 m, on May 13, 2004. Voucher specimens were identified and deposited in the Medicinal Plants

Table I. Information on three *Salvia* species localities and essential oils.

Species	Locality	Voucher number	Oil yield (%)	Major components	Percentage
S. mirzayanii	Fars: Darab, Kase-tarashan, 1500 m, May 8, 2004	MP-889	0.8	α-terpinenyl acetate 1,8-cineol linalool linalyl acetate γ-cadinene	22.6 21.2 8.9 5.4 5.2
S. hydrangea	Fars: Abadeh, Dahaneh 2250 m, May 13, 2004	MP-761	0.1	β-caryophyllene 1,8-cineol caryophyllene oxide α-pinene borneol	25.1 15.2 11.5 5.5 5.2
S. santolinifolia	Hormozgan: Hajiabad, Golzar, 950 m, May 9, 2004	MP-745	0.5	α-pinene β-pinene limonene borneol	72.4 6.6 5.3 2.5

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Isolation procedure

Air-dried aerial parts (100 g) were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulphate and stored in sealed vials at 4 °C until analysis and testing.

Analytical procedure

GC-MS analyses of the oils were performed on a Thermoquest-Finnigan Trace GC-MS system equipped with a fused silica Rtx-1 capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Helium was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was 60 °C rising to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min; transfer line temperature was 250 °C; split ratio was 1/50. The quadrupole mass spectrometer was scanned over the 45–465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively. Retention indices (RI) for all constituents were calculated according to Van den Dool approach, using *n*-alkanes (C_6–C_{24})

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

Microorganism	*S. mirzayanii*		*S. hydrangea*		*S. santolinifolia*		Standard	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	Ampicillin[c]	Nystatine[d]
Bacillus subtilis	27 ± 0.3	1.25 ± 0.4	17 ± 0.4	15.0 ± 0.4	17 ± 0.3	7.5 ± 0.5	14 ± 0.4	nt
Enterococcus faecalis	14 ± 0.4	10.0 ± 0.2	10 ± 0.1	15.0 ± 0.6	10 ± 0.4	>15 ± 0.2	11 ± 0.3	nt
Staphylococcus aureus	16 ± 0.5	2.5 ± 0.3	14 ± 0.2	15.0 ± 0.3	12 ± 0.5	15.0 ± 0.5	13 ± 0.3	nt
Staphylococcus epidermidis	22 ± 0.3	1.25 ± 0.4	16 ± 0.3	7.5 ± 0.5	18 ± 0.5	7.5 ± 0.1	19 ± 0.5	nt
Escherichia coli	16 ± 0.2	2.5 ± 0.2	8 ± 0.5	>15 ± 0.7	12 ± 0.4	>15 ± 0.4	12 ± 0.2	nt
Klebsiella pneumoniae	10 ± 0.3	20.0 ± 0.6	–	nt	11 ± 0.6	15.0 ± 0.3	–	nt
Pseudomonas aeruginosa	9 ± 0.6	20.0 ± 1.0	–	nt	–	nt	9.7 ± 0.2	nt
Aspergillus niger	20 ± 0.4	2.5 ± 0.5	14 ± 0.3	10.0 ± 0.5	13 ± 0.3	>10 ± 0.5	nt	16 ± 0.4
Candida albicans	19 ± 0.3	2.5 ± 0.3	12 ± 0.3	>10 ± 0.4	14 ± 0.3	10.0 ± 0.5	nt	18 ± 0.5
Saccharomyces cerevisiae	17 ± 0.6	5.0 ± 0.4	11 ± 0.3	>10 ± 0.5	17 ± 0.3	5.0 ± 0.5	nt	18 ± 0.2

[a] Zone of inhibition includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml.
[c] Tested at 10 μg/disc.
[d] Tested at 30 μg/disc.
(–), Inactive; (7–13), moderately active; (> 14), highly active; nt, not tested.
Values are given as mean ± standard deviation.

as standards and the essential oils on a DB-1 column under the same chromatographic conditions. The identification of the components was made based on comparison of their mass spectra with those of the internal computer reference mass spectra libraries (Wiley 7.0 and Nist), as well as by comparison of their retention indices with published data (Shibamoto, 1987; Adams, 1995), and in some cases by co-injection of authentic compounds.

Antimicrobial activity

The preliminary antimicrobial activity of the essential oils was evaluated by the disc diffusion method using Mueller-Hinton agar for bacteria and Sabourod Dextrose agar for fungi (Baron and Finegold, 1990) with determination of inhibition zones. Three Gram-negative and four Gram-positive bacteria as well as three fungi were used: *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, *Klebsiella pneumoniae* ATCC 3583, *Candida albicans* ATCC 5027, *Saccharomyces cerevisiae* ATCC 9763 and *Aspergillus niger* ATCC 16404. Ampicillin for bacteria and nystatine for fungi were used as positive standards in order to control the sensitivity of the microorganisms. The incubation conditions used were 24 h at 37 °C for bacteria and 48 h at 24 °C for fungi. Minimum inhibitory concentration (MIC) was measured using the microdilution broth susceptibility assay as recommended by NCCLS (1999).

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

Microorganism	α-Pinene		β-Pinene		Limonene	
	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC
Bacillus subtilis	10.1 ± 0.6	7.5 (55.1) ± 0.2	15.2 ± 0.4	1.87 (13.8) ± 0.4	18.1 ± 0.4	0.6 (4.4) ± 0.1
Enterococcus faecalis	–	nt	8.3 ± 0.1	15 (110.4) ± 0.3	–	4.8 (36.8) ± 0.9
Staphylococcus aureus	8.3 ± 0.4	> 15 (> 55.1) ± 0.4	9.4 ± 0.2	15 (110.4) ± 0.2	10.2 ± 0.5	2.4 (18.4) ± 0.2
Staphylococcus epidermidis	9.4 ± 0.5	15 (110.2) ± 0.3	12.1 ± 0.3	7.5 (55.2) ± 0.4	–	0.6 (4.4) ± 0.2
Escherichia coli	11.5 ± 0.1	15.0 (110.2) ± 0.4	10.6 ± 0.1	7.5 (55.2) ± 0.3	10.3 ± 0.6	4.8 (36.8) ± 0.4
Klebsiella pneumoniae	–	nt	–	nt	8.4 ± 0.4	1.2 (9.2) ± 0.3
Pseudomonas aeruginosa	–	nt	–	nt	–	nt
Aspergillus niger	–	nt	–	nt	–	nt
Candida albicans	–	nt	–	nt	8.2 ± 0.5	4.8 (36.8) ± 0.4
Saccharomyces cerevisiae	–	nt	–	nt	10.3 ± 0.2	9.6 (73.6) ± 0.4

Table III. (cont.)

Linalool		β-Caryophyllene		1,8-Cineol	
IZ	MIC	IZ	MIC	IZ	MIC
29.1 ± 0.4	0.2 (1.3) ± 0.1	10 ± 0.8	7.5 (36.8) ± 0.3	25 ± 0.6	0.93 (6.1) ± 0.2
12.3 ± 0.5	3.2 (20.8) ± 0.4	–	nt	10 ± 0.4	7.5 (48.8) ± 0.2
18.5 ± 0.2	0.8 (5.2) ± 0.3	–	nt	15 ± 0.1	3.75 (24.4) ± 0.3
27.2 ± 0.3	0.2 (1.3) ± 0.2	–	nt	18 ± 0.2	1.87 (12.2) ± 0.4
22.2 ± 0.6	0.4 (2.6) ± 0.3	8 ± 0.7	> 15 (> 73.6) ± 0.4	20 ± 0.1	1.87 (12.2) ± 0.5
14.5 ± 0.2	0.8 (5.2) ± 0.3	–	nt	8 ± 0.1	7.5 (48.8) ± 0.1
–	nt	–	nt	–	nt
14.3 ± 0.1	4.8 (31.2) ± 0.3	–	nt	–	nt
30.1 ± 0.3	0.6 (3.9) ± 0.5	–	nt	11 ± 0.5	7.5 (48.8) ± 0.2
26.2 ± 0.5	1.2 (7.8) ± 0.2	–	nt	14 ± 0.4	> 15 (> 97.6) ± 0.4

[a] Zone of inhibition includes diameter of disc (6 mm).
[b] Minimum inhibitory concentration values in mg/ml (mM).
Main compounds tested at 10 μl/disc on bacteria and 20 μl/disc on fungi.
(–), Inactive; (7–13), moderately active; (> 14), highly active; nt, not tested.
Values are given as mean ± standard deviation.

Results and Discussion

The hydrodistillation of the aerial parts of three *Salvia* species, *i.e. S. santolinifolia, S. hydrangea* and *S. mirzayanii*, gave oils in 0.5, 0.1 and 0.8% (w/w) yields, based on the dry weight of the plants. The main components of the essential oils, their percentages, voucher numbers and localities of the studied species are presented in Table I.

All essential oils showed antimicrobial activity by the disk diffusion assay. However, the best results were obtained with the oil of *S. mirzayanii* which was active not only against the bacterial strains but also produced good zones of inhibition against the fungal test organisms (Table II). The most susceptible microbial strains were: *Bacillus subtilis* and *Staphylococcus epidermidis* (MIC values of 1.25 mg/ml) followed by *Aspergillus niger* and *Candida albicans* (MIC values of 2.5 mg/ml). *Klebsiella pneumoniae* exhibited little susceptibility against the oils of *S. santolinifolia* and *S. mirzayanii*, while *Pseudomonas aeruginosa* was almost resistant to essential oils except for *S. mirzayanii* oil with the MIC value of 20.0 mg/ml.

Table III shows the antimicrobial activity of the major components of the essential oils tested. Among these, linalool and 1,8-cineol had the highest antimicrobial activity against all test organisms except for *P. aeruginosa*. The antimicrobial activity of the essential oils from studied *Salvia* species may well be due to the presence of synergy between the tested major components and other constituents of the oils with various degrees of antimicrobial activity. Considering the fact that *S. mirzayanii* oil contained 1,8-cineol (21.2%) and linalool (8.9%), and *S. hydrangea* oil contained 1,8-cineol (15.2%), the results obtained may be attributed for the presence of these compounds.

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