Research

Assessment of different solvents effect on bioactive compounds, antioxidant activity and nutritional formation of red seaweed, *Gracilariopsis longissima*, from Bay of Bengal, Bangladesh

Mousumi Akhter¹ · Md. Rahamat Ullah² · Abu Bakker Siddique Khan¹ · Md. Monjurul Hasan² · Farhana Yasmin² · Aovijite Bosu² · Md. Ashraful Haque² · Md. Mohidul Islam¹ · Md. Amirul Islam³ · Yahia Mahmud⁴

Received: 6 September 2023 / Accepted: 18 December 2023 Published online: 04 January 2024 © The Author(s) 2024 OPEN

Abstract

Seaweed is known to empower a mass embodiment of bioactive compounds with potent health convenience. Current perusals intended to explore the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant dynamism of red seaweed, *Gracilariopsis longissima* as pervaded by different solvents and solvent fractions (methanol, ethanol, acetone, and water). The extracts dynamism to antioxidant were assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2, 2[']-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Here, 100% methanolic and acetonic extract asserted maximal extent of total phenolic and flavonoid content (88.70 mg of gallic acid/g and 75.06 mg of quercetin/g respectively) in a sufficiently great way. However, 50% methanolic DPPH and ABTS solution (74.32%, IC_{50} = 0.027 mg/ml and 70.51%, IC_{50} = 0.033 mg/ml) significantly demonstrated the highest percentage of inhibition and antioxidant activity compared to other solvents. *Gracilariopsis longissima* also contains a high amount of protein (30.63%) and minerals especially potassium (4.47%) and calcium (2.54%). According to the results of this study, *G. longissima* extracts prepared using 100% methanol as a solvent showed higher total phenolic and total flavonoid quantity and 50% methanol as a solvent showed higher antioxidant potential. These properties will be valuable for nutritional food and medicinal uses.

Keywords Red algae · Gallic acid · Quercetin · DPPH · ABTS · Nutrition

1 Introduction

Marine algae extracts have been pointed out as bearing antioxidant landmarks [9, 11, 13, 19, 32, 56]. Seaweed contributes significantly to hydrophyte ecology by creating the foundation for the food web [21]. Worthy products and stuff propagate from the manifold types of macroalgae as the blue economy goes into recession [53]. Studies also indicated that diverse swatches with isolated diluents have several antioxidant features concerning compounds with distinct polarities [19, 23]. Phenolic constituents play an important role in the oxidative features of umpteen shrub-realized antioxidants [7]. It is exigent for an antioxidant to parry the leash commencing leap by scavenging the inciter radical as gratis radicals propagated at the whole step of a chain recompose [42]. Compounds consisting of one or more aromatic rings coupled to one or more hydroxyl groups are typically referred to as phenolic. With over 8,000 known structures, they are the most

Mousumi Akhter, mousumiakhter.fs@gmail.com | ¹Bangladesh Fisheries Research Institute, Marine Fisheries and Technology Station, Cox's Bazar 4700, Bangladesh. ²Bangladesh Fisheries Research Institute, Riverine Sub-Station, Khepupara, Patuakhali 8650, Bangladesh. ³Bangladesh Fisheries Research Institute, Riverine Station, Chandpur 3602, Bangladesh. ⁴Bangladesh Fisheries Research Institute, Headquarter, Mymensingh 2201, Bangladesh.



https://doi.org/10.1007/s44187-023-00069-1



prevalent secondary plant metabolites. They range from basic phenolic compounds, such as phenolic acids, to complex phenolic compounds, such as tannins. The compounds contribute to the plant's defense against ultraviolet light (UV), pathogens, and other predators. Their presence in all plant organs renders them an indispensable component of the human diet [4, 46]. Their inclusion in every organ of a plant renders them an indispensable nutritive component. Utilizing organic antioxidants as an alternative form of calories protects the body from degenerative diseases. Since chemically produced antioxidants such as butylated hydroxyl toluene (BHT), the synthetic form of butylated hydroxyanisole (BHA), and terbutylhydroquinone (TBHQ) pose health hazards, it is prudent to pursue natural antioxidants [8]. In turn, there is an increasing fascination with substances with antioxidant properties that can be provided via food or as prophylactic medication [43]. Seaweed possesses the potential to be a rich source of highly beneficial secondary metabolites, which may be employed to develop unique medicinal products. Recent studies highlighted the health benefits of algae consumption and the bio functional activities of seaweed extracts and compounds, such as cholesterol-lowering, antithrombotic, antioxidant, and anti-diabetic effects, along with improved gut bacteria efficiencies and estrogen combustion [22, 33–36, 51, 55]. Despite Bangladesh's enormous potential, however, there are only a few investigations into the prospecting of seaweed resources. The preponderance of research has concentrated on molecular and mechanistic analyses of seaweed's constituents [15]. [35, 48, 49] are among the very few researchers who have analysed the phytochemical composition and antioxidant activity of seaweeds collected along the coast of Bangladesh. Season, maturity, creatures, and place of residence alter crop yield and makeup [14]. Using various in vitro spectroscopic assays, we assessed the biologically active total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capabilities of crude extracts of Gracilariopsis longissima for the very first time in Bangladesh. Likewise, we verified the dietary significance of G. longissima by analysing its approximate composition with an assortment of mineral density analyses.

2 Materials and procedures

2.1 Substances and reactants

Methanol, ethanol, acetone, gallic acid, quercetin, Folin-Ciocalteu's phenol reagent, sodium carbonate, aluminium chloride, potassium acetate, DPPH (2, 2-diphenyl-1-picrylhydrazyl), ascorbic acid, potassium persulfate, ABTS (2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid), phenol–sulphuric acid, perchloric acid, concentrated nitric acid, and hydrochloric acid were purchased from Merck, Germany. The experiment incorporated only substances and reagents of analytical purity.

2.2 Acquisition of samples and processing

In December 2022, mature Gracilariopsis longissima was garnered from the Bakkhali river, Cox's Bazar (21 25 44.7" N and 91 58 31.6" E). To maintain the specimen's scientific longevity, it was shipped in a refrigerator (4 °C) to the research facility. To flush out salt, sand, and other pollutants from the samples, they were thoroughly flushed with distillate water. After being meticulously cleansed, seaweed samples were freeze-dried, leveraging a freeze dryer as the drying approached. Samples of seaweed were frozen for 48 h at – 80 °C in an analytical freeze dryer (LFD-BT-101, Labocon, United Kingdom). By means of a grinder machine, seaweed samples were pulverized and scrutinized (300–500 μm). The samples were subsequently stored at 4 °C until biological solvent mining.

2.3 Synthesis of seaweed extract

Based on their polarisation, methanol, ethanol, acetone, and water were used as solvents. Four grammes of finely pulverised seaweed were steeped in 100 millilitres of solvent (50, 70, 100% methanol, ethanol, acetone, and water) for solvent extraction. For 24 h, the sample was spun on and off at 45 °C and 100 rpm in a wobbling incubator (KC121, Labstac, United Kingdom) at 45 °C and 100 rpm to speed up extraction. Regarding ethical circumstances, the resulting mixture was funneled through Whatman filter paper No. 4 (20–25 µm) after incubating. To extract as much powder as feasible from the sample, every bit of supple powder was simmered in their pertinent solvents for 12 h with vigorous stirring and then strained. The methanol, ethanol, and acetone were evaporated using a rotary vacuum evaporator (SCI100-pro, Labocon, Manchester, United Kingdom) at 36 °C and 80 rpm, in contrast to the water solvent, which underwent drying using a freeze dryer at – 80 °C for 48 h and was then retained at 4 °C until the experiment culminated. Ultimately, efficacious solutions of 5 mg/ml were assembled for each extract.





2.4 Quantitative phytochemical analysis

Total phenolic content (TPC). The concentration of total phenols in the pure extracts was quantified using Folin-Ciocalteu Phenol kits and externally calibrated with gallic acid, with a little tweaking [20]. In simple terms, 0.5 ml of retrieve solution was infused with 0.1 ml of 10% FC reagent solution. 75 g/l of sodium carbonate (Na₂CO₃) was tossed in a solution comprising 2.5 ml of sodium carbonate (Na₂CO₃) after 15 min, and the index of absorption was tracked at 760 nm using a spectrophotometer. It was determined that the concentration of total phenolics corresponds to mg of gallic acid per gramme. With a correlation coefficient of 0.9906, linearity spanning the 5–25 mg/ml range originated. (Fig. 1). The algorithm for gallic acid validation was:

Y = 0.0067x + 0.0338(R2 = 0.9906)

2.5 Total flavonoid content (TFC)

The entire flavonoid spectrum of plain extracts was assessed using a modified version of the aluminium chloride colorimetric method outlined by [31]. 1 millilitre of extract solution was neutralised with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, and 0.2 ml of 1 M potassium acetate. After 30 min of exposure at the exterior temperature, the absorbance was taken into account at 420 nm. The total flavonoid concentration emerged to be identical to mg of quercetin per gramme. Correlation coefficient 0.9988, was the metric employed to validate linear behavior regarding 5–25 mg/ml range (Fig. 2). The equation for quercetin calibration was:

$$Y = 0.0497x + 0.0119(R2 = 0.9988)$$



(2024) 4:3

2.6 Evaluation of total antioxidant capacity

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. The DPPH free radical scavenging experiment was carried out in triplicate with dime swapping, according to [6]. Conversely, 4.75 ml of 0.25 mM DPPH was synthesised in methanol, ethanol, acetone, and water. Numerous extract solutions (1, 3, and 5 mg/ml) and ascorbic acid (the standard) were wholeheartedly mingled with 0.25 mM of methanolic, ethanolic, acetonic, and water DPPH solutions. The resultant solution was subsequently allowed to incubate at 350 °C in a darkened area at a steady temperature for 20 min prior to its spectroscopic density (OD) being tracked at 517 nm. The simulation was replicated with three sets containing 50, 70, and 100% of each solvent, and the resultant mean value was factored into consideration. Implementing the aforementioned calculation, the percent radical-scavenging activity of the cleansed extract was quantified.

DPPH radical scavenging activity (percentage) = $[(A0 - A1)/A0] \times 100$

Where A_0 represents the ascorbic acid absorbance in the control and A_1 represents the DPPH radical absorbance in the corresponding seaweed extract or standard in the sample to be examined. Through the use of the "y = mx + c" algorithm in conjunction with the trajectory of the graph, the inhibitory concentration (IC_{50}) was figured out from the normative ascorbic acid graph utilizing the gradient.

ABTS radical scavenging assay (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid). The antioxidant capacity of distinct extracts was taken note of with a slight rewrite of the colorimetric method of ABTS radical removal pioneered by [39]. In an effort to yield a volunteer solution, 7 mM ABTS solution and 2.45 mM potassium persulfate (K₂0₈S₂) were infused into water in a 1:1 ratio. 15 h were dedicated to maintaining the mélange in a pitch-black environment at 300 degrees. Afterwards the incubation procedure, the radical reaction was further diminished with water (1 ml of ABTS reagent and 25 ml DW). Then, 30 L of marine algae extract with a variety of concentrations (1, 3, and 5 mg/ml) were incorporated into 1000 L of working solution. Featuring a spectrophotometer (T80 + UV/VIS spectrophotometer, UK), the absorbed radiation of every sample at 734 nm was analyzed. Ascorbic acid was deployed as the standard. The hindering percentage of synthesized extracts was calculated using the following method:

ABTS scavenged (%) =
$$\left[\frac{Abs_{control} - Abs_{sample}}{Abs_{sample}}\right] \times 100$$

Abs sample is the absorbance of the sample extracts, while Abs control is the absorbance of the control.

2.7 Dietary composition of G. longissima

Estimative compositional evaluation. The official method of the Association of Official Analytical Chemists (AOAC) was put into effect to figure out all dietary metrics [52]. To ascertain the protein content, nitrogen was equated into basic protein using the Kjeldahl method and a mitigating factor of 6.25. Total carbohydrate became apparent using the phenol-sulfuric acid assay. The crude lipid makeup was ascertained using a segregation funnel gadget. The total percentage of ash was determined by igniting mummified instances in a muffle furnace at 600 °C, and the level of moisture was determined by depleting samples in an oven and noting the discrepancy in weight.

Determination of elemental mineral. The seaweed (4 g) was placed in a glass container, 10 ml of perchloric acid was added, and it was left undisturbed for five minutes (to eradicate any organic components). The mixture was then treated with 10 ml of concentrated nitric acid, incubated for 5 min, and 10 ml of HCl added. The mixture was permitted to evaporate, and the resulting residue was dissolved in 10 ml of HCl solution. The filtrate was analyzed with a spectrophotometer for atomic absorption. Potassium, magnesium, sodium, calcium, phosphorus, and sulphate were the minerals analyzed. Total nitrogen was calculated using the micro Kjeldahl method [18], and phosphorus was calculated using the [12] method.

2.8 Statistical examination

Using the standard statistical method, the obtained experimental data were analysed. Using SPSS software (IBM Co., Chicago, IL), data were analysed. Using analysis of variance (ANOVA) and Duncan's multiple range method, solvents and



Table 1The aggregatephenolic content (mg GAE/gsample) of the multiplesolvents and particulates ofsolvents (methanol, ethanol,acetone, and water) ofGracilariopsis longissima (n=4)

Solvent	Total phenolic content (mg of GA/g dw)
50% methanol	27.13±0.85 ^g
70% methanol	33.62 ± 1.76^{e}
100% methanol	88.70 ± 2.19^{a}
50% ethanol	29.82 ± 0.98^{f}
70% ethanol	37.43 ± 1.52^{d}
100% ethanol	$43.02 \pm 1.38^{\circ}$
50% acetone	36.76 ± 0.92^{d}
70% acetone	$39.89 \pm 1.05^{\circ}$
100% acetone	70.34 ± 1.78^{b}
Water	$40.56 \pm 1.36^{\circ}$

^{a, b, c, d, e, f, g} There is a significant difference in every fraction of solvents. Groups that do not share a common letter indicate a significant difference (P < 0.05)

samples were compared. The values were expressed in terms of means and standard deviations. When *P* < 0.05, differences were considered significant.

3 Results

3.1 Empirical scrutiny of phytochemicals

Total phenolic content (TPC). The organoleptic features of vegetative consumption are dictated by the phenolic substances, which principally find their homes in fruits, legumes, vegetables, beverages, and liquor. In a comparable vein, the metallic flavour of fruits is exacerbated by phenolic molecules functioning alongside sublingual fibronectin. Countless fruits and vegetables are capable of being coloured with phenolic substances [2].

The TPC composition was portrayed as mg analogous gallic acid (GAE) per gram of wilted weight. The data are depicted as the mean standard variance. Entities that deficit a prevalent prefix recommend a computationally noteworthy disparity (P < 0.05).

By means of Folin-Ciocalteu phenol reactants and independent calibration with gallic acid, the total extent of phenol in the raw extract of *Gracilariopsis longissima* was tenacious. From 88.70 mg of GA/g (100% methanol) to 27.13 mg of GA/g (50% methanol), the TPC of various solvent-derived extracts diverged markedly. Table 1 implied that 100% methanolic extract encompassed the most noticeable span of TPC (88.70 mg of GA/g), traversed by ethanol, acetone, and water extracts (43.02, 70.34, and 40.56 mg of GA/g, respectively) (P < 0.05).

Total flavonoid content (TFC). Flavonoids are one of the most prevalent kinds of polyphenols that lurk in plant-based substances. Classical flavonoid methods of recovery have been substituted by contemporary methods that seek to lessen vitality and solvent usage, augment competence and precision, and are compatible with expanding market demand and safety standards. Flavonoids are an entourage of organically generated phenolic elements that are manufactured by flora as second-generation metabolites conferring aroma, hue, and pharmaceutical effects [45]. They are potent antioxidants that hedge greenery from deleterious outside stimuli [27].

The TFC composition was portrayed as mg analogous gallic acid (GAE) per gram of wilted weight. The data are depicted as the mean standard variance. Entities that deficit a prevalent prefix recommend a computationally noteworthy disparity (P < 0.05).

The aluminium chloride protocol was used to come up with the degree of total flavonoid content across numerous plain extracts at a dosage of 5 mg/ml. The TFC proportion climbed remarkably to 75.06 mg of quercetin/g (in 100% acetone) and 17.51 mg of quercetin/g in 50% ethanol. The 100% acetonic extract encompassed the greatest amount of TFC (75.06 mg of quercetin/g) among the four *Gracilariopsis longissima* instances, preceded by the methanol, ethanol, and water extracts (71.46, 42.51, and 42.51 mg of quercetin/g, consecutively). (P < 0.05; Table 2).



https://doi.org/10.1007/s44187-023-00069-1

Table 2Total flavonoidcontent (mg quercetin/gsample) of the differentsolvent and solvent fractions(methanol, ethanol,acetone, and water) of thered seaweed, Gracilariopsislongissima (n=4)

Solvent	Total flavonoid content (mg of quercetin/g dw)
50% methanol	22.76 ± 0.78^{d}
70% methanol	32.89 ± 1.14^{d}
100% methanol	71.46±2.17 ^a
50% ethanol	17.51±0.83 ^e
70% ethanol	23.76±0.91 ^d
100% ethanol	42.51±1.42 ^c
50% acetone	23.08 ± 0.96^{d}
70% acetone	41.37±1.15 ^d
100% acetone	75.06±2.47 ^a
Water	49.27 ± 1.56^{b}

^{a, b, c, d, e} There is a significant difference in every fraction of solvents. Groups that do not share a common letter indicate a significant difference (P < 0.05)

3.2 Evaluation of total antioxidant capability

DPPH radical scavenging inspection. The DPPH assay was integrated because it is a simple test mechanism that's capable of offering a fontal portent of the free radical elimination potential of a test molecule. Inevitably, the streamlined form of the robust DPPH radical impedes its interaction with radical scavengers. UV–Vis spectroscopy can ascertain the degree of diminished DPPH by virtue of the alteration in colour owing to the diminution of the free radical. The DPPH assay is undertaken with multiple levels of the antioxidant with the goal of identifying the IC₅₀, which signifies the volume obligated to offset 50% of the free radical (DPPH). Low IC₅₀ values will henceforth denote an elevated degree of antioxidant activity, as well as the opposite.

The results pointed out that a refined infusion of the seaweed *G. longissima* has antioxidant qualities. *G. longissima* astronomically rose as seaweed extract saturation was boosted (p < 0.05) (Table 3).

In the current inquiry, the average percentage of inhibitory activity of methanolic extracts tended to be more intense (P < 0.05) than that of ethanolic, acetonic, and water extracts. In accordance with the array of solvents, 50% methanolic extracts of different strengths (1, 3, and 5 mg/ml) showcased a greater coefficient of resistance (49.66, 55.76, and 74.32%, respectively) than 70% methanol (43.22, 52.77, and 69.43%, respectively) and 100% methanol (44.22, 50.4%, and 65.32%, respectively). (Fig. 3).

The IC₅₀ value of the extracts was assigned using the "y = mx + c" algorithm, and the resultant figure for typical ascorbic acid turned out to be 0.0032 mg/ml. All crude extracts clarified an inferior IC₅₀ value for DPPH radical scavenging than the positive benchmark.

Table 3	Values of IC ₅₀ (mg/ml)
resultar	nt from various crude
extracts	of DPPH antioxidant
assay	

Antioxidant assay	IC ₅₀ (mg/ml) values of differen	t crude extracts
DPPH assay	Ascorbic acid	0.0032 ± 0.0018^{e}
	50% methanol	$0.027 \pm 0.71^{\circ}$
	70% methanol	0.038 ± 0.045^{d}
	100% methanol	0.046 ± 0.028^{d}
	50% ethanol	0.076 ± 0.051^{d}
	70% ethanol	$1.541 \pm 0.11^{\circ}$
	100% ethanol	0.094 ± 0.047^{d}
	50% acetone	2.189 ± 0.98^{b}
	70% acetone	0.031 ± 0.042^{d}
	100% acetone	0.049 ± 0.013^{d}
	Water	3.462 ± 0.671^{a}

^{a, b, c, d, e} There is a significant difference in every fraction of solvents. Groups that do not share a common letter indicate a significant difference (P < 0.05)



Table 4 IC₅₀ (mg/ml) values for the ABTS antioxidant assay combining distinguished crude extracts

Antioxidant assay	IC ₅₀ (mg/ml) values of differen	t crude extracts
ABTS Assay	Ascorbic acid	0.0056 ± 0.043^{e}
	50% methanol	0.033 ± 0.047^{d}
	70% methanol	$0.174 \pm 0.014^{\circ}$
	100% methanol	0.086 ± 0.058^{d}
	50% ethanol	0.039 ± 0.025^{d}
	70% ethanol	$0.094 \pm 0.069^{\circ}$
	100% ethanol	1.102 ± 0.29^{b}
	50% acetone	0.042 ± 0.042^d
	70% acetone	0.064 ± 0.016^{d}
	100% acetone	1.249 ± 0.57^{b}
	Water	4.221 ± 0.47^{a}

^{a, b, c, d, e} There is a significant difference in every fraction of solvents. Groups that do not share a common letter indicate a significant difference (P < 0.05)

A noteworthy discrepancy is apparent by clusters that do not share an identifiable letter. (P < 0.05)

C 1 mg/ml = 3 mg/ml ≈ 5 mg/ml

Fig. 4 ABTS (2, 2[']-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) scavenging activity (%) of diffrent concentrated crude extracts of the red seaweed, *Gracilariopsis longissima*



(2024) 4:3

% of dry weight

 89.24 ± 2.4 30.63 ± 0.9 1.49 ± 0.05 22.25 ± 1.1

 30.45 ± 0.8

Table 5 Proximate composition analysis of dried seaweed G. longissima	Nutritional constituents
	Moisture
	Protein
	Lipid
	Ash

Carbohydrate

Table 6 Minerals incorporation of dried seaweed G. longissima

Minerals constituents	% of dry weight
Sodium	0.82±0.02
Potassium	4.47±0.05
Calcium	2.54 ± 0.04
Magnesium	0.58 ± 0.02
Phosphorus	0.31±0.03
Sulfur	1.56±0.05

Radical scavenging assay of ABTS. The total antioxidant capacity (TAC) of naturally occurring substances like basic extracts, polyphenols, phenolic substances, and flavonoids is frequently approximated by implementing the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS +), a free radical cation with long stability [17, 37].

The unilateral integration of the green or blue ABTS chromophore for ABTS scavenging necessitated the liaison of ABTS with an H-donating oxidizing agent, in the present instance potassium persulfate (K₂S₂0₈). 5 mg/ml of seaweed extract turned out to have the finest ABTS-scavenging capacity as the level of the extract stood up. ABTS radical scavenging activity was significantly elevated at 50% concentrations of methanolic, ethanolic, acetonic, and watery seaweed extracts (70.51%, IC₅₀=0.032 mg/ml; 60.12%, IC₅₀=0.039 mg/ml; 61.88%, IC₅₀=0.042 mg/ml; and 47.11%, IC₅₀=4.221 mg/ml, correspondingly). (Table 4, Fig. 4).

The inhibitory concentrations (IC₅₀) of all extracted compounds conveyed less ABTS radical scavenging activity if juxtaposed to the positive control, ascorbic acid ($IC_{50} = 0.0056$ mg/ml).

3.3 Proximate constituent analysis

Table 5 represents the theoretical molecular makeup of G. longissima retrieved from the Kuakata coast. In accordance with research carried out by [5], the nutrient profile of seaweeds shifts based on the species, geographical context, and terrestrial circumstances pertaining to growth.

The degree of warmth and accumulation of critical nutrients in the water surrounding it affect the basic energy expenditure of seaweed [29]. On a dry-weight basis, moisture (89.24%), protein (30.63%), and fat (1.49%) contrasted drastically with ash (22.25%) and carbohydrate (30.45%).

3.4 Mineral composition analysis

The mineral constitution of G. longissima from the aforesaid research is shown in Table 6. The most ingrained component that comprises G. longissima is potassium (4.47%).

In the present inquiry, sodium (0.82%), calcium (2.54%), magnesium (0.58%), phosphorus (0.31%), and sulphur (1.56%) were scrutinised, as well as more volunteers' micronutrients (Table 6).



4 Discussion

The emergence of mainstream therapeutics is significantly simplified by phytochemicals synthesized from medicinal herbs. The overwhelming number of phytochemicals executed, as specified by [54], are phenolic and flavonoid compounds, which have an assortment of advantageous implications for longevity and combating the spread of carcinoma. Scientific inquiry into botanical medicines has surged because of the swap of renewable resources for synthetic substances in the inception and development of aesthetic and grooming goods [26]. Pre-extraction and extraction procedures are the primordial marches in the seclusion of biologically active ingredients from vegetation [3]. The bipolar nature of the solvent matters enormously when phenolic molecules are pulled out of the foliage or fruit [28]. Methanol tends to be a particularly efficacious solvent for polyphenolic separation, on top of boasting a willingness to suppress polyphenol oxidase activity [57]. According to independent inquiries, marine macro and diatoms manufacture copious quantities of phenolic materials upon exposure to adverse conditions [24]. With pertinent GAE levels of 88.70, 43.02, and 70.34 mg/g, the latest research indicated that, as opposed to other extracts, 100% methanol, ethanol, and acetone encompassed considerable quantities of phenolics (Table 1). [44, 48], and [49] all furnished irrefutable testimony for the customary espial. In sharp contrast to our study, [41] articulated G. longissima possessed major amounts of TPC in methanol (150.37 ± 8.07 mg of GA/g), ethanol (96.35 ± 4.87 mg of GA/g), and acetone (94.21 ± 0.40 mg of GA/g). The highest detected amount of total phenolic compound (205.5 mg of GA/g) was uncovered by [8] in red seaweed, Hypnea musciformes. The here and now inspection outweighed 45.1 ± 0.01 mg of GA/g for G. persica [58], 10.7 ± 0.02 mg of GA/g for H. flagelliformis [58], 9.84±0.03 mg of GA/g for H. musciformes [8], 20.4±0.13 mg of GA/g for Gracilariopsis tenuifrons [59], and 11.37±0.92 mg of GA/g for Hypnea sp. [21]. In an opposite fashion to [1, 38] verified a greater variety of TPC $(99.62 \pm 1.2 \text{ mg of GA/g})$ with ethanol than the preceding investigation, and the same comes out of TPC $(42.2 \pm 0.09 \text{ mg})$ of GA/g) with water in G. burso-pastoris. According to [47], G. corticata acetonic extract had a total phenolic strength of 59.85 mg of GA/g, which is analogous to the outcomes of this investigation. The TFC that we presently discovered was reminiscent of the TFC of 70% ethanol (11.76 \pm 0.91 mg of guercetin/g), which was in accordance with a summary in [49]. [38] disclosed the TFC results in G. bursa-pastoris (36.6 ± 10.3 mg of quercetin/g), which corresponded to the TFC of 100% ethanol (42.51 ± 1.42 mg of quercetin/g) and water extract (27.47 ± 0.04 mg of quercetin/g), which came up with a result a bit lower than the forward-thinking result (49.27 ± 1.56 mg of guercetin/g). Other red seaweeds' TFCs have been conveyed in a handful of literary works, but the insights were scanty: 43.12±0.98 with methanol, 32.25±1.65 with ethanol, and 25.48 ± 1.44 with water in the case of H. pannosa [48], 28.2 ± 0.01 in the scenario of G. persica [58], and 18.3 ± 1.36 in the instance of *H. elongata*. Compositions that are likely to act as free radical ambassadors or hydrogen volunteers tend to be prepared and tallied in convoluted biological networks employing the DPPH free radical scavenging exertion. The antioxidant activity of crude G. longissima extracts was assessed via an assortment of in vitro antioxidant assays, including DPPH and ABTS. A surge in antioxidant activity that is correlated with concentration may be due to the repercussions of the variety of bioactive phytochemicals. The prevailing DPPH radical scavenging results were akin to [44]. Even so, [47] and [48] perceived a greater volume of DPPH (2.21 ± 0.15 mg/ml for G. cortica). The DPPH imagery for G. tenuistipitata was outstanding for both DPPH and ABTS radical scavenging activity (IC_{50} value = 2.59 ± 0.08 mg/ml for DPPH methanolic extract). As stated by [1], 100% acetonic DPPH had a DPPH (IC_{50} = 0.054 ± 0.01 mg/ml) level that was remarkably comparable to the DPPH (IC₅₀ = 0.049 ± 0.067 mg/ml) magnitude. The proportion of restraint by ABTS (80%) and DPPH (79.34%) for H. musciformes in the current scrutiny (% suppression for ABTS 50% methanol = 70.51% and DPPH 50% methanol = 74.32 mg/ml, respectively) The IC₅₀ values for DPPH and ABTS water extract transpired to surpass those spotted in the present experiment (3.46 ± 0.67 and 4.221 ± 0.44 mg/ml, respectively) (IC₅₀ values: 5.04 ± 0.10 and 5.91 ± 0.03 mg/ml, respectively). Red algae had an excessive degree of moisture considering that it predominantly consists of water. Contrary to the degree of moisture uncovered in the current study (89.24±2.4%), [16] stipulated a level of moisture of 64.60 ± 5.69%. Red algae, as distinct from a great deal of green and brown algae, have been depicted in multiple investigations to possess more substantial protein. The protein uncovered in this ongoing study $(30.63 \pm 0.9\%)$ dried weight) was identical to those documented for G. tenuistipitata (21.6%), G. gracilis (22.5%), Gracilaria sp. (23.6%), and Hypnea [10]. Lipids are plentiful in Gelidium sp., as stated by [16]. Hypnea sp. (1.460.30%) turned out to be notably similar to the current study (1.49 \pm 0.05%) by (1.11 \pm 0.11%) and [10]. The proportion of rubble in G. fisheri according to [5] (22.2%) matched up to the earlier research (22.25%) and mirrored [40] to a lesser degree. With a marginally superior ash concentration (26.31±1.049%), [16] honed G. pusilum. More carbohydrates were generated than in the current study $(30.45 \pm 0.8\%)$ by [10] and [30]. Potassium and calcium are particularly plentiful in red phytoplankton. While [5] verified that G. gracilis entailed 2.9% sodium, [25] stumbled upon sodium levels of 0.2%, which were lower, and G. tenuistipitata,



https://doi.org/10.1007/s44187-023-00069-1

which exceeded the assessment of this investigation $(0.82 \pm 0.02\%)$. In juxtaposition with [50] (5.22%) and [5] (5.72%), this investigation witnessed a potassium concentration (4.47 ± 0.05%) that was lower. Likewise, to our study's prevalence, phosphorus (0.31 ± 0.03%) was spotted in *G. changii* (0.30%) and *G. tenuistipitata* (0.25%) by [25] and [50].

5 Conclusions

According to the results presented here, the choice of extraction solvent and variety of seaweed have a substantial effect on the availability of bioactive compounds. It has also been discovered that there are significant differences between the phenolic content of extracts and their antioxidant activity, as measured by various antioxidant assays such as DPPH and ABTS. *G. longissima* could be utilized as a potent natural antioxidant source as a nutritional supplement or functional feed. Using various solvents, additional research should be conducted on the identification, isolation, and characterization of bioactive principles.

Acknowledgements The authors would like to thank Bangladesh Fisheries Research Institute (BFRI) for providing financial support for this study.

Author contributions MA: conceptualization, methodology, software, and writing; MRU, ABS Khan: formal analysis, data curation and software; MMH, FY, AB: writing-review and editing; MAH, MMI, MAI and YM: Supervision and validation. All authors have read and approved the final manuscript for publication.

Data availability Data will be supplied upon request.

Declarations

Competing interests The authors declare that they have no pecuniary, personal, or other relationships with other individuals or organizations that could inappropriately influence or be perceived as inappropriately influencing the current work.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

- 1. Abdullah AR, Bakhari NA, Osman H. Study on the relationship of the phenolic, flavonoid and tannin content to the antioxidant activity of *Garcinia Atroviridis*. Univ J Appl Sci. 2013;1(3):95–100.
- 2. Alara OR, Abdurahman NH, Ukaegbu CI. Extraction of phenolic compounds: a review. Curr Res Food Sci. 2021;4:200–14.
- 3. Azwanida NN. A review on the extraction methods used in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4:196–9.
- 4. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem. 2006;99(1):191–203.
- 5. Benjama O, Masniyom P. Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. Songklanakarin J Sci Technol. 2012;34(2):230–223.
- 6. Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT- Food Sci Technol. 1995;28:25–30.
- 7. Canadanovic-Brunet SJ, Djilas G, Cetkovic V, Tumbas A, Mandic V. Antioxidant activities of different *Teucrium montanum L*. extracts. Int J Food Sci Technol. 2006;41:667–73.
- 8. Chakraborty K, Joseph D, Praveen NK. Antioxidant activities and phenolic contents of three red seaweeds (Division: Rhodophyta) harvested from the Gulf of Mannar of Peninsular, India. J Food Sci Technol. 2015;52:1924–35.
- 9. Chew Y, Lim Y, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in Southeast Asia. LWT- Food Sci Technol. 2008;41:1067–72.
- 10. Chowdhury KN, Ahmed MK, Akhter KT, Alam MJ, Rani S, Khan MI. Proximate composition of some selected seaweeds from coastal areas of Cox's Bazar and the St. Martin's Island, Bangladesh. Dhaka Univ J Earth Environ Sci. 2022;10(3):113–22.
- 11. Elalla MA, Shalaby A. Antioxidant activity of extract and semi-purified fractions of marine red macroalgae, *Gracilaria verrucosa*. Aust J Basic Appl Sci. 2009;3(4):3179–85.
- 12. Fiske CH, Subbarow YJ. The colorimetric determination of phosphorus. Biol Chem. 1925;66:375–400.
- 13. Ganesan P, Kumar C, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Biores Technol. 2008;99:2717–23.
- 14. Graham LE, Wilcox LW. Algae. Upper Saddle River: Prentice Hall; 2000. p. 640–52.



- 15. Haque KF, Chy SY, Akter S, Wahab MA, Nath KK. Collection, identification and biochemical analyses of different seaweeds from Saint Martin's Island, Bangladesh. J Agric Res. 2009;34(1):59–65.
- Hossain MT, Sohag AAM, Haque MN, Tahjib-Ul-Arif M, Dash R, Chowdhury MTH, Hossain MA, Moon IS, Hannan MA. Nutritional value, phytochemical profile, antioxidant property and agar yielding potential of macroalgae from Coasts of Cox's Bazar and St. Martin's Island of Bangladesh. J Aquat Food Prod Technol. 2021;30(2):217–27.
- 17. Holdt SL, Kraan S. Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol. 2011;23(3):543–97.
- 18. Humphries EC. Mineral components and ash analysis. In: Paech K, Tracey MV, editors. Moderne methoden der pflanzenanalyse/modern methods of plant analysis. Berlin: Springer; 1956.
- 19. Ismail A Jr, Tan S. Antioxidant activity of selected commercial seaweed. Malays J Nutr. 2002;8(2):177–167.
- 20. Karydas C, latrou M, Kouretas D, Patouna A, latrou G, Lazos N, Gewehr S, Tseni X, Tekos F, Zartaloudis Z, Mainos E, Mourelatos S. Prediction of antioxidant activity of cherry fruits from UAS multispectral imagery using machine learning. Antioxidants. 2020;9(2):156–62.
- Khan ABS, Islam MA, Ullah MR, Akhter M, Yasmin F, Bosu A, Hasan MM, Hasan KR, Mahmud Y. Yield enhancement of seaweed, Gracilaria tenuistipitata (Chang and Xia, 1976) cultured in different raft models and water depths on the Kuakata coast of the Bay of Bengal, Bangladesh. Sustain Aquat Res. 2023;2(1):40–50.
- 22. Kim EY, Rafiquzzaman SM, Lee JM, Noh G, Jo G, Lee JH, Kong IS. Structural features of glycoprotein purified from Saccharina japonica and its effects on the selected probiotic properties of *Lactobacillus plantarum* in Caco-2 cell. J Appl Phycol. 2014;27:965–73.
- 23. Marinova EM, Yanishlieva NV. Antioxidative activity of extract from selected species of the family Lamiaceae in sunflower oil. Food Chem. 1997;58(3):245–8.
- 24. Minhas AK, Hodgson P, Barrow CJ, Adholeya A. A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids. Front Microbiol. 2016;7:1–19.
- 25. S.M. Mohy El-Din, "Temporal variation in chemical composition of *Ulva lactuca* and *Corallina mediterranea*." International Journal of Environmental Science and Technology, vol. 16, pp. 5783–5796, 2019.
- 26. Mukherjee PK, Maity N, Nema NK, Sarkar BK. Bioactive compounds from natural resources against skin aging. Phytomedicine. 2011;19(1):64–73.
- Nabavi SM, Šamec D, Tomczyk M, Milella L, Russo D, Habtemariam S, Suntar I, Rastrelli L, Daglia M, Xiao J, Giampieri F, Battino M, Sobarzo-Sanchez E, Nabavi SF, Yousefi B, Jeandet P, Xu S, Shirooie S. Flavonoid biosynthetic pathways in plants: Versatile targets for metabolic engineering. Biotechnology Advance. 2020;38: 107316.
- 28. Naczk M, Shahidi F. Phenolics in cereals, fruits, and vegetables: Occurrence, extraction, and analysis. J Pharm Biomed Anal. 2006;41:1523–42.
- 29. Nazni P, Deepa S. Evaluation of proximate and pigment composition of red seaweed in South Coastal Regions of Tamilnadu. International Journal of Research and Marine Science. 2015;4(2):38–41.
- 30. R. T. Neto, C. Marçal, A. S. Queirós, H. Abreu, A. Silva, and S. Cardoso, "Screening of *Ulva rigida*, *Gracilaria* sp., *Fucus vesiculosus* and *Saccharina latissima* as functional ingredients." *International Journal of Molecular Science*, vol. 19, no.10, p. 2987, 2018.
- 31. Padhan B, Nayak V, Panda D. Natural antioxidant potential of selected underutilized wild yams (*Dioscorea* spp.) for health benefit. J Food Sci Technol. 2020;57:2370–6.
- 32. Pise N, Jena K, Maharan D, Gaikwad D, Jagtap T, Paula D. Free radical scavenging potential, reducing power, phenolic and biochemical constituent of *Porphyra* species from India. Journal of Algal Biomass Utilization. 2010;1(3):29–42.
- 33. S. M. Rafiquzzaman, E. Y. Kim, Y.R. Kim, T. J. Nam., and I. S. Kong, "Antioxidant activity of glycoprotein purified from *Undaria pinnatifida* measured by an in vitro digestion model." *International Journal of Biological Macromolecules*, vol. 62, pp. 265–272, 2013.
- 34. S. M. Rafiquzzaman, J. M. Lee, R. Ahmed, J. H. Lee, J. M. Kim, and I. S.Kong, "Characterisation of the hypoglycaemic activity of glycoprotein purified from the edible brown seaweed, *Undaria pinnatifida*. *International Journal of Food Science & Technology*, vol. 50, no. 1, 2013.
- 35. Rafiquzzaman SM, Ahmed R, Lee JM, Noh G, Jo G, Kong IS. Improved methods for isolation of carrageenan from *Hypnea musciformis* and its antioxidant activity. J Appl Phycol. 2015;28(2):1265–74.
- 36. Mohibbullah M, Talha MA, Baten MA, Newaz AW, Choi JS. Yield optimization, physicochemical characterizations, and antioxidantproperties of food grade agar from Gracilaria tenuistipitata of Cox's Bazar coast, Bangladesh. Food Sci Nutr. 2023;11:2852–63.
- 37. Rajauria G, Jaiswal AK, Abu-Gannam N, Gupta S. Antimicrobial, antioxidant, and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from western coast of Ireland. J Food Biochem. 2010;37:322–35.
- Ramdani M, Elasri O, Elkhiati N, Taybi FA, Mostareb M, Haloui B. Evaluation of antioxidant activity and total phenol content of *Gracilaria* bursa-pastoris harvested in Nador lagoon for an enhanced economic valorization. Chemical and Biological Technologies in Agriculture. 2017;4:28–32.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol Med. 1999;26(9–10):1231–7.
- 40. Rodrigues D, Freitas AC, Pereira L, Rocha-Santos T, Vasconcelos MW, Roriz M, Rodríguez-Alcalá LM, Gomes A, Duarte A. Chemical composition of red, brown, and green macroalgae from Buarcos bay in Central West Coast of Portugal. Food Chem. 2015;183:197–207.
- 41. A. Rusli, M. Metusalach, M. Muhammad Tahir, S. Salengke, and S. Syamsuar, "Analysis of bioactive compounds of *Caulerpa recemosa*, *Sargassum* sp. and *Gracillaria verrucosa* using different solvents." *Jurnal Teknologi*, vol. 78, pp. 2–4, 2016.
- 42. Saha L, Lajis NH, Israf DA, Hamzah AS, Khozirah S, Khamis S, Syahida A. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J Ethnopharmacol. 2004;92:263–7.
- 43. Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Aabali G, Mete E. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus castes* L. Fruits from Turkey. Food Chem Toxicol. 2009;47(10):2479–83.
- 44. Sasadara MMV, Wirawan IGP, Jawi IM, Sritamin M, Dewi NNA, Adi AAAM. Anti-inflammatory effect of red macroalgae Bulung Sangu (Gracilaria sp.) extract in UVB-Irradiated mice. Pak J Biol Sci. 2020;24(1):80–9.
- 45. Scarano A, Butelli E, De Santis S, Cavalcanti E, Hill L, De Angelis M, Giovinazzo G, Chieppa M, Martin C, Santino A. Combined dietary anthocyanins, flavanols, and stilbenoids alleviate inflammatory bowel disease symptoms in mice. Front Nutr. 2018;4:75–81.
- 46. Shah SR, Ukaegbu CI, Hamid HA, Alara OR. Evaluation of antioxidant and antibacterial activities of the stems of *Flammulina velutipes* and *Hypsizygus tessellatus* (white and brown var.) extracted with different solvents. J Food Meas Charact. 2018;12(3):1–15.



- 47. Sharma G, John J. Evaluation of total phenolic content and antioxidant activities of three species of marine algae from Thirumullavaram, Kerala, Southwest coast of India. Int J Pharm Sci Drug Res. 2022;14(4):493–8.
- Sobuj MKA, Islam MA, Haque MA, Islam MA, Alam MJ, Rafiquzzaman SM. Evaluation of bioactive chemical composition, phenolic, and antioxidant profiling of different crude extracts of *Sargassum coriifolium* and *Hypnea pannosa* seaweeds. J Food Meas Charact. 2020;5(2):1653–65.
- 49. Sobuj MKA, Islam MA, Islam MS, Islam MM, Mahmud Y, Rafiquzzaman SM. Effect of solvents on bioactive compounds and antioxidant activity of *Padina tetrastromatica* and *Gracilaria tenuistipitata* seaweeds collected from Bangladesh. Sci Rep. 2021;11:19082.
- 50. Syad AN, Shunmugiah KP, Kasi PD. Seaweeds as nutritional supplements: analysis of nutritional profile, physicochemical properties, and proximate composition of *G. Acerosa* and *S. Wightii*. Biomed Prev Nutr. 2013;3(2):139–44.
- 51. Teas J, Hurley TG, Hebert JR, Franke AA, Sepkovic DW, Kurzer MS. Dietary seaweed modifies estrogen and phytoestrogen metabolism in healthy postmenopausal women. J Nutr. 2009;139(5):939–44.
- 52. AOAC. The association of official analytical chemists. In: Horwitz William, editor. Agricultural chemicals, contaminants, drugs. Gaithersburg: AOAC International; 2010.
- 53. Ullah MR, Islam MA, Khan ABS, Bosu A, Yasmin F, Hasan MM, Islam MM, Rahman MA, Mahmud Y. Effect of stocking density and water depth on the growth and production of red seaweed, *Gracilaria tenuistipitata* in the Kuakata coast of Bangladesh. Aquacult Rep. 2023;29:101509.
- 54. Venugopal R, Liu RH. Phytochemicals in diets for breast cancer prevention: the importance of resveratrol and ursolic acid. Food Sci Human Wellness. 2012;1:1–13.
- 55. Wong KH, Sam SW, Cheung PCK, Ang PO. Changes in lipid profiles of rats fed with seaweed-based diets. Nutr Res. 1999;19(10):1519–27.
- Yangthong M. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. Plant Food Human Nutr. 2009;64:218–23.
 Yermilov V, Rubio J, Becchi M, Friesen MD, Pignatelli B, Ohshima H. Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite in vitro. Carcinogenesis. 1995;16:2045–50.
- Zarei Jeliani Z, Yousefzadi M, Sohrabi Pour J, Toiserkani H. Growth, phytochemicals, and optimal timing of planting *Gracilariopsis persica*: an economic red seaweed. J Appl Phycol. 2017;30:525–33.
- 59. Zubia M, Robledo D, Freile-Pelegrin Y. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J Appl Phycol. 2007;19:449–58.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

