Turk J Biol
34 (2010) 89-95

doi:10.3906/biy-0809-2

Characterization of essential oils of some *Salvia* species and their antimycobacterial activities

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Received: 02.09.2008

Abstract: The compositions of the essential oils of 5 Turkish *Salvia* species, namely *Salvia aucheri* Bentham var. *aucheri* (endemic for Turkey), *Salvia aramiensis* Rech. fil., *Salvia fruticosa* Mill., *Salvia tomentosa* Mill., and *Salvia verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm., were studied.

Water distilled essential oils from the aerial parts of *Salvia* species from Turkey were analysed by GC and GC/MS. *Salvia aucheri* var. *aucheri, Salvia aramiensis,* and *Salvia fruticosa* oils *have the same main constituent: 1,8-cineole (39.2%, 55.6%, and 52.8% respectively)*. α-*Pinene (25.1%), camphor (14.9%), and borneol (13.2%)* were identified as the major components of *Salvia tomentosa.* The main constituents, β-*pinene (21.4%) and 1,8-cineole (16.1%), were also the major constituents in the oil of Salvia verticillata* subsp. *amasiaca.*

S. verticillata subsp. *amasiaca*, *S. aucheri* subsp. *Aucheri*, and *S. tomentosa* showed activity (MIC 196 µg/mL), while *S. aramiensis* and *S. fruticosa* did not. This is the first study of the antimycobacterial activity of these 5 plants.

Key words: Antimycobacterial activity, fungi, *Salvia*, essential oil

Bazı *Salvia* türlerinin uçucu yağlarının karakterizasyonu ve antimikobakteriyel aktivitesi

Özet: Türkiyede yetişen 5 *Salvia* türünün; *Salvia aucheri* Bentham var. *aucheri* (Türkiye için endemik), *Salvia aramiensis* Rech. fil., *Salvia fruticosa* Mill., *Salvia tomentosa* Mill. ve *Salvia verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm.'nın uçucu yağ kompozisyonu çalışıldı.

Salvia türlerinin topraküstü kısımlarından elde edilen uçucu yağların GC ve GC/MS ile analizleri yapıldı. *Salvia aucheri* var. *aucheri*, *Salvia aramiensis* ve *Salvia fruticosa* yağları ana bileşik olarak 1,8-sineol (sırası ile % 39,2, % 55,6, % 52,8) içermektedir. *Salvia tomentosa* uçucu yağının ana bileşenleri α-pinene (% 25,1), kafur (% 14,9) ve borneol (% 13,2) olarak bulunmuştur. *Salvia verticillata* subsp. *amasiaca*'nın başlıca bileşenleri β-pinene (% 21,4) ve 1,8-sineol (% 16,1)'dür.

S. verticillata subsp. *amasiaca*, *S. aucheri* subsp. *aucheri* ve *S. tomentosa* antimikobakteriyel aktivite gösterirken (MIC 196 µg/mL) *S. aramiensis* ve *S. fruticosa* aktivite göstermedi. Beş *Salvia* türüne ait antimikobakteriyel aktivite çalışması burada ilk defa verilmektedir.

Anahtar sözcükler: Antimikobakteriyel aktivite, fungi, *Salvia,* uçucu yağ

Introduction

Salvia L. is the largest genus of the family Labiatae, including over 900 species in the world and represented in Turkey by 94 taxa belonging to 89 species with 50% endemism (1,2).

Since ancient times, species of *Salvia* have been used in folk medicine for the treatment of diabetes (3) and skin diseases such as psoriasis and eczema (4). Numerous species of the genus *Salvia* (Labiatae) have been used since ancient times in folk medicine and subjected to extensive pharmacognosic research intended to identify biologically active compounds (5-7).

Salvia species are commonly used in Anatolia for colds, stomach aches, and sore throats. A solution of *Salvia tomentosa* is also used by pouring onto the open cuts and called "Tentürdiyot otu (Iodine tincture herb), "Moşabla" or "Boş yaprak". In addition to *S. fruticosa* tea, called "adaçayı", "elmaçayı" is commonly used to cure colds and stomach aches and other species are used as herbal tea locally (8-10).

Salvia species contain various secondary metabolites such as sterols, flavonoids, sesquiterpenoids, sesterpenoids (11), diterpenoids (11-13), triterpenoids (11,14-19), essential oils (13-20), and flavonoids (12).

In a previous study, the essential oils of *S. aucheri* subsp. *aucheri* from a different locality in Turkey were shown to contain α-pinene (7.6% to 4.3%), *β*-pinene (6.1% to 4.0%), and 1,8-cineole (39.2% to 20.3%) (24).

Particular interest has been shown in the members of the genus *Salvia* due to a wide range of biological activities such as antifungal activities (25-30), antitumor activities (31-34), antibacterial activities (35-39), antiviral activities (40), cytotoxic activities (41,42), antioxidant activities (36,43), treatment of heart disease (44), and antimycobacterial activity (13).

In addition to these activities, their capability to scavenge free radicals and to inhibit the growth of pathogenic microorganisms (21,45) and antiplatelet aggregation (46), and to inhibit acetyl choline esterase in vitro and in vivo were investigated. The last of these may help explain its traditional use for ailing memory (47,48). *Salvia* species also have some useful compounds to preserve raw and processed food (49) and some of them are used as a drink (50).

To eliminate pathogenic microorganisms, researchers are interested in studying new biologically active compounds isolated from plant species. New studies have shown that some essentials oils could safely be used as antifungal and antibacterial agents to partially or completely inhibit the growth of fungi and bacteria (26,51).

In this study, compositions and antimicrobial activity of the oils of *Salvia aramiensis* Rech. f., *Salvia aucheri* Bentham subsp. *aucheri* (endemic to Turkey), *Salvia fruticosa* Mill., *Salvia tomentosa* Mill., and *Salvia verticillata* L. subsp. *amasiaca* (Freyn & Bornm.) Bornm. were studied. Antimycobacterial activity of the oils is given here for the first time.

Our aim of the study was to determine the major chemicals of the essential oils of *Salvia* species and research their antimycobacterial activity.

Materials and methods

Plant materials

Aerial parts of *S. aucheri* subsp. *aucheri, S. aramiensis, S. fruticosa, S. tomentosa*, and *S. verticillata* subsp. *amasiaca* were collected from different parts of Turkey. Locality, altitude, collection time, and herbarium number are given for *Salvia* species in Table 1.

Isolation of essential oil

Air-dried aerial parts (90-150 g) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to produce the oil. Oil yields are shown in Table 1.

GC and GC/MS Conditions

The oils were analyzed by capillary GC and GC/MS using an Agilent GC-MSD system.

GC/MS

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. An Innowax FSC column (60 m × 0.25 mm, 0.25 m film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C/min. Split ratio was adjusted to 40:1. The injector temperature was 250 °C. MS was

Table 1. Herbarium data of plants and oil yields.

***Salvia* species**	**Locality**	**Altitude (m)**	**Collection date**	**Oil Yield (%)**	**Herbarium number**
S. aucheri Bentham subsp. *aucheri*	Mut, Mersin	850 m	13/06/2006	1.8	FS 1543
S. aramiensis Rech. f.	Hatay	350 m	28/06/2006	3.0	FS 1441
S. fruticosa Mill.	Marmara Adası	600 m	01/06/2005	2.3	FS 1423
S. tomentosa Mill.	Kazdagı, Balikesir	850 m	06/07/2006	1.0	FS 1422
S. verticillata L. subsp. *amasiaca* (Freyn & Bornm.) Bornm	Bitlis, Tatvan, Hizan	1300 m	21/08/2005	0.22	FS 1480

performed at 70 eV. Mass range was from m/z 35 to 450.

GC

The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain the same elution order with GC/MS, simultaneous injection was done using the same column and appropriate operational conditions. FID temperature was 300 °C.

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, and MassFinder Library, and confirmed by comparison of their retention indices. Alkanes were used as reference points in the calculation of relative retention indices (RRIs). Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of the analysis are shown in Tables 2-6.

Microorganism Used

The oils were tested against the reference strain, *Mycobacterium tuberculosis* H37Ra (ATCC 25177), in duplicate. Inoculums were prepared with 3- to 5-day-old culture of *M. tuberculosis* by diluting 1:5 from MGIT broth, which showed positive.

Antimycobacterial Activity

A *Mycobacteria* Growth Indicator Tube (MGIT) containing 4 mL of modified Middlebrook 7H9 Broth Base was used. The assay was done according to the instructions in the MGIT manual fluorometric susceptibility test procedure recommended by the

Table 2. The main compounds of essential oils of *Salvia aucheri* subsp. *aucheriç*.

RRI	**Compounds***	**%**
1032	*α*-Pinene	7.6
1076	Camphene	7.3
1118	*β*-Pinene	6.1
1203	Limonene	1.9
1213	**1,8-cineole**	**39.2**
1532	**Camphor**	**20.7**
1719	Borneol	4.9
2008	Caryophyllene oxide	1.7
2130	Spathulenol	1.1

* Only the percentages over 1% are indicated in this table.

Table 3. The main compounds of essential oils of *Salvia aramiensis*.

RRI	**Compounds***	**%**
1032	*α*-Pinene	4.3
1076	Camphene	4.3
1118	***β*-Pinene**	**10.2**
1174	Myrcene	1.2
1203	Limonene	1.5
1213	**1,8-cineole**	**55.6**
1532	**Camphor**	**5.7**
1611	Terpinen-4-ol	1.1
1706	*α*-Terpineol	1.5
1719	Borneol	4.4

* Only the percentages over 1% are indicated in this table.

Table 4. The main compounds of essential oils of *Salvia fruticosa*.

RRI	Compounds*	%
1032	**α-Pinene**	**5.8**
1076	Camphene	3.1
1118	β-Pinene	4.5
1174	Myrcene	3.8
1203	Limonene	2.1
1213	**1,8-cineole**	**52.8**
1280	*p*-Cymene	1.4
1437	α-Thujone	1.4
1451	β-Thujone	1.1
1532	**Camphor**	**5.8**
1612	β-Caryophyllene	2.1
1687	α-Humulene	2.6
1706	α-Terpineol	2.1
2008	Caryophyllene oxide	1.1
2104	Viridiflorol	1.1

* Only the percentages over 1% are indicated in this table.

Table 5. The main compounds of essential oils of *Salvia tomentosa*.

RRI	Compounds*	%
1032	**α-Pinene**	**25.1**
1076	Camphene	4.1
1118	β-Pinene	1.6
1174	Myrcene	4.6
1203	Limonene	2.3
1213	**1,8-cineole**	**7.0**
1497	α-Copaene	1.0
1532	**Camphor**	**14.9**
1590	Bornyl acetate	2.1
1612	β-Caryophyllene	2.2
1687	α-Humulene	2.3
1704	γ-Muurolene	2.6
1719	**Borneol**	**13.2**
1773	δ-Cadinene	1.6
2104	Viridiflorol	1.8

* Only the percentages over 1% are indicated in this table.

Table 6. The main compounds of essential oils of *Salvia verticillata* subsp. *amasiense*.

RRI	Compounds*	%
1032	α-Pinene	3.3
1118	**β-Pinene**	**21.4**
1132	Sabinene	1.2
1174	Myrcene	1.2
1203	Limonene	1.4
1213	**1,8-cineole**	**16.1**
1497	α-Copaene	5.4
1535	β-Bourbonene	1.7
1544	α-Gurjunene	4.6
1612	β-Caryophyllene	2.3
1661	Alloaromadendrene	5.1
1704	- γ-Muurolene	1.1
1726	Germacrene D	1.2
1755	Bicyclogermacrene	1.6
1773	δ-Cadinene	2.5
2069	Germacrene D-4-ol	1.2
2145	Valeranone	2.5
2187	T-Cadinol	1.2
2208	Copaborneol	1.5
2255	α-Cadinol	2.6
2931	Hexadecanoic acid	2.7

* Only the percentages over 1% are indicated in this table.

manufacturer (Becton Dickinson). OADC enrichment (0.5 mL), a mixture of oleic acid, albumin, dextrose, and catalase, was added to each tube. Oil was added in a volume of 0.1 ml to an MGIT. Then 500 µL of bacterial suspension was dispersed in the tubes. The final concentrations of the oil were 196, 98, 49, and 24 µg/mL. An uninoculated MGIT tube was used as a negative control. The control tube contained organisms only and not the oil. Blood Agar was used for checking the growth of other bacteria. The vials were incubated at 37 °C and MIC was determined to be the lowest dilution that gives a negative result by MicroMGIT Fluorescence reader within 2 days when the controls turned positive. Tubes were read daily starting on the second day of incubation using a MicroMGIT Fluorescence reader with a long wave UV light (52).

Results and discussion

In this study, essential oils of 5 *Salvia* spp., namely *S. aucheri* subsp. *aucheri* (endemic), *S. aramiensis, S. fruticosa, S. tomentosa*, and *S. verticillata* subsp. *amasiaca*, were used (Table 1).

Chemical compositions of these oils were elucidated by GC and GC/MS analysis (Tables 2-6) and the results were evaluated for their in vitro antimycobacterial activity against *M. tuberculosis* (Table 7).

Essential oils of *Salvia species* were screened for antimycobacterial susceptibility testing, and 3 to 5 essential oils, namely *S. aucheri* subsp. *aucheri, S. tomentosa,* and *S. verticillata* subsp. *amasiaca,* exhibited antimycobacterial activity (MIC 196 μg/mL). *S. aramiensis* and *S. fruticosa* were ineffective.

Table 7. Susceptibility test results against *Mycobacterium tuberculosis* H37Ra (ATCC 25177) obtained by MGIT flourometric manual method.

Plant no.	Extracts	Concentrations (μg/mL)
1	*Salvia aramiensis*	n.a.
2	*Salvia aucheri* subsp. *aucheri*	196
3	*Salvia tomentosa*	196
4	*Salvia fruticosa*	n.a.
5	*Salvia verticillata* subsp. *amasiaca*	196
Standard Drugs	Streptomycin	0.8
	Rifampin	1.0
	Ethambuthol	3.5
	Isoniasid	0.1

n.a. not active

Among the plants that showed antimycobacterial activity, the major components of oils were 1,8-cineole (39.2%), camphor (20.7%), α-pinene (7.6%), and β-pinene (6.1%) for *S. aucheri* subsp. *aucheri*; α-pinene (25.1%), camphor (14.9%), borneol (13.2%), and 1,8-cineole (7.0%) for *S. tomentosa*; and β-pinene (21.4%) and 1,8-cineole (16.1%) for *S. verticillata* subsp. *amasiaca*.

The in vitro results obtained in this study provided evidence that some sage oils include chemicals that may have potential as a source of antimycobacterial agents against *M. tuberculosis.*

Acknowledgements

This study was supported by a grant from the Scientific and Technological Research Council of Turkey (TÜBİTAK), TBAG (Research grant no. 104T336).

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