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Original article

A Comparative Evaluation of Phytochemical and Antimicrobial Properties of Selected Aquaticand Terrestrial Halophyte Plants Growing in Egypt

Fatma Sh. Abd El-Gwaid*, Albaraa S. El Saied, Zeinab A. El-Swaify*, Rawheya A. Salah El Din***

*Botany and Microbiology Department, Faculty of Science (Girls Branch), Al–Azhar University, Cairo, Egypt.
**Botany and Microbiology Department, Faculty of Science (Boys), Al –Azhar University, Cairo, Egypt.

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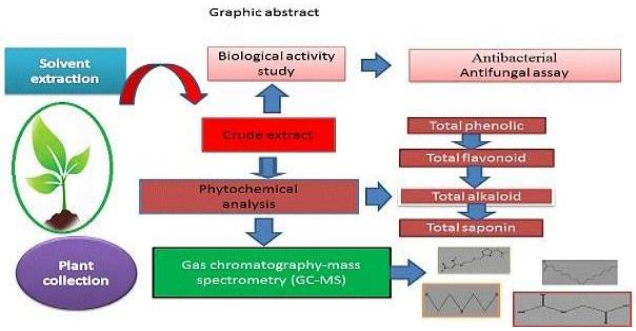
ABSTRACT

Halophytes are plants that have significant economic importance with, the potential for use in therapeutic medicine and environmental restoration. This study compiles a comparison of the chemical composition of ethanol extracts from aquatic and terrestrial halophyte plants (*Halophila stipulacea, Halodule uninervis, Thalassodendron ciliatum, Spergularia marina, Suaeda aegyptiaca* and *Arthrocnemum fruticosum*) using gas chromatography –Mass spectrometry (GC-MS) analysis also demonstrated antimicrobial properties. GC-MS analysis is the first step in identifying the nature of the active compounds synthesis plants. In comparison, total phenolics, flavonoids, and alkaloids (mg/g) contents are higher in *H. stipulacea* than in other species, with a quantity of 17.02, 28.17, and 12.79 mg/g, respectively. 132 compounds were confirmed by GC/MS qualitatively and quantitatively in all plants. The major compounds of these six halophytic plants extract (n-Hexadecanoic acid 19.83%, 6-Octadecenoic acid 26.72, 28.01%, Phytol 14.85%, 13.32%, and Tridecane 2 phenyl 11.06%) Rhodopin and 2,6-dimethyl-N-(2-methyl-à-phenylbenzyl) aniline are found only in *S. aegyptiaca*, as is benzedrex, which is found only in *H. stipulacea*. The halophyte extracts were subjected to antimicrobial assays by using the agar diffusion method against four species of fungi, three species of Gram-positive bacteria, and three species of Gram-negative bacteria. *H. uninervis* and *S. aegyptiaca* showed the highest inhibition zones against fungi, G+ve bacteria, and G-ve bacteria (9, 9, 11, 12, and 12 mm), respectively. The obtained data showed these plants are promising sources of natural compounds with antimicrobial properties that could be suitable for future applications.

Graphical abstract



∗ *Corresponding author*

E-mail address: fatma86shaaban@gmail.com

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1. Introduction

Halophytes are described as plants that can tolerate harsh conditions including high salinity. They are common in areas where other types of plants cannot grow. In such harsh environmental conditions, halophytes exhibit certain strategies to adapt, whether morphologically or physiologically, to such conditions. Among these strategies, the most important is the synthesis of biologically active metabolites with antioxidant potential. Such valuable metabolites could be effective chemicals used in the food industry to protect against food oxidation or as medicinal drugs used for the treatment of many diseases [1-2]. Some halophyte species have traditionally been used as herbs and vegetables, feed, and fodder due to their high phytonutrient content. As a result, they are regarded as one of the alternative solutions to problems such as food safety, freshwater shortages, and salinization [3]. In recent years people looking for innovative antimicrobial treatments and pharmacological therapeutics have be- come more interested in natural products [4]. Currently,halophytes have become an interesting subject for qualitative and quantitative investigation of their meta- bolic content. Polyphenols, such as phenolic acids, fla- vonoid glycosides, tannins, and saponins are among the most frequent secondary metabolites detected in halophytes [3]. Numerous secondary metabolites, including alkaloids, flavonoids, phenolics, saponins, terpenoids, and a wide range of other substances, are stimulated by the harsh conditions that plants grow in, such as salinity stress, light, temperature, etc. [5]. Halo- phyte species are divided into 33 families including(terrestrial and aquatic plants). Among terrestrial spe- cies, *Spergularia marina, Suaeda aegyptiaca,* and *Ar- throcnemum fruticosum*) aquatic species, (*Halodule uninervis, Halophila stipulacea* and *Thalassodendron ciliatum*) are significant for their economic and thera- peutic characteristics. Marine natural products have an abundant supply of therapeutically active constituents that are universally dispensed everywhere in the coastal regions. Nowadays, the pharmaceutical industries worldwide still depend on several pharmacologically approved marine-based products for the latest drug pro- grams [6]. The marine habitats (>70% of the planet's surface) have exceptional biological and chemical traits that play significant roles in the identification of nu- merous therapeutic leads. Numerous marine-living or- ganisms are soft-bodied and/or sessile. Therefore, they have produced toxic secondary metabolites to protect themselves from predators [7]. Seagrasses are a group of monocotyledon flowering plants in the marine envi- ronment. Seagrasses have rhizomes, leaves, and true roots and survive submerged in the sea, where they ad- just to extremely salinized environments [8]. Anti- oxidant, anti-bacterial, anticancer, anti-inflammatory, antiviral, and bioactivities are among the therapeutic qualities of seagrass metabolites [9-10-11-12-13]. The antimicrobial potential of many halophytes was studied by [14]. They showed the availability of certain phyto- constituents such as phenols and fatty acids, with poten-tial antimicrobial activity, in these salt-tolerant plants. Halophytes manufacture antifungal chemicals as a de- fense mechanism against phytopathogenic infections [15]. Promising research has revealed that these halophytic plants have antifungal activities, which are at- tributable mostly to their essential oils and phenolic- rich, extracts [16]. Halophytic plants have bioactive secondary metabolites that have antibacterial, antiviral, anticancer, and anti-inflammatory activities [17]. There- fore, halophyte plants are attracting attention due totheir pharmaceutical and therapeutic medicinal role. The application of mass spectrometry techniques is encourage for future studies in preclinical examinations besides determining the potential bioactive constituents in plants [18]. The present study aims to provide, for thefirst time a comparative of the potential of hydroalco- holic extracts of aquatic and terrestrial halophyte plants in the production of bioactive compounds and evaluate of their antimicrobial potential.

2. Materials and methodsCollecting of samples

Three fresh seagrasses from order *Alismatales* were collected by hand at 0.5-1 m depth from Hurghada Coast, Red Sea, Samples were transferred to a labeled plastic bag in seawater for preservation. Samples were numbered, and transferred to the laboratory. One *spe- cies* belonging to the family *Hydrocharitaceae* was *Halophila stipulacea* (Forsk.) Aschers (*H. stipulacea*) (Fig.1A) and two were belonging to the family *Cymo- doceaceae Thalassodendron ciliatum* (Forsskål) den Hartog (*T. ciliatum*) (Fig. 1B) and *Halodule uninervis* (Forsskål) Ascherson *(H. uninervis*) (Fig. 1C) (30.0°40 8" E, 31.2°64 7" N) and three terrestrial plants were chosen based on their dominance in Bahariya Oases are one *species* of *Caryophyllaceae (Spergularia marina*) (*S. marina*) (Fig. 1D) (28°25 24.8"E, 28°55 57.7"N), one *species* of *Amaranthaceae* (*Suaeda aegyptiaca*) (*S. aegyptiaca*) (Fig 1F) (28°21 38.4E", 28°56 10.4N") and one *species* of *Chenopodiaceae* (*Arthrocnemum fruticosum*) (*A. fruticosum*) site (1) (28°22 16.7"E,28°52 24.7"N) and site (2) (28°21 57.8"E, 28°52 11.2"N) (Fig. 1E) were, collected from four natu-rally growing population of Bahariya Oases, Egypt, dur- ing the period April 2021 then identified by Ass. Prof.Al baraa Salah El-Din from the Al- Azhar Univer- sity, Faculty of Science, Cairo, Egypt. The plants were washed under tap water to remove the adhered sedi- ments and impurities, dried the shade, and subsequently grinded.

1- Plant extracts preparation.

Two hundred grams of air-dried powder of eachstudied plants were extracted with ethanol (500 ml X 3 times) by cold percolation method for 72 hr. The etha- nol extract was filtered in a Buchner funnel. The filtrate evaporated in a rotary evaporator at a temperature be- low 70°C and the residue was dried in dissector and then saved in storage vials for phytochemical analysis and biological activity study. [19]

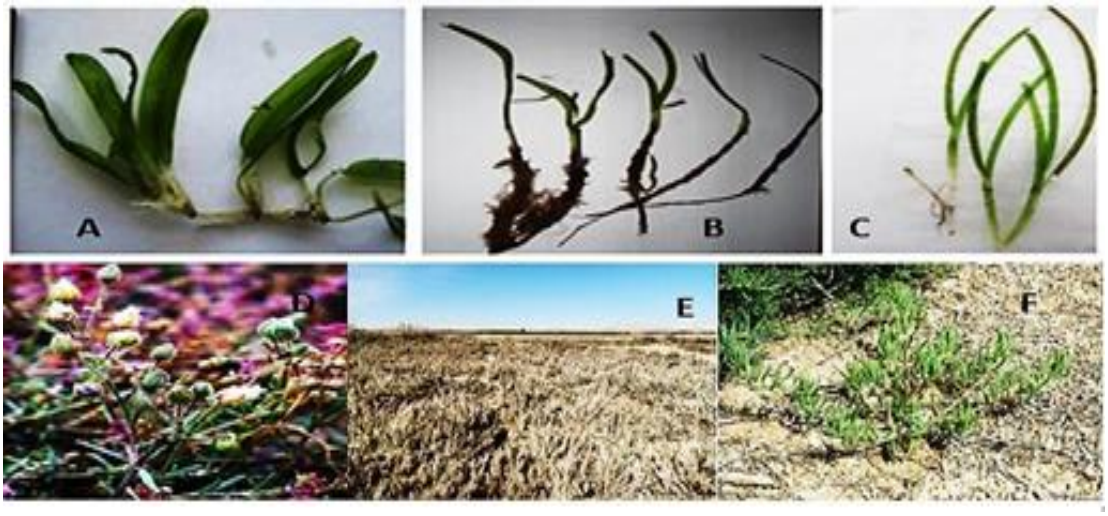

Fig. 1(A) ***Halophila stipulacea*****, (B)** ***Thalassodendron ciliatum*****, (C)** ***Halodule uninervis*****, (D)** ***Spergularia marina*** **(E)** ***Arthrocnemum fruticosum*** **(F)** ***Suaeda aegyptiaca***

2-Phytochemical screening

Test for carbohydrates and/ or glycosides, resins, saponins and tannins by [20**]**

Carbohydrates and/ or glycosides (Molish's test): 50ml of alcoholic extract were concentrated under reduced pressure until free from alcohol and were tested for carbohydrates and reducing sugars using a −naphthol and H_2SO_4 to give a violet ring .

Resins: To five grams of the dry plant were extracted by 50 ml of 70% ethyl alcohol on water bath for 20 min. and filtered. 200 mls of distilled water were added to the filtrate, where a white precipitate was formed in the presence of resins

Saponins (Frothing test): About 2.5mg of the plant extract was allowed to be reacted with 5ml water and shaken properly in a test tube. Samples showing froth were warmed. Persistent foam formation indicates the presence of saponin

Tannins: Ferric chloride solution 1 % was added to the concentrated alcohol extract, where a yellowish green colour can be obtained in the presence of tannins**.**

Terpens and sterols by [**21]** Libermann-Burchard's test by adding 1 ml acetic acid anhydrous followed by few mls of concentrated sulfuric acid poured down the side of test tube to form two separate layers, where a red ring was formed indicating the presence of sterols and terpens.

Flavonoids by [**22]** carried out by adding concentrated HCl drop wise to one ml of alcoholic extract containing a fragment of magnesium ribbon Positive result gave pinkish color.

Alkaloids by [**23]** the alcoholic extract was concentrat- ed under vacuum till dryness. The dried extract was dissolved in 2N HCl on a water bath, shaken well and filtered. The filtrate was extracted with chloroform to remove undesirable matters. The acidic aqueous layer was adjusted to alkaline pH with ammonia, and the lib- erated alkaloid bases were extracted by chloroform till exhaustion. The chloroform extract was concentratedtill least volume and tested by Mayer's, Wagner's and Dragendorff's reagents

3- Determination of total phenolics, flavonoids, alka-loids, and saponins contents

The total phenolics contents were determined by the Folin-Ciocalteu method using gallic acid as standard calibration curve this method described by [**24]** . The flavonoids content was calculated using the regression equation obtained from the quercetin standard calibra- tion curve **[25].** On the other hand, total alkaloids were detected as mentioned by **[26].** Total saponins content was detected as stated by [**27]**

4-GC-MC (gas chromatography –Mass spectrometry) analysis

A Thermo Scientific Trace GC1310-ISQ mass spec- trometer with a direct capillary column TG-5MS (30mx0.25m film thickness) was used to analyze the chemical components of each plant extracts. The temperature of the column oven was initially maintained at 50 C, then increased by 5°C/min to 230 °C and held for 2 minutes, and then increased to final temperature of290 °C by 30°C/min and kept for 2 min. Helium was employed as the carrier gas, with a constant flow rate of 1 ml/min, and temperatures of the injector and MS transfer line were maintained at 250 and 260°C, respectively. Autosampler AS1300 combined with GC in split mode automatically injected diluted samples of1 l with a 3 minute solvent delay. Full scan EI mass spectra covering the m/z range of 40–1000 were collected at 70 Ev ionization voltages. The temperature of the ion source had been set at 200°C.By comparing it's retention times and mass spectra to those from the WILEY09 and NIST 11 mass spectral databases, the constituents were identified. The experiment was conducted at AL-Azhar University's Regional Centre of Mycology and Biotechnology in Cairo,Egypt.

5-Antimicrobial activities

Test microorganisms

Six tested bacterial strains were (three Gram-positive bacteria *Micrococcus sp.* RCMP 028 (1), *Bacil- lus cereus* RCMP 027 (1) and *Enterococcus faecalis* (ATCC29212) and three Gram-negative bactria *Proteus vulgaris* RCMP 004(1) ATCC13315, *Pseudomonas aeruginosa* ATCC27853 and *Enterobacter cloacae* RCMP001(1)ATCC 23355 and four fungi *Grotricum candidum, Syncephalastrum racemosum, Penicillium marneffeii* and *Cryptococcus neoformas* RCMP 0049001]. The biological studies were conducted in the AL-Azhar University,s Regional Centre of Mycology and Biotechnology in Cairo,Egypt.

Culture medium

The stock cultures of microorganisms used in this study were maintained on plate count agar slants at40C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10 ml of nutrient agar broth and was incubated at 370C for 24 h. About 60 µl of bacterial suspensions adjusted to 106-107 colony form- ing units (CFU)/ml were taken and poured into Petri plates containing 6 ml sterilized nutrient agar medium. Bacterial suspensions were spread to get a uniform lawn culture.

Agar disc diffusion method

The disc diffusion method was followed to evaluate antimicrobial activities using a range of microorgan-isms. Sterile Discs (Whatman, 6 mm) were impregnatedwith 10 µl of reconstituted crude extracts (1 mg/ml) andplaced on the surface of Muller-Hilton agar dispersion plates inoculated with microbes. Control discs con- tained 10 µl of solvent DMSO was used as a negative control. Standard antibiotics, Gentamycin (Antibacterial agent) 4 µg/ml and Amphotericin B (Antifungal agent) 100 µg/ml served as positive control. Agar plates con- taining bacteria were incubated at 37 0C for 24-48 hours. Blank paper disks (Schleicher &Schuell, Spain) with a diameter of 8.0 mm were impregnated 10 µl of tested concentration of the stock solution. Inhibition zones were recorded as the diameter of growth-free Zones (IZ), including the diameter of the discs, in mm, at the end of the incubation period [28]

3. Results and discussion

The medicinal significance of plants can be correlated to various phytochemicals, as they offer a wide variety of pharmacological activities. Due to these pharmacological properties, a great attention has been derived toward the medicinal plants [29].

The results of the qualitative analysis of phytochemicals are shown in **Table (2).** It was observed the presence of tannins, phenols, carbohydrates, terpenoids and alkaloids in all plants that under investigation and absence of saponins from aquatic plants but present in all tested terrestrial plants under investigation**.** The previous study also supported the presence of different phytochemicals bioactive compounds in plants that under investigation [30, 31, 11, 12, 13]

Table (2) Preliminary phytochemical screening of aquatic and terrestrial halophyte plants

Phyto-constituents	***H. uninervis***	***H. stipulacea***	***T. ciliatum***	***S. marina***	***S. aegyptiaca***	***A. fruticosum* site (1)**	***A. fruticosum* site (2)**
Carbohydrates	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+
Resins	-	-	-	-	-	--	-
Tannins	+	+	+	+	+	+	+
Saponins	-	-	-	+	+	+	+
Wt. of ethanolic extract/100g Dry weight	9.1	8.5	9.25	16.67	19.83	18.01	17.65

(-) absent ; (+) present ;

Total phenolics, flavonoids, alkaloids and saponins contents

Table 3 makes it evident that *H. stipulacea* has higher amounts of total phenolics, flavonoids, and alka- loids (17.02, 28.17, and 12.79 mg/g, respectively) than any other plants under study. On the other hand, *S. ma- rina* showed the highest total saponins (36.7 mg/g), followed by S. aegyptiaca (20.98 mg/g), while totalsaponins was absent in all aquatic plants that were un- der investigation. The value of total phenolics is the contents of *A. fruticosum* site (1) (15.10 mg/g), fol-lowed by S. aegyptiaca (14.03 mg/g). [30] Comparedthe chemical composition of *Spergularia diandra* and *Spergularia marina*. Chemical compounds were detect- ed in the aerial part: phenols, saponins, glycosides, fla- vonoids, and tannins were found in them except for the alkaloids that appeared only in *Spergularia diandra*.Also quantitatively estimated phenols and Spergularia marina increased its quantity by 515.4 mg/50 g plant, while in *Spergularia diandra* it reached 461.75 mg/50 g plant. These variations in the quantities of the biologi- cally active constituents and the bioactivities might be ascribed to the influence of the environment, climate, genetics, or soil nutrients [32]. Bioactivities of second- ary metabolites and polyphenols have valued halo- phytes as a significant source of such bioactive com- pounds [33].

Gas chromatography Mass analysis of ethanol ex- tracts of studied halophyte plants

It is clear from the results using gas chromatog- raphy-mass spectrum analysis for aquatics (*H. uninervis H. stipulacea, and T. ciliatum*) and terrestrialshalophyte plants (*S. marina, S. aegyptiaca, A. fruti- cosum* site (1) ,*and A. fruticosum* site (2)) ethanolic extracts The active principles with their retention time (RT) and concentration (peak area %) are tabulated in **Table 4**, & **Fig. 2 (2 a, 2b, 2c, 2 d, 2e, 2f, 2g)** that con- tain different chemical classes of compounds are pre- sent ; ranged from fatty acids ,fatty acid esters ,sterols, terpenes, flavoniods and alkaloids. The major compo- nents in terrestrial plants (*A. fruticosum* site (1), *A. fru- ticosum* site (2), *S. marina and S. aegyptiaca*) ethanol extracts were 6-Octadecenoic acid (28.01%, 22.65%, 14.36%, 26.72%) respectively, n-Hexadecanoic acid (23.59 %, 17.24%, 19.83%, 20.62%) respectively, and Octadecanoic acid (stearate) (5.20%, 7.11%, 5.09%, 3.45%) respectively. Hexadecanoic acid methyl ester is present in all aquatics and terrestrial plants except *H. stipulacea and S. marina*. The major components in aquatics *H. uninervis and T. ciliatum* extracts are phytol 14.85% and 13.32% respectively, this compound is not found in other plants and has antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti- inflammatory, immune-modulating, and antimicrobialeffects [34] and hexadecanoic acid methyl ester 8.81% and9.34%, this compound reveals antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive and lipoxygenase inhibitor [35]. Nizatidine (3.49%) is an alkaloidal compound, is detected for the first time in *H. uninervis*. This compound is used in the treatment of peptic ulcer disease and gastroesophageal reflux disease (36). Also, *H. uninervis* contains neophytadiene ; is a diterpene (3-methylidenehexadec-1-ene substituted at positions 7 ,11 and 15 by methyl group). It has a role as an anti-inflammatory agent, antimicrobial agents cardi- oprotective properties, and benzedrex (propylhexedrine or Methamphetamine, 3.72%) is an alkaloidal com- pound; a temporarily relieves nasal congestion due to a cold, hay fever, or other upper respiratory allergies. [37]. Also vitamin E (2.02 %) that found in *S. marina* might be considered as a promising antibacterial agent particularly in form of an adjuvant for various antibiotic compounds , potential immune-modulatory agent in- hancing the host immune responses upon bacterial chal- lenges as well as it rise antimicrobial sensitivity by bac- terial lipocalin antibiotic linking [38, 39,40]. The bio- logical activity of some active compounds such as pal- matic acid has antimicrobial [41] and antioxidant activi- ty [42]. Eicosane exhibits antitumour [43], antimicrobi- al and cytotoxic activity [44], Dodecane –phenyl has antibacterial activity [45]. Oleic acid has antibacterial activity [46]. Cis-Vaccenic acid has antimicrobial [47] According to the mentioned halophyte plants are a great source of therapeutically relevant chemicals with a wide range of structural variety. In the present study, these phytomedicinal plants showed a wide range of thera- peutic potentials.

Table 4: GC/MS of ethanol extracts of studied halophyte plants

N	Compounds name	Structure	*H. uninervis*		*H. stipulacea*		*T. ciliatum*		*A. fruticosum* site (1)		*A. fruticosum* site (2)		*S. marina*		*S. aegyptiaca*	
			R.T	%	R.T	%	R.T	%	R.T	%	R.T	%	R.T	%	R.T	%
1	Benzene chloromethyl	C7H7Cl	7.04	0.67	7.04	0.84	7.03	1.46	-	-	-	-	-	-	-	-
2	Nizatidine	C12H21N5O2S2	19.86	3.49	-	-	-	-	-	-	-	-	-	-	-	-
3	Undecane 6phenyl	C18H30	22.68	0.47	22.67	1.02	-	-	-	-	-	-	-	-	-	-
4	Undecane5 phenyl	C17H28	22.76	1.06	-	-	-	-	-	-	-	-	-	-	-	-
5	Undecane4 phenyl	C17H28	22.98	1.11	22.98	2.17	22.98	1.07	-	-	-	-	-	-	-	-
6	Undecane3 phenyl	C17H28	23.44	1.32	23.43	2.30	23.44	1.41	-	-	-	-	--	-	-	*
7	Undecane2 phenyl	C17H28	24.27	2.60	24.27	3.62	24.27	2.61	-	-	-	-	-	-	-	-
8	3(N,NDimethylmyristylammonio)propanesulfonate	C19H41NO3S	24.49	1.12	-	-	-	-	-	-	-	-	-	-	-	-
9	Dodecan6 phenyl	C18H30	24.87	2.96	24.87	3.89	24.88	3.10	-	-	-	-	-	-	-	-
10	Dodecane5 phenyl	C18H30	24.98	2.51	22.75	6.45	22.76	3.33	-	-	-	-	-	-	-	-
11	Dodecane4 phenyl	C18H30	25.24	2.45	25.23	3.99	25.24	2.23	-	-	-	-	-	-	-	-
12	Dodecane3 phenyl	C18H30	25.69	2.14	25.68	4.35	25.69	2.46	-	-	-	-	-	-	-	-
13	Dodecane2 phenyl	C18H30	26,51	4.87	26.51	7.10	26.51	4.57	-	-	-	-	-	-	-	-
14	Tridecane6 phenyl	C19H32	26.98	4.43	26.98	7.93	22.68	0.51	-	-	-	-	-	-	-	-
15	Tridecane5 phenyl	C19H32	27.12	2.88	27.12	5.54	-	-	-	-	-	-	-	-	-	-
16	Neophytadiane	C20H38	27.22	3.52	-	-	22.75	2.22	-	-	-	-	-	-	-	-
17	Eicosane,4-phenyl	C26H46	27.37	5.57	27.37	8.19	27.38	4.00	-	-	-	-	-	-	-	-
18	3,7,11,15 -tetramethyl-2hexadecenol 1	C20H40O	27.74	3.69	-	-	-	-	-	-	-	-	-	-	-	-
19	Tridecane3 phenyl	C19H32	27.85	4.06	27.84	6.82	27.85	3.61	-	-	-	-	-	-	-	-
20	Tridecane2 phenyl	C19H32	28.65	7.16	28.64	11.06	28.65	6.13	-	-	-	-	-	-	-	-
21	1,3,5- Triazine-2,4-diamine 6-chloro-N-ethyl	C5H8ClN5	28.78	0.45	-	-	-	-	-	-	-	-	24.79	0.55	-	-
22	Cis -11-Eicosenoic acid	C20H38O2	28.90	1.31	-	-	-	-	-	-	-	-	-	-	-	-
23	Hexadecanoic acid,methyl ester(palmitic methyl ester	C17H34O2	29.01	8.81	29.01	4.09	29.02	9.34	-	-	-	-	26.46	8.31	-	-
24	Oleic acid	C18H34O2	29.92	1.53	32.47	0.37	-	-	-	-	-	-	-	-	29.38	4.28
25	ethyl hexadecanoate	C18H36O2	30.36	3.08	-	-	-	-	-	-	-	-	-	-	-	-
26	2,2-dideutero octadecanal	C18H34D2O	32.06	0.64	32.06	0.49	-	-	-	-	-	-	-	-	-	-
27	9,12-Octadecadienoic acid (Z,Z)-, methyl ester(Linoleic acid, methyl ester)	C19H34O2	32.23	1.82	-	-	32.23	5.85	-	-	-	-	-	-	-	-
28	Linoleoyl chloride	C18H31ClO	32.35	4.43	-	-	33.16	2.38	-	-	-	-	-	-	-	-
29	Oleic acid, methyl ester	C19H36O2	32.47	0.48	32.35	2.71	32.36	6.15	29.84	6.15	-	-	29.74	8.98	-	-
30	Phytol	C20H40O	32.60	14.85	-	-	32.60	13.32	-	-	-	-	-	-	-	-
31	Methyl isostearate	C19H38O2	32.85	0.98	-	-	-	-	-	-	-	-	-	-	-	-
32	cis-Vaccenic acid	C18H34O2	33.13	1.88	-	-	-	-	-	-	-	-	-	-	-	-
33	(9E,12E)-9,12-Octadecadienoyl chloride	C18H31ClO	33.46	0.51	-	-	-	-	-	-	-	-	-	-	-	-
34	7,11-Hexadecadienal	C16H28O	33.58	1.00	-	-	-	-	-	-	-	-	-	-	-	-
35	1-Chlorooctadecane	C18H37Cl	-	-	8.90	0.64	-	-	-	-	-	-	-	-	-	-
36	Benzedrex	C10H21N	--	-	19.85	3.72	-	-	-	-	-	-	-	-	-	-
37	Decane 5phenyl	C16H26	-	-	20.45	0.42	-	-	-	-	-	-	-	-	-	-
38	10,12-Octadecadiynoic acid	C18H28O2	-	-	20.65	0.39	-	-	-	-	-	-	-	-	-	-
39	12,15-Octadecadiynoic acid, methyl ester	C19H30O2	-	-	21.07	0.40	-	-	-	-	-	-	-	-	-	-

40	1-Methylnonadecyl)- ethanol, 2,2'-iminobis	C4H11NO2	-	-	21.94	0.55	-	-	-	-	-	-	-	-	-	-
41	Hydrochloride2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one,9-[[[2-(dimethylamino)ethyl]amino] methyl] octahydro-2,5a-dimethyl-	C19H32N2O3	-	-	24.51	1.17	-	-	-	-	-	-	-	-	-	-
42	2-cis-9-Octadecenyloxyethanol	C20H40O2	-	-	27.22	1.02	-	-	-	-	-	-	-	-	-	-
43	17-Octadecynoic acid	C18H32O2	-	-	28.10	0.57	28.10	0.46	-	-	-	-	-	-	-	-
44	Cholestan-3-ol, 2-methylene-, (3á,5à)-	C28H48O	-	-	28.78	0.39	-	-	-	-	-	-	-	-	-	-
45	3,5-Triazine-2,4-diamine 6-chloro-N-ethyl	C5H8ClN5	-	-	28.89	0.79	-	-	-	-	-	-	-	-	-	-
46	Estra-1,3,5(10)-trien-17á-ol	C18H24O	-	-	29.92	0.66	-	-	-	-	-	-	-	-	-	-
47	Palmitic acid, ethyl ester	C18H36O2	-	-	30.36	2.29	30.36	2.56	-	-	-	-	-	-	-	-
48	7,10-Octadecadienoic acid, methyl Ester	C19H34O2	-	-	32.23	0.58	-	-	-	-	-	-	-			
49	2-[12-(2-oxiranyl)dodecyl] oxirane	C16H30O2	-	-	32.59	2.15	-	-	-	-	-	-	-			
50	octadecanoic acid, methyl ester	C19H38O2	-	-	32.85	0.82	-	-	30.31	2.06	28.90	2.49	30.16	2.25	31.15	0.80
51	Ergosta-5,22-dien-3-ol, acetate, (3á,22E)-	C30H48O2	-	-	38.24	0.53	-	-	-	-	-	-	-			
52	1-Dodecanamine, N,N-dimethyl	C14H31N	-	-	-	-	19.70	5.70	-	-	-	-	-			
53	Dimethyl myristamine	C16H35N	-	-	-	-	24.42	2.38	-	-	-	-	-			
54	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	C20H40O2	-	-	-	-	27.22	0.57	-	-	-	-	-			
55	2,2,3,3,4,4 hexadeutero octadecanal	C18H30D6O	-	-	-	-	28.47	0.44	-	-	-	-	-			
56	2-Aminoethanethiol hygrogen sulfate ester	C2H7NO3S2	-	-	-	-	28.90	0.68	-	-	-	-	-			
57	n-Hexadecanoic acid	C16H32O2	-	-	-	-	29.85	2.50	28.33	23.59	25.69	17.24	28.06	19.83	26.66	20.62
58	Methyl stearate	C19H38O2	-	-	-	-	32.85	0.98	-	-	-	-	-			
59	Linoleic acid ethyl ester	C20H36O2	-	-	-	-	33.46	0.75	-	-	-	-	30.71	0.78		
60	9,12,15-Octadecatrienoicacid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-(Linolenin, 1-mono-)	C21H36O4	-	-	-	---	33.59	0.90	-	-	-	-	-	-	-	-
61	á-Sitosterol(Stigmast-5-en-3-ol, (3á)-)	C29H50O	-	-	-	-	44.00	2.4	-	-	-	-	-	-	-	-
62	3,5-Heptadienal, 2-ethylidene-6-methyl NE-1,2-Diol	C10H14O	-	-	-	-	-	-	13.17	0.92	-	-	-	-	-	-
63	2-Ethylcyclohexylamine, N-(2-chloropropylidene)-, N-oxide	C11H20ClNO	-	-	-	-	-	-	14.20	1.08	-	-	-	--	-	-
64	8-Azabicyclo[3.2.1]octaN-3- OL, 8-methyl-, benzoate (ester), exo-	C15H19NO2	-	-	-	-	-	-	16.55	0.37	-	-	-	-	-	-
65	Ethanone, 2-chloro-1-(3,4-dihydroxyphenyl)-	C8H7ClO3	-	-	-	-	-	-	18.43	1.38	-	-	-	-	-	-
67	4-(2-Hydroxyethyl)-2-methoxyphenol	C9H12O3	-	-	-	-	-	-	19.25	1.03	-	-	-			
68	Alanine,3(benzyloxy)-, L-	C10H13NO3	-	-	-	-	-	-	22.15	0.30	-	-	-			
69	Tetradecanoic acid	C14H28O2	-	-	-	-	-	-	23.96	1.08	-	-	23.65	0.62		
70	Ethanol,2-(9octadecenyloxy)-, (Z)-	C20H40O2	-	-	-	-	-	-	24.78	0.35	-	-	24.64	1.33	24.65	0.48
71	9-Octadecenoicacid, (2-phenyl-1,3-dioxolan-4-YL)methyl ester, cis	C28H44O4	-	-	-	-	-	-	26.13	0.57	-	-	-	-	-	-
72	Hexadecanoic acid, methyl ester	C17H34O2	-	-	-	-	-	-	26.59	4.58	22.33	6.03	-	-	26.45	4.79
73	2-Acetyl-3-(2-*cinnamido)ethyl-7-methoxyindole*	C22H22N2O3	-	-	-	-	-	-	27.36	3.98	-	-	32.92	2.11	-	-
74	Butanoic acid, heptafluoro-, methyl Ester	C5H3F7O2	-	-	-	-	-	-	27.46	0.29	-	-	-	-	-	-

75	9,12-Octadecadienoic acid, methyl ester, (E,E)-(Linolelaidic acid, methyl ester)	C19H34O2	-	-	-	-	-	-	29.70	3.66	-	-	29.55	2.48	-	-
76	10-Octadecenoic acid, methyl ester	C19H36O2	-	-	-	-	-	-	29.95	1.95	28.38	4.88	29.82	2.38	-	-
77	Stigmast-5-EN-3-OL, (3á,24S)-	C29H50O	-	-	-	-	-	-	30.62	0.87	-	-	43.37	1.10	43.73	1.17
78	6-Octadecenoic acid	C18H34O2	-	-	-	-	-	-	31.56	28.01	29.82	22.65	31.26	14.36	31.27	26.72
79	Octadecanoic acid(stearate)	C18H36O2	-	-	-	-	-	-	31.85	5.20	30.59	7.11	31.51	5.09	31.63	3.45
80	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	-	-	-	-	-	-	32.15	4.31	-	-	-	-	-	-
81	9-Octadecenoic acid(Z)-		-	-	-	-	-	-	-	-	30.86	1.80	30.82	1.11	-	-
82	Oxiraneoctanoic acid, 3-octyl-, methyl ester	C19H36O3	-	-	-	-	-	-	33.44	1.10	-	-	-	-	-	-
83	DI-2-benzothiazole Disulfane	C14H8N2S4	-	-	-	-	-	-	33.74	0.28	-	-	-	-	-	-
84	2-Hydroxy-3-[(9E)-9-octadec enoyloxy]propyl (9E)-9-octadecenoate #	C39H72O5	-	-	-	-	-	-	35.36	0.95	-	-	-	-	-	-
85	Prostaglandin A1-biotin	C35H58N4O5S	-	-	-	-	-	-	36.32	1.97	-	-	-	-	-	-
86	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Palmitin, 1,2-di-)	C35H68O5	-	-	-	-	-	-	36.88	0.82	-	-	-	-	-	-
87	3',8,8'-Trimethoxy-3-piperidyl-2,2'-b inaphthalene-1,1',4,4'-tetrone	C28H25NO7	-	-	-	-	-	-	37.15	1.43	-	-	-	-	-	-
88	Prostaglandin F2à-biotinamide	C35H60N4O6S	-	-	-	-	-	-	38.99	0.50	-	-	-	-	-	-
89	Flavone 4'-OH,5-OH,7-DI-O-glucoside	C27H30O15	-	-	-	-	-	-	39.67	0.87	39.38	2.39	39.29	3.93	38.73	0.75
90	Hahnfett	N/A	-	-	-	-	-		42.11	0.32	33.23	6.23	36.11	2.62	36.15	4.56
91	2-Methoxy-4-vinylphenol	C9H10O2	-	-	-	-	-	-	-	--	12.98	1.62	--	--	--	--
92	1,3-Bis(2-chloroethyl)urea (15N(1),15N(3)) Benzene	C5H10Cl2N2O	-	-	-	-	-		-	--	18.28	7.13	--	--	--	--
93	Ethanol,2-(3-MethoxybIcyclo[2.2.1]Hept-2-ylidene)-	C10H16O2	-	-	-	-	-		-	-	19.02	4.38	--	--	--	--
94	Octanal, 2-(phenylmethylene) (Cinnamaldehyde, à-hexyl-)	C15H20O	-	-	-	-	-	-	-	-	22.85	1.81	--	--	--	--
95	9-Oximino-2,7-diethoxyfluorene	C17H17NO3	-	-	-	-	-		-	-	27.18	1.16	--	--	22.38	0.84
96	11-Octadecenoic acid, methyl ester, (Z)-	C19H36O2	-	-	-	-	-	-	-	-	28.21	6.48	--	--	29.68	6.97
97	Methyl 9,9-dideutero-Octadecanoate	C19H36D2O2									29.82	1.05				
98	Androstan-17-one3-ethyl-3-hydroxy-, (5à)-	C21H34O2	-	-	-	-	-	-	-	-	31.72	0.89	-	-	-	-
99	cis-5,8,11,14,17-Eicosapentaenoic acid	C20H30O2	-	-	-	-	-	-	-	-	30.71	1.08	--	--	30.24	0.26
100	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z)-	C21H36O4	-	-	-	-	-	-	--	--	32.26	0.78	--	-	--	-
101	Pregn-4-ENE-3,20-Dione, 11,21-Dihydroxy-, (11á)-	C21H30O4	-	--	-	-	-		-	-	33.57	0.98	-	-	-	-
102	icosyl (Z)-octadec-9-enoate	C38H74O2	-	-	-	-	-	-	-	-	34.40	0.98	-	-	-	-
103	Doosanoic acid, methyl ester	C23H46O2	-	--	-	-	-	-	-	-	36.71	1.16	-	-	-	-

	4H-1-benzopyran-4-ONE, 2-(3,4-Dihydroxyphenyl)-6,8 -Di-á-D-Glucopyranosyl-5, 7-Dihydroxy-	C27H30O16									36.11	1.33				
104	Docosanoic acid, 1,2,3-propanetriyl ester	C69H134O6	-	-	-	-	-	-	-	-	36.96	0.74	-	-	-	-
105	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	C21H38O2	-	-	-	-	-	-	-	-	-	-	25.48	0.60	-	-
106	9-Hexadecenoic acid, methyl ester, (Z)- (Methyl palmitoleate)	C17H32O2	-	-	-	-	-	-	-	--	-	-	25.97	0.56	-	-
107	Ethyl iso-allocholate	C26H44O5	-	-	-	-	-	-	-	-	-	-	29.92	1.21	38.09	1.02
108	1,25-Dihydroxyvitamin D3, TMS Derivative	C30H52O3Si							-	-	-	-	30.44	0.43	-	-
109	i-Propyl 5,8,11,14,17-eicosapentaenoate	C23H36O2	-	-	--	-	-	-	-	-	-	-	31.64	1.07	31.77	3.73
110	Cis-2-phenyl-1,3dioxolane-4-methyl octadce-9, 12, 15-Trienoate	C28H40O4	--	--	--	-	-	-	---	--	--	-----	31.79	2.46	-	-
111	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans	C19H36O3	-	-	-	-	-	-	-	-	-	-	33.75	0.84	-	-
112	Dotriacontane	C32H66	-	-	-	-	-	-	-	-	-	-	33.95	3.93	-	-
113	17-Pentatriacontene	C35H70	-	-	-	-	-	-	-	-	-	-	35.15	1.83	-	-
114	Vitamin E	C29H50O2	-	-	--	-	-	-	-	-	-	-	36.55	2.02	-	-
115	Diisooctyl phthalate	C24H38O4	-	-	-	-	-		-	-	-	-	36.97	2.91	-	-
116	Isochiapin B	C19H22O6	-	-	-	-	-		-	-	-	-	37.45	1.65	37.51	1.07
117	1-Heptatriacotanol	C37H76O	-	-	-	-	-		-	-	-	-	40.87	1.31	24.45	0.47
118	Glycodeoxycholic acid	C26H43NO5	-	-	-	-	-	-	-	-	-	-	42.73	1.16	-	-
119	2-Cyclohexyl-2,5-cyclohexadiene-1,4-dione, 4-oxime	C12H15NO2	-	-	-	-	-	-	-	-	-	-	-	-	16.97	1.23
120	Pentadecanoic Acid, 14-methyl-, methyl ester	C17H34O2	-	-	-	-	-	-	-	-	-	-	-	-	26.16	0.34
121	Octadecanoic Acid, 2,3-Dihydroxypropyl Ester	C21H42O4	-	-	-	-	-	-	-	-	-	-	-	-	29.97	0.73
122	Ursodeoxycholic acid	C24H40O4	-	-	-	-	-	-	-	-	-	-	-	-	30.43	0.47
123	Oxiraneoctanoic Acid, 3-Octyl-, Cis-	C18H34O3	-	-	-	-	-		-	-	-	-	-	-	33.24	0.28
124	DI-2-Benzothiazole Disulfane	C14H8N2S4	-	-	-	-	-		-	-	-	-	-	--	33.60	2.83
125	cis-13-Eicosenoic acid	C20H38O2	-	-	-	-	-	-	-	-	-	-	-	-	34.04	0.58
126	2-Hydroxy-3-[(9E)-9-Octadec Enoyloxy]propyl (9E)-9-octadecenoate #	C39H72O5	-	-	-	-	-	-	---	-	-	-	-	-	35.19	0.27
127	2,6-Dimethyl-N-(2-methyl-à -phenylbenzyl)aniline	C22H23N	-	-	-	-	-		-	-	-	-	-	-	36.99	1.78
128	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl Ester	C21H40O4	--	--	--	--	--	--	-	-	-	-	-	-	39.40	2.25
129	Rhodopin	C40H58O	--	--	--	--	--	--	-	-	-	-	-	-	40.60	0.78
130	Cholesterol margarate	C44H78O2	--	--	--	--	--	--	-	-	-	-	-	-	41.55	0.76
131	7,8-Epoxylanostan-11-ol, 3-acetoxy	C32H54O4	--	--	--	--	--	--	-	-	-	-	--	-	42.36	1.33
132	Stigmasterol	C29H48O	--	--	--	--	--	--	-	-	-	-	-	-	42.67	1.56

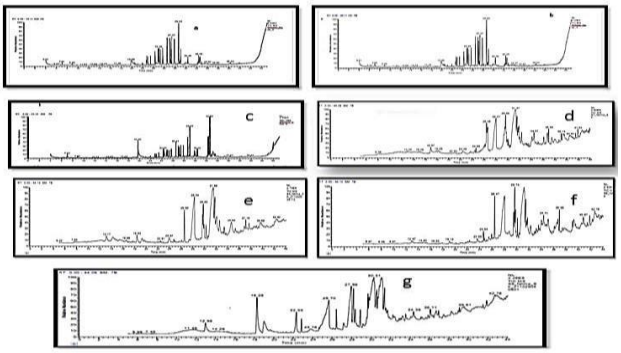

Fig. (2) Gas chromatography mass spectrometry spectra of ethanol extracts of (a) *H. stipulacea* (b) *Halodule uninervis* (c) *T. ciliatum* (d) *S. aegyptiaca* (e) *A. fruticosum* site (2) (f) *A. fruticosum* site (1) (g) *S. marina*

Antimicrobial activity

Antibiotic resistance is currently regarded as one of the most pressing concerns to humanity [48]. Several research programs are being directed toward the discovery of novel antibiotic sources. In this regard, the authors are interested in exploring Bahariya Oases and Red Sea at Hurghada Coast plants, particularly thosewith limited or no previous reports as promising sources for searching for new medicinal constituents. The ethanolic extracts of *H. uninervis*, *A. fruticosum* site *(2), Suaeda and S. marina* have shown inhibition effects growth on four species of fungi; *Cryptococcus neoformas, Grotricum candidum and Penicillium marn- effeii*, meanwhile there is no activity against the species of fungi, the growth of fungi that were inhibited by *H. uninervis A. fruticosum site (2), Suaeda and S .marina* have shown (9, 13 ,12, 12mm) respectively, inhibition diameter zone (IDZ) while *T. ciliatum , H. stipulacea* and *A. fruticosum* site (1) have shown no inhibition effects on the growth of all tested fungi **Table (5**). On the other hand, the ethanolic extract of *T. ciliatum* has shown activities against G +ve bacteria; *Bacillus cereus* and *Enterococcus faecalis* (9mm and 8mm) respective- ly, also *H. uninervis* has an inhibition effect on thegrowth of Bacillus cereus that was 9 mm while, *H. stipulacea* has shown no inhibition effects on the growth of all tested G +ve bacteria. On the other hand, ethanolic extracts of all terrestrial plants (*A. fruticosum* site (1*), A. fruticosum* site (2), *Suaeda and S. marina*) have inhibition effect on the growth of *Micrococcus sp* that were (8, 7, 12 and 7mm) respectively. The *H. stipu- lacea, T. ciliatum and H. uninervis* have shown high activities against G-ve bacteria; *Proteus vulgaris* (11, 11 and 13 mm; respectively) and not reveal any effect on other species of G-ve bacteria; *Pseudomonas aerugino- sa and Enterobacter cloacae*. Generally, the ethanolic extracts of *H. uninervis* and *Suaeda* have activities ef- fect against fungi, G+ve bacteria and G-ve bacteria (9, 9, 11 mm, 12, 12 and 12 mm) respectively. The *H. uninervis* and *Suaeda* have activities effect against fungi, G+ve bacteria, and G-ve bacteria (9, 9, 11 mm, 12, 12, and 12mm) respectively. The antimicrobial activities may be related to the presence of fatty acids. The results showed that ethanol extracts of each plants that under investigation were rich in saturated fatty acids and unsaturated fatty acids both with long carbon chains16 and more.[46] reported that G-ve bacteria are less sensitive to fatty acids that G+ev bacteria. Also, they stated that fatty acids carbon chain lengths play a very important role in their antimicrobial properties. Fatty acids having 6 and fewer carbons inhibit Gram-negative bacteria while Gram-positive bacteria are inhibited by fatty acids that contain carbon chains longer than 12. Numerous studies reported that unsaturated fatty acids with long carbon chains as linoleic acid, and oleic acid have bactericidal ,but saturated fatty acids with long carbon chain as stearic acid and palmitic acid, are less active [49]. Flavonoid compounds have the ability to inhibit bacterial growth with many various mechanisms, by, interaction between flavonoid substances and bacterial DNA, it causes damage to the bacterial wall permeability, microsomes, and lysosomes [50]. Also, alkaloids have the function as antibacterial by disrupting the peptidoglycan constituent of the bacterial cell therefore, that the cell wall layer is not completely formed and causes the cells death [51]. Also, our results agree with [52] Who indicated that plant pathogenic fungi are more resistant to plant extracts than pathogenic bacteria. Only five extracts inhibited fungal growth among thirteen different plant extracts that inhibited the growth of bacteria. There are many reports issued regarding the investigation of antimicrobial activity of some species of this plants [53] investigated the antibacterial of *Suaeda australis* and *Suaeda maritime* extracts aganist *P. aeruginosa, P. mirabilis* and *A. baumannii*. [54] evaluated the antibacterial activity of *Halophila stipulacea (H. stipulacea), Cymodocea serrulata (C. serrulata) and Halodule pinifolia (H. pinifolia)* against seven human bacterial pathogens. Antibacterial activity of three seagrass screened, was in the order of *H. pinifolia > H. stipulacea > C. serrulata.* [55] Investigated the antibacterial activity of *Halodule uninervis* against seven bacterial pathogens. [56] He studied antibacterial and fungicidal activity of methanolic extracts from different parts of *S. marina* against *Escherichia coli, Bacillus subtilis*, and *Candida albicans* andshowed no antibacterial activity against *Escherichia coli and Bacillus subtilis*, and weak fungicidal activity of stem extracts and inflorescences grown on soils, with high levels of salinities, was detected against *Candida albicans*.

Table (5) Antimicrobial activity of studied halophyte plants

Sample code	*H. uninervis*	*H .stipulacea*	*T. ciliatum*	*A. fruticosum* site (1)	*A. fruticosum* site (2)	*Suaeda*	*S. marina*	Control
Tested microorganisms								Ketocnazole
FUNGI								
Grotricum candidum	NA	NA	NA	NA	NA	NA	12	15
Syncephalastrum race-mosum	NA	NA	NA	NA	NA	NA	NA	26
Penicillium marneffeii	NA	NA	NA	NA	13	12	NA	
Cryptococcus neoformas	9	NA	NA	NA	NA	NA	NA	25
Gram positive bactria:								**Gentamycin**
Micrococcus sp.	NA	NA	NA	8	7	12	7	21
Bacillus cereus	9	NA	9	NA	NA	NA	NA	25
Enterococcus faecalis	NA	NA	8	NA	NA	NA	NA	26
Gram Negative bactria:								**Gentamycin**
Proteus vulgaris	11	11	13	NA	NA	NA	NA	25
Pseudomonas aeruginosa	NA	NA	NA	NA	NA	NA	10	27
Enterobacter cloacae	NA	NA	NA	NA	NA	12	NA	30

The test was done using the diffusion agar technique, Well diameter; 6.0 mm (100µl was tested), Inhibition zone diameter (mm/mg sample). Positive control for fungi Ketocnazole 100µg/ml positive control for bacteria Gentamycin 4µg/ml. NA;No activity .the sample was tested at 10mg/ml concentration.

4. Conclusion

This study aims at evaluating and assembling and compares the chemical composition of aquatic and terrestrial halophyte plants extracts. With the display of antimicrobial activities, these plants show ability to pro- duce variety of bioactive secondary metabolites that can be used for therapeutic purposes. The present results determine the total alkaloids in Egyptian, Red Sea *H. uninervis* and *H. stipulacea* seagrasses and define new alkaloids; Nizatidine *in Halodule uninervis* and benzedrex (propylhexedrine) in *Halophila stipulacea* for the first time. Also Ne-1,2-diol 2-Ethylcyclohexylamine,N-(2-chloropropylidene)-, N-oxide, 2-Acetyl-3-(2-Cinnamido)ethyl-7-Methoxyindole and Alanine, 3-(Benzyloxy)-, L found only in *A. fruticosum* (site1) and Rhodopin , Cholesterol margarate found only in *S. aegyptiaca*, Egyptian, Red sea *Halodule uninervis* contains wide variety of secondary metabolites like diterpene neophytadiene, Phytol, Methyl isostearate, Oleic acid and 1,3,5-Triazine-2,4-Diamine, 6-Chloro-nethyl that have anti-inflammatory antioxidant, cardioprotective, antibacterial and antifungal properties. As a result, this kind of GC-MS analysis serves as the initial step towards understanding the nature of the active ingredients in these plants, and will be significant for future research. Halophyte plants are considered an excellent source of natural biologically active secondary metabolites that may be used as potential alternatives in the therapeutic industry to substitute synthetic medication with natural biologically active constituents.

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