

Tropical Journal of Pharmaceutical Research May 2018; 17 (5): 857-864

ISSN: 1596-5996 (print); 1596-9827 (electronic)

© Pharmacotherapy Group, Faculty of Pharmacy, University of Benin, Benin City, 300001 Nigeria.

Available online at <http://www.tjpr.org><http://dx.doi.org/10.4314/tjpr.v17i5.16>

Original Research Article

Phytochemical profile and antimicrobial properties of volatile compounds of *Satureja calamintha* (L) Scheel from northern Algeria

Khaled Boudjema^{1,2*}, Amel Bouanane², Souad Gamgani², Mourad Djeziri^{1,3}, Mohamed Abou Mustapha³, Fathia Fazouane^{1,2}

¹Research Laboratory in Food Technology, ²Laboratory of Applied Biochemistry, Department of Biology, University of M'Hamed, Bougara, Boumerdes, 35000, ³Research Centre in Physicochemical Analysis, Bousmail, 42000, Algeria

*For correspondence: **Email:** boudjemasidi@yahoo.fr; **Tel:** +213552553941

Sent for review: 4 January 2018

Revised accepted: 22 April 2018

Abstract

Purpose: To undertake the phytochemical screening of *Satureja calamintha* (L.) Scheel., and evaluate the antimicrobial activities of its volatile compounds.

Methods: Qualitative phytochemical analysis of the plant was performed using standard methods. The extraction of essential oils (EOs) was conducted using steam distillation, while the chemical composition was determined using gas chromatography-mass spectrometry (GC-MS). The antimicrobial activities of the oils were evaluated against ten bacterial and six fungal strains using disc-diffusion assay and poisoned food technique, respectively.

Results: After steam distillation, the extraction yield was 0.54 ± 0.11 %. GC-MS analysis identified approximately 99.99 % of the EOs. The three most abundant compounds identified were l-menthone (32.10 %), neo-menthol (32.07 %) and pulegone (22.35 %). The oils had significant ($p < 0.05$) antimicrobial activities against the tested bacterial and fungal strains, except *Bacillus cereus* and *Candida albicans*. The lowest minimum inhibitory concentration (MIC) for bacteria was 0.007 % (v/v) against *Enterococcus faecalis* and *Klebsiella pneumoniae*, whereas for fungi, it was 0.500 % (v/v) against *Candida albicans*. Moreover, *Enterococcus faecalis* and *Listeria innocua* had the lowest minimum bactericidal concentration (MBC) at 0.125 % (v/v), in contrast to the lowest fungicidal concentration (MFC) for *Candida albicans* at 0.500 % (v/v).

Conclusion: These results demonstrate that EOs from *Satureja calamintha* (L.) Scheel. possess significant antimicrobial activities which might be useful for therapeutic and pharmaceutical applications.

Keywords: *Satureja calamintha*, Phytochemicals, Essential oils, Antimicrobial activity, Steam distillation

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Fungal infections have become increasingly recognized as important threats in critically-ill patients. They are characterized by easy

transmission, wide prevalence and a large variety of human and animal reservoirs [1]. Reports on food-borne diseases caused by the consumption of food contaminated with pathogenic bacteria are increasing worldwide [2].

Each year, an estimated 30 % of people in industrialized nations suffer from these diseases [3].

Synthetic antimicrobial substances are undeniably one of the most important therapeutic discoveries of the 20th century; they are effective against serious bacterial and fungal infections. However, most of these pathogenic microorganisms have developed new resistance to several antibiotics. There has been renewed interest in plants and their natural antimicrobial agents as alternatives for reducing the problem of resistance [4]. Among these, essential oils (EOs) have received special attention as natural agents with great potential for food preservation and other pharmacological effects such as antispasmodial, carminative, hepatoprotective, antiviral and anti-cancer activities [5].

Algeria is well-known for its biodiversity, with about three hundred species of plants, 15 % of which are endemic and belong to several botanical families.

The aim of this study was to perform phytochemical screening on the aerial parts of *Satureja calamintha* (L.), and to evaluate the antimicrobial activities of its essential oils.

EXPERIMENTAL

Plant material

Aerial parts of *Satureja calamintha* (L.) Scheel. were collected from the Jijel region of Algeria (Eastern Algeria) and identified by Prof C. Baali of the Department of Botany, Higher National Agronomic School, Algeria. A specimen of the plant was prepared and deposited in the ENSA Herbarium. The aerial parts of the plant were washed and shade-dried in a well-ventilated room.

Phytochemical screening

The qualitative screening for tannins, saponosides, glycosides, alkaloids, quinones, reducing sugars, coumarins and flavonoids were carried out using standard methods [6].

Extraction of essential oils

Extraction of essential oils (EOs) from the aerial parts of the plant were extracted using steam distillation. A weighed portion (100 g) of dried aerial parts was suspended in 300 mL of distilled water with intermittent stirring for 3 h. The oils obtained after extraction were dried over

anhydrous sodium sulfate (Na₂SO₄) and kept in the refrigerator at 4 °C in sealed opaque bottles.

Test microorganisms

Ten bacterial and six fungal strains were purchased from the American-Type Culture Collection (ATCC), and were used for testing the antimicrobial activities of the EOs. The strains were *Bacillus cereus* ATCC 10876, *Enterococcus faecalis* ATCC 49452, *Listeria innocua* CLIP 74915, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica* ATCC 43972, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 853, *Candida tropicalis* DIV13-Z087D0VS, *Candida tropicalis* DIV13-Z087B0VS [7], *Candida albicans* ATCC 1024, *Aspergillus niger* 1, *Aspergillus niger* 2 and *Aspergillus flavus*.

Analysis of essential oils by GC-MS

The identification and quantification of essential oils present in the aerial parts of the plant were carried out using HP 6890 gas chromatography coupled to an HP5973 mass spectrometer. Sample volume of 0.2 µL was injected in split mode (split ratio of 50:1) into a column capillary HP-5MS (30 m x 0.25 mm x 0.25 µm), containing a mixture of phenyl polysiloxane (5 %) and methyl polysiloxane (95 %). The separation was carried out using helium as the carrier gas at a flow rate of 0.5 ml/min under the following conditions: First, the oven temperature was set at 60 °C for 8 min, increased further to 250 °C at a rate of 2 °C/min, and finally maintained at 250 °C for 10 min. The injection and detector temperatures were maintained at 250 and 230 °C, respectively. Individual components were identified by comparing their mass spectra with those of standards, and also by comparing their GC-retention indices with those recorded in literature. The components of the EOs were expressed as percentages determined by integration of GC peak areas without using correction factors.

Evaluation of antibiotic sensitivity and antibacterial activities

The pathogenic bacterial strains were tested for their sensitivities against a range of antibiotics: ampicillin (Amp30), chloramphenicol (C30), cefotaxime (Ct30), gentamycin (Gen10), vancomycin (Va30), cefoperazone (Cep30) and tetracycline (Te30), using the Kirby-Bauer method [8]. The antibacterial activities of the EOs

were determined using disc-diffusion assay. A single colony from an overnight bacterial culture plate was transferred into tubes containing 5 mL of sterile saline solution to obtain the bacterial suspension. This was adjusted to 0.5 McFarland standard, approximately corresponding to an initial inoculum size of 10^8 colony-forming units per milliliter (CFU/mL). The bacterial suspension was diluted 1:100 to obtain 10^6 CFU/mL.

Using a sterile swab, 200 μ L of fresh culture was spread on Muller Hinton agar plates, and 20 μ L of EO was dispensed on the sterile paper disc 6 mm in diameter. The EO disc and antibiotic-containing discs were deposited separately on the surface of each inoculated agar plate and incubated at 37 °C for 24 h. Chloramphenicol (15 μ g/disc) was used as a positive control. The antibacterial activity was estimated by measuring the diameter of the inhibition zone surrounding each disc.

Evaluation of antifungal activity

The antifungal activity was evaluated using the disc-diffusion method for yeast, while poisoned food technique was used for mold [9]. In the disc-diffusion method, a colony from a two-day yeast culture plate was transferred to tubes containing 5 mL of sterile saline solution to obtain a yeast suspension corresponding approximately to an initial inoculum size of 10^4 CFU/mL. A portion of the fresh culture (200 μ L) was inoculated on Sabouraud Dextrose agar plates. This was followed by the injection of 20 μ L of EOs on a sterile paper disc 6 mm in diameter, which was further deposited on the surface of each inoculated agar plate and incubated at 25 °C for 48 h. Griseofulvin (15 μ g/disc) was used as a positive control and the antifungal activity was estimated by measuring the diameter of the inhibition zone surrounding each disc.

In the poisoned food technique, 30 μ L of EOs and two drops of Tween 80 were transferred to a sterile tube containing 13.5 mL of molten Sabouraud Dextrose agar medium and cooled at 45 °C. The mixture obtained was shaken manually and dispensed on sterile petri dishes. After solidification of the medium, a mycelial disc measuring approximately 6 mm in diameter, cut from a fungal culture of 5 days, was deposited in the center of each petri plate, and further incubated at 25 °C. Control plates without EOs, were treated following the same procedure with nystatin (30 μ L) as the positive control. The diameter of mycelial growth was recorded after five days of incubation and the percentage inhibition of mycelial growth (M) was calculated as in Eq 1.

$$M (\%) = \{1 - (D_{EOs}/Dc)\}100 \dots\dots\dots (1)$$

where Dc is the mean diameter of mycelial growth in the control sample, and D_{EOs} is the mean diameter of mycelial growth treated with EOs.

Determination of minimum inhibitory concentrations (MIC)

The MICs of the EOs were determined using the micro-dilution method [10]. Stock solutions of EOs were prepared at concentration of 1.000 % (v/v) and diluted in Muller Hinton broth and Sabouraud Dextrose broth to obtain concentrations in the range of 0.007 to 1.000 % (w/v). Portions of each concentration (100 μ L) were transferred to wells and inoculated separately with 10 μ L of the microbial suspensions (10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi). The incubation temperatures for bacteria and fungi were 37 °C and 25 °C, respectively. Chloramphenicol, nystatin and griseofulvin were used as positive controls. The MIC was defined as the lowest concentration of EOs that resulted in no visible growth of microorganisms.

Evaluation of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

After MIC determination, the wells that showed no visible growth of microorganisms were used for determination of MBC for bacteria, and MFC for fungi. Different concentrations of EOs ranging from 0.007 to 1.000 % (w/v) were prepared in Muller Hinton broth and Sabouraud Dextrose broth. Portions of each concentration (100 μ L) were transferred to the wells and were inoculated separately with 10 μ L of the microbial suspensions (10^6 CFU/mL for bacteria and 10^4 CFU/mL for fungi), at 37 °C for bacteria, and at 25 °C for fungi. Chloramphenicol, nystatin and griseofulvin were used as positive controls. The MBC and MFC were defined as the lowest concentrations of EOs that completely killed the initial inoculums of bacteria and fungi, respectively.

Statistical analysis

Statistical analyses were performed with Microsoft Excel 2010 and the results are presented as mean \pm standard deviation (SD) of three replicates. All the data were statistically evaluated using Student's *t*-test and significant difference between the means was set at $p < 0.05$.

RESULTS

Phytochemical profile

From Table 1, twelve groups of chemical compounds were detected, namely flavonoids, anthocyanins, total and gallic tannins, glycosides, saponosides, reducing compounds, holosides, alkaloids, coumarins, O-heterosides and C-glycosides. Leucoanthocyanins and quinones were not detected, whereas catechic tannins and saponosides were present in minimal amounts.

Table 1: Phytochemical profile of aerial parts of *Satureja calamintha*

Phytochemical	Result
Flavonoids	+++
Anthocyanins	++
Leucoanthocyanins	-
Total tannins	+++
Catechic tannins	+
Gallic tannins	+++
Glycosides	++
Saponosides	+
Quinones	-
Reducing compounds	+++
Oses and holosides	+
Alkaloids	+++
Coumarins	+++
O-heterosides	+++
C-heterosides	+++

(-): Absent, (+): Present in minimal quantity, (++): Present in medium quantity, (+++): Present in appreciable quantity

Yield extraction and chemical profile of the essential oils

The extracted volatile substances had aromatic odor and yellow color. The average yield of EOs was about 0.54 ± 0.11 %. The aerial parts of the plant contained three major compounds: l-menthone (32.10 %), neo-menthol (32.07 %) and pulegone (22.35 %).

Antibacterial and antifungal activities

The bacterial strains reacted differently to the tested antibiotics; with inhibition zones varying from 6 to 45 mm. *Enterococcus faecalis* seemed resistant to Gen10 (12 mm) and susceptible to the other antibiotics, whereas MRSA appeared resistant to Te30 and Va30 (6 mm). Cep30 had a larger spectrum of inhibition than Gen10, Te30 and Va30. It exhibited a smaller inhibition zone, varying from 6 to 10.5 mm. Amp30 and Ct30 seemed to be the most efficient antibiotics against all the tested bacterial strains (6 - 15 mm), whereas C30 appeared to be the most

effective with an inhibition zone varying from 25 to 39 mm.

Table 2: Extraction yield and compositional analysis of volatile fractions of *Satureja calamintha*.

No.	Constituent	Retention time (min)	Content (%)
1	Alpha.-Thujene	10.28	0.01
2	Alpha.-Pinene	10.68	0.45
3	Camphene	11.55	0.04
4	Sabinene	13.13	0.28
5	Beta.-Pinene	13.32	0.57
6	Beta.-Myrcene	14.33	0.30
7	3-Octanol	14.69	0.16
8	Pseudolimonene	15.12	0.02
9	Alpha.-Terpinene	16.00	0.01
10	Benzene	16.59	0.01
11	1,8-Cineole	17.08	3.33
12	Beta.-Ocimene	18.31	0.01
13	Gamma.-Terpinene	18.99	0.03
14	4-Thujanol	19.64	0.03
15	Alpha.-Terpinolene	21.10	0.04
16	L-Linalool	22.22	0.07
17	Camphor	25.14	0.03
18	l-Menthone	26.56	32.10
19	neo-Menthol	27.59	32.07
20	Menthol	27.75	0.13
21	Isopulegone	27.91	0.57
22	Menthol	28.30	0.25
23	2-Carene	28.84	0.11
24	Cumene	31.33	0.03
25	Pulegone	32.59	22.35
26	Piperitone Oxide	33.41	3.31
27	Seudonone	34.29	0.04
28	Thymol	36.02	0.06
29	Diosphenol	36.14	0.07
30	Cyclohexane	36.39	0.02
31	Artemisia triene	38.56	0.01
32	Pulespenone	38.84	0.24
33	Eugenol	40.04	0.04
34	Piperitenone Oxide	40.57	0.32
35	Copaene	41.06	0.03
36	Beta. Bourbonene	41.64	0.02
37	Beta.-Elemene	42.15	0.04
38	Cinrolon	42.70	0.03
39	Alpha.-Gurjunene	43.20	0.01
40	Caryophyllene	43.81	0.15
41	Geranylacetone	46.06	0.03
42	Beta.-Farnesene	46.30	0.10
43	Beta.-Cubebene	47.73	1.46
44	Bicyclogermacrene	48.63	0.62
45	Delta-Cadinene	50.24	0.02
46	Palustrol	52.81	0.05
47	(+) Spathulenol	53.43	0.05
48	d-Ledol	54.86	0.02
49	Isobicyclogermacrene	57.81	0.01

The results of antibacterial activity are presented in Table 4. The essential oils had significant antibacterial effects against all tested bacteria, with inhibition diameters between 10.33 and 30 mm, except for *Salmonella enterica*, which had low sensitivity (7.33 ± 2.62 mm).

Table 3: Selected antibiotics and their antimicrobial activities

Test strain	Antibiotics						
	Gen10	C30	Te30	Va30	Amp30	Ct30	Cep30
Gram-positive bacteria							
<i>Bacillus cereus</i> ATCC 10876	27.5±0.5	25±0.0	16.5±0.5	19.5±0.5	6±0.0	6±0.0	6±0.0
<i>Enterococcus faecalis</i> ATCC 49452	12±0.0	30±0.0	30±0.0	22±0.0	6±0.0	7±0.0	6±0.0
<i>Listeria innocua</i> CLIP 74915	32±0.0	33.5±0.5	19.5±1.5	27±10	14.5±0.5	6±0.0	40±0.0
RMSA ATCC 43300	28±0.0	27.5±0.5	6±0.0	6±0.0	8±0.0	6±0.0	10.5±0.5
<i>Staphylococcus aureus</i> ATCC 25923	32.5±0.5	33±0.0	21±1	25.5±0.5	8.5±0.5	7±0.0	37.5±0.5
Gram-negative bacteria							
<i>Escherichia coli</i> ATCC 25922	37.5±2.5	45±0.0	35±0.0	8.5±0.5	6±0.0	9.5±0.5	30±0.0
<i>Klebsiella pneumoniae</i> ATCC 700603	17.5±0.5	31±0.0	38.5±1.5	25.5±0.5	6±0.0	7±0.0	6±0.0
<i>Salmonella enterica</i> ATCC 43972	17.5±0.5	35.5±0.5	32.5±0.5	23.5±0.5	6±0.0	9.5±0.5	30±0.0
<i>Salmonella typhimurium</i> ATCC 13311			30±0.0		15±0.0		
<i>Pseudomonas aeruginosa</i> ATCC 853	34.5±0.5	39±0.0	22±10	26.5±0.5	13.5±0.5	6.5±0.5	39±10

Data are inhibition diameters (mm) of selected antibiotics

The antibacterial activities of the oils were significantly higher ($p < 0.05$) against *Enterococcus faecalis*, *Listeria innocua*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica* and *Pseudomonas aeruginosa*, when compared to the effects of chloramphenicol.

The oils had moderate antifungal activities, ranging from 10.66 to 39.66 mm. All results were statistically significant ($p < 0.05$), with the exception of *Candida tropicalis* DIV13-Z087D0VS. The fungal strains reacted differently to the oils, with variable percentages of inhibition between 54.44 and 71.25 %.

MIC, MBC and MFC

The minimum inhibitory concentration (MIC),

minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of EOs against all tested strains are shown in Table 5. The MIC ranged from 0.007 to 1.000 % (v/v) for bacteria and from 0.250 to 1.000 % (v/v) for fungi. The lowest MIC for the EOs in the case of bacteria was observed with *Enterococcus faecalis* and *Klebsiella pneumoniae* corresponding to 0.007 % (v/v), whereas it was 0.500 % (v/v) for *Candida albicans* in case of fungi. Two bacterial stains (i.e. *Enterococcus faecalis* and *Listeria innocua*), showed a bactericidal effect with a low MCB of 0.125 % (v/v), whereas *Candida albicans* was considered a sensitive fungal strain with an MFC of 0.500 % (v/v).

Table 4: Antibacterial and antifungal activities of EOs from *Satureja calamintha* and standard antibiotics

Test strain	ZI (mm) essential oils	ZI(mm) Chloramphenicol	ZI(mm) Griseofulvin	PI(%) Nystatin
Gram-positive bacteria				
<i>Bacillus cereus</i> ATCC 10876	24 ± 1.41	25±0.0	-	-
<i>Enterococcus faecalis</i> ATCC 49452	18.33 ± 20 ^{****}	30±0.0	-	-
<i>Listeria innocua</i> CLIP 74915	10.33±1.24 ^{****}	33.5±0.5	-	-
MRSA ATCC 43300	30 ± 1.63 ^{****}	27.5±0.5	-	-
<i>Staphylococcus aureus</i> ATCC 25923	13.66 ± 20 ^{****}	33±0.0	-	-
Gram-negative bacteria				
<i>Escherichia coli</i> ATCC 25922	12 ± 1.63 ^{****}	43.66±1.33	-	-
<i>Klebsiella pneumoniae</i> ATCC 700603	11 ± 0.81 ^{****}	31±0.0	-	-
<i>Salmonella enterica</i> ATCC 43972	7.33 ± 2.62 ^{****}	35.5±0.5	-	-
<i>Salmonella typhimurium</i> ATCC 13311	22 ± 1.63 ^{****}	24.5±1.5	-	-
<i>Pseudomonas aeruginosa</i> ATCC 853	10.3 ± 20 ^{****}	39±0.0	-	-
Yeast				
<i>Candida albicans</i> ATCC 1024	20 ± 0.0 ^{**}	-	26.5 ± 0.5	-
<i>Candida tropicalis</i> DIV13-Z087D0VS	10.66 ± 1.69 ^{****}	-	8.33 ± 0.47	-
<i>Candida tropicalis</i> DIV13-Z087B0VS	39.33 ± 4.71 ^{****}	-	7.66 ± 0.94	-
Mold				
PI (%)				
Essential oils				
<i>Aspergillus niger</i> 1	54.44	-	-	3.33
<i>Aspergillus niger</i> 2	48.88	-	-	28.88
<i>Aspergillus flavus</i>	71.25	-	-	17.5

ZI: zone of inhibition, PI: % inhibition, -: not determined

Table 5: Minimum inhibitory concentrations of EOs on test microorganisms

Test strain	Essential oils		Standard (Chloramphenicol)	
	MIC (% v/v)	MBC or MFC (% v/v)	MIC (% v/v)	MBC or MFC (% v/v)
Gram-positive bacteria				
<i>Bacillus cereus</i> ATCC 10876	>1.000	-	0.250	1.000
<i>Enterococcus faecalis</i> ATCC 49452	<0.007	0.125	0.250	0.500
<i>Listeria innocua</i> CLIP 74915	0.062	0.125	0.250	>1.000
MRSA ATCC 43300	1.000	1.000	1.000	>1.000
<i>Staphylococcus aureus</i> ATCC 25923	0.125	1.000	0.500	>1.000
Gram-negative bacteria				
<i>Escherichia coli</i> ATCC 25922	>1.000	-	0.500	>1.000
<i>Klebsiella pneumoniae</i> ATCC 700603	< 0.007	-	0.250	>1.000
<i>Salmonella enterica</i> ATCC 43972	0.015	1.000	0.500	0.500
<i>Salmonella typhimurium</i> ATCC 13311	>1.000	-	0.500	>1.000
<i>Pseudomonas aeruginosa</i> ATCC 853	0.500	1.000	0.250	1.000
Fungi				
Yeast				
<i>Candida albicans</i> ATCC 1024	0.500	0.500	Nystatin 1.000	1.000
<i>Candida tropicalis</i> DIV13-Z087D0VS	>1.000	-	>1.000	-
<i>Candida tropicalis</i> DIV13-Z087B0VS	1.000	1.000	1.000	1.000
Mold				
<i>Aspergillus niger</i> 1	>1.000	-	Griseofulvin >1.000	-
<i>Aspergillus niger</i> 2	>1.000	-	>1.000	-
<i>Aspergillus flavus</i>	1.000	1.000	0.250	1.000

DISCUSSION

The phytochemical compositions of plants vary from one species to another and sometimes within the same species. These differences in composition confer important biological properties on plants. Twelve phytochemicals were detected in the aerial part of *Satureja calamintha*, namely: flavonoids, anthocyanins, total and gallic tannins; glycosides, saponosides, reducing compounds, holosides, alkaloids, coumarins, O-heterosides and C-glycosides. Leucoanthocyanins and quinones were not detected. Although catechic tannins and saponosides were present, they had minimal relative abundance.

A similar study carried out on *Satureja calamintha* (L.) Scheel. from Western Algeria showed the presence of saponosides, coumarins, total tannins and free flavonoids, but anthocyanins, leucoanthocyanins and reducing compounds were absent [11]. In another study on the same plant, the presence of flavonoids, total tannins, alkaloids, saponosides and quinones was reported [12]. These observed differences in chemical composition may be linked to variations in climatic and geographical conditions.

The extract yield from steam distillation was comparable to that reported in a previous study [13]. In contrast, a higher yield was obtained from the same species from Morocco [14]. Different extraction yields varying from 1.27 to 2.52 %

have been reported on the same plant grown in different parts of Algeria [12,15]. The three most abundant compounds identified in the EOs were 1-menthone, neo-menthol and pulegone.

The chemical compositions of oils extracted from *Satureja calamintha* differed from one region to another. In Algeria, studies on oils of this plant species collected from two different sites demonstrated the presence of twenty phytochemical components [15]. The sample from the first site was characterized by the predominance of piperitone oxide, cyclohexanone 2- (methylethylidene), and menthone, whereas the major compounds in the oils from the second site were menthone, piperitone oxide, pulegone, and cyclohexanone 2- (1-ethylthylidene). Studies carried out on oils from *Satureja calamintha* (L.) Scheel. from Belgium showed that the major compounds were pulegone, piperitenone oxide, and menthone [16]. In a study on the same plant from Italy, the dominant constituents were pulegone, piperitenone oxide, piperitone oxide, and piperitenone [13]. These differences in composition are due seasonal and environmental factors such as soil type, geographic location, harvest period and extraction method [17].

In the present study, the essential oils (EOs) from *Satureja calamintha* exhibited potent antibacterial activities. Gram-positive bacteria were more sensitive to the EOs than Gram-negative bacteria. These differences in susceptibilities indicate a possible effect of the

EOs on the plasma membrane, since the walls of Gram-negative bacteria may be less permeable to antimicrobial agents. The outer layer of polysaccharides, proteins and lipids acts as a barrier to the entry of different chemical agents [18].

These results are in agreement with those obtained from previous studies [19]. However, they are better, when compared to the results obtained by Bensouici et al, [20] who reported inhibition zones ranging from 14 to 25 mm against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. The inhibition zones obtained in the present study are smaller than those reported by Gormez et al, [21]. The antifungal activities are also in agreement with previous reports [13,19], but they differ from those obtained by Kerbouche et al, [19] who reported inhibition zone of 11.33 mm against *Candida albicans*; and also from values reported by Abdoune [22] who reported inhibition zone of 47 mm against the same fungal strain. The antibacterial and antifungal activities of the EOs may be linked to their richness in l-menthone, neo-menthol and pulegone. However, Monforte et al, [23] had earlier attributed the potent antimicrobial activities to carvone content. Some authors have suggested that minor components of the EOs may have some synergistic effects [24].

CONCLUSION

The phytochemical profile of aerial parts of *Satureja calamintha* (L.) Scheel. shows that the plant is rich in diverse secondary metabolites, which confer on it several biological properties. The essential oils demonstrated potent antimicrobial activities against different pathogenic strains of bacteria and fungi. These findings suggest that essential oils of the plant could be used as potential natural antimicrobial agents in pharmaceutical processes.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. Hennequin C. *Épidemiologie des mycoses invasives. L'expérience d'un centre hospitalo-universitaire parisien. Rev Med Interne* 1996; 17:754-760.
2. Diao WR, Hu QP, Feng SS, Li WQ, Xu JG. *Chemical composition and antibacterial activity of the essential oils from green huajiao (Zanthoxylum schinifolium) against selected foodborne pathogens. J Agric Food Chem* 2013; 61: 6044-6049.
3. Burt S. *Essential oils: their antibacterial properties and potential applications in foods: a review. Inter J Food Microbiol* 2004; 94: 223-253.
4. Jassim SAA, Limoges RG. *Natural solution to antibiotic resistance: bacteriophages 'The Living Drugs'. World J Microbiol Biotechnol* 2014; 30: 2153-2170.
5. Lahlou M. *Essential oils and fragrance compounds: bioactivity and mechanisms of action. Flavour frag J* 2004; 19(2): 159-165.
6. Harborne JB. *Phytochemical methods, London Chapman and Hall, Ltd, 1973: 49-88.*
7. Boudjema K, Fazouane-Naimi F, Hellal A. *Isolation, identification of yeast strains producing bioethanol and improvement of bioethanol production on cheese whey, Turk J Biochem* 2016; 41(1): 157-166.
8. Bauer AW, Kirby WMM, Sherris JCT, Turck M. *Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol* 1966; 45: 493-496.
9. Adjou ES, Kouton S, Dahouenon-Ahoussi E, Sohounhloue CK, Soumanou MM. *Antifungal activity of Ocimum canum essential oil against toxinogenic fungi isolated from peanut seeds in post-harvest in Benin. Int Res J Biological Sci* 2012; 1(7): 20-26.
10. Erkmen O and Ozcan M. *Antimicrobial effects of essential oils on growth of bacteria, yeasts and molds. J Essent Oil Bear PI* 2004; 7(3): 279-287.
11. Bougandoura N, Bendimerad N. *Evaluation of the antioxidant activity of aqueous and methanolic of Satureja calamintha ssp. Nepeta (L.) Briq. Nature & Technology* 2003; 9: 14-19.
12. Benkhedimallah R, Kismoun S. *Phytochemical and biological studies of Satureja calamintha. Master degree, University of Constantine 1, Algeria, 2014; p 88.*
13. Panizzi L, Flamini G, Cioni PL, Morelli I. *Composition and antimicrobial properties of essential oils of four Mediterranean Lamiaceae. J Ethnopharmacol* 1993; 39(3): 167-170.
14. Satrani B, Ghanmi M, Farah A, Aafi A, Fougrach H, Bourkhiss B, Talbi M. *Composition chimique et activité antimicrobienne de l'huile essentielle de Cladanthus mixtus. Bull Soc Pharm Bord* 2007; 146: 85-96.
15. Labiod R. *Valorization of essential oils and extracts of Satureja calamintha nepeta: antibacterial antioxidant and antifungal activities. PhD Thesis. University of Badji Mokhtar, Algeria, 2016; p 115*
16. De Pooter HL, Goetghebeur P, Schamp N. *Variability in composition of the essential oil of Calamintha nepeta. Phytochem* 1987; 26(12): 3355-3356.

17. Gende LB, Mendiara S, Fernández NJ, Van Baren C, Di Leo Lira A, Bandoni A, Eguaras M. Essentials oils of some *Mentha* spp. and their relation with antimicrobial activity against *Paenibacillus* larvae, the causative agent of American foulbrood in honey bees, by using the bioautography technique. *Bull Insectol* 2014; 67(1): 13-20.
18. Cao G, Sofic E, Prior RL. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radic Biol Med* 1997; 22(5): 749-760.
19. Kerbouche L, Hazzit M, Baaliouamer A. Essential Oil of *Satureja calamintha* subsp. *nepeta* (L.) Briq. from Algeria: Analysis, Antimicrobial and Antioxidant Activities. *Journal Biologically Active Products from Nature* 2013; 3(4): 266-272.
20. Bensouici C, Benmerache A, Chibani S, Kabouche A, Abuhamdah S, Semra Z, Kabouche Z. Antibacterial activity and chemical composition of the essential oil of *Satureja calamintha* ssp. *sylvatica* from Jijel, Algeria. *Pharm Lett* 2013; 5(2): 224-227.
21. Gormez A, Bozari S, Yanmis D, Gulluce M, Fikrettin Sahin F, Agar G. Chemical composition and antibacterial activity of essential oils of two Species of Lamiaceae against phytopathogenic bacteria. *Pol J Microbiol* 2015; 64(2): 121–127.
22. Abdoune MN. Preventive effect of some essential oils against growth and biofilm formation of some oral pathogens. Magister degree, University of Tlemcen, Algeria, 2013; p 93.
23. Monforte MT, Tzakou O, Nostro A, Zimbalatti V, Galati EM. Chemical composition and biological activities of *Calamintha officinalis* Moench essential oil. *J Med Food* 2011; 14: 297-303.
24. Wang W, Wu N, Zu YG, Fu YJ. Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components. *Food chem* 2008; 108(3): 1019-1022.