

Title

**Chemical Composition of Essential Oil and
Antibacterial Activity of *Salvia Glutinosa* L.
Growing Wild in Iran**

Authors

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Abstract

The chemical composition of the volatile oil obtained by hydro-distillation from the whole aerial parts of *Salvia glutinosa* L. was analyzed by GC and GC/MS. Thirty-three constituents were identified. Trans-caryophyllene (20.9%), germacrene D (18.0%) and α -caryophyllene (9.4%) were found to be the main components. Furthermore, the oil was tested against Gram-positive and Gram-negative bacteria.

Key words

Salvia Glutinosa; Lamiaceae; Essential oil composition; Antimicrobial activity; Trans-caryophyllene; Germacrene D; α -caryophyllene; Phytol.

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Introduction

Salvia is a fascinating plant genus and one of the wide- spread members of the Labiatae (Lamiaceae) family, which comprises about 900 herbs and shrubs, growing in the temperate and warmer zones of the world.

Fifty-eight species of the genus are found in Iran, seventeen of which are endemic [1,2]. There are a number of literature reports essential oils of this genus [3-6]. Dried leaves of some species of *Salvia* are commonly used as herbal tea, food flavoring and as sources of an essential oil. The oil is utilized mainly in the food, cosmetic, perfumery and pharmaceutical industries [7], for their antimicrobial activities [8,9]. Other species of *Salvia* have been studied for their protective effects such as anti-lipidic peroxidation [10], cardioactive [11], antispasmodic [12] and antioxidant activity [13]. The most well-known of this genus is *Salvia officinalis*, which has been credited with a long list of medicinal uses: spasmolytic, antiseptic, astringent [14–17]. Antioxidant and antitumor activities were also found in *Salvia miltiorrhiza* [18, 19].

Tanshen, the rhizome of *Salvia miltiorrhiza* Bunge., has been used in Chinese traditional medicine for multiple therapeutic remedies. Tanshen has been used primarily for the treatment of coronary heart disease, particularly angina pectoris and myocardial infarction [20]. It has also been included for the treatment of hemorrhage, dysmenorrhea, miscarriage, swelling and insomnia [21,22], as well as inflammatory diseases such as edema, arthritis and endangitis [23]. Chronic hepatitis and liver fibrosis have also been treated with Tanshen for centuries [24]. The genus *Salvia*, including some Iranian species, has been studied chemically and the presence has been reported of terpenoids, even the rare sesterterpenes [25,26],

essential oils [27] and flavonoids [28].

In this paper, we describe the analysis of essential oils obtained from the aerial parts of *S. glutinosa* and the antibacterial activity of methanolic extract of this plant.

Experimental

Plant material: Aerial parts of *Salvia glutinosa* L. were collected during blossoming from wild populations. The plants were collected in August 2007 at an altitude of ca. 1750 m near the city of Amol, Mazandaran, Iran; Voucher being deposited in the Herbarium. The herb materials were kept immediately after harvesting in a shady and well-aired place for 15 days. Then, the dry plant materials were packed in paper bags and kept in a dark, dry and cool place. Before being used, the aerial parts were cut into small pieces and subjected to hydrodistillation for 3 h using Clevenger apparatus. The oils were obtained using n-pentane as a collecting solvent and subsequently were dried over anhydrous sodium sulfate and stored in amber vials at 4 °C until they were analyzed.

Analysis: GC analysis was performed on a Shimadzu GC-15A equipped with a split/splitless injector (250 °C) and a flame ionization detector (250 °C). Nitrogen was used as carrier gas (1 mL/min). The capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 mm). The column temperature was kept at 60 °C for 3 min and then heated to 220 °C with a 5 °C/min rate and kept constant at 220 °C for 5 min. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

GC/MS: Analysis was carried out using a Hewlett-Packard 6890-5973 GC-MS operating in EI at 70 eV, equipped with a Hp-5MS capillary Silica column (30m×0.25 mm, film thickness 0.25 µm) and split/

splitless injector (250 °C). The initial temperature of the column was 60 °C for 3min and was heated to 220 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a flow rate of 1ml/min.

The retention indices for all the components were determined according to the van den Dool method, using n-alkanes as standards [35]. The compounds were identified by RRI, DB5, with those reported in the literature and by comparison of their mass spectra with the Wiley library or with the published mass/spectra [36-38].

Antibacterial activity: The whole aerial parts of the plant were dried and coarsely ground before extraction. A known amount of aerial parts was extracted at room temperature by percolation method using methanol.

The resulting extract was concentrated over rotary vacuum until a crude solid extract was obtained, which was then freeze-dried for complete solvent removal.

Antibacterial activity was determined by disc diffusion method in which cellulose discs with diameter of 6.4mm were used [39]. The cultures of the following bacteria were used: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Mueller Hinton Agar medium was used in this study and the concentration of the bacteria used was 3×10^8 per ml, which was equal to 0.5 Macfarland. Extracts were added with different concentrations (2 mg, 1 mg, 500 µg and 100 µg in per disc) to sterile discs and dried extracts. The discs were placed on the medium and incubated at 37 °C for 24 h; inhibition zone diameters were measured. Controls included discs of Amoxicillin (25), Ampicillin (10), cephalexin (30), and Erythromycin (15) and 100 µl of solvent (methanol) was added to empty discs and the discs

were placed on the medium plate for evaluation of effect of the solvent on bacteria. The solvents had no effect on bacteria.

Results and Discussion

Chemical components identified in the oils of aerial parts of *S. glutinosa* and their percentage compositions are listed in Table 1. Thirty seven volatile constituents were identified in the oil of the aerial parts of *S. glutinosa*, representing 87.0% of the total oil. Monoterpene hydrocarbon compounds were 16.3% in the oil, whereas sesquiterpene hydrocarbon compounds constituted 64.2%. The major compound was trans-Caryophyllene (20.9%), Germacrene D (18.0%), α-Caryophyllene (9.4%) and Phytol (5.2%), followed by β- Elemene (3.2%), Caryophyllene oxide (2.6%) and Bornyl acetate (2.4%). According to the results of GC-MS analysis of *S. glutinosa* oil from Serbia [29-31] Caryophyllene oxide represented the major compound (22.3-33.3%). In the oil from Yugoslavia [32], the major constituent was Bornyl acetate (11.7%), whereas in the oil from Italy [33], the main constituents in fresh leaves were γ-Murolene (18.7%) and β-Bourbonene (5.9%) and in fresh flowering tops γ-Murolene (15.1%) and Bornyl formate (6.3%). In the oil from Greece [34] the major compound was Butyl butyryl lactate (26.7%). The previously reported oils from Serbia [34-36], Italy [33] and Greece [34] were found to be rich in sesquiterpenes. According to Senatore *et al.* [33] various factors, both endogenous and exogenous, can affect the composition of essential oil of *S. glutinosa*. The time of flowering, besides the geographical and climatic factors, is important in determining the composition of oil.

Antibacterial activity of methanolic extract of *S. glutinosa* showed by disc diffusion method using

Table 1- Chemical composition (%) of essential oil of *Salvia glutinosa*

Compound	RI	Salvia glutinosa
α -Pinene	940	0.4
Sabinene	980	0.7
Myrcene	995	0.4
<i>m</i> -Mentha-1(7),8-diene	999	0.5
δ -3-Carene	1011	2.0
α -Terpinene	1018	0.9
Limonene	1031	0.8
(Z) - β -Ocimene	1040	0.7
α -Terpinolene	1088	0.2
Linalool	1100	1.5
Nonanal	1105	2.6
Isoborneol	1156	0.7
Terpin-1-ol	1134	0.5
Terpin-4-ol	1177	1.0
α -Terpineol	1189	1.0
Bornyl acetate	1285	2.4
β -Elemene	1391	3.2
γ -Caryophyllene	1404	9.4
trans-Caryophyllene	1418	20.9
Aromadendrene	1439	0.6
Germacrene D	1480	18.0
Bicyclgermacrene	1494	1.7
α -Bourbonene	1505	1.6
α -(E,E)-Farnesene	1508	1.8
γ -Cadinene	1513	0.4
δ -Cadinene	1524	1.2
Germacrene B	1551	0.5
Nerolidol	1564	1.0
Caryophyllene oxide	1581	2.6
α -Muurolol	1645	1.3
n-Heptadecane	1700	0.7
6,10,14-Trimethyl-2-pentadecanone	1846	0.6
Phytol	1949	5.2
Total		87.0

* Retention indices as determined on a DB-5 column using the homologous series of n-alkane

four bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* in four different concentrations of the extract is presented in Table 2. It was observed that the plant had its most effect on Gram (+) bacteria *Staphylococcus aureus*, with 2mg per disc concentration, and its effect was less on Gram (-) bacteria

Escherichia coli with 2 mg per disc concentration, and it had almost weak effects on *Bacillus subtilis* and *Pseudomonas aeruginosa*. The results showed that *Salvia glutinosa* has antibacterial activity. The effect was weak, but further investigation of other microorganisms on their antimicrobial activities is needed.

According to the results of antimicrobial activity of *Salvia glutinosa* from Serbia [19], the extract of *S. glutinosa* has antimicrobial activity on Gram (+) and Gram (-) bacteria.

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Table 2- Antibacterial activity of the whole aerial parts of methanolic extract of *Salvia glutinosa* and antibiotic control. Values are the mean diameter of inhibitory zones (mm).

Bateria Species	Extracts, controls and solvent								
	2mg/ disc	1mg/ disc	500µg disc	100µg disc	Methanol 100µg disc	Amoxicillin (25)	Ampicillin (10)	Cephalexin (30)	Erythromycin (15)
<i>Staphylococcus aureus</i> ATCC 25923 +	14	13	10	9	6.4	16	22	30	20
<i>Bacillus subtilis</i> ATCC 6633 +	9	9	8	7	6.4	8	8	22	26
<i>Escherichia coli</i> ATCC 25922 -	11	10	8	8	6.4	18	20	14	-
<i>Pseudomonas aeruginosa</i> ATCC 27853 -	8	8	8	8	6.4	-	-	12	-

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