# Antimicrobial Activity of Essential Oil and Major Constituents of *Salvia chloroleuca*

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The aerial parts of *Salvia chloroleuca* were collected at full flowering stage at Shahrestanak (Tehran province of Iran). The essential oil was isolated by hydrodistillation and analyzed by combination of capillary GC and GC-MS. Thirty-four components were identified, representing 98.5% of the total oil. $\beta$-Pinene (10.6%), $\alpha$-pinene (9.0%), $\beta$-caryophyllene (9.0%), 1,8-cineole (9.0%) and carvacrol (7.9%) were the main components. The *in vitro* antimicrobial activity of the essential oil of *S. chloroleuca* was studied against seven Gram-positive and Gram-negative bacteria (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus, S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus niger*); the disc diffusion method and MIC values indicated that the oil exhibited moderate to high antimicrobial activity.

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

## Introduction

Essential oils obtained from many plants have recently gained popularity and scientific interest. Many plants have been used for different purposes, such as food, drugs and perfumery. Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics (Tepe *et al*., 2005).

*Salvia*, the largest genus of Lamiaceae, includes about 900 species, widespread throughout the world. This genus is represented in Iranian flora by 58 species, 17 of which are endemic (Mozaffarian, 1996). Some of these species are used as medicinal, aromatic and ornamental plants. *Salvia officinalis* is one of the most widespread species and, since ancient times, has been used in the treatment of various disorders, such as tuberculosis and psoriasis. It has shown strong antibacterial and antifungal activities (Rustaiyan *et al.*, 1999). Other members of this genus have been used in folk medicine around the world for their antibacterial (Ulubelen *et al.*, 1997) and antitumour (Topcu, 2006) activities and as flavouring agent in perfumery and cosmetics (Tzakou *et al*., 2001).

A literature survey revealed that no chemical composition and biological studies had been performed on the essential oil of *S. chloroleuca.* The aim of our study was to evaluate the chemical composition of *S. chloroleuca* essential oil and its antimicrobial activity.

## Material and Methods

### *Plant material*

The aerial parts of *S. chloroleuca* Rech. f. & Aell. were collected at Shahrestanak, Tehran province of Iran, at an altitude of 2300 m. A voucher specimen (AS-85106) has been deposited at the herbarium of Ecology and Systematic Department, Research Institute of Applied Science, Shahid Beheshti University, Tehran, Iran.

*Isolation of the essential oil*

Air-dried aerial parts of the plants (250 g) were hydrodistilled for 4 h using a Clevenger type apparatus. The resulting essential oil was dried over anhydrous sodium sulfate and stored at 4 °C until analyzed and tested.

*GC and GC-MS analyses*

GC-FID analysis of the oil was 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 rate 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 analysis. 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 oil were identified by calculation of the retention indices of *n*-alkanes ($C_6$–$C_{24}$) under temperature-programmed conditions 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 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.

*Antimicrobial activity*

Ten microbial strains were used which included: *Bacillus subtilis* (ATCC 465), *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 oil and its main components was determined by the disc diffusion method (Baron and Finegold, 1990). Briefly, 0.1 ml of a suspension of the test microorganism ($10^8$ cells/ml) was spread on Mueller-Hinton Agar plates for bacterium and Sabouraud Dextrose Agar for the fungi. Sterile 6 mm discs, each containing 10 $\mu$l of essential oil, were placed on the microbial lawns. Discs containing 10 $\mu$l of carvacrol, $\beta$-caryophyllene, 1,8-cineole, $\alpha$-pinene and $\beta$-pinene were used to study the antimicrobial activity of the major oil components. The plates were incubated at 37 °C for 24 h for the bacteria

Table I. Composition of the essential oil of *Salvia chloroleuca.*

| Compound | RI[a] | % of the oil |
|---|---|---|
| Tricyclene | 926 | 1.4 |
| $\alpha$-Pinene | 936 | 9.0 |
| Camphene | 949 | 3.2 |
| Sabinene | 970 | 5.0 |
| $\beta$-Pinene | 978 | 10.6 |
| Myrcene | 982 | 0.9 |
| $\varrho$-Cymene | 1015 | 3.7 |
| 1,8-Cineole | 1025 | 9.0 |
| (*E*)-$\beta$-Ocimene | 1037 | 0.7 |
| $\gamma$-Terpinene | 1052 | 2.7 |
| Terpinolene | 1081 | 0.2 |
| Linalool | 1084 | 1.2 |
| *trans*-Pinocarveol | 1128 | 0.8 |
| *trans*-Verbenol | 1132 | 0.5 |
| Borneol | 1155 | 2.7 |
| Terpinen-4-ol | 1166 | 2.0 |
| Myrtenal | 1175 | 1.1 |
| Myrtenol | 1182 | 0.6 |
| Thymol | 1265 | 0.3 |
| Carvacrol | 1281 | 7.9 |
| Tridecane | 1285 | 1.0 |
| Eugenol | 1322 | 0.7 |
| $\alpha$-Copaene | 1385 | 0.7 |
| Tetradecane | 1395 | 4.9 |
| $\beta$-Caryophyllene | 1430 | 9.0 |
| $\alpha$-Humulene | 1460 | 0.6 |
| Germacrene D | 1485 | 6.4 |
| Bicyclogermacrene | 1499 | 1.5 |
| Spathulenol | 1573 | 3.3 |
| Caryophyllene oxide | 1581 | 3.8 |
| Epoxy allo-aromadendrene | 1619 | 1.1 |
| $\beta$-Eudesmol | 1645 | 1.0 |
| $\alpha$-Bisabolene oxide | 1661 | 0.5 |
| Platambin | 1842 | 0.5 |
| Monoterpene hydrocarbons | | 37.4 |
| Oxygenated monoterpenes | | 26.1 |
| Sesquiterpene hydrocarbons | | 18.2 |
| Oxygenated sesquiterpenes | | 9.7 |
| Others | | 7.1 |
| Total | | 98.5 |

Compounds are listed in the order of their elution from a DB-1 column.
[a] RI, retention index relative to *n*-alkanes ($C_6$–$C_{24}$).

and 30 °C for 48 h for the fungi. The diameters of the zones of inhibition were measured and reported in mm. Triplicate tests were carried out for each sample.

MIC (minimum inhibitory concentration) values were determined by a broth microdilution assay (NCCLS, 1997, 1999). Serial two-fold dilutions of the essential oil 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 $5 \cdot 10^5$ 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 the bacteria and 30 °C for 48 h for the fungi. The first dilution with no microbial growth was recorded as MIC.

## Results and Discussion

The essential oil composition of *S. chloroleuca* is presented in Table I, where all compounds are listed in the order of their elution from a DB-1 column. The oil yield was 0.3% (w/w) based on the dry weight of the plant. Thirty-four components were identified, representing 98.5% of the total oil. Monoterpene hydrocarbons were the major compounds group and constituted 37.4% of the oil. Among them, $\beta$-pinene (10.6%), and $\alpha$-pinene (9.0%) were identified as the main compounds. Oxygenated monoterpenes comprised 26.1% of the oil; 1,8-cineole (9.0%) and carvacrol (7.9%) were determined as their principal components. $\beta$-Caryophyllene (9.0%) and germacrene D (6.4%) were characterized as the main constituents among the sesquiterpene hydrocarbons.

The essential oil of *S. chloroleuca* was tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The result of the bioassay (Table II) showed that the oil exhibited moderate to high antimicrobial activity against all the fungi and bacteria tested, except for two microorganisms, *Pseudomonas aeruginosa* and *Aspergillus niger.* The most sensitive microorganisms were *Bacillus subtilis*, *Staphylococcus epidermidis* and *S. aureus* with inhibition zones of 21, 19, 15 mm and MIC values of 3.75, 3.75 and 7.5 mg/ml, respectively. Five microbial strains, *Escherichia coli*, *Enterococcus faecalis, Klebsiella pneumoniae*, *Saccharomyces cerevisiae* and *Candida albicans*, were found to be less sensitive to the oil.

Table III shows the antimicrobial activity of five major components of the oil. Among them the antimicrobial activity of carvacrol was superior compared to the other components. 1,8-Cineole exhibited high to moderate antibacterial activity against the test bacteria, while its antifungal properties were moderate. $\alpha$-Pinene and $\beta$-caryophyllene showed moderate antibacterial activity, except for *K. pneumoniae* and *P. aeruginosa*, with inhibition zones ranging from 10 to 15 and 9 to 15 mm, respectively. No antifungal activity was determined for these two components. $\beta$-Pinene showed no

Table II. Antimicrobial activity of the essential oil of *Salvia chloroleuca*.

| Microorganism | *S. chloroleuca* | | Tetracycline (30 $\mu$g/disc) | | Gentamicin (10 $\mu$g/disc) | | Nystatine (30 $\mu$g/disc) | |
|---|---|---|---|---|---|---|---|---|
| | IZ[a] | MIC[b] | IZ | MIC | IZ | MIC | IZ | MIC |
| *B. subtilis* | 21 ± 0.2 | 3.75 | 21 ± 0.8 | 3.2 | – | nt | nt | nt |
| *E. faecalis* | 13 ± 0.4 | 15 | 9 ± 0.4 | 6.4 | – | nt | nt | nt |
| *S. aureus* | 15 ± 0.3 | 7.5 | 20 ± 0.4 | 3.2 | – | nt | nt | nt |
| *S. epidermidis* | 19 ± 0.8 | 3.75 | 34 ± 0.8 | 1.6 | – | nt | nt | nt |
| *E. coli* | 14 ± 0.2 | 15 | – | nt | 23 ± 0.8 | 3.2 | nt | nt |
| *K. pneumoniae* | 12 ± 0.4 | 15 | – | nt | 20 ± 0.8 | 3.2 | nt | nt |
| *P. aeruginosa* | – | nt | – | nt | 12 ± 0.4 | 6.4 | nt | nt |
| *A. niger* | – | nt | nt | nt | nt | nt | 16 ± 0.4 | 6.4 |
| *C. albicans* | 10 ± 0.4 | >10 | nt | nt | nt | nt | 18 ± 0.4 | 3.2 |
| *S. cerevisiae* | 11 ± 0.2 | >10 | nt | nt | nt | nt | 18 ± 0.8 | 1.6 |

Values are given as means ± standard deviation.
[a] Zone of inhibition (in mm) includes diameter of the disc (6 mm).
[b] Minimum inhibitory concentration in mg/ml. –, Inactive; 7–13, moderately active; >14, highly active; nt, not tested.

Table III. Antimicrobial activity of the main compounds of the essential oil of *Salvia chloroleuca*.

| Microorganism | α-Pinene | | β-Pinene | | 1,8-Cineole | | β-Caryophyllene | | Carvacrol | |
|---|---|---|---|---|---|---|---|---|---|---|
| | IZ[a] | MIC[b] | IZ | MIC | IZ | MIC | IZ | MIC | IZ | MIC |
| *B. subtilis* | 15 ± 0.2 | 3.7 (27.6) | 9 ± 0.4 | >15 (110.3) | 31 ± 0.3 | 0.9 (6.0) | 15 ± 0.5 | 3.7 (18.1) | 40 ± 0.3 | 0.2 (1.3) |
| *E. faecalis* | – | nt | – | nt | 10 ± 0.4 | 7.5 (48.7) | 9 ± 0.4 | >15 (73.6) | 23 ± 0.2 | 0.8 (5.2) |
| *S. aureus* | 13 ± 0.4 | 7.5 (55.1) | – | nt | 23 ± 0.5 | 1.9 (12.1) | 12 ± 0.6 | 15 (73.6) | 36 ± 0.4 | 0.4 (2.6) |
| *S. epidermidis* | 14 ± 0.2 | 7.5 (55.1) | – | nt | 27 ± 0.3 | 0.9 (6.0) | 14 ± 0.2 | 7.5 (36.8) | 41 ± 0.6 | 0.2 (1.3) |
| *E. coli* | 10 ± 0.1 | 15 (110.3) | 11 ± 0.2 | >15 (110.3) | 22 ± 0.4 | 0.9 (6.0) | 15 ± 0.4 | 7.5 (36.8) | 35 ± 0.5 | 0.4 (2.6) |
| *K. pneumoniae* | – | nt | – | nt | 12 ± 0.4 | 7.5 (48.7) | – | nt | 28 ± 0.3 | 0.8 (5.2) |
| *P. aeruginosa* | – | nt | – | nt | – | nt | – | nt | 25 ± 0.2 | 0.8 (5.2) |
| *A. niger* | – | nt | – | nt | – | nt | – | nt | 42 ± 0.3 | 0.8 (5.2) |
| *C. albicans* | – | nt | – | nt | 10 ± 0.4 | >10 | – | nt | 31 ± 0.4 | 0.4 (2.6) |
| *S. cerevisiae* | – | nt | – | nt | 13 ± 0.2 | 10 | – | nt | 25 ± 0.3 | 0.4 (2.6) |

Main compounds tested at 10 μl/disc.
Values are given as means ± standard deviation.
[a] Inhibition zone (in mm) includes diameter of the disc (6 mm).
[b] Minimum inhibitory concentration in mg/ml (mM). –, Inactive; 7–13, moderately active; >14, highly active; nt, not tested.

considerable antimicrobial activity towards the test microorganisms.


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

Baron E. J. and Finegold S. M. (1990), Methods for testing antimicrobial effectiveness. In: Diagnostic Microbiology (Stephanie M., ed.). Mosboy, Baltimore, MD, pp. 171–194.

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, PA, USA.

National Committee for Clinical Laboratory Standard (NCCLS) (1999), Performance standards for antimicrobial susceptibility testing. 9th International Supplement. M100-S9. Wayne, PA, USA.

Rustaiyan A., Masoudi S., Monfared A., and Komeilizadeh H. (1999), Volatile constituents of three *Salvia* species grown wild in Iran. Flavour Fragr. J. **14**, 276–278.

Tepe B., Daferera D., Sokmen A., Sokmen M., and Polissiou M. (2005), Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomontosa*. Food Chem. **90**, 333–340.

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

Tzakou O., Pitarokili B., Chinou L. B., and Harvala C. (2001), Composition and antimicrobial activity of the essential oil of *Salvia vingens*. Planta Med. **67**, 81–83.

Ulubelen A., Tan N., and Topcu G. (1997), Terpenoid from *Salvia candidissima* subsp. *candidissima*. Phytochemistry **45**, 1221–1223.