ЕКОЛОГІЧНА ФІЗІОЛОГІЯ І БІОХІМІЯ ВОДНИХ РОСЛИН

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ФАРМАКОЛОГІЧНІ АСПЕКТИ І ХРОМАТОГРАФІЧНИЙ ПРОФІЛЬ ФЕНОЛЬНИХ СКЛАДОВИХ ТРЬОХ ВИДІВ МАКРОВОДОРОСТЕЙ ЄГИПТУ. ДОСЛІДЖЕННЯ ЗВ'ЯЗКУ СТРУКТУРИ І АКТИВНОСТІ

Метою роботи було порівняльне дослідження загального вмісту фенолів (ЗФ), танінів (Т) і флавоноїдів (Ф) в Ulva compressa, Pterocladia capillacea *і* Colpomenia sinuosa, *екстрагованих трьома різними розчинниками. Проведено якісну і кількісну оцінку фенольних речовин у різних екстрактах водоростей.*

Найбільший вміст ЗФ і Ф зареєстровано у етилацетатному екстракті C. sinuosa *(відповідно 49,99 і 38,68 мг/г). В експерименті з екстрагуванням метанолом зареєстровано найбільший вміст дубильних речовин у всіх трьох видів водоростей, найвищий у* C. sinuosa *(19,36 мг/г). Натомість при екстрагуванні гексаном вміст фенольних речовин і біологічна активність були найнижчими. Аналіз екстрактів водоростей за допомогою високоефективної рідинної хроматографії (HPLC) дозволив встановити наявність 16 фенольних кислот і флавоноїдів, включаючи галову і коричну кислоти і катехін, у більшості екстрактів.*

Дослідження дали змогу припустити, що етилацетатні і метанольні екстракти C. sinuosa *і* P. capillacea *можуть слугувати важливим джерелом природних поліфенолів з антиоксидантними, протидіабетичними, протиартритними і протизапальними властивостями. Відмічено значну кореляцію між вмістом флавоноїдів і антиоксидантною і протидіабетичною активністю. Відношення структури і активності флавоноїдів, як антиоксидантів і протидіабетичних речовин, регулюється через переніс атома гідрогену та послідовної втрати протону/переносу електро-*

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ну. Метанольні і етилацетатні екстракти водоростей мали антикоагулянтні властивості. Однак необхідні подальші токсикологічні і клінічні дослідження.

Ключові слова: *макроводорості, біологічна активність, флавоноїди, феноли, таніни, затока Абу Кір,Середземне море, Єгипет.*

Introduction

Macroalgae «seaweeds» have attracted attention in the search for bioactive substances to create novel medications and nutritious diets [17, 25, 26, 35]. Phenolics are among the most abundant and important bioactive molecules produced by seaweeds, especially brown seaweeds which play a significant role in algal growth and development. They are also created to protect against a range of abiotic and biotic stresses, such as UV radiation, high temperatures, salinity, pathogenic infections, and herbivory [30]. On the other side, stress such as high salinity, microbial attack, and ultraviolet rays induces an elevation in polyphenol production in seaweeds, leading to the development of more intricate forms [1, 26, 59]. Seaweeds contain phenol up to 20 % of their dry weight. Most of the secondary metabolites found in plants are classified into two groups: phenolic acids and polyphenols [35].

Seaweed phenolics contain a basic structure consisting of the hydroxy group connected to aromatic ring. They are classified based on the number of carbon atoms and benzene rings, as well as solubility [30]. Polyphenols comprise a broad set of about 8000 structurally different substances that can be grouped into various families, such as flavonoids, phenolic acids, stilbenes, lignans, and tannins. The number of hydroxyl groups, the position of the hydroxy group, and the number of phenyl rings in the structure all affect how bioactive phenolic compounds are [30, 74].

Polyphenols in seaweeds vary arrays of phytochemicals that have garnered significant interest owing to their therapeutic qualities and beneficial impacts on health [51]. These compounds are characterized by a wide spectrum of bioactivities such as antimicrobial, anticancer, anti-inflammatory, anti-Alzheimer, anti-aging, and beneficial antioxidant activities [27, 28, 67]. Numerous studies have shown that macroalgal polyphenols are more stable than those found in terrestrial plants, making them more effective at lowering oxidative stress [25, 26, 34]. They are consumed raw or processed worldwide due to their high mineral and antioxidant content, including polyphenols [14]. Phenols have significant commercial potential that could lead to a better future for humans, such as anti-inflammatory, anticoagulant, anti-arthritic, anti-cancer, anti-diabetic drugs, as well as functional foods [48, 75]. Moreover, seaweed-derived phenols have been found to reduce blood glucose levels [44]. In recent decades, seaweed phenolics have emerged as a desirable and sustainable source of antioxidants with a variety of biofunctional qualities. They may eventually replace current aquafeed additives [22].

Among the polyphenols present in seaweeds are flavanols, catechins, and phlorotannins which have incredibly wide range of possible biological functions [28, 29]. Flavonoids with their distinct structural features are naturally occurring secondary metabolites of phenolic compounds that possess a variety of

biofunctional qualities, including the ability to scavenge free radicals and act as antioxidants [67]. Additionally, phenolic acids, flavonoids, and tannins are the main dietary phenolic compounds [68]. Seaweeds contain both soluble and binding forms of tannins, which can make up to 14 % of their dry biomass [65]. The brown algae were found to be able to store polyphenolic chemicals up to 25—30 % of their dry weight [55]. The main phenolic compounds found in green and red algae are flavonoids, phenolic acids, and bromophenols [77]. Phenolic compounds are believed to have antioxidant properties because they can scavenge free radicals, donate electrons or hydrogen atoms, or bind metal cations. The ability of phenolic compounds to neutralize free radicals is attributed to their molecular structures, specifically the number and positions of hydroxyl groups and the types of substitutions on aromatic rings. This concept is known as the structure-activity relationship (SAR) [58]. Also, flavonoids are a significant group of polyphenols found in seaweeds. They have protective effects in biological systems by transferring hydrogen or electrons to free radicals, activating antioxidant enzymes, chelating metal catalysts, reducing α-tocopherol radicals, and inhibiting oxidases [69]. Future views and research approaches are given to encourage further investigation of seaweed phenolics, foster a better knowledge of their medicinal potential, and promote sustainable extraction and purifying procedures [62].

The goal of this study was to examine how three different solvents (ethyl acetate, hexane, and methanol) affected the extraction yields of the phenolic compounds. A detailed chromatographic analysis of algal extract was performed. The study sought to assess the antioxidant, antidiabetic, anti-arthritic, anti-inflammatory, and anticoagulant properties of the extracts *in vitro*. The findings of these investigations will help us better understand the possible health benefits of *U. compressa, P. capillacea,* and *C. sinuosa* from Abu Qir Bay in Egypt. The study keeps track of structure-activity correlations to support biological processes with possible uses in the pharmaceutical industry.

Material and Methods

Chemicals: Sigma-Aldrich (Darmstadt, Germany) supplied all of the chemicals and solvents utilized in the study.

Collection and identification of seaweeds. Three different species were freshly collected during autumn 2023 from Abu Qir beach (30º05'—30°22' E, 31º16'—31°21' N), Alexandria, Egypt (Fig. 1). They were washed with seawater to remove epiphytes and sand attached to the surface, then transported in the iced condition to the laboratory of Taxonomy and Biodiversity of Aquatic Biota at the National Institute of Oceanography and Fisheries, Alexandria, Egypt. The specimens were carefully rinsed with tap water and some of the fresh thallus was preserved in seawater containing 5 % solution of formaldehyde for taxonomic identification. The other clean thallus was shade dried at room temperature and powdered, then preserved at -20 ºC for further analysis. The collected seaweed species were identified based on [5] and confirmed using the Algae Base website [21] as *Ulva compressa* Linnaeus from Chlorophyceae, *Pte-*

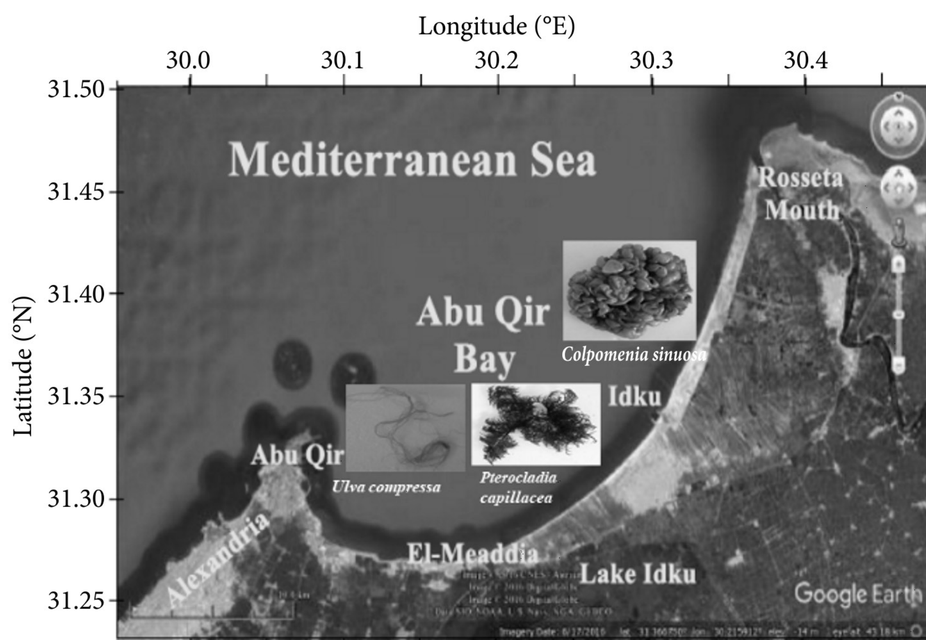


Fig. 1. Sampling locations and seaweed species

rocladia capillacea (S.G. Gmelin) Borne from Rhodophyceae and *Colpomenia sinuosa* (Mertens ex Roth 1806) Derbès & Solier from Phaeophyceae.

Preparation of algal extracts. Dry seaweed extracts were prepared using a Soxhlet apparatus. Approximately 10 g of finely powdered seaweed were placed in a thimble and extracted with approximately 100 mL of ethyl acetate, methanol, and hexane separately for about 48 hours. The resulting extracts were concentrated under lower pressure using a rotary vacuum evaporator (40—60 °C, 135—150 rpm, for 2 h) and then freeze-dried. The freeze-dried sample was weighed for yield calculations (mg/g dry weight) and stored at -20 °C until use. The crude extracts were utilized for qualitative and quantitative analysis of phenolic compounds and the selected bioactivities *in vitro*.

Qualitative analysis. Phytochemical analysis of the algal crude extracts was subjected to nine qualitative assays such as coumarins, flavonoids, phenols, quinones, saponins, tannins, terpenes, steroids, and cardiac glycosides. These tests were performed on the tested extracts following established protocols; basic responses in these studies enabled the identification of these substances [27, 57].

Test for phenol. A small amount of each extract was mixed with 1 mL of water, and 1 to 2 drops of $FeCl_3$ were added to a test tube. A blue, green, red, or purple color indicates a positive result.

Test for flavonoids. A small amount of each algal extract was mixed with one to five drops of concentrated HCl. The presence of flavonoids is indicated by the rapid development of a red color.

Test for tannins. 2 mL of 5 % $FeCl_3$ solution was added to a test tube containing 5 mL of each extract. The presence of a greenish-black precipitate suggests the presence of tannins.

Test for steroid. Each algal extract was mixed with 2 mL of chloroform, and then concentrated H_2SO_4 was added at the same time. The presence of steroids is indicated by the red coloration that develops in the lower chloroform layer.

Test for saponins. Dilute 1 mL of the tested extracts with distilled water to 20 mL and shake in a graduated cylinder for 15 minutes. The formation of a 1 cm layer of foam indicates the presence of saponins.

Test for terpenoids. 2 mL of chloroform and 0.5 mL of each algal extract were placed in a test tube. Then, 3 mL of concentrated H_2SO_4 was added, forming a distinct layer. Terpenoids are indicated by reddish-brown interface coloration.

Test for quinones. Concentrated sulfuric acid should be added to 1 mL of each algal extract. If red coloration appears, it indicates the presence of quinones.

Test for coumarins. 2 mL of crude algal extract were combined with 3 mL of 10 % sodium hydroxide (NaOH); the presence of coumarins is indicated by the yellow coloration of the mixture.

Test for cardiac glycosides. Glacial acetic acid was mixed with a few drops of 2 % $FeCl_3$ solution, and then 2 mL of the resulting solution were combined with the crude extract. Following this, the mixture was transferred to another vessel along with 2 mL of concentrated H_2SO_4. The presence of cardiac glycosides in the samples is indicated by the formation of a brown ring forms at the interface.

Quantitative analysis. Total phenol content. The total phenolic content (TPC) of the algal extracts was determined using the Folin-Ciocalteu reagent. For each algal extract, 1.0 mL mixed with 5 mL of distilled H_2O and 0.5 mL of Folin-Ciocalteu reagent applied afterwards. After mixing and letting the mixture incubate for 30 minutes at room temperature, 1.0 mL of 1.8 M Na_2CO_3 was added and incubation in the dark for 2 hrs. The absorbance at 760 nm was measured and data were expressed as milligrams of gallic acid equivalent per gram extract dry weight (mg GAE/g).

Total tannins concentration. After combining 1 mL of the algal extract with 0.5 mL of Folin-Ciocalteau's reagent, as reported by [32], 1 mL of saturated Na_2CO_3 solution and 8 mL of distilled water were added. The reaction mixture was left to stand at room temperature for half an hour. The absorbance was measured at 725 nm using a UV-Visible Spectrophotometer after the supernatant was collected by centrifugation. A standard graph was created by plotting the absorbance of different tannic acid concentrations as increasing quantities of standard tannic acid were added. Tannic acid equivalent as milligrams per gram of the sample was used to express the tannin content (mg TAE/g) [50].

Total flavonoids content (TFC). Using the technique [76], which involve the formation of a complex flavonoid-aluminum with a peak absorbance at 430 nm, the TFC in various algal extracts was measured spectrophotometrically. A 2 % $AlCl_3$ in methanol solution was combined with a volume of the algal extracts. The absorbance of the resulting reaction mixture was then recorded at

430 nm after 10 minutes of incubation at room temperature. The calibration curve was created using catechin and the results were reported as mg of catechin equivalents per gram of dry extract (mg CE/g).

High-performance liquid chromatography (HPLC) analysis of phenolic compounds. The HPLC method was used for the quantification and identification of individual phenolics. An Agilent 1260 series HPLC was used to analyze the phenolic components in the examined algal extracts. A C18 Eclipse column (4.6×250 mm i.d., 5 μm) was utilized for the separation at 35 °C. At a flow rate of 1 mL per minute, the mobile phase was composed of 0.1 % trifluoroacetic acid in acetonitrile (B) and water (A). The following linear gradient was used to program the mobile phase: 12—14 min (80 % A), 14—16 min (80 % A), 0—5 min (80 % A), 5-8 min (40 % A), 8—12 min (50 % A), and 12—14 min (80 % A). The wavelength of the multi-wavelength detector was calibrated at 280 nm. An injection volume of 10 μL was used for each sample solution. The phenolic compounds in extracts were identified by comparing retention times and absorption spectra with standards, as well as by spiking samples. Results are expressed as μg of compound per mL of extract (μg/mL).

Pharmacological aspects in vitro. Anti-inflammatory activity. The basic of this test is to create an orange Fe^{3+}/xylenol combination, that reaches its maximum absorbance at 560 nm. 20 μL algal extracts (500 μg/mL) and 40 μL glycine max enzyme 15-lipoxygenase were incubated for five minutes at 25 °C. The mixture was then further incubated at 25 °C for 20 min in the dark after adding linoleic acid to a 50 mM Tris-HCl buffer (pH 7.4) at a final concentration of 140 μM. To stop the reaction, 100 μL of the FOX reagent (9:1 v/v methanol/water solution containing 30 mM sulfuric acid, 100 μM xylenol orange, and 100 μM iron (II) sulfate) was added. In the control wells, only the buffer and LOX solution were pipetted. The LOX enzyme was present in the blanks during the incubation time, and the FOX reagent was added followed by the addition of the substrate, linoleic acid [52]. To measure the inhibitory action of lipoxygenase, the percentage suppression of hydrogen peroxide generation was determined by calculating changes in absorbance values at 560 nm after 30 minutes at 25 °C. The inhibition % was determined using the following equation (Eq. 1);

$$\text{Anti-inflammatory activity} = \frac{((A_{control} - A_{blank}) - (A_{sample} - A_{blank}))}{(A_{control} - A_{blank})} \times 100 \qquad \text{(Eq. 1)}$$

where $A_{control}$ represents the absorbance value of the control well, A_{blank} represents the absorbance value of the blank well, and A_{sample} represents the absorbance value of a sample well.

Anti-arthritic activity (anti-denaturation activity). The efficiency of protein denaturation in the examined extracts was assessed using the assay [63]. 0.05 mL of algal extracts (500 μg/mL) and 0.45 mL of 5 % w/v bovine serum albumin in an aqueous solution made up 0.5 mL of the reaction mixture. To adjust the pH to 6.3, 1 N HCl was used. The samples were incubated for 20 min at

37ºC and then heated for 3 min at 57ºC. After cooling the sample, 2.5 mL of phosphate buffer was added and then the turbidity generated was measured at 416 nm.

Equation (2) was used to calculate the inhibition percentage,

$$\text{Anti-arthritic activity} = \frac{(A_{sample} - A_{blank})}{(A_{control})} \times 100 \quad \text{(Eq. 2)}$$

Antioxidant activity. Total antioxidant capacity (TAC) was determined using the techniques [54]. Specifically, 0.3 mL of each algal extract (500 μg/mL) was added to 3 mL of the working solution (28 mM sodium phosphate, 4 mM ammonium molybdate, 0.6 M sulfuric acid) in three replicates. After 90 minutes of incubation at 95 °C, the absorbance of the mixture was measured at 695 nm. The data obtained were expressed as milligrams of ascorbic acid equivalent per gram of extract dry weight (mg AAE/g).

Ferric reducing power (FRP) activity. Ferric chloride-potassium ferricyanide was used to assess the ability of different algal extracts to remove Fe^{3+} [47]. After adding 1 mL of algal extracts to 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % $K_3[Fe(CN)_6]$, the mixture was incubated for 20 minutes at 50 ºC. The 10 % $C_2HCl_3O_2$ was added in 2.5 mL increments. An absorbance measurement was recorded at 700 nm after combining a portion of the solution (2.5 mL) with 2.5 mL of distilled H_2O and 0.5 mL of 0.1 % $FeCl_3$. A calibration curve was plotted using ascorbic acid, and the results were expressed in mg of ascorbic acid equivalent per gram of extract dry weight (mg AAE/g).

DPPH radical scavenging capacity. Following the assay [38], the DPPH radical scavenging capacity of various algal extracts was evaluated. Approximately 3 mL of DPPH solution (0.1 mM) was mixed with 0.3 mL of each algal extract in triplicate. The absorbance of the samples was measured at 517 nm. Ascorbic acid was used as the standard free radical scavenger. The degree of inhibition was calculated using the following equation (Eq. 3):

$$\text{DPPH scavenging activity \%} = \frac{(A_{control} - A_{sample})}{(A_{control})} \times 100 \quad \text{(Eq. 3)}$$

where the absorbance of the DPPH radical plus methanol is the control and the absorbance of the DPPH radical plus sample extract or standard is the sample.

Antidiabetic activity (α-amylase inhibition assay). Anti-diabetic ability using α-amylase inhibition was measured using the method [15]. Phosphate buffer (500 μL, 0.20 mM, pH 6.9) containing α-amylase (0.5 mg/mL) solution was mixed with test algal extracts (500 μL) and a reference drug (acarbose) (500 μg/mL). The mixture was then incubated for 10 minutes at 25 ºC. Following the addition of a starch solution (500 μL, 1 % w/v in 0.02 M sodium phosphate buffer pH 6.9), the reaction mixtures were incubated for 10 minutes at

25°C. After heating in a boiling water bath for five minutes and cooling to room temperature, the reaction was quenched with 3 mL of dinitrosalicylic acid reagent (1.0 mL). Following a 10 mL dilution of the reaction mixture with distilled water, the absorbance was determined at 540 nm and the enzyme inhibition percentage by the crude extracts was determined using the following equation (Eq. 4)

$$\% \text{ inhibition} = \frac{\text{Enzyme activity of control} - \text{Enzyme activity of sample}}{\text{Enzyme activity of control}} \times 100 \qquad \text{(Eq. 4)}$$

The doses of the extract resulting in 50 % scavenging activity (IC50) were calculated using GraphPad Prism 6 software.

Anticoagulant activity. All clotting assays were done using normal citrated human plasma from three individual healthy donors with no history of bleeding or thrombosis. The anticoagulant activity was assessed using three assays [78]: prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT). In the APTT assay, 90 µL of citrated normal human plasma and 10 µL of algal extracts were incubated at 37 °C for 60 seconds. Then, 100 µL of prewarmed APTT reagent were added and allowed to react at 37 °C for 2 min. After that, 100 µL of 0.25 mol/L pre-warmed calcium chloride was added and APTT was recorded as the clot formation time. To perform the PT clotting experiment, 90 µL of citrated normal human plasma was added to 10 µL of algal extracts (500 µg/mL) and incubated at 37 °C for 1 min. After that, 200 µL of pre-incubated PT test reagent (37 °C, 10 min), was added and the clotting time was recorded. Heparin was used as a positive control, while saline (0.9 % NaCl) was used as the negative control. For the TT assay: 90 µL of citrated normal human plasma was mixed with 10 µL of each sample (500 µg/mL) and incubated at 37 °C for 60 seconds. Following the addition of 200 µL of TT test reagent, the clotting time was then measured.

Statistical analysis. The means ± standard deviation of three replicates was employed to present the results. One-way analysis of variance (ANOVA) was utilized for statistical comparisons, with *p*-values less than 0.05 considered significant. IBM-SPSS Statistics version 22 was used to conduct a Pearson correlation analysis between the phenolic contents and the biological activities of all extracts under investigation.

Results and Discussion

Extraction yields. Yields of different extracts of the selected species were examined and presented in Table 1. With respect to other solvent extracts; the methanol extract (polar solvent) of every species exhibited the maximum yield followed by ethyl acetate then hexane. It is commonly known that the yield of chemical extraction is affected by the pH, extraction time, temperature, chemical composition of a sample, type and polarities of solvents [39]. In this connection, it was stated [61] that methanol produced the highest yield when extracting from *Gracilaria* species and is regarded as one of the best solvents. In addi-

Table 1

Yield and qualitative analysis of phytochemical components in the tested algal extracts

Species	Extracts	Yieldmg/ g DW	Steroids	Terpenes	Saponins	Quinones	Couma-rins	Tannins	Phenols	Flavono-ids	Cardiac glycosides
Colpomenia sinuosa	M	18.41	**	**	*	**	**	**	**	**	nd
	H	13.12	*	*	nd	nd	nd	*	*	*	nd
	EA	16.98	*	**	*	*	*	**	**	**	nd
Ulva compressa	M	16.24	**	**	*	*	**	**	**	**	nd
	H	14.2	*	nd	nd	nd	nd	*	*	*	nd
	EA	15,81	*	*	*	nd	nd	**	**	**	nd
Pterocladia capillacea	M	18.43	**	**	**	*	**	**	**	**	nd
	H	14.5	*	*	nd	nd	nd	*	*	*	nd
	EA	15.87	**	*	*	nd	*	**	**	**	nd

Note. M — methanol; H — hexane; EA — ethyl acetate; nd — not detected; ** — high; * — moderate based on the intensity of the color produced from the reactions.

tion, it was reported [73] that methanol was shown to be the most efficient extraction solvent, producing the highest extraction yield (16.24—18.43 %).

Qualitative analysis of preliminary phytochemical composition in the tested algal extracts. The nine crude extracts from three macroalgal species were screened for the presence of nine secondary biocompounds: steroids, saponins, terpenes, quinones, coumarines, tannins, cardiac glycosides, phenolics, and flavonoids as shown in Table 1. The pharmacological action of the plant is demonstrated by the presence of these metabolites [23]. Most of the extracts tested contained beneficial secondary chemicals, including phenols, terpenes, flavonoids, and tannins. However, terpenes, coumarins, quinones, and saponins were frequently detected in methanol and ethyl acetate extracts. Cardiac glycosides were not found in any of the extracts. In this context, it is stated [72] that ethyl acetate is the most effective solvent for extracting phenolic compounds from plants. Also, it is suggested [67] that methanol was more effective for extracting the bioactive phytochemicals than the other solvents. The presence or absence of phytoconstituents in seaweeds depends on the solvent medium used for extraction and the physiological properties of the seaweeds [67]. The solvent employed in the extraction process was the main factor influencing the existence of any phytoconstituents [53]. Additionally, the extraction of phenolic compounds from plants or fruits is significantly affected by the polarity of the solvent, which can suppress polyphenol oxidase activity [43].

Quantitative analysis of phenol, flavonoid and tannins. Polyphenols, including total phenolics, flavonoids, and tannin content, were detected in the selected extracts. In the present study, a significant difference in the phenolic compounds in the algal extracts was observed at $p<0.05$ (Table 2). Ethyl acetate (a semipolar solvent) showed the highest extraction efficiency for TPC and TFC from all the tested species. However, low levels of both components were obtained in the hexane extracts, which is expected since most phenolic compounds are polar in nature; semipolar and polar organic solvents are commonly used for their extraction [56]. The lowest TPC in the hexane extract (non-polar) of *Turbinaria* spp. compared to all methanol and ethyl acetate extracts was also reported [9]. It was detected [2] that most phenolic compounds from different algal species are more extracted in ethyl acetate solvent than methanol and hexane solvents. All of the tested *Colpomenia sinuosa* extracts exhibited a higher concentration of phenolic compounds than the other two species. Numerous studies demonstrated the highest phenolic content in brown seaweeds [28, 55].

Methanol (a polar solvent) was found to be the most effective solvent for tannins, with values ranging from 9.09 mg/g to 19.36 mg/g for *P. capillacea* and *C. sinuosa*, respectively. This could be attributed to the higher solubility of tannins in methanol compared to the other solvents tested. Within the ethyl acetate extracts, tannin content ranges between 9.09 and 14.63 mg TAE/g in *Ulva compressa* and *P. capillacea*, respectively (Table 2). The lowest value was observed in the *P. capillacea* hexane extract (7.27 mg TAE/g) ($p<0.05$). The variance in extraction efficiency could be linked to the algae species, the extraction solvent used, and environmental stressors.

Table 2

One-Way ANOVA regarding phenolic compounds content in the tested algal extracts

Species	Solvent	Phenolic compounds (mg/g)		
		flavonoids	phenols	tannins
Colpomenia sinuosa	M	32.52a	33.027b	19.36a
	H	15.31d	34.603b	9.09c
	EA	38.68b	49.997a	12.15b
Ulva compressa	M	28.57b	23.18c	17.04a
	H	19.97c	20.91c	10.91c
	EA	32.58a	25.45c	14.63b
Pterocladia capillacea	M	24.41c	23.18c	18.09a
	H	12.67d	20.91c	7.27c
	EA	37.01a	36.81b	9.09c

Note. M — methanol; H — hexane; EA — ethyl acetate; letters are significantly different at $p < 0.05$. Average values marked with the same letter are non-significantly different.

HPLC profile based on polyphenolic compounds. HPLC is an appropriate procedure to detect polyphenols, which can be used to assay the potential phenolic constituents. In this study, we report here the rapid determination of 16 polyphenols in crude extracts derived from three macroalgal species (Fig. 2, Table 3), to compare the ability of each solvent to dissolve a selected group of polyphenols. The 16 phenolics, namely gallic acid (RT: 3.297 min), chlorogenic acid (RT 3.936 min), catechin (RT 4.358 min), methyl gallate (RT 5.554 min), caffeic acid (RT 5.883 min), syringic acid (RT 6.386 min), pyro catechol (RT 7.088 min), rutin (RT 7.343 min), ellagic acid (RT 7.978 min), coumaric acid (RT 9.004 min), vanillin (RT 9.835 min), ferulic acid (RT 10.044 min), naringenin (RT 10.223 min), taxifolin (RT 12.440 min), cinnamic acid (RT 14.298 min) and kaempferol (RT 14.716 min) were detected. While the compounds were well resolved, not all of them were presented in the algal extracts, which may depend on the solvents used and the algal species (Table 3). The presence of polyphenols in the samples was verified by comparing the retention periods and overlaying UV spectra of the extracts with those of reference substances. In total, sixteen polyphenols were targeted for quantification by HPLC, including twelve phenolic acids (gallic acid, chlorogenic acid, catechin, caftaric acid, chlorogenic acid, caffeic acid, syringic acid, rutin, ellagic acid, vanillin, ferulic acid, naringenin, cinnamic acid, and coumaric acid) and four flavonoids (kaempferol, methyl gallate, pyro catechol, and taxifolin). The variance of individual phenolic compounds is influenced by the extraction solvents utilized in this study. This can be clarified by identifying flavonoids (pyro catechol and methyl gallate) in the ethyl acetate extract of *C. sinuosa* only, rutin in the hexane extract of both *C. sinuosa* and *P. capillacea* only, and ferulic acid in the ethyl acetate extract of *P. capillacea* only. Gallic acid was the predominant

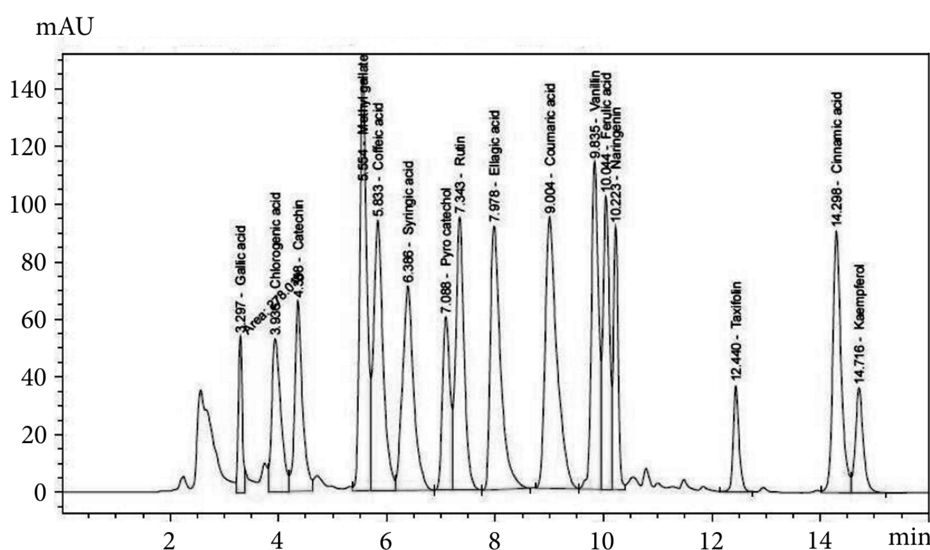


Fig. 2. HPLC chromatograms of polyphenol compounds types, retention times and concentrations in standard polyphenols

polyphenol. The HPLC results showed that the ethyl acetate extract from *C. sinuosa* had the highest extraction capacity for polyphenolic content, especially taxifolin (7.53 μg/mL), gallic acid (7.22 μg/mL), coumaric acid (6.35 μg/mL), and syringic acid (5.95 μg/mL) compared to other algal extracts. In addition, the highest values of flavonoids, such as taxifolin and kaempferol were detected in the ethyl acetate and methanol extracts of both *C. sinuosa* and *P. capillacea* in the range of 0.63—7.53 μg/mL and 0.45—2.03 μg/mL, respectively. This study showed that most of the tested algal extracts could be considered as an important source of natural agents for use in the pharmaceutical and food industries.

Pharmacological aspects. Anti-inflammatory activity. Seaweeds have an anti-inflammatory impact associated with their bioactive contents, like tannins, phenols, and flavonoids, highlighting their potential for novel therapeutic applications. Polyphenols impact numerous inflammatory cellular pathways, including the Nuclear factor kappa B (NF-κB) cellular pathway, which controls gut microbiota makeup and immunological responses. Polyphenol compounds exhibited anti-inflammatory properties by suppressing AP-1 transcription and NF-kB [10]. Additionally, polyphenols can reduce inflammation by regulating inflammatory cell activity, modifying the actions of enzymes action involved in arachidonic acid metabolism (phospholipase A2, cyclooxygenase (COX), lipoxygenase (LOX), arginine metabolism (NOS)), and modulating the release of other molecules that facilitate inflammation [36].

Figure 3 depicts that, depending on the algal species and their bioactive content in different extracts, all selected seaweed extracts exhibited varying inhibitory efficacy on lipoxygenase, as previously documented in [73]. They stated that extraction solvents affect extraction yield and the bioactive compound

Table 3

Concentration of polyphenolic compounds (μg/ml) **in the tested algal extracts using HPLC chromatograms**

Polyphenolic compounds	*Colpomenia sinuosa*			*Ulva compressa*			*Pterocladia capillacea*		
	M	H	EA	M	H	EA	M	H	EA
Gallic acid	1.20	0.09	7.22	0.56	0.00	2.02	1.60	0.00	5.54
Chlorogenic acid	0.21	0.00	4.91	0.45	0.30	0.87	0.77	0.00	1.21
Catechin	3.21	1.03	4.21	0.00	0.90	0.00	0.00	0.00	0.00
Methyl gallate	0.00	0.00	0.29	0.00	0.00	0.00	0.00	0.00	0.00
Caffeic acid	0.84	0.16	1.37	0.00	0.00	0.00	0.00	0.00	0.01
Syringic acid	0.10	0.12	5.95	0.00	0.00	2.15	0.05	0.00	3.56
Pyro catechol	0.00	0.00	1.43	0.00	0.00	0.00	0.00	0.00	0.00
Rutin	0.00	0.15	0.00	0.00	0.00	0.00	0.00	4.56	0.00
Ellagic acid	0.82	0.09	1.07	0.00	0.00	0.00	0.12	0.00	0.87
Coumaric acid	4.00	0.04	6.35	0.00	0.28	0.31	0.00	0.00	0.08
Vanillin	0.24	0.17	0.37	0.00	0.00	0.29	0.15	0.00	3.21
Ferulic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32
Naringenin	0.75	0.00	2.77	0.41	0.00	0.00	0.21	0.00	1.12
Taxifolin	2.98	0.00	7.53	0.00	0.00	0.63	3.17	0.00	4.21
Cinnamic acid	8.07	14.64	11.30	0.19	0.00	0.25	3.54	0.33	6.54
Kaempferol	1.55	0.00	2.03	0.00	0.64	0.00	0.45	0.00	0.55

Note. M — methanol; H — hexane; EA — ethyl acetate.

content, significantly impacting the biological activity of extracts. Methanol extracts exhibited the maximum inhibition of lipoxygenase as detected by [13]; the methanolic extract of *Matricaria chamomilla* demonstrated effective suppression of lipoxygenase. Additionally, the crude methanolic extracts of both *Coriaria intermedia* stem and *Dracontomelon dao* bark exhibited high levels of activity against α-15-lipoxygenase compared to hexane and ethyl acetate extracts. The anti-lipoxygenase activity of *C. sinuosa* methanolic extract was the highest at 65.24 %, compared to 55.17 % for quercetin. In comparison with [66] (35.02-42.5 %), the inhibition range for nine extracts from three distinct species in our investigation is significantly higher (41.98—65.24 %).

Anti-arthritic activity. Denaturation of proteins and the synthesis of autoantigens are the main causes of inflammation and rheumatoid arthritis [4]. The pathophysiological elements of rheumatoid arthritis can be influenced by polyphenolic substances, which can delay the development and alleviate symptoms [11]. The highest anti-denaturation activity was obtained for the methanol extracts of *C. sinuosa* (73.87 %), followed by *P. capillacea* at 68.13 % and the

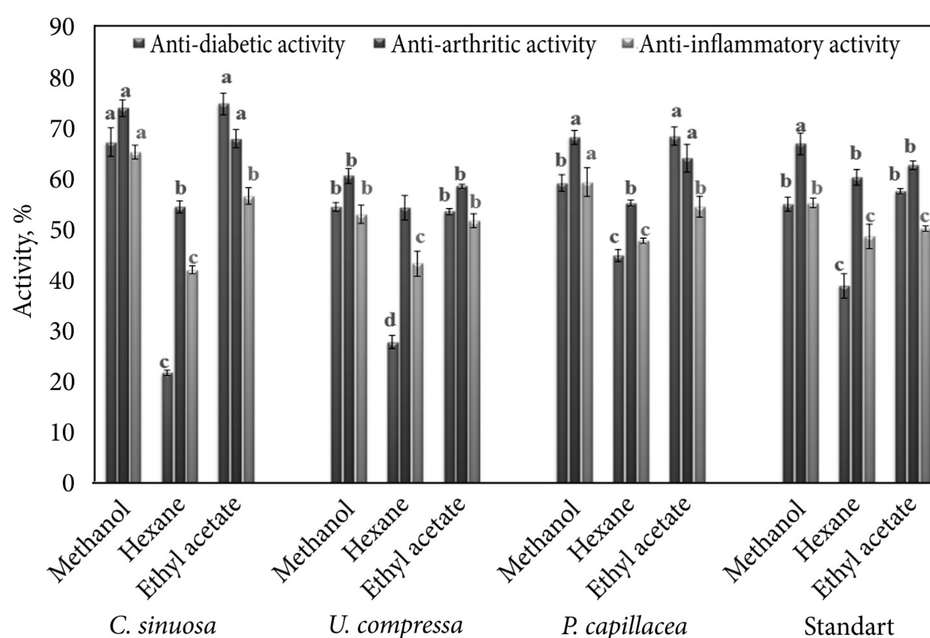


Fig. 3. Bioactivities of the tested algal extracts applying three solvents

ethyl acetate extract of *C. sinuosa* at 67.82 % compared to diclofenac sodium «standard drug» at 66.85 % (Fig. 3). These extracts demonstrated their capacity to regulate the synthesis of autoantigens, inhibiting the denaturation of proteins. Our results were compatible with those reported in [66], which reported a range of anti-arthrtic activity for different algal solvents (62.5 to 75 %) and were higher than those reported in [70], with the maximum anti-arthritic activity of 49.33±0.60 %. The anti-arthritic properties of these extracts may be attributed to their concentration of flavonoids, which have the ability to regulate the transcription factor NF-kB in the production of TNF-a. This regulating pathway helps prevent the release of the proinflammatory cytokine TNF-a. Additionally, inhibiting lipoxygenase and the COX pathway limits leukocyte accumulation and reduces the production of proinflammatory cytokines. Flavonoids preferentially inhibit the formation of the proximal signal complex IL1R, with the exception of TNFR-1. They also inhibit TACE, thereby preventing the synthesis of TNF-a, a proinflammatory cytokine, by binding to proteins with negative bond energy. This reduction in TNF-a and IL-6 expression can help decrease inflammation in rheumatoid arthritis [20].

Antidiabetic activity. Figure 3 shows the inhibitory activity of the tested extracts against α-amylase *in vitro*. The highest antidiabetic ability was recorded for the ethyl acetate extract of both *C. sinuosa* (74.73 %; IC50 6.69 mg/mL) and *P. capillacea* (68.36 %; IC50 7.31 mg/mL), followed by the methanolic extracts of the same species. The antidiabetic action of the macroalgal extracts may be attributed to the presence of various bio-compounds, such as polyphenols.

These compounds bind to the active sites of the enzymes involved in diabetes, modifying their catalytic activity. The antidiabetic effect of these extracts was attributed to their unique content of taxifolin and kaempferol, as shown in Table 3. Both compounds displayed antidiabetic, hepatoprotective, neuroprotective, and regenerative actions in cells [71]. Taxifolin has been shown to be effective as an antidiabetic drug, demonstrating a reduction in glycated hemoglobin levels [33]. Kaempferol functions similarly to insulin secretagogues by stimulating the release of insulin. It also decreases blood glucose levels and increases plasma insulin levels in rats with diabetes caused by streptozotocin [3]. Moreover, it has been suggested that polyphenols from edible seaweeds may impact reactions related to diabetes by inhibiting enzymes at μM concentrations that break down starch and by modifying the oxidative stress induced by glucose [46]. The results presented in Table 6 demonstrate a significant correlation between flavonoids, antioxidant activity and antidiabetic activity at $p<0.05$. This could be because flavonoids are highly dynamic natural phenolic molecules with a wide range of chemical and biological activities [60]. This result aligns with previous research [16, 27]. Plus, the main defense against the consequences of diabetes is provided by flavonoids. Their ability to act as antioxidants is essential for maintaining endothelial function in individuals with diabetes [8]. The inhibitory effects of nine extracts in our study were lower than those reported in [27], which observed exceptionally high values of 93.68 %, 84.96 %, and 77.99 %.

Antioxidant potency. The present investigation employed three distinct assays, namely TAC, FRAP and DPPH assays, to evaluate the antioxidant properties of seaweed extracts. These three techniques demonstrated various antioxidant activity pathways. It has recently been discovered that seaweeds are an interesting source of naturally occurring bioactive chemicals that may have antioxidant properties [25, 26, 28, 67]. According to reports, phenolic compounds possess antioxidant properties depending on their ability to scavenge free radicals, which mitigates the harmful effects of advanced glycation end products (AGEs). AGEs are the toxic consequences of hyperglycemia in diabetes and cause damage to a variety of tissues and organs in patients [19]. As illustrated in Table 6, there is a significant correlation between TFC content and DPPH (0.842), FRAP (0.759). Flavonoids react with the radical's reactive component to stabilize the reactive oxygen species [45]. Ferric-reducing antioxidant power (FRAP) plays an important role in flavonoid structure to resist the effect of energetic oxidants like free radicals. Flavonoids can reduce Fe^{3+} to Fe^{2+} at pH 5.5, which can be explained by their ability to donate protons and electrons as illustrated in Figure 4 [6]. The structural characteristics play a significant role in assessing flavonoids' capacity as antioxidants [24].

The primary mechanisms involved in the initial stage of flavonoid and phenolic compounds oxidation are single electron transfer-proton transfer (SET-PT) (Eq. 5), sequential proton loss electron transfer (SPLET) (Eq. 6), and hydrogen atom transfer (HAT) (Eq. 7) [42].

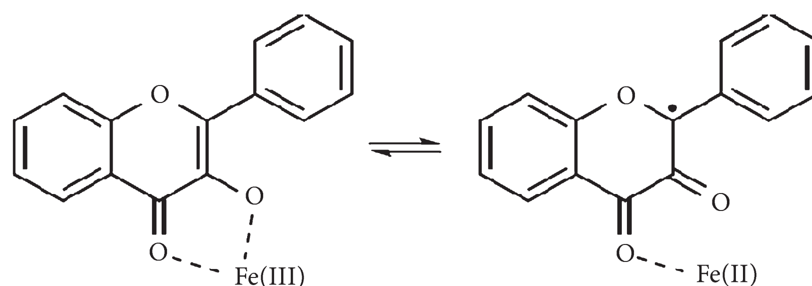
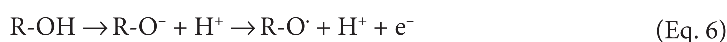


Fig. 4. Proposed route for generating the ferrous complex from the Fe(III) flavonoid complex [6]

$$\text{R-OH} \rightarrow \text{R-OH}^{+} + e^{-} \rightarrow \text{R-O}^{\cdot} + H^{+} + e^{-} \quad \text{(Eq. 5)}$$



$$\text{R-OH} \rightarrow \text{R-O}^{-} + H^{+} \rightarrow \text{R-O}^{\cdot} + H^{+} + e^{-} \quad \text{(Eq. 6)}$$

$$\text{R-OH} \rightarrow \text{R-O}^{\cdot} + \text{H} \quad \text{(Eq. 7)}$$

The O-H bond dissociation enthalpy (BDE) controls this process. The most significant aspect of BDE is its contribution to antioxidant activity; the lower the BDE, the easier it is to break O-H bonds. The phenolic hydroxyl group with the lowest BDE determines the compound's free radical scavenging capability when it has several phenolic hydroxyl groups [42].

The second pathway involves the transfer of electrons from the antioxidant to the free radical, resulting in the formation of a radical cation. The ionization potential (IP) and the reactivity of the ROH^{+} cation control this reaction. A high IP reduces electron transfer rate, while a low IP facilitates electron abstraction. An extract's or compound's diminished power is associated with its capacity to transfer electrons or to donate hydrogen atoms, thereby disrupting the free radical chain [61].

Significant variations in antioxidant activity are demonstrated based on the type of algal species, extraction method, and solvent employed as shown in Table 4. The variations in the antioxidant activities among the different algal extracts suggest that the choice of extracting could impact the scavenging ability of these extracts. This could be due to differences in the physiochemical properties of the soluble phenolic compounds in each solvent extract, as noted in [12]. Overall, *P. capillacea, U. compressa,* and *C. sinuosa* extracts using ethyl acetate demonstrated the highest DPPH scavenging abilities at 63.23 %, 65.33 %, and 69.89 %, respectively. These results for brown algae are consistent with those obtained previously [27]. The highest TAC was observed in ethyl acetate extracts of *C. sinuosa* (3.49 mg AAE/g extract), and *U. compressa* (3.44 mg AAE/g extract), while the lowest activity was detected in the hexane extract of *U. compressa* (0.469). According to [18], the maximum TAC for the green alga *Enteromorpha prolifera* is 1.837 mg AAE/g.

Ferric-reducing power is a crucial indicator of the antioxidant potential of a compound [25, 26]. The methanol (38.69 mg AAE/g) and ethyl acetate

Table 4

Antioxidant activity of the tested algal extracts using three techniques

Species	Solvent	DPPH %	TAC (mg AAE/g DW)	FRAP (mg AAE/g DW)
Colpomenia sinuosa	M	65.208a	3.069a	38.69a
	H	8.944c	1.84a	4.91c
	EA	69.888a	3.491a	38.4a
Ulva compressa	M	55.848b	2.469a	30.69b
	H	8.736c	0.577b	4.8c
	EA	65.325a	3.44a	34.4a
Pterocladia capillacea	M	52.104b	3.025a	35.77a
	H	8.528c	1.396b	4.69c
	EA	63.232 a	3.12a	31.46b
Ascorbic acid	M	55.52b	3.14a	31.2b
	H	4.25c	0.83b	2.1c
	EA	65.12 a	3.16a	30.25b

Note. M — methanol; H — hexane; EA — ethyl acetate; letters are significantly different at $p<0.05$. Average values marked with the same letter are non-significantly different.

(38.4 mg AAE/g) extracts of *C. sinuosa* followed by the methanolic extract of *P. capillacea* (35.77 mg AAE/g), exhibited the maximum FRAP activity (Table 4). These findings are consistent with the data obtained by [25, 26] showing that the reducing power activity of different seaweed methanolic extracts ranged from 3.06 mg AAE/g for *U. lactuca* to 31.10 mg AAE/g DW for *Sargassum wightii*. This wide variation in reducing power activity is influenced by the polarity of the extraction solvent and the total phenolic content. Plus, the higher polyphenol content also tends to have higher antioxidant activity [7], as demonstrated by correlation analysis (Table 6). The tested extracts showed a significant correlation between total flavonoids content and DPPH (0.842) and FRAP (0.759) (Table 6).

Anticoagulant activity. Heparin is a commercial anticoagulant that has been known and used for over 50 years. It is frequently used to prevent venous thromboembolic diseases. However, heparin has been documented to have a number of side effects, including the emergence of avian influenza, bovine spongiform encephalopathy, thrombocytopenia, hemorrhagic impact, and the inability to inhibit thrombin bound to fibrin [41]. Consequently, the search for novel anticoagulants to replace heparin is becoming more intense. Previous research [49] has demonstrated the anticoagulant properties of methanolic extracts of the red alga *Acanthaphora spicifera*, whether they are naturally occurring or chemically engineered.

Prothrombin time (PT), thrombin time (TT) and activated partial thromboplastin time (APTT) analysis are frequently used tests to evaluate blood coagulation. APTT quantifies the impact of substances on intrinsic and common coagulation pathways. Based on the standard range of APTT (28—38 s), which can vary depending on the reagents used, a ratio (APTT measured/APTT control) greater than 1.2 results in a significant anticoagulant effect. Therefore, methanol and ethyl acetate extracts are suitable solvents for extracting anticoagulant compounds from both *P. capillacea and C. sinuosa*, affecting the intrinsic system of the coagulation cascade. Conversely, all hexane extracts show no anticoagulant ability (Table 5).

The PT and TT tests showed the same APTT pattern (Table 5), indicating that these extracts act as reactive anticoagulant agents [31]. In this connection, it has been reported [40] that the APTT and PT effects of the brown alga *Sargassum cristaefolium* extract are due to its phenolic contents especially flavonoid. The findings of our study demonstrated that the solvent types and algal species had an anticoagulant effect, resulting in an extension of both PT and APTT. According to [36], prolonged PT indicates inhibition of the extrinsic pathway of coagulation, while a prolonged APTT indicates inhibition of the intrinsic and/or common pathway.

Conclusion

There is a growing emphasis on using bioactive chemicals from natural sources as functional foods to enhance human health and well-being. Results

Table 5

Anticoagulant efficiency of the tested algal extracts

Species	Solvents	APTT (s)	%	PT(s)	TT
Colpomenia sinuosa	M	50	1.471	23	33
	H	32	0.941	17	24
	EA	44	1.294	19	27
Ulva compressa	M	39	1.147	16	23
	H	nd	nd	nd	nd
	EA	33	0.971	14	20
Pterocladia capillacea	M	44	1.294	19	27
	H	nd	nd	nd	nd
	EA	36	1.059	16	23
Standard (heparin)	M	45	1.324	18	24
	H	30	0.882	nd	nd
	EA	34	1.000	13	19

Note. M — methanol; H — hexane; EA — ethyl acetate; nd — not detected; APTT — activated partial thromboplastin time; PT — prothrombin time; TT — thrombin time. Ratio was calculated by the formula: Ratio = APTT measured / APTT control «34 second».

Table 6

Correlation matrix of estimated parameters

Estimated parameters	DPPH %	FRAP	TAC	Flavonoids	Phenols	Tannins	Anti-arthritic	Anti-inflammatory	Anti-diabetic	APTT (s)	PT(s)	TT
DPPH %	1											
F R P	0.981	1										
TAC	-0.255	-0.236	1									
Flavonoid	0.842	0.759	-0.123	1								
Phenol	0.456	0.399	-0.211	0.391	1							
Tannins	0.565	0.562	-0.161	0.298	0.058	1						
Anti-arthritic	0.774	0.844	-0.156	0.414	0.455	0.478	1					
Anti-inflammatory	0.812	0.878	-0.207	0.438	0.285	0.536	0.965	1				
Anti-diabetic	0.893	0.887	-0.443	0.688	0.487	0.353	0.830	0.867	1			
APTT (s)	0.496	0.662	-0.324	-0.095	0.164	0.456	0.949	0.902	0.645	1		
PT (s)	0.132	0.301	-0.038	-0.432	0.276	0.325	0.824	0.696	0.334	0.892	1	
TT	0.158	0.323	-0.046	-0.407	0.266	0.350	0.837	0.716	0.356	0.901	0.999	1

Note. $p<0.05$, confidence limit 95%

show that selected seaweeds are an excellent source of phenolic contents with various biological activities. The findings indicate that seaweeds, particularly brown, followed by red and then green species, have antioxidant and antidiabetic properties. This makes them a good source of natural antioxidants. Hence, they may be employed as functional food and nutraceutical ingredients in the food industry due to their high flavonoid content. The extraction solvent significantly influenced the overall polyphenol content and their bioactivities. Methanol and ethyl acetate have high priorities as extracting solvents for phenolic compounds with various biological activities. Furthermore, HPLC investigation indicates the existence of several phenolic compounds that could account for the bioactivity in this species. Therefore, it is advised to conduct additional research to identify and refine the phenolic compositions accountable for the various actions and detect their cytotoxicity.

Literature Cited

1. Aina, O. 2022. Phenolic compounds in growth promotion and stress alleviation in plants. *Life* **12**: 1548. https://doi.org/ 10.3390/life12101548

2. Akbar, A., N.H. Soekamto, Firdaus & Bahrun. 2021. Antioxidant of n-hexane, ethyl acetate and methanol extracts of *Padina* sp. with DPPH method. *A IOP Conf. Series: Earth and Environ. Sci*. **800**: 012019

3. Al-Numair, K.S., G. Chandramohan, C. Veeramani, & M.A. Alsaif. 2015. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. *Redox Rep*. **20**(5): 198—209. doi:10.1179/135 1000214Y.0000000117

4. Alamanos, Y., P.V. Voulgari, & A.A. Drosos. 2006. Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology criteria: A systematic review. *Seminars in Arthritis and Rheumatism* **36**(3): 182—188. https://doi.org/ 10.1016/j.semarthrit.2006.08.006.

5. Aleem, A.A. 1993. *The marine algae of Alexandria, Egypt.* Faculty of Science, University of Alexandria, Egypt. Oxford, Blackwell Publ.

6. Amić, D., D. Bešlo, V. Rastija, et al. 200. 7SAR and QSAR of the Antioxidant Activity of Flavonoids. *Curr. Med. Chemistry* **14**: 827—845.

7. Bambang, B.S., S. Kumalaningsih, W. Susinggih, & Hardoko. 2013. Polyphenol Content and Antioxidant Activities of Crude Extract from Brown Algae by Various Solvents. *J. Life Sci. Biomedicine* **3**(6): 439—443.

8. Bouyahya, A., Y. Bakri, E. Khay, et al. 2024. Antibacterial, antioxidant and antitumor properties of Moroccan medicinal plants: A review. *Asian Pacif. J. Trop Dis*. **7**(1): 57—64.

9. Chakraborty, K., K. Praveen, K. Kizekadath, et al. 2013. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to *Turbinaria* spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. *Asian Pacif. J. Trop. Biomed*. **3**(1): 8—16

10. Chao, P.Y., Y.P. Huang, & W.B. Hsieh. 2013. Inhibitive effect of purple sweet potato leaf extract and its components on cell adhesion and inflammatory response in human aortic endothelial cells. *Cell Adhesion & Migration* **7**(2): 237—245. https://doi.org/10.4161/ cam.23649

11. Christman, L.M., & L. Gu. 2020. Efficacy and mechanisms of dietary polyphenols in mitigating rheumatoid arthritis. *J. Function. Foods* **71**: 104003, https://doi.org/10.1016/ j.jff.2020.104003

12. Chun, S.S., D.A. Vattem, Y.T. Lin, & K. Shetty. 2005. Phenolic antioxidants from clonal oregano (*Origanum vulgare*) with antimicrobial activity against *Helicobacter pylori*. *Proc. Biochemistry* **40**(2): 809—816.

13. Danciu, C., I. Zupko, A. Bor, et al. 2018. Botanical Therapeutics: Phytochemical Screening and Biological Assessment of Chamomile, Parsley and Celery Extracts against A375 Human Melanoma and Dendritic Cells. *Int. J. Molecular Sci.* **19**: 3624.

14. Datta, S., S. Bhattacharjee, & T. Seal. 2022. Anti-diabetic, anti-inflammatory and anti-oxidant properties of four underutilized ethnomedicinal plants of West Bengal, India: An in vitro approach. *South Afr. J. Botany* **149**: 768—780. https://doi.org/ 10.1016/ j.sajb.2022.06.029

15. Dong, H.Q., M. Li, F. Zhu, et al. 2012. Inhibitory potential of trilobatin from *Lithocarpus polystachyus* Rehd against α-glucosidase and α-amylase linked to type 2 diabetes. *Food Chemistry* **130**(2): 261—266. https://doi.org/10.1016 /j.foodchem.2011.07.030

16. Dou, B., Y. Zhu, M. Sun, et al. 2024. Mechanisms of flavonoids and their derivatives in endothelial dysfunction induced by oxidative stress in diabetes. *Molecules* **29**: 3265. https://doi.org/10.3390/molecules29143265

17. El-Said, Gh. F, G.M. El Zokm, A. El-Sikaily, & M.M. Ismail. 2024. Comparison of crude nano-hydroxyapatite extracted from calcified seaweed in terms of composition, antibacterial activity, and cytotoxicity. *Environ. Nanotechnol., Monit. Manag.* **21**: 100908. https://doi.org/10.1016/j.enmm.2023.100908.

18. El Zokm, G.M., M.M. Ismail, & G.F. El-Said. 2021. Halogen content relative to the chemical and biochemical composition of fifteen marine macro and micro algae: nutritional value, energy supply, antioxidant potency, and health risk assessment. *Environ. Sci. Pollution Res. Int.* **28**: 14893—14908. https://doi. org/10.1007/s11356-020-11596-0

19. Farias de Paulo D., F.F. de Araújo, I.A.N. Numa, & G.M. Pastore. 2021. Antidiabetic potential of dietary polyphenols: a mechanistic review. *Food Res. Int.* **145**: 110383

20. Gazali, M., A.N. Fatimah, A.Husni, et al. 2024. Antioxidant and anti-arthritic activities of green seaweed *Halimeda tuna* methanolic extract. *Squalen Bull. Marine and Fisheries Postharvest & Biotechnology* **19**(1): 45—54.

21. Guiry M.D., Guiry G.M. 2023. *AlgaeBase. World-Wide Electronic Publication*. Retrieved August 22, 2021, https://www.google.com /search?q=Guiry+and+Guiry+2021&oq=Guiry+and+Guiry+2021&aqs=chrome.69i57.503j0j4&sourceid=chrome&ie=UTF-8

22. Gunathilake, T., T.O. Akanbi, H.A.R. Suleria, et al. 2022. Seaweed phenolics as natural antioxidants, aquafeed additives, veterinary treatments and cross-linkers for microencapsulation. *Mar. Drugs* **20**: 445. https: //doi.org/10.3390/md20070445

23. Hamid, A.A., M.A. Aliyu, L.Z. Abubakar, et al. 2017. *Thaumatococcus daniellii* leaves: its chemical compositions, antioxidant and antimicrobial activities. *Ife J. Sci.* **19**(2): 409. doi: 10.4314/ijs.v19i2.21

24. Hassanpour, S.H., & A. Doroudi. 2023. Review of the antioxidant potential of flavonoids as a subgroup of polyphenols and partial substitute for synthetic antioxidants. *Avicenna J. Phytomedicine* **13**(4): 354—376. https://doi.org/10.22038/ AJP.2023.21774.

25. Ismail, M.M., G.M. El Zokm, & A.A.M. El-Sayed. 2017. Variation in biochemical constituents and master elements in common seaweeds from Alexandria coast, Egypt with special reference to their antioxidant activity and potential food uses: prospective equations. *Env. Monit. Assessment* **189**(12): 648.

26. Ismail, G.A., & M.M. Ismail. 2017. Variation in oxidative stress indices of two green seaweeds growing under different heavy metal stresses. *Env. Monit. Assessment* **189**(2): 68.

27. Ismail, M.M., M.H. Mostafa, & A.M. El Sikaily. 2022. Phytochemical variation, antioxidant and antidiabetic capacity of extracts of common brown seaweeds from the Red Sea, Egypt. *Egyptian J. Aquatic Biology and Fisheries* **26**(5): 445—462.

28. Ismail, M.M, G.M. El Zokm, A.M. El Sikaily, et al. 2023. Chemodiversity and bioactivity assessment of phlorotannins from some Phaeophyta species from the Red Sea. *J. Appl. Phycolog.* **35**: 1769—1788. https://doi. org/10.1007/s10811-023-03000-4.

29. Ismail, M.M., G.M. El Zokm, & J.M. Miranda Lopez. 2023. Nutritional, bioactive compounds content, and antioxidant activity of brown seaweeds from the Red Sea. *Frontiers in Nutrition* **10**: 1210934. doi:10.3389/fnut. 1210934

30. Jimenez-Lopez, C., A.G. Pereira, C. Lourenço-Lopes, et al. 2021. Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits. *Food Chemistry* **341**: 128262.

31. Karaki, N., C. Sebaaly, N. Chahine, et al. 2013. The antioxidant and anticoagulant activities of polysaccharides isolated from the brown algae *Dictyopteris polypodioides* growing on the Lebanese coast. *J. Appl. Pharmaceutical Sci.* **3**: 043—051.

32. Kim, D.-O., S.W. Jeong, & C.Y. Lee. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chemistry* **81**(3): 321—326. https://doi.org/ 10. 1016/ S0308- 8146(02) 00423-5

33. Kondo, S., S. Adachi, F. Yoshizawa, & K. Yagasaki. 2021. Antidiabetic effect of taxifolin in cultured L6 Myotubes and Type 2 diabetic model KK-Ay/Ta mice with hyperglycemia and hyperuricemia. *Curr. Iss. Molecular Biology* **43**: 1293—1306.

34. Kumar, Y., A. Tarafdar, & P.C. Badgujar. 2021. Macroalgae as a source of natural antioxidants: Therapeutic activity and food applications. *J. Food Quality* 5753391.

35. Kumar, Y., A. Tarafdar, D. Kumar, et al. 2022. Polyphenols of edible macroalgae: estimation of *in vitro* bio-accessibility and cytotoxicity, quantification by LC-MS/MS and potential utilization as an antimicrobial and functional food ingredient. *Antioxidants* **11**: 993. https://doi.org/10.3390/ antiox11050993

36. Liu, W., X. Cui, Y. Zhong, et al. 2023. Phenolic metabolites as therapeutic in inflammation and neoplasms: molecular pathways explaining their efficacy. *Pharmacology Res.* **193**: 106812. https://doi.org/10.1016/j. phrs.2023.106812.

37. Liu, X., S. Wang, S. Cao, et al. 2018. Structural characteristics and anticoagulant property *in vitro* and *in vivo* of a seaweed sulfated rhamnan. *Marine Drugs* **16**: 243-256.

38. Liyana-Pathirana, C.M. & F. Shahidi. 2005. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J. Agricult. Food Chemistry* **53**(7): 2433—2440. https://doi.org/ 10.1021/JF049320I

39. López, A., M. Rico, A. Rivero, & M.S. de Tangil. 2011. The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. *Food Chemistry* **125**: 1104—1109

40. Manggau, M., M. Hamzah, S. Mamada, et al. 2019. Anticoagulant activities of brown seaweed *Sargassum cristaefolium* extract. *J. Physics: Conference Ser.* **1341**: 072006. doi:10.1088/1742-6596/1341/7/072006.

41. Mendes, S.F., O. dos Santos, Jr, A.M. Barbosa, et al. 2009. Sulfonation and anticoagulant activity of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 grown on fructose. *Int. J. Biol. Macromolecules* **45**: 305—309.

42. Miličević, A. 2024. Flavonoid oxidation potentials and antioxidant activities –theoretical models based on oxidation mechanisms and related changes in electronic structure. *Int. J. Molecular Sci.* **25**: 5011. https://doi.org/10.3390/ ijms2509501

43. Naczk, M., & F. Shahidi. 2006. Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *J. Pharmaceutical and Biomedical Analysis* **41**: 1523—1542. https://doi. org/ 10.1016/j. jpba.2006.04.002.

44. Namvar, F., S. Mohamed, F.S. Ghasemi, et al. 2012. Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction. *Food Chemistry* **130**(2): 376—382. DOI:10.1016/j.foodchem.2011.07.054

45. Nijveldt, R.J., E. van Nood, D.E. van Hoorn, et al. 2001. Flavonoids: a review of probable mechanisms of action and potential applications. *Am. J. Clin. Nutrition* **74**(4): 418—25. doi:10.1093/ ajcn/74.4.418.

46. Nwosu, F., J. Morris, V.A. Lund, et al. 2011. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chemistry* **126**: 1006—1012

47. Oyaizu, M. 1986. Studies on products of browning reactions: antioxidative activities of product of browning reaction prepared from glucosamine. *Jap. J. Nutrition* **44**(6): 307—315.

48. Palma-Wong, M., J.A. Ascacio-Valdés, N. Ramírez-Guzmán, et al. 2023. Exploration of phenolic content and antioxidant potential from plants used in traditional medicine in Viesca, Mexico. *Horticulture* **9**: 1252. https://doi.org/10.3390/ horticulturae9121252.

49. Pandian, P. 2016. *In vitro* anticoagulant activity of marine algae species *Acanthaphora spicifera* (Rhodophyceae). *Int. J. Pharm. Sci. Res*. **7**(11): 471—476.

50. Parikh, B., & V.H. Patel. 2018. Total phenolic content and total antioxidant capacity of common Indian pulses and split pulses. *J. Food Sci. Technol.* **55**(4): 1499—1507. doi: 10.1007/s13197-018-3066-5.

51. Petchidurai, G., N.J. Amruthraj, M.S. John, et al. 2019. Standardization of quantification of total tannins, condensed tannin and soluble phlorotannins extracted from thirty-two drifted coastal macroalgae using high performance liquid chromatography. *Bioresource Technology Rep*. **7**(1): 100273 https://doi.org/10.1016/j.biteb.2019.100273

52. Pinto, M.C., A. Tejeda, A.L. Duque, & P. Macias. 2007. Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation-xylenol orange assay. *J. Agricult. Food Chemistry* **55**(15): 5956—5959. https://doi.org/10.1021/jf070537x

53. Prabakaran, G., M. Moovendhan, A. Arumugam, & P. Sampathkumar. 2018. Quantitative analysis of phytochemical profile in marine microalgae *Chlorella vulgaris*. *Online IJPBS*. 8: 565. www.ijpbs.comorwww. ijpbsonline.com/

54. Prieto, P., M. Pineda, & M. Aguilar. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochemistry* **269**(2). https://doi.org/10.1006/abio.1999.4019

55. Priyanka, K.R., R. Rajaram, & S.R. Sivakumar. 2022. A critical review on pharmacological properties of marine macroalgae. *Biomass Conversion & Biorefinery*. doi: 10.1007/s13399-022-03134-4

56. Purbosari, N., E. Warsiki, K. Syamsu, & J. Santoso. 2020. Effect of harvest age and solvents on the phenolic content of *Eucheuma cottonii* extract. *Makara J. Sci*. **24**(3). DOI: 10.7454/mss.v24i3.1177

57. Raman, N. 2006. *Phytochemical techniques.* New Delhi. 19—22.

58. Rice-Evans, C.A., N.J. Miller, & G. Paganga. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med*. **20**(7): 933-56. doi: 10.1016/0891-5849(95)02227-9.

59. Rodríguez, P.F., L. Murillo-González, E. Rodríguez, & A.M. Pérez. 2023. Marine phenolic compounds: Sources, commercial value, and biological activities. Pp. 47—86 in: *Marine Phenolic Compounds: Science and Engineering*. J.R. Pérez-Correa, R. Mateos, H. Domínguez (Eds.). Amsterdam, Elsevier.

60. Sobuj, M.K.A., M.S. Shemul, I.S. Shoebul, et al. 2024. Qualitative and quantitative phytochemical analysis of brown seaweed *Sargassum polycystum* collected from Bangladesh with its antioxidant activity determination. *Food Chemistry Advances* **4**: 100565. https://doi.org/10.1016/j.focha.2023.100565.

61. Sadasivam K. & R. Kumaresan. 2011. Theoretical investigation on the antioxidant behavior of chrysoeriol and hispidulin flavonoid compounds. *A DFT Study* **963**(1): 227—235. doi: 10.1016/j.comptc.2010.10.025

62. Sadeghi, A., A. Rajabiyan, N. Nabizade et al. 2024. Seaweed-derived phenolic compounds as diverse bioactive molecules: A review on identification, application, extraction and purification strategies. *Int. J. Biol. Macromolecules* **266**: 131147. https://doi.org/10.1016/j.ijbiomac.2024.131147.

63. Sakat, S.S., A.R. Juvekar & M.N. Gambhire. 2021. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Lin. *Int. J. Pharm. Sci*. **2**(1): 146—155.

64. Sasadara, M.M.V. & I.G.P. Wirawan. 2021. Effect of extraction solvent on total phenolic content, total flavonoid content, and antioxidant activity of Bulung Sangu (*Gracilaria* sp.) Seaweed. *3rd international conference on bioscience and biotechnology IOP Conf. Series: Earth and Environ. Sci*. **712**: 012005.

65. Senapati, S.R., B. Singh, A. Hassan, et al. 2016. Effect of different solvents on total phenolics and antioxidant activity of extracts from *Sargassum tenerrimum* (J. Agardh, 1848). *J. Biosci*. **30**(2): 415—419.

66. Shobier, A.H., M.M. Ismail, & S.W.M. Hassan. 2022. Variation in anti-inflammatory, anti-arthritic, and antimicrobial activities of different extracts of common Egyptian seaweeds with an emphasis on their phytochemical and heavy metal contents. *Biol. Trace Elem. Res*. 201(4): 2071–2087. https://doi.org/10.1007/s12011 022 03297 1.

67. Sobuj, M.K.A., A. Islam, Sh. Islam, et al. 2021. Effect of solvents on bioactive compounds and antioxidant activity of *Padina tetrastromatica* and *Gracilaria tenuistipitata* seaweeds collected from Bangladesh. *Sci. Rep*. **11**: 19082

68. Soto-Hernandez, M., M. Palma-Tenango & R. Garcia-Mateos. 2017. Phenolic compounds — biological activity phenolic compounds: functional properties, impact of processing and bioavailability. *Intech. Publ*. 10.5772/63693 (Chapter 1). doi:10.5772/66368

69. Sroka, Z., A. Sowa, & A. Dryś. 2017. Inhibition of lipoxygenase and peroxidase reaction by some flavonols and flavones: the structure-activity relationship. *Nat. Prod. Commun*. **12**(11). doi:10.1177/1934578X17012 01111

70. Sumanya, H., R. Lavanya, & R.C. Umamaheswara. 2015. Evaluation of *in vitro* antioxidant and anti-arthritic activity of methanolic extract of marine green algae *Caulerpa racemosa*. *Int. J. Pharmacy and Pharm. Sci*. **7**(7): 340—34.

71. Taldaev, A., A.D. Savina, V.V. Olicheva, et al. 2023. Protective properties of spheroidal taxifolin form in streptozotocin-induced diabetic rats. *Int. J. Mol. Sci*. **24**(15):11962. https://doi.org/10.3390/ijms 2415 11962

72. Tavanappanavar, A.N., S.I. Mulla, C.S. Seth, et al. 2024. Phytochemical analysis, GC—MS profile and determination of antibacterial, antifungal, anti-inflammatory, antioxidant activities of peel and seeds extracts (chloroform and ethyl acetate) of *Tamarindus indica* L. *Saudi J. Biol. Sci*. **31**(1): 103878. https://doi.org/10.1016/j.sjbs.2023.103878.

73. Truong, D.H., D.H. Nguyen, N.T.A. Ta, et al. 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-infammatory activities of *Severinia buxifolia*. *J. Food Quality*. Article ID 817829.

74. Vicente-Zurdo, D., E. Gómez-Mejía, N. Rosales-Conrado, & M.E. León-González. 2024. A comprehensive analytical review of polyphenols: Evaluating neuroprotection in Alzheimer's disease. *Int. J. Mol. Sci*. **25**: 5906. https://doi.org/10.3390/ijms25115906

75. Xie, J., S. Xiong, Y. Li, et al. 2024. Phenolic acids from medicinal and edible homologous plants: a potential anti-inflammatory agent for inflammatory diseases. *Frontiers in Immunology* **15**: 1345002. doi: 10.3389/fimmu. 2024.1345002

76. Yang, J., K.J. Meyers, J. Van Der Heide, & H.L. Rui. 2004. Varietal differences in phenolic content and antioxidant and antiproliferative activities of onions. *J. Agricult. Food Chemistry* **52**(22). https://doi. org/10. 1021/jf0307144

77. Yuan, Y., J. Zhang, J. Fan, et al. 2018. Microwave assisted extraction of phenolic compounds from four economic brown macroalgae species and evaluation of their antioxidant activities and inhibitory effects on α-amylase, α-glucosidase, pancreatic lipase and tyrosinase. *Food Res. Int*. **113**: 288—297. https://doi. org/10.1016/ J. FOODRES.2018.07.021

78. Zhang, H.-J., W.-J. Mao, F. Fang, et al. 2008. Chemical characteristics and anticoagulant activities of a sulfated polysaccharide and its fragments from *Monostroma latissimum*. *Carbohydrate Polymers* **71**: 428—434.

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PHARMACOLOGICAL ASPECTS AND HPLC PROFILE OF THREE EGYPTIAN SEAWEEDS VIA PHENOLIC COMPOUNDS; UNRAVELING STRUCTURE-ACTIVITY RELATIONSHIPS

This study was designed to give comparative investigations on the total phenols (TPC), tannins (TTC), and flavonoids (TFC) content in *Ulva compressa*, *Pterocladia capillacea*, and *Colpomenia sinuosa* using three different solvents. Phenolic compounds were qualitatively and quantitatively estimated in various algal extracts. The maximum values of TPC and TFC were found in the ethyl acetate extract of *C. sinuosa*, with contents of 49.997 mg/g and 38.68 mg/g, respectively. The methanolic extract of all three selected species showed the highest yield of TTC, with a maximum yield of 19.36 mg/g for *C. sinuosa*. Conversely, the hexane extracts exhibited the lowest phenolic content and bioactivity. Algal extracts were also identified using high-performance liquid chromatography (HPLC), revealing sixteen phenolic acids and flavonoids, including important compounds like gallic acid, cinnamic acid, and catechin in most algal extracts, particularly in methanol and ethyl acetate extracts.

The study suggests that ethyl acetate and methanol extracts of *C. sinuosa* and *P. capillacea* could serve as valuable sources of natural polyphenols with antioxidant, antidiabetic, anti-arthritic, and anti-inflammatory properties. Strong correlations were observed between flavonoid content and antioxidant and antidiabetic activities. Additionally, flavonoids were supported by structure-activity relationships (SARs) as antioxidants and antidiabetic agents through hydrogen atom transfer (HAT) and sequential proton loss electron transfer (SPLET). The methanol and ethyl acetate extracts from the species studied exhibited an anticoagulant effect, indicating that they could be used as natural anticoagulants. However, further toxicological and clinical research is necessary before considering these algal extracts as nutraceuticals and pharmaceuticals.

Keywords: *macroalgae, biological activities, flavonoids, phenols, tannins, Abu Qir Bay, the Mediterranean Sea, Egypt.*