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ORIGINAL PAPER

Antibacterial and Antifungal Activities of the Essential Oils of Two Saltcedar Species from Tunisia

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Abstract The chemical composition of the volatile constituents from the flowering parts of *Suaeda fructicosa* and *Limonium echioides* were analysed by GC-FID and GC-MS. Sixty-five compounds were identified in *L. echioides* aerial parts. 48 out of 65 were found common to the aerial part of *S. fructicosa.* Palmitic acid was found as a predominant compound in both tested halophytic oils. Furthermore, the essential oil was tested against six bacteria and four fungi at different concentrations. Both oils, tested at 0.5 and 0.8 mg ml^{-1}, inhibited the visible growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Escherichia coli* and *Salmonella typhimurium*, but no antibacterial effect was detected against *Pseudomonas aeruginosa.* Additionally, both halophytic oils failed to show antifungal activity against all the test fungi when applied at 80, 200 and 500 µg/disc.

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Keywords Chemical composition · Volatile constituents · Halophytes · GC-MS · Antibacterial profile · Antifungal activity

Introduction

Salinity is reported to affect about one billion hectares of land [1], that are mostly located in arid and semiarid regions. It is estimated that 20 million hectares, in addition to that already affected, deteriorate to zero productivity each year [2]. Hence, 23% of the whole cultivated soils have deteriorated [3]. The Mediterranean-type salt marsh is one of the most affected by environmental degradation and erosive processes which occur due to its climatic characteristics, such as, a scarce and irregular rainfall and a long, dry and hot summer [4]. Saline soils of various natures and degrees of salinity make up over 80 million hectares in the Mediterranean basin [5], out of which 5,140 hectares are located in Tunisia and represent 31% of the total area [6].

Planting saline habitats with halophytic species is profitable and provides many additional benefits. There are about 6,000 species of terrestrial and tidal halophytes in the world, 700 halophytic species in the Mediterranean climate area [7] out of which, 215 species are located in Tunisia [8–10].

The saltcedars *Suaeda fructicosa* and *Limonium echioides*, respectively, belong to the Chenopodiaceae and Plumbaginaceae families. Species of both families show a wide range of biological activity, suggesting a great pharmacological and biotechnological potential. They hold promise as sources of chemical leads for the development of new drugs. Indeed, *S. fructicosa* has an hypoglycemic effect [11] and it is specific with its black wool dyeing [12]. *Suaeda salsa* exhibits, however, an antioxidant activity

scavenging free radicals [13]. Furthermore, *Limonium* spp. are employed as an antioxidant medicinal herb, such as, *Limonium wrightii* [14] and *Limonium brasiliense* [15]. *Limonium sinense* and *Limonium tetragonum* show antiviral activity [16], whereas, *Limonium axillare* and *Limonium californicum* show cytotoxic and antibacterial activities [17–19].

This paper reports the results of GC-MS analyses and the antibacterial and antifungal perspectives of the essential oil from aerial parts (leaves, stems and flowers) of *S. fructicosa* and *L. echioides*, the activities which had not been studied previously.

Materials and Methods

Plant Material

The saltcedars *S. fructicosa* and *L. echioides* were collected, respectively, from Sebkhet Monastir (35°46′0″ North and 10°59′0″ East) and Elkalaa, Sousse region, at the flowering stage. The fresh plant aerial parts were divided into small pieces and weighed before the extraction of volatile compounds. Voucher specimens were deposited in the herbarium of the Faculty of Sciences, Monastir, Tunisia.

Volatile Compounds Extraction

Steam distillation (8 h) was adopted for the extraction of volatile compounds from both plants. The recovered solution was extracted with chloroform. Yields based on fresh weight of the sample were calculated.

Analysis of the Volatile Compounds by Gas Chromatography

An HP 5890-series II gas chromograph was used, it was equipped with: flame ionization detectors (FID), 0.25-μm film thickness fused capillary column, type HP-5, having a dimension of 30 m × 0.25 mm ID, and 0.25-μm film thickness fused capillary column, type HP Innowax, having a dimension of 30 m × 0.25 mm ID. The carrier gas was nitrogen (1.2 ml/min). The oven temperature program was 1 min isothermal at 50 °C, then 50–280 °C at rate of 5 °C/min and held isothermally for 1 min. The injection report temperature was 250 °C, detector 280 °C. Volume injected: 0.1 μl of 1% solution (diluted in hexane). Percentages of the constituents were calculated by electronic integration of the FID peak areas without the use of response factor correction.

GC-MS

The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP-5 MS capillary column (30 m × 0.25 mm ID, film thickness of 0.25 μm) was directly coupled to the MS. Oven temperature was programmed (50 °C for 1 min, then 50–280 °C at 5 °C/min) and subsequently, held isothermally for 20 min. Injector port: 250 °C, detector: 280 °C, split ratio 1:50. Volume injected: 0.1 μl of 1% solution (diluted in hexane).

Mass Spectrometer

An HP5972 recording at 70 eV; scan time 1.5 s; mass range 40–300 amu. The software adopted to handle mass spectra and chromatograms was a Chem. Station (HPGC Chem Station Rev.A.06.03 (509), Copyright© Hewlett-Packard 1990–1998).

Identification of the Compounds

The identification of the components was based on comparison of their mass spectra with those of a computer library (Wiley 275 library). Further confirmation was done by referring to retention index data generated from a series of alkanes (C_9–C_{28}) [20, 21].

Antibacterial Activity

Organisms

The strains chosen for investigation were: a reference strain Gram-positive cocci *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* NCIMB 8853 and *Micrococcus luteus* NCIMB 8166 and Gram-negative bacilli *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028. All bacteria were subcultured from −70 °C stock cultures into 5 ml Mueller–Hinton broth and were incubated for 24 h at 37 °C. For use as inoculum, the turbidity of the bacterial suspension was adjusted to the McFarland standard (0.5) [22].

Two methods were used to assess the antibacterial activity: a diffusion method on agar plates [23, 24] and microdilution method in liquid medium [24, 25].

Diffusion Method

The determination of bacterial susceptibility to antibiotics using disc diffusion on Mueller–Hinton agar (beef maceration 300 ml, hydrolysis of casein 17.5 g, starch 1.5 g,

Agar 10 g). The pH of Mueller–Hinton agar was adjusted to 7.2–7.4.

Two to three millilitres of the inoculums were spread over plates containing Mueller–Hinton agar, however, the excess fluid was removed as much as possible by aspiration and the plates were dried at +35 °C for 15 min. The paper filter discs (5 mm) impregnated, under aseptic conditions, with the volatile fractions at different concentrations were applied to the agar surface. Whichever method used, the discs must completely adhere to the agar surface and should be arranged in such a way that overlapping of the inhibition zones is avoided and that the distance between the discs and plate edges does not exceed 15 mm. The plates were inverted and incubated after 15 min at 35 ± 2 °C. After 18 h, the inhibitory diameters were measured.

In our study, we added Tween-80 at 5% to chloroform to dissolve the volatile fractions [26, 27]. Several substances have been used as solvents to dissolve essential oils or to stabilise them in water-based culture media [28].

Two controls were also included in the test. The first was a control involving the presence of microorganisms without the test material (chloroform added to Tween-80). The second was a standard antibiotic (ampicillin) which was used in order to control the sensitivity of the tested microorganisms, and the developing inhibition zones were compared with those of reference discs.

Organisms, media components and ampicillin were provided by the Laboratory of Environment Microbiology, Faculty of Pharmacy.

MIC and MBC Determinations

The minimum inhibitory concentration (MIC) was defined as the lowest concentration that prevented visible growth (lowest concentration without turbidity) [26, 29, 30]. The minimum bactericidal concentration (MBC) was determined as a concentration where 99.9% or more of the initial inoculum was killed [26, 31, 32]. MIC and MBC of tested volatile fractions were determined using the Mueller–Hinton broth (MHB) dilution method [28, 33]. All tests were performed in MHB supplemented with Tween-80 (5%) [34]. To confirm the results of MBC the experimental suspensions were sub-cultured at TSA agar plates [31] which were incubated at 30 °C for 18–24 h.

Antifungal Activity

Test Organisms

Four phytopathogenic fungal species were used for the antifungal testing, namely: *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium* sp. and *Alternaria* sp. These were obtained from the Phytopathology Laboratory, Regional Pole of Agriculture Research-Development, Chott Mariem, Sousse, Tunisia.

Determination of Antifungal Activity of Volatile Oils

The disc diffusion method was used for antifungal screening [35]. Fungal broth culture aliquots adjusted to 10^4–10^5 CFU/ml were added to potato dextrose agar medium and distributed uniformly in 9-cm Petri plates. Different dilutions of the oils were made with chloroform mixed with Tween-80 at a concentration of 2%. Under aseptic conditions, paper discs (6 mm, Whatman No. 1 filter paper) were impregnated with 20 μl of volatile oils at different concentrations and placed on the culture plates after removing the chloroform by evaporation. The antifungal agent, carbendazine, was provided by the phytopathology laboratory and used as a positive control and chloroform was used as a negative control. The diameter of the zone of inhibition (mm) around the disc was measured after cultivation at 28 °C for 4 days and was compared with the control. The test was performed in triplicate.

Results and Discussion

Chemical Composition of the Essential Oil

Although, chemical composition of the investigated samples exhibited a certain resemblance, significant differences were revealed in the compound proportions. Indeed, 65 compounds were identified in *L. echioides* aerial parts out of which 48 were also found in the aerial parts of *S. fructicosa*. According to Table 1, the main components of *L. echioides* were: hexacosane (10.7%), palmitic acid (9.76%), nonacosane (8.44%), (*E*,*E*)-farnesyl acetate (7%) and vanillin (6.51%), numbered, respectively, as 64, 51, 67, 46, 20 in Fig. 1. While for *S. fructicosa*, they were palmitic acid (15.23%), methyl linoleate (10.8%), phytyl acetate (8.78%), hexacosane (7.41%) and methyl decanoate (6.97%), numbered, respectively, as 51, 59, 60, 64 and 15 in Fig. 1. Thus, palmitic acid and hexacosane (peak numbers 51 and 64 in the Fig. 1) were found as predominant compounds in both the tested halophyte oils. Palmitic acid also represented the main component in the seed oil of *S. fructicosa* (17%) [36].

Tricyclene, octanal, 2-methyl decanal, vanillin, calarene, germacrene D, farnesal, ethyl tetradecanoate and 1-docosene were only identified in the aerial parts of *L. echioides*. Whereas, α-fenchol, 2,4-nonadienal and fenchyl acetate appeared to be unique to *S. fructicosa* oil. In addition, hexanoic acid, *β*-cubebene, *γ*-cadinene, elemol, *δ*-cadinol, manool and phytol were present at a higher

Table 1 Aerial part volatile-compound compositions of *Suaeda fructicosa* and *Limonium echioides*

No.	Compounds	RI apolar	RI polar	*L.e.* (%)	*S.f.* (%)
1	Tricyclene	930	1,004	0.49	–
2	Camphene	962	1,076	0.01	–
3	Octanal	1,010	1,294	1.30	–
4	Hexanoic acid	1,020	1,848	0.01	0.54
5	Heptanoic acid	1,085	1,959	0.02	0.01
6	2-Ethyl hexanoic acid	1,126	1,950	0.01	0.01
7	α-Fenchol	1,130	1,577	–	0.02
8	Camphene hydrate	1,147	1,442	0.67	0.02
9	Octanoic acid	1,180	2,048	0.39	0.01
10	2,4-Nonadienal	1,210	1,738	–	0.02
11	Fenchyl acetate	1,219	1,476	–	0.01
12	Isocarveol	1,239	1,620	0.39	0.01
13	2-Methyl decanal	1,251	1,521	0.99	–
14	Nonanoic acid	1,280	2,158	0.02	–
15	Methyl decanoate	1,325	1,595	0.39	6.97
16	Benzyl butyrate	1,344	1,851	0.54	0.01
17	Decanoic acid	1,354	2,280	0.01	–
18	Eugenol	1,364	2,153	0.75	0.40
19	α-Copaene	1,375	2,182	0.02	–
20	Vanillin	1,385	2,567	6.51	–
21	β-Cubebene	1,395	1,543	0.01	1.64
22	Methyl eugenol	1,405	1,985	0.01	0
23	Calarene	1,421	1,725	0.89	0
24	β-Caryophyllene	1,433	1,598	0.60	0.36
25	Alloaromadendrene	1,476	1,648	0.02	0.02
26	Germacrene D	1,488	1,714	1.24	–
27	γ-Elemene	1,495	1,642	0.01	–
28	γ-Cadinene	1,526	1,785	0.01	0.57
29	Elemol	1,545	2,076	0.03	0.94
30	Germacrene B	1,555	1,811	0.01	0.02
31	Dodecanoic acid	1,565	2,502	1.90	0.95
32	*(E)*-Nerolidol	1,572	2,039	3.15	1.02
33	Tridecanol	1,585	2,087	0.01	0.02
34	Caryophyllene oxide	1,590	1,997	0.01	0.01
35	Tetradecanal	1,610	2,168	0.02	0.01
36	γ-Eudesmol	1,628	2,191	0.01	0.01
37	α-Cadinol	1,648	2,225	0.01	0.01
38	δ-Cadinol	1,666	2,143	0.02	0.47
39	α-Bisabolol	1,682	2,268	0.64	0.01
40	Heptadecane	1,700	1,700	0.01	0.02
41	Chamazulene	1,710	1,922	0.02	0.01
42	*(E,E)*-Farnesal	1,735	2,351	0.01	–
43	Farnesal	1,760	2,287	2.70	–
44	Benzyl benzoate	1,775	2,632	0.70	5.32
45	Ethyl tetradecanoate	1,796	2,043	0.37	–
46	*(E,E)*-Farnesyl acetate	1,844	2,271	7	4.98
47	Hexadecanol	1,886	2,333	0.68	1.02
48	Nonadecane	1,900	1,900	2.01	0.01
49	Methyl palmitate	1,931	2,203	0.01	0.01
50	Isophytol	1,945	2,278	0.55	5.11
51	Hexadecanoic acid (Palmitic acid)	1,978	2,912	9.76	15.23
52	Ethyl hexadecanoate (Palmitic acid, ethyl ester)	1,990	2,246	0.03	0.01
53	Octadecanal	2,018	2,582	0.01	–
54	Manool	2,055	2,663	0.02	0.64
55	Heneicosane	2,100	2,200	0.01	0.01
56	Phytol	2,135	2,603	0.01	1.02
57	Benzyl cinnamate	2,152	2,769	2.60	0.72
58	1-Docosene	2,192	2,198	1.39	–
59	Methyl linoleate = linoleic acid methyl ester	2,200	2,200	5.26	10.8
60	Phytyl acetate	2,225	2,292	1.10	8.78
61	Tricosane	2,300	2,300	3.68	4.50
62	Tetracosane	2,400	2,400	1.11	0.348
63	Pentacosane	2,500	2,500	3.44	5.83
64	Hexacosane	2,600	2,600	10.70	7.41
65	Heptacosane	2,700	2,700	6.37	3.94
66	Octacosane	2,800	2,800	1.63	0.01
67	Nonacosane	2,900	2,900	8.44	2.46
68	Triacontane	3,000	3,000	1.88	0.01
	Yield %			0.06	0.14
	Hydrocarbons				
	Alkanes, alkenes			40.65	24.55
	Alcohols			1.26	7.17
	Aldehyde			5.02	0.03
	Terpenoids				
	monoterpenes hydrocarbons			0.5	0
	oxygenated monoterpenes			1.05	0.05
	Sesquiterpene hydrocarbons			2.81	2.61
	oxygenated sesquiterpenes			3.87	2.48
	Diterpenoids			0.02	0.64
	Aromatic compounds			11.13	6.46
	Fatty acids and fatty acid esters			26.25	48.26
	Total			92.56	92.25

L.e. Limonium echioides, *S.f. Suaeda fructicosa*, – compound absent in the volatile fraction, *RI polar* and *RI apolar* Retention indices given for the first and second eluting isomer (without assignments for *syn* or *anti* configuration) on apolar (RI-5) and polar (RI-wax) separation columns

percentage in the aerial parts of *S. fructicosa* compared to *L. echioides*. Thus, the quantitative composition and the relative proportions of the oil components are widely influenced by the genotype, ontogenic development and the environmental and growing conditions [37, 38].

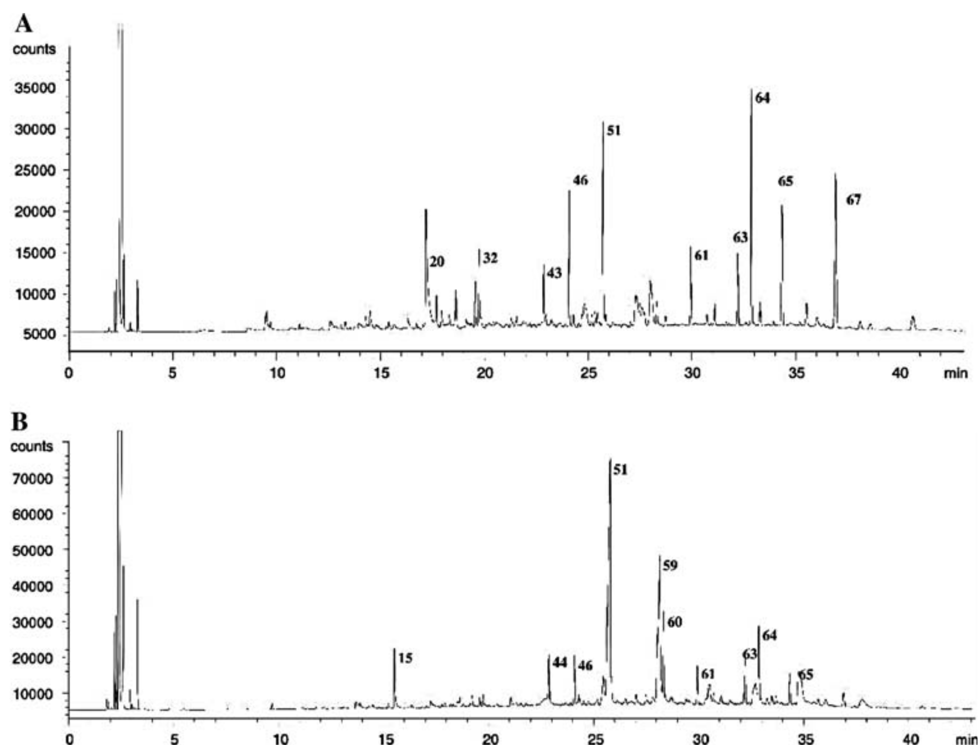

Fig. 1 Chromatograms of the volatile oils extracted from *Limonium echioides* (**a**), and *Suaeda fructicosa* (**b**) aerial parts

L. echioides was richer than *S. fructicosa* in oxygenated sesquiterpenes (3.87%) and in aldehydes (5.02%), (E)-Nerolidol was the main oxygenated sesquiterpene present (3.15%). While, *S. fructicosa* accumulated more fatty acid esters in its aerial parts (31.5%), the main components were: linoleic acid methyl ester (10.8%), phytyl acetate (8.78%), methyl decanoate (6.97%) and (*E*,*E*) farnesyl acetate (4.98%).

Antibacterial Activity

The in vitro antibacterial activity of the volatile oils against the employed microorganisms was qualitatively and quantitatively assessed depending on the presence or absence of inhibition zones, zone diameters, MIC and MBC values. According to the results given in Tables 2 and 3, the volatile oils of the tested aerial part halophytes exhibited an interesting antibacterial activity against all pathogenic bacteria tested except *P. aeruginosa*. According to several authors, these bacilli Gram-negative bacteria appear to be least sensitive to the action of many other plants essential oils [39–44]. The antibacterial activity of the volatile oils tested was more pronounced against Gram-positive than against Gram-negative bacteria. This result was in agreement with many studies carried out on other plant species [45–47]. This generally higher resistance among Gram-negative bacteria could be ascribed to the presence of the outer membrane, surrounding their cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide cover [48]. The absence of this barrier in Gram-positive bacteria allows direct contact of the essential oil's hydrophobic constituents with the phospholipid bilayer of the cell membrane, causing either an increase in ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems [49, 50].

The antibacterial activity noted against the majority of the tested bacteria (all bacteria tested except *P. aeruginosa*) could be attributed to the presence of eugenol known for its powerful antibacterial capacity [28, 51–53]. It is, respectively, concentrated as 0.75 and 0.4% in the aerial parts of *L. echioides* and *S. fructicosa.* In the same way, eugenol found in the leaf essential oil of *Cinnamomum osmophloeum* exhibited a bacteriostatic effect against *E. coli* at a concentration of 1 mg ml^{-1} but no activity was revealed against *P. aeruginosa* and *S. aureus* [54]. Eugenol has been found to inhibit production of amylase and proteases by the susceptible bacteria, cause deterioration to the cell wall and cause a high degree of cell lysis [55]. The hydroxyl group on eugenol is thought to bind to proteins, preventing enzyme activity in the bacteria [50].

Additionally, β-caryophyllene present, respectively, as 0.6 and 0.36% in the aerial parts of *L. echioides* and *S. fructicosa* is known to exhibit an important antibacterial activity [56, 57]. Indeed, the presence of a high concentration of β-caryophyllene in the essential oils of *Salvia triloba* (11.8%) applied at concentrations of 5 and 10 mg ml^{-1}, contributed to a strong antibacterial activity against *S. epidermidis*, *S. aureus*, *S. typhimurium*, *E. coli* and

Table 2 Antibacterial activity of the volatile oils for oil concentrations of 0.5 and 2 mg/disc of *Limonium echioides* and *Suaeda fructicosa*

Bacterial species	Source	Inhibition zone diameter (mm)						
			L.e.		*S.f.*		Negative control chloroform +Tween-80 (5%)	Positive control ampicillin
Oil concentration (mg/disc)			0.5	2	0.5	2		0.01
Gram (+)								
Staphylococcus aureus	*ATCC 29213*	Presence or absence of inhibition zones	(+)	(+)	(+)	(+)	(−)	(+)
		Zone diameters (mm)	7	7	7	7		17
Staphylococcus epidermidis	*NCIMB 8853*	Presence or absence of inhibition zones	(−)	(−)	(−)	(−)	(−)	(+)
		Zone diameters (mm)						18
Micrococcus luteus	*NCIMB 8166*	Presence or absence of inhibition zones	(+)	(+)	(+)	(+)	(−)	(+)
		Zone diameters (mm)	7	7	7	7		17
Gram (−)								
Escherichia coli	*ATCC 35218*	Presence or absence of inhibition zones	(−)	(−)	(−)	(−)	(−)	(+)
		Zone diameters (mm)						10
Pseudomonas aeruginosa	*ATCC 27853*	Presence or absence of inhibition zones	(−)	(−)	(−)	(−)	(−)	(+)
		Zone diameters (mm)						9
Salmonella typhimurium	*ATCC 14028*	Presence or absence of inhibition zones	(+)	(+)	(−)	(+)	(−)	(+)
		Zone diameters (mm)	6	6		6		12

L.e. Limonium echioides, *S.f. Suaeda fructicosa*, *ATCC American type culture collection*, *NCIMB national collections of industrial*, *marine and bacteria*

P. aeruginosa [58]. In our research, *S. fructicosa* and *L. echioides* oils were, however, ineffective against *P. aeruginosa*. This result may be explained by the low proportion of this compound in the tested oils, in addition to the lower applied concentration of the oils (0.5 and 0.8 mg ml^{-1} comparatively to *S. triloba* oil concentrations). In addition, possible antagonistic effects of the compounds may play an important role in bacterial inhibition [59, 60]. α-Caryophyllene has shown in-vitro antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus* [61, 62]. A combination of hydrogen bonding and size parameters could influence the antimicrobial activities of monoterpenes [63].

Table 3 Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the volatile oils of *Limonium echioides* and *Suaeda fructicosa* against some pathogenic bacteria (mg ml^{-1})

Bacterial species	Oil concentration (mg ml^{-1})		
Gram (+)		*S. f.*	*L.e.*
Staphylococcus aureus ATCC 29213	MIC	0.5	0.8
	MBC	>0.8	–
Staphylococcus epidermidis NCIMB 8853	MIC	0.8	0.8
	MBC	–	–
Micrococcus luteus NCIMB 8166	MIC	0.5	0.8
	MBC	>0.8	–
Gram (−)			
Escherichia coli ATCC 35218	MIC	0.5	0.8
	MBC	>0.8	–
Pseudomonas aeruginosa ATCC 27853	MIC	–	–
	MBC	–	–
Salmonella typhimurium	MIC	0.5	0.5
	MBC	>0.8	>0.8

L.e. Limonium echioides, *S.f. Suaeda fructicosa*, *– inactive*

Volatile compound, such as caryophyllene are likely to be the precursors of the complex menthols or resins which have been also claimed to contain antibacterial properties [64–67].

Suaeda fructicosa prevented visible growth of the majority of the tested bacteria at a lower concentration (MIC = 0.5 mg ml^{-1}). The antibacterial properties of this plant may also be related to the percentage of aliphatic alcohols, especially isophytol (5.11%), phytol (1.02%) and hexadecanol (1.02%). Indeed, fractions rich in long chain alcohols were active against bacteria [30]. The antimicrobial properties of alcohols were known to increase with molecular weight [68, 69].

Palmitic acid was found to be the major compound in a mixture of fatty acids from *L. echioides* (9.76%) and *S. fructicosa* (15.23%). This acid had been reported to exhibit antibacterial activity against Gram-positive and Gram-negative bacteria comparable to ampicillin and in the case of *Streptococci*, greater than that of ampicillin [70]. In addition, palmitic acid was considered as the major antibacterial compound in the ethyl acetate root extract of *Pentanisia prunelloides* [71].

Only aerial part volatile fractions of *L. echioides* exhibited antimicrobial activity against *S. typhimurium* (Gram-negative) at a concentration of 500 μg/disc. This activity may be attributed to the presence of the high concentration of aldehydes comparatively to the other tested halophyte (5%). The aldehyde group was known to have the best antibacterial activity [54]. Also, the eugenol which is concentrated especially in the plant aerial parts may contribute to this activity.

Moreover, the antibacterial profile of the volatile fractions of the tested halophytic plants were suspected to be associated with the oxygenated sesquiterpenes, especially α-bisabolol and the fatty acid esters farnesyl acetate and methyl linoleate which act powerfully against Gram-positive bacteria, moulds and dermatophytes [69].

Some studies have proved that the whole volatile fractions have a greater antibacterial activity compared to the major component [72, 73]. This suggests that the compounds present in the greatest proportions (palmitic acid, farnesyl acetate, methyl linoleate …) are responsible for a share of the total activity. In addition, the involvement of the less abundant constituents should be considered. And then, the activity could be attributed also to the presence of minor components such as Eugenol, β-caryophyllene and α-bisabolol, or at least to a synergistic effect between all components. In fact, the synergistic effects of the diversity of major and minor constituents present in the essential oils should be taken into consideration in order to account for their biological activity [28]. Accordingly, it has been proved that α-bisabolol have a synergistic effect with 125 ppm eugenol or menthol or octan-3-ol [69].

Results of both methods employed for the antibacterial activity are comparable (diffusion method, micro-dilution method). However, MIC and MBC values were the lowest. This suggests that the size of the inhibition zone does not reflect the real antibacterial effectiveness of a compound, since it is affected by the solubility of the oil, the diffusion range in the agar, the evaporation, etc. [52, 74].

Furthermore, both volatile oils did not show any effect on the proliferation of *E. coli*, using diffusion method. However, using broth dilution method, these bacteria have been inhibited and showed minimal inhibitory concentrations equal to 0.5 and 0.8 mg ml^{-1}, respectively, for *S. fructicosa* and *L. echioides* extracts. The negative response of *E. coli*, when using the diffusion method, may be explained by the high resistance of these Gram-negative bacteria. Indeed, *E. coli* are known to have multi-resistance to many drugs [75–77]. Additionally, the diffusion method was very delicate and could vary greatly according to the molecules [78], the organisms tested [79] and the inoculum size. Then, physical and chemical properties of the drugs as well as biological behaviour of the bacteria could be put in competition, with a rather unpredictable outcome [80].

Antifungal Activity

The volatile oils of *L. echioides* and *S. fructicosa* aerial parts failed to show antifungal activity when applied at 80, 200 and 500 μg/disc against all the test fungi. In spite of the presence of eugenol and β-caryophyllene in halophyte volatile oil compositions, which are known to possess antifungal activity [64–67, 81–83], no antifungal activity was detected. Perhaps, this result was due either to possible antagonistic effects of such compounds which block fungi inhibition [59, 60], or to the low proportions of these compounds in the halophyte volatile oil compositions. The inherent activity of an oil can be expected to relate to the chemical configuration of the components, the proportions in which they are present and the interactions between them [30, 39, 84]. A test with a higher concentration of the tested halophyte volatile oils is needed for possible antifungal activity against the selected fungi.

Our study can be considered as the first report on the antimicrobial properties of *L. echioides* and *S. fructicosa* volatile oils. Our results are a contribution to a better valorisation of these medicinal plants growing in Tunisia. Several other biological tests would be worthwhile carrying out to search for more possible activities of these plants. Phytochemical investigations would be planned to identify and characterize active principles, and assess toxicity by laboratory experiments.

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