

ISSN 1682-296X (Print)

ISSN 1682-2978 (Online)



# Bio Technology



**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Chemical Composition and Antimicrobial Activity of Essential Oil of *Salvia verbenaca*

Tawfeq A. Al-Howiriny

Medicinal, Aromatic and Poisonous Plants Research Center,  
Department of Pharmacognosy, College of Pharmacy, King Saud University,  
P.O. Box 2457, Riyadh 11451, Saudi Arabia

---

**Abstract:** The aerial parts of *Salvia verbenaca* L. (Labiatae) afforded an essential oil on steam distillation was analyzed by gas chromatography-mass spectrometry (GC/MS) using direct injection. Out of fifty-two peaks (representing 99.2% of the oil), forty-one components were identified representing 96.3% of the total oil composition. The major components were sabinene (16.0%),  $\delta$ -cadinene (7.9%),  $\alpha$ -pinene (7.3%), 4-terpeniol (7.4%) and limonene (6.7%). The oil was also screened for its anti-microbial activity, which exhibited a significant anti-bacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Mycobacterium smegmatis*, and a marked anti-fungal activity against *Candida albicans*.

**Key words:** *Salvia verbenaca* L., Labiatae, essential oils, anti-microbial activity

---

### Introduction

*Salvia verbenaca* L. (Labiatae) is widely distributed in tropical regions including Saudi Arabia. The alcoholic extract of the aerial parts has been reported to potentiate smooth muscle contractions induced by acetylcholine, histamine, BaCl<sub>2</sub>, and serotonin (Todorov *et al.*, 1984). Previous reports on the plant have shown the presence of abietane diterpene quinone namely 6 $\beta$ -hydroxy-7 $\alpha$ -acetoxy royleanone (Sabri *et al.*, 1989) and 5-hydroxy 7, 4'-methoxy flavone (Camarasa *et al.*, 1982) from the leaves of the plant. The literature survey revealed that no phytochemical and pharmacological work has been done so far on the essential oil of the aerial parts of the plant. We report herein the composition and anti-microbial activity of the essential oil, wherein forty-one components were identified by gas chromatography-mass spectrometry (GC/MS) using direct injection (Table 1). The anti-microbial activity was screened against *Bacillus subtilis*, *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Table 2).

### Materials and Methods

**Plant material:** The aerial parts of *Salvia verbenaca* L. were collected on 18<sup>th</sup> February 2001 from Fifa mountains, 18 Km south of Sabia city, Southern Province, Saudi Arabia. The taxonomist of the center, Dr. Atiqur-Rehman identified the plant, and a voucher specimen No. 14182 has been

deposited at the herbarium, College of Pharmacy, King Saud University, for future reference.

**Analysis of the essential oil:** Fresh aerial parts (500gm) were crushed to coarse powder and steam distilled in a Clevenger apparatus for about four hours to obtain the yellow coloured oil (0.6 ml, 0.12% v/w), which was subjected to analysis by GC/MS using direct injection in the split mode under the following conditions:

Hewlett-Packard 5973 MSD GC/MS equipped with a quartz capillary column: 30 x 0.32 mm x 1.0  $\mu$  Rtx- 5 sil MS (Restek); oven temperature: 40 °C (hold 3 min) to 200 °C at 8 °C/ min then to 320 °C at 6 °C/min (hold 4 min); Injector temperature: 320 °C; Sample size: 0.2  $\mu$  L, split 1: 100; mass range: 39- 500 amu, 3.17 scans /sec; carrier gas: He; ionization energy: 70 eV. The qualitative identification of different constituents was performed by comparison of their retention times and mass spectra with those of the library.

**Pharmacological screening:** The anti-microbial activity was tested according to the National Committee of Clinical Laboratory Standards using American type of Culture Collection (ATCC) standard (Ferraro *et al.*, 2000) against various microorganisms namely: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium smegmatis* and *Candida albicans*. The positive antibacterial and antifungal activities were established by the presence of measurable zones of inhibition after 24 hrs incubation for *Bacillus subtilis* and *Staphylococcus aureus*, and 48 hrs for *Mycobacterium smegmatis* and *Candida albicans*.

### Results and Discussion

The results of the analysis of the essential oil are qualitative and semi-quantitative. The fresh aerial parts of *Salvia verbenaca* yielded 0.12% v/w of essential oil. The constituents identified by GC/MS analysis, their retention times and area percentages are summarized in Table 1. Out of fifty-two peaks (representing 99.2% of the oil), forty-one components were identified representing 96.3% of the total oil composition. Monoterpenes hydrocarbons were the major constituents in the oil. In addition to Sabinene (16.0%), that was the major monoterpene hydrocarbon, 4-terpeniol (7.4%),  $\alpha$ -pinene (7.3%), limonene (6.7%) and  $\delta$ -3-carene (4.0%) were present in fairly good amount.

On the other hand,  $\alpha$ -cadinene (7.9%) was the major sesquiterpene hydrocarbon present in the oil. Other sesquiterpene hydrocarbons such as  $\beta$ -caryophyllene (5.0%) and  $\beta$ -elemene (3.4%) were also detected in appreciable amounts. Moreover, some minor components were also detected of which camphene (1.5%),  $\delta$ -guaiene (1.2%), germacrene (1.1%) and  $\beta$ -selinene (0.8%) were identified.

The oil showed a significant anti-microbial potential on various microorganisms tested. The minimum inhibitory concentration (MIC) of the oil was 2.0 mg/ml against *Bacillus subtilis* and *Staphylococcus aureus*, and 3.0 mg/ml against *Mycobacterium smegmatis*. In addition, the minimum inhibitory concentration against the fungus *Candida albicans* was 2.0 mg/ml. Other microorganisms that were tested such as *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to the oil (Table 2).

Tawfeq A. Al-Howiriny: Essential oil of *Salvia verbenaca*

Table 1: Identified chemical constituents in the essential oil of *Salvia verbenaca*

Peak No.	Compounds area	Retention time (min)	%
1.	$\alpha$ -Thujene	6.55	1.0
2.	$\alpha$ -Pinene	6.72	7.3
3.	Camphene	7.17	1.5
4.	Sabinene	7.88	16.0
5.	$\beta$ -Pinene	7.97	6.3
6.	1-Octen-3-ol	8.23	0.3
7.	$\beta$ -Myrcene	8.41	0.3
8.	$\alpha$ -Phellandrene	8.80	0.3
9.	$\delta$ -3-Carene	8.87	4.0
10.	$\alpha$ -Terpinene	9.11	1.1
11.	o-Cymene	9.24	0.3
12.	p-Cymene	9.35	1.7
13.	Limonene	9.47	6.7
14.	$\gamma$ -Terpinene	10.26	2.7
15.	Sabinene hydrate	10.59	0.2
16.	Terpinolene	11.00	0.7
17.	Linalool	11.42	3.1
18.	Trans-1-Methyl-4-isopropyl-2-cyclohexene-1-ol	12.01	0.4
19.	Trans-Pinocarveol	12.43	0.3
20.	Cis-1-Methyl-4-isopropyl-2-cyclohexene-1-ol	12.49	0.4
21.	Borneol	13.20	1.2
22.	4-Terpineol	13.43	7.4
23.	$\alpha$ -Terpineol	13.80	1.2
24.	Bornyl acetate	15.91	0.3
25.	$\alpha$ -Copaene	17.91	3.0
26.	Bourbonene	18.09	0.8
27.	$\beta$ -Elemene	18.24	3.4
28.	$\beta$ -Caryophyllene	18.86	5.0
29.	$\gamma$ -Elemene	19.10	1.0
30.	$\delta$ -Guaiene	19.21	0.5
31.	$\alpha$ -Humulene	19.61	1.4
32.	$\alpha$ -Amorphene	20.04	0.7
33.	Germacrene-D	20.16	0.5
34.	$\beta$ -Selinene	20.31	0.8
35.	$\delta$ -Guaiene	20.60	1.2

Table 1: Continued

36.	$\delta$ -Cadinene	20.95	7.9
37.	$\alpha$ -Calacorene	21.41	0.6
38.	Germacrene-B	21.72	1.1
39.	Caryophyllene oxide	22.22	2.4
40.	Humulene epoxide II	22.75	0.3
41.	$\beta$ -Eudesmol	23.60	0.9

Table 2: Antimicrobial activity of the essential oil of *Salvia verbenaca*

Microorganisms	Activity	Time duration	MIC*
<i>Bacillus subtilis</i>	+ ve	24 hours	2.0 mg/ml
<i>Staphylococcus aureus</i>	+ ve	24 hours	2.0 mg/ml
<i>Escherichia coli</i>	- ve	48 hours	Nil
<i>Pseudomonas aeruginosa</i>	- ve	48 hours	Nil
<i>Mycobacterium smegmatis</i>	+ ve	48 hours	3.0 mg/ml
<i>Candida albicans</i>	+ ve	48 hours	2.0 mg/ml

\*Minimum inhibitory concentration (MIC)

The above results indicate that the oil may be used in the treatment of skin diseases and diarrhea etc. caused by microorganisms tested. Further toxicological and clinical studies are required to prove the safety of the oil as a medicine.

#### Acknowledgements

The author is thankful to Mr. Mohammad Mukhair, the technician of the center, for technical assistance. In addition, the author gratefully acknowledge the financial support of the Research Center at College of Pharmacy, King Saud University.

#### References

- Camarasa, J., S. Canigual, J. Iglesias and E. Marin, 1982. Flavonic aglycone from the leaves of *Salvia verbenaca* L.: 5-hydroxy 7, 4'-methoxy flavone. *Plant. Med. Phytother.*, 16: 192-196.
- Ferraro, M.J., W.A. Craig, M.N. Dudley, G.M. Eliopoulos, D.W. Hecht, J. Hindler, L.B. Reller, A.T. Sheldon, J.M. Swenson, F.C. Tenover, R.T. Testa, M.P. Weinstein and M.A. Wikler, 2000. Performance standard for antimicrobial disc susceptibility tests; Approved standard, 7<sup>th</sup> edition. National Committee for Clinical Laboratory Standards, 20: 1- 26.
- Sabri, N.N., A.A. Abou-Donia, A.M. Assad, N.M. Ghazy, A.M. El-Lakany, M.S. Tempesta and D.R. Sanson, 1989. Abietane diterpene quinones from the roots of *Salvia verbenaca* and *S. lanigera*. *Planta Medica*, 55: 582
- Todorov, S., S. Philianos, V. Petkov, C. Harvala, R. Zamfirova and H. Olimpiou, 1984. Experimental pharmacological study of three species from genus *Salvia*. *Acta Physiol. Pharmacol. Bulg.*, 10: 13-20.