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Screening of Antifungal Activity of Bioactive Chemical Constituents in Some Brown Marine Macroalgae from the Red Sea, Egypt

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SOME mycological strains have been reported to be resistant to commonly used drugs. As a result, the development of new and important antifungal drugs has become a global endeavor. This study investigated the antifungal activity of three brown marine macroalgae, *Sargassum cinereum, Padina boergesenii*, and *Cystoseira myrica* against three fungal dermatophytes, namely *Candida albicans, Candida glabrata*, and *Candida tropicalis* and two nondermatophytic, *Fusarium oxysporum* and *Alternaria alternata* using the disc diffusion assay. Algal extraction was performed using three organic solvents (acetone, ethanol, and methanol). The results showed that the seaweed extracts exhibited different patterns of antifungal activities. The ethanolic extract of *Sargassum cinereum* was the most active as compared to other organic extracts. The maximum antifungal activity of the *S. cinereum* ethanolic extract was 21.3 ± 0.32 and 18.8 ± 0.16mm against *F. oxysporum* and *C. glabrata*, respectively, followed by the methanolic extract of *C. myrica* (16.5 ± 0.21mm) against *F. oxysporum*, and ethanolic extract of *P. boergesenii* (16.3 ± 0.16mm) against *C. glabrata.* The minimal inhibitory concentrations (MICs) of the algal extracts for inhibiting the tested fungi were in a range of 8.5–70µg/mL. The ethanolic extract of *S. cinereum* had the lowest MIC value (8.5 ± 0.12µg/mL) against *F. oxysporum.* The gas chromatography–mass spectrometry of the ethanolic extracts revealed the presence of chemical constituents that could have significant antifungal effects in the brown marine macroalgae. The main constituents were the hexadecanoic acid methyl ester (palmitic acid); 3,7-dimethylocta-2,6-dienal; and octadecanoic acid methyl ester.

Keywords: Antifungal activity, Brown seaweeds, GC-MS analysis, Minimum inhibitory concentrations.

Introduction

Marine habitats are a puddle of bioactive natural compounds that are not present in terrestrial natural products. Marine organisms such as macroalgae are sources of various natural antimicrobial compounds with biological and pharmacological activities (Ismail et al., 2016) as fungal infections are responsible for an extremely high degree of mortality in humans and aquaculture organisms (Rajkumar et al., 2018).

A variety of solvents with different polarities can be used to extract different biologically active compounds from marine algae (Khelil-Radji et al., 2017). These compounds are fatty acids, flavonoids, peptides, acrylic acid, crude polysaccharides, sulfated polysaccharides, phenolic compounds, steroids, ketones, phlorotannins, and alkanes (Omar et al., 2018). Antifungal, antibacterial, antioxidant, antiviral, and anthelmintic properties are among the reported pharmacological actions of these bioactive substances (El-Sheekh et al., 2020, 2021). Recently, there has been increased attention on naturally produced active compounds as replacements for synthetic substances. Although

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these compounds frequently exhibit low activity, they are harmless and do not leave deposits. This indicates that there is a need to establish new and safe products of biological sources, with properties similar to those of synthetic sources, in specific antimicrobial, antifungal, and antioxidizing compounds. These natural composites are found in seaweed extracts (El Zawawy et al., 2020).

Approximately 16000 marine natural products have been isolated from marine organisms and reported in approximately 6800 publications. Over 9000 reports exist on the subject of marine natural products, including syntheses, reviews, biological activity investigations, and ecological studies. Some of these compounds are only found in marine organisms (Bhakuni & Rawat, 2005), indicating that marine microorganisms are an important source for novel antibiotic, antitumor, and antiinflammatory agents. Thus, marine organisms appear to be an environmentally friendly source of new pharmaceuticals, and the potential of algae to provide a variety of primary and secondary metabolites has been intensively explored (Turney et al., 2006). The maximum antifungal activities of seaweeds such as red, brown, and green algae are 37.33, 33.3, and 8.3%, respectively (Pandian et al., 2011).

Candida species cause opportunistic diseases and enormous disease rates, which lead to hospital visits and expensive treatments (Gholampour-Azizi et al., 2015). The genus *Candida* belongs to yeasts. *Candida* species exist as commensal organisms in the oral microbiota in approximately 20–60% of the general population (Aggarual et al., 2018). It is also the leading cause of opportunistic mycoses worldwide. It is a recurrent colonizer of mucous membranes and the human skin (Steenkamp et al., 2007).

In many algal species with antifungal activity against *Candida albicans*, heptadecane and tetradecane are important volatile constituents (Ozdemir et al., 2004). El-Sheekh et al. (2014) stated that significant antifungal activity was identified in brown seaweeds (*Padina gymnospora*, *Cystoseira myrica*, and *Sargassum ramifolium*). *Sargassum* sp. has been confirmed as an oriental medical primer; rudimentary medicines were documented and used as food in East Asia (Lee et al., 2010). *Cystoseira crinita* extracts exhibited antifungal activity against *Candida krusei* (Berber et al., 2015).

This study investigated the antifungal activities of various solvent extracts of marine brown seaweeds sampled off the Red Sea in Hurghada region, Egypt, against five human and plant fungal pathogens. Furthermore, gas chromatography–mass spectrometry (GC–MS) was performed to investigate the composition of the most efficient extracts.

Materials and Methods

Seaweed samples collection

Three species of brown seaweeds, *Sargassum cinereum*, *Padina boergesenii*, and *Cystoseira myrica* were collected in September 2016 from Hurghada city (27°17′03″ N, 33°46′21″ E) off the Red Sea coast of Egypt. The collected algae were identified according to Guiry & Guiry (2011). The samples were properly washed with seawater to remove any epiphytes and sand particles, transported in sterile containers to the laboratory, and washed with tap and distilled water. The algal samples were spread on plates, dried in a shade, and processed using an electric blender (El-Sheekh et al., 2020).

Preparation of seaweed extracts using different solvents

The algae powder (10g) was soaked for 7 days in 100mL of each solvent (acetone, ethanol, and methanol) on a rotary shaker at 120rpm at room temperature. The solution was filtered through Whatman No. 1 sterile filter paper. The obtained filtrate was extracted from the solvent by evaporation under reduced pressure. The residues (crude extracts) obtained were suspended in dimethyl sulfoxide (DMSO, 1g/mL) to a final concentration and placed in a cooler for further use (Patra et al., 2008). The algal extracts were tested at three concentrations: 300, 200, and 100mg/mL.

Tested fungi

Vaginal dermatophytic fungal strains such as *Candida albicans, Candida glabrata,* and *Candida tropicalis* were obtained from the Department of Medical Microbiology, Faculty of Medicine, South Valley University, Qena, Egypt. Nondermatophytic fungal species such as *Fusarium oxysporum* and *Alternaria alternata* were isolated from tomato plants. The pathogenic fungi were maintained on a suitable medium at 4°C and subcultured on Sabouraud's agar medium, while *Fusarium oxysporum* and

Alternaria alternata were maintained on a potato dextrose agar (PDA) medium.

Antifungal activities of the seaweed extracts

The antifungal activities of the algal extracts were screened using the agar disc diffusion assay, s described in our earlier studies (Farghl et al., 2019). The standard antifungal medicine, amphotericin B (5mg/mL), as a positive control, and the algal extracts (300mg/ mL) were injected into the discs. The experiment was done in triplicate, and the plates were carefully checked for the presence or absence of fungal growths and the sizes of the inhibitory zones (mm). The data were presented as mean values with the standard deviation (SD).

Minimal inhibitory concentrations (MICs) of algal extracts

Serial dilutions of the algal extracts (µg/mL) were applied to sterilized plates containing the freshly prepared media with a standard number of cells for fungal isolates. The MIC was determined to be the lowest concentration that did not show any visible growth of microorganisms (Da Silva Barros et al., 2007).

GC–MS analysis

The seaweed extracts were analyzed by GC–MS at the National Research Center, Dokki in Cairo, Egypt. The sample was injected into a column of HP-5 (30m × 0.25µM film thickness) for GC–MS. The PerkinElmer Clarus 580/560 S model system was used for the transportation gas at a flux rate of 0.8mL/min. The temperature of the GC oven was programmed at a rate of 2°C/min from 60 to 250°C. The total ion current directly obtained relative area values (as a percentage of the total volatile composition).

Statistical analyses

One-way ANOVA tests were used to analyze the data, followed by Tukey's multiple comparison post hoc test. Differences were considered significant at $P< 0.05$. Graph Pad Prism version 5 was used for the statistical analyses. The mean ± SD of three replicates is presented for the antifungal experiments.

Results

Antifungal activities of seaweed extracts

The antifungal activity of the brown seaweed extracts is shown in Fig.1. Three concentrations of each brown seaweed extract were tested: 300, 200, and 100mg/mL. The sample with a concentration of 300mg/mL exhibited the highest antifungal activity. The results demonstrated that the *S. cinereum*, *C. myrica*, and *P. boergesenii* extracts (acetone, ethanol, and methanol) exhibited antifungal efficacy against all the tested pathogenic fungi with varying potencies. *F. oxysporum, C. glabrata,* and *C. tropicalis* were the fungal isolates most susceptible to the algal extracts, compared with other fungi and amphotericin B, with average inhibition zone values of 28.7±0.81, 25.8±0.84, and 23.3±0.74mm, respectively (Fig.1).

For *S. cinereum*, the maximum inhibition zones were observed in the ethanolic extract (21.3±0.32mm, 74.21%, compared with the positive control) against *F. oxysporum* and 18.8±0.16mm, 72.86% against *C. glabrata*. The acetone extract demonstrated the minimum activity against *C. albicans* (3.6±0.11mm, Fig. 1A).

The inhibition zone of the *P. boergesenii* extracts ranged between 16.3 and 4.2 mm against the pathogenic fungi (Fig. 1B). The highest inhibition zone was observed in the ethanolic extract (16.3±0.16mm, 63.17%) against *C. glabrata,* followed by the ethanolic extract (14.1±0.07mm, 60.51%) against *C. tropicalis.* The acetone extract exhibited the minimum activity against *C. albicans and A. alternata.*

For *C. myrica*, the inhibition zone of the extracts ranged between 16.5 and 3.5mm against the fungal strains (Fig. 1C). The methanolic extract demonstrated the highest inhibition activity (16.5±0.21mm, 57.49%) against *F. oxysporum*, followed by the ethanolic extract (12.6± 0.60mm, 48.83%) against *C. glabrata*, whereas the acetone extract exhibited the minimum activity (3.5± 0.31mm, 20.0%) against *A. alternata*.

MICs of the brown seaweed extracts

The MIC value is critical in estimating the extract dose required to inhibit the growth of specific microorganisms. The minimum inhibitory concentrations (MICs) of the seaweed extracts are shown in Table 1. The results showed that the algal extracts exhibited significant and potent antifungal activity against all the tested fungal isolates at low concentrations. The MIC values of seaweed extracts for inhibiting tested

fungi ranged from 8.5-70µg/mL. The ethanolic extract of *S. cinereum* had the lowest MIC value (8.5±0.12µg/mL) against *F. oxysporum*, followed by the ethanolic extract of *S. cinereum and P. boergesenii* against *C. glabrata* and *C. tropicalis*, respectively, and the methanolic extracts of *P. boergesenii* against *F. oxysporum* (10µg/mL). Thus, the ethanolic extract of the three species of brown algae was determined to be the best algal extract.

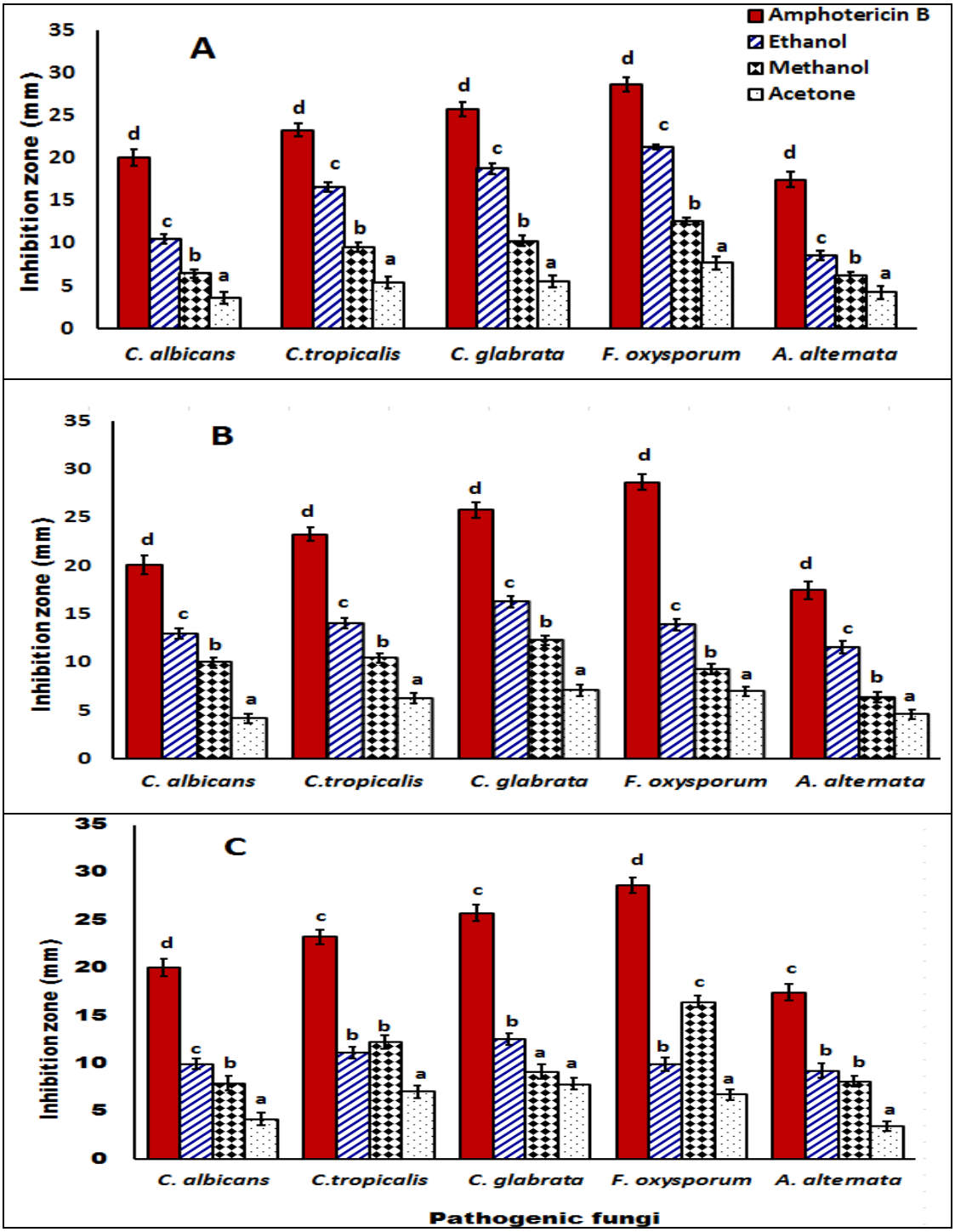

Fig. 1. The antifungal activity of seaweed extracts of *S. cinereum* (A), *P. boergesenii* (B), and *C. myrica* (C) against pathogenic fungi. Values are the mean of three replicates ± stander deviations (SD). Different letters refer to significant differences between the data sets (P< 0.05)

TABLE 1. Minimum inhibitory concentrations (MICs µg/ mL) of brown seaweed extracts against pathogenic fungi

Fungal strains / Algal extracts		*C. albicans*	*C.tropicalis*	*C .glabrata*	*F .oxysporum*	*A. alternata*
Algal species	**Organic solvents**					
S. cinereum	Ethanol	15±0.05	30±0.10	10±0.15	8.5±0.12	40±0.21
	Methanol	25±0.08	26±0.16	26±0.05	15±0.02	50±0.06
	Acetone	26±0.11	20±0.15	30±0.17	13±0.15	70±0.12
P. boergesenii	Ethanol	20±0.21	10±0.13	30±0.11	25±0.15	30±0.08
	Methanol	25±0.11	25±0.05	11±0.07	26±0.09	32±0.05
	Acetone	70±0.13	28±0.07	30±0.05	27±0.07	67±0.07
C. myrica	Ethanol	19±0.05	25±0.07	12±0.14	15±0.13	40±0.09
	Methanol	69±0.12	25±0.05	14±0.07	10±0.05	65±0.11
	Acetone	69±0.05	30±0.11	30±0.12	30±0.11	70±0.12

GC–MS analysis

The GC–MS analysis results of the most biologically active extract *in S. cinereum, C. myrica,* and *P. boergesenii* are recorded in Tables 2–4 and Fig. 2. In total, 20, 17, and 14 bioactive compounds were recognized in the ethanolic extracts of *S. cinereum*, *P. boergesenii*, and *C. myrica*, respectively.

The most abundant chemical constituents of the ethanolic extract of *S. cinereum* were 3,7-dimethylocta-1,6-diene-3-ol (63%); 3,7-dimethylocta-2,6-dienal (48.4%); hexadecanoic acid methyl ester (palmitic acid, 43.8%); 5-hexyloxolan-2-one (39.8%); 4,6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-ol (37.5%); octadecanoic acid methyl ester (31.8%); and (+–)-trans-1-isopropenyl-4-methyl-1,4-cyclohexanediol (24.9%) (Table 2 and Fig. 2A).

The major components in the ethanolic extract of *P. boergesenii* were the hexadecanoic acid methyl ester (palmitic acid, 60.21%); 3,7-dimethylocta-1,6-diene-3-ol (52.3%); 3,7- dimethylocta-2,6-dienal (40.2%); 4,6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-ol (37.11%); and 9-octadecenoic acid (Z)- methyl ester (31.7%) (Table 3 and Fig. 2B).

The predominant compounds of the ethanolic extract of *C. myrica* were 1,6-octadien-3-ol, 3,7-dimethyl-,formate (50%); 3,7-dimethylocta-2,6-dienal (39.3%); 4,6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-ol (30.8%); 5-hexyloxolan-2-one (22.68%); hexadecanoic acid methyl ester (palmitic acid, 20.4%); and 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol (18.2 %) (Table 4 and Fig. 2C).

Discussion

Seaweeds (marine macroalgae) play a vital role in the ocean; they are a valuable source of food, pharmaceuticals, and industrial products. Bioactive compounds isolated from seaweeds have a high value in pharmaceutical and biomedical applications (Krishnamoorthi & Sivakumar, 2019). Many studies have confirmed the safety of these seaweeds. Naidu et al. (1993) stated that marine algae could potentially be considered safe for human use. Abd Elmegeed et al. (2014) reported that the methanolic extract of *U. lactuca* (0.25–1mg conc.) exhibited a non-hemolytic effect on red cells, which can use for safe therapeutic purposes.

In this study, among the seaweeds tested, the highest rate of antifungal activity was detected in the ethanolic extract of *S. cinereum* (21.3±0.32mm; MIC, 8.5µg/mL) against *F. oxysporum*, while the lowest activity (3.5±0.51mm; MIC, 70µg/mL) was observed in the acetone extract of *C. myrica* against *A. alternata*. The fungal species most sensitive to the seaweed extracts was *F. oxysporum,* followed by *C. glabrata*, and *C. tropicalis* (MIC= 8.5–30µg/mL). These results correlated with those of Ambreen et al. (2012) who discovered that *Sargassum ilicifolium* was efficient against *Fusarium oxysporum*.

TABLE 2. Bioactive compounds of *S. cinereum* ethanolic extract as determined by GC/ MS analysis

NO	RT (min)	Compounds identified	Peak area %	MF	MW (g/mol)	Compound nature
1	7.455	Hexanoic acid, 2-propenyl ester	1.04	$C_9H_{16}O_2$	156.22	Fatty acid esters
2	7.648	Alpha – Methyl – alpha –[4-methyl-3-pentenyl] oxirane methanol	22	$C_{10}H_{18}O_2$	170.25	Fatty acid ester with methanol
3	7.974	3,7-Dimethylocta-1,6-dien-3-ol	63	$C_{10}H_{18}O$	154.25	Oxygenated monoterpen
4	9.845	2,2,6-Trimethyl-6-vinyltetrahydro-2H-pyran-3-ol	1.28	$C_{10}H_{18}O_2$	170.249	Terpenes
5	10.073	(3Z)-3,7-dimethylocta-3,6-dienal	0.52	$C_{10}H_{16}O$	152.233	Monoterpenoid
6	10.481	Benzene, 1-methoxy-4-(2-propenyl)	22.25	$C_{10}H_{12}O$	148.20	Phenylpropene
7	11.646	4,6,6-trimethyl bicycle [3.1.1] hept-3-en-2-ol	37.5	$C_{10}H_{16}O$	152.237	Bicyclic monoterpenoids
8	11.757	3-Hydroxy-D-tyrosine	1.78	$C_9H_{11}NO_4$	197.19	Amino acids
9	12.439	3,7-dimethylocta-2,6-dienal	48.4	$C_{10}H_{16}O$	152.24	Terpenoids
10	13.622	(+-)-trans-1-Isopropenyl-4-methyl-1,4-cyclohexanediol	24.9	$C_{10}H_2O(C_{10}H_{18}O_{2)}$	140.27	Monoterpene
11	14.217	5-Hexyloxolan-2-one	39.8	$C_{10}H_{18}O_2$	170.25	Lactone
12	14.87	Geranyl vinyl ether	1.5	$C_{12}H_{20}O$	180.287	Alkene
13	15.103	(2,2,6-Trimethyl-bicyclo [4.1.0] hept-1-yl)-methanol	1.36	$C_{11}H_{20}O$	168.2759	Monoterpene
14	23.263	Tetradecanoic acid, methyl ester	1.71	$C_{15}H_{30}O_2$	242.20	Fatty acid methyl ester
15	27.395	Hexadecanoic acid, methyl ester(Palmitic acid)	43.8	$C_{17}H_{34}O_2$	270.5	Fatty acid methyl ester
16	30.648	9-Octadecenoic acid, methyl ester	21.18	$C_{19}H_{36}O_2$	296.487	Fatty acid methyl ester
17	30.834	9-Octadecenoic acid (Z)-, methyl ester	1.47	$C_{19}H_{36}O_2$	296.5	Unsaturated fatty acid methyl ester
18	31.132	Octadecanoic acid, methyl ester	31.8	$C_{19}H_{34}O_2$	294.472	Fatty acid methyl ester
19	35.13	9-Octadecenamide, (Z)	23.51	$C_{18}H_{35}No$	281.48	Amide
20	41.373	13-Docosenamide, (Z)	2.48	$C_{22}H_{43}NO$	337.592	Fatty amide

RT, retention time of the compounds identified. **MF,** molecular formula of the compounds identified. **MW**, molecular weight of the compounds identified.

TABLE 3. Bioactive compounds of *P. boergesenii* ethanolic extract as determined by GC/ MS analysis

NO	RT (min)	Compounds identified	Peak area %	M F	M W	Compound nature
1	7.962	3,7-Dimethylocta-1,6-dien-3-ol	52.3	$C_{10}H_{18}O$	154.25	Monoterpenoid
2	9.857	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl	0.25	$C_{10}H_{18}O_2$	170.49	Olefinic compound
3	10.492	Benzene, 1-methoxy-4-(2-propenyl)	21.65	$C_{10}H_{12}O$	148.20	Phenylpropene
4	11.646	4,6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-ol	37.11	$C_{10}H_{16}O$	152.237	Monoterpene
5	12.433	3,7-dimethylocta-2,6-dienal	40.2	$C_{10}H_{16}O$	152.24	Terpenoids
6	12.742	Farnesene epoxide, E	0.2	$C_{15}H_{24}O$	220.350	Sesquiterpenes
7	13.622	Bicyclo [3.1.1] hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	2.12	$C_{12}H_{18}O_2$	194.270	Monoterpenes
8	13.791	Geranyl vinyl ether	0.65	$C_{12}H_{20}O$	180.291	Alkene
9	14.205	5-Hexyloxolan-2-one	18.56	$C_{10}H_{18}O_2$	170.25	Lactone
10	14.887	trans-1,3,5-Trimethylcyclohexane	0.32	C_9H_{18}	126.239	Cycloalkane
11	15.114	1-Methyl-4-(1-methylethenyl)-cyclohexene	0.8	$C_{10}H_{16}O_2$	168.232	Monoterpene
12	23.274	Tetradecanoic acid, methyl ester	14.11	$C_{15}H_{30}O_2$	242.20	Fatty acid methyl ester
13	27.401	Hexadecanoic acid, methyl ester (Palmitic acid)	60.21	$C_{17}H_{34}O_2$	270.5	Fatty acid methyl ester
14	30.648	9-Octadecenoic acid (Z)-, methyl ester	31.77	$C_{19}H_{36}O_2$	296.5	Unsaturated fatty acid methyl ester
15	31.137	Octadecanoic acid, methyl ester	31.2	$C_{19}H_{38}O_2$	298.511	Fatty acid methyl ester
16	35.136	9-Octadecenamide, (Z)Ionization	27.09	$C_{18}H_{35}No$	281.48	Fatty acid methyl ester
17	41.373	13-Docosenamide, (Z)	2.58	$C_{22}H_{43}nO$	337.592	Fatty amide

RT, retention time of the compounds identified. **MF,** molecular formula of the compounds identified. **MW**, molecular weight of the compounds identified.

TABLE 4. Bioactive compounds of *C. myrica* ethanolic extract as determined by GC /MS analysis

NO	RT (min)	Compounds identified	Peak area %	MF	MW	Compound nature
1	7.91	1,6-Octadien-3-ol, 3,7-dimethyl-, format	50	$C_{11}H_{18}O_2$	182.259	Terpene
2	10.481	1-Methoxy-4-(prop-2-en-1-yl) benzene	15.84	$C_{10}H_{12}O$	148.20	Unsaturated ether
3	11.017	Cathine (psychoactive drug)	3.79	$C_9H_{13}NO$	151.209	Phenethylamine
4	11.611	4,6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-Ol	30.8	$C_{10}H_{16}O$	152.237	Monoterpenes
5	11.551	D-Tyrosine, 3-hydroxy (Alanine))	4.06	$C_9H_{11}NO_4$	197.19	Amino acid
6	11.751	D-Tyrosine, 3-hydroxy	20.21	$C_9H_{11}nO_4$	197.19	Amino acid
7	12.392	3,7-dimethylocta-2,6-dienal	39.3	$C_{10}H_{16}O$	152.24	Mono terpenoid
8	13.611	2-Methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol ((Z)	18.2	$C_{10}H_{16}O$	152.24	Mono terpenoid
9	14.193	5-Hexyloxolan-2-on	22.68	$C_{10}H_{18}O_2$	170.25	Ester
10	25.758	2-Pentadecanone, 6,10,14-trimethyl (Phytone)	0.38	$C_{18}H_{38}O$	270.493	Diterpene
11	27.372	Hexadecanoic acid, methyl ester (Palmitic acid)	20.4	$C_{17}H_{34}O_2$	270.5	Fatty acid methyl ester
12	30.642	9-Octadecenoic acid (Z)-, methyl ester(Oleic acid)	12.45	$C_{19}H_{36}0_2$	296.5	Fatty acid methyl ester
13	31.125	methyl octadecenoate (Stearic acid)	15.09	$C_{19}H_{38}O_2$	298.511	Saturated fatty acid
14	41.367	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl Ionization	0.56	$C_{13}H_{22}OSi_2$	250.484	Tropone

RT, retention time of the compounds identified. **MF,** molecular formula of the compounds identified. **MW**, molecular weight of the compounds identified.

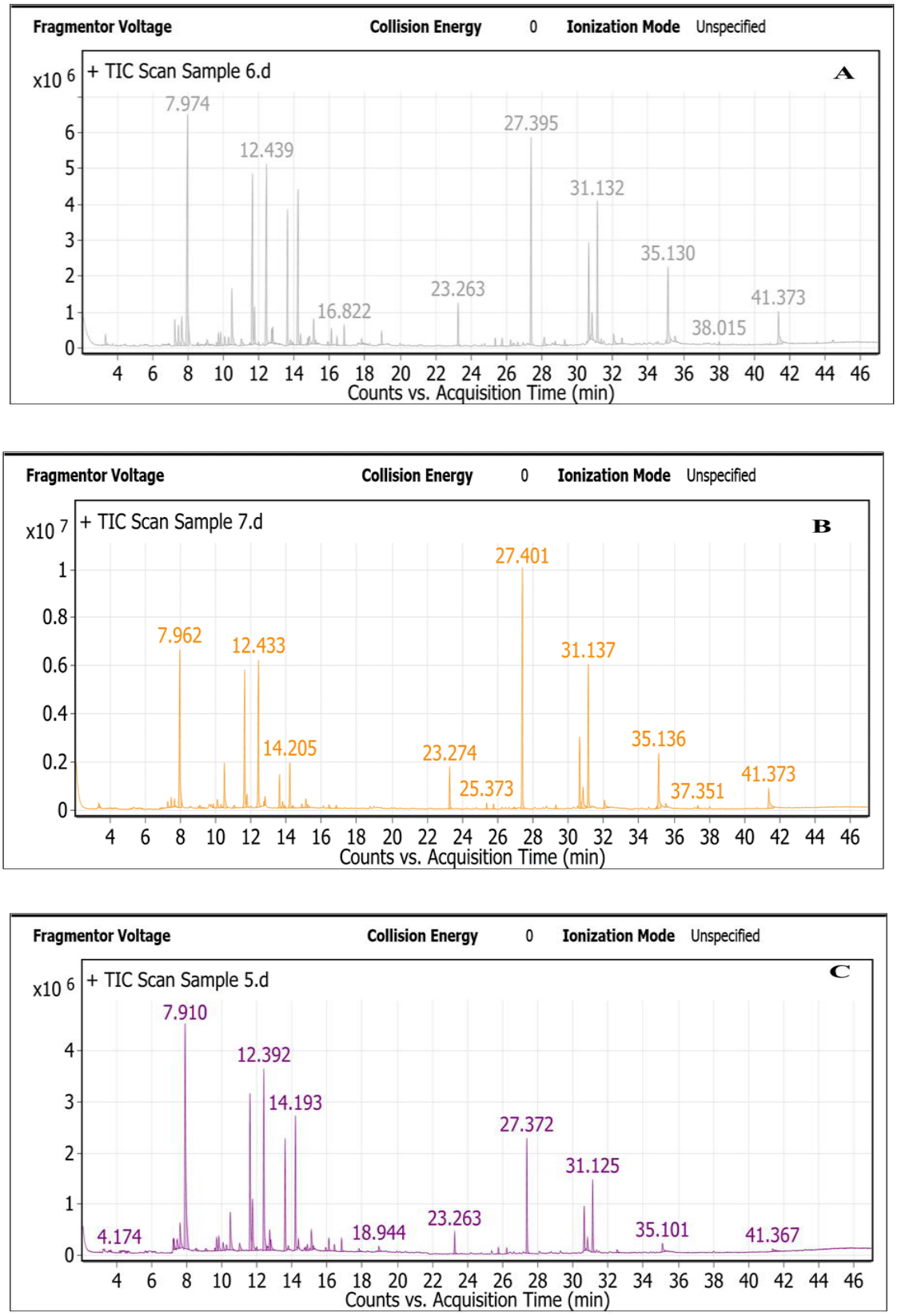

Fig. 2. Total ion chromatograms (TICS) obtained in the GC-MS analysis of the ethanol extract of *S. cinereum* (A), *P. boergesenii* (B) and *C. myrica* (C)

According to Aruna et al. (2010) stated that among the marine algae tested, the brown alga, *Sargassum wightii*, exhibited the highest rate of antifungal activity, followed by the red alga, *K. alvarezii*. Oppositely, Sheikh et al. (2018) reported that acetone was the most effective solvent for extracting bioactive chemicals from seaweeds with the highest antifungal activity. Our results showed that *C. albicans* and *A. alternata* were more resistant to the seaweed extracts (MIC= 15–70µg/mL).

The MICs of the ethanolic fractions of the algal species were observed to be 125–500 µg/mL according to Mickymaray & Alturaiki (2018). El-Sheekh et al. (2014) observed that the *Cystoseira myrica* extract exhibited poor antifungal activity against *Candida* species (MICs= 200mg/mL). In the current study, the methanolic extract of the brown seaweed (*C. myrica*) exhibited the highest inhibition activity against *F. oxysporum* (nondermatophyte). Contrarily, Pandithurai et al. (2015) demonstrated that the methanolic extract of the brown alga, *Spatoglossum asperum*, was more effective on dermatophytes.

According to the findings of the current study, ethanol was observed to be the most effective solvent for extracting antifungal compounds from the tested algae, followed by methanolic extract. Moreover, *S. cinereum* was the marine algae that was most effective against the tested fungal strains, followed by *P. boergesenii* and *C. myrica*. In general, the antifungal efficacy of the seaweed extracts was dependent on the used solvent, algal species, and characteristic resistance of the tested fungal species. According to a previous study, the antimicrobial activity of bioactive chemicals from marine algae against pathogenic fungi was primarily dependent on the type of extraction solvent and algal species, according to Mohamed & Saber (2019) and Lotfi et al. (2021). It was supported by Guedes et al. (2012), who reported that various solvent extracts from the same seaweed could be distinguished by different antimicrobial activities based on the solvent polarity and the chemical structure of the extracted bioactive material. Marine organisms are rich sources of functional materials, such as polyunsaturated fatty acids (PUFAs), vitamins, polysaccharides, antioxidants, natural pigments, enzymes, essential minerals, and bioactive peptides (Radhika & Mohaideen, 2015).

Many chemical distinctive compounds have been isolated from seaweeds with antimicrobial activity, and many of them are being developed as new pharmaceuticals, including polysaccharides, terpenoids, sterols, peptides, proteins, terpenes, acrylic acid, phenols, chlorophyllides, and heterocyclic carbons (Mickymaray & Alturaiki, 2018; Saeed et al., 2020).

In this study, the most abundant phytochemical constituents of the ethanolic extract were the hexadecanoic acid methyl ester; 3,7-dimethylocta-1,6-diene-3-ol; 6,6-trimethyl bicyclo [3.1.1] hept-3-en-2-ol; 5-hexyloxolan-2-one; and octadecanoic acid methyl ester.

Most of these components exhibited biological activity with certain pathogenic substances. The rationales for the traditional application of this species may be found in the antioxidant (Kumar et al., 2010), antimicrobial (Rahuman et al., 2000), and antiinflammatory activities reported for n-hexadecanoic acid, which may suggest a justification for the traditional use of the species (Aparna et al., 2012). Previous studies have verified the pharmacological efficacy of α-terpineol, such as antifungal (Giuseppe & Baratta, 2000), anticancer (Hassan et al., 2010), hypotensive (Ribeiro et al., 2010), gastroprotective (Souza et al., 2011), and antiviral activities (Zaid et al., 2016).

The organic behavior of hexadecanoic acid products and the macroalgae isolated octadecanoic acid derivatives were considered (Ali et al., 2016). *Falkenbergia hillebrandii* crude methanol extracts exhibit microbicidal actions, reportedly in n-hexadecanoic and oleic (Manilal et al., 2009). Stirk et al. (2007) concluded that marine algae have a rich source of many active secondary metabolites that are used by organisms for defense against herbivores and pathogens, such as terpenes, alkaloids, polyphenols, and acetogenins.

A variety of factors influence the antimicrobial activity of these compounds, including their distribution, molecular weight, charge density, sulfate content (in polysaccharide sulfates), and structural and conformational characteristics. These factors help plants resist viral, fungal, and bacterial infections (Vera et al., 2011).

The antimicrobial properties of these

compounds are attributable to the presence of fatty acids, fatty acid esters, and aliphatic chains (long-chain alkanes and alkenes), typically accumulated in the lipid layer of the cell membrane and mitochondria. Thus, the integrity of the cell structure is disrupted, and the cell becomes permeable (Belakhdar et al., 2015). Unsaturated fatty acids have also been shown to decrease cholesterol levels in the blood (Okwu & Morah, 2006). Phenol derivatives also disrupt cell homeostasis and cause cell death and growth inhibition (Devi et al., 2010). The ability of these compounds to change efflux pumping and increase the pH permeability of the membrane causes this phenomenon (Srivastava et al., 2014).

Conclusion

Brown algae (*S. cinereum*, *P. boergesenii*, and *C. myrica)* obtained from the Red Sea coast is greatly recommended as an environmentally friendly and promising source of bioactive composites that can be beneficial in the treatment or prevention of numerous human pathogenic fungal diseases, as well as in the resistance against plant-infecting fungal pathogens. This is to ensure that Egypt's agricultural development is more sustainable. However, more research is required to determine the impact of each bioactive component on the tested fungi.

Conflicts of interest: The authors declare that there is no conflict of interest.

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فحص النشاط المضاد للفطريات للمواد الفعالة في بعض الطحالب البحرية البنية من البحر الأحمر - مصر

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بعض السلالات الفطرية تكون مقاومة للأدوية شائعة الاستخدام. لذلك أصبح اكتشاف/ تطوير أدوية جديدة مضادة للفطريات مطلبًا عالميًا و من أولويات الدراسات في هذا المجال. لذلك تهدف الدراسة الحالية إلى تقييم الأنشطة المضادة للفطريات لثلاث انواع من الطحالب البحرية البنية:

Sargassum cinereum, Padina boergesenii, and *Cystoseira myrica*

بإستخدام المذيبات القطبية: الايثانول و الميثانول والاسيتون ضد ثلاثة انواع من الفطريات الجلدية (كممرضات بشرية): *Candida albicans, Candida glabrata*, and *Candida tropicalis* واثنان من الفطريات غير الجلدية (كفطريات ممرضة للنبات):

Fusarium oxysporum and *Alternaria alternata*

بأستخدام إختبار نشر الآجار. أظهرت النتائج التي توصلنا إليها أن مستخلصات الأعشاب البحرية أظهرت أنماطًا مختلفة من الأنشطة المضادة للفطريات. كان مستخلص الإيثانول من طحلب *S.cinereum* هو المستخلص الأكثر نشاطًا مقارنة بالمستخلصات العضوية الأخرى. أقصى نشاط مضاد للفطريات لمستخلص الإيثانول من طحلب *Sargassum cinereum* (21.3مم) ضد فطر *F. oxysporum* و (18.8 مم) ضد فطر *C.glabrata*, يلية مستخلص الميثانول من طحلب *C. myrica* (16.5مم) ضد *F. oxysporum* ثم مستخلص الإيثانول من طحلب *P.boergesenii* (16.3مم) ضد فطر *C. glabrata*. تراوحت التركيزات المثبطة الدنيا (MICs) لمستخلصات الطحالب لتثبيط الفطريات المختبرة من 70-8.5 ميكروجرام / مل. كان للمستخلص الإيثانول من طحلب *S. cinereum* أقل قيمة 8.5 ميكروجرام / مل ضد فطر *F. oxysporum*. كشف تحليل كروماتوجرافيا الغاز -مطياف الكتلة لمستخلصات الإيثانول عن وجود مركبات كيميائية نشطة بيولوجيا في الطحالب البحرية البنية منها حمض هيكساديكانويك ، إستر الميثيل (حمض النخيل) وايضا حمض Octadecanoic، إستر الميثيل وغيرها من المركبات النشطة بيولوجيا .