Phytochemical composition and cytotoxic potential of Red Sea macroalgae from Egypt

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Received: April 15, 2025
Accepted: July 23, 2025
DOI: 10.21608/egyjs.2025.375907.1045

ABSTRACT: Bioactive compounds from macroalgae have gained considerable attention for their therapeutic potential. This study focuses on four macroalgae *Polycladia myrica* Draima *et al*., *Sirophysalis trinodis* Kütz., *Sargassum aquifolium* C. Agardh, and *Digenea simplex* C. Agardh collected from the Egyptian Red Sea. Methanolic extracts were screened for phytochemical constituents and cytotoxicity against colon cancer (Caco-2), breast cancer (MCF-7), and normal fibroblast (WI-38) cell lines. Phytochemical screening confirmed the presence of carbohydrates, terpenoids, flavonoids, steroids, and cardiac glycosides, with varying levels of positivity. Saponins were detected in *P. myrica* Draima *et al*. and *S. aquifolium* C. Agardh but were absent in the other two species. Tannins were absent only in *D. simplex* C. Agardh. None of the examined algae contained alkaloids. *D. simplex* C. Agardh and *P. myrica* Draima *et al*. showed the strongest anticancer activity with minimal toxicity to normal cells. Their IC_{50} values were 21.71 ± 1.9, 18.92 ± 1.6, and 41.88 ± 2.8 µg/mL for *D. simplex* C. Agardh, and 32.49 ± 2.3, 39.13 ± 2.6, and 85.28 ± 4.3 µg/mL for *P. myrica* Draima *et al*. against Caco-2, MCF-7, and WI-38 cell lines, respectively. These two species were selected for further chemical analysis. Their extracts were saponified and analyzed using GC-MS. The identified compounds support the observed bioactivity, suggesting a link between chemical profile and cytotoxic effect. This study highlights the potential of Red Sea macroalgae, particularly *D. simplex* C. Agardh and *P. myrica* Draima *et al*., as promising natural anticancer agents, and encourages investigation of their active constituents.

Keywords: *Polycladia myrica*, cytotoxicity, *Digenea simplex*, GC-MS, Macroalgae

INTRODUCTION

Seaweeds, or macroalgae, are a broad class of multicellular marine algae that are essential to aquatic ecosystems. Macroalgae range in size from multicellular, microscopic filamentous species to massive, complex structures that resemble plants. Most of these marine algae can be found near the world's coasts (Dorhoi *et al.*, 2020). Egypt's macroalgae are found mainly in the Red and Mediterranean Seas. The Mediterranean Sea is home to about 660 species belonging to the brown and red macroalgae.

The broad category of brown algae is of multicellular marine algae. Fucoxanthin, a brown pigment, and chlorophyll a and c work together to give brown algae their distinctive color (Din *et al.*, 2022). The *Cystoseira* genus are the long-living brown macroalgae; they are especially significant to the benthic ecosystem of the Mediterranean because they exhibit a three-dimensional structure that serves as a home and shelter for fish, invertebrates, and smaller algae (Rashad and El-Chaghaby, 2020).

Macroalgae contain a variety of bioactive metabolites such as phlorotannins, amino acids, steroids, terpenoids, phenolic compounds, fatty acids and polysaccharides (Kharkwal *et al.*, 2012). The extracted Compounds from macroalgae have been used in a variety of industries, such as prebiotics, coating in active packaging, antibiofilm, antifouling, antibiotics in the pharmaceutical sector, and preservatives in the food or cosmetics sectors (Silva *et al.*, 2020). In addition, brown seaweeds belonging to the *Cystoseiraceae* family, *Polycladia myrica* Draima *et al*. (formerly known as *Cystoseira myrica* C. Agardh) and *Sirophysalis trinodis* Kütz. (formerly known as *Cystoseira trinodis* C. Agardh), are widely distributed along the shores of the Egyptian Red Sea (Guiry *et al.*, 2014). They have elevated levels of protein, carbohydrates, vitamins, and minerals that assist their possible application in nutritional and therapeutic settings. Furthermore, these seaweeds offer new opportunities as sources of bioactive compounds such as flavonoids, phenols, and ascorbic acid, which have potent antioxidant properties, so they are used in a variety of pharmaceutical, medical, and nutraceutical uses (El-Shazoly and Fawzy, 2018). *Sargassum aquifolium* C. Agardh (formerly known as *Sargassum cinereum* J. Agardh) is a brown seaweed belonging to the *Sargassaceae* family (Guiry *et al.*, 2014), has numerous biological activities such as cholinesterase inhibitory, hepatoprotective, analgesic, anti-inflammatory, antipyretic, antioxidant, and antiviral qualities (Rinu *et al.*, 2017).

The morphologies of red algae are highly diverse, ranging from simple filamentous forms to more complex structures like branched or sheet-like thalli (Pereira, 2021). *Digenea simplex* C. Agardh is a red seaweed belonging to the *Rhodomelaceae* family (Guiry *et al.*, 2014), was used as a source of agar, anthelmintic, laxative, vermifuge, and medication to treat parasitic roundworms (Ascaris), whipworms

(Trichuris), and tapeworms (Taenia) (Carpenter and Niem, 1998). Nowadays, instead of chemically manufactured medications, people worldwide are searching for natural sources of active ingredients because most synthetic drugs have several adverse effects on people. Macroalgae is one of these natural sources.

This study aims to explore the phytochemical composition and evaluate the cytotoxic potential of the four macroalgae *P. myrica* Draima *et al*., *S. trinodis* Kütz*., S. aquifolium* C. Agardh*, and D. simplex* C. Agardh from the Egyptian Red Sea, contributing to the discovery of marine derived compounds with selective anticancer activity and expanding current knowledge on their chemical and pharmacological profiles. The cytotoxic activity of the four macroalgae against malignant cell lines Caco-2, MCF-7 and the regular WI-38 cell lines was determined. The two most effective macroalgae were extracted and saponified to determine the saponifiable (SAP) and unsaponifiable (UNSAP) compounds. They were subjected to gas chromatography-mass spectrometry (GC-MS) analysis for additional examinations.

MATERIALS AND METHODS

Collection of Macroalgae Samples

Seaweed samples were collected from the Red Sea coast at the mangrove area, which is located 17 km south of Safaga City at latitude 26º 36` 55``N and longitude 34º 00` 43``E. The seaweeds were identified based on morphological characteristics according to taxonomic references (Aleem, 1978; Børgesen, 1957; Dawson, 1962; El-Manawy *et al.*, 2019; Sahoo, 2001) identification was further supported by comparison with herbarium specimens authenticated by Prof. Islam El-Manawy, available at the Marine Botany Laboratory, Faculty of Science, Suez Canal University. The currently accepted names used for the macroalgal species follow Algae Base (Guiry and Guiry 2022). The collected seaweed was washed with sea water to remove epiphytes, animal castings, sand, and other adhering detritus matters, followed by another wash with fresh tap water to remove excess salt. The algal materials were then shade-dried under an air jet, then the dried materials were ground coarsely in a mechanical grinder, weighed, and stored in sealed plastic pages for further use.

Chemicals and Reagents

Methanol (MeOH), hexane, and diethyl ether were used for extraction and fractionation: All solvents of analytical grade were sourced from Iso-Chem Pharmaceutical Chemical Co., Egypt. 10% alcoholic potassium hydroxide (KOH) concentrated sulfuric acid (H_2SO_4) (Iso-Chem Pharmaceutical Chemical Co., Egypt), and concentrated hydrochloric acid (HCl) (Horus Co., Egypt). 1% aqueous ferric chloride ($FeCl_3$), alcoholic α-naphthol, glacial acetic acid, picric acid (PIOCHEM Pharmaceutical Chemical Co., Egypt), Molisch's Dragendorff's and Mayer's reagents (prepared in the Pharmacognosy Department laboratories, Faculty of Pharmacy, Tanta University), sodium hydroxide (NaOH) (Inter. Trade Co., Egypt), and dinitrobenzoic acid (ADWIC Co., Egypt). The cytotoxicity assay utilized MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Co., St. Louis, USA), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, USA), and doxorubicin (Dox) (Sigma Co., St. Louis, USA) as a reference anticancer drug. Cell culture and preservation were conducted using Roswell Park Memorial Institute (RPMI)-1640 medium (GIBCO, UK), fetal bovine serum (FBS) (GIBCO, UK), and a combination of penicillin (100 units/mL) and streptomycin (100 µg/mL) (Sigma Co., St. Louis, USA).

Preparation of the Methanolic Extract

One kilogram of dried powder for each of the four macroalgae was separately extracted using the cold maceration method till exhaustion. The extracts were separately dried. The residues were washed with absolute ethanol to remove the salt that was unfortunately present.

Phytochemical analysis of Algal Methanolic Extract

Phytochemical screening was carried out according to the reference procedures to identify the major natural chemical groups such as Carbohydrates (Saha *et al.*, 2020), saponins (Alhaithloul, 2023), tannins (Alhaithloul, 2023), cardiac glycosides (Yadav, 2011; Godlewska *et al.*, 2023; Kumara and Bulugahapitiya, 2004), steroids (Saha *et al.*, 2020), terpenoids (Yadav, 2011), flavonoids (Yadav, 2011), and alkaloids (Saha *et al.*, 2020; Smith *et al.*, 2012).

Cytotoxicity Assay

Human colorectal adenocarcinoma (Caco-2), breast cancer (MCF-7), and normal lung fibroblast (WI-38) cell lines were obtained from the American Type Culture Collection (ATCC) through the Holding Company for Biological Products and Vaccines (VACSERA), Cairo, Egypt. Cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin, and incubated at 37 °C in a humidified atmosphere with 5% CO_2.

For the cytotoxicity assay, cells were seeded in 96-well plates at a density of 1.0×10^4 cells/well and allowed to adhere for 48 hours, reaching approximately 70–80% confluency before treatment. Cells were then exposed to serial concentrations (1.56, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL) of the algal methanolic extracts and incubated for an additional 24 hours. Doxorubicin was used as a positive control, and untreated cells served as negative controls. Following treatment, 20 µL of MTT solution (5 mg/mL) was added to each well, and plates were incubated for 4 hours to allow the formation of formazan crystals (Cree, 2011). Subsequently, 100 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the crystals, and absorbance was measured at 570 nm using a microplate reader (EXL 800, USA). Cell viability was calculated as a percentage relative to the untreated control: (A_{570} of treated / A_{570} of control) × 100 (Denizot, 1986; Mosmann, 1983).

Statistical Analysis

Statistical analysis of the cytotoxicity data was evaluated using one-way analysis of variance (ANOVA) at $p < 0.05$ with Minitab® statistical software (version 17.1, Minitab, LLC., State College, Pennsylvania, USA) for the various extracts. Tukey's post hoc test was applied to determine statistically significant differences among the tested algal extracts. All data are presented as mean ± SD (n = 3).

Saponification

For the saponification process, according to (Nagappan *et al.*, 2019) 30 mL of 10% (v/v) alcoholic potassium hydroxide (KOH) was combined with two grams of each of *Digenea simplex* C. Agardh and *P. myrica* Draima *et al*. methanolic extract on a boiling water bath for 6 h. Each resulting residue was suspended in 20 mL of distilled water and extracted with ether. The ether layer, UNSAP fraction, was concentrated under reduced pressure and then subjected to GC-MS analysis. The UNSAP fraction for *D. simplex* C. Agardh weighed 0.06 g, while that for *P. myrica* Draima *et al.* weighed 0.09 g. To the aqueous layer, SAP fraction, 2.5 mL strong hydrochloric acid (HCL) was added. The oily layer was mixed with an equivalent volume of methanol and 1 mL of concentrated sulfuric acid (H_2SO_4), and after 2 h of reflux, the mixture was extracted with diethyl ether. The extracts were washed separately with water, dehydrated and concentrated then subjected to GC-MS analysis. The SAP fraction for *D. simplex* C. Agardh weighed 0.05 g, while that for *P. myrica* Draima *et al.* weighed 0.08g.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed for *Digenea simplex* C. Agardh and *P. myrica* Draima *et al*. extracts using the PerkinElmer model Clarus 580/560S at the Scientific Research Center & Measurements (SRCM) in Tanta. This approach is consistent with standard analytical procedures used in similar investigations for SAP and UNSAP compounds (Ali *et al.*, 2015).

RESULTS AND DISCUSSION

Qualitative Phytochemical Screening

Our qualitative phytochemical screening in (Table 1) confirmed the presence of carbohydrates in all four macroalgal species investigated in this study. This observation is consistent with previous quantitative reports, which recorded carbohydrate contents of 26% in *P. myrica* Draima *et al*., 25% in *S. trinodis* Kütz., 47% in *S. aquifolium* C. Agardh, and 35.7% in *D. simplex* C. Agardh (Aly *et al.*, 2023; Salosso, 2019). Notably, carbohydrates in *D. simplex* C. Agardh are primarily composed of alginates (El-Rafie *et al.*, 2023), while *P. myrica* Draima *et al*. and *S. trinodis* Kütz., which are similar to other *Cystoseira* species, are rich in both fucoidan and alginates (Aly et al., 2023; Dhahri, 2023). Saponins are present in both *P. myrica* Draima *et al*. and *S. aquifolium* C. Agardh but absent in both *S. trinodis* Kütz. and *D. simplex* C. Agardh.

On the other hand, tannins were not found in *D. simplex* C. Agardh but in the other three macroalgae, as noted by (Hagaggi and Abdul-Raouf, 2022; Ranjani *et al.*, 2018). They were found in *S. trinodis* Kütz. as phlorotannin (Sathya *et al.*, 2017). The samples contain cardiac glycosides of the cardenolide type, according to moderately positive results from the Baljet and Kedde tests and negative results from the Keller-Kilani test. Both flavonoids and sterols provide trace-positive findings, while terpenoids offer more.

The four species were free from alkaloids. This absence is consistent with earlier reports on related brown macroalgae, particularly *Cystoseira* species (Aly *et al*., 2023; Sathya et al., 2017). In contrast, the presence of flavonoids, terpenoids, and steroids aligns with previous phytochemical profiles of *Polycladia*, *Sargassum*, and *Digenea* species (El-Shazoly and Fawzy, 2018; Ranjani et al., 2018), confirming both the taxonomic specificity and the potential bioactivity of these compounds.

Cytotoxicity

The cytotoxicity level of the methanolic extracts obtained from *P. myrica* Draima *et al*., *S. trinodis* Kütz*., S. aquifolium* C. Agardh*, and Digenea simplex* C. Agardh were evaluated against Caco-2, MCF-7. Least against WI-38 cell lines, as shown in (Table 2). Based on IC_{50} values, cytotoxicity was categorized as non-cytotoxic (>100 µg/mL), weak (51–100 µg/mL), moderate (21–50 µg/mL), strong (11–20 µg/mL), and very strong (1–10 µg/mL) (Suffness and Pezzuto, 1990). The results illustrated that the methanolic extract of *D. simplex* C. Agardh exhibited the highest cytotoxic effect against Caco-2, MCF-7, and least against WI-38 with IC_{50} values of 18.92 ± 1.6, 21.71 ± 1.9, and 41.88 ± 2.80, respectively, followed by the *P. myrica* Draima *et al*. methanolic extract which exhibited the highest cytotoxic effect against Caco-2, MCF-7 and least against WI-38 with IC_{50} values of 39.13 ± 2.6, 32.49 ± 2.3, and 85.28±4.3, respectively. Based on previous data, *D. simplex* C. Agardh and *P. myrica* Draima *et al*. were selected because they required higher doses than doxorubicin to affect normal cells while effectively targeting cancer cells.

Statistical Analysis

D. simplex C. Agardh showed the most significant and consistent cytotoxicity across all cell lines, with the lowest Caco-2 and MCF-7 values indicating high potency. *P. myrica* Draima *et al.* also exhibited significant activity against cancer cells while maintaining minimal effect on WI-38, suggesting a potentially favorable safety profile. These results position *D. simplex* C. Agardh as a broad-spectrum anticancer candidate and support *P. myrica* Draima *et al*. as a selective and possibly safer alternative. According to these findings, *D. simplex* C. Agardh and *P. myrica* Draima *et al.* were selected for further investigation (Table 3). To confirm the statistical significance of observed differences among treatments, one-way ANOVA was performed for each cell line and revealed highly significant differences (Caco-2: $p = 0.000087$; MCF-7: $p = 0.000071$; WI-38: $p = 0.000066$). These results were followed by Tukey's post hoc test, which indicated distinct groupings among algal extracts and doxorubicin.

To further evaluate the therapeutic relevance of these findings, selectivity indices (SI) were calculated for *D. simplex* C. Agardh and *P. myrica* Draima *et al*. The SI was determined by dividing the IC_{50} value for the normal cell line (WI-38) by the IC_{50} value for the cancer cell line (Caco-2 or MCF-7), where higher SI values indicate greater selectivity toward cancer cells. *D. simplex* C. Agardh demonstrated SI values of 2.21 (Caco-2) and 1.93 (MCF-7), while *P. myrica* Draima *et al*. showed SI values of 1.35 and 1.63, respectively. These values highlight the relatively safer cytotoxic profile of *D. simplex* C. Agardh and support its potential as a selective anticancer candidate.

GC-MS Analysis

The GC-MS analysis of *D. simplex* C. Agardh and *P. myrica* Draima *et al*. methanolic extracts for SAP and UNSAP for both species provide detailed information for each extracted compound.

Table 1. Qualitative phytochemical constituents detected in methanolic extracts of the four red sea macroalgae

Phytochemical Constituents	Test Name	Macroalgae			
		P. myrica Draima *et al.*	*S. trinodis* Kütz.	*S. aquifolium* C. Agardh	*D. simplex* C. Agardh
Carbohydrates	Molisch test	+++	+++	+++	+++
Saponins	Froth test	+	-	+	-
Tannins	Ferric test	+	+	+	-
Cardiac Glycosides	Keller-kilani test for deoxy sugars	-	-	-	-
	Baljet test for cardiac glycosides	+	+	+	+
	Kedde test for cardenolides	++	++	++	++
Steroids	Liebermann- Burchard's test	+	+	+	+
Terpenoids	Salkowski test	+	++	++	+++
Flavonoids	Alkaline reagent test	+	+	+	+
Alkaloids	Mayer's Test	-	-	-	-
	Wagner's test	-	-	-	-

(+++) Present in high concentration, (++) Present in moderate concentration, (+) Present in traces, (-) Absent.
Note: This analysis was qualitative; the symbols (+++), (++), (+), and (–) indicate relative abundance based on visual intensity of standard colorimetric reactions.

Table 2. IC_{50} values (µg/mL) of doxorubicin and macroalgae methanolic extracts against cell lines.

No.	Comp.	*In vitro* Cytotoxicity IC_{50} (µg/mL)		
		Caco-2	MCF-7	WI-38
••	Dox	12.49±1.1	4.17±0.2	6.72±0.5
1	*D. simplex* C. Agardh	21.71±1.9	18.92±1.6	41.88±2.8
2	*S. aquifolium* C. Agardh	73.20±3.9	56.64±3.4	35.23±2.4
3	*P. myrica* Draima *et al*.	32.49±2.3	39.13±2.6	85.28±4.3
4	*S. trinodis* Kütz.	48.56±2.9	62.89±3.5	52.87±3.2

•• doxorubicin (Dox) as a reference anticancer drug. Note: Results are expressed as mean ± SD (n = 3).

Table 3. Cytotoxicity (IC_{50}, µg/mL) of algal extracts against Caco-2, MCF-7, and WI-38 cell lines, with statistical significance assessed by Tukey's HSD following one-way ANOVA ($p < 0.05$)

Macroalgae	Caco-2	MCF-7	WI-38
D. simplex C. Agardh	$21.71 \pm 1.9^{c,d}$	18.92 ± 1.6^{c}	$41.88 \pm 2.8^{b,c}$
S. aquifolium C. Agardh	73.20 ± 3.9^{a}	56.64 ± 1.6^{a}	35.23 ± 2.4^{c}
P. myrica Draima *et al.*	32.49 ± 2.3^{c}	39.13 ± 2.6^{b}	85.28 ± 4.3^{a}
S. trinodis Kütz	48.56 ± 2.9^{b}	62.89 ± 3.5^{a}	52.87 ± 3.2^{b}

* Values are expressed as mean ± SD (n = 3), * Statistical analysis was performed using one-way ANOVA for each cell line. Groups sharing the same superscript letters are not significantly different, while those with different letters indicate significant differences based on post-hoc Tukey grouping at $p < 0.05$.

GC-MS of SAP Fractions: The GC-MS analysis of *D. simplex* C. Agardh SAP (Figure 1) and (Table 4) revealed the presence of a significant concentration of esterified fatty acids and associated compounds. The total identified saponifiable matter in *D. simplex* C. Agardh was 73.58% from which Hexadecanoic acid methyl ester and 9,12-Octadecadienoic acid, methyl ester, (E,E) showed a higher percentage of 17.87% and 16.15%, respectively. Also, it comprises 25.44% saturated fatty acids**,** 35.12% unsaturated fatty acids**,** and 13.02% other compounds. The GC-MS analysis of *P. myrica* Draima *et al*. SAP (Figure 2) and (Table 5) dominated saturated and unsaturated fatty acids with 64.28% and 12.91% of the total recognized components, which comprise 77.19%. Octadecanoic acid, methyl ester was the most prevalent chemical followed by Heptadecanoic acid, methyl ester, with percentages of 28.458% and 24.200%, respectively.

GC-MS of UNSAP Fractions: A complex profile of chemicals was found in the examination of *Digenea simplex* C. Agardh by GC-MS analysis (Figure 3) and (Table 6) UNSAP. 77.19% of the measured peaks were recognized. With pentadecane (27.83%), heptacosane (20.71%), and Phytol (4.57%) as the main ingredients, hydrocarbons predominate, making up 54.32% of all detected chemicals. Furthermore, 12.11% of the profile was made up of oxygenated chemicals. On other hand, methanolic extract from *P. myrica* Draima *et al*. UNSAP (Figure 4) and (Table 7) as investigated by GC-MS indicated a broad spectrum of chemical components, with 68.24% of them identified. Cholesterol was the most abundant component, with the most significant area percentage of 16.25%, Phytol (10.27%), 2-piperidinone, N-[4-bromo-n-butyl] (7.00%), and hexadecane, 2,6,11,15-tetramethyl- (5.12%) were other notable peaks that made substantial contributions. A significant number of hydrocarbons (25.16%) and oxygenated compounds (23.89%) were also detected with steroids making up 16.25%.

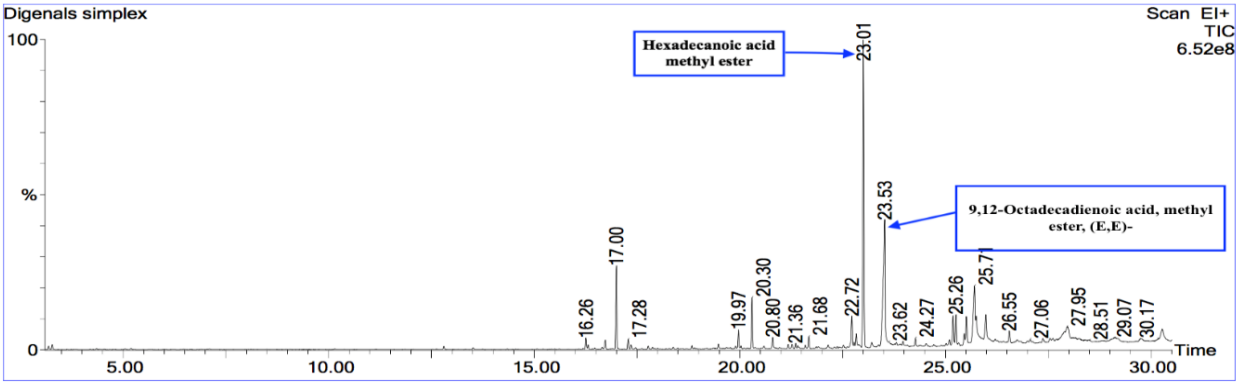

Figure 1. GC-MS chromatogram post derivatization for *D. simplex* C. Agardh SAP represents percent area versus time

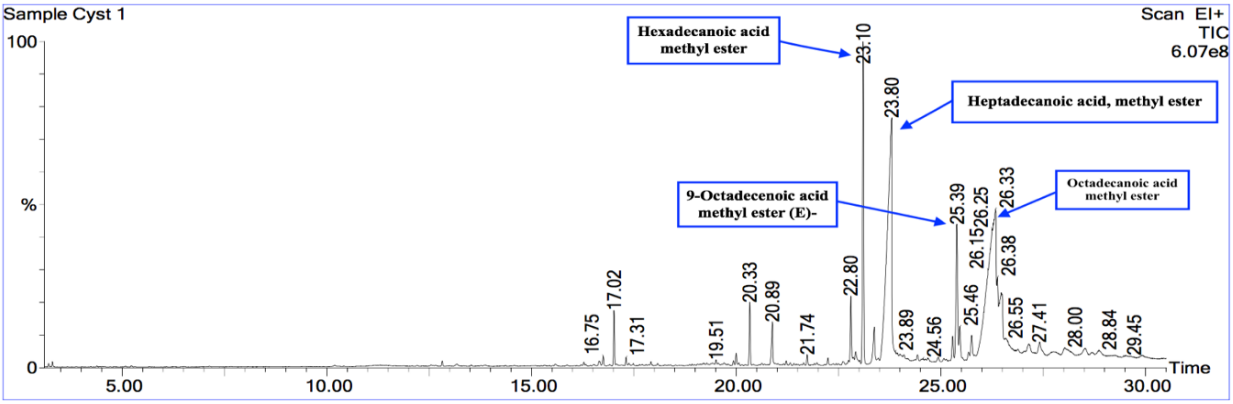

Figure 2. GC-MS chromatogram post derivatization for *P. myrica* Draima *et al*. SAP represents percentage area versus time

Table 4. Composition of methanol extracts of *D. simplex C. Agardh* SAP as investigated by GC-MS

Peaks	Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	RRT	Area %
1	Ethylene glycol monoisobutyl ether	$C_6H_{14}O_2$	118.18	3.278	0.14	0.262
5	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.34	17.284	0.75	0.568
6	Octadecanoic acid, 3-hydroxy-, methyl ester	$C_{19}H_{38}O_3$	318.51	19.480	0.85	0.296
8	Tridecanoic acid, 12-methyl-, methyl ester	$C_{14}H_{28}O_2$	228.37	20.296	0.88	3.000
9	Sulfurous acid, hexyl pentadecyl ester	$C_{21}H_{44}O_2S$	368.66	20.586	0.89	0.272
11	Tetradecanoic acid, 12-methyl-, methyl ester	$C_{16}H_{32}O_2$	256.42	21.176	0.92	0.245
14	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.42	21.676	0.94	0.739
17	9-Hexadecenoic acid, methyl ester	$C_{17}H_{32}O_2$	268.43	22.722	0.99	1.879
19	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	23.007	1.0	17.870
21	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.48	23.527	1.02	16.150
23	Tridecanoic acid, methyl ester	$C_{14}H_{28}O_2$	228.37	23.807	1.03	0.353
24	Cyclopentaneundecanoic acid, methyl ester	$C_{16}H_{30}O_2$	254.41	23.912	1.04	0.422
26	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.48	24.272	1.05	0.580
27	Hexadecanoic acid, 2-hydroxy-, methyl ester	$C_{17}H_{34}O_3$	286.45	24.527	1.07	0.269
28	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.49	25.098	1.09	0.390
29	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	25.188	1.09	1.776
30	*trans*-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	25.258	1.1	1.756
32	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.50	25.508	1.11	1.717
33,34	Oleic Acid, methyl ester	$C_{19}H_{36}O_2$	296.49	25.731	1.12	8.039
35	*cis*-10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	310.52	25.983	1.13	2.529
36	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	$C_{18}H_{34}O_2$	282.46	26.553	1.15	0.936
37	17-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294.48	26.748	1.16	0.687
39	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	278.44	27.068	1.18	0.394
40	Hexadecanoic acid, 3-hydroxy-, methyl ester	$C_{17}H_{34}O_3$	286.46	27.373	1.19	0.387
42,43,44	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.49	27.974	1.22	7.573
45	6-Hexadecenoic acid, 7-methyl,methyl ester (Z)	$C_{18}H_{34}O_2$	282.46	28.379	1.23	0.745
49	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.49	29.754	1.29	0.776
50	glyceryl monopalmitate	$C_{19}H_{38}O_4$	330.50	30.275	1.32	2.968
Total identified compounds						73.58%
Total Saturated Fatty Acids						25.44%
Total Unsaturated Fatty Acids						35.12%
Other						13.02%
Total unidentified compounds						26.42%

*RRT: Retention time relative to Hexadecanoic acid, methyl ester

Table 5. Composition of methanol extracts of *P. myrica Draima et al.* SAP as investigated by GC-MS

Peak	Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	RRT	Area %
17	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.40	20.331	0.88	1.447
18	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.42	20.886	0.9	1.414
23	9-Hexadecenoic acid methyl ester	$C_{17}H_{32}O_2$	268.43	22.802	0.99	1.791
26	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	23.102	1.0	8.764
28	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.48	23.802	1.03	24.200
35	9-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296.49	25.393	1.1	4.767
39	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.50	26.333	1.14	28.458
40	Octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	$C_{22}H_{44}O_4$	372.59	26.478	1.15	6.352
Total identified compounds						77.19%
Total Saturated Fatty Acids						64.28%
Total Unsaturated Fatty Acids						12.91%
Total unidentified compounds						22.81%

*RRT relative to Hexadecanoic acid, methyl ester

Table 6. Composition of methanol extracts of *Digenea simplex C. Agardh* UNSAP as investigated by GC-MS

Peak	Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	RRt	Area %
1	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212.41	12.807	0.53	0.231
2	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198.39	16.263	0.68	0.379
3	Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	$C_{15}H_{24}O$	220.35	16.523	0.69	0.436
4	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.41	16.743	0.7	0.368
5	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, methylcarbamate	$C_{19}H_{29}NO_2$	303.44	17.009	0.71	0.359
6	Card-20(22)-enolide	$C_{23}H_{34}O_4$	374.52	17.934	0.75	0.178
7	Phenol, 2,6-bis(1,1-dimethylethyl)-4-(methoxymethyl)-	$C_{18}H_{28}O_2$	276.41	19.600	0.82	0.250
8,9	Heptadecane	$C_{17}H_{36}$	240.47	19.950	0.83	1.841
10	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	282.55	20.625	0.86	0.160
13	Pentadecanal	$C_{15}H_{30}O$	226.40	21.610	0.9	0.169
14	Tetradecanal	$C_{14}H_{28}O$	212.37	21.650	0.9	0.221
15,16	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.48	21.969	0.92	0.595
17,18	Andrographolide	$C_{20}H_{30}O_5$	350.45	22.059	0.92	0.337
20	1-Eicosanol	$C_{20}H_{42}O$	298.55	22.516	0.94	0.475
21	2-Tetradecanone	$C_{14}H_{28}O$	212.37	22.791	0.95	0.276
22	Hexadecane	$C_{16}H_{34}$	226.44	22.926	0.96	1.405
24	Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240.38	23.386	0.98	0.906
26,27	Pentadecane	$C_{15}H_{32}$	212.41	23.94	1.0	27.83
28	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	312.5	24.392	1.02	0.371
30	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{38}O$	294.52	24.672	1.03	0.218
31	Octadecane	$C_{18}H_{38}$	254.49	24.727	1.03	0.264
33	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242.44	25.252	1.05	0.217
35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$	296.53	25.577	1.07	4.565
36	Eicosane	$C_{20}H_{42}$	282.55	25.732	1.07	0.688
37	Heptacosane	$C_{27}H_{56}$	380.74	26.392	1.1	20.71
42	4,14-Retro-retinol	$C_{20}H_{30}O$	286.45	27.818	1.16	0.848
44	2-Propenoic acid, 3-(4-methoxyphenyl)-	$C_{10}H_{10}O_3$	178.18	28.378	1.19	0.709
45	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	240.47	28.688	1.2	0.628
47	Bicyclo[4.4.0]dec-2-ene derivative	$C_{15}H_{24}O$	220.35	30.369	1.27	0.226
50	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336.55	32.225	1.35	1.201
Total identified compounds						67.06%
Total identified Hydrocarbons						54.32%
Total identified Oxygenated Compounds						12.11%
Other Compounds						0.63%
Total unidentified compounds						32.94%

*RRT relative to Pentadecane

Table 7. Composition of methanol extracts of *P. myrica Draima et al.* UNSAP as investigated by GC-MS

Peak	Compound Name	Molecular Formula	Molecular Weight (g/mol)	RT (min)	RRt	Area %
2	Stigmastan-6,22-dien, 3,5-dedihydro-	$C_{29}H_{48}$	396.70	6.124	0.24	1.971
3	Dodecane, 2,7,10-trimethyl-	$C_{15}H_{32}$	212.41	12.777	0.51	0.731
4	Butylated Hydroxytoluene (BHT)	$C_{15}H_{24}O$	220.35	16.238	0.64	0.41
6	Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.41	16.708	0.66	2.31
7	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, methylcarbamate	$C_{19}H_{29}NO_2$	303.44	16.974	0.67	0.65
8	Decane, 2,3,5,8-tetramethyl-	$C_{14}H_{30}$	198.39	17.444	0.69	0.52
9	Octadecane	$C_{18}H_{38}$	254.49	18.874	0.75	0.639
11	Nonadecane	$C_{19}H_{40}$	268.52	19.640	0.78	0.439
13	Eicosane	$C_{20}H_{42}$	282.55	19.870	0.79	0.454
14	Heptadecane	$C_{17}H_{36}$	240.48	19.940	0.79	3.28
15	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212.41	20.00	0.79	0.507
16	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	296.58	20.555	0.81	1.092
19	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268.48	21.881	0.87	1.440
22	Eicosane, 2-methyl-	$C_{21}H_{44}$	296.58	22.796	0.9	2.26
25	Heptacosane	$C_{27}H_{56}$	380.74	23.336	0.92	0.558
26	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	282.55	23.456	0.93	5.12
27	Octadecyl trifluoroacetate	$C_{20}H_{39}F_3O_2$	366.52	24.962	0.99	0.561
29	Phytol	$C_{20}H_{40}O$	296.53	25.267	1.0	10.27
30	Heneicosane	$C_{21}H_{44}$	296.58	25.402	1.01	1.912
34	2-Piperidinone, N-[4-bromo-n-butyl]-	$C_9H_{16}BrNO$	234.13	27.013	1.07	7.00
35	Retinol	$C_{20}H_{30}O$	286.45	27.308	1.08	1.24
37	Tetracosane	$C_{24}H_{50}$	338.66	27.868	1.1	1.540
38	Andrographolide	$C_{20}H_{30}O_5$	350.45	28.253	1.12	0.411
39	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.71	28.358	1.12	0.467
40	Oxirane, [(hexadecyloxy)methyl]-	$C_{19}H_{38}O_2$	298.50	28.633	1.13	0.792
43	Hexadecane	$C_{16}H_{34}$	226.44	29.989	1.19	2.101
44	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.56	30.209	1.2	0.720
45	Docosane	$C_{22}H_{46}$	310.61	30.344	1.2	1.234
48	Cholesterol	$C_{27}H_{46}O$	386.65	32.955	1.3	16.25
49	1-Heptatriacotanol	$C_{37}H_{76}O$	536.99	34.211	1.35	0.64
50	Doconexent	$C_{22}H_{32}O_2$	328.49	34.376	1.36	0.73
Total identified compounds						68.24%
Total identified Hydrocarbons						25.16%
Total identified Oxygenated Compounds						23.89%
Total identified Steroids						16.25%
Other Compounds						2.94%
Total unidentified compounds						31.76%

*RRT relative to Phytol

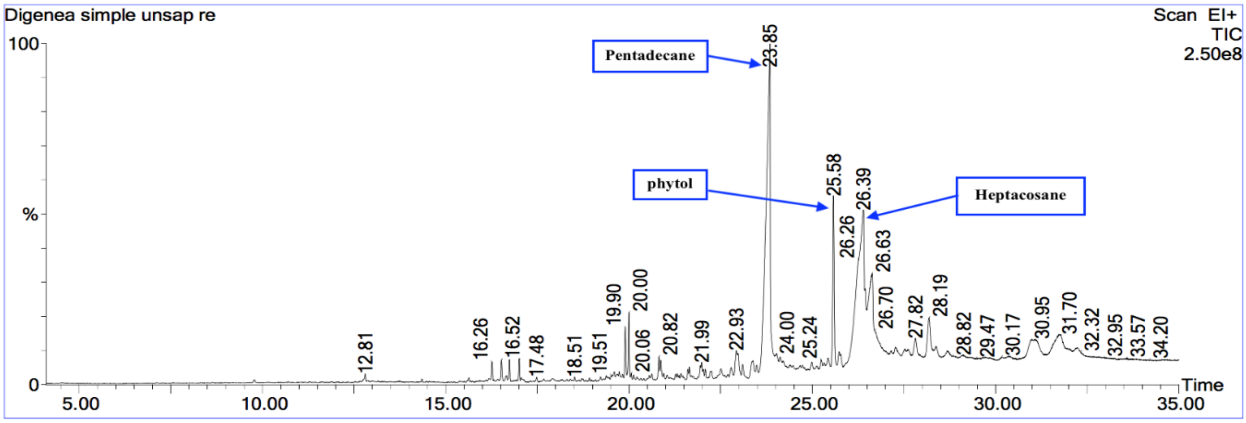

Figure 3. GC-MS chromatogram post derivatization for *D. simplex C. Agardh* UNSAP represents percent area versus time

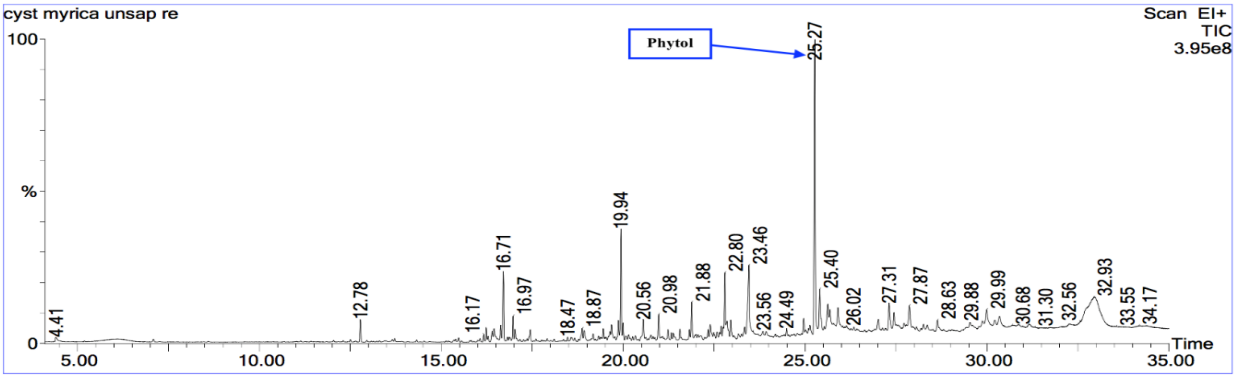

Figure 4. GC-MS chromatogram post derivatization for *P. myrica Draima et al.* UNSAP represents percent area versus time

CONCLUSION

The results of this study demonstrate the marine macroalgae's potential as a source of bioactive substances with strong cytotoxic effects. The phytochemical screening of *P. myrica* Draima *et al., S. trinodis* Kütz., *S. aquifolium* C. Agardh, and *D. simplex* C. Agardh indicated that they are rich sources of bioactive compounds. All four macroalgae contain a high carbohydrate content: *P. myrica Draima et al.* and *S. aquifolium* C. Agardh contain saponins, while the latter includes tannins.

Cardiac glycosides were also identified in *D. simplex* C. Agardh and *P. myrica* Draima *et al*. methanolic fractions showed especially significant cytotoxic effects against the cancerous cell lines CaCo-2 and MCF-7 while demonstrating the least cytotoxicity against the standard cell line WI-38 proving their safety. The compounds present in the methanol extracts were thoroughly analyzed using GC-MS, revealing a diverse range of saturated and unsaturated fatty acids in the SAP fraction. In contrast, the UNSAP fraction contained hydrocarbons, steroids, and other oxygenated compounds.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Sarah Hamdy Rashedy, Researcher in Aquatic Plants at the National Institute of Oceanography and Fisheries (NIOF), Red Sea Branch, Hurghada, Egypt, for her assistance in collecting and identifying macroalgae.

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