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Research Article

Chemical Composition and Cytotoxic and Antioxidant Activities of *Satureja montana* L. Essential Oil and Its Antibacterial Potential against *Salmonella* Spp. Strains

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The present study describes chemical composition as well as cytotoxic, antioxidant, and antimicrobial activities of winter savory *Satureja montana* L. essential oil (EO). The plant was collected from south France mountain, and its EO was extracted by hydrodistillation (HD) and analysed by gas chromatography/mass spectrometry (GC/MS). Thirty-two compounds were identified accounting for 99.85% of the total oil, where oxygenated monoterpenes constituted the main chemical class (59.11%). The oil was dominated by carvacrol (53.35%), γ-terpinene (13.54%), and the monoterpenic hydrocarbons *p*-cymene (13.03%). Moreover, *S. montana* L. EO exhibited high antibacterial activities with strong effectiveness against several pathogenic food isolated *Salmonella* spp. including *S. enteritidis* with a diameter of inhibition zones growth ranging from 21 to 51 mm and MIC and MBC values ranging from 0.39–1.56 mg/mL to 0.39–3.12 mg/mL, respectively. Furthermore, the *S. montana* L. EO was investigated for its cytotoxic and antioxidant activities. The results revealed a significant cytotoxic effect of *S. montana* L. EO against A549 cell line and an important antioxidant activity. These findings suggest that *S. montana* L. EO may be considered as an interesting source of components used as potent agents in food preservation and for therapeutic or nutraceutical industries.

1. Introduction

Salmonella species are responsible for causing foodborne bacterial illnesses. Human salmonellosis is generally increasing worldwide because of contaminated foods consumption, especially those of animal origin. Foodborne salmonellosis is characterized by gastrointestinal disorders manifested predominantly by diarrhea, fever, and abdominal cramps. Since the disease has not only an economic impact on individuals and countries but also affects people's health and well-being, many efforts have been spent to find out approaches reducing or eliminating *Salmonella* that contaminates foods [1]. In this context, food safety has become a complex problem related to food products frequently introduced into the market. Indeed, these require generally a longer shelf life and a greater safety assurance, that is, lack of foodborne pathogenic microorganisms [2, 3]. Alternative preservation techniques based on the use of naturally derived ingredients are under investigation for their application in food products. Because of negative consumer perceptions of chemical preservatives, attention is shifting toward natural products used as alternatives, especially plant extracts, including the essential oils (EOs) and essences of plant extracts [4]. However, plants EOs are a source of bioactive molecules and have been widely traditionally used and commercialized to increase the shelf life and food safety [5, 6]; these are gaining significance for their potential as preservative [4, 7]. EOs are volatile, natural and complex compounds characterized by a strong odor

which is the result of plants aromatic secondary metabolites [8]. In addition, demand is growing for natural and high-quality products as well, and are used in food preservation and in aromatherapy, the EOs have multiple antimicrobial, that is, antifungal and antiviral [8–11], as well as anticancer effects [8].

Satureja montana L., commonly known as winter savory or mountain savory, belongs to the Lamiaceae family, Nepetoideae subfamily, and Mentheae tribe and is a perennial semishrub (20–30 cm) that inhabits arid, sunny, and rocky regions. *S. montana* L. is native to the Mediterranean and is found throughout Europe, Russia, and Turkey [4]. *S. montana* L. is a well-known aromatic plant, frequently used as traditional medicinal herb [12] and spice for food and teas. It is used in Mediterranean cooking, mainly as a seasoning for meats and fish and a flavoring agent for soups, sausages, canned meats, and spicy sauces [4, 13]. The EOs derived from plants of *Satureja* species possess strong antibacterial activities of different extents against organisms of importance to food spoilage and/or poisoning, as well as to those of interest to the medical field such as *Salmonella*, *Listeria*, and *Staphylococcus*. In view of their broad activity, these EOs may find industrial applications as natural preservatives and conservation agents in the cosmetic and/or food industries and as active ingredients in medical preparations [3]. In this context, *S. montana* L. has biological properties that are related to the presence of its major EO chemical compounds which are carvacrol and *p*-cymene [12].

The aims of the present study were to assess the chemical composition and antioxidant and cytotoxicity activities of *Satureja montana* L. EO and to evaluate the antimicrobial effect of this winter savory against several foodborne pathogens especially the most common causative agent of foodborne salmonellosis.

2. Materials and Methods

2.1. Plant Material and Essential Oil Extraction. *S. montana* L. plants were freshly collected in 2011 during the period of full flowering on the mountain in the south of France (Mediterranean climate country and mountainous region). The species was identified according to the forester flora of France [14]. Aerial parts of the winter savory were dried at room temperature. Then EO was extracted by hydrodistillation (HD) for 3 h with 500 mL distilled water using a Clevenger-type apparatus according to the European Pharmacopoeia [15]. The EO was collected and dried over anhydrous sodium sulfate and then stored in sealed glass vials in a refrigerator at 4°C prior to analysis. EOs yield was calculated based on dry weight.

2.2. Essential Oil Analyses

2.2.1. Gas Chromatography (GC). An Agilent Technologies 6890N GC equipped with HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Hewlett-Packard) and connected to a FID was used. The column temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and finally left at 250°C for 5 min. The injection port temperature was 240°C, while that of the detector was 250°C (split ratio: 1/60).

The carrier gas was helium (99.995% purity) with a flow rate of 1.2 mL/min. The analysed EO volume was 2 μL. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Mean percentage of *S. montana* L. volatiles compounds represented the average calculated on three individuals. Retention indices (RIs) were calculated for separate compounds relative to C_8–C_{26} n-alkanes mixture (Aldrich Library of Chemicals Standards) [16].

2.2.2. Gas Chromatography/Mass Spectrometry (GC/MS). The volatile compounds isolated by HD were analysed by GC/MS, using an Agilent Technologies 6890N GC. The fused HP-5MS capillary column (the same as that used in the GC/FID analysis) was coupled to an Agilent Technologies 5973B MS (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was programmed as before (50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min). The injection port temperature was 250°C and that of the detector was 280°C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 mL/min. The MS conditions were as follows: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50 to 550 m/z.

2.2.3. Volatile Compounds Identification. The volatile compounds of *S. montana* L. aerial parts were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00). Further identification confirmations were made referring to RI data generated from a series of known standards of n-alkanes mixture (C_8 to C_{26}) [16] and to those previously reported in the literature [17–19].

2.3. Antioxidant Activity

DPPH Radical Method. The free-radical scavenging activity of *S. montana* L. EOs was measured by 2,2-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, France) using the method described by Hatano et al. [20]. One milliliter of the EO at known concentrations was added to 0.25 mL of a DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm and corresponded to the ability of the EO to reduce the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The antiradical activity was expressed as IC_{50} (μg/mL), the extract dose required to cause a 50% inhibition. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as negative control. All determinations were performed in triplicate. The ability to

scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect } (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100, \quad (1)$$

where A_0 was the absorbance of the control at 30 min and A_1 was the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

2.4. Cytotoxic Activity. The *S. montana* L. EOs was screened for cytotoxic activity using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphynyltetrazolium bromide] colorimetric assay against the human lung adenocarcinoma epithelial cell line (A549) as described previously [21]. Briefly, cells were treated with concentrations of EOs ranging from 12.5 to 800 μg/mL and seeded in 96-well microplates. The EO was first dissolved in DMSO and then in RPMI 1640 supplemented with 2% foetal calf serum (FBS). The final DMSO concentrations in the test medium and controls were 1% (v/v). Each concentration was tested in quadruplicate together with that in the control and repeated two times in separate experiments.

After incubation for 24, 48 and 72 hours, the medium in each well was collected and the cytotoxic effect was measured with the MTT colorimetric assay. To determine the cell viability, 20 μL of MTT (5 mg/mL) was added to each well and cells were cultured in additional incubation for 4 h. After washing the supernatant out, the insoluble formazan product was dissolved in acidified isopropanol. Then, optical density (OD) of 96-well culture plates was measured using an enzyme-linked immunosorbent assay (ELISA) reader at 540 nm. The OD of formazan formed in untreated control cells was taken as 100% of viability.

2.5. Antimicrobial Activity

2.5.1. Microorganisms. The tested microorganisms included the following Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CIP 106510, *Micrococcus luteus* NCIMB 8166, *Bacillus cereus* ATCC 11778, *Bacillus cereus* ATCC 14579, and *Listeria monocytogenes* ATCC 19115 and Gram-negative bacteria: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC27853, *Enterococcus faecalis* ATCC 29212, *Vibrio alginolyticus* ATCC 17749, *Vibrio alginolyticus* ATCC 33787, *Salmonella typhimurium* ATCC 1408, and *Salmonella typhimurium* LT2 DT104. The antibacterial effect was also tested against 31 strains belonging to *Salmonella* genus, including 12 species of *enteritidis* responsible for collective food intoxication isolated in hospital Fattouma Bougruiba Monastir (Tunisia) in June 2000. These microorganisms were kindly provided by Prof. Rhim Amel from the Regional Laboratory of Public Health of Monastir (Tunisia), and the serotyping of the strains was performed at the Pasteur Institute, Tunisia.

2.5.2. Disc-Diffusion Assay. Antimicrobial activity testing was done according to the Clinical and Laboratory Standards Institute [22] guidelines (CLSI, 2006). For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 9 mL of Mueller-Hinton (MH) broth and then incubated at 37°C for 18–24 h. The overnight cultures were used for the *S. montana* L. EO antimicrobial activity test, and the optical density was adjusted to 0.5 McFarland turbidity standards with a DENSIMAT (Biomérieux). The inoculums were streaked onto MH agar plates at 37°C.

Sterile filter discs (diameter 6 mm, Whatman Paper no. 3) were impregnated with 10 μL of EO placed on the MH agar mediums. The treated Petri dishes were placed at 4°C for 1-2 h and then incubated at 37°C for 18–24 h under anaerobic condition. The antibacterial activity was evaluated by measuring the growth inhibition diameter zone around the disk. Standard disks of the antibiotic ciprofloxacin (5 μg) served as the positive antibacterial controls according to the committee of the French Society of Antimicrobial for all strains except *L. monocytogenes* which standard disks of the antibiotic gentamycin (10 μg/disc), served as the positive antibacterial controls [23]. Each experiment was carried out in triplicate and the mean diameter of the inhibition zone was recorded.

2.5.3. Microwell Determination of MIC and MBC. The minimal inhibition concentration (MIC) and the minimal bactericidal concentration (MBC) values were determined for all bacterial strains used in this study as described by Güllüce et al. [24]. The inoculums of the bacterial strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. The *S. montana* L. EOs dissolved in 10% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (50 mg/mL) to be tested, and then serial twofold dilutions were made in a concentration range from 0.0488 to 50 mg/mL in 5 mL sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 μL of nutrient broth and 5 μL of the inoculum. A 100 μL aliquot from the stock solutions of each EO was added to the first wells. Then, 100 μL from the serial dilutions was transferred into 100 μL consecutive wells. The last well containing 195 μL of nutrient broth without EO and 5 μL of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μL. The plates were incubated at 37°C for 18–24 h.

After incubation, bacterial growth was evaluated by the presence of turbidity and a pellet on the well bottom. The MIC was defined as the lowest concentration of the compounds to inhibit the microorganism growth. The BMC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no turbidity development and without visible growth. All tests were performed in triplicate.

2.6. Statistical Analysis. Values were expressed as means ± standard deviation. Analysis of variance was conducted, and differences between variables were tested for significance by one-way ANOVA with an SPSS 11 (Statistical Package for the Social Sciences) programme. Differences at $P < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1. Essential Oil Composition. The *S. montana* L. EO chemical composition was investigated using both GC and GC/MS techniques. The volatile compounds composition was based on the chemical functions (acids, alcohols, aliphatics, aromatics, sulfurs, and terpenes). The oil yield of the French variety of the winter savory *Satureja montana* was 1.56% while that of the Croatian variety was 1.7% [12]. The identified components, their percentages, their calculated RI, and their comparison according to the RI values previously published in the literature are listed in Table 1, considering the compounds elution order on the HP-5MS column. A total of 32 compounds were identified, accounting for 99.85% of the total oil content (Table 1). These compounds were divided into five classes that are monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others. This oil was characterized by very high percentage of monoterpenes (92.31%) and especially the oxygenated ones (59.11%) which constituted the predominant class as was found for the majority of *S. montana* L. [12].

Furthermore, *S. montana* L. EO is characterized by a high content of the phenolic carvacrol (53.35%). Other important compounds were γ-terpinene (13.54%) and the monoterpenic hydrocarbons *p*-cymene (13.03%). Moreover, low percentages of β-caryophyllene (2.23%), linalool (1.84%), α-terpinene (1.7%), myrcene (1.3%), β-bisabolene (1.3%), and the oxygenated compounds borneol (1.14%) were evidenced. The EOs of the aerial parts of *S. montana* L. collected in Croatia contained an important percentage of carvacrol (45.7%) [12]. Twenty-six chemical compounds were identified in the EO of winter savory spice (*S. montana* L.) originating from Albania [4], and the major constituents were thymol (28.99%), *p*-cymene (12.00%), linalool (11.00%), and carvacrol (10.71%).

In fact, the composition of the EO of *S. montana* L. depends on many factors, such as environmental conditions, harvest time, geographic origin, and storage conditions which seem to have a significant influence on the EOs relative compounds concentration in *S. montana* [25, 26]. Various isolates of winter savory from Croatia, Bosnia, and Herzegovina have carvacrol (up to 84.19%) as the main constituent [27], and a review of the published literature [25] reveals that the composition of *S. montana* oil shows large variations in the relative concentration of major components: carvacrol (5%–69%) was found to be the major component among the oxygenated monoterpenes, linalool (1%–62%), γ-terpinene (1%–31%), and *p*-cymene (3%–27%) arising from the existence of different chemotypes, and environmental conditions seem to have a significant influence on the relative amounts of EO components of *S. montana* [25, 26].

The monoterpene hydrocarbons fraction accounting for 33.2% of the total oil was represented by 11 compounds: the most important were γ-terpinene (13.54%) and *p*-cymene (13.03%). In contrast, the sesquiterpene hydrocarbons fraction (5.78%) was less important. Among sesquiterpenes hydrocarbons, β-caryophyllene (2.23%) and β-bisabolene (1.3%) were the major compounds found in *S. montana* L. originating from Albania [4].

3.2. Antioxidant Activity. The antioxidant properties of *S. montana* L. bioactive extracts were evaluated by DPPH radical scavenging, one of the most used methods to evaluate evaluate EOs, and phenolic extracts. The antioxidant activity in this study showed that the IC_{50} value was 410.5 ± 4.27 μg/mL.

In this study, *S. montana* L. EO IC_{50} was higher than that found by Güllüce et al. [24] for *Satureja hortensis* EO (350 ± 5 μg/mL). In contrast, Cavar et al. [28] reported a higher IC_{50} for *S. montana* L. EO with 13-folds that of the present study (5490 μg/mL). More recently, Serrano et al. [29] found an IC_{50} value of 508.45 ± 5.80 μg/mL for the same species.

Table 1 shows that EO of *S. montana* L. was markedly rich in oxygenated terpenes which may act as radical scavenging agents. However, Tepe et al. [30] indicate that EOs containing oxygenated monoterpenes and/or sesquiterpenes have greater antioxidative properties. This EO antioxidant property (DPPH radical-scavenging activity) is well needed for food industry as possible alternative to synthetic preservatives. In this context, *S. montana* L. EO gave interesting results, indicating the presence of potent antioxidant compound(s) that can be exploited as alternatives for use in the food and cosmetic industries and/or as nutraceuticals.

3.3. Cytotoxic Activity. Cell viability, determined by the ability of the cells to metabolically reduce MTT to a formazan dye, was performed after 24, 48, and 72 h exposure to EO at different concentrations ranging from 12.5 to 800 μg/mL. A concentration- and time-dependent inhibitory effect on human respiratory epithelial cell line (A549) was observed. After 24 h of incubation, cytotoxicity was considered whenever cell survival percent was less than 50. The extract was not cytotoxic towards A549 cell line in all tested concentrations. But after 48 and 72 h EO exposure, the *S. montana* L. IC_{50} is 400.00 ± 0.02 μg/mL and 11.00 ± 0.01 μg/mL, respectively (Figure 1).

Koparal and Zeytinoglu [31] demonstrate that carvacrol, the predominant monoterpene in *S. montana* L., was very potent cell growth inhibitor of A549 cell line.

The cytotoxicity is likely due to the relatively high phenolic compounds concentrations, particularly carvacrol as described previously [31]. The cytotoxic effects of carvacrol have been previously described in different cellular models, especiallyin tumor cell lines. Comparative evaluation of its components' cytotoxicity (generally recognized as safe) showed that this type of oil and its major component carvacrol (which constitutes 53.35% of the oil) were highly cytotoxic against human metastatic breast cancer cells, MDA-MB 231, and that the compound could have a potential therapeutic significance in treating cancer [32].

3.4. Antimicrobial Activity. In the present study, *S. montana* L. EO antimicrobial activities against microorganisms examined and its potency were qualified by inhibition zone diameter and quantified by MIC and MBC values. The results given in Table 2 showed that the EO of *S. montana* L. had substantial antimicrobial activity against 13 reference strains and 31 strains belonging to *Salmonella* genus; with 12

Table 1: Mean percentage of the *Satureja montana* L. (France) essential oil components and RI comparison according to the literature.

Peak number	Compound[a]	Percentage	Retention index (RI)		
			Calculated[b]	Literature (reference)	
1	α-Thujene	1.14	927	927 (Mimica-Dukic et al., 2003)	[33]
2	α-Pinene	0.73	934	934 (Basta et al., 2007)	[34]
3	Camphene	0.31	949	949 (Baranauskiene et al., 2003)	[35]
4	β-Pinene	0.15	978	979 (Hajlaoui et al., 2010)	[36]
5	1-Octen-3-ol	0.86	981	980 (Forero et al., 2009)	[37]
6	β-Myrcene	1.3	992	992 (Hazzit et al., 2006)	[38]
7	α-Phellandrene	0.22	1006	1006 (Wannes et al., 2009)	[39]
8	α-Terpinene	1.76	1018	1020 (Yu et al., 2007)	[40]
9	p-Cymene	13.03	1027	1027 (Roussis et al., 2000)	[41]
10	Limonene	0.73	1030	1030 (Harzallah-Skhiri et al., 2006)	[42]
11	Eucalyptol	0.42	1033	1033 (Juliani et al., 2004)	[43]
12	Trans-β-ocimene	0.29	1039	1039 (Jalali-Heravi et al., 2006)	[44]
13	γ-Terpinene	13.54	1060	1061 (Hajlaoui et al., 2010)	[36]
14	Trans-sabinene hydrate	0.87	1070	1070 (Ogunwande et al., 2010)	[45]
15	Linalool	1.81	1101	1101 (Zhao et al., 2005)	[46]
16	Borneol	1.14	1170	1167 (Singh et al., 2006)	[47]
17	Terpinen-4-ol	0.5	1181	1181 (Mehrabani et al., 2004)	[48]
18	α-Terpineol	0.13	1194	1194 (Sajjadi and Ghannadi, 2005)	[49]
19	Linalyl acetate	0.19	1255	1255 (Afsharypuor and Jahromy, 2003)	[50]
20	Thymol	0.89	1298	1298 (Ghannadi et al., 2004)	[51]
21	Carvacrol	53.35	1309	1311 (Saroglou et al., 2006)	[52]
22	Carvacrol acetate	0.19	1374	1377 (Zouari et al., 2007)	[53]
23	β-Bourbonene	0.15	1390	1391 (Baranauskiene et al., 2003)	[35]
24	β-Caryophyllene	2.23	1426	1426 (Zhao et al., 2005)	[46]
25	α-Amorphene	0.3	1482	1494 (Jalali-Heravi et al., 2006)	[44]
26	Germacrene-D	0.52	1488	1488 (Novak et al., 2005)	[54]
27	β-Patchoulene	0.49	1502	1503 (Pripdeevech et al., 2010)	[55]
28	β-Bisabolene	1.3	1511	1511 (Asuming et al., 2005)	[56]
29	γ-Cadinene	0.25	1521	1521 (Zouari et al., 2011)	[57]
30	δ-Cadinene	0.48	1529	1528 (Zouari et al., 2011)	[57]
31	Spathulenol	0.15	1588	1590 (Mehrabani et al., 2004)	[48]
32	Caryophyllene oxide	0.43	1594	1594 (Zouari et al., 2011)	[57]
Total identified		99.85			
Yield (g/100 g dry weight)		1.56			
Grouped components (%)					
Monoterpene hydrocarbons		33.2			
Oxygenated monoterpenes		59.11			
Sesquiterpene hydrocarbons		5.72			
Oxygenated sesquiterpenes		0.58			
Others		1.24			

[a]Compounds are listed in order of their elution from the HP-5MS column.
[b]Retention index calculated against C_8–C_{26} n-alkanes mixture on HP-5MS column.

belonging to the species *enteritidis* and being responsible for collective food intoxication in June 2000 in hospital Fattouma Bourguiba Monastir (Tunisia). In fact, the data obtained of zones of growth inhibition (mm) scored in Mueller-Hinton agar demonstrated that Gram-positive bacteria exhibited the highest diameters of growth inhibition (between 20 and 51 mm). Winter savory EO was particularly effective against *M. luteus* NCIMB 8166 and *L. monocytogenes* ATCC 19115 with inhibition diameter exceeding that of the ciprofloxacin. On the other hand, Gram-negative bacteria were less sensitive to *S. montana* L. EO with a diameter of growth inhibition ranging from 17 (*E. feacalis* ATCC 29212) to 30 mm (*S. typhimurium* ATCC 1408 and *S. typhimurium* LT2 DT104).

The maximum activity of this oil was observed against Gram-positive *L. monocytogenes,* but this oil had poor activity on the growth of *Enterococcus faecium* resistant to

Table 2: Antibacterial activity of *S. montana* L. essential oil against human pathogenic bacteria using agar disc diffusion method and determination of MIC (mg/mL) and MBC (mg/mL) values.

Microorganisms	Origin	IZ (mm ± SD)	Antibiotics (Cip)	MIC	MBC
Bacterial strains					
Gram-positive bacteria					
Staphylococcus aureus	ATCC 25923	23.33 ± 0.58	32.33 ± 0.58	0.78	0.78
Staphylococcus epidermidis	CIP 106510	24.00 ± 0.50	33.67 ± 0.58	0.39	0.78
Micrococcusluteus	NCIMB 8166	36.00 ± 0.00	29.00 ± 0.57	0.78	0.78
Bacillus cereus	ATCC 11778	20.67 ± 0.58	40.00 ± 0.00	0.39	0.78
Bacilluscereus	ATCC 14579	20.33 ± 0.58	40.00 ± 0.00	1.56	3.12
Listeria monocytogenes	ATCC 19115	51.00 ± 1.00	37.67 ± 0.58 (Gen)	0.39	0.78
Gram-negative bacteria					
Escherichia coli	ATCC 35218	24.00 ± 0.00	40.00 ± 0.00	0.78	0.78
Pseudomonas aeruginosa	ATCC 27853	22.33 ± 0.58	24.32 ± 0.58	1.56	3.12
Enterococcusfaecalis	ATCC 29212	17.00 ± 0.00	18.00 ± 0.58	0.78	0.78
Vibrioalginolyticus	ATCC 17749	30.00 ± 0.00	35.00 ± 0.00	0.78	0.78
Vibrioalginolyticus	ATCC 33787	26.33 ± 0.58	40.00 ± 0.00	1.56	3.12
Salmonella typhimurium	ATCC 1408	30.00 ± 0.00	30.00 ± 0.00	0.78	0.78
Salmonella typhimurium	LT2 DT104	30.00 ± 0.00	33.38 ± 0.58	0.78	0.78
Salmonella spp. strains	Samples (isolation date)				
S. enteritidis (1127)	Manipulator (06-09-2000)	29.00 ± 0.00	34.00 ± 1.00	0.39	0.39
S. enteritidis (1128)	Manipulator (06-09-2000)	50.00 ± 1.00	28.33 ± 0.57	0.39	0.39
S. enteritidis (1129)	Manipulator (05-09-2000)	21.33 ± 0.58	22.33 ± 0.58	0.39	0.39
S. enteritidis (1130)	Patient (06-09-2000)	20.00 ± 0.50	28.00 ± 0.00	0.39	0.39
S. enteritidis (1131)	Manipulator (05-09-2000)	23.00 ± 0.00	32.33 ± 0.58	0.39	0.39
S. enteritidis (1133)	Patient (06-09-2000)	35.00 ± 1.00	25.00 ± 0.00	0.39	0.39
S. enteritidis (1136)	Patient (05-09-2000)	26.33 ± 0.58	25.00 ± 0.00	0.39	0.39
S. enteritidis (1137)	Patient (06-09-2000)	38.00 ± 0.00	39.00 ± 1.00	0.39	0.78
S. enteritidis (1138)	Meatball (05-09-2000)	35.00 ± 0.50	24.00 ± 0.00	0.39	0.39
S. enteritidis (1141)	Patient (06-09-2000)	35.33 ± 0.58	40.00 ± 0.00	0.39	0.39
S. enteritidis (1142)	Sandwich (06-09-2000)	22.00 ± 0.00	25.33 ± 0.57	0.39	0.39
S. enteritidis (1143)	Salad (06-09-2000)	30.00 ± 0.00	34.33 ± 0.58	0.39	0.39
S. spp. (3654)	Fermented milk: Lben (14-07-2010)	18.33 ± 0.58	29.66 ± 0.58	0.78	0.78
S. spp. (3777)	Turkey (21-07-2010)	17.00 ± 1.00	30.00 ± 0.00	0.39	0.78
S. spp. (3907)	Sandwich (26-07-2010)	35.00 ± 0.00	22.33 ± 0.57	0.78	0.78
S. spp. (3915)	Cooked meat (26-07-2010)	19.00 ± 0.00	26.00 ± 1.00	0.78	6.25
S. spp. (4091)	Turkey soup (02-08-2010)	15.00 ± 1.00	0.00 ± 0.00	0.78	0.78
S. spp. (5213)	Salad (15-10-2010)	16.33 ± 0.58	30.33 ± 0.58	0.78	0.78
S. spp. (5545)	Salad (01-11-2010)	25.00 ± 0.00	26.00 ± 1.00	0.39	0.78
S. spp. (5687)	Lettuce Salad (08-11-2010)	30.00 ± 0.00	26.33 ± 0.57	0.78	0.78
S. spp. (5240)	Sausage (28-01-2011)	24.33 ± 0.58	31.00 ± 1.00	0.39	0.78
S. spp. (5481)	Salad (22-02-2011)	21.00 ± 1.00	26.00 ± 0.00	0.78	0.78
S. spp. (3912)	Tajine (22-02-2011)	17.00 ± 0.00	24.33 ± 0.58	0.78	0.78
S. spp. (1063)	Cheese (29-03-2011)	15.00 ± 0.00	30.00 ± 0.00	0.78	0.78
S. spp. (5235)	Cheese (21-04-2011)	29.00 ± 0.00	30.00 ± 0.00	0.78	0.78
S. spp. (2591)	Sausage (19-06-2011)	23.33 ± 0.58	34.33 ± 0.58	0.39	0.39
S. spp. (2543)	Sausage (28-06-2011)	26.67 ± 0.58	30.00 ± 0.00	0.39	0.78
S. spp. (2586)	Sausage (29-06-2011)	22.00 ± 1.00	24.33 ± 0.58	0.39	0.78
S. spp. (2786)	Salad (08-07-2011)	16.00 ± 0.00	30.00 ± 0.00	1.56	3.12
S. spp. (2925)	Tomato (19-07-2011)	18.00 ± 0.00	32.33 ± 0.57	0.78	1.56
S. spp. (3907)	Sausage (05-09-2011)	30.00 ± 0.00	24.00 ± 1.00	0.78	0.78

IZ: inhibition zone in diameter (mm ± SD) around the discs impregnated with 10 μL of essential oil. SD: standard deviation; Cip: ciprofloxacin (5 μg/disc); Gen: gentamycin (10 μg/disc).

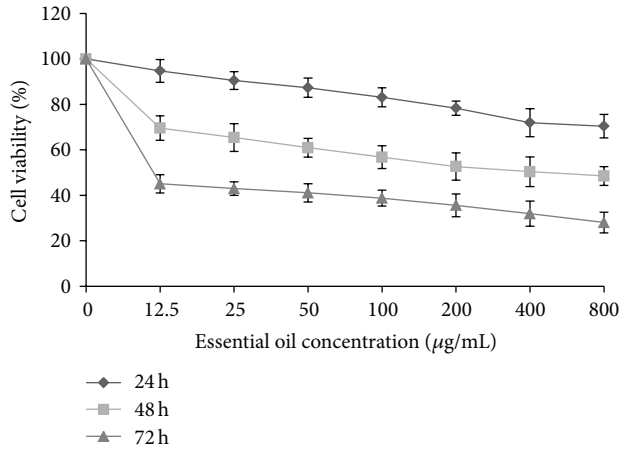

Figure 1: *Satureja montana* L. essential oil cytotoxicity on A549 cells.

ciprofloxacin. The oil of *S. montana* L. was generally active against the majority of food intoxitcation isolated *S. enteritidis*. The diameters of growth inhibition were ranging from 20 mm to 50 mm. Concerning 19 food isolated strains of *Salmonella* spp., the EO (7.9 mg/disc) was very active showing a clear zone of inhibition ranging from 15 to 35 mm.

The MIC and MBC values of *S. montana* L. EO against all the studied strains were summarised in Table 2. The EO was efficient against all tested bacteria with MIC about 0.39 mg/mL to 1.56 mg/mL for Gram-positive bacteria and from 0.78 to 1.56 mg/mL for Gram-negative bacteria. For *Salmonella* spp. strains, MIC values were ranging from 0.39 to 0.78 mg/mL. The MBC values were also important, and low concentration of *S. montana* L. EO was sufficient to eliminate the growth of Gram-positive bacteria (MBC: 0.78 mg/mL) and *E. coli*, *E. feacalis*, *V. alginolyticus,* and *S. typhimurium* (MBC: 0.78 mg/mL). It has shown also that 0.39 mg/mL of EO was sufficient to stop the growth of several pathogenic *Salmonella* species including *S. enteritidis* responsible for collective food intoxication.

A number of reports indicate that EOs containing carvacrol, eugenol, or thymol have the highest antimicrobial properties [58, 59]. The chemical composition of this sample of *S. montana* L. oil demonstrates this relationship between high antimicrobial activity and the presence of phenolic components since savory oil was active against methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus faecium,* and multidrug-resistant *Serratia marcescens* [12]. However, the antimicrobial activities of *Satureja* species do not arise only from the carvacrol and thymol content since the oil of *S. cuneifolia*, which is relatively rich in β-cubebene, limonene, α-pinene, spathulenol, and β-caryophyllene, also displayed relatively good activity. It has also to be considered that minor components, as well as a possible interaction between the substances, could also affect the antimicrobial properties. It has also been reported that Gram-positive bacteria are more susceptible to EOs than Gram-negative bacteria [60]. The relative tolerance of Gram-negative bacteria to EOs has been ascribed to the presence of a hydrophilic outer membrane which can block the penetration of hydrophobic components through the target cell membrane [61]. We conclude that the oils investigated can be used as a complementary therapy where a single antimicrobial agent is often ineffective; however, it is most important that the composition of the oil is known for maximum efficacy.

4. Conclusion

In conclusion, *S. montana* L. EOs represent a source of natural antibacterial and antioxidant substances with potential applicability in food systems to prevent the growth of foodborne pathogenic and spoilage bacteria, as well as oxidation, and to extend food shelf life. However, further research is still required to confirm such applicability and to evaluate the safety of *S. montana* L. EO.

Conflict of Interests

The authors declare that they do not have any financial relations with any of the commercial entities mentioned in the paper that could lead to a conflict of interests.

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