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Comparative Chemical and Biological Study of Roots and Aerial Parts of *Halocnemum strobilaceum* Growing Wildly in Egypt.

M. M. M. Abdel Razek[1], A. Y. Moussa*[2,3], M. A. El-Shanawany[1], A. B. Singab[2,3]

[1] *Faculty of Pharmacy, Badr University in Cairo, Egypt*

[2] *Faculty of Pharmacy, Ain Shams University, Cairo, Egypt*

[3]*Center for Drug Discovery Research and Development, Ain Shams University, Cairo, Egypt*

Abstract

Halocnemum strobilaceum is a terrestrial halophytic plant with reported folk medicinal uses. RAPD-PCR was used to amplify the DNA fragments where 83 amplified DNA fragments result from the RAPD-PCR, and primer P2 was the best sequence dominating them. GC/MS analysis identified the components of both the root and the aerial parts hexane extracts as hexadecanoic acid methyl ester (38.88%) and 9-octadecenoic acid (Z)- methyl ester (23.25%) in the former and dodecane (9.83%), heptadecane (8.26%) and octadecane (8.2%) in the latter. MTT cell viability assay was carried out on three different human cell lines, MCF-7, Hep-G2 and Caco-2, to evaluate the cytotoxic potential of *H. strobilaceum*; furthermore, the cup well diffusion and serial dilution methods were utilized to screen for the anti-infective potential. Marked activity was recorded for the aerial part against the MCF-7 cell line after 24h, 48h and 72h, with IC_{50} values of 227.40, 184.45 and 135.68 µg/ml, respectively, and moderate cell killing effect was manifested by the root part with IC_{50} values of 341.98, 308.77 and 218.99 µg/ml. *H. strobilaceum* root extract exhibits significant effect against *Bacillus subtilis* and *Staphylococcus aureus*, with MIC values of 6.25 and 50 µg/ml respectively. *H. strobilaceum* is a promising candidate for further future biological studies.

Keywords: Antimicrobial activity, cytotoxicity, GC/MS analysis, *Halocnemum strobilaceum*, genetic profiling, root hexane extract.

INTRODUCTION

Exploration of lead drugs' natural resources has been always a global and national need [1]. Medicinal plants were highlighted for their unusual and unexpected chemical diversity and lead structures[2]. Family Chenopodiaceae was reported to have many biological activities; especially, in the Algerian ethnobotanical literature where it represents 23.33% of folk medicinal uses compared to other families such as Asteraceae and Fabaceae, which show 16.66% only of tribal herbal remedies [3]. Chenopodiaceae, the goosefoot family, contains about 103 genera and 1300 species with cosmopolitan distribution but largely centered in arid regions; particularly, on saline soils [4]. In the flora of Egypt, the family was represented by 77 wild species in 20 genera and few cultivated ones [5]. *Halocnemum strobilaceum*, the terrestrial halophytic plant, growing wildly in Egypt was represented by only one specie along the North Coast and the Red Sea region where it is largely distributed [6-7]. Even though no reported data were traced for *H. strobilaceaum* medicinal use in the Egyptian folk medicine, it was utilized in Algeria and Iran as a digestive, stimulant and cure for fever and headache [3-8]. In Syria, the Bedouins used *H. strobilaceum* to feed camels until they noticed that the fed animals developed a lung disease later on [9].

Although some studies revealed the chemical and biological prospects of the aerial part of *H. strobilaceum*, scanty work was reported about the root part. Due to the high amounts of unsaturated fatty acids in *H. strobilaceum*, 65-74%, that were known for their health benefits, it was reported as a potentially good source of edible oil [10]. The non-polar fraction of the plant's aerial part contains a series of famous hydrocarbons; palmitic acid, campesterol, stigmasterol, phytosterols and alpha-amyrin. Additionally, 63% of the volatile oil content was identified and manifested high hydrocarbon content, minor oxygenated hydrocarbons, sesquiterpene hydrocarbons and oxygenated sesquiterpenes [11]. *H. strobilaceum* different extracts exhibited no cytotoxic activity against the Ehrlich ascites carcinoma cell line, and the volatile oil showed moderate activity in the same assay. The non-polar extract of the aerial parts exhibited significant antimicrobial activity at the concentration of 100 µg/ml; however, no appreciable activity was reported on lower concentration [11-12].

The study herein aims to genetically profile *H. strobilaceum* growing wildly in Egypt and to characterize comparatively the different components of its n-hexane extracts of root and aerial parts. Screening its uses to achieve an economical value application in the pharmaceutical and medical fields.[13–15]. hence, abiotic stresses on medicinal halophytes was reported as potent source of health promoting medical biomolecules [16].

MATERIALS AND METHODS

Plant Material

Fresh plant roots and aerial parts of *H. strobilaceum* were collected from a wild location on El-Gameel Lake; Cairo Port Said international costal road (GPS location 31˚12'13.9''N 32˚16'13.0''E), Egypt on January 2017. Plant samples were air dried, reduced to fine powder and stored in sealed zip bags in the refrigerator for further studies. Samples of the fresh plant were freeze-dried at -70˚C under liquid nitrogen prior to DNA profiling steps. A total of 200 g of each of the aerial and root parts of *H. strobilaceum* were extracted with n-hexane to yield 4.035 g of aerial part and 2.913 g of the root part.

Plant authentication

The plant was identified by Prof. Dr. Abdel-Halim Abdel-Magly, Horticulture Research Institute, Flora and Phytotaxonomy Research Unit, Egypt. Voucher specimen of the authenticated plant was coded PHG-P-HS-210 and deposited in the department of pharmacognosy herbarium at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

Plant DNA Extraction

DNA extraction procedures, using the modified sodium dodecyl sulfate (SDS) method, was performed in the cotton disease department, Plant Pathology Research Institute, Giza, Egypt. A fresh plant pelt was homogenized in 400 µl sterile salt homogenizing buffer comprised of 200 mM Tris-HCl, pH 8.5, 250 mM NaCl, 25 mM EDTA and 0.5% SDS; subsequently, 6 µl of 20 µg/ml RNase A was added. Samples were incubated at 65°C for 10 min; then, 130 µl of 3 M sodium acetate at pH 5.2 was added to each of them; eventually, they were vortexed for 30 min at maximum speed and incubated at -20°C for 10 min. The lysate was centrifuged at 13,000 rpm at 4°C for 15 min, and the supernatant was transferred to fresh tubes. An equal volume of isopropanol was added to each sample and mixed well before the samples were incubated at -20°C for 10 min. After the sample centrifugation, which took 20 min at 4°C, 6000 rpm, DNA pellets were washed twice using 700 µl of a washing solution formed of 100% and 70% ethanol, respectively. The DNA pellets were, subsequently, air dried in an oven at 40°C for at least 10 min. The resultant DNA pellets were resuspended in 100 µl 1X TE buffer, consisting of 10 mM Tris-HCl and 1 mM EDTA at pH 8.0 [17].

Plant DNA quantification and gel documentation

To check the quality of the DNA, 7 µl of the isolated DNA and 3 µl of 10X loading dye were loaded in a lane of 1.5% (w/v) agarose gel containing 0.05 µg/ml ethidium bromide. For quantitative measurements, images were captured using a charge-coupled device camera imaging system and UVI soft analysis (Gel Documentation and Analysis Systems, Uvitec, Cambridge, UK) to calculate the band intensities [17].

RAPD-PCR analysis

Random Amplified Polymorphic DNA polymerase Chain Reaction (RAPD-PCR) analysis was launched, using 10 decemer primers shown in (Table 1), in 25-µl reaction volumes containing PCR buffer, 0.2 mmol/l dNTPs, 0.5 mmol/l of each primer, 4.0 mmol/l $MgCl_2$, 1.25 units of Taq polymerase and 10–20 ng of genomic DNA. The PCR reaction were carried out in a T-Gradient thermo-cycler using the following profile: 94 °C for 1 min, 36 °C for 1 min, 72 °C for 1 min for 30 cycles, and a final extension at 72 °C for 5 min. Following amplification, samples were separated by electrophoresis in 1.4 % agarose gel, stained with 0.5 µg/ml of ethidium bromide, viewed under ultra-violet light, and 300- to 1500-bp ladder was used as a molecular mass marker. [17]. The banding pattern generated by RAPD-PCR marker analysis was compared to 100 bp DNA marker (Promeaga) to determine the genetic relatedness of clear and distinct amplification products, scored as (+) for presence and (-) for absence of bands; finally, bands of the same mobility were scored as identical [18–20].

Gas Chromatography Mass Spectroscopy (GC/MS)

Roots and aerial parts n-hexane extracts were analyzed using Shimadzu GCMS-QP2010 equipped with Rtx-5MS fused bonded column (30m x 0.25 mm x 0.25 µm film thickness) equipped with a split–splitless injector. The initial column temperature was kept at 50 °C for 3 min (isothermal) and programmed to 300 °C at a rate of 5 °C/min and kept constant at 300 °C for 10 min (isothermal) with an injector temperature of 280 °C. Helium carrier gas flow rate was 1.37 ml/min, and all the mass spectra were recorded applying the following conditions; equipment current, filament emission current, 60 mA; ionization voltage, 70 eV; and ion source, 220°C. Diluted samples dissolved in n-hexane (1% v/v) were injected with split mode (split ratio 1: 15). Compounds were identified using the National Institute of Standards and Technology (NIST) MS spectral database and Kovats index [21–25].

Antimicrobial Activity

Standard tested microorganisms were obtained from the Fermentation Biotechnology & Applied Microbiology Center (FBAMC), Azhar University, Egypt. Antimicrobial activity was carried using cup well diffusion method with 1 cm well diameter enclosing 100 µl of each tested sample dissolved in DMSO in three different concentrations, 30, 50, 100 and 200 µg/ml. Samples activity were tested on; Gram-positive bacterial strains, *Bacillus subtilis* (ATCC-6633), and *Staphylococcus aureus* (ATCC–6538); Gram-negative bacterial strains, *Pseudomonas aeruginosa* (ATCC-9027), and *Escherichia coli* (ATCC-8739); and fungal strains, *Candida albicans* (ATCC-90028, and *Aspergillus niger* (ATTC-7966) [26-27]. The minimal inhibitory concentrations (MIC) were determined using serial dilutions compared to the reference antibiotic Chloramphenicol [28-29].

MTT cytotoxicity assay

Cells were added to a 96-well plate with a concentration of 1 X 10^5 cells/ml (100 µl/well) and incubated at 37°C for 24 hours to be treated with three different samples, doxorubicin as control, root n-hexane extract, and aerial n-hexane extract. Roswell Park Memorial Institute (RPMI) medium with 2% serum as maintenance medium was added to the wells of each tested plate. Subsequently, a serial dilution was prepared from each sample, and 0.1 ml of each dilution was added to 93 wells leaving three wells as control on each plate before incubation was conducted at 37°C. Each sample's effect was observed on each cell line at three incubation periods 24, 48, and 72 hours. Cells were observed for any physical signs of toxicity such as partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. 20ul MTT solution were added to each well using a shaking table at 150 rpm for 5 minutes to mix the MTT into the media. Furthermore, cells were incubated at 37C with 5% CO_2 for 1-5 hours to allow the MTT to be metabolized. After rinsing the media, formazan dye was resuspended and dissolved in 200ul of DMSO using a shaking table at 150 rpm for 5 minutes in order to add it to all the wells. Optical density was measured using spectrophotometer at 560nm and the

background was subtracted at 620nm [30-31].

RESULTS AND DISCUSSION

Plant DNA profiling

H. strobilaceum was subjected to PCR-RAPD assay of its genomic DNA using ten different primers (table 1). The number of RAPD-PCR fragments indicated that ten primers were reproduced (Figure 1 and 2). While the DNA amplified with RAPD technique using the P2 primer (5'- TGCCGAGCTG-3') was the most characteristic, showing 13 fragments, the DNA amplified with the P4 primer (5'- AATCGGGCTG-3') was the least, showing only 4 fragments. It is worth mentioning that primers P1, P3, P6, and P8 showed good domination in *H. strobilaceum* producing between 8 and 9 amplified DNA fragments. In the same vein, primers P7 and P10 produced intermediate, 6 and 7, amplified DNA fragments, respectively (Figure 3). The ten primers of arbitrary sequences generated a total of 83 fragments in *H. strobilaceum*. (Table 2)

Plant genetic profiling using RAPD-PCR analysis with 10 primers produced totally 83 amplified DNA fragments, and primer P2 (5'- TGCCGAGCTG-3') yielded the best sequence dominating *H. strobilaceum* with the highest number of hits.

Table 1: RAPD polymorphic 10 decemer primers.

Primer Code	5'-Sequence-3'
P1	GGTCCCTGAC
P2	TGCCGAGCTG
P3	GGGTAACGCC
P4	AATCGGGCTG
P5	AGGGGTCTTG
P6	CAGGCCTTCA
P7	GTGATCGCAG
P8	GTGACGTAGG
P9	GAAACGGGTG
P10	AGTCAGCCAC

Table 2: Total number of RAPD-PCR fragments in *H. strobilaceum*.

Primer Code	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	Total
RAPD Fragments	**9**	**13**	**9**	**4**	**8**	**8**	**6**	***11***	***8***	***7***	***83***

Table 3*:* The identified compounds of the aerial part n-hexane extract of *H. strobilaceum* using GC /MS analysis. Compounds were identified using Kovats indexes and mass spectrum fragmentation chromatograms in NIST library with range ±5, (*) range ±10.

	Rt (min)	Area %	KI_{Cal}	KI_{Lit}	Identified Compounds	Formula
1	11.463	0.68	1062	1068*	Decane, 2-methyl-	$C_{11}H_{24}$
2	**12.611**	**6.01**	**1098**	**1100**	**Undecane**	**$C_{11}H_{24}$**
3	13.466	1.12	1125	1130	Oxirane, octyl-	$C_{10}H_{20}O$
4	13.704	0.86	1133	1136	Cyclohexane, pentyl-	$C_{11}H_{22}$
5	14.615	2.43	1161	1164	Undecane, 2-methyl-	$C_{12}H_{26}$
6	14.821	1.17	1168	1169	Undecane, 3-methyl-	$C_{12}H_{26}$
7	**15.731**	**9.83**	**1197**	**1200**	**Dodecane**	**$C_{12}H_{26}$**
8	16.148	0.59	1211	1210	Undecane, 2,6-dimethyl-	$C_{13}H_{28}$
9	24.111	3.15	1498	1500	Pentadecane	$C_{15}H_{32}$
10	**26.595**	**6.25**	**1596**	**1600**	**Hexadecane**	**$C_{16}H_{34}$**
11	27.748	1.06	1645	1649	Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$
12	27.967	0.61	1655	1659	Hexadecane, 4-methyl-	$C_{17}H_{36}$
13	28.092	0.84	1660	1665	Hexadecane, 2-methyl-	$C_{17}H_{36}$
14	**28.952**	**8.26**	**1697**	**1700**	**Heptadecane**	**$C_{17}H_{36}$**
15	29.073	2.87	1703	1705	Pentadecane, 2,6,10,14-tetramethyl-	$C_{19}H_{40}$
16	30.368	0.84	1759	1764	Heptadecane, 2-methyl-	$C_{18}H_{38}$
17	**31.190**	**8.20**	**1795**	**1800**	**Octadecane**	**$C_{18}H_{38}$**
18	31.384	1.84	1804	1808	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$
19	32.224	1.17	1844	1843	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$
20	32.540	0.58	1859	1864	Octadecane, 2-methyl-	$C_{19}H_{40}$
21	**33.322**	**6.93**	**1896**	**1900**	**Nonadecane**	**$C_{19}H_{40}$**
22	**33.916**	**4.89**	**1925**	**1926**	**Hexadecanoic acid, methyl ester**	**$C_{17}H_{34}O_2$**
23	**35.354**	**5.26**	**1993**	**2000**	**Eicosane**	**$C_{20}H_{42}$**
24	**37.295**	**4.05**	**2099**	**2100**	**Heneicosane**	**$C_{21}H_{44}$**
25	39.155	2.71	2201	2200	Docosane	$C_{22}H_{46}$
26	40.937	1.93	2298	2300	Tricosane	$C_{23}H_{48}$
27	42.037	0.73	2359	2364	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$
28	42.648	1.18	2393	2400	Tetracosane	$C_{24}H_{50}$
29	44.296	2.04	2497	2500	Pentacosane	$C_{25}H_{52}$
30	45.256	7.45	2559	2551	Bis(2-ethylhexyl) phthalate	C24H38O4
31	45.878	0.67	2599	2600	Hexacosane	$C_{26}H_{54}$
32	47.405	1.82	2697	2700	Heptacosane	$C_{27}H_{56}$
33	50.298	1.07	2883	2900	Nonacosane	$C_{29}H_{60}$
34	53.000	0.89	3056	3060	2-Methyltriacontane	$C_{31}H_{64}$

Table 4: The identified compounds of the root n-hexane extract of *H. strobilaceum* using GC /MS analysis. Compounds were identified using Kovats indexes and mass spectrum fragmentation chromatograms in NIST library with range ±5, (*) range ±10.

	Rt (min)	Area %	RI Cal	RI Lit	Identified compounds	Formula
1	21.497	0.53	1397	1400	Tetradecane	$C_{14}H_{30}$
2	24.134	1.32	1499	1500	Pentadecane	$C_{15}H_{32}$
3	25.158	0.39	1539	1535	Decane, 5-phenyl-	$C_{16}H_{26}$
4	25.374	0.31	1548	1550	Decane, 4-phenyl-	$C_{16}H_{26}$
5	26.625	1.78	1597	1600	Hexadecane	$C_{16}H_{34}$
6	26.724	0.3	1601	1595*	Decane, 2-phenyl-	$C_{16}H_{26}$
7	27.473	0.52	1633	1625*	Undecane, 6-phenyl-	$C_{17}H_{28}$
8	27.557	1.36	1637	1633	Undecane, 5-phenyl-	$C_{17}H_{28}$
9	27.794	1.23	1647	1643	Undecane, 4-phenyl-	$C_{17}H_{28}$
10	28.284	0.63	1668	1670	Undecane, 3-phenyl-	$C_{17}H_{28}$
11	28.987	1.12	1699	1700	Heptadecane	$C_{17}H_{36}$
12	29.146	1	1706	1715*	Undecane, 2-phenyl-	$C_{17}H_{28}$
13	29.744	0.77	1732	1727	Dodecane, 6-phenyl-	$C_{18}H_{30}$
14	29.853	0.75	1737	1734	Dodecane, 4-phenyl-	$C_{18}H_{30}$
15	30.123	0.52	1748	1743	Decane, 4-phenyl-	$C_{16}H_{26}$
16	30.607	0.43	1769	1767	Dodecane, 3-phenyl-	$C_{18}H_{30}$
17	31.224	0.8	1796	1800	Octadecane	$C_{18}H_{38}$
18	31.460	0.64	1807	1813*	Dodecane, 2-phenyl-	$C_{18}H_{30}$
19	31.918	0.54	1829	1819*	Tridecane, 6-phenyl-	$C_{19}H_{32}$
20	32.065	0.39	1836	1826*	Tridecane, 5-phenyl-	$C_{19}H_{32}$
21	32.334	0.29	1849	1840*	Tridecane, 4-phenyl-	$C_{19}H_{32}$
22	32.834	0.22	1873	1865*	Tridecane, 3-phenyl-	$C_{19}H_{32}$
23	33.355	0.64	1898	1900	1-Nonadecene	$C_{19}H_{38}$
24	33.660	0.31	1912	1911	Tridecane, 2-phenyl-	$C_{19}H_{32}$
25	**33.999**	**38.88**	**1929**	**1927**	**Hexadecanoic acid, methyl ester**	**$C_{17}H_{34}O_2$**
26	35.387	1.03	1995	2000	Eicosane	$C_{20}H_{42}$
27	37.335	3.54	2102	2101	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
28	**37.469**	**23.25**	**2108**	**2106**	**9-Octadecenoic acid (Z)-, methyl ester**	**$C_{19}H_{36}O_2$**
29	37.545	1.03	2113	2110	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$
30	37.897	0.75	2133	2134	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$
31	39.187	0.64	2202	2200	Docosane	$C_{22}H_{46}$
32	39.537	0.35	2222	2230*	Nonadecanoic acid, methyl ester	$C_{20}H_{40}O_2$
33	40.973	0.39	2301	3000	Tricosane	$C_{23}H_{48}$
34	41.137	2.15	2309	2306	cis-11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$
35	42.069	1.25	2361	2356	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$
36	42.680	0.31	2394	2400*	Tetracosane	$C_{24}H_{50}$
37	44.324	0.25	2499	2500	Pentacosane	$C_{25}H_{52}$
38	45.292	3.43	2561	2551*	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$
39	47.433	0.25	2699	2700	Heptacosane	$C_{27}H_{56}$
40	48.903	0.37	2793	2800*	Octacosane	$C_{28}H_{58}$
41	50.326	0.31	2884	2886	4,10-Dimethyloctacosane	$C_{30}H_{62}$
42	51.698	0.29	2973	2972	Nonacosane, 3-methyl-	$C_{30}H_{62}$
43	53.031	0.24	3058	3060	2-Methyltriacontane	$C_{31}H_{64}$
44	57.145	0.55	3323	3325	5α-Stigmastan-3β-ol	$C_{29}H_{52}O$
45	57.895	1.52	3371	3378*	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	$C_{32}H_{52}O_2$
46	58.476	1.47	3408	3398*	9,19-Cyclolanost-24-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$
47	59.665	0.43	3484	3477*	Lanosta-7,9(11)-diene-3β,18,20-triol, 3,18-diacetate, (20R)-	$C_{34}H_{54}O_5$
48	60.000	0.53	3506	3505	Pentatriacontane	$C_{35}H_{72}$

GC/MS characterization *of H. strobilaceum* roots and aerial parts

GC/MS chemical analysis has identified 34 compounds in the n-hexane extract of *H. strobilaceum* aerial parts, which present 100 % of its total composition (table 3) and 48 compounds in the n-hexane extract of the root part, which embody 100 % of its total extract composition (table 4). Data were investigated by comparing both the calculated and the literature Kovat's index, in the online NIST library, with a maximum deviation of ± 5; moreover, the fragmentation patterns of the chromatograms were matched. On the one hand, the major compounds of the root extract were saturated fatty acids, namely, hexadecanoic acid, methyl ester (38.88%) and 9-octadecenoic acid (Z)-methyl ester (23.25%); nevertheless, long chain hydrocarbons; hexadecane (1.78%), pentadecane (1.32%), heptadecane (1.12%) and octadecane (0.8%), were present as minorities. In the same extract, alkyl hydrocarbons such as ecosane (1.03%), docasane (0.64%), tricosane (0.39%), tetracosane (0.31%), pentacosane (0.25%) and heptacosane (0.25%) were detected. On the other hand, the major components of the aerial part n-hexane extract were hydrocarbons such as dodecane (9.83%), heptadecane (8.26%) and octadecane (8.2%). Hexadecanoic acid, methyl ester (4.89%) is found in moderate abundance, and alkyl hydrocarbons as ecosnae (5.26%), heneicosane (4.05%), docosane (2.71%), tricosane (1.93%) and tetracosane (1.18%) were identified in higher percentages in the aerial part.

Antimicrobial Screening

The antimicrobial activity of the n-hexane root and aerial part extracts was tested using the concentrations 30, 50, 100 and 200 mg /ml [12]. The n-hexane extract of the root showed significant effect against Gram-positive tested bacterial strains; *Bacillus subtilis* ATCC-6633 (P<0.0001, SD 0.8339, mean 7.141, SE 0.3152) with MIC value of 6.25 µg/ml; and against *Staphylococcus aureus* ATCC – 6538 (P<0.0001, SD 6.682, mean 56.10, SE 2.728) with MIC value of 50 µg/ml compared to the reference antibiotic whose MIC was 7.81 and 62.5 µg/ml, respectively. The aerial part's hexane extract showed weak activity against *Bacillus subtilis* ATCC-6633 (P>0.0001 = 0.0592, SD 102.7, mean 90.18, SE 38.83) with MIC value of 200µg/ml and no activity against *Staphylococcus aureus* ATCC–6538 (P>0.0001 = 0.0781, SD 33.41, mean 26.79, SE 12.63). Detectable activity was neither observed against the Gram-negative bacteria; *Pseudomonas aeruginosa* (ATCC -9027) and *Escherichia coli* (ATCC -8739) nor against tested fungal strains; *Candida albicans* (ATCC-90028) and *Aspergillus niger* (ATTC-7966) in the tested concentrations. Chloramphenicol antibiotic was used as the control at a concentration of 1µg/ml. While the antimicrobial activity of the n-hexane extract of the root part showed potent effectiveness with p-value <0.0001, the aerial parts' extract didn't reveal any discernable effect on Gram-positive bacteria. Meanwhile, both extracts showed no activity on Gram-negative bacterial and fungal strains (Table 5) (Figure 4).

Because 9,12-octadecadienoic acid (Z,Z)- methyl ester and hexadecanoic acid, methyl ester are reported for their antimicrobial activities, and they serve as minor components in the aerial part extract and major components in the root part extract, the root part shows a surpassing antimicrobial potential over the aerial counterpart [32-33]. Our results are in accordance with Galbraith and Miller report, which indicated the effectiveness of long chain fatty acids against Gram-positive organisms than the Gram-negative ones due to the nature of the bacterial outer membrane and its lower permeability towards lipophilic components[34]. GCMS analysis revealed the presence of bioactive compounds that promote the plant as a natural health product, and some compounds may reflect the role of abiotic stresses on *H. strobilaceum.* There was a remarkable antimicrobial activity on Gram-positive bacteria; therefore, the non-polar fractions of *H. strobilaceum* are promising candidates for further future biological studies.

Moreover, minor quantities of benzene derivatives are traced in the root extract without looming in the aerial part in spite of using the same solvents during the extraction process such as benzene-1-butylheptyl (0.39%) and 1-propylheptyl benzene, reported as a novel natural product in the degradation of bitumen by *Providencia stuartii*, a previously studied Gram-negative bacterium [35–38]. This denotes that *H. strobilaceum* root exudates can gradually alter the sedimental conditions to select and enrich a specific rhizo-microbiome, which is capable of promoting the plant's growth in its halophytic environment [39].

MTT antiproliferative cytotoxicity assay

H. strobilaceum root and aerial part n-hexane extracts show cytotoxic effect against MCF-7, Hep-G2 and Caco-2 cell lines at different time periods of 24,48 and 72 h, compared to the reference antibiotic (Figure 5). This is reported in terms of IC_{50} (Inhibitory concentration of 50%) values where the n-hexane root extract showed less effectiveness than the aerial part (Figure 6) (Table 6). However, compared to the standard doxorubicin, both extracts showed weak activity on both Hep-G2 and Caco-2 cell lines. The aerial part n-hexane extract demonstrates strong cytotoxic activity against MCF-7 cell line in different time periods, 24h, 48h, and 72h with IC_{50} of 227.40, 184.45 and 135.68 µg/ml, respectively, compared to the standard control used in the same time periods whose IC_{50} values were 130.84, 90.42 and 67.23 µg/ml, or presented as 2.1, 2.03 and 2.01 times the effect of doxorubicin. On the other hand, the root n-hexane extract showed moderate cytotoxicity against the MCF-7 cell line in the same time periods with IC_{50} values of 341.98, 308.77 and 218.99 µg/ml, presented as 2.6, 3.4 and 3.25 times the effect of doxorubicin.

Although previous research revealed the cytotoxic potential of the free hexadecenoic acid against some human leukemia cells [40], our assays unfold that the aerial part extract of *H. strobilaceum* was more effective than the root extract against the breast (MCF-7), colon (Caco-2) and liver (Hep-G2) human cancer cell lines through different time periods; 24, 48 and 72h despite documenting hexadecenoic acid higher percentage in the former than the later. Consequently, the relevance of other

hydrophobic constituents of the root part and their probable interaction with each other and with the biological membranes should be highlighted here. Future prospective work is considered to evaluate the plant's root extract against different cell lines in different time intervals and doses as well as with normal cell control to assess the effectiveness of the minor extract components.

The investigation of *H. strobilaceum* aerial and root parts, carried out by GC/MS analysis, detected the presence of numerous bioactive molecules putting the plant forward as a potential candidate for future study of its traditional use causes. To the best of our knowledge, this is the first report of the lipoidal profile of *Halocnemum strobilaceum*.

Table 5: Minimum inhibitory concentration (MIC) of *H. strobilaceum* root and aerial parts n-hexane extracts compared to reference antibiotic. (-) no noticeable activity.

Sample	Gram Positive Bacteria	
	B. subtilis (ATCC-6633)	***S. aureus*** (ATCC-6538)
Root part	6.25 µg/ml	50 µg/ml
Aerial part	200 µg/ml	--
Chloramphenicol control	7.81 µg/ml	62.5 µg/ml

Table 6: IC_{50} values of *H. strobilaceum* root and aerial parts n-hexane extracts.

	MCF-7 IC_{50} (µg/ml)			Hep-G2 IC_{50} (µg/ml)			Caco-2 IC_{50} (µg/ml)		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Doxorubicin	130.84	90.42	67.23	49.06	41.12	47.358	41.66	30.59	25.15
Root part	341.98	308.77	218.99	423.45	256.35	259.03	266.87	328.08	208.47
Aerial part	277.40	184.45	135.68	247.68	170.97	184.85	238.19	200.82	177.58

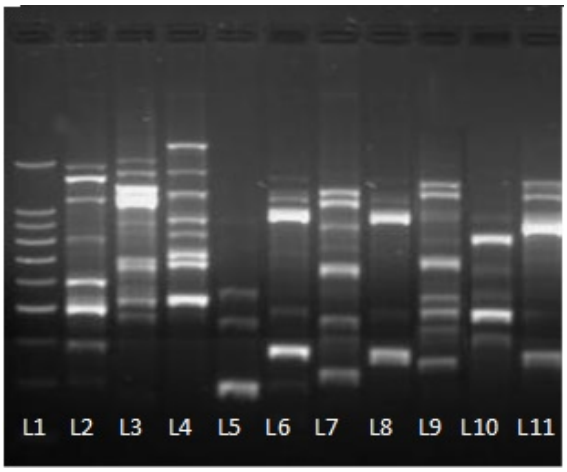

Figure 1: RAPD-PCR products using ten decemer primers. Lanes; L1, 100 bp DNA marker (Promeaga); L2, sample with primer 1; L3, sample with primer 2; L4, sample with primer 3 till lane 11, which carries sample with primer 10.

	MW-RF										
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11
1	135.000	150.556	166.111	147.963	80.836	134.825	136.296	134.439	134.641	150.556	162.222
2	100.000	134.825	147.963	134.208	31.294	132.825	127.202	132.825	131.044	134.208	131.727
3	75.000	132.825	137.593	125.918	22.551	121.142	117.095	119.211	125.918	91.877	122.897
4	63.000	121.142	133.941	87.979	16.739	95.906	75.000	87.979	95.906	65.849	73.732
5	48.000	67.219	128.347	69.878		55.206	61.422	58.286	65.849	42.260	49.366
6	35.000	53.699	121.142	53.699		19.210	42.260	18.924	46.839	29.874	24.067
7	25.000	35.946	114.785	46.839		17.000	22.890		29.186	24.067	18.787
8	20.000	25.515	80.836	28.517		14.391	20.173		24.518	20.352	
9	17.000	19.670	71.176	19.833			17.672		20.000		
10		17.111	27.867						18.393		
11			23.647						16.478		
12			19.833								
13			16.348								

Figure 2: Lanes; L1, 100 bp DNA marker (Promeaga); L2, sample with primer 1; L3, sample with primer 2; L4, sample with primer 3 till lane 11, which carries sample with primer 10.

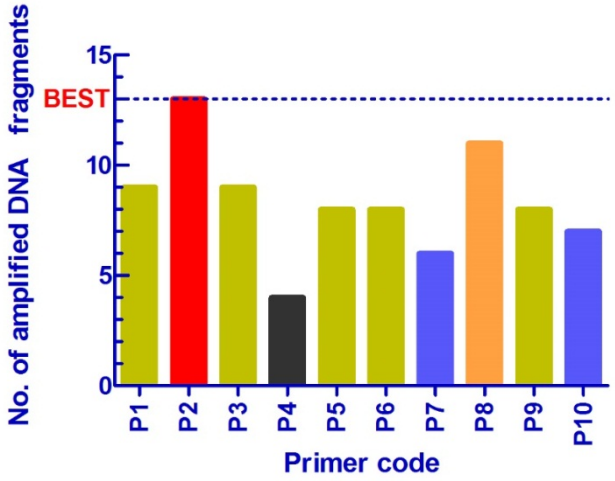

Figure 3*:* Alignment of matching amplified DNA fragments from 10 primers.

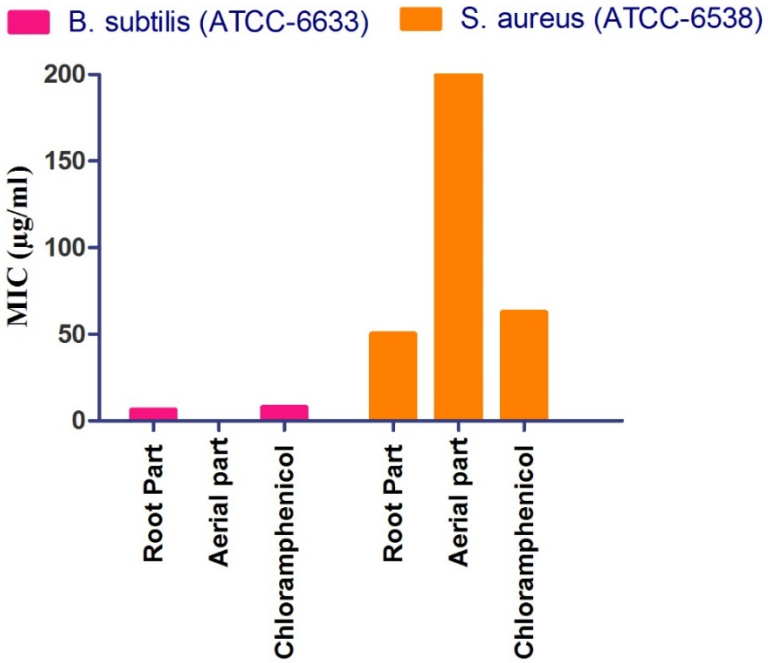

Figure 4*:* MIC values of the root and aerial parts n-hexane extracts compared to the control antibiotic chloramphenicol .

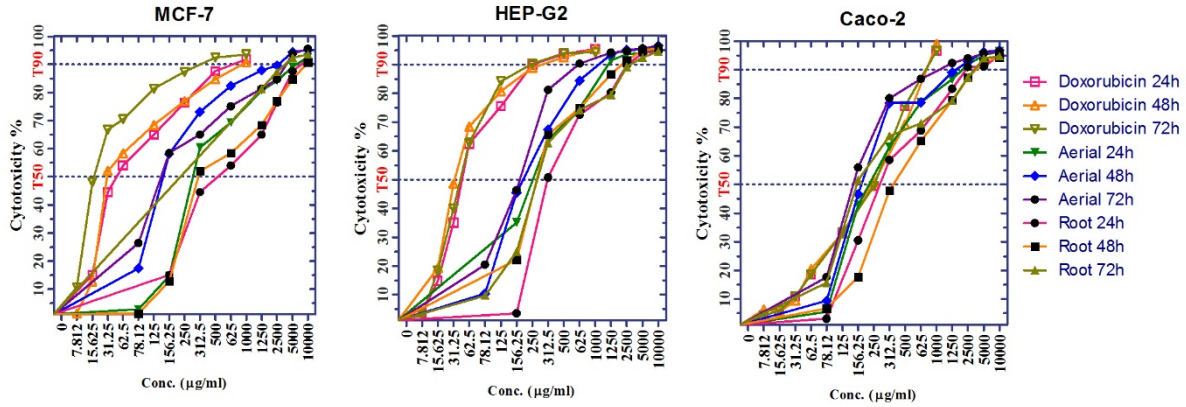

Figure 5*:* Percentage of cytotoxicity of the *H. strobilaceum* root and aerial part n-hexane extracts against MCF-7, Hep-G2 and Caco-2 cell lines compared to the control doxorubicin.

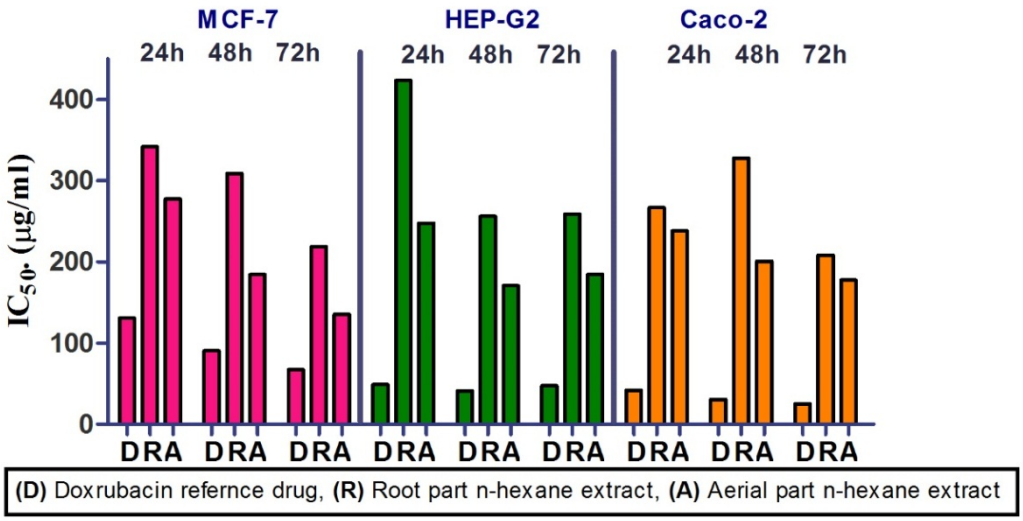

Figure 6*:* IC_{50} of the *H. strobilaceum* root and aerial part n-hexane extracts against MCF-7, Hep-G2 and Caco-2 cell lines, compared to the control doxorubicin at three different time periods 24h, 48h and 72h.

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CONFLICT OF INTEREST

There is no conflict of interest

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