European Journal of Chemistry

Journal webpage: www.eurjchem.com

Chemical composition, antimicrobial and antioxidant activities of the essentials oils from flowers of *Salvia sharifii*

Hallouma Bilel [1], Lamia Boubakri [1], Fethi Zagrouba [1] and Naceur Hamdi [1,2,*]

[1] *Higher Institute of Sciences and Technology of Environment of Borj Cedria, University of Carthage, Hammam-Lif, B.P. 1003, Tunisia*
[2] *College of Science and Arts, Qassim University, Al-Rass, 51477, The Kingdom of Saudi Arabia*

** Corresponding author at: Higher Institute of Sciences and Technology of Environment of Borj Cedria, University of Carthage, Hammam-Lif, B.P. 1003, Tunisia. Tel.: +966.55.6384839. Fax: +966.63.339351. E-mail address: naceur.hamdi@isste.rnu.tn (N. Hamdi).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.6.3.301-304.1264

Received: 01 April 2015
Accepted: 02 May 2015
Published online: 30 September 2015
Printed: 30 September 2015

KEYWORDS

Essential oil
Salvia sharifii
Green leaf volatiles
Antioxidant activity
Antimicrobial activity
Chemical composition

ABSTRACT

The present study is the first investigation of chemical composition, antioxidant and antimicrobial activities of the volatile oils from *Salvia sharifii* in Tunisia. The obtained results show that essential oils from Tunis locality were most complex and present 35 compounds representing 96.83% of the total oil composition. The major components of the studied oils in this site are Linalool (32.9%) and the green leaf volatiles; hexyl isolaverate (15.4%) and hexyl-2-methyl butanoate (10.9%) were detected as the major constituents of the oil. Considerable levels of antioxidant activities of the investigated essential oils were highlighted. Variations in antioxidant activities may be attributed to the concentrations of major components and the presence of some phenolic compounds like linalool. Our results showed strong activities of the investigated oils against all tested microorganisms. The antimicrobial test results showed that the oils had a great potential antimicrobial activity against all bacteria and fungal strains. Gram-positive bacteria are more sensitive to the investigated oil, with a range of 0.09 to 6.25 µL/mL than Gram-negative bacteria in the range, which is significantly higher from 1.56 to 25.00 µL/mL. The oil showed moderate antioxidant activities, (IC_{50} = 16.8 µg/mL) but good to moderate antimicrobial activity against most of the tested microorganisms.

Cite this: *Eur. J. Chem.* **2015**, *6(3)*, 301-304

1. Introduction

For the past few decades, the essential oils and various extracts of plants have been of great interest as they have been the sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases and the preservation of the foods from the toxic effects of the oxidants. Particularly, the antimicrobial activities of plant oils and extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [1-3]. Furthermore, plant products are also known to possess potential as natural agents for food preservation [4-8]. In order to prolong the storage stability of foods, synthetic antioxidants are mainly used in industrial processing.

Antimicrobial activity of the genus *Salvia* has been well established in the literature [9]. But, as far as our literature survey could as certain, biological properties of *Salvia sharifii* evaluated here had not previously been reported. From this point of view, this study could be assumed as the first report on the antioxidant and antimicrobial activities of *Salvia sharifii*. The aim of this study was to identify the chemical compositions of the oils of *Salvia sharifii* and to study the antioxidant and antimicrobial activities of them, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation. The antimicrobial activity was evaluated against a diverse range of food-borne pathogens. The antioxidant potential was evaluated by scavenging of DPPH radicals.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

2.2. Collection of plant material

The whole plant, *Salvia sharifii* was collected during its flowering season. They were identified by Dr. Anis Benhsouna from Gafsa University, Department of Biology, Tunisia.

The plant was identified with comparison to a voucher specimen (No. 01) that was deposited at the Herbarium of Department of Life Science, College of Science and Arts, Al-Rass Qassim University, The Kingdom of Saudi Arabia. The

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.6.3.301-304.1264

aerial parts of plant were air-dried at room temperature (25 °C) in the shade for 5 days before isolation of the oil.

2.3. Extraction of the essential oil

The air-dried aerial parts of the plant were cut into small pieces (100 g) and subjected to hydrodistillation using a Clevenger-type apparatus until there was no significant increase in the volume of the oil collected (5 h). The oil obtained was separated from water and dried over anhydrous Na_2SO_4 and was stored at 4 °C until analysis. The yield of the yellow oil was 0.37% (*v*:*w*) based on the dry weight of the plant.

2.4. Antimicrobial activity

2.4.1. Microbial strains

Authentic pure cultures of bacteria and fungi were obtained from international culture collections (ATCC), the local culture collection of the Center of Biotechnology of Sfax, Tunisia, the Microorganisms Collection of the Laboratory of Microbiology, University Hospital Center (CHU) of Habib Bourguiba, Sfax, Tunisia and food isolates from the Laboratory of Parasitology-Mycology, Sfax Faculty of Medicine, Tunisia. They included Gram-positive bacteria: *Listeria monocytogenes* (Food isolate 2132), *Kocuria varians* (ATTC 15306), *Bacillus pumilus* (ATTC 14884) and Gram-negative bacteria: *Escherichia coli, Salmonella typhi*, *Pseudomonas aeruginosa*. The following fungal strains were also tested: *Aspergillus niger* (CTM 10099), *Aspergillus flavus* (Food isolate), *Candida glabrata*.

The bacterial strains were grown on Mueller Hinton broth (Bio-Rad, France) at 37 °C for 12-14 h while potato dextrose agar (PDA) (1.5% agar) at 28 °C for 4 days were used for fungi. Inocula were prepared from an overnight broth culture by their dilution in saline solution to approximately 10^7 colony-forming units CFU/mL for bacteria and 10^5 spores/mL for fungus.

2.4.2. Disc diffusion method

Agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils [10]. Briefly, a suspension of the tested microorganism (0.1 mL of 10^8 cells per mL) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 µL of the oil and placed on the inoculated plates. These plates, after staying at 4 °C for 2 h, were incubated at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The diameters of the inhibition zones were measured in millimeters. Tests were carried out in triplicate. Values are presented as means ± SD of three parallel measurements.

2.4.3. Determination of MIC

A broth microdilution broth susceptibility assay was used, as recommended by NCCLS, for the determination of the MIC [11]. All tests were performed in Mueller Hinton Broth (MHB; BBL) supplemented with Tween 80 detergent (final concentration of 0.5% (*v*:*v*), with the exception of the yeasts (Sabouraud dextrose broth-SDB + Tween 80). Bacterial strains were cultured overnight at 37 °C in MHA and the yeasts were cultured overnight at 30 °C in SDB. Test strains were suspended in MHB to give a final density of 5×10^5 cfu/mL and these were confirmed by viable counts. Geometric dilutions ranging from 0.036 mg/mL to 72.00 mg/mL of the essential oils were prepared in a 96-well microtiter plate, including one growth control (MHB + Tween 80) and one sterility control (MHB + Tween 80 + test oil). Plates were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and at 30 °C for 48 h for the yeasts. The bacterial growth was indicated by the presence of a white ''pellet'' on the well bottom.

2.4.4. Gas chromatography

Essential oils from flowers of *Salvia sharifii* were analysed using a Thermo Electron (Courtaboeuf, France) gas chromatograph equipped with flame ionization detection (FID) detector and DB-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 200 °C and detector temperature 270 °C, respectively oven temperature gradually raised from 60 °C to 260 °C at 5 °C/min, held for 15 min and finally raised to 340 °C at 40 °C/min. Helium (purity 99.99%) was the carrier gas, at a flow rate of 1 mL/min. Total analysis time was 57 min. Diluted sample (1/100 in petroleum ether, *v*:*v*) of 1.0 µL was injected in the split mode (ratio 1:10). Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

2.4.5. Gas chromatography/mass spectrometry (GC-MS)

Analysis of essential oils from flowers of *Salvia sharifii* was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a Thermo Electron (Courtaboeuf, France) DSQ II GC-MS single quadrupole mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 200 and 300 °C, respectively. MS was adjusted for an emission current of 10 µA and electron multiplier voltage at 1500 V. Trap temperature was 250 °C and mass scanning was from 40 to 650 amu. The components were identified based on the comparison of their retention time and mass spectra with those of standards, Wiley 2001 library data (NIST 02 version 2.62) of the GC-MS system and literature data [12]. All determinations were performed in duplicate and averaged.

2.5. Antioxidant activity by DPPH radical scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was determined using Blois method [13] with minor modifications [14]. Briefly, stock solutions (10 mg/mL each) of the essential oil and the well-known natural standard antioxidants, quercetin were prepared in methanol. Dilutions are made to obtain concentrations ranging 2.5, 5.0, 10.0 mg/mL for essential oil. Diluted solutions (1 mL each) were mixed with 1 mL of a freshly prepared 80 µg/mL DPPH radical methanol solution and allowed to stand for 30 min in the dark at room temperature for any reaction to take place. Absorbance values of these solutions were recorded on an ultraviolet and visible (UV-Vis) spectrometer (Bio-Tek, Model UvikonXL) at 517 nm.

Inhibitions of DPPH radical in percent (I%) was calculated as follow:

$$I\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100 \quad (1)$$

where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance values of the test compounds. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

3. Results and discussion

3.1. Chemical Composition of the essential oil

Hydrodistillation of the aerial parts of the plant yielded 0.37 (*v*:*w*) of oil. The oil was subjected to GC and GC-MS analyses. The composition of the oil was determined by comparison of their mass spectra and the calculated GC

retention indices to those reported in the literature for the standard compounds (Table 1 and 2).

Table 1. Chemical composition of *S. Sharifii* essential oils.

Compounds	KI *	Composition (%)
(*Z*)-Hexanal	855	0.73
Hexanol	871	2.55
α-Pinene	939	0.43
1-Octen-3-one	977	0.43
β-Pinene	979	0.63
Hexyl acetate	1008	0.49
1,8-Cineole	1031	1.77
3-Methyl butanoic acid	1042	0.40
n-Octanol	1068	0.61
Linalool	1097	32.95
Comphor	1146	0.40
Hexyl isobutanoate	1152	5.18
α-Terpineol	1189	1.02
Hexyl 2-methyl butanoate	1236	10.99
Hexyl isolaverate	1244	15.44
Geraniol	1267	0.48
δ-Elemene	1338	0.26
Octyl 2-methyl propanoate	1341	0.56
Hexyl hexanoat	1384	1.73
β-Elemene	1391	2.87
(*E*)-Caryophyllene	1419	0.95
Octyl 2- methyl butanoate	1426	0.70
Pentyl 2-methyl butanoate	1430	1.17
Aromadendrene	1441	0.46
Germacrene D	1485	1.13
δ-Selinene	1493	0.64
α-Selinene	1498	1.63
Hexyl octanoate	1573	2.96
Spathulenol	1578	0.57
Hexyl benzoate	1580	0.49
Caryophyllene oxide	1583	0.67
β- Eudesmol	1651	1.03
Longifolol	1715	0.95
Guaiol acetate	1727	1.02
6,10,14-Trimethyl-2-pentadecanone	1832	0.79
Total (%)		95.01
Yield (%)		0.37

* KI = Kovat's indices literature [16].

Table 2. Class composition of *S. Sharifii* essential oils.

Class	Percentage
Monoterpene hydrocarbons	1.06
Oxygenated monoterpenes	41.80
Sesquiterpene hydrocarbons	7.94
Oxygenated sesquiterpenes	4.24
Aliphatic compounds	40.04
Total identified	95.01

Thirty five constituents including the 2,6-dimethyl octane monoterpenes; linalool (32.9%), and green leaf volatile derivatives; hexyl isolaverate (15.4%) and hexyl-2-methyl butanoate (10.9%) were the major components representing of 59.2% of the oil.

However, the reports on the chemical compositions of the oils isolated from the other plants of the genus *S. macrosiphon*, *S. atropatana*, *S. reuterana* and *S. spinosa*, which show patterns of essential oil compositions similar to our present study for the investigated plant [15]. All of the plants synthesize green leaf volatiles the low molecular weight (C6) esters, aldehydes, and alcohols, linear monoterpenes with 2,6-dimethyl octane carbon skeletons or fatty acid derivatives as their major constituents.

As can be seen from Table 1, oil samples were found to be rich in Linalol, hexyle-2-methyl butanoate and hexyl isoaverate. To the best of our knowledge, there are many reports on the chemical composition of the oils isolated from the plants belonging to the genus *Salvia* [17,18]. Most of these reports indicate that 1,8-cineole (eucalyptol) and borneol are the main and/or characteristic constituents of *Salvia* oils. Chemical compositions of the essential oils of the plant species studied had been reported before [19].

3.2. Antioxidant activity and total phenolic contents

DPPH radical scavenging activity of the essential oil of *Salvia sharifii* was evaluated for the assessment of its antioxidant potential. Free radical scavenging capacities of the corresponding oils were measured by DPPH assay and the results are shown in Table 3.

Table 3. Antioxidant activity and total phenolic contents of *Salvia sharifii* *.

Sample	DPPH IC_{50} (mg/mL)	Catechin equivalent/g extract
Salvia sharifii	16.8±6.9	0.390±0.004
Quercetin	0.005±0.001	-

* Values represent the mean of three experiments ± SD. Values with different letters in the same column were significantly different.

DPPH radical scavenging activity of the positive standard, quercetin and plant sample were expressed as IC_{50} values, the concentrations of analytes required for the conversion of the half of the DPPH radicals to their more stable molecular counterparts 2,2-diphenyl-1-picrylhydrazines. The mild anti-oxidant capacity of the essential oil of the plant may be a consequence of its weak phenolic compounds content which was reflected in its Folin-Ciocalteu test result. A good linear correlation was observed between the IC_{50} values in the DPPH assay and the total phenolic content (r^2 = 0.822, data not shown).

3.3. Antimicrobial activity

The antimicrobial activity of *Salvia sharifii* essential oil was evaluated against a panel of nine microorganisms and its potency was assessed qualitatively and quantitatively by the presence or absence of inhibition zones and MIC values. The disc diffusion method for antibacterial activity was showed significant reduction in bacterial growth in terms of zone of inhibition around the disc (Table 4). Among bacterial strains tested, *Escherichia coli*, *Salmonella typhi* and *Listeria monocytogenes* were found to be more sensitive to oil. Other bacterial forms were inhibited by the plant oil. The zone of inhibition increased on increasing the concentration of essential oil in disc. This was showed the concentration dependent activity.

The antibacterial activity of the plant oil against the bacterial strains was showed high values of MIC (Table 4). Our findings showed that the essential oil from aerial part of *Salvia sharifii* had interesting activity against both Gram-negative and Gram-positive bacteria. The plant oil proved to be active against 3 out of the 6 bacterial strains used and was particularly active against *Escherichia coli*, *Pseudomonas aeroginosa* and *Salmonella typhi* (MIC values of 32 µg/mL for the first and 64 µg/mL for the others, respectively).

As for *Listeria monocytogenes* and *Kocuria varians* an MIC value of 128 µg/mL was found, while *Bacillus pumilus* was the least affected with an MIC value of 256 µg/mL. This oil showed an inhibiting activity on disease causing Gram-negative and Gram-positive bacteria, especially *Escherichia coli*. This is particularly interesting from a medical point of view because this microbial agent is responsible for severe opportunistic infections.

We also screened the antifungal activity of the essential oil of *Salvia sharifii*. This oil showed mildly significant activity against fungal.

4. Conclusions

Antioxidant and antimicrobial properties of the essential oils and various extracts from many plants are of great interest in both academia and the food, cosmetic and pharmaceutical

Table 4. Antimicrobial activity of the essential oil of *Salvia sharifii.*

Microorganism	MIC of essential oil	MIC of reference [a]	Zone of inhibition of the essential oil in mm (Mean±SD)	Zone of inhibition of the reference mm (Mean±SD) [b]
Bacillus pumilus	256	64	11.6±1.1	16.3±0.3
Kocuria varians	128	32	10.6±1.5	17.6±0.5
Listeria monocytogenes	128	16	14.5±0.5	14.3±0.5
Escherichia coli	32	16	15.4±0.5	16±0
Salmonella typhi	64	32	16.3±0.5	21.3±0.5
Pseudomonas aeruginosa	64	8	13.6±0.5	16.3±0.1
Aspergillus flavus	256	64	11.3±1.1	19.3±0.5
Candida glabrata	256	64	9.1±0.3	19±0
Aspergillus niger	128	32	10±1	22±0

[a] Ampicillin, tetracycline and fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively.
[b] The values represent the mean of four experiments ± SD. Ampicillin, gentamicin and ketoconazole (10 µg/disc) were used as references.

industries, since their possible use as natural additives emerged from a growing tendency to replace synthetic preservatives by natural ones.

In this respect, studying with the endemic species may be of great interest since their bioactive properties and secrets could be lost forever without being tapped. Owing to their excellent protective features exhibited in antioxidant and antimicrobial tests, the essential oil of *Salvia sharifii* could be concluded as a natural source that can be freely used in the food industry as a culinary herb, but, firstly, immediate and necessary measurements should be taken for the protection of *Salvia sharifii*. In conclusion, our study can be considered as the first detailed document on the *in-vitro* antimicrobial and antioxidant features of *Salvia sharifii.*

Acknowledgements

Authors are thankful to Dr. Anis Ben Hsouna (Gafsa University, Gafsa, Tunisia) for performing the biological activity tests.

References

[1]. Ahmadi, L.; Mirza, M. *J. Essent. Oil Res.* **1999**, *11*, 289-290.
[2]. Ames, B. M. *Science* **1983**, *221*, 1256-1263.
[3]. Baardseth, P. *Food Add. Contam.* **1989**, *6*, 201-207.
[4]. Baratta, M. T.; Dorman, H. J. D.; Deans, S. G.; Figueiredo, A. C.; Barroso, J. G.; Ruberto, G. *Flav. Frag. J.* **1998**, *13*, 235-244.
[5]. Baratta, M. T.; Dorman, H. J. D.; Deans, S. G.; Biondi, D. M.; Ruberto, G. *J. Essent. Oil Res.* **1998**, *10*, 618-627.
[6]. Baser, K. H. C.; Ozek, T.; Kirimer, N.; Tumen, G. *J. Essent. Oil Res.* **1993**, *5*, 347-348.
[7]. Baser, K. H. C.; Beis, S. H.; Ozek, T. *J. Essent. Oil Res.* **1995**, *7*, 113-114.
[8]. Baser, K. H. C.; Kurkcuoglu, M.; Ozek, T.; Sarikardasoglu, S. *J. Essent. Oil Res.* **1995**, *7*, 229-230.
[9]. Kelen, M.; Tepe, B. *Bioresour. Tech.* **2008**, *99*, 4096-4104
[10]. Davies, N. W. *J. Chromatogr.* **1990**, *503*, 1-24.
[11]. Deans, S. G. Essential Oils and Waxes, vol. 12. Springer-Verlag, Berlin, 1991, pp. 309-320.
[12]. Marta, B.; Marek, G. *Chem. Pap.* **2012**, *66*, 85-91
[13]. Deans, S. G.; Ritchie, G. *Int. J. Food Microbiol.* **1987**, *5*, 165-180.
[14]. Demirci, B.; Baser, K. H. C.; Tumen, G. *Flav. Frag. J.* **2002**, *17*, 23-25.
[15]. Demirci, B.; Baser, K. H. C.; Yildiz, B.; Bahcecioglu, Z. *Flav. Frag. J.* **2003**, *18*, 116-121.
[16]. Adams, R. P. Identification of essential oil components by gas chromatography/mass spectroscopy. Illinois: Allured Publishing Corporation, 1995, pp. 469.
[17]. Dorman, H. J. D.; Deans, S. G. *J. Appl. Microbiol.* **2000**, *88*, 308-316.
[18]. Helander, I. M.; Alakomi, H. L; Latva-Kala, K.; Mattila-Sandholm, T.; Pol, I.; Smid, E. J.; Gorris, L. G. M.; Wright, A. *J. Agric. Food Chem.* **1998**, *46*, 3590-3595.
[19]. Hammerschmidt, F. J.; Clark, A. M.; Soliman, F. M.; El-Kashoury, E. S.; Abd El-Kawy, M. M.; El-Fishawy, A. M. *Planta Med.* **1993**, *59(1)*, 68-70.