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Phytochemical and Biological Investigation of *Spergularia marina* (L.) Griseb. Growing in Egypt

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Abstract – A phytochemical investigation of *Spergularia marina* (L.) Griseb. growing in Egypt, has been carried out, which resulted in the isolation of seven compounds from the different extracts of the plant namely; β-sitosterol glucoside, tricin (1) dihydroferulic acid (2), vanillic acid (3), 4-hydroxybenzoic acid (4), uracil (5) and 8-hydroxy cuminoic acid (6) Structure elucidation of the isolated compounds was carried out using different spectroscopic techniques. This is the first report for the isolation of these compounds from genus *Spergularia*. Furthermore, 8-Hydroxy cuminoic acid and uracil were isolated for the first time from family Caryophyllaceae. The chemical composition of the volatile components present in the petroleum ether extract of *Spergularia marina* (L.) Griseb. using combined gas chromatography-mass spectrometry (GC-MS) is reported here for the first time. Of the 97 components present, 59 were identified including three sulfur containing compounds which represented about 1.8% of the volatiles of the total petroleum ether extract. This prompted us to study and report its possible antimicrobial activity. In addition, the antibacterial and antifungal screening of different extracts of *Spergularia marina* (L.) Griseb. as well as some isolates have been performed using agar diffusion method.
Keywords – Caryophyllaceae, *Spergularia marina* (L.) Griseb., Antimicrobial activity, Gas chromatography-mass spectrometry

Introduction

Family Caryophyllaceae contains many economic plants, it is the source of many ornamental and fragrant decorative plants.[1,2] Genus *Spergularia* belongs to family Caryophyllaceae, subfamily Paronychioidea, tribe polycarpeae.[3] It includes 25 species distributed especially in cosmopolitan.[4,5] Literature survey revealed that some of the *Spergularia* species are found to have several biological activities. The aerial parts of *S. rubra* L. have a diuretic effect and have been used internally, as a liquid extract for cases of cystitis, dysuria and urinary calculus. The leaves of *S. ramosa* Cambess. are used in the form of a decoction, as a remedy for respiratory ailments, tuberculosis, and rickets.[6] For many years, the water extract of the whole plant of *S. purpurea* has been traditionally used by Moroccan population against many diseases, like diabetes, hypertension, renal disorders and cardiac diseases without much scientific basis of its therapeutic effect.[7,8]

The work presented in this paper includes the phytochemical investigation of *Spergularia marina* (L.) Griseb., the most common *Spergularia* species in Egypt, which resulted in the isolation of seven compounds from the different extracts of the plant. These are β-sitosterol glycoside and tricin (**1**) which were isolated from the chloroform extract, dihydroferulic acid (**2**), vanillic acid (**3**), 4-hydroxybenzoic acid (**4**), uracil (**5**) and 8-hydroxy cuminoic acid (**6**) which were isolated from the ethyl acetate extract. Structure elucidation of the isolated compounds was carried out using different spectroscopic techniques.

The volatile oil analysis of some caryophyllaceous plants are reported in the literature. The volatile constituents of *Cerastium candidissimum* Correns (Caryophyllaceae) were extracted by steam distillation and analyzed by gas chromatography-electron impact mass spectroscopy "GC-*ei*MS". Floral scent of 13 *Silene* species (Caryophyllaceae) was analyzed by gas chromatography and mass spectrometry; benzenoids together with isoprenoids dominated the scent in all species.[9] Meanwhile, the essential oil of air-dried *Minuartia meyeri* (Boiss.) Bornm. (Caryophyllaceae) was obtained by hydro-distillation and analyzed by GC-MS. Fifty-two components were identified in the oil. The antimicrobial activity of the isolated

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essential oil of the plant was also investigated and showed moderate antibacterial activity against Gram-positive and Gram-negative bacteria, but no antifungal activity was observed against two yeast-like fungi.[10]

To our knowledge, there are no published reports on the chemical composition of the volatile constituents of *Spergularia*. In addition the antimicrobial activity of the petroleum ether extract of *Spergularia marina* (L.) Griseb. has been studied. Furthermore, the antibacterial and antifungal screening of different extracts, as well as some isolates from *Spergularia marina* (L.) Griseb., has been performed using agar diffusion method.

Experimental

General experimental procedures – Mps. were determined on a Stuart SMP heating stage microscope and are uncorrected. UV spectra were determined on Pye Unicam SP8-100 UV/VIS Spectrophotometer. 1D-NMR and 2D-NMR (COSY, HSQC and HMBC) spectra were recorded on Jeol 500 MHz spectrometer (Japan) and- Bruker 500 MHz spectrometer (Germany). EI/MS were taken at Finnigan-Mat SSQ 7000 at 70 eV (Germany) and JEOL JMS AX 500 mass spectra (Japan). Analytical and preparative TLCs were performed on silica gel (Merck, Kieselgel, 60 F_{254}, 0.25 and 0.50 mm, respectively). Spots were visualized by exposure to UV radiation, anisaldehyde/H_2SO_4. GC-MS analysis was carried out using Clarus 600 Gas Chromatograph/Mass spectrometer (Perkin Elmer, USA) provided with Turbomass 5.03 software fitted with Rtx®-5MS (a fused silica, low-polarity phase; 5% diphenyl/95% dimethyl polysiloxane from Restek) column 30 m × 0.32 mm ID and 0.25 μm film thickness. Helium used as carrier gas at flow rate 1.1 mL/min.

Plant material – *Spergularia marina* (L.) Griseb. was collected in April 2006 during flowering stage from Rosetta 40 km East of Alexandria, Egypt. The plant was identified at the Department of Botany, Faculty of Science, University of Alexandria, Egypt.

Extraction and isolation – Fresh aerial parts (10 kg) of *S. marina* (L.) Griseb., collected at the flowering stage, were chopped and exhaustively extracted with 70% ethanol (40 L) at room temperature. The extract was concentrated under reduced pressure to small volume then fractionated successively with petroleum ether (4 × 500 mL), chloroform (3 × 500 mL), ethyl acetate (3 × 500 mL) and finally *n*-butanol (3 × 500 mL). The solvents were distilled off and the corresponding residues were weighed. Five grams of the residue of the chloroform extract was chromatographed on a column (64 × 3.5 cm) packed with silica gel (200 g) in chloroform. Elution was started by chloroform with increasing polarity of methanol. Fractions (200 mL each) were collected, screened by TLC systems (chloroform : methanol 9 : 1 and ethyl acetate : methanol : water : acetic acid 60 : 5 : 4 : 1) and similar fractions were combined. β-sitosterol *O*-β-D-glucopyranoside was identified by direct comparison with reference sample through m.p., mixed m.p., and co-chromatography. Chromatographic separation resulted in the isolation of tricin (**1**). Structure elucidation of the isolated compound was carried out using different spectroscopic techniques.

The residue (3 g) of ethyl acetate extract was chromatographed on a column (31 × 9 cm, negative pressure) packed with silica (400 g) in methylene chloride using vacuum liquid chromatography. Elution was started by methylene chloride with increasing polarity of methanol. Fractions, 400 mL each were collected, screened by TLC systems (chloroform : methanol 9 : 1, ethyl acetate : methanol : water 30 : 5 : 4 and ethyl acetate : methanol : water : acetic acid 60 : 5 : 4 : 1) and similar fractions were combined. Chromatographic separation resulted in the isolation of dihydroferulic acid (**2**), vanillic acid (**3**), 4-hydroxybenzoic acid (**4**), uracil (**5**) and 8-hydroxy cuminoic acid (**6**) from the ethyl acetate extract. Structure elucidation of the isolated compounds was carried out using different spectroscopic techniques.

GC-MS analysis of the petroleum ether extract of *Spergularia marina* (L.) Griseb. – The fresh flowering plant (250 g) of *Spergularia marina* (L.) Griseb. was extracted with petroleum ether (1 L) by percolation till exhaustion at room temperature. The solvent was evaporated under reduced pressure at 30 ºC to give yellowish brown residues (520 mg). A weight of 100 mg of the residue was used for GC/MS analysis. GC/MS analysis was carried out under the following conditions Column Rtx®-5MS (30 m × 0.32 mm i.d. and 0.25 μm film thickness), carrier gas: helium, mass mode: EI, 70 eV, injection port temperature 280 ºC, column temperature programme 40 ºC for 0 minute (rate 5 ºC/minute) up to 300 ºC for 15 minutes. The identity of the components was established by logical and analogical interpretation of their retention times, and mass fragments with those available in the literature in addition to computer searches at the National Research Center, Cairo, Egypt MS Data Library. Components were quantified as relative area percentage of total ion current generated.

Antibacterial and antifungal activities of different extracts of *Spergularia marina* (L.) Griseb. – Antibacterial and antifungal assays were carried out using the agar diffusion technique[11] against three Gram-positive bacteria;

Staphylococcus aureus, *Micrococcus luteus* and *Bacillus subtillus*, two Gram-negative bacteria; *Esherichia coli* and *Pseudomonas aeroginosea*, and the fungus *Candida albicans*. The used organisms are local isolates provided from the Department of Microbiology, Faculty of Pharmacy, University of Alexandria. One mL of 24 hours broth culture of each of the tested organisms was separately inoculated into 100 mL of sterile molten nutrient agar maintained at 45 ºC. The inoculated medium was mixed well and poured into sterile 10 cm diameter Petri-dishes, receiving 15 mL. After setting, ten cups, each 8 mm in diameter, were cut in the agar medium (Oxoid). A portion (200 mg) of the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb. in addition to the chloroform, ethyl acetate and *n*-butanol extracts, were accurately weighed, dissolved in 3 mL DMSO, the solutions were inserted in the cups and incubated at 37 ºC for 24 hours. 3 mg of each of tricin, *p*-hydroxybenzoic acid and 8-hydroxy cuminoic acid were accurately weighed, dissolved in 3 mL DMSO, the solutions were inserted in the cups and incubated at 37 ºC for 24 hours. The Minimum inhibitory concentration (MIC) and the Minimum bactericidal concentration (MBC) were carried out using the agar dilution method.[12]

Tricin (1) – Yellow crystals, mp 291 - 292 ºC, UV λ_{max}, nm (abs.): MeOH 349, 270, MeOH/ NaOMe 419, 263, 278 (sh), MeOH/ $AlCl_3$ 369 (sh), 392, 259 (sh), 278, MeOH/ $AlCl_3$/HCl 363 (sh), 389, 257 (sh), 279, MeOH/ NaOAc 418, 264, 278 (sh); EI-MS (rel. int) *m/z*: 331 (14.8), 330 $[M]^+$(62.2), 178 (17.8), 153 (24.4), 152 (8.9), 135 (18.5), 124 (20.0), 119 (11.9), 116 (7.4), 107 (17.0), 108 (31.9), 105 (22.2), 91 (33.3), 74 (22.2); ^{1}H-NMRδ: 7.21 (2H,s, H-2', 6'), 6.63 (1H,s, H-3), 6.45 (1H,br.s, H-8), 6.19 (1H,br.s, H-6), 3.93 (6H,s, 3',5'-OCH_3), ^{13}C -NMR δ: 182.38 (C-4), 164.75 (C-2), 164.61 (C-7), 161.30 (C-5), 157.80 (C-9), 148.29 (C-3', 5'), 139.70 (C-4'), 120.80 (C-1'), 103.98 (C-2', 6'), 103.84 (C-10), 103.12 (C-3), 98.89 (C- 6, 8), 55.68 (3',5'-OCH_3).

Dihydroferulic acid (2) – Orange crystals, mp 89 - 90 ºC,; UV λ_{max}, nm (abs.) MeOH: 280 (0.978); EI-MS (rel. int) *m/z*: 197 (8.35), 196 $[M]^+$ (30.47), 195 $[M^+ - H]$ (33.99), 180 $[M^+ - H - CH_3]$ (42.9), 178 $[M^+ - H_2O]$ (35.31), 151 $[M^+ - COOH]$ (41.83), 138 $[M^+ + H - CH_2COOH]$ (100), 123 $[M^+ - CH_2\ CH_2COOH]$ (38.01), 119 $[M^+ - H_2O - CH_2COOH]$ (61.6), 107 $[M^+ + H - OCH_3 - CH_2COOH]$ (68.59), 91 $[C_7H_7]$ (23.63), 78 $[C_6H_6]$ (50.24); ^{1}H NMR, δ: 6.80 (1H, d, H-2), 6.66 (1H, d, H-5), 6.63 (1H, dd, H-6), 3.82 (3H, s, 3-OCH_3), 2.80 (2H, t, H-7), 2.42 (2H, t, H-8); ^{13}C- NMR, δ: 177.3 (s, C-9), 147.7(s, C-3), 144.7 (s, C-4), 134.1(s, C-1), 120.6 (d, C-6), 115.6 (d, C-5), 112.9(d, C-2), 55.9 (q, 3-OCH_3), 32.3 (t, C-7), 29.5 (t, C-7).

Vanillic acid (3) – Pale orange crystals, mp 210 - 212 ºC,; UV λ_{max}, nm (abs.) MeOH: 287(0.994); EI-MS (rel. int) *m/z*: 169 (28.38), 168 $[M^+]$ (100), 154 $[M+H-CH_3]$ (16.24), 139 [M-H-CO] (44.92), 93$[M-CO_2-OCH_3]$ (34.07); ^{1}H NMR, δ: 7.44 (1H, br.d, H-2), 7.37 (1H, dd, H-6), 6.74 (1H, d, H-5), 3.76 (3H, s, 3-OCH_3); ^{13}C- NMR, δ: 171.35 (s, C-7), 149.25 (s, C-4), 147.15 (s, C-3), 126.53 (s, C-1), 115.06 (d, C-5), 123.66 (d, C-6), 113.07 (d, C-2), 55.85 (q, 3-OCH_3).

4-Hydroxybenzoic acid (4) – Pale yellow crystals, mp 213 - 214 ºC; UV λ_{max}, nm (abs.) MeOH: 278 (1.064); EI-MS (rel. int) *m/z*: 139 (21.0), 138 $[M^+]$ (100), 121 [M - OH] (86.41), 105 [M+H-OH-OH], (35.58), 93 [M-COOH] (44.28), 78 $[C_6H_6]$ (93.43); ^{1}H NMR, δ: 7.73 (2H, d, H-2, 6), 6.72 (2H, d, H-3, 5); ^{13}C- NMR, δ: 169.22 (s, C-7), 159.46 (s, C-4), 130.63 (d, C-2, 6), 127.55 (s, C-1), 113.96 (d, C-3, 5).

Uracil (5) – Yellowish white amorphous residue, mp 335 ºC; UV λ_{max}, nm (abs.) MeOH: 278 (1.189); IR ν_{max} cm^{-1} (KBr): 3410.1(N-H), 3103.8 (=C-H), 1722.4 (C=O); CHN: C% 42.47, H%3.33, N%18.93, O% 35.27; EI-MS (rel. int) *m/z*:112 $[M^+]$ (48.7%), 96 (20.5), 86 (21.8), 73(100); ^{1}H NMR, δ: 10.93 (2H, br.s, NH), 7.39 (1H, d, H-6), 5.45 (1H, d, H-5); ^{13}C- NMR, δ: 164.26 (s, C-4), 151.45 (s, C-2), 142.09 (d, C- 6), 100.18 (d, C-5).

8-Hydroxy cuminoic acid (6) – Pale yellow crystals, mp 156 - 157 ºC; UV λ_{max}, nm (abs.) MeOH: 280 (1.028); EI-MS (rel. int) *m/z*: 180 $[M]^+$ (7.66%), 166 (100%), 165 (18.21%),163 (13.17%), 150 (14.87%), 135 (14.79%), 121 (35.22%), 118 (16.26%), 105 (51.14%); ^{1}H NMR, δ: 7.83 (2H, d, H-2, 6), 7.43 (2H, d, H-3, 5), 1.43 (6H, s, CH_3-9, 10); ^{13}C- NMR, δ: 169.12 (s, C-7), 152.16 (s, C-4), 134.78 (s, C-1), 128.42 (d, C-2, 6), 123.47 (d, C-3, 5), 70.44 (s, C-8), 31.64 (q, C-9, 10).

Result and Discussion

The phytochemical investigation of *Spergularia marina* (L.) Griseb. resulted in the isolation of seven compounds (Fig. 1) from the different extracts of the plant. They were identified as *β*-sitosterol glycoside and tricin (**1**)[13] which were isolated from the chloroform extract. Meanwhile, dihydroferulic acid (**2**),[14] vanillic acid (**3**),[15] 4-hydroxybenzoic acid (**4**),[16] uracil (**5**),[17] and 8-hydroxy cuminoic acid (**6**)[18] were isolated from the ethyl acetate extract. Structure elucidation of the isolated compounds was carried out using different spectroscopic techniques. It is worth mentioning that this is the first report for the isolation of these compounds from genus *Spergularia*.

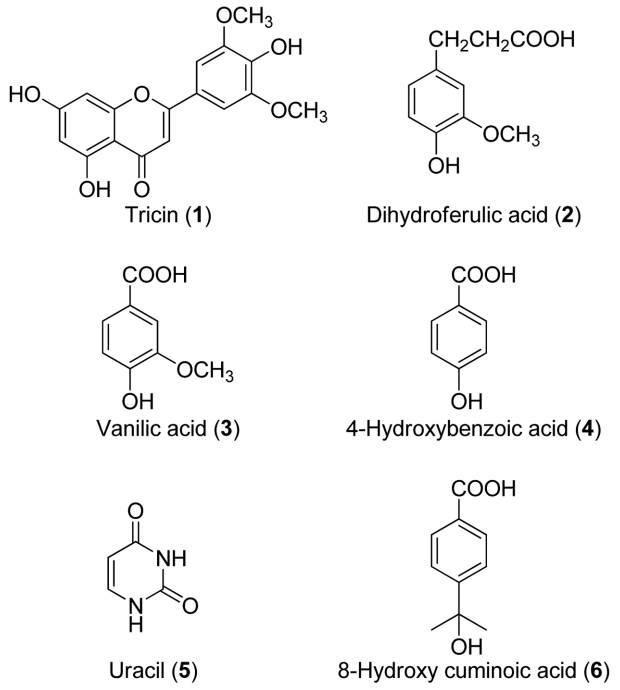

Fig. 1. Compounds isolated from *Spergularia marina* (L.) Griseb.

Furthermore, 8-hydroxy cuminoic acid and uracil were isolated for the first time from family Caryophyllaceae.

EI-MS spectrum of uracil (**5**) showed a molecular ion peak at m/z 112 $[M]^+$ calculated for a molecular formula $C_4H_4O_2N_2$ which indicated four degree of unsaturation, supported by data from the elemental analysis. The ^1H, ^{13}C-NMR and DEPT experiment spectral data revealed the presence of 2, 4-pyrimidinedione through the appearance of two aromatic doublets each integrated for one proton, at 5.45 (J = 7.5 Hz) and 7.39 (J = 7.5 Hz), and two N-H proton through the appearance of a highly deshielded broad singlet integrated for 2 protons at δ 10.93. ^{13}C-NMR spectrum and DEPT experiment showed two carbonyl functional groups at δ 151.45 and 164.26, and two methines carbon at δ 100.18 and 142.09. The HMQC and the HMBC experiments allowed the assignment of the protons to the corresponding carbons. From the previously mentioned discussion, the structure of **5** is identified as 2,4(1H, 3H)-pyrimidinedione (uracil) which was confirmed by comparing its spectral data with those reported in the literature.[17]

The EI-MS spectrum of 8-hydroxy cuminoic acid showed a molecular ion peak at *m/z* 180 suggesting a molecular formula $C_{10}H_{12}O_3$ which indicated five degree of unsaturation, supported by data from UV spectrum. The ^1H and ^{13}C-NMR spectral data revealed the presence of one 1, 4-disubstituted benzene ring through the appearance of two aromatic doublets each integrated for two protons, at δ 7.43 (J = 7.5 Hz) and 7.83 (J = 7.5 Hz), two tertiary methyls through the appearance of a singlet integrated for six protons at δ 1.43, one hydroxylated quaternary carbon at δ 70.44 and one carboxyl functional group at δ 169.12. The DEPT experiment confirmed the presence of two methyl, four methines and four quaternary carbons. The HMQC and the HMBC experiments allowed the assignment of the protons to the corresponding carbons. From the previously mentioned discussion the structure of (**6**) is identified as 4-(1-hydroxy-1-methylethyl) benzoic acid (8-hydroxy cuminoic acid) which was confirmed by comparing its spectral data with those of 8-hydroxycuminyl β-D-glucopyranoside reported in the literature.[18]

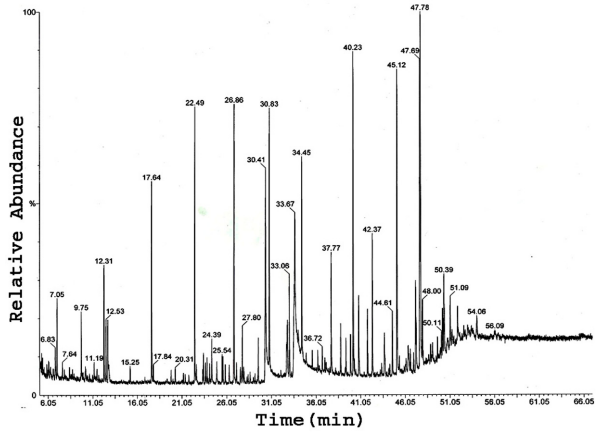

Fig. 2. GC chromatogram of the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb.

The chemical composition of the volatile components present in the petroleum ether extract of *Spergularia marina* (L.) Griseb. was investigated by combined GC/MS spectrometry. The GC chromatogram (Fig. 2) showed 97 peaks corresponding to 97 components. Only 59 Components were identified by GC/MS that almost constitutes about 86.25% of the volatiles of the total petroleum ether extract of *Spergularia marina* (L.) Griseb. The identification of the previous peaks was established through National Institute of Standards and Technology (NIST) mass data search libraries and the highest REV similarity index hits. Results of qualitative and quantitative analysis of petroleum ether extract of *Spergularia marina* (L.) Griseb. are given in Table 1. Results showed that the percentage of: the hydrocarbons constituted about 44.2%, the oxygen-containing compounds constituted about 39.9%, the sulphur-containing compounds constituted about 1.8%, the nitrogen-containing compounds constituted about 0.51% and the unidentified compounds was 13.75%. The

Table 1. Results of GC/MS analysis of the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb.

Peak Number	Retention time Min.	Relative % composition	M^+ peak	Base peak	REV	Component
1	7.045	0.70%	184	57	941	Undecane, 4, 6-dimethyl $C_{13}H_{28}$
2	9.751	0.64%	156	57	969	Undecane $C_{11}H_{24}$
3	11.529	0.22%	178	32	869	Disulfide, (1,1-dimethylethyl)(1-methylpropyl) $C_8H_{18}S_2$
4	12.31	1.56%	196	69	975	3-Tetradecene $C_{14}H_{28}$
5	12.532	0.70%	184	57	960	Tridecane $C_{13}H_{28}$
6	12.74	1.13%	120	41	964	Tetrahydrothiophene 1,1-dioxide $C_4H_8O_2S$
7	15.249	0.27%	212	32	930	Dodecane,2,6,11-trimethyl $C_{15}H_{32}$
8	17.644	2.82%	224	69	979	3-hexadecene $C_{16}H_{32}$
9	22.488	3.47%	252	32	961	3-Octadecene $C_{18}H_{36}$
10	23.404	0.22%	232	32	916	Benzene, (1-pentylhexyl) $C_{17}H_{28}$
11	23.471	0.36%	232	91	947	Benzene, (1- butylheptyl) $C_{17}H_{28}$
12	24.138	0.19%	232	32	930	Benzene, (1- ethylnonyl) $C_{17}H_{28}$
13	24.393	0.55%	208	32	899	2-Cyclohexen-1-one,3,5,5-trimethyl-4-(3-oxobutyl)-$C_{13}H_{20}O_2$
14	24.947	0.48%	232	105	948	Benzene, (1-methyldecyl) $C_{17}H_{28}$
15	25.540	0.37%	246	91	956	Benzene, (1- pentylheptyl) $C_{18}H_{30}$
16	25.641	0.35%	246	91	957	Benzene, (1- butyloctyl) $C_{18}H_{30}$
17	25.889	0.25%	246	91	955	Benzene, (1-propylnonyl) $C_{18}H_{30}$
18	26.335	0.38%	246	32	944	Benzene, (1-ethyldecyl) $C_{18}H_{30}$
19	26.859	3.65%	280	69	982	3-Eicosene $C_{20}H_{40}$
20	27.134	0.30%	246	105	940	Benzene, (1-methylundecyl) $C_{18}H_{30}$
21	27.593	0.20%	260	91	933	Benzene, (1-pentyloctyl) $C_{19}H_{32}$
22	27.805	0.60%	296	68	934	3,7,11,15-Tetramethyl-2-hexadecen-1-ol $C_{20}H_{40}O$
23	27.962	0.24%	268	32	816	2-Pentadecanone,6, 10, 14-trimethyl-$C_{18}H_{36}O$
24	28.432	0.24%	260	32	913	Benzene, (1-ethylundecyl) $C_{19}H_{32}$
25	28.663	0.20%	252	32	883	14-Heptadecenal $C_{17}H_{32}O$
26	29.576	0.79%	228	74	927	Methyl tridecanoate ester $C_{14}H_{28}O_2$
27	30.41	5.61%	312	73	937	Eicosanoic acid $C_{20}H_{40}O_2$
28	30.827	3.31%	280	69	973	9-Eicosene $C_{20}H_{40}$
29	32.782	1.02%	296	57	963	Heptadecane,2,6,10,15-tetramethyl $C_{21}H_{44}$
30	32.849	0.70%	320	32	929	11, 14, 17-Eicosatrienoic acid, methyl ester $C_{21}H_{36}O_2$
31	33.06	1.38%	296	71	945	Phytol $C_{20}H_{40}O$
32	33.671	8.47%	224	79	921	6,9-pentadecadien-1-ol $C_{15}H_{28}O$
33	33.88	1%	252	32	926	14-methyl-8-hexadecyn-1-ol $C_{17}H_{32}O$
34	34.04	1.17%	266	32	875	2-Octylcyclopropene-1-heptanol $C_{18}H_{34}O$
35	34.45	2.87%	284	83	973	1-Nonadecanol $C_{19}H_{40}O$
36	36.72	0.51%	213	32	887	1-Hexyl-2-nitrocyclohexane $C_{12}H_{23}O_2N$
37	37.77	1.55%	340	83	971	1-heneicosyl formate $C_{22}H_{44}O_2$
38	38.84	0.66%	450	71	931	Docosane, 11-decyl- $C_{32}H_{66}$
39	39.41	0.44%	402	71	903	Disulfide, di-tert-dodecyl $C_{24}H_{50}S_2$
40	39.92	0.69%	286	32	901	Hexadecanoic acid, 11-hydroxy-,methyl ester $C_{17}H_{34}O_3$
41	40.23	4.44%	278	149	975	1,2-Benzenedicarboxylic acid, Mono(2-ethylhexyl) ester $C_{16}H_{22}O_4$
42	40.85	1.56%	322	83	965	9-Tricosene $C_{23}H_{46}$
43	41.83	1.23%	478	57	941	Tetracosane, 11-decyl- $C_{34}H_{70}$
44	42.36	2.2%	296	71	935	Heptadecane, 2, 6, 10, 15-tetramethyl $C_{21}H_{44}$

Table 1. continued

Peak Number	Retention time Min.	Relative % composition	M^+ peak	Base peak	REV	Component
45	43.707	1.1%	490	32	958	17-Pentatriacontene $C_{35}H_{70}$
46	44.61	1.28%	436	71	932	n- Hentriacontane $C_{31}H_{64}$
47	45.12	6.33%	618	71	937	Tetratetracontane $C_{44}H_{90}$
48	46.40	0.96%	604	32	906	Tritetracontane $C_{43}H_{88}$
49	47.21	1.30%	506	57	943	Hexatriacontane $C_{36}H_{74}$
50	47.29	0.78%	718	32	924	1-Pentacontanol $C_{50}H_{102}O$
51	47.70	4.82%	478	71	940	Tetratriacontane $C_{34}H_{70}$
52	47.78	5.21%	592	57	974	1-Hentetracontanol $C_{41}H_{84}O$
53	48.00	0.9%	282	59	888	2-Nonadecanone $C_{19}H_{38}O$
54	48.897	0.40%	648	32	785	Docosanoic acid, docosyl ester $C_{44}H_{88}O_2$
55	49.125	0.52%	506	32	791	D-mannitol, 1-O-(22-hydroxydocosyl)- $C_{28}H_{58}O_7$
56	49.659	0.39%	366	32	834	Octadecane, 3-ethyl-5-(2-ethylbutyl)- $C_{26}H_{54}$
57	50.111	0.25%	380	32	815	Octadecane, 9-ethyl-9-heptyl $C_{27}H_{56}$
58	50.24	0.61%	336	32	880	Cyclodocosane, ethyl- $C_{24}H_{48}$
59	50.39	1.71%	398	81	840	Ergosta-7,22-dien-3-ol $C_{28}H_{46}O$

Table 2. Results of antibacterial and antifungal screening of the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb.

Microorganisms	Inhibition zone (IZ) in mm				
	Volatile fraction	DMSO	Ciprofloxacin (5 µg/disc)	Clotrimazole (10 mg/mL)	Rifampicin (5 µg/disc)
Gram-positive bacteria					
Staphylococcus aureus	11	–	30	–	32
Micrococcus luteus	12	–	32	–	30
Bacillus subtillus	18	15	31	–	30
Gram-negative bacteria					
Pseudomonas aeroginosea	18	18	33	–	–
Esherichia coli	–	–	38	–	–
Fungi					
Candida albicans	13	13	–	40	–

DMSO and commercial discs of ciprofloxacin, clotrimazole and rifampicin were used as negative and positive controls.

main components in the volatile fraction of *Spergularia marina* (L.) Griseb. were 6,9-pentadecadien-1-ol (8.47%), tetratetracontane (6.33%), eicosanoic acid (5.61%), 1-hentetracontanol (5.21%), tetratriacontane (4.82%), 1,2-benzenedicarboxylic acid- mono (2-ethylhexyl) ester (4.44%), 3-micosene (3.65%), 3-octadecene (3.47%), 9-eicosene (3.31%), 1-nonadecanol (2.87%), 3-hexadecene (2.82%) and heptadecane, 2, 6, 10, 15-tetramethyl (2.2%), whereas, three sulfur-containing compounds were identified and represent about 1.8% of the volatiles of the total petroleum ether extract of *Spergularia marina* (L.) Griseb. It is important to mention that this is the first report for the GC/MS analysis of the volatile constituents of *Spergularia marina* (L.) Griseb.

Table 3. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb.

Gram-positive bacteria	MIC (mg/mL)	MBC (mg/mL)
Staphylococcus aureus	0.83	3.34
Micrococcus luteus	3.34	3.34
Bacillus subtillus	3.34	0.83

Multiple drugs resistance has become a very real issue in pharmaco-therapeutics as there are an increasing number of diseases which are exhibiting various levels of drug resistance, including bacterial infections. The search

Table 4. Results of antibacterial and antifungal screening of the different extracts of *Spergularia marina* (L.) Griseb. and some of its isolates

Extract/Isolate	Inhibition zone diameter (IZD) in mm						
	Bacteria						Fungi
	Gram-positive bacteria				Gram-negative bacteria		
	Staphylococcus aureus	*Sarcina*	*Micrococcus luteus*	*Bacillus subtillus*	*Pseudomonas aeroginosea*	*Esherichia coli*	*Candida albicans*
Petroleum ether	12	13	12	18	11	–	13
Chloroform	12	14	15	17	11	–	13
Ethyl acetate	12	14	15	19	11	–	13
n-Butanol	–	11	11	17	11	–	15
Tricin	–	–	11	18	18	–	15
p-Hydroxybenzoic acid	–	–	11	18	18	–	15
8-Hydroxy cuminoic acid	–	–	11	18	18	–	15
DMSO	–	–	–	14	11	–	12
Ciproflaxacin (5 μg/disc)	30	–	32	31	33	38	–
Ampicillin (10 μg/disc)	30	31	31	30	–	–	–
Clotrimazole (0.01 g/mL)	–	–	–	–	–	–	40
Imipenam (10 μg/disc)	30	32	32	31	30	–	–

Table 5. Minimum inhibitory concentration (MIC) and inimum bactericida concentration (MBC) of different extracts of *Spergularia marina* (L.) Griseb. and some of its isolates

Extract/Isolate	Gram-positive bacteria								Fungi
	Staphylococcus aureus		*Sarcina*		*Micrococcus luteus*		*Bacillus subtillus*		*Candida albicans*
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	(mg/mL)
Petroleum ether	3.33	6.66	0.83	3.33	0.83	3.33	0.105	0.21	–
Chloroform	0.21	1.67	0.21	1.67	0.21	0.83	0.21	0.21	–
Ethyl acetate	1.1	1.1	0.28	0.55	0.28	1.1	0.07	1.1	–
n-Butanol	–	–	3.33	–	3.33	–	3.33	–	1.67
Tricin	–	–	–	–	0.1	–	0.1	–	0.05
p-Hydroxybenzoic acid	–	–	–	–	0.1	–	0.1	–	0.1
8-Hydroxy cuminoic acid	–	–	–	–	–	–	–	–	0.1

for new drugs to combat this problem is receiving much attention. The presence of sulfur containing compounds in the volatile fraction of *Spergularia marina* (L.) Griseb. prompted us to study its possible antimicrobial activity. The results of antibacterial and antifungal activities screening (Tables 2 and 3) showed that the volatile fraction of petroleum ether extract of *Spergularia marina* (L.) Griseb. has only antibacterial activity against Gram-positive bacteria; *Staphylococcus aureus*, *Micrococcus luteu*s and *Bacillus subtillu*s.

Antimicrobial activity testing was carried out for the different fractions in an attempt to define the biologically active fraction. The results of antibacterial and antifungal activities screening (Tables 4 and 5) showed that all the tested extracts and isolates didn't exhibit antibacterial activity against the Gram-negative *Esherichia coli* and *Pseudomonas aeroginosea*. All the tested extracts of *Spergularia marina* (L.) Griseb. exhibited activity against all the tested Gram-positive bacteria, except the *n*-butanol which didn't exhibit activity against *Staphylococcus aureus*. On the other hand, all the tested extracts except, *n*-butanol, didn't exhibit activity against the fungus *Candida albicans*. The ethyl acetate and chloroform fractions were the most active and therefore they were chosen for further investigation which resulted in the isolation of the previously mentioned seven compounds. The major com-

pounds isolated were also tested to compare their results with the extracts. The extracts were more active. Tricin and *p*-hydroxybenzoic acid showed activity against Gram-positive bacteria: *Bacillus subtillus*, *Micrococcus luteus* and the fungus *Candida albicans*. 8-hydroxy cuminoic acid showed activity only against the fungus *Candida albicans*.

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