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Der Pharmacia Lettre, 2013, 5 (3):310-314
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ISSN 0975-5071
USA CODEN: DPLEB4

Chemical composition and biological activities of the essential oil of *Salvia officinalis* from Batna (Algeria)

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ABSTRACT

The hydrodistilled essential oil of fresh aerial parts of Salvia officinalis, collected at Batna (Eastern Algerian) was analyzed by GC and GC/MS. 35 components representing 98.39 *% of the essential oil were detected with α-thujone (24.52%) ,1,8- cineole (15.92%), camphor (16.86%) , β-thujone (6.50%) and veridiflorol (6.35%), as the major components. The antibacterial activity of the essential oil was tested against eighth gram-positive and gram-negative bacteria by the use of the disc diffusion method. The antioxidant activity was also investigated by the use of β-carotene bleaching method.*

Keywords: *Salvia officinalis*, Antioxidant activity, Linoleic acid, β-carotene, Antibacterial activity.

INTRODUCTION

The genus *Salvia* (commonly known as sage) belongs to the family Lamiaceae, which is a large cosmopolitan family comprising about 252 genera and 7200 species [1,2]. Various species of *Salvia* are used as flavorings, food condiments, cosmetics, perfume additives and as herbal medicine e.g antibacterial, antiviral, antitumor, spasmolytic, antioxidant and anti-inflammatory [3-5]. In continuation of our works on Lamiaceae [6-25], we report here the GC and GC/MS analyses, the antibacterial and the antioxidant activities of the essential oil of *Salvia officinalis* collected from Batna (Eastern Algerian) which is traditionally used as emmenagogue and antispasmolytic.

MATERIALS AND METHODS

Plant Material

Fresh aerial parts of *Salvia officinalis*, collected from Batna (Eastern Algerian) was collected in May 2013. A voucher specimen was deposited at the herbarium of the University of Constantine 1, Algeria (LOSTSo /05/13).

Extraction of the essential oil

The hydrodistillation of fresh aerial parts (100 g) of *Salvia officinalis*, collected from Batna, for 3h in a Clevenger-type apparatus, according to the British Pharmacopeia, yielded 2.1 % of a yellow good smell essential oil.

Gas chromatography

GC analysis was performed on a Shimadzu GC17A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). Retention times for comparison with authentic compounds were measured using a cross-linked DB5-MS column (40 m × 0.18 mm, film thickness 0.18 µm). The oven temperature was programmed as isothermal at 60°C for 5 min, then raised to 275°C at 5°C/min and held at this temperature for 5

min. Helium was used as the carrier gas at a rate of 1 ml/min. Relative percentage amounts were calculated from peak area without the use of correction factors.

Gas Chromatography-Mass spectrometry

Gas chromatography-mass spectrometry: GC-MS was performed using a Shimadzu QP5050 mass selective detector using a cross-linked DB5-MS column (40 m × 0.18mm, film thickness 0.18 µm). The oven temperature was programmed as isothermal at 60°C for 5 min, then raised to 275°C at 5°C/min and held at this temperature for 5 min. Helium was used as the carrier gas at a rate of 1 ml/min. 0.1 µl oil was introduced directly into the source of the MS via a transfer line (280°C) with a split ratio of 1:50 and a linear velocity of 30.0 cm/sec. Ionization was obtained by electron impact (70 eV, source temperature 200°C, resolution 1000).

Identification of components

Essential oil components were identified based on their retention indices (determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [26,27] and with authentic compounds.

Antibacterial activity

The antibacterial activity of the essential oil was tested against a range of microorganisms, namely *Escherichia coli* ATCC 25922, *Escherichia coli, Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa, Klebsiella pneumonia ATCC, Klebsiella pneumonia, Streptococus.* The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation) [28].

Antioxidant activity: β-carotene bleaching test

The antioxidant activity of the essential oil was evaluated by β-carotene–linoleic acid model system [29]. 0.5 mg of β-carotene in 1 mL of chloroform was added to 25 µL of linoleic acid and 200 mg of Tween 40 emulsifier mixture. After chloroform was evaporated under vacuum, 100 mL of distilled water saturated with oxygen were added by vigorous shaking. Four thousand microliters of this mixture were transferred into deferent test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50°C. A blank, devoid of β-carotene, was prepared for background subtraction. Vitamin E was used as standards. The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \ln (a/b)/t$$

Where ln is the natural log, a is the absorbance at time 0, b is the absorbance at time t (120 min) [30]. The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using following equation:

$$AA = [(Rcontrol - Rsample/Rcontrol] \times 100$$

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The hydrodistillation of flowering *Salvia officinalis,* collected from Batna, yielded 2.1% (w/w) of a yellowish good smell oil. 35 compounds were identified by GC and GC/MS, representing 98.39% of the total essential oil characterized by the main presence of α-thujone (24.52%), camphor (16.86%), 1,8-cineole (15.92%), β-thujone (6.50%) and veridiflorol (6.35%), The chemical composition of the present oil was found to be nearly similar to those of species collected from Algeria [31], Italy [32],Tunisia [33] and India [34], which were mainly represented by α-thujone, 1,8-cineole and camphor. Great amounts of α-thujone (34.42 %, 31.23-52%, 31.56%) were found in oil compositions of species collected from of Tunisia [33], Romania [35] and Georgia [36], respectively while camphor (20.4%, 20.69-54%, 29%, 8,58-31,89%, 5.10 -16.82%) [31,37], 1,8-cineole (32-35%) [32,38], and viridiflorol (16.42-9.92%, 9.94-19.46%) [39-41] were found to be high-contents of *S. officinalis* oils from Algeria, Italy, Algeria, Morocco, Tunisia and Egypt, respectively.

Antibacterial activity

The antibacterial activity of *Salvia officinalis* essential oil was tested against several microorganisms (Table 2). The essential oil exhibited the best antibacterial activity against, ATCC 25922, *E. coli* (HS) and *and Streptococus homeolytic* with 20, 21 mm and 23 mm inhibition zone diameters, respectively.

Table 1: Chemical composition, Retention indices and percentage composition of the essential oil of *Salvia officinalis* collected from Batna.

Pic	Compounds[a]	RI[b]	(%)
1	2,4-Dimethylhexane	729	0.51
2	α- Thujone	809	**24.52**
3	4-Methylpentanol	838	1.88
4	α-Pinene	930	3.72
5	Camphene	939	3.28
6	β-Pinene	954	0.23
7	*p*-Cymene	979	3.60
8	Tert-butylbenzene	990	1.09
9	β-Phellandrene	1024	1.60
10	Limonene	1025	1.70
11	1,8- Cineole	1029	**15.92**
13	γ-Terpinene	1060	0.43
14	Terpinolene	1089	0.20
15	Linalool	1095	0.20
16	*trans*- Sabinene hydrate	1097	0.46
17	β-Thujone	1114	**6.50**
18	*o*-Isopropenyltoluene	1118	0.2 1
19	β-Fenchyl alcohol	1127	0.37
20	1-Terpinol	1134	0.10
21	Camphor	1146	**16.86**
22	Borneol	1169	1.89
23	(Z)- Pinocamphone	1172	0.20
24	*p*-Cymene-9-ol	1205	1.64
25	Bornyl acetate	1289	0.37
26	*Z*-Caryophyllene	1409	0.94
27	*E*-Caryophyllene	1420	0.67
28	α-Humulene	1455	0.67
29	β-Cadinene	1583	0.10
30	Caryphyllene oxide	1593	1.29
31	Viridiflorol	1596	**6.35**
32	Humulene epoxide	1608	0.10
33	□-Eudesmol	1651	0.22
34	Manool	2057	0.54
35	Linolenic acid	2105	0.20
	Identified compounds	**Total**	**98.39**

[a]Compounds listed in order of their RI

[b]RI (retention index) measured relative to n-alkanes (C6-C24) using DB5-MS column

Table 2: Antibacterial activity (inhibition zones and MIC) of the essential oil of *S. officinalis* collected from Batna.

Microrganism	Inhibition zone [a] (mm)	MIC (µg/ml)
***Escherichia coli* ATCC 25922**	20	80
***Escherichia coli* (HS)**	21	80
***Pseudomonas aeruginosa* ATCC 2785**	-	-
***Pseudomonas aeruginosa* (HS)**	-	-
***Staphylococcus aureus* ATCC 43300**	-	-
***Streptococus homeolytic* (HS)**	23	20
Klebsiella pneumoniae ATCC	-	-
***Klebsiella pneumoniae* (HS)**	-	-

[a]: (128 µg/ml)

HS : Hospital strain

Antioxidant activity

Salvia species have been reported as potent natural antioxidants [37]. In this study, total antioxidant activity, by β-carotene bleaching method [25] assays, of the essential oil of *Salvia officinalis* collected from Batna, was carried out. The activity was increased as dose dependent. The essential oil exhibited a good activity, the best inhibition (55.46 %) was measured at 4 mg/ml (Figure 1)

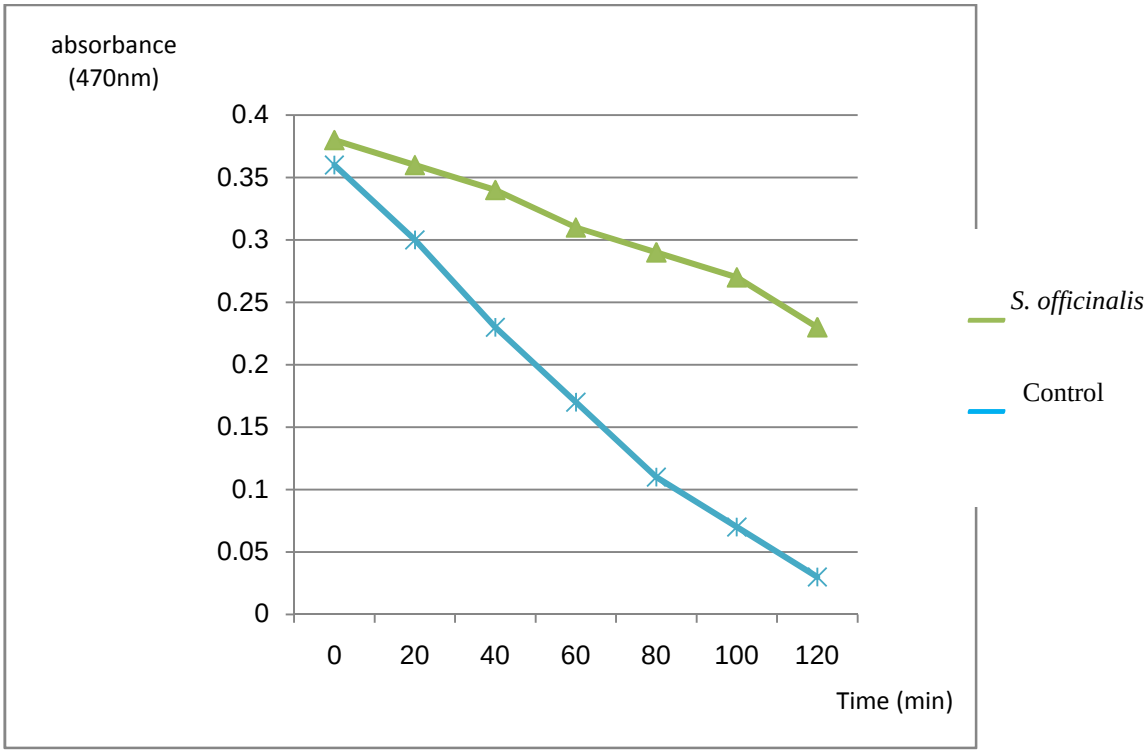

Figure (1): Inhibition of lipid peroxidation of *Salvia officinalis* essential oil and Vitamin E by the β-carotene bleaching method

CONCLUSION

The essential oil of hydrodistilled fresh aerial parts of *Salvia officinalis*, collected at Batna, was mainly represented by α-thujone, 1,8-cineole, Camphor, β-thujone and veridiflorol.

The antibacterial activity of this essential oil against several microorganisms was investigated. The best antibacterial activity was obtained against *E. coli and Streptococus hemolytic* strains. The essential oil exhibited a good antioxidant activity by the use of β-carotene bleaching method which justifies its use in Algerian food chemistry.

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