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*Full Length Research Paper*

# Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp. from Turkey

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**Water-distilled essential oils from dried aerial parts of *Salvia cryptantha and Salvia heldreichiana* (Lamiaceae), endemic in Turkey, were analysed using gas chromatography-mass spectrometry (GC-MS). Camphor (19.1%), 1,8-cineole (16.4%), borneol (11.9%), viridiflorol (11.5%) and bornyl acetate (2.4%) were found to be the major constituents in the oil of *S. cryptantha*. The major constituents in the oil of *S. heldreichiana* were linalool (9.4%), α-pinene (5.6%), 1,8-cineole (5.6%), borneol (5.6%), cryptone (5.3%), linalyl acetate (4.9%), α-terpineol (4.4%), camphor (3.9%), terpinen-4-ol (3.3%), *trans*-linalool oxide (*Furanoid*) (2.9%), *trans*-verbenol (2.2%), geranyl acetate (2.2%) and *cis*-linalool oxide (*Furanoid*) (2.1%). Essential oil of *S. heldreichiana* exhibited antimicrobial activity using the disc diffusion method against *Escherichia coli*, *Sarcinia lutea* and *Salmonella typhimurium*. The oil of *S. cryptantha* inhibited the growth of *S. lutea*.**

**Key words:** *Salvia cryptantha*, *Salvia heldreichiana*, Lamiaceae, essential oil composition, antibacterial activity.

## INTRODUCTION

In recent years, an upsurge of interest in the use of natural substances as phytomedicines has resulted in a more thorough investigation of plant resources. Aromatic plants and their essential oils, used since antiquity in folk medicine and for the preservation of food, are known sources of natural secondary metabolites having biological activity such as antimicrobial and antioxidant action among many others (Deans and Svoboda, 1990).

The genus *Salvia* (*Lamiaceae*) includes nearly 900 species spread throughout the world. This genus is represented in Turkey by 89 species with a total of 94 taxa, of which 45 are endemic in Turkey. The ratio of endemism in the genus *Salvia* in Turkey is about 45% (Davis, 1982; Davis, et al., 1988; Guner et al., 2000). Some members of the genus are of economic importance since they have been used as flavouring agents and in perfumery and cosmetics.

Until the discovery of antibiotics, *Salvia* was a frequent component of herbal tea mixtures, recommended to patients with tuberculosis to prevent sudation and was found to be an active ingredient in combined plant preparations for the treatment of chronic bronchitis. It has also been used as medication against perspiration, fever, rheumatism, sexual debility and in treating mental and nervous conditions as well as an insecticidal (Watt and Breyer-Brandwijk, 1962; Baricevic and Bartol, 2000).

The analysis of the essential oil composition of several *Salvia* species indicates that 1,8-cineole (eucalyptol), camphor and borneol are its main constituents. However, several authors have documented significant species specific variations in the concentration of these compounds and/or presence of others in high concentrations (Ahmadi and Mirza, 1999; Baser et al., 1993, 1995, 1997; Baser, 2002; Haznedaroglu et al., 2001; Holeman et al., 1984; Perry et al., 1999; Putievsky et al., 1990; Sivropoulou et al., 1997; Torres et al., 1997). Moreover, the essential oil composition of *Salvia* species, as occurs with other medicinal and aromatic plants, is highly influenced by genetic and environmental factors (Deans and Ritchie, 1987; Piccaglia and Marottu, 1993).

Other than the major compounds, α-pinene (a monoterpene hydrocarbon) and borneol (an oxygenated monoterpene), as well as other minor constituents of the essential oils of *Salvia officinalis* and *Salvia triloba* have

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**Table 1.** *Salvia* sp. used in this study.

| Code | *Salvia* sp. | Plant part | Collection site | Collection date | Essential oil yield (%) | KNYA |
|---|---|---|---|---|---|---|
| A | *S. heldreichiana* | Aerial parts | Kayseri; Yahyali | 09.07.1995 | 0.5 | 824 |
| B | *S. cryptantha* | Aerial parts | Kayseri; Yahyali | 06.07.1994 | 0.4 | 321 |

antimicrobial activity (Dorman and Deans, 2000). In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity.

In part of our continuing research into *Salvia* species, we have investigated *Salvia cryptantha* and *Salvia heldreichiana* essential oil compositions as well as their antimicrobial activity.

## MATERIALS AND METHODS

### Plant materials

The plants used were *S. cryptantha* Montbret and Aucher ex Bentham. and *S. heldreichiana* Boiss. ex Bentham (Davis, 1982). These were gotten from the aerial parts of the plants and were collected from Kayseri in Turkey. Voucher specimens are kept at the herbarium of the Faculty of Science, Selcuk University in Konya (KNYA), Turkey (Bagci 321 and 824) (Table 1).

### Distillation method

The air-dried plant materials were subjected to hydrodistillation for 3 h using a Clevenger type apparatus to give yellow oils in 0.4% (*S. cryptantha*) and 0.5% (*S. heldreichiana*) yield.

### Analysis of essential oils

The oils were analysed using a Hewlett-Packard G1800A GCD system. Innowax FSC column (60 m x 0.25 mm Ø, with 0.25 µm film thickness) was used as stationary phase while helium (0.8 ml/min) was used as carrier gas. Gas chromatography (GC) oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and then kept constant at 220°C for 10 min to 240°C at a rate of 1°C/min. Mass range was recorded from m/z 35 to 425. Split ratio was adjusted to 50:1. Injector temperature was 250°C. MS was taken at 70 eV.

Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogrammes. Library search was carried out using Wiley GC/MS Library and Baser Library of Essential Oil Constituents. n-Alkanes were used as reference points in the calculation of relative retention indices (RRI). The compounds identified in the oil are shown in Table 2.

### Biological activities

### Test microorganisms and culture media

The microorganisms cultures (*Staphylococcus aureus* ATCC 6538, *Sarcinia lutea* ATCC 9341, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*) used in this study were taken from the culture collection of Selcuk University, Faculty of Veterinary Medicine, Department of Bacteriology.

Disc diffusion method were used for the determination of antimicrobial effects of volatile oils (Shelef et al., 1984). Volatile oil prepared with suspension of 1/8 sterile Tween 20 (Merck) was absorbed on to commercial "oxoid" empty sterilized antibiotic discs of 6 mm diameter (SchleicherandShull No:2668, Germany) in an amount of 20 µl.

As culture medium, Mueller Hinton Agar (Oxoid) was used for identification of antibacterial activities to volatile oil. Chloramphenicol was used as standard control in the experiment. All tests were performed in triplicate.

## RESULTS AND DISCUSSION

### Chemical composition

Water-distilled essential oils from aerial parts of *Salvia* sp. were analysed by GC-MS. The identified compounds and their percentages are given in Table 2.

### *S. cryptantha* Montbret and Aucher ex Bentham

Eighty-six (86) components were identified in the oil, representing 94.7%, of the total oil. Camphor (19.1%), 1,8-cineole (16.4%), borneol (11.9%), viridiflorol (11.5%) and bornyl acetate (2.4%) were found to be the major constituents.

### *S. heldreichiana* Boiss. ex Bentham

Ninty-six (96) components were identified in the oil, representing 97.4% of the total oil. Linalool (9.4%), α-pinene (5.6%), 1,8-cineole (5.6%), borneol (5.6%), cryptone (5.3%), linalyl acetate (4.9%), α-terpineol (4.4%), camphor (3.9%), terpinen-4-ol (3.3%), *trans*-linalool oxide (*Furanoid*) (2.9%), *trans*-verbenol (2.2%), geranyl acetate (2.2%) and *cis*-linalool oxide (*Furanoid*) (2.1%) were found to be the major constituents.

Major components of the oil of *S. cryptantha* were generally in agreement with previous reports (Dogan, 1972; Bayrak and Akgul, 1987; Dortunc 1990; Baser, 2002; Tepe et al., 2004). The observed differences may be due to edaphic factors, chemotypic variations and different collection times (Perry et al., 1999).

### Antibacterial activity

The oil obtained from *S. heldreichiana* showed maximum

**Table 2.** The composition of the essential oils of *Salvia* species.

| RRI | Compound | *S. heldreichiana* (%) | *S. cryptantha* (%) |
|---|---|---|---|
| 1014 | Tricyclene | 0.1 | - |
| 1032 | α-Pinene | 5.6 | 0.7 |
| 1035 | α-Thujene | 0.3 | - |
| 1048 | 2-Methyl-3-buten-2-ol | 0.1 | - |
| 1076 | Camphene | 1.0 | 1.1 |
| 1093 | Hexanal | 0.1 | - |
| 1118 | β-Pinene | 1.2 | 0.4 |
| 1132 | Sabinene | 0.8 | - |
| 1174 | Myrcene | 1.3 | 0.1 |
| 1183 | *p*-Mentha-1,7(8)-diene (=*Pseudolimonene*) | 1.0 | - |
| 1188 | α-Terpinene | 0.3 | - |
| 1203 | Limonene | 1.9 | 0.1 |
| 1213 | 1,8-cineole | 5.6 | 16.4 |
| 1220 | *cis*-Anhydrolinalool oxide | 0.2 | - |
| 1224 | *o*-Mentha-1(7),5,8-triene | 0.1 | - |
| 1225 | (*Z*)-3-Hexenal | 0.2 | - |
| 1246 | (*Z*)-β-Ocimene | 0.2 | - |
| 1253 | *trans*-Anhydrolinalool oxide | 0.2 | - |
| 1255 | γ-Terpinene | 0.8 | - |
| 1265 | 5-Methyl-3-heptanone | 0.3 | - |
| 1266 | (*E*)-β-Ocimene | 0.5 | - |
| 1280 | *p*-Cymene | 3.4 | 0.2 |
| 1290 | Terpinolene | 0.4 | - |
| 1345 | 3-Octyl acetate | 0.2 | - |
| 1348 | 6-Methyl-5-hepten-2-one | 0.1 | - |
| 1360 | Hexanol | - | 0.1 |
| 1386 | Octenyl acetate | 0.3 | - |
| 1391 | (*Z*)-3-Hexenol | 0.1 | - |
| 1393 | 3-Octanol | 0.2 | 0.2 |
| 1400 | Nonanal | - | 0.3 |
| 1415 | Rose furan | 0.1 | - |
| 1439 | γ-Campholene aldehyde | 0.1 | - |
| 1450 | *trans*-Linalool oxide (*Furanoid*) | 2.9 | 0.1 |
| 1452 | α,*p*-Dimethylstyrene | 0.1 | - |
| 1452 | 1-Octen-3-ol | 0.3 | 0.3 |
| 1465 | Eucarvone | 0.2 | - |
| 1466 | α-Cubebene | - | 0.2 |
| 1478 | *cis*-Linalool oxide (*Furanoid*) | 2.1 | 0.1 |
| 1493 | α-Ylangene | - | 0.3 |
| 1497 | α-Copaene | - | 1.7 |
| 1499 | α-Campholene aldehyde | 1.2 | - |
| 1532 | Camphor | 3.9 | **19.1** |
| 1535 | β-Bourbonene | 0.1 | - |
| 1549 | β-Cubebene | - | 0.1 |
| 1553 | Linalool | **9.4** | 1.0 |
| 1562 | Isopinocamphone | - | 0.2 |
| 1565 | Linalyl acetate | 4.9 | 0.3 |
| 1571 | *trans*-*p*-Menth-2-en-1-ol | 0.2 | 0.1 |
| 1586 | Pinocarvone | 0.2 | 0.1 |
| 1588 | Bornyl formate | 0.2 | - |

**Table 2.** Contd.

| | | | |
|---|---|---|---|
| 1589 | β-Ylangene | - | 0.1 |
| 1597 | Bornyl acetate | 0.4 | 2.4 |
| 1602 | 6-Methyl-3,5-heptadien-2-one | 0.2 | - |
| 1611 | Terpinen-4-ol | 3.3 | 1.9 |
| 1616 | Hotrienol | 0.2 | - |
| 1626 | 2-Methyl-6-methylene-3,7-octadien-2-ol | 0.3 | 0.1 |
| 1638 | *cis-p*-Menth-2-en-1-ol | 0.3 | 0.2 |
| 1642 | *Thuj-3-en10-al* | 0.1 | - |
| 1648 | *Myrtenal* | 0.4 | 0.1 |
| 1651 | *Sabinaketone* | 0.2 | - |
| 1661 | *Alloaromadendrene* | 0.2 | 0.2 |
| 1663 | *cis-Verbenol* | 0.2 | - |
| 1664 | *Nonanol* | - | 0.1 |
| 1664 | *trans-Pinocarveol* | 0.8 | 0.4 |
| 1674 | *p-Mentha-1,5-dien-8-ol* | 0.3 | - |
| 1682 | *δ-Terpineol* | - | 0.2 |
| 1683 | *trans-Verbenol* | 2.2 | - |
| 1684 | *Isoborneol* | 0.1 | 0.1 |
| 1687 | *α-Humulene* | - | 0.7 |
| 1690 | *Cryptone* | 5.3 | 0.9 |
| 1700 | *p-Mentha-1, 8-dien-4-ol (=Limonen-4-ol)* | - | 0.1 |
| 1704 | *γ-Muurolene* | - | 2.0 |
| 1706 | *α-Terpineol* | 4.4 | 0.6 |
| 1719 | *Borneol* | 5.6 | 11.9 |
| 1725 | *Verbenone* | 0.8 | - |
| 1733 | *Neryl acetate* | 1.3 | - |
| 1740 | *α-Muurolene* | - | 0.9 |
| 1740 | *Valencene* | - | 0.3 |
| 1741 | *β-Bisabolene* | - | 0.1 |
| 1744 | *α-Selinene* | - | 0.4 |
| 1744 | *Phellandral* | 0.4 | - |
| 1748 | *Piperitone* | 0.1 | - |
| 1751 | *Carvone* | 0.7 | - |
| 1758 | *cis-Piperitol* | - | 0.1 |
| 1765 | *Geranyl acetate* | 2.2 | 0.2 |
| 1773 | *δ-Cadinene* | 0.3 | 1.5 |
| 1776 | *γ-Cadinene* | 0.9 | 0.9 |
| 1783 | *Campholene alcohol* | - | 0.3 |
| 1786 | *ar-Curcumene* | 1.8 | 0.2 |
| 1796 | *Selina-3,7(11)-diene* | - | 0.3 |
| 1797 | *p-Methyl acetophenone* | 0.3 | - |
| 1802 | *Cumin aldehyde* | 3.3 | - |
| 1804 | *Myrtenol* | - | 0.9 |
| 1808 | *Nerol* | 0.8 | - |
| 1810 | *3,7-Guaiadiene* | - | 0.2 |
| 1811 | *p-Mentha-1,3-dien-7-al* | 0.1 | - |
| 1827 | *(E,E)-2,4-Decadienal* | - | 0.1 |
| 1838 | *2-Phenylethyl acetate* | 0.4 | - |
| 1845 | *trans-Carveol* | 0.6 | 0.1 |
| 1853 | *Calamenene* | - | 1.0 |
| 1857 | *Geraniol* | 1.5 | - |

**Table 2.** Contd.

| | | | |
|---|---|---|---|
| 1864 | *p-Cymen-8-ol* | 0.5 | 0.1 |
| 1868 | *(E)-Geranyl acetone* | 0.1 | 0.1 |
| 1882 | *cis-Carveol* | 0.1 | - |
| 1900 | *epi-Cubebol* | 0.1 | 0.3 |
| 1940 | *4-Isopropyl salicylaldehyde* | 0.2 | - |
| 1941 | *α-Calacorene* | 0.2 | 0.4 |
| 1957 | *Cubebol* | 0.1 | 0.3 |
| 1969 | *cis-Jasmone* | - | 0.1 |
| 1981 | *Cuminyl acetate* | 0.1 | - |
| 1984 | *γ-Calacorene* | - | 0.1 |
| 2008 | *Caryophyllene oxide* | 0.9 | 0.8 |
| 2030 | *Methyl eugenol* | - | 0.1 |
| 2037 | *Salvial-4(14)-en-1-one* | - | 0.1 |
| 2045 | *Humulene epoxide-I* | - | 0.2 |
| 2050 | (E)-Nerolidol | - | 1.5 |
| 2057 | Ledol | - | 0.1 |
| 2071 | Humulene epoxide-II | - | 0.8 |
| 2073 | p-Mentha-1,4-dien-7-ol | 0.1 | - |
| 2080 | Cubenol | 0.1 | 0.2 |
| 2088 | 1-epi-Cubenol | - | 0.2 |
| 2104 | Viridiflorol | - | 11.5 |
| 2113 | Cumin alcohol | 0.5 | - |
| 2131 | Hexahydrofarnesyl acetone | - | 0.2 |
| 2144 | Spathulenol | 0.2 | 1.2 |
| 2186 | Eugenol | - | 0.1 |
| 2187 | T-Cadinol | 0.4 | 0.2 |
| 2192 | Nonanoic acid | - | 0.1 |
| 2209 | T-Muurolol | - | 0.1 |
| 2214 | ar-Turmerol | 0.3 | 0.1 |
| 2241 | p-Isopropyl phenol | 0.2 | - |
| 2250 | α-Eudesmol | - | 0.3 |
| 2255 | α-Cadinol | 0.3 | - |
| 2257 | β-Eudesmol | 0.3 | 1.4 |
| 2289 | Oxo-α-Ylangene | 0.1 | - |
| 2324 | Caryophylla-2(12),6(13)-dien-5 α-ol (=Caryophylladienol II) | - | 0.2 |
| 2389 | Caryophylla-2(12),6-dien-5 α-ol (=Caryophyllenol I) | - | 0.3 |
| 2392 | Caryophylla-2(12),6-dien-5 α-ol (=Caryophyllenol II) | - | 0.1 |
| 2396 | 13-epi-Manoyl oxide (=8 α-13-oxy-14-en-epilabdane) | - | 0.8 |
| | Total | 97.4 | 94.7 |

RRI: relative retention indices; tr: trace (< 0.1%).
*Correct isomer not identified.

antibacterial activity with 16 mm inhibition zone against *E. coli* strain among the test microorganisms used. Slightly lower antibacterial effects were observed against *S. lutea* and *S. typhimurium* (5 mm inhibition zone). No antibacterial activity was observed on the other test microorganisms (Table 3).

Essential oil obtained from *S. cryptantha* inhibited *S. lutea* (6 mm inhibition zone) (Table 3). It did not inhibit the growth of *S. aureus* ATCC 6538. Earlier reports had claimed the growth-inhibitory effect of this essential oil against *S. aureus* ATCC 6538 p.

Eucalyptol (1,8-cineole) and camphor are well-known chemicals having antimicrobial activity (Pattnaik et al., 1997; Tzakou et al., 2001). Based on a report, α-pinene (a monoterpene hydrocarbon) and borneol (an oxygenated monoterpene) had slight activity against a panel of microorganisms (Dormans and Deans, 2000). The antimicrobial effects of borneol were also reported

**Table 3.** Antibacterial activity of *Salvia* oils against microorganisms.

| Plant | Inhibition zone (mm) | | | | |
|---|---|---|---|---|---|
| | ***S. aureus*** | ***E. coli*** | ***S. lutea*** | ***S. typhimurium*** | ***P. aeruginosa*** |
| *Salvia cryptantha* | - | - | 6 | - | - |
| *Salvia heldreichiana* | - | 16 | 5 | 5 | - |
| Chloramphenicol (30 µg/disc) | 15 | 18.3 | 8.4 | 10 | 9 |

- : No inhibition.

elsewhere (Knobloch et al., 1989; Tabanca et al., 2001; Vardar-Unlu et al., 2003). As a result of these findings, antimicrobial activities of *S. cryptantha* oil could be attributed to 1,8-cineole, camphor and borneol.

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