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THE CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OIL OF *SALVIA FRUTICOSA* GROWING WILD IN LIBYA

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Abstract - The composition of essential oil isolated from *Salvia fruticosa,* wild growing in Libya, was analyzed by GC and GC-MS. Forty-five compounds could be identified. The essential oil contained 1,8-cineole (49.34%), camphor (7.53%), β-pinene (7.38%), myrcene (7.38%), α-pinene (5.15%), β-caryophyllene (4.13%) and α-terpineol (3.25). Antioxidant activity was analyzed using the DPPH free radical scavenging method and low antioxidant activity was found (IC_{50} = 15.53 mg/ml). The oil was also screened for its antimicrobial activity against eight bacteria (four Gram-negative and four Gram-positive) and eight fungi. The essential oil of *S. fruticosa* showed minimal inhibitory activity (MIC) at 0.125-1.5 mg/ml and bactericidal (MBC) at 0.5-2.0 mg/ml. In addition, it exhibited fungistatic (MIC) at 0.125-1.0 mg/ml and fungicidal effect (MFC) at 0.125-1.5 mg/ml.

Key words: Salvia fruticosa, essential oil, antimicrobial, antioxidant activity, 1,8-cineole.

INTRODUCTION

The genus *Salvia*, belonging to Lamiaceae, is considered one of the largest genera in this family. It comprises about 900 species that grow in the Oriental Mediterranean, South-West Asia, South Africa and America (Standley and Williams, 1973; Kelen et al., 2008; Maksimovic et al., 2007). In Libya, the genus *Salvia* is represented by ten species, out of which three are cultivated (Jafri and El-Gadi, 1985).

High attention has been paid to *Salvia* species due to the wide range of its biological activities (Askun et al., 2010). Many authors have focused on the biological properties of the essential oils obtained from *Salvia* species and their major compounds such as antibacterial, cytostatic (Janssen et al., 1987; Gonzalez et al., 1989; Darias et al., 1990), antiviral (Tada et al., 1994) and antioxidant activities (Weng and Wang, 2000). Moreover, they are frequently used in traditional medicine to treat diarrhea, eye diseases, gonorrhea; they possess antiseptic and antispasmodic activities. Also, the essential oils of *Salvia* species are used as cosmetics and as flavoring agents in perfumery (Basaif et al., 2004; Longaray Delamaer et al., 2007; Kelen et al., 2008).

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(Tada et al., 1994). Plant products that have antimicrobial activity have gained special interest due to the resistance of some microorganisms to antibiotics (Essawi and Srour, 2000).

Salvia fruticosa Mill. (Syn. *S. triloba* L.) is a native species of the eastern Mediterranean basin (Carmona et al., 2005; Ali-Shtayeh et al., 2000). It is an aromatic, perennial herb and, commercially, one of the most important *Salvia* species for culinary and medicinal purposes (Kosar et al., 2005). This herb (especially the leaves) has a folk standing in the eastern Mediterranean region for the treatment of various skin, blood, and infectious ailments as well as ailments of the digestive, circulatory, respiratory, and osteomuscular systems (Carmona et al., 2005; Ali-Shtayeh et al., 2000). It is also used as a hypoglycemic herb and against inflammations, hepatitis, and tuberculosis (Pitarokili et al., 2003).

Evidence suggests that the essential oil from *S. fruticosa* has antimicrobial properties. Longaray Delamare et al. (2007) reported that the essential oil obtained from *S. fruticosa* possessed good antimicrobial activity against foodborne bacteria. Also, Pitarokili et al. (2003) found that *S. fruticosa* has antifungal activity. There are many studies carried out on the chemical composition, antimicrobial and antioxidant activities of the essential oil of *S. fruticosa*.

However, to the best of our knowledge there is no report on the chemical composition of *S. fruticosa* from Libya. The aims of the present work were to evaluate the essential oil composition, antifungal, antibacterial and antioxidant activities of this wild growing species in Libya.

MATERIALS AND METHODS

Plant collection

The samples from wild growing *Salvia fruticosa* were collected in March 2010 during the flowering stage from Biadda city, which is located on the Green Mountain (eastern Libya). The plant was identified by Dr A. Felaly (Faculty of Science, Al-Gabel Al-Garbi University Zentan-Libya), and later confirmed by one of the authors (P. D. M.). The samples were dried in the shade at room temperature for 10 days. Voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac" (BEOU).

Isolation of the essential oil

The essential oil was obtained by hydrodistillation of 100 g of air-dried aerial parts of *S. fruticosa* using a Clevenger-type apparatus for 3 h according to the British Pharmacopoeia specifications (British Pharmacopoeia, 1980). The oil was dried over anhydrous sodium sulphate and stored in a vial at low temperature until analysis. The oils yield (v/w) on a dry weight basis is given in Table 1.

Gas chromatography and gas chromatography-mass spectrometry analysis

Qualitative and quantitative analyses of the oils were performed using GC and GC-MS. The GC analysis of the oil was carried out on a GC HP-5890 II apparatus, equipped with a split-splitless injector, attached to HP-5 column (25 m x 0.32 mm, 0.52 μm film thickness) and fitted to FID. Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250°C, detector temperature 300°C, while column temperature was linearly programmed from 40-240°C (at rate of 4°/min). The same analytical conditions were employed for GC-MS analysis, where a HP G 1800C Series II GCD system equipped with HP-5MS column (30 m x 0.25 mm, 0.25 μm film thickness) was used. The transfer line was heated at 260°C. Mass spectra were acquired in EI mode (70 eV) in an m/z range of 40-400. Identification of the individual oil components was accomplished by comparison of the retention times with standard substances and by matching mass spectral data with those held in the Wiley 275 library of mass spectra. Confirmation was performed using AMDIS software and literature (Adams, 2007). For the purpose of quantitative analysis, area percents obtained by FID were used as a base.

Table 1. Chemical composition of essential oil of *Salvia fruticosa.*

Constituents	[a] **KIE**	[b] **KIL**	**%**		
tricyclene	911.7	921	0.08	±	0.0181
α-thujene	917.6	924	0.25	±	0.0005
α-pinene	922.9	932	5.15	±	0.0376
camphene	936.9	946	2.72	±	0.0200
sabinene	965.2	969	0.35	±	0.0014
β-pinene	970.8	974	7.38	±	0.0529
1-octen-3-ol	979.9	974	0.11	±	0.0014
myrcene	982.9	988	7.38	±	0.0530
α-phellandrene	995.1	1002	0.12	±	0.0036
α-terpinene	1007.6	1014	0.41	±	0.0041
p-cymene	1017.6	1020	0.40	±	0.0033
limonene	1019.1	1024	0.85	±	0.0031
1,8-cineole	1023.4	1026	49.34	±	0.6247
cis-β-ocimene	1031.1	1032	0.04	±	0.0022
γ-terpinene	1049.5	1054	0.77	±	0.0067
cis-sabinene hydrate	1059.8	1058	0.16	±	0.0021
α-terpinolene	1079.0	1086	0.28	±	0.0024
trans-sabinene hydrate	1090.5	1098	0.09	±	0.0059
linalool	1093.6	1095	0.58	±	0.0562
β-thujone (cis-thujone)	1096.1	1101	0.66	±	0.0058
α-thujone (trans-thujone)	1107.2	1112	0.48	±	0.0176
cis-p-menth-2-en-1-ol	1114.0	1118	0.05	±	0.0013
camphor	1133.9	1141	7.53	±	0.0543
borneol	1156.3	1165	1.71	±	0.0221
terpinen-4-ol	1168.0	1174	0.63	±	0.0223
α-terpineol	1182.1	1186	3.25	±	0.0239
bornyl acetate	1276.0	1287	0.19	±	0.0009
α-copaene	1364.1	1374	0.03	±	0.0015
α-burbonene	1373.1	1387	0.04	±	0.0022
α-gurjunene	1397.0	1409	0.03	±	0.1521
β-caryophyllene	1406.8	1417	4.13	±	0.0192
β-gurjunene	1415.7	1431	0.07	±	0.0109
selina-4(15),5-diene	1421.7	1433	0.05	±	0.0056
aromadendrene	1426.2	1439	0.43	±	0.0098
selina-5,11-diene	1430.7	1444	0.05	±	0.0071
α-humulene	1441.0	1452	0.97	±	0.0219
alloaromadendrene	1448.2	1458	0.10	±	0.0188
γ-muurolene	1465.1	1478	0.05	±	0.0084
viridiflorene	1483.1	1496	0.21	±	0.0211
α-selinene	1487.1	1498	0.05	±	0.0113
γ-cadinene	1502.2	1513	0.05	±	0.0174
trans-calamenene	1510.8	1521	0.15	±	0.0174
caryophyllene oxide	1569.1	1582	0.49	±	0.0120
viridiflorol	1578.2	1592	1.73	±	0.0346
humulene epoxide II	1594.7	1608	0.15	±	0.0171
Total			99.76%		
Yield (v/w) %			1.56 %		
Number of constituents			45		
Monoterpene hydrocarbons			25.89%		
Oxygenated monoterpene			64.89%		
Sesquiterpene hydrocarbons			6.90%		
Oxygenated hydrocarbons			1.88%		
other			0.19%		

[a] KIE=Kovats (retention) index experimentally determined (AMDIS);
[b] KIL=Kovats (retention) index - literature data;

Antioxidant activity (DPPH assay)

Radical scavenging using DPPH radical is considered to be one of the main mechanisms by which antioxidant act in a food system. The DPPH method used in this study was as described earlier (Bios, 1958). The stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the oil. A solution (0.04 mg/ml) was prepared, and then 1800 µl of this solution was added to 200 µl of essential oils in methanol at different concentrations. The absorbance of remaining DPPH radical was measured spectrophotometrically at 517 nm using a Jenway 6305 UV/Vis spectrophotometer (Sineks Laboratory, UK) after 30 min at room temperature for all samples. Methanol was used as a blank, while methanol with DPPH solution was used as a control. All determinations were taken in triplicate and special care was taken to minimize loss of the free radical activity of the DPPH. Butylated hydroxyanisole (*BHA)* was used as a positive control. The DPPH scavenging capacity expressed in percentage (%) was calculated using the following equation:

% inhibition = [$(A_0 - A_1 A_0 - A_1)/A_0 A_0$] × × 100

where A_0 A_0 is the absorbance of control sample (without essential oils), and $A_1 A_1$ is the absorbance of the samples with essential oils at different concentrations. Oil concentrations (mg/ml) providing 50% inhibition (IC_{50}) were calculated from a graph in which scavenging activity was plotted against oil concentration.

Antibacterial activity

The following Gram-negative bacteria were used: *Escherichia coli* (ATCC 35210), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 13311), *Listeria monocytogenes* (NCTC 7973), *Proteus mirabilis* (human isolate) and the following Gram-positive bacteria: *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), and *Staphylococcus aureus* (ATCC 6538). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia. The antibacterial assay was carried out by microdilution method (Daouk et al., 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001) in order to determine the antibacterial activity of compounds tested against the human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x 10^5 CFU/ml. The inocula were prepared daily and stored at +4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

Microdilution test

The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0 x 10^5 cfu/ml. Compounds that were investigated were dissolved in LB medium (100 µl) with a bacterial inoculum (1.0 x 10^4 cfu per well) to achieve the wanted concentrations (1 mg/ml). The microplates were incubated for 24 h at 48°C. The lowest concentrations without visible growth (observed through a binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial subcultivation of 2 µl into microtiter plates containing 100 µl of broth per well and further incubation for 72 h. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by Microplate Manager 4.0 (Bio-Rad Laboratories) and compared with the blank and positive controls. Streptomycin was used as a positive control (1 mg/ml DMSO). Two replicates were done for each compound.

Antifungal activity

For the antifungal bioassays, eight fungi were used:

Aspergillus flavus (ATCC 9643), *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112), *Trichoderma viride* (IAM 5061) and *C. albicans* (human isolate). The organisms were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia.

The micromycetes were maintained on malt agar, and the cultures stored at 4°C and subcultured once a month (Booth, 1971). In order to investigate the antifungal activity of the extracts, a modified microdilution technique was used (Daouk et al. 1995; Hanel and Raether, 1988; Espinel-Ingroff, 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10^5 in a final volume of 100 µl per well. The inocula were stored at 4°C for further use. Dilutions of the inocula were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum.

Determination of the minimal inhibitory concentration (MIC) was performed using a serial dilution technique in 96-well microtiter plates. The compounds investigated were dissolved in DMSO (1 mg/ml) and added to the broth malt medium with inoculum. The microplates were incubated for 72 h at 28°C, respectively. The lowest concentrations without visible growth (at the binocular microscope) were defined as the MICs.

The fungicidal concentrations (MFCs) were determined by the serial subcultivation of 2 µl of culture in microtiter plates containing 100 µl of broth per well and further incubation 72 h at 28 °C. The lowest concentration with no visible growth was defined as the MFC, indicating 99.5% killing of the original inoculum. DMSO was used as a negative control, the commercial fungicide Bifonazole, was used as positive control (1-3000 µg/ml).

RESULTS AND DISCUSSION

Chemical composition

Forty-five compounds were identified in *S. fruticosa* essential oil, accounting for 99.76% of the whole essential oil. The yield of essential oil was 1.56% (v/w). The composition of the essential oil and oil yield are listed in Table 1. It can be seen that 64.89% was oxygenated monoterpene, the high percentage of this class due to the high percentage of 1,8-cineole (49.34%), camphor (7.53%) and α-terpineol (3.25%). Other major compounds belong to monoterpene hydrocarbons, such as β-pinene 7.38%, myrcene (7.38%), α-pinene (5.15%) and camphene (2.72%), and β-caryophyllene (4.13%) belongs to sesquiterpene hydrocarbons. Other compounds were detected in trace amounts (less than 1%).

In our sample of *S. fruticosa* oil, 1,8-cineole (49.34%) was the most abundant compound, followed by camphor (7.53%), β-pinene 7.38%, myrcene (7.38%), and α-pinene (5.15%). These results are in full agreement with Kosar et al. (2005) who reported that 1,8-cineole and camphor are the predominant compounds of *S. fruticosa* essential oil from Turkey; the same applies for *S. fruticosa* from Greece (Pitarokili et al., 2003). Al-Kalaldeh et al. (2010) reported that *S. fruticosa* collected from Amman, Jordan, was dominated by 1,8 cineole as the major compound, and Skoula et al. (2000) also reported that 1,8-cineole was the major constituent. However, Sivropoulou et al. (1997) reported 1,8-cineole α- and β-thujone as the main constituents. We reported a small amount of β-thujone that did not exceed 0.25%. On the other hand, Longaray Delamare et al. (2007) reported that α-thujone (20.1%) was the major compound in the essential oil of *S. fruticosa* cultivated in south Brazil, and Pierozan et al. (2009) found α-thujone to be the main compound, which is in contrast with our results.

According to literature data and our results, it is obvious that the essential oil composition of *S. fruticosa* differs according different geographic locations. The variation in the essential oil compositions could

be due to several environmental conditions (climatic, seasonal, geographical) and genetic differences (Perry et al., 1999). Moreover, it has been reported that the oil quantitative composition is related to the plant habitat (Karousou et al., 2005).

In most of the investigations into the essential oil composition of *S. fruticosa* 1,8-cineole was the main compound (Skoula et al., 2000; Pitarokili et al., 2003; Kosar, et al., 2005; Papageoriou et al., 2008; Longaray Delamare et al., 2007).

Antioxidant activity

Antioxidant activity was analyzed using the DPPH free radical scavenging method. The results of DPPH scavenging assay of *S. fruticosa* essential oil and synthetic antioxidant butylated hydroxyanisole (BHA) are shown in Fig. 1. The antioxidant activity of the *S. fruticosa* essential oil is very low (IC_{50} = 15.53 mg/ml) compared to BHA. This could be attributed to the absence of phenolic compounds such as thymol and carvacrol. Papagergiou et al. (2008) reported that 1,8-cineole was not active. Also, Burits et al. (2001) studied the antioxidant activity of 1,8-cineole and camphor and found that they did not exhibit strong antioxidant activity.

The activity of the essential oil of *S. fruticosa* could not be attributed to the main compounds nor to the other minor components, such as β-caryophyllene 4.13%, which has been reported to have antioxidant activity with IC_{50} = 18.6 g/L (Papagergiou et al., 2008).

Antimicrobial activity

The results of antibacterial activity in the tested essential oil and its main compounds are presented in Table 2. The oil showed antibacterial activity against all the species tested. The essential oil from *S. fruticosa* possessed antibacterial activity, and showed minimal inhibitory concentration (MIC) effect at 0.125-1.5 mg/ml and minimal bactericidal concentration (MBC) at 0.5-2.0 mg/ml. The monoterpenic hydrocarbon β-pinene showed antibacterial activity with a MIC of 0.05-0.10 mg/ml and MBC of 0.05-0.13 mg/ml. Camphor exhibited inhibitory activity at 0.05-0.07 mg/ml and bactericidal effect at 0.06-0.10 mg/ml. The best activity among the components and essential oil tested was achieved for 1,8-cineole (bacteriostatic activity at 0.04-0.07 mg/ml and bactericidal at 0.04-0.09 mg/ml). Streptomycin showed an inhibitory effect at 0.0005-0.001 mg/ml and bactericidal activity at 0.0005-0.002 mg/ml. The tested oil showed lower antibacterial activity than all the tested compounds and streptomycin. It can be seen that the most sensitive bacteria to essential oil was *Pseudomonas aeruginosa* (MBC = 0.25 mg/ml) and the lowest bactericidal activity (MBC = 2.0 mg/ml) was against *Escherichia coli.* It is obvious that among the components tested, hydrocarbon monoterpenes show the lowest antibacterial activity, while oxygenated compounds possess a higher potential.

Results of the antifungal activity of the essential oil of *S. fruticosa* and the main compounds tested are presented in Table 3. As in the case of antibacterial activity, the oil exhibited antifungal potential. *S. fruticosa* oil possessed good activity with MIC 0.125-1.0 mg/ml and showed minimal fungicidal concentration (MFC) at 0.125-1.5 mg/ml; for β-pinene it is 0.03-0.06 mg/ml and it is fungicidal at 0.04-0.09 mg/ml, while camphor showed MIC at 0.04-0.06 mg/ml and MFC at 0.04-0.10 mg/ml. The best activity was obtained for 1,8-cineole (MIC at 0.05-0.07 mg/ml and MFC at 0.05-0.11 mg/ml). Antifungal potential could be presented as follows: essential oil < β-pinene < camphor < 1,8-cineole. Bifonazole showed the lowest antifungal activity: MIC was 1.5-2 mg/ml and MFC 2-2.5 mg/ml. Thus it can be seen that the antifungal capacity was the same as in the case of its antibacterial activity. The most sensitive fungus to the essential oil was *Aspergillus flavus* with MFC of 0.125 mg/ml, and the most resistant species was *A. niger*. However, the *S. fruticosa* essential oil had an antifungal activity lower than its main compounds; it showed activity better than the Bifonazole.

The effectiveness of the essential oil of *S. fruticosa* (MIC and MBC) against susceptible bacteria was higher than that previously reported for this species

Table 2. Antibacterial activity of *Salvia fruticosa* essential oil and their main compounds tested by microdilution method (MIC and MBC in mg/ml).

Bacteria	*S. fruticosa*		1,8-cineole		Camphor		β-pinene		streptomycin	
	MIC	MBC	MIC	MBC	MIC		MIC	MBC	MIC	MBC
Gram (+) bacteria										
Bacillus cereus	0.5	1	0.04	0.05	0.05	0.06	0.05	0.06	0.0005	0.0005
Micrococcus flavus	0.25	0.5	0.04	0.05	0.05	0.06	0.05	0.05	0.0005	0.001
Staphylococcus aureus	0.25	0.5	0.05	0.06	0.06	0.07	0.06	0.08	0.001	0.001
Listeria monocytogenes	0.5	1	0.05	0.06	0.07	0.07	0.09	0.10	0.001	0.002
Gram (-) bacteria										
Escherichia coli	1.5	2.0	0.06	0.08	0.7	0.08	0.08	0.10	0.0005	0.001
Pseudomonas aeruginosa	0.125	2.0	0.07	0.09	0.07	0.10	0.10	0.13	0.001	0.002
Proteus mirabilis	0.25	0.5	0.06	0.08	0.07	0.09	0.09	0.10	0.001	0.002
Salmonella typhimurium	0.5	1	0.05	0.06	0.06	0.07	0.08	0.09	0.001	0.001

Table 3. Antifungal activity of *Salvia fruticosa* essential oil and their main compounds tested by microdilution method (MIC and MFC in mg/ml).

Fungi	*Salvia fruticosa*		1,8 cineole		Camphor		β-pinene		Bifonazole	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Penicillium funiculosum	0.5	0.5	0.03	0.04	0.04	0.05	0.06	0.06	2.0	2.5
Penicillium ochrochloron	0.15	0.25	0.04	0.04	0.04	0.05	0.05	0.05	1.5	2.0
Aspergillus fumigatus	0.15	0.3	0.04	0.05	0.04	0.06	0.05	0.07	1.5	2.0
Aspergillus niger	1.0	1.5	0.06	0.07	0.06	0.08	0.07	0.09	1.5	2.0
Aspergillus flavus	0.125	0.125	0.05	0.09	0.05	0.10	0.06	0.11	1.5	2.0
Aspergillus ochraceus	0.125	0.25	0.05	0.07	0.05	0.08	0.06	0.09	1.5	2.0
Candida albicans	0.25	0.5	0.05	0.06	0.05	0.07	0.06	0.08	1.5	2.0
Trichoderma viride	0.25	0.5	0.03	0.05	0.04	0.04	0.07	0.09	2.0	2.5

(Sivropoulou et al., 1997; Longaray Delamare et al., 2007), and for *Salvia, S. pratensis, S. glutinosa,* and *S. aethiopis* (Velickovic et al., 2002), and *S. tomentosa* (Tepe et al., 2005). 1,8-cineole and camphor are monoterpenes with a well-documented antibacterial and antifungal potential (Jalsenjak et al., 1987; Sivropoulou et al., 1997; Sur et al., 1991). The stronger activity of the essential oil of *S. fruticosa (=S. triloba*) against almost all the susceptible bacteria may be due to the presence of a high concentration of β-caryophyllene. The antimicrobial properties of caryophyllene and caryophyllene oxide were described previously (Azaz et al., 2002). Other than the major compounds, α-pinene (a monoterpene hydrocarbon) and borneol (an oxygenated monoterpene), as well as other minor constituents of the essential oils of *S. officinalis* and *S. triloba* have antimicrobial activity (Dorman and Deans, 2000). In addition,

the essential oil of *Artemisia afra* Jacq., which has a qualitative composition similar to that of *S. fruticosa* (α- and *β*-thujone, 52%; 1,8-cineole, 13%; and camphor, 15%), showed moderate antimicrobial and antifungal activities (Graven et al., 1992) comparable in magnitude to those reported here for *S. fruticosa*. In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration to account for their biological activity.

In general, Gram-positive bacteria were more sensitive than Gram-negative strains; these results are in accordance with Longaray Dealamare et al., 2007 and Marino et al., 2001. This resistance of Gram-negative bacteria could be attributed to the presence of their outer phospholipidic membrane that is almost impermeable to lipophilic compounds (Nikaido and Vaara, 1985). In contrast, the absence of this wall in Gram-positive bacteria allows the essential oil and hydrophobic constituents to be in direct contact with the phospholipid bilayer of the cell membrane, where they bring about their effect, causing either an increase in ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Cowan, 1999; Wendakoon and Sakaguchi, 1995). Overall, fungi were more sensitive than the bacterial strains (Tables 1, 2).

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