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The impact of seasonal variation on the volatile oil profile of leaves of *Severinia buxifolia* (Poir.) and its antimicrobial activity

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The effect of the time of collection on the quality and quantity of the oil obtained from *Severinia buxifolia* leaves as well as its antimicrobial activity was studied. The chemical composition of the hydro-distilled oils of the leaves of *S. buxifolia* (Poir.)Tenore, collected at the four seasons was determined by GC/MS analysis. Moreover, antimicrobial activity was studied, for the oil sample regarding the yield and quality, against selected bacteria and yeast. The highest oil yield was obtained from the leaves collected during winter (0.5%) followed by autumn (0.308%), however, those collected in spring and summer scored almost the same yield; (0.26%) and (0.283%) respectively. Limonene was the most abundant hydrocarbon in winter (35.5%), and amounted to 29.3% in summer, whereas, spring and autumn samples constituted 21.15% and 19.17% of limonene respectively. α-Santalene, accounted to 20.87% in autumn sample followed by the winter sample (18.93%), then 13.56% in the spring sample and recorded its lowest concentration in the summer sample (8.1%). Furthermore, γ-elemene was detected in a lesser extent amounting to 7.75% in the spring sample, 7.33% in autumn sample, 6.28% in the winter sample and 5.54% in the summer sample. Based on the above results, as regards to limonene content, *S. buxifolia* leaf oil collected in winter was chosen for further antimicrobial study. The agar disc diffusion method was adopted for screening the antibacterial activity of the selected oil sample. Results show moderate effect against *Escherichia coli*, and *Listeria monocytogenes*. Nevertheless, it showed weak activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilus,* MRSA, and *Candida albicans.* The MIC of the volatile oil against *L. monocytogenes was* 4 and 1 µl/ ml against *S. aureus, P. aeruginosa, B. subtilus,* MRSA and *C. albicans.*

Key words: *Severinia buxifolia* (Poir.) Tenore, Rutaceae, GC/MS analysis, limonene, *in vitro* antimicrobial activity.

INTRODUCTION

Rutaceae is best known for the exotic genus citrus, because of its commercially consumed fruits. Other groups of the same subfamily of Citrus are commonly cultivated as ornamentals in America, including species of *Atalantia*, *Clausena*, *Murraya*, and *Swinglea*.

Genus *Atalantia* includes approximately 20 species

(Roskov et al., 2017). Amongst *Atalantia* species, *Atalantia buxifolia* or *Severinia buxifolia* (Poir.) Tenore is the plant of our interest. *S. buxifolia* (*Atalantia buxifolia*) is commonly known as the thorny, evergreen shrub; it is also known as Chinese box-orange or Box-leaved *Atalantia*. The Flora of China has moved this species to *A. buxifolia* (Poir.) Oliv. (Wu et al., 2008). In Hainan province of China, the roots of *A. buxifolia* are used in folk medicine for the treatment of influenza, cough, malaria, and stomachache by the people of Li nationality (Yang, 2012).

Essential oils are complex mixtures including important compounds where each contributes to the beneficial or adverse effects of these oils. Essential oils have massive consumptions as raw materials in several areas, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition Buchbauer, 2000). Accordingly, it was of deep interest to have strong background regarding the essential oil composition since this permits for a better and specially directed application.

Infectious diseases are major causes of death worldwide. Infections with bacteria are associated with high morbidity and mortality especially with immuno-compromised patients (Driscoll et al., 2007; Del Toro et al., 2006). The proposed strategies to avoid and govern infectious diseases include public health improvements in sanitation and hygiene, as well as the use of antimicrobial agents (WHO, 2001). The resistance of microorganisms to antibiotic has become an important alarm to the patients as well as a scientist (Westh et al., 2004) in addition to the side effects of these antibiotics. This directed researchers to explore new chemotherapeutic agents to combat the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotics (Bocanegra-Garcı´a et al., 2009; Giamarellou, 2006). Volatile oils obtained from plants have been recognized for many years as antimicrobial agents.

Direct addition of essential oil from aromatic plants to food products evidenced antimicrobial effect (Costa et al., 2015). Nowadays, due to consumer complaint from artificial preservatives, attention to volatile oils and their application in food preservation has been considered. Many reports dealt with the wide range of application for essential oils as antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal agents (Burt, 2004; Dorman and Deans, 2008). Hence, essential oils can serve as a powerful device to minimize the bacterial resistance (Stefanakis et al., 2013).

This work was carried out to evaluate the oil produced from the plant and the effect of seasonal fluctuation on the yield and composition of the essential oil of the leaves, furthermore comparing the identified components and the major constituents detected in the studied samples, with the aim to rationalize the effect of climate conditions on the yield and quantity. The antimicrobial activity of the oil sample giving the highest yield and quality was studied.

MATERIALS AND METHODS

Plant material

Sample leaves of *S. buxifolia* (Poir.) Tenore used in this study were collected in May to June during the years 2013 to 2014 (collected in four different seasons throughout the year) from the Orman Garden, Giza, Egypt. The plant was authenticated by Dr. Mohamed El-Gebaly, senior botany specialist at the Orman Garden, Giza, Egypt. Voucher specimens (26-05-2015) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

Microbial strains

The tested bacteria and fungi were supplied by the Antimicrobial Unit, National Research Center, Egypt. Bacterial strains were *Bacillus subtilus* (ATCC6051), *Staphylococcus aureus* (ATCC 6538), *Methicillin-resistant Staphylococcus aureus* (MRSA) (laboratory collection), *Listeria monocytogenes* (ATCC2180-1A) representing pathogenic gram positive bacteria, and *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa*(ATCC 9027) representing pathogenic gram negative bacteria and *Candida albicans* (RCMB 05035) representing fungi. Ciprofloxacin (Pharco Pharm. Cairo Egypt, and Amphotericin B obtained from Sigma-Aldrich (Merk) was used as reference drugs.

Preparation of the volatile oil

For preparation of essential oil, fresh leaf samples were collected at three months intervals along the four seasons (winter, spring, summer and autumn) between May 2013 to June 2014 and separately subjected to hydro distillation in a Clevenger's apparatus for 3 h, according to the procedure described in the Egyptian Pharmacopeia. The obtained oils were separately dried over anhydrous sodium sulphate and carefully stored in a refrigerator for further chemical and biological studies. The percentage yields were calculated on a dry weight basis, and the oils were kept in a refrigerator for further analysis. The specific gravities and refractive indices were determined according to the Egyptian Pharmacopoeia (CAPA, 2005) procedures. All stated values were the average of three determinations.

Determination of percentage yield of the volatile oil

The yield of the volatile oils was calculated as weight/weight (g/kg), on fresh weight basis.

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Table 1. Yield and physical characters of essential oil of the leaves of *S. buxifolia* (Poir.) Ten. at four different seasons.

Physical properties	Seasons (%)			
	Winter	Spring	Summer	Autumn
Odour	Lemon-like odour			
Colour	Pale yellow			
Yield % (w/w, g/kg)	0.5	0.283	0.26	0.308
Specific gravity (g/cm^3 at 25°C)	*0.86	*0.84	*0.81	*0.83
Refractive index (recorded at 20°C)	*1.4694	*1.4653	*1.4624	*1.4642

*Results are average of three determinations.

GC/MS analysis of the volatile oil content

Volatile oil prepared from *S. buxifolia* leaves were subjected to GC/MS analysis. Volatile oil prepared was subjected to GC/MS analysis. The injection volume was 1 µl/ml and the instrument was controlled by the Shimadzu Class-5000 Version 2.2 software containing a NIST62 (National Institute of Standards and Technology) MS library. Volatiles were separated on a DB5-MS column (30 m length, 0.25 mm inner diameter, and 0.25 ml film (J&W Scientific, Santa Clara, California). Injections were made in the split mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220°C and column oven 40°C for 3 min, then programmed at a rate of 12°C/min to 180°C, kept at 180°C for 5 min, and finally ramped at a rate of 40°C/min to 220°C and kept for 2 min; He carrier gas was at 1 mL/min. The transfer line and ion–source temperatures were adjusted at 230 and 180°C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at 40 to 500 m/z. The percentages of different components in each oil sample were determined by computerized peak area measurements relative to each other. Volatile components were identified using the procedure described (Farag and Wessjohann, 2012). The peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to *n*-alkanes (C6–C20), mass spectrum matching to NIST, WILEY library database.

Evaluation of the antimicrobial activity

In-vitro qualitative screening susceptibility test

The selected volatile oil sample of the leaves of *S. buxifolia* under investigation was screened for their antimicrobial activity against representatives of G+ bacteria: *B. subtillus*, *S. aureus*, Methicillin-resistant *S. aureus* (MRSA), L. *monocytogenes*, and G$^-$*E. coli*, *P. aeruginosa*, and yeast (*C. albicans*) applying the agar disc diffusion according to CLSI guidelines (2009).

The selected volatile oil was tested by impregnating sterile discs of Whatmann filter paper 1 (5 mm diameter) in 20 µl of the oils. 20 µl of dimethyl sulfoxide was used as a negative control. The reference standards ciprofloxacin and amphotericin-B were dissolved separately in dimethyl sulfoxide at a concentration of 20 µg/µl. The discs were then placed onto the surface of the plates containing the solid bacterial medium (Mueller–Hinton agar) or the fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested microorganisms. The plates were incubated at 37°C for 25 h in case of bacteria and at 25°C for 48 h in case of fungi. After incubation, the inhibition zones were measured in mm.

Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (quantitative assay) was evaluated for the volatile oil of *S. buxifolia*, based on the results obtained for the antimicrobial screening. Accordingly, *B. subtilus, S. aureus,* MRSA*, L. monocytogenes, E. coli, P. aeruginosa* and yeast (*C. albicans*) for *S. buxifolia* leaf oil. In brief, stationary phase cultures of bacterial strains were prepared at 37°C and used to inoculate fresh 5.0 ml culture to an OD600 of 0.05. The 5.0 ml culture was then incubated at 37°C until an OD600 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6 105 CFU/ml. Serial dilutions from the volatile oils were prepared and mixed with 5.0 ml of the standardized bacterial suspension then added to the plates and incubated for 24 h at 37°C. The colony forming units (CFU) were counted for each dilution (NCCLS, 2000).

Agar dilution method

The tested samples were serially diluted in molten medium equilibrated at 50°C with 2% glucose. One ml was added to each well in a 24-well plate with a flat bottom and allowed to solidify. The centre of each well was inoculated with 10 ml of the bacterial suspension. Drug free growth control was included. MIC was determined after 48 h at 35°C. MICs were defined as the lowest concentration that had granular appearing micro-colonies of growth instead of filamentous radiating colonies on solid agar.

RESULTS AND DISCUSSION

This is the first report dealing with the effect of seasonal variation of the essential oils obtained from the fresh leaves of *S. buxifolia* (Poir.) Tenore.

Investigation of the oil obtained from the leaves of *S. buxifolia* at different seasons; winter, spring, summer and autumn revealed qualitative and quantitative differences. The highest oil yield was obtained from the leaves collected during winter (0.5%) followed by autumn (0.308%), while, those collected in spring and summer counted almost the same yield; 0.26 and 0.283% respectively (Table 1).

GC/MS analysis of the essential oil obtained from *S. buxifolia* leaves at four different seasons (Table 2) indicated that the identified constituents varied as regards to their abundance and concentration. The total identified

Table 2. Chemical composition of the essential oils of *S. buxifolia* leaves at four different seasons and their mass data.

S/N	Rt	KI	M^+	BP	Main fragments m / z	Compound	Area%			
							Autumn	Winter	Spring	Summer
1	7.680	913.3	136	93	41, 53, 67, 79, 105, 121	*β* - trans-Ocimene	0.44	0.45	0.65	0.82
2	7.7712	936.2	136	93	41, 53, 79, 107, 121	*α*-Thujene	–	0.3	0.41	0.55
3	7.9672	936.5	136	93	41, 53, 79, 107, 121	*β*-Thujene	0.23	0.66	0.43	0.36
4	8.251	937.7	136	93	41, 53, 67, 77, 105, 121	*α*-Pinene	0.1	–	–	–
5	8.530	975.8	136	93	41, 53, 69, 77, 121	Sabinene	–	0.08	0.1	0.07
6	8.6432	989	136	41	53, 67, 69, 79, 91	*β*-Myrcene	0.31	0.72	0.35	0.07
7	8.7910	1011	136	93	41, 53, 65, 77, 105, 121	*δ*-3-Carene	–	–	0.03	0.05
8	9.052	1027.7	134	119	41, 51, 65, 77, 91, 103	*p*-Cymene	–	0.03	0.04	0.1
9	9.1976	1033	154	59	43, 67, 81, 96	Santolina alcohol	–	–	–	0.05
10	9.3675	1034	136	68	41,53,68,79, 93,107, 121	Limonene	19.17	35.5	21.15	29.3
11	9.5390	1047.4	134	119	41, 51, 65, 77, 91, 103, 119	Octen-1-ol	–	–	0.19	–
12	9.8175	1061.9	136	93	41, 51, 65, 77	*α*-Phellandrene	0.13	–	–	–
13	10.1151	1062.1	136	93	43, 58, 65, 77, 105, 121	*δ*-Terpinene	–	–	–	0.16
14	10.5283	1063	136	93	41, 53, 65, 77, 121	*β*-Phellanderene	0.11	0.23	–	–
15	10.7522	1101	154	71	43, 55, 80, 93, 107, 121	*β*-Linaloo	0.04	–	0.16	0.15
17	10.9910	1169	156	71	41, 55, 81, 95, 109, 123	Menthol	0.3	0.25	0.19	0.1
18	11.2071	1172	154	71	43, 55, 77, 86, 93, 111, 136	Terpinen-4-o	0.13	–	0.17	0.2
19	11.5259	1173.0	170	71	43, 55, 82, 128, 141	Hexenyl butanoate	–	0.18	–	–
20	11.7814	1206	94	81	41, 54, 67, 95	Norbornene	–	–	0.8	–
21	11.940	1207	156	41	57, 70, 82, 95, 112, 128	Decana	0.96	0.93	0.84	1.17
22	12.5582	1274.6	152	41	53, 59, 69, 84, 94, 109, 119, 137	Neral	0.05	0.08	–	–
23	13.463	1345	204	121	41, 53, 67, 77, 93, 105, 136, 161, 175, 189	*δ*-Elemene	0.04	–	0.1	0.2
24	13.796	1359	202	145	41, 55, 65, 77, 91, 105, 119, 131, 145, 159, 174, 187	Silphiperfol-5,7(14)-diene	0.05	0.09	0.09	–
25	14.0061	1401.5	96	67	43, 57, 85	Norbornane	0.1	–	0.16	0.18
26	14.537	1411	184	67	43, 55, 79, 110, 123, 138, 166	Z-2-Dodecenol	0.13	0.15	0.17	0.13
27	14.7733	1431	204	94	41, 55, 69, 79, 107, 121, 133, 147, 161, 176, 189	*α*-Santalene	20.87	18.93	13.56	8.1
28	14.8725	1439	204	41	55, 69, 79, 93, 105, 120, 133, 147, 161, 175, 189	*β*-Caryophyllene	5.08	5.07	3.78	2.98
29	14.9125	1442	204	119	41, 55, 69, 77, 93, 105, 133, 147, 161	Zingiberene	0.43	0.37	0.3	0.22
30	15.0371	1446	164	164	41, 55, 65, 77, 91, 103, 121, 131, 149	Isoeugenol	0.4	–	0.13	0.19
31	15.1192	1459	204	94	41, 55, 67, 79, 107, 122, 133, 147, 161, 189	Epi-*β*-Santalene	2.07	1.6	1.96	0.83
32	15.2725	1472	204	94	41, 55, 67, 79, 107, 122, 133, 147, 161, 189	*β*-Santalene	2.52	1.98	–	1.23
33	15.323	1475	204	93	41, 53, 67, 80, 107, 121, 136, 147, 161, 189	*α*-Humulene	0.87	0.9	0.75	0.65
35	15.5634	1511.3	204	161	41,55, 67, 79, 91, 105, 119, 133, 147, 175, 189	[+]-Valencene	0.55	–	–	–

Table 2. Contd.

36	15.834	1515	204	121	41, 55, 67, 79, 93, 107, 136, 147, 161, 175, 189	*γ* -Elemene	7.33	6.28	7.75	5.54
37	15.895	1519	204	161	41, 53, 81, 93, 107, 119, 133, 147, 161, 189	Germacrene A	–	–	0.18	0.2
38	16.032	1530	204	105	41, 55, 69, 81, 91, 119, 133, 161, 175, 189	*α* -Cadinene	0.75	–	0.94	0.8
39	16.219	1531.6	204	119	41, 55, 69, 79, 93, 105, 133, 148, 161, 189	γ-Cuprenene	–	0.62	–	0.45
40	16.330	1534.5	222	69	41, 55, 69, 81, 93, 107, 136, 161, 189	Nerolidol	–	–	0.13	–
41	16.475	1550	222	59	43, 67, 81, 93, 107, 121, 135, 149, 161, 189	Elemol	0.21	–	0.28	0.1
42	16.55	1562	204	41	55, 69, 79, 93, 107, 119, 134, 147, 161, 189	(Z)-*α*-Farnesene	0.48	–	0.7	0.74
43	16.782	1575	220	43	55, 67, 79, 91, 105, 119, 131, 147, 159, 177, 187, 205	Spathulenol	0.28	–	–	–
44	16.903	1588	220	41	55, 69, 77, 93, 109, 121, 135, 149, 161, 177, 187,205	Caryophylline oxide	–	–	–	0.52
45	17.198	1600	220	159	41, 55, 67, 79, 91, 105, 118, 131, 177, 187, 202	cis-*α*-Copaene-8-ol	1.41	0.69	1.88	1.65
46	17.210	1608.2	222	41	55, 69, 81, 93, 107, 121, 135, 161, 179	Z-Sesquilavandulol	–	–	0.91	0.76
48	17.273	1609.3	204	69	41, 55, 79, 93, 107, 120, 133, 147, 161	(Z)- *β*-Farnesene	0.77	–	–	–
49	17.4195	1609.8	202	91	41, 53, 67, 79, 105, 119, 131, 145, 159, 187	*β*-Atalantol	0.77	–	–	0.43
50	17.6391	1617.5	222	95	43 ,55, 69, 81, 107, 119, 135, 150, 150, 161, 189, 204	*Epi* Cedrol	0.25	–	0.1	0.11
52	17.736	1669.9	222	93	41, 59, 67, 81, 107, 121, 136, 161, 175, 189	*E*-Bisabol-11-ol	0.11	–	–	–
54	17.7985	1675.6	220	41	55, 67, 81, 98, 109, 125, 161, 179, 207	Z-*α*-Santalol	24.44	13.73	29.19	22.2
56	17.8157	1688.0	222	43	55, 69, 79, 93, 109, 119, 134, 161, 189	*α*-Bisabolol	–	–	–	0.36
57	17.892	1687.7	222	69	41, 55, 81, 93, 107, 121, 136, 161, 191	(2Z,6Z)-Farnesol	3.72	–	1.35	3.54
58	18.001	1866.0	262	94	43, 55, 67, 79, 107, 122, 134, 159, 187	E-*β*-Santalol acetate	–	–	–	1.49
59	18.5500	1925.5	228	213	128, 141, 157, 185	Seselin	0.33	–	0.87	0.52
	%Total identified compounds						95.93	89.82	90.79	87.27
	% unidentified compounds						4.07	10.18	9.21	12.73
	% of Hydrocarbons constituents						93.3	74.16	54.91	54.66
	% of Oxygenated constituents						3.62	16	37.7	33.78

Rt= retention time; K.I. = kovat's index; M+= molecular weight; BP= Base peak.

compounds were 95.93, (89.82, 90.16 and 88.44% in autumn, spring, winter and summer respectively. The total number of constituents identified under the adopted conditions was 59 among which 15 were detected in the four oil samples under investigation. The rest of constituents appeared, however, unevenly distributed in the analyzed oils.

Hydrocarbons were the most abundant compounds detected, amounting the highest concentration in autumn (93.3%), followed by those collected in winter (74.16%). Nevertheless, spring and summer samples constituted the same amount of hydrocarbons; 54.91 and 54.66%, respectively. Monoterpene hydrocarbons constituted the most dominant chemical group among the four studied seasons; limonene was the most abundant in winter (35.5%), and amounted to 29.3% in summer, whereas, spring and autumn samples constituted 21.15 and 19.17% of limonene respectively. Next to monoterpenoids, sesquiterpenoids were the most detected compounds in the volatiles studied at different seasons from the leaves of *S. buxifolia*,

Table 3. Antibacterial and antifungal activity of the winter sample (essential oil of the leaves) of *S. buxifolia* (Poir.) Ten.

Microorganism	Diameter of zone of inhibition (mm)		
	Essential oil (4 µL / disc)	Ciprofloxacin	Amphotericin-B
Bacillus Subtillus (ATCC6051)	16	31	-
Staphylococcus aureus (ATCC 6538)	17	30	-
MRSA (Laboratory Collection)	10	28	-
Listeria monocytogenes (ATCC2180-1A)	13	-	-
Escherichia Coli (ATCC 8739)	19	29	-
Pseudomonas aeruginosa (ATCC 9027)	12	29	-
Candida albicans (yeast) (RCMB 05035)	13	-	29

Values are average of three determinations.

with α-santalene accounting up to 20.87% in autumn sample followed by the winter sample (18.93%), then reaching 13.56% in the spring sample and finally, recorded the lowest concentration in the summer sample(8.1%) relative to the other samples.

Furthermore, γ- elemene was found but in a lesser extent than α-santalene amounting to (7.75%) in the oil collected in spring, (7.33%) in the autumn sample, (6.28%) in the winter sample and (5.54%) in the summer sample. Throughout our study in the four seasons; the number of oxygenated compounds ranged from 16 to 37.7%; spring sample recorded the highest content of oxygenated compounds (37.7%) with Z-α-santalol constituting the major sesquiterpene alcohol (29.19%), the oxygenated compounds amounted up to 3.62 % in autumn, where Z-α-santalol was the major compound (24.44%), followed by summer (33.78%) with same major sesquiterpene (22.2%), however winter showed the lowest oxygenated compounds recorded in all studied oil samples (16%), Z-α-santalol amounting to 13.37%.

These findings support the idea that the seasonal variation can influence the quantifier of oxygenated compounds present in the oil. Comparing the results to that reported (Scora and Ahmed, 1994), there is no great difference between the number of compounds identified; 59 components were also identified, however, the difference was clear in the detected compounds. The major reported components were α -santalene (24%), trans β -santalol (21%), germacrene-B (10%), and β-caryophyllene (7%) and the monoterpene limonene (19%) in one tested sample. On the other hand, another report showed variation (Dongxu et al., 2011) from our study as major identified components were isocyclocitral (41.598 %), guaiacol (15.234%), β-eudesmol (10.790%), thujopsene (2.581%), 1,7,7-trimethyl-bicyclohept-2-one (5.841%), santalol (3.702%), ferruginol (2.657%) at one sample. Herein, we have evidenced variation in the tested samples as regards to yield and components which were affected by the seasonal effect. Furthermore, there was an obvious impact on the climax of each season on the volatile constituents of each cultivated plant.

Previous reports evidenced that limonene possesses antifungal, bacteriostatic and bactericidal activities (Dorman and Deans, 2000; Dambolena et al., 2008; Jaroenkit et al., 2011; Chee et al., 2009). Moreover, it was also suggested to be used as a food preservative (Vuuren and Viljoen, 2007). Since winter sample recorded the maximum yield and constituted the highest percentage of limonene, therefore, winter sample was selected as a representative to undergo further *in the vitro* antimicrobial study.

There was a correlation between the inhibition zone diameter of the agar diffusion method (qualitative method) (Rashed and Butnariu, 2014a) and MIC (quantitative method) values of broth dilution method (Rashed and Butnariu, 2014b). The selected essential oil showed a maximum zone of inhibition with minimum MIC value among all tested microorganisms. The selected volatile oil sample of *S. buxifolia* leaves exhibited moderate antibacterial and antifungal activity at the given concentrations when compared to ciprofloxacin and amphotericin B respectively as a standard (Table 3). Moreover, the oil exhibited activity against most of the tested bacteria with a MIC of 1% v/v except for *L. monocytogenes.* This activity might be attributed to the high concentration of limonene in the sample; the mechanism of action for the antibacterial activity was previously reported; the inactivation of certain strain of *E. coli* by (+)-limonene, in a medium adjusted at certain pH, the effect of the synergistic lethal effect on combining (+)-limonene with heat and Pulse Electric field (PEF) treatments to inactivate *E. coli* (Espina et al., 2013). Referring to Table 4, the MIC of the volatile oil of the leaves of *S. buxifolia* against *L. monocytogenes* recorded 4 µl/ml, while 1 µl/ml against other bacterial strains. Therefore, this volatile oil could be considered as moderate antibacterial and antifungal agent.

Conclusion

Our findings support the concept that collecting oil samples from the fresh leaves of *S. buxifolia* (Poir.)

Table 4. MIC of the winter sample (essential oil of the fresh leaves) of *S. buxifolia* (Poir.) Ten. on bacteria and fungi.

Tested microorganism	Minimum inhibitory concentration (µl/ml)of essential oil
Gram-positive bacteria	
Bacillus Subtillus	1
Staphylococcus aureus	1
MRSA	1
Listeria Monocytogenes	4
Gram-negative bacteria	
Escherichia Coli	1
Pseudomonas aeruginosa	1
Fungi *Candida albicans (yeast)*	1

Tenore at different seasons had an impact on their yield and composition. Winter sample was the best with the high content of limonene. There is variation in the antimicrobial activity of MIC of winter oil sample against the entire tested microorganism. The data indicated that the oil has antimicrobial activity against gram +ve and gram –ve bacteria and even Candida in close range.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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