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Characterization of Bioactive Compounds from Egyptian Coastal Seaweeds

Mona M. Ismail[1], Mostafa E. Elshobary[2*], Ahmed S. El Newehy[3], Saly F. Gheda[2]

[1]National Institute of Oceanography and Fisheries, Cairo 4262110, Egypt
[2]Botany and Microbiology Department, Faculty of Science, Tanta University, Tanta 31527, Egypt
[3]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh 11433, Saudi Arabia

***Corresponding Author: mostafa_elshobary@science.tanta.edu.eg**

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ABSTRACT

This study investigated the phytochemical composition of 10 different species of marine macroalgae collected from the Mediterranean and the Red Sea, Egypt. The collected species taxonomically belong to three major groups: Ochrophyta (3 species), Rhodophyta (5 species), and Chlorophyta (2 species). Phytochemical extraction of each seaweed, using water, methanol, ethanol and acetone, was carried out. Qualitative and quantitative analyses were conducted to detect phenolics, flavonoids, tannins, steroids, terpenoids, and saponins. Among the studied species, *Sargassum cinereum* exhibited the highest phytochemical yield, particularly in ethanolic extracts, while Rhodophyta species generally showed lower phytochemical contents. Quantitative analyses revealed significant variation in total phenolic, flavonoid, and tannin contents depending on the algal species and extraction solvent. Gas chromatography–mass spectrometry (GC-MS) profiling identified numerous bioactive compounds, with palmitic acid and its methyl ester, and stearic acid methyl ester are being the predominant fatty acids. The findings highlight the substantial potential of Egyptian coastal seaweeds as a sustainable source of biologically active compounds for pharmaceutical, nutraceutical, and environmental applications. This work emphasizes the need to explore marine algal resources in Egypt for novel bioactive compounds and suggests that the solvent type significantly influences the extraction efficiency of different phytochemicals.

INTRODUCTION

Seaweeds produce a wide range of metabolites (phenol, tannins, flavonoids) to protect themselves from biotic and abiotic factors like herbivory or mechanical aggression at sea because they are frequently exposed to extreme conditions of the environment without showing any signs of damage **(Gómez-Guzmán *et al.*, 2018)**.

Ochrophyta have been shown to provide a variety of health benefits. The dominant brown species, such as *Polycladia myrica*, *Dictyota spiralis*, and *Sargassum euryphllum* from the Red Sea, Egypt, contain various phenolic contents that have the potential to treat a wide range of serious diseases. These compounds may also be valuable in the pharmaceuticals, cosmeceuticals, and functional food industries **(Ismail *et al.*, 2022)**. The brown alga *Padina tetrastromatica* is a member of the Dictyotales order and is found along the Egyptian coast. *P. tetrastromatica* contains numerous chemical compounds that are responsible for the plant's diverse pharmacological and traditional qualities **(Yende *et al.*, 2020)**. Alkaloids, triterpenoids, steroids, tannins, flavonoids, and saponins are all positively detected in Tricleocarpa fragile exhibiting antibacterial activity against *V. harveyi* bacterium **(Singkoh *et al.*, 2019)**. The Egyptian marine red macroalga *Digenea simplex* has a wide variety of phytochemicals with distinct bioactivities **(El-Rafie *et al.*, 2023)**. *D. simplex* chloroform extract's cytotoxic and antioxidant properties have not received enough attention, and the majority of the species' published data are from waters other than the beaches of the Red Sea in Egypt **(Sobuj *et al.*, 2021; Ghosh *et al.*, 2022)**.

Ulva linza and *U. fasciata* are common green seaweed that are extensively found throughout the Mediterranean coast, Egypt **(Mohy El-Din & Alagawany, 2019; El Zokm *et al.*, 2021)**. According to the study conducted by **Mohy El-Din and Alagawany (2019)**, *U. linza* has been reported as a promising antioxidant resource. Therefore, this species might be useful as natural anticoagulants and antioxidants. Besides, the most studied species like *Ulva fasciata*, *U. linza*, *U. lactuca*, *Corallina officinalis*, and *Sargassum vulgaris* from the Alexandria coast in Egypt may be considered as alternatives and supplements to help reduce the risk of obesity. This is because they have a low energy content (≤ 1.76 KJ/g DW) and caloric values (≤ 0.357 kcal), which are lower than the standard levels recommended for women (2 kcal) and men (2.5 kcal) **(Ismail *et al.*, 2017)**.

Phenolic compounds are well-established bioactive compounds (BCs) that are significant secondary metabolites of both plants and algae. They have been shown to have anti-inflammatory, anti-cancer, and antioxidant properties **(Getachew *et al.*, 2020)**. Three categories constitute the majority of algal sources: phenolic acids, phenylpropanoids, and flavonoids **(Carreira-Casais *et al.*, 2021)**.

Egypt needs to take advantage from a high richness of marine macroalgal species, owing to the presence of macroalgae near its coasts in the Mediterranean Sea, Red Sea, and Suez Canal. This wealth of marine macroalgae serves as an important source of bioactive chemical compounds **(Ahmed *et al.*, 2023)**.

The objective of this research was to evaluate the variation in phytochemical contents and concentrations of several seaweeds extracts from the Egyptian coast. Additionally, the study aimed to identify the bioactive compounds in the collected

ethanolic extracts using gas chromatography-mass spectrometry for future applications in medicine, in pharmacology, dietary supplements, cosmetics or in the food industries.

MATERIALS AND METHODS

1. Collection and identification of macroalgae samples

Ten species of seaweeds (5 Rhodophyta, 3 Ochrophyta and 2 Chlorophyta) were handpicked at depths of 0.2m or less for Chlorophyta and 1m for Rhodophyta and Ochrophyta from the submerged rocks at Abu Qir (N 31º 19`, E 030º 03`) and from the front of the National Institute of Oceanography and Fisheries (NIOF), Hurghada, Red Sea (latitudes 27° 17′ 13″ N and longitudes 33° 46′ 21″E) during the summer season of 2023. The collected samples were cleaned with seawater to remove sand, conditioned in plastic bags containing seawater to prevent evaporation, and kept on ice until transported to the laboratory **(Thomas & Harrison, 1987)**. All samples were brought to the Taxonomy and Biodiversity Laboratory, NIOF, Alexandria in plastic bags and were taxonomically identified. The seaweed specimens were cleaned well from epiphytes and rock debris. Thereafter, they were given a quick freshwater rinse to remove surface salts, and then a part of each species was preserved in 5% formalin in seawater. The remaining cleaned seaweeds were spread on blotting paper at 25 to 30°C to remove excess water and were then shade-dried for about 4-7 days at room temperature until a constant weight was obtained. The shade-dried material was crushed in an electric mixer to obtain a coarse powder **(Ibtissam *et al.*, 2009)** that was stored in airtight dark bottles at -20°C. The names of the species were confirmed according to the Algae Base website **(Guiry & Guiry, 2022)**.

2. Extraction of phytochemical compounds

The extraction of bioactive compounds was performed using the method described by **Sarker (2008)**. Five grams of dried powder from each algae species was extracted using four different solvents: water, methanol, ethanol, and acetone. The extraction was carried out using a shaker for 24 h at room temperature (25±2°C), with a solvent-to-algal sample ratio of 15:1. After extraction, the mixtures were filtered to remove the solid residue using Whatman No. 1 filter paper. The filtrates were then evaporated under reduced pressure using a rotary evaporator to obtain the crude extracts. The resulting extracts were weighed and stored at 4°C until further analysis.

3. Qualitative analysis for bioactive compounds in algal extracts

The standard procedure outlined by **Savithramma *et al*. (2011)** was followed to assess the presence or absence of phytochemical content, including tannins, flavonoids, phenols, terpenoids, saponins, and steroids. For steroids, 0.5mL of the algal extract was mixed with 2mL of chloroform and 1 mL of sulfuric acid (H_2SO_4). Steroids are present when a reddish-brown ring forms at the contact.

Flavonoids identification was performed by mixing 1mL of 2N sodium hydroxide (NaOH) with 2mL of algal extract. The formation of yellow color indicates the presence of flavonoids.

In order to identify the phenols, two milliliters of distilled water and a few drops of 10% ferric chloride were added to one milliliter of the algal extract, when phenols are present, a blue or green tinge is formed. For qualitative estimation of tannins, 1mL ferric chloride (5%) was mixed to 1mL of the algal extract. When tannins are present, dark blue or greenish-black color are formed.

Salkowski test for terpenoids, five milliliters of each extract were mixed with two milliliters of chloroform and three milliliters of concentrated H_2SO_4. The reddish-brown color was produced at the contact due to the presence of terpenoids **(Harborne, 1998).** Frothing test for saponin estimation; after treating around 3 milliliters of each extract with 3 milliliters of distilled water, the sample was rapidly shaken for approximately one minute to produce a stable, long-lasting froth. By shaking the mixture vigorously with three drops of olive oil, the presence of saponins in the extracts was determined by observing the production of an emulsion **(Trease & Evans, 1989).**

4. Quantitative analysis for bioactive compounds

4.1 Estimation of total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method as described by **Chang *et al*. (2020)**. Briefly, 0.5mL of each extract was mixed with 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate, and 2.8mL of distilled water. The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 415nm using a UV-visible spectrophotometer. Quercetin was used as a standard, and the results were expressed as mg of quercetin equivalents (QE) per gram of dry weight (DW) of the sample.

4.2 Estimation of total phenolic content

The total phenolic content was determined using the Folin-Ciocalteu method described by **Taga *et al.* (1984)**. Briefly, 100µL of each extract was mixed with 2mL of 2% Na_2CO_3 and the mixture was allowed to stand for 2 minutes at room temperature. Then, 100µL of 50% Folin-Ciocalteu phenol reagent was added. The mixture was incubated for 30 minutes at room temperature in darkness, and the absorbance was measured at 720 nm using a UV-visible spectrophotometer. Gallic acid was used as the standard, and the results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight (DW) of the sample.

4.3 Estimation of total tannin content

The total tannin content was determined using the method described by **Julkunen-Tiitto (1985)**. In this procedure, 50µL of each extract was mixed with 1.5mL of 40% vanillin solution in methanol, followed by the addition of 750µL of concentrated HCl. The mixture was shaken vigorously and was left to stand at room temperature for 20

minutes in darkness. The absorbance was measured at 500nm using a UV-visible spectrophotometer. Catechin was used as a standard, and the results were expressed as mg of catechin equivalents (CE) per gram of dry weight (DW) of the sample.

4.4 Gas chromatography-mass spectrum (GC-MS)

The ethanol extracts of 10 algal samples were examined utilizing an Agilent GC 7890A gas chromatograph used with a 5975C triple-axis detector single quadrupole mass spectrometer. Chromatographic separation utilized an Agilent HP-5MS column (30 m × 0.25mm × 0.25µm film thickness), employing high-purity helium as the carrier gas at a steady flow rate of 1mL/ min. The injector temperature was sustained at 250°C, employing a splitless injection mode with a 20:1 split ratio. The ion source temperature of the mass spectrometer was established at 230°C, while the quadrupole temperature was kept at 150°C. The oven temperature protocol commenced at 40°C (maintained for 1 minute), then escalated to 150°C at a rate of 20°C/min (sustained for 1 minute) and subsequently increased to 300°C at the same rate, held for another 1 minute. A 1µL sample was injected, and the mass spectrometer operated within a scan range of 50–600amu at an electron energy of 70eV. The solvent delay was established at 3 minutes. Compounds were identified by comparing their mass spectra with the NIST 2008 collection (National Institute of Standards and Technology). The cumulative analytical duration for each sample was 16 minutes.

5. Statistical analysis

Data represented as mean ± standard deviation of three replicates was employed to present the results. The statistical analysis was conducted using SPSS software (version 23, IBM Corp., Armonk, NY, USA). All experiments were performed with three independent replicates. Analysis of variance (ANOVA) was used to determine the statistical significance of the differences among the experimental groups. A post hoc analysis was carried out using the Tukey test to compare the means between the groups. Statistical significance was set at $P< 0.05$

RESULTS

1. Identification of the collected macroalgae species from Egyptian Coast

The collected seaweeds were identified into the following species *Sargassum cinereum* J. Agardh, *Padina tetrastromatica* Hauck, *Turbinaria decurrens* Bory from Ochrophyta, *Tricleocarpa fragilis* (Linnaeus) Huisman & Townsend, *Gracilaria armata* (Agardh) Greville, *Corallina officinalis* Linnaeus, *Amphiroa rigida* Lamouroux, *Digenea simplex* (Wulfen) Agardh from Rhodophyta, *Ulva fasciata* Delile, and *Ulva linza* Linnaeus from Chlorophyta (Fig. 1). The taxonomical identification of the collected algal species is represented in Table (1).

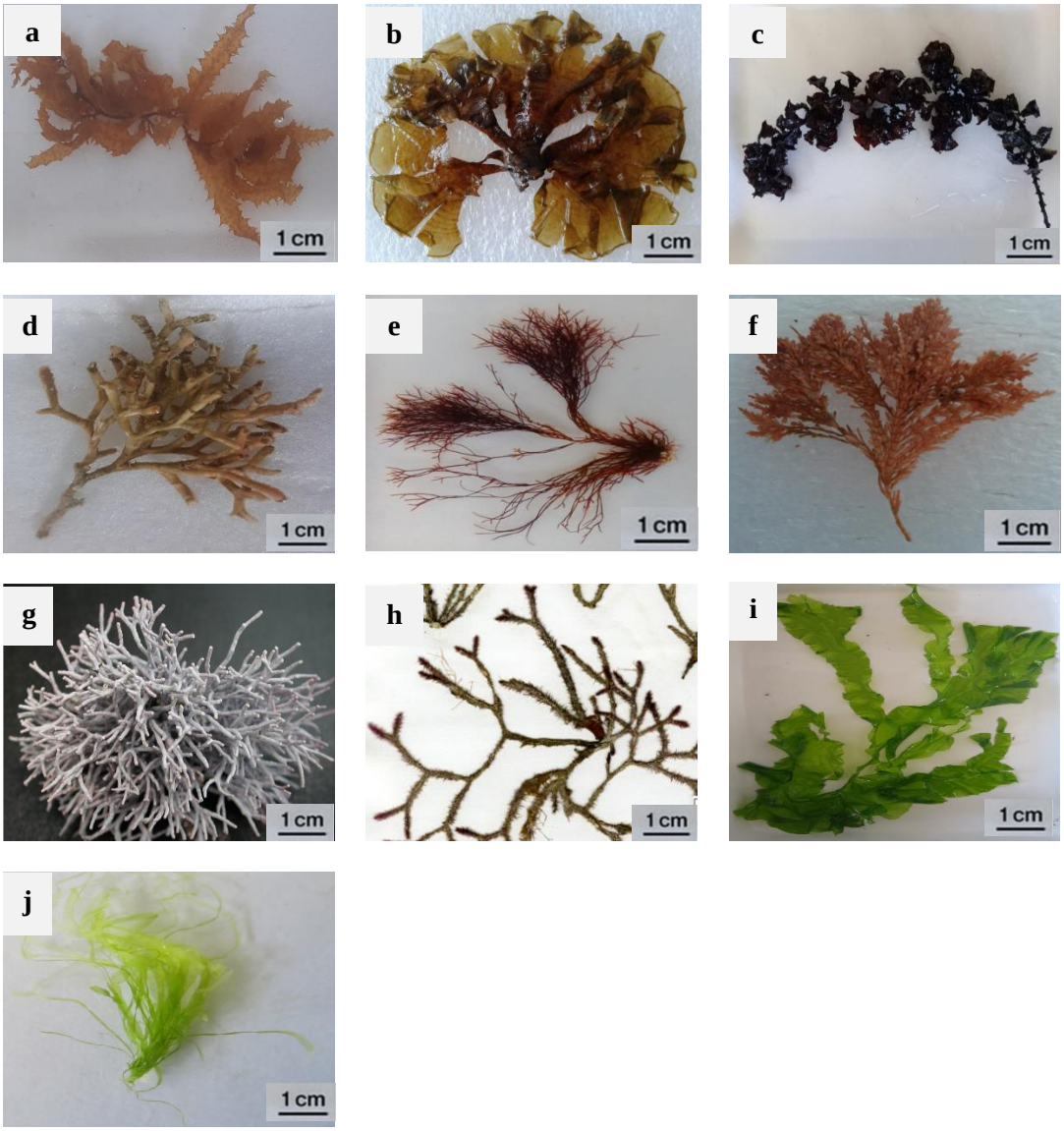

Fig. 1. Photos of the studied seaweeds: (a) *Sargassum cinereum* (b) *Padina tetrastromatica* (c) *Turbinaria decurrens* (d) *Tricleocarpa fragilis* (e) *Gracilaria armata* (f) *Corallina officinalis* (g) *Amphiroa rigida* (h) *Digenea simplex* (i) *Ulva fasciata* (j) *U. linza* Linnaeus.

Table 1. Taxonomical identification and the collection sites of the selected seaweeds

Seaweed	Empire	Kingdom	Subkingdom	Phylum	Class	Subclass	Order	Family	Genus	**Collection Site**
Sargassum cinereum	Eukaryota	Chromista	Harosa	Ochrophyta	Phaeophyceae	Fucophycidae	Fucales	Sargassaceae	*Sargassum*	*Hurghada, Red Sea*
Padina tetrastromatica	Eukaryota	Chromista	harosa	Ochrophyta	Phaeophyceae	Dictyotophycidae	Dictyotales	Dictyotaceae	*Padina*	*Hurghada, Red Sea*
Turbinaria decurrens	Eukaryota	Chromista	Harosa	Ochrophyta	Phaeophyceae	Fucophycidae	Fucales	Sargassaceae	*Turbinaria*	*Hurghada, Red Sea*
Tricleocarpa fragilis	Eukaryota	Plantae	Biliphyta	Rhodophyta	Florideophyceae	Nemaliophycidae	Nemaliales	Galaxauraceae	*Tricleocarpa*	*Hurghada, Red Sea*
Gracilaria armata	Eukaryota	Plantae	Biliphyta	Rhodophyta	Florideophyceae	Rhodymeniophycidae	Gracilariales	Gracilariaceae	*Gracilaria*	*Abo Qir, Mediterranean Sea*
Corallina officinalis	Eukaryota	Plantae	Biliphyta	Rhodophyta	Florideophyceae	Corallinophycidae	Coralliinales	Corallinaceae	*Corallina*	*Abo Qir, Mediterranean Sea*
Amphiroa rigida	Eukaryota	Plantae	Biliphyta	Rhodophyta	Florideophyceae	Corallinophycidae	Coralliinales	Lithophyllaceae	*Amphiroa*	*Abo Qir, Mediterranean Sea*
Digenea simplex	Eukaryota	Plantae	Biliphyta	Rhodphyta	Florideophyceae	Corallinophycidae	Ceramiales	Rhodymeniaceae	*Digenea*	*Hurghada, Red Sea*
Ulva fasciata	Eukaryota	Plantae	Viridiplantae	Chlorophyta	Ulvophyceae		Ulvales	Ulvaceae	*Ulva*	*Abo Qir, Mediterranean Sea*
Ulva linza	Eukaryota	Plantae	Viridiplantae	Chlorophyta	Ulvophyceae		Ulvales	Ulvaceae	*Ulva*	*Abo Qir, Mediterranean Sea*

2. The yield of phytochemical compounds

Phytochemical compounds were extracted from collected species using different solvents including ethanol, acetone, water, and methanol. The obtained results represented in Fig. (2) indicate that the *Ochrophyta* species recorded the highest phytochemical yield with ethanolic extract as *S. cinereum* recorded 0.94 g/g DW, and *T. decurrens* recorded 0.815 g, also *Ulva linza*) water extract recorded 0.904 g. Among all the studied algal groups, Rhodophyta algal group recorded the lowest phytochemical amount. Among most tested algal extracts, methanol recorded the lowest phytochemical yield, while water extract showed the highest yield (Fig. 2). Phytochemical amounts were significantly different ($P< 0.05$) between algal species and ranged from 0.014g in *G. armata* methanol extracts to 0.94g in *S. cinereum* ethanol extract.

The highest levels of phytochemical yield in the Chlorophyta algal group were observed in water extracts of *U. linza* and *U. fasciata* 0.904 and 0.544g, respectively.-The

lowest levels of phytochemical compounds were detected in methanolic extracts of *U. linza* (0.051g) and *U. fasciata* (0.041g. Ethanol extract of *U. linza* and *U. fasciata* recorded phytochemical components (0.115 and 0.216g, respectively), while acetonic extract recorded 0.084 and 0.179g, respectively.

In the Rhodophyta group, the aqueous extract recorded the highest phytochemical yield with *T. fragilis* (0.431g), *A. rigida* (0.332g) and *D. simplex* (0.328g). The ethanol extract of *T. fragilis* recorded (0.246g), while its acetone extract recorded (0.23g) phytochemical compounds. The methanolic extract of the Rhodophyta group contained 0.039, 0.048, 0.041, 0.014, and 0.058g of phytochemical compounds with *C. officinalis*, *D. simplex*, *A. rigida*, *G. armata*, and *T. fragile*, respectively.

Acetone extract recorded (0.034, 0.073, 0.091, and 0.059g) phytochemical yield with *C. officinalis*, *D. simplex*, *A. rigida*, and *G. armata*, respectively. While ethanol extract recorded (0.058, 0.1, and 0.106g) phytochemical amount with *C. officinalis*, *D. simplex*, *A. rigida*, respectively, water extract of *C. officinalis*, and *G. armata* recorded low yield (0.09 and 0.046g) of phytochemical compounds, respectively**.**

The yield of phytochemical compounds was estimated in the Ochrophyta group. *S. cinereum* had phytochemical compounds 0.941g with ethanol, 0.713g with acetone, and 0.772 with water extract. *T. decurrens* had phytochemical yield (0.815, 0.689, and 0.721g) with ethanol, acetone, and water extract, respectively. The yield of phytochemical compounds of *P. tetrastromatica* were (0.546, 0.422, and 0.656 g) recorded with ethanol, acetone, and water extract, respectively. Among the different solvents used, the methanol extract recorded the lowest phytochemical amount of 0.051, 0.048, and 0.047 g with Ochrophyta species including *S. cinereum*, *P. tetrastromatica* and *T. decurrens*, respectively.

3. Qualitative estimation of phytochemical compounds in various seaweed extracts

Preliminary phytochemical screenings of six different chemical compounds (flavonoids, phenols, tannins, saponins, steroids, and terpenoids) were tested in the ten different collected seaweed. In the present study, phytochemical screening was evaluated using different solvents; ethanol, methanol, acetone, and distilled water in different seaweed. Water extract of *G. armata*, *A. rigida*, *U. fasciata*, and *U. linza* did not show any positive result for the presence of any phytochemical compounds, as illustrated in Table (2). The methanol extract of *S. cinereum* and *G. armata* did not show any positive result for their presence of flavonoids and tannin. Similarly, the methanol extract of *P. tetrastromatica* and *U. fasciata* did not show any positive results for the presence of flavonoids and tannins, respectively.

Among the four different solvents used extracts, acetone extract showed the presence of the maximum number of phytochemical compounds, followed by ethanol

extracts and the other two solvents (acetone and ethanol) extracted from all tested phytochemical compounds.

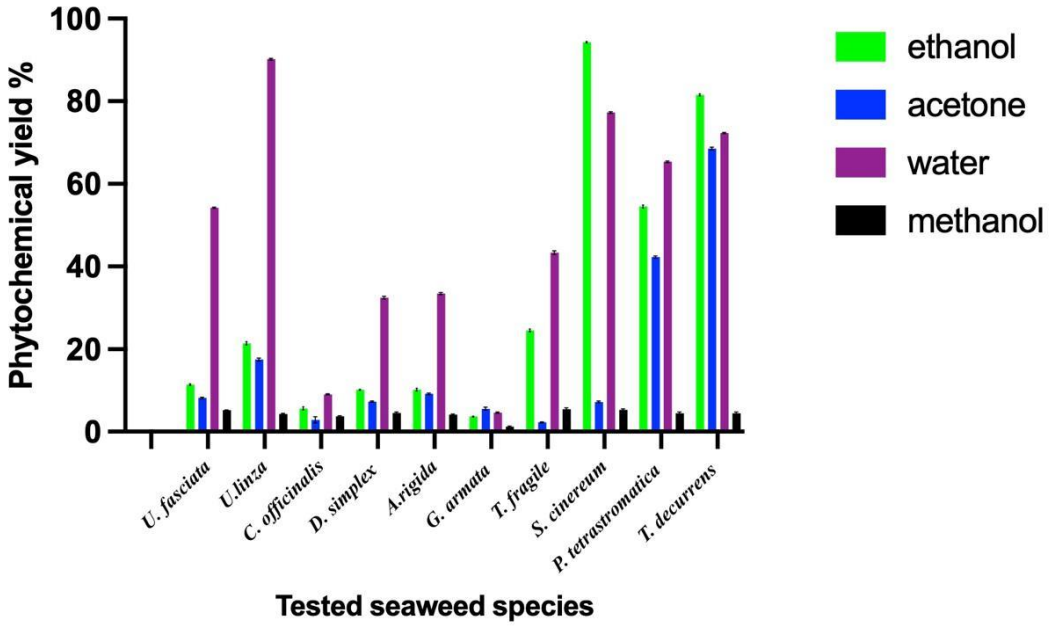

Fig. 2. Phytochemical compounds yield % of tested seaweed species using various solvents. Data points represent means ± standard deviation (n = 3)

Table 2. Preliminary phytochemical screening of different extracts of the studied species

Seaweed	*Extract*	*Flavonoids*	*Phenol*	*Tannin*	*Saponin*	*Steroids*	*Terpenoids*
S. cinereum	Ethanol	+	++	+	+	+	++
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	+	+
	Acetone	+	+++	+	+	++	++
P. tetrastromatica	Ethanol	+	++	+	+	+	+
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	+	+
	Acetone	++	++	++	+	++	++
T. decurrens	Ethanol	++	+++	+	+	+	++
	Methanol	+	+	+	+	+	+
	Water	+	++	+	-	+	+
	Acetone	++	+++	+	+	++	++
T. fragilis	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	+	+
	Acetone	++	++	+	+	++	++
G. armata	Ethanol	+	++	+	+	+	+

	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	-	-
	Acetone	++	++	+	+	++	++
C. officinalis	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	+	+
	Acetone	+	+	+	+	++	++
A. rigida	Ethanol	++	+	++	+	+	+
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	-	-
	Acetone	+	+	+	+	++	++
D. simplex	Ethanol	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+
	Water	++	++	+	-	+	+
	Acetone	++	++	+	+	++	++
U. fasciata	Ethanol	++	++	+	+	+	++
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	-	-
	Acetone	++	++	+	+	++	++
U. linza	Ethanol	++	++	++	+	+	++
	Methanol	+	+	+	+	+	+
	Water	+	+	+	-	-	-
	Acetone	++	++	+	+	++	++

+++ (very high), ++ (medium), + (low), and – (not detected).

4. Quantitative estimation of phytochemical compounds

Total phenolic content (TPC), total tannin content (TTC), and total flavonoid content (TFC) of the tested species were estimated in different solvents (Ethanol, acetone, water, and methanol).

4.1. Total phenolic content (TPC) of different seaweed extracts

The total phenolic content varied significantly ($P< 0.05$) among the different species, ranging from 0.04mg/ g DW in the water extract of *A. rigida* to 1.02mg/ g DW in the acetone extract of *D. simplex*. The maximum amounts of TPC in different algae extracts were found in the Ochrophyta algal group acetone extraction and ranged from 0.19mg/ g DW in *D. simplex* to 1.02mg/ g DW in *T. decurrens*, as represented in Fig. (3).

In the algal group Chlorophyta (*U. fasciata*, *U. linza*); *U. linza* recorded the highest amount of total phenolic (0.49±0 mg/g DW) with ethanolic extract, followed by *U. fasciata* with acetone, and ethanolic extract with total amounts of phenolic (0.47±0.02 mg/g DW), (0.48±0.01 mg/g DW), respectively. Water extracts showed the lowest TPC in both Chlorophyta species (*U. fasciata*, *U. linza*) at 0.05±0mg/ g DW. Methanol extracts of Chlorophyta algae recorded total phenolic contents of 0.19±0, and 0.23±0mg/ g DW with *U. linza* and *U. fasciata*, respectively.

In the Rhodophyta group, *G. armata* acetone extract recorded the highest TPC (0.55±0mg/ g DW), while *G. armata* ethanolic extract recorded (0.49±0.01 mg/g DW)

total amount of phenolics. *T. fragile* (Linnaeus) ethanolic extract recorded (0.38±0mg/ g DW) total amount of phenolic. *A. rigida* acetone extract recorded (0.31±0mg/ g DW) total amount of phenolics. Water and methanolic extract recorded the lowest TPC (0.12±0, 0.19±0, 0.04±0, 0.09±0, and 0.11±0mg/ g DW) for water extract; 0.16±0, 0.11±0, 0.09±0, 0.19±0, and 0.13±0mg/ g DW for methanol with *C. officinalis*, *D. simplex*, *A. rigida*, *G. armata*, and *T. fragile*, respectively. The ethanolic extract recorded (0.23, 0.21, 0.14, and 0.23 mg/g DW) total amount of phenolic with *C. officinalis*, *D. simplex*, *A. rigida*, and *T. fragile*, respectively. While the acetone extract recorded (0.2, 0.19, and 0.31mg/ g DW) total amount of phenolics with *C. officinalis*, *D. simplex*, *A. rigida*, respectively.

In the Ochrophyta group, the highest TPC was recorded in acetonic extract of *T. decurrens* (1.02±0.03 mg/g DW) followed by the ethanolic extract of *T. decurrens* (0.71 mg/g DW) and the acetonic extract of *S. cinereum* (0.71 mg/g DW). The lowest TFC was detected in methanolic extract of *P. tetrastromatica* (0.07 mg/g DW) and *T. decurrens* (0.08 mg/g DW), respectively, followed by the water extract for 0.17, 0.18, and 0.31mg/ g DW for the same species. On the other hand, *P. tetrastromatica* acetone extract recorded 0.4mg/ g DW. While, *S. cinereum*, and *P. tetrastromatica* ethanolic extract recorded 0.41, and 0.37mg/ g DW.

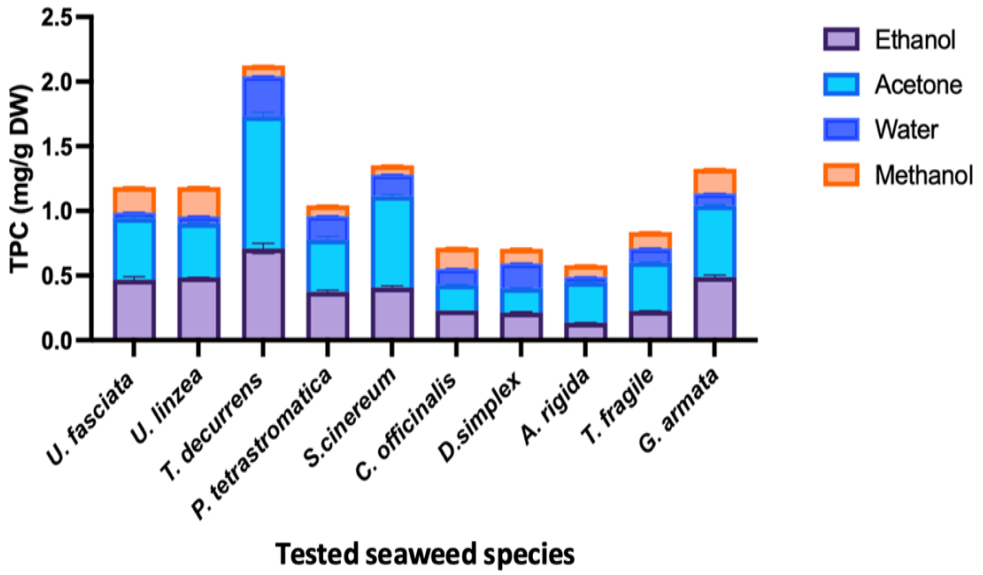

Fig. 3. Total phenolic content (TPC) of the tested seaweed species using various solvents. Data points represent means ± standard deviation (n = 3).

4.2. Total flavonoid content (TFC) of different algal extracts

Within the Chlorophyta algal group, the acetonic extract of *U. linza* showed the highest level of TFC (10.69mg/ g DW), followed by the ethanol extract of *U. fasciata*

(7.57mg/ g DW), and the acetonic extract of *U. fasciata* (6.85mg/ g DW). Among the various solvents used for extraction, the aqueous and methanolic extracts exhibited the lowest TFC values: 0.16 and 0.13mg/ g DW for *U. fasciata*, and 0.92 and 0.88mg/ g DW for *U. linza*, respectively.

The acetonic extract of *A. rigida* recorded the highest TFC (14.95mg/ g DW) among the Rhodophyta species, followed by the acetonic extracts of *G. armata* and *T. fragile* (12.23 and 7.16mg/ g DW, respectively). Conversely, water and methanol extracts showed the lowest TFC values across all Rhodophyta species. The water extracts recorded TFC values of 0.18, 0.28, 0.10, 0.07, and 0.27mg/ g DW for *C. officinalis*, *D. simplex*, *A. rigida*, *G. armata*, and *T. fragile*, respectively. Methanolic extracts showed TFC values of 0.94, 0.87, 0.89, 0.50, and 0.49mg/ g DW for the same species, respectively.

Using ethanolic extracts, the TFC values were 0.59, 2.94, 2.82, 2.24, and 2.22 mg/g DW for *C. officinalis*, *D. simplex*, *A. rigida*, *G. armata*, and *T. fragile*, respectively.

The acetonic extract of *S. cinereum* showed the highest TFC (18.21mg/ g DW), followed by *P. tetrastromatica* (16.87 mg/g DW), and *T. decurrens* (8.71mg/ g DW). As observed with both the Chlorophyta and Rhodophyta groups, the lowest TFC values were obtained from water and methanolic extracts. Water extracts of *S. cinereum*, *P. tetrastromatica*, and *T. decurrens* recorded TFC values of 0.13, 0.19, and 0.45mg/ g DW, respectively. Methanolic extracts showed TFC values of 0.19, 0.73, and 0.11mg/ g DW for the same species, respectively. When using ethanolic extracts, TFC values were 3.97, 2.45, and 3.17mg/ g DW for *S. cinereum*, *P. tetrastromatica*, and *T. decurrens*, respectively.

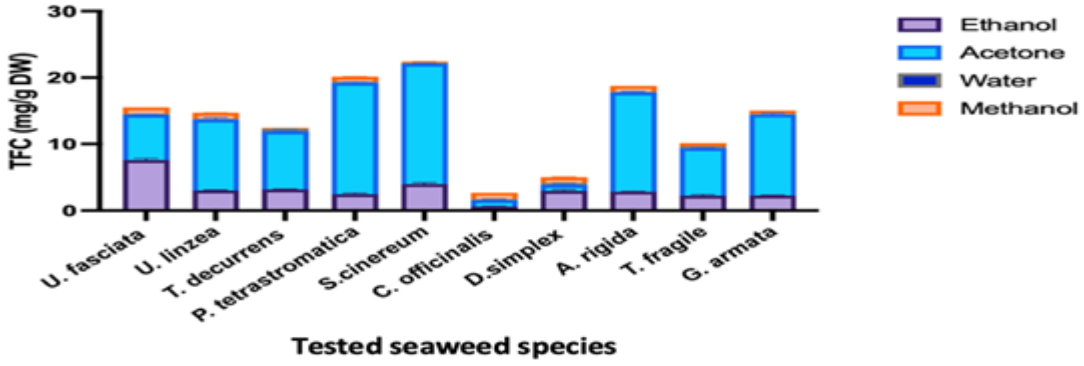

Fig. 4. Total flavonoid content (TFC) (mg/g DW) of the tested seaweed species using various solvents. Data points represent means ± standard deviation (n = 3).

4.3. Total tannin content (TTC) among different seaweeds extracts

The results of the quantitative tannin content (TTC) showed that the different types of seaweed had significantly different levels of tannins ($P< 0.050$). Tannin levels varied significantly among solvent extracts, ranging from 0.04 in *T. decurrens* to 0.56mg/

g DW in *U. fasciata*). Aqueous extract of *U. fasciata* exhibited the highest tannin level (0.56mg/ g DW), followed by the ethanolic extract of *A. rigida* (0.41mg/ g DW), as shown in Fig. (5). Tannin levels in the selected species vary significantly depending on the variety of seaweeds and the solvents used.

The maximum tannin level in the Chlorophyta algal group was detected in *U. fasciata* water extract (0.56mg/ g DW) followed by acetonic extract of *U. linza* (0.46 mg/g DW), while the ethanol extract of *U. fasciata*, and *U. linza* recorded close tannin levels of (0.13mg/ g DW) and (0.19mg/ g DW), respectively. Additionally, methanol extract of *U. fasciata*, and *U. linza* recorded 0.06 and 0.1mg/ g DW.

Among the different groups of Rhodophyta algae, the ethanolic extract of *A. rigida* exhibited the highest tannin level value (0.41mg/ g DW), followed by the water extract of *A. rigida* (0.32mg/ g DW), and the acetonic extract of *T. fragile* (0.3mg/ g DW). The lowest tannin level was recorded with aqueous extract of *G. armata* (0.04mg/ g DW). *C. officinalis* and *D. simplex* exhibited the lowest tannin level with all solvents. *C. officinalis* recorded values of 0.09, 0.08, 0.08 and 0.05mg/ g DW with ethanol, acetone, water, and methanol, respectively, while *D. simplex* recorded 0.05, 0.09, 0.08 and 0.08mg/ g DW with ethanol, acetone, water, and methanol, respectively. *G. armata* ethanolic and acetonic extract recorded close tannin level values with 0.2±0.0 and 0.25mg/ g DW, respectively. In addition, water and methanolic extract of *G. armata* recorded similar tannin level values of 0.04 and 0.05mg/ g DW, respectively. Whereas *T. fragile* exhibited tannin level values of 0.1, 0.05, and 0.06mg/ g DW with ethanol, water, and methanol extract, respectively.

The aqueous extract of *P. tetrastromatica* exhibited the highest tannin level value (0.48mg/ g DW) among the different Ochrophyta groups, while *T. decurrens* methanolic, acetonic, and ethanolic extracts had tannin level values close to each other at 0.29, 0.27, and 0.26mg/ g DW.

In the Ochrophyta group, *P. tetrastromatica* acetonic extract had a tannin level of 0.23 mg/g DW. *S. cinereum* exhibited tannin levels of 0.15, 0.14, 0.12, and 0.08mg/ g DW with ethanolic, acetonic, water, and methanolic extracts, respectively. In this context, *P. tetrastromatica* ethanolic and methanolic extracts had tannins levels of 0.16 and 0.06mg/ g DW, respectively.

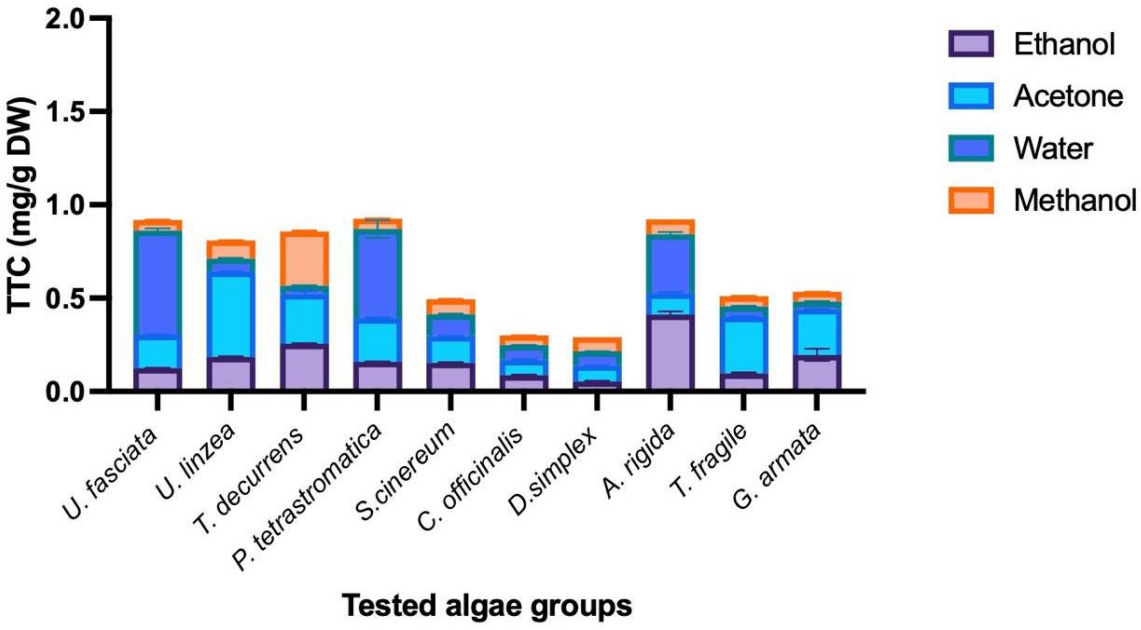

Fig. 5. Total tannin content (TTC) (mg/g DW) of tested seaweed species using various solvents. Data points represent means ± standard deviation (n = 3).

4.4 Gas chromatography–mass spectrometry analysis

Gas chromatography–mass spectrometry (GC–MS) is an authentic reliable tool commonly employed for the identification of chemical constituents. In the current investigations, the maximum number of bioactive compounds were found in ethanolic extract of *S. cinereum* (15 compounds) followed by 12 compounds in both *P. tetrastromatica* and *T. decurrens* extract. The investigation revealed various peaks of compounds, which were tentatively determined by comparing their height in percentage, peak retention time and other data reported in the literature and the National Institute of Standards and Technology library (NIST), as listed in Table (3) and Fig. (S1). The major compounds in *S. cinereum* were palmitic acid (37.43); Palmitic acid, methyl ester (17.75). The major compounds in the ethanolic extract of *P. tetrastromatica* were stearic acid, methyl ester (26.05); Palmitic acid (23.3); palmitic acid, methyl ester (20.61), and cholesterol (14.44). GC/MS analysis of *T. decurrens* extract identified different compounds, with the palmitic acid (56.09) being the major one; 13-docosenamide (Z) (11.06), cis-vaccenic acid (9.98) and palmitic acid, methyl ester (7.35).

GC-MS analysis of Rhodophyta showed that eighteen main constituents were identified in *D. simplex*; seventeen bioactive compounds were found in *G. armata*; fifteen peaks were present in the extract of *A. rigida*; fourteen bio compounds-were elucidated in *T. fragile*; and *C. officinalis* contained 11 constituents. The major constituents of *C. officinalis* were palmitic acid, methyl ester (20.01); palmitic acid (12.52664262); bicyclo [3.1.1] heptane, 2,6,6-trimethyl-, (1. alpha.,2. beta.,5. alpha.)- (10.84341219) and 2-butenoic acid, 3-(methylamino)-, ethyl ester (8.922992623). While the main constituents

of *D. simplex* were palmitic acid (49.4). palmitic acid (35.46) and palmitic acid, methyl ester (11.75) represented the high area in *G. armata* extract. The GC-MS chromatogram analysis of the *A. rigida* extract revealed that the major compounds were palmitic acid (19.17%) and palmitic acid, methyl ester (31.995%). Similarly, the GC-MS analysis of *T. fragile* identified palmitic acid (22.17%), bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, (1.α,2.β,5.α) (15.25%), palmitic acid, methyl ester (9.36%), and tetradecanoic acid (14.75%) as the predominant compounds, as shown in Table (4) and Fig. (S2).

Among the Chlorophyta algal group, the GC-MS analysis of *U. linza* revealed the presence of 11phytoconstituents while *U. fasciata* contained nine constituents. The major compounds found in *U. fasciata* were palmitic acid (55.94) and palmitic acid methyl ester (19.16), while the main compounds in *U. linza* extract were palmitic acid (38.18); palmitic acid methyl ester (28.41) and 9-octadecenoic acid (Z)-, methyl ester (10.85), as illustrated in Table (5) and Fig. (S3).

DISCUSSION

The pronounced influence of the extraction solvent on crude yield and phytochemical recovery reflects trends reported for the tested brown species. *Sargassum cinereum* and *Turbinaria decurrens* exhibited the highest extractable material with ethanol, consistent with **Ismail *et al.* (2019)**, who observed that moderately polar ethanol preferentially solubilizes phenolic phlorotannins in Phaeophyceae. Additionally, **Sujatha *et al.* (2019)** demonstrated that ethanolic fractions of *Sargassum swartzii* contain richer bioactive profiles. On the other hand, **Mahendran *et al.* (2021)** showed that methanolic extraction of the red seaweeds *Gracilaria edulis* and *Hypnea valentiae* maximizes total phenolic content, highlighting the importance of matching solvent polarity to target metabolite classes. The variation in extraction yield of bioactive compounds from algal species may be related to solvent type, age, algal species, and differences in habitat **(Gazali *et al.*, 2023)**.

Qualitative screening revealed the consistent presence of phenols, flavonoids, tannins, steroids, terpenoids, and saponins across most species, with alkaloids detected more variably. This echoes **Ganesan *et al.* (2008)**, who found that Ochrophyta often display the widest suite of secondary metabolites. The absence of certain compounds in high-polarity (water, methanol) extracts of Rhodophyta and Chlorophyta aligns with the "polarity window" hypothesis of **Herawati and Pudjiastuti (2021)**, whereby intermediate solvents such as acetone and ethanol co-extract both polar and moderately nonpolar constituents simultaneously. Additionally, qualitative phytochemical screening by **Al-Hashdy *et al.* (2025)** detected phenolic and tannin compounds in the methanolic extract of *Sargassum vulgare*, whereas these were absent in its nonpolar n-hexane fraction, further underscoring the critical role of solvent polarity in recovering flavonoids and condensed tannins from brown macroalgae.

Quantitatively, total phenolic, flavonoid, and tannin contents varied significantly ($P< 0.05$) among species and solvents. The highest total phenolic content (1.02 ± 0.03 mg GAE/g DW) was found in the acetone extract of *Turbinaria decurrens*. Regarding total phenolic content, **Nguyen and Tran (2025)** reported that the ethanolic extract of *Dictyota implexa* contained 85.95 ± 1.21 mg GAE/g extract, a value substantially higher than many brown algal extracts and closely mirroring the elevated phenolic levels we observed in our ethanol fractions. This concordance not only validates the reliability of ethanol as an extraction solvent for phenolic compounds in Phaeophyceae but also suggests that the intrinsic biochemical composition of *D. implexa* and related species shares a consistent propensity for phenolic accumulation under similar collection and processing conditions. Therefore, our data, in conjunction with Nguyen and Tran's findings, underscore the critical influence of solvent polarity and algal physiology on maximizing phenolic recovery across diverse brown macroalgae. **Nagah *et al.* (2023)** reported that the cold-water extract of *Sargassum swartzii* (SCWE) contained 5,749.04 ± 118.2µg GAE/mL, compared with 4,559.09 ± 79.8µg GAE/mL in the autoclaved water extract and only 662.21 ± 26.3µg GAE/mL in the ethanolic extract, underscoring how solvent polarity dramatically influences phenolic recovery.

The highest flavonoid content was recorded in acetone extracts of *Ulva linza* (10.7 ± 0.2mg QE/g DW) and *Sargassum cinereum* (18.2 ± 0.04mg QE/g DW), confirming similar solvent biases noted by **Koivikko *et al.* (2005)** and **Lee *et al.* (2022)** for green and brown seaweeds. In this respect, **Subbiah *et al.* (2025)** reported that *Phyllospora comosa* exhibited a peak flavonoid content of 0.73mg QE/g at 0h during colonic fermentation, further emphasizing the strong influence of solvent polarity and species-specific chemistry on flavonoid recovery in marine algae. The cold-water extract of *Sargassum swartzii* (SCWE) contained 352.22 ± 7.7µg QE/mL, compared with only 39.00 ± 1.7µg QE/mL in its ethanolic extract, nearly a tenfold difference, underscoring the profound impact of solvent polarity on flavonoid recovery.

Tannin distributions, peaking in aqueous *Ulva fasciata* (0.56 ± 0.01mg CE/g DW), further illustrate how solvent polarity governs the leaching of condensed tannins **(Koivikko *et al.*, 2005)**.

Comparative GC-MS profiling of ethanolic extracts from ten Egyptian seaweeds revealed a phytochemical suite dominated by fatty acids, esters, alcohols, and hydrocarbons. Four core metabolites—palmitic acid, palmitic-acid methyl ester, cis-vaccenic acid, and 9-octadecenoic-acid methyl ester—recurred at high abundance, with the palmitic-acid pair alone contributing ≥30% of the total peak area in *T. decurrens*, *S. cinereum*, *P. tetrastromatica*, *U. fasciata*, and *G. armata*. Our results are in good accordance with **Al-Hashdy *et al.* (2025)**, who stated that the prevalence of palmitic acid derivatives mirrors earlier GC-MS reports for the brown alga *S. cinereum* and the red alga *Corallina officinalis* **(Hassan & Shobier, 2018)**. Saturated medium-chain lipids such as

lauric and myristic acids, both prominent in many marine algal extracts, have been shown to scavenge free radicals, attenuate lipid peroxidation, suppress a wide range of Gram-positive skin pathogens, and competitively inhibit type-1 and type-2 5-α-reductase, the key enzyme in androgen activation **(Nakatsuji *et al.*, 2009)**. Red-algal extracts displayed lineage-specific markers: *Dichotomaria simplex* accumulated phenobarbital; *Acanthophora rigida* and *Tetraclada fragile* contained 2-pentadecanone, 6,10,14-trimethyl- (hexahydrofarnesyl acetone); and *Corallina officinalis* was enriched in tetradecanoic (myristic) acid metabolites. These compounds have been linked to antinociceptive/anti-inflammatory (hexahydrofarnesyl acetone) and NF-κB-mediated anti-inflammatory (myristic acid) activities **(Huang *et al.*, 2023)**. Such chemovariation likely reflects interacting environmental drivers (climate, seasonality, geography) and intrinsic genetic diversity that modulate secondary-metabolite biosynthesis and, consequently, medicinal quality **(Briskin, 2000)**. Collectively, the dominance of bioactive fatty acids, esters, and alkenes, supplemented by species-specific compounds, underscores the therapeutic promise of Egyptian seaweed extracts and rationalizes their strong antioxidant and cytotoxic activities observed *in vitro*.

CONCLUSION

Phytochemical screening of various seaweed extracts revealed the presence of phenolic compounds with varying concentrations depending on the algal species and the solvent used. The study clearly indicates that *Sargassum cinereum* is a promising source of phytochemical compounds. This research also supports the health benefits of common Egyptian seaweed extracts in medicinal applications by emphasizing their significance as possible sources of natural phenolic compounds with a variety of bioactivities. According to the results obtained, it can be said that the solvent used had a significant effect on the total phytochemical content and concentrations. However, further studies are needed to isolate and purify the bioactive compounds from the most promising and dominant species responsible for the biological activity. This will allow them to be utilized as natural ingredients in the nutritional and pharmaceutical industries.

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