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Research article

Evaluation of Total Phenolic Content and Antioxidant Activities of Three Species of Marine Algae from Thirumullavaram, Kerala, Southwest Coast of India

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ABSTRACT

Antioxidants from natural sources are gaining demand in various industries since they are the safest alternatives to synthetic antioxidants. Marine macroalgae are rich sources of antioxidants and polyphenols. In the present study, *in-vitro* antioxidant activities of the acetone extract, aqueous extract, and acetone: water extracts (7:3 v/v) of three macroalgae (*Ulva fasciata*, *Gracilaria corticata*, and *Hypnea valentiae*) collected from Thirumullavaram, Kerala, Southwestern coast of India were evaluated using three *in-vitro* assays, *viz.,* 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), NO^* radical scavenging activities. The total phenolic content of the extracts was determined using the folin-ciocalteau method and the results are expressed in milligrams of phloroglucinol equivalents per gram of dry weight (mg PGA g^{-1} DW). The highest DPPH radical scavenging was shown by acetone: water extract of *U. fasciata* (IC_{50} 2.21 mg. mg mL^{-1}) and the highest $ABTS^+$ radical scavenging was shown by the aqueous extract of *G. corticata* (IC50 0.13 mg mL^{-1}) followed by the acetone extract of *H (mg mL^{-1})valentiae* (IC_{50} 0.14 mg. mg mL-1). The highest NO^* radical scavenging was exhibited by *H. valentiae* (IC_{50} 0.06 mg mL^{-1}). The acetone: water extracts of *U. fasciata* and *G. corticata* exhibited very high phenolic content (59.13 mg PGA g^{-1} DW and 59.85 mg PGA g^{-1} DW, respectively). The study indicated that all three species showed increasing radical scavenging activities corresponding to the concentration of the extract and the solvent used for extraction. The presence of secondary metabolites like phenols, and terpenes also seem to have contributed to the radical scavenging activities.

Introduction

Free radicals and reactive oxygen species (ROS) are considered slow-moving poisons because they adversely affect biologically important molecules. To combat harmful free radicals, all organisms have their own antioxidant system. It is composed of several characteristic enzymatic and non-enzymatic antioxidants. Excessive ROS accumulation due to the inefficiency of the antioxidant system causes serious damage to various biomolecules in the body, which in turn causes cancer, brain aging, diabetes, atherosclerosis and arthritis. This leads to several life-threatening illnesses, including aging and neurodegenerative diseases.[1] Algae are the largest and oldest photoautotrophic taxonomic group, with a myriad of therapeutically potent antioxidant compounds.[2] Macroalgae, or seaweeds, are among the most important primary producers and ecological engineers in the rocky shores across the world.[3] Secondary metabolites produced by seaweeds have a wide range of biological functions, including anti-coagulation, anti-virus, antioxidant, anti-allergy, anti-cancer, anti-inflammation, anti-obesity, anti-diabetes, anti-hypertension, neuroprotection, and immunomodulation.[4] As a result, marine algae are considered a prospective source of biologically active molecules for pharmaceutical research and necessary compounds for human nutrition.[5] According to recent research, several algal species have high levels of antioxidants and phenolic chemicals. Natural antioxidants

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in algae protect cells from oxidative damage, which helps prevent many diseases and aging processes. Red, brown, and green algae and cyanobacteria have been found to have high radical scavenging activities.[6] Polyphenols generated by seaweeds have received special attention due to their pharmacological action and diversity of health-promoting advantages, since polyphenols play a vital role in a wide range of biological activities exhibited by seaweeds.[7]

Synthetic antioxidants are now found in almost all food and medicinal items. These compounds are added to food to extend the product's shelf life, primarily by inhibiting the oxidation of unsaturated double bonds in fatty acids. Antioxidants are used in pharmaceutical goods to improve the stability of therapeutic substances prone to oxidative chemical destruction. Butylated hydroxyanisole (BHA) and butylated hydroxyl-toluene (BHT) are the two most commonly used synthetic antioxidants. The pharmaceutical industry frequently uses ascorbic acid derivatives such as ascorbic acid and erythorbic acid, thiol derivatives such as thioglycerol, cysteine, dithiothreitol, and glutathione, and sulfurous-acid salts such as sodium sulfite, sodium formaldehyde sulfoxylate, and tocopherols.[6]

The most extensively used artificial antioxidants, BHA and BHT, have unrivaled potency in a variety of food systems, in addition to their high stability, low cost, and other practical benefits. However, their use in food has decreased because of their carcinogenic concerns and a general rejection of synthetic food additives. The tocopherol and ascorbic acid derivatives employed as BHA and BHT substitutes are substantially less effective as food antioxidants. Hence, the development of more effective natural antioxidants is important, even if they may not be as efficient as synthetic agents since the new antioxidants would be beneficial in the fight against carcinogenesis and aging.[8]

Ulva fasciata, *Gracilaria corticata*, and *Hypnea valentiae* are among the various macroalgae found in the Thirumullavaram Coast, Kerala, along India's southwest coast. Although antioxidant qualities of macroalgae have been scientifically demonstrated, information on their potential from this location is limited. This study aims to determine the phytochemicals, "phenolic content, and antibacterial capability of the crude" acetone extract, aqueous extract, and acetone: water extract (7:3 v/v) of these species. The findings of this research will aid in developing a new generation of antioxidants for use in nutritional supplements and/or functional foods.

Material and Methods

Collection and Identification of Algae

Marine algae from two classes *Chlorophyceae* (*Ulva fasciata* Delile) and *Rhodophyceae* (*Gracilaria corticata* (J. Agardh) J. Agardh, *Hypnea valentiae* (Turner) Montagne were collected from the intertidal zone of Thirumullavaram coast (8.89974° N, 76.55044° E), Kerala, Southwest coast of India. The collected samples were washed thoroughly in seawater, freshwater, and distilled water to remove epiphytes, sand, and debris. The samples were identified using the standard algal identification manuals and books.[9-12] Fresh algae were chopped and ground into fine powder using liquid nitrogen. The powdered samples were stored in a -18°C freezer for further analysis.

Crude Extraction

The crude extracts were prepared using a process already described with minor modifications.[13] In brief, 10 g of powder of thalli was extracted using 50 mL of acetone, aqueous, and acetone: water (7:3 v/v) (Aq A) in the dark at room temperature for 2 hours under orbital agitation. The sample was centrifuged at 4000 g at 10°C for 10 min. The supernatant was collected and pellet was extracted twice with 50 mL solvent in the dark at room temperature for 1-hours in an orbital shaker and centrifuged. The collected supernatants were pooled (150 mL) and evaporated at 40°C under vacuum. The dried extract was stored at -18°C for further analysis.

Phytochemical Screening

The preliminary screening of phytochemicals in various extracts (Acetone, Aqueous and Aq A) of *U. fasciata*, *G. corticata* and *H. valentiae* was carried out by standard procedures.[14]

Total Phenolic Content

The total phenolics were determined using Folin-ciocalteau assay.[15,16] Folin-ciocalteau phenol reagent (0.5 mL) was added to the crude extracts (1-mL). To which 2 mL of 20% Sodium carbonate solution was added and 6.5 mL of distilled water was added. The mixture was vortexed and incubated at 70°C in dark for 10 min. The absorbance was measured at 650 nm using a Shimadzu UV visible spectrophotometer after the mixture turned blue. The total phenolic content was expressed in milligrams of phloroglucinol equivalents per gram of dry weight (mg Phloroglucinol (PGA) g^{-1} DW. The calibration of curve of phloroglucinol was used as a standard ($y = 0.0078x - 0.0423$), $R^2 = 0.9975$ (Fig 1).

In-vitro Antioxidant Assays

2,2- Diphenyl 2- picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was determined by using standard methods.[17–19] Briefly, 60 µM solution of DPPH in methanol was prepared fresh and a 200 µL of this solution was mixed with 50 µL of the test sample at various concentrations (1.56–1000 ug mL^{-1}). The plates were incubated in the dark at room temperature for 15 min, and the absorbance decreased at 515 nm. The assay

was done in triplicates for each sample. The positive control was prepared with DPPH solution, without any extract or ascorbic acid and the negative control was 95% methanol. The percentage of inhibition was calculated using the following equation:

DPPH scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the test compound.

2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) assay

The $ABTS^+$ assay was determined by a method already described.[20,21] The reaction was initiated by the addition of 1-mL of diluted $ABTS^+$ to different concentrations (20 - 100 µg mL^{-1}) of the algal extract. Positive control of 100 µL methanol with $ABTS^+$ was used and 95% Methanol was used as a negative control. The absorbance was read at 734 nm. The experiment was done in triplicates and the inhibition was calculated according to the equation:

$ABTS^+$ scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the test compound.

Nitric Oxide (NO^*) Radical Scavenging Assay

Nitric oxide radical scavenging activity was determined using a standard method.[22,23] The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL saline phosphate buffer, 0.5 mL of standard solution, and various concentrations of samples (6.25 - 200 ug mL^{-1}) were incubated at 25°C for 150 min. After incubation, 1-mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33 % in 20% glacial acetic acid) and allowed to stand for 5 min for the completion of the reaction. Further, 1-mL of the naphthyl ethylenediamine dihydrochloride was added, mixed, and incubated for 30 min at 25°C. The absorbance was measured at 546 nm, where the buffer was used as a negative control and ascorbic acid was a positive control. The percentage of inhibition was calculated as follows:

NO^* radical scavenging activity (%) = $(A_0 - A_1)/A_0 \times 100$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the test compound.

Statistical Analysis

All the experiments were performed in triplicates and the values were expressed as mean ± standard deviation of the mean. The data were analyzed using the statistical software package IBM SPSS version 25. Analysis of variance (ANOVA) and Duncans multiple range tests were used to find the difference in significance between solvents and samples. The differences were significant at ($p < 0.05$).

Results

Phytochemical Screening

The results of phytochemical screening of the three species showed that the extracts contain various primary and secondary metabolites such as carbohydrates, proteins, saponins, flavonoids, coumarins, phenols, terpenes, quinones and glycosides (Table 1).

Total Phenolic Content

Of the selected species and solvents, the total phenolic content was higher in Aq A and acetone extracts (Fig 2). The lowest concentration of phenols was found in the aqueous extracts (Table 2). The Aq A extracts of *U. fasciata* and *G. corticata* showed the highest phenolic content with 59.13 mg PGA g^{-1} DW and 59.85 mg PGA g^{-1} DW compared to the acetone extracts (52.65 mg PGA g^{-1} DW and 40.23 mg PGA g^{-1} DW). The acetone extract of *H. valentiae* showed 51.82 mg PGA g^{-1} DW which was higher than the Aq A extract which showed 46.75 mg PGA g^{-1} DW. The aqueous extract had the least concentration in all three species with *H. valentiae > G. corticata >U. fasciata* having 12.74 mg PGA g^{-1} DW, 7.89 mg PGA g^{-1} DW, and 5.27 mg PGA g^{-1} DW, respectively.

Table 1: Phytochemical screening of *U. fasciata*, *G. corticata*, *H. valentiae*

	U. fasciata			*G. corticata*			*H. valentiae*		
Phytochemicals	***A***	***Aq***	***AqA***	***A***	***Aq***	***AqA***	***A***	***Aq***	***AqA***
Carbohydrates	+	+	+	+	+	+	+	+	+
Proteins	-	+	+	-	+	+	-	+	+
Saponins	-	+	-	-	+	-	-	+	-
Flavonoids	+	+	+	+	-	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-
Coumarins	+	-	+	+	-	+	+	-	+
Quinones	+	+	+	+	+	+	+	+	+
Terpenes	+	-	+	+	-	+	+	-	+
Phenols	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+

+ indicates presence and – indicates absence of the phytochemicals

A – Acetone extract, Aq – Aqueous extract, Aq A – acetone: water (7:3 v/v)

DPPH Radical Scavenging Assay

The results of the DPPH radical scavenging activity of ascorbic acid (control) and the extracts of three species are shown (Table 3). The Inhibitory Concentration (IC_{50}) for ascorbic acid was 0.026 mg mL^{-1}. Among the three species, Aq A extract of *U. fasciata* showed the highest radical scavenging with an IC_{50} of 2.21 mg mL^{-1} followed by the acetone extract of *H. valentiae* with an IC_{50} of 3.15 mg mL^{-1}, whereas the minimum radical scavenging was shown in Aq A extract of *H. valentiae* with an IC_{50} of 19 mg mL^{-1}. Of the different solvents, the highest radical scavenging was shown by Aq A and acetone extracts. The aqueous extract of *G. corticata* showed the highest radical scavenging compared with its acetone and Aq A extracts.

$ABTS^+$ Radical Scavenging Assay

$ABTS^+$ radical scavenging activity of the extracts and control (ascorbic acid) are summarized (Table 4). The extent of inhibition on $ABTS^+$ cation was found to be the highest in the acetone extract of *H. valentiae* followed by Aq A and aqueous extract. The Aq A extract of *G. corticata* showed the highest scavenging activity followed by aqueous and the lowest inhibition was shown by the acetone extracts. The $ABTS^+$ scavenging activity was less in *U. fasciata* compared with the other two species with acetone > aqueous > Aq A.

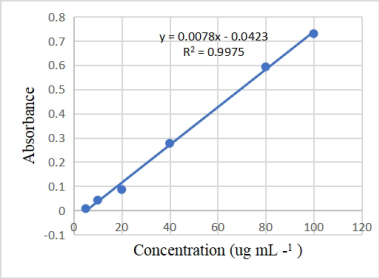

Fig 1: Calibration curve for phloroglucinol

Table 2: Total phenolic content (mg PGA g^{-1} DW) of *U. fasciata*, *G. corticata* and *H. valentiae.*(n = 3)

Species	*Solvent*	*Total phenolic content (mg PGA g^{-1} DW)*
U. fasciata	Acetone	52.65 ± 4.57
	Aqueous	5.27 ± 0.56
	Aq A	59.13 ± 1.27
G. corticata	Acetone	40.23 ± 7.48
	Aqueous	7.89 ± 0.83
	Aq A	59.85 ± 2.12
H. valentiae	Acetone	51.82 ± 0.41
	Aqueous	12.74 ± 1.17
	Aq A	46.75 ± 1.05

NO^* Radical Scavenging Assay

The nitrite inhibition was measured in various concentrations of extracts and control in percentage of inhibition. The highest nitrite inhibition was shown by the acetone extract of *H. valentiae* followed by Aq A extract of *U. fasciata* with an IC_{50} of 0.06 mg mL^{-1} and 0.17 mg mL^{-1}, respectively, which is comparable to that of the control ascorbic acid with an IC_{50} of 0.06 mg mL^{-1} (Table 4). The IC_{50} of aqueous extract of *U. fasciata* and *G. corticata* was significantly higher than that of *H. valentiae,* which showed lower nitrite inhibition.

Discussion

Recently, phenolic compounds produced from marine algae have been investigated for their anticancer, antibacterial, anti-inflammatory, and antioxidant properties.[24] In this study, we analyzed the antioxidant activities of polyphenols from three marine algae using different solvents. The results indicate that the highest phenolic content was found in the Aq A extracts of *U. fasciata* and *H. valentiae* whereas in *G. corticata*, the highest phenolic content was found in the acetone extract. The presence of phenols in acetone extract of *G. corticata* has been reported in a similar study.[25]

Recent studies have reported the total phenolic content of methanolic extracts of *G. corticate.*[26–28] Studies have also reported the total phenolic content of *U. fasciata* in various solvents.[29,30] In another study by Divya and

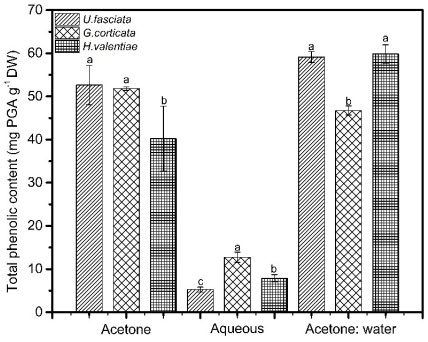

Figure 2: The total phenolic content of the three algal species was expressed as mg PGA g^{-1} DW. Bars represent mean ± standard deviation (n = 3). Bars with the same letters in each group do not differ significantly ($p < 0.05$)

Table 3: DPPH radical scavenging activity of *U. fasciata*, *G. corticata* and *H. valentiae*. Values followed by various letters shows a significant difference at $p < 0.05$ (n=3)

	DPPH IC50 (mg mL^{-1})			
Species	*Acetone*	*Aqueous*	*Aq A*	*Ascorbic acid*
U. fasciata	2.49 ± 0.01^c	4.84 ± 0.00^c	2.21 ± 0.01^c	0.026 ± 0.00^d
G. corticata	11.96 ± 0.17^a	6.08 ± 0.00^a	6.70 ± 0.05^b	
H. valentiae	3.15 ± 0.02^b	5.21 ± 0.01^b	19.90 ± 0.00^a	

Table 4: $ABTS^+$ and NO^* radical scavenging activity of *U. fasciata*, *G. corticata* and *H. valentiae*. values followed by various letters shows a significant difference at $p < 0.05$ (n=3)

	$ABTS^+$ IC_{50} (mg mL^{-1})			NO^* IC_{50} (mg mL^{-1})		
Species	***Acetone***	***Aqueous***	***Aq A***	***Acetone***	***Aqueous***	***Aq A***
U. fasciata	0.32 ± 0.01^b	0.46 ± 0.03^b	0.56 ± 0.04^a	2.57 ± 0.23^a	0.66 ± 0.42^b	0.17 ± 0.00^b
G. corticata	0.35 ± 0.00^a	0.13 ± 0.00^c	0.15 ± 0.00^b	0.48 ± 0.02^b	0.72 ± 0.01^b	1.67 ± 0.34^a
H. valentiae	0.14 ± 0.00^c	0.79 ± 0.10^a	0.55 ± 0.20^a	0.06 ± 0.00^c	10.97 ± 2.8^a	1.87 ± 0.82^a
Ascorbic acid	0.06 ± 0.00^d			0.06 ± 0.00^c		

Chandramohan, the total phenolic content of *H. valentiae* from the ethyl acetate extracts was higher than the ethanol extract.[31] A recent study has reported the total phenolic content of *H. valentiae* in methanolic extracts.[7] Diverse phenolic contents observed in marine algae could be related to solvent variability, methodological variances in estimating derived phenolic content analyses, or genuine variances in polyphenol accumulation based on environmental conditions.[30] Higher phenolic content in Aq A is in line with similar studies.[32–34]

Macroalgae in the high intertidal zone of marine ecosystems are exposed to strong, unfiltered UV light for lengthy periods, causing the generation of active oxygen species and free radicals. The generation of secondary metabolites like phenolic chemicals in marine algae has also been associated with numerous protective mechanisms, including protection against the oxidative and cytotoxic effects of UV damage.[35] Three assays (DPPH, $ABTS^{+,}$ and NO^*) were used to measure the antioxidant activities of the polyphenols from the algal species. The results indicate that the highest radical scavenging of the DPPH radical was shown by the Aq A extract of *U. fasciata* followed by the acetone extract of *H. valentiae*. Because it can handle a large number of samples in a short time and detect active components at low concentrations, the DPPH assay is the most widely used approach for assessing the antioxidant activity of natural products. The presence of an antioxidant caused a decrease in DPPH radical absorbance by scavenging the radical due to hydrogen donation. Some studies have reported improved effects of *U. fasciata* on DPPH and NO^* in other solvents.[30,36]

The $ABTS^+$ assay is based on antioxidant radical scavenging activity against the long-lasting radical anion $ABTS^+$. In this assay, peroxy radical or another oxidant oxidises $ABTS^+$ to its radical cation, $ABTS^+$, which is brightly colored, and antioxidant capacity is assessed by the ability of compounds to reduce color reaction directly with $ABTS^+$ free radicals.[26] This study's ABTS+ radical scavenging activity increased with increasing concentration ($p < 0.05$).

The reduction in NO^* release can be due to a direct NO^* scavenging effect, as all of the seaweed extracts decreased the quantity of nitrite formed *in-vitro* after the breakdown of sodium nitroprusside. Nitroprusside in aqueous solution at physiological pH produces nitric oxide, which interacts with molecular oxygen to produce nitrite ions in this approach. Nitric oxide generation is scavenged by the antioxidant.[27] *H. valentiae* showed the highest inhibition on $ABTS^+$, and NO^* radical with an IC_{50} comparable to the control. Compared to a similar study, our data suggest that the IC_{50} values for the selected solvents in this study are significantly more efficient.[37] Another study reported significant antioxidant activities of *H. valentiae* against DPPH, $ABTS^+$, and NO^*.[7] Since many antioxidant activity studies on seaweed reported in the literature have used different extraction, measurement methods, and units, a direct comparison of our data on radical scavenging activity of seaweed extracts with other studies was not achievable.[32]

Interesting radical scavenging results were obtained for the extracts of *G. corticata* where aqueous extracts showed high DPPH, $ABTS^+$, and NO^* inhibition compared to acetone and Aq A extracts. Similar works have shown an increased inhibition percentage for aqueous extracts of *G. corticata*.[27] A similar study reported an IC_{50} value of 1.93 mg mL^{-1} for ethanolic extract of *G. corticata* in the DPPH assay.[38]

In conclusion, the Aq A extracts of *U. fasciata* and *H. valentiae* had the highest overall phenolic content. The amount of total phenolic content in algae changes throughout the year and around the world. A range of abiotic and biotic variables influence the synthesis of phenolic compounds.[39] As a result, more research should be done on the variance of factors impacting total phenolic content over time and space. Against several assays in this study, all three algal species have considerable antioxidant activity on the increasing concentration of the sample and hence show dose-dependency. The antioxidant properties of these algae can be used in various pharmacological and industrial applications. The synergistic effects of many chemicals contained in the crude extracts could explain their antioxidant properties. To gain insight into the active molecules responsible for antioxidant actions, more studies such as characterization and isolation of compounds are required.

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References

1. Sonani RR, Rastogi RP, Madamwar D. Natural Antioxidants From Algae: A Therapeutic Perspective. Algal Green Chemistry: Recent Progress in Biotechnology. Elsevier; 2017.pp. 91–120 p. http://dx.doi.org/10.1016/B978-0-444-63784-0.00005-9
2. Rastogi RP, Sinha RP. Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnol Adv. 2009; 27(4): 521–39.
3. Domettila C, Sarasabai T, Brintha S, Sukumaran S, Jeeva S. Diversity and distribution of seaweeds in the Muttom coastal waters, southwest coast of India. Biodivers J. 2013; 4(1): 105–10.
4. El Gamal AA. Biological importance of marine algae. Saudi Pharm J [Internet]. 2010; 18(1): 1–25. http://dx.doi.org/10.1016/j.jsps.2009.12.001
5. Lincoln R., Strupinski K, Walker J. Bioactive Compounds from Algae. Life Chemistry Reports Vol. 8, Scientific Research Publishing. 1991.
6. Shebis Y, Iluz D, Kinel-Tahan Y, Dubinsky Z, Yehoshua Y. Natural Antioxidants: Function and Sources. Food Nutr Sci. 2013; 04(06): 643–9.
7. Mahendran S, Maheswari P, Sasikala V, Rubika J jaya, Pandiarajan J. In vitro antioxidant study of polyphenol from red seaweeds dichotomously branched gracilaria *Gracilaria edulis* and robust sea moss *Hypnea valentiae*. Toxicol Reports. 2021; 8(6):1404–11. https://doi.org/10.1016/j.toxrep.2021.07.006
8. Namiki M. Antioxidants/antimutagens in food. Crit Rev Food Sci Nutr. 1990; 29(4): 273–300.
9. Desikachary T., Krishnamurthy V, Balakrishnan M. Rhodophyta Vol. II. Part- II(B). Chennai: Madras Science Foundation; 1998. 91–123, pp. 154–160.
10. Krishnamurthy V. Algae of India and neighbouring countries I. Chlorophycota. New Delhi: Oxford & IBH Publishing Co Pvt Ltd; 2000. pp. 98–104.
11. Jha B, Reddy CR., Thakur M. ., Rao U. Seaweeds of India – The Diversity and Distribution of Seaweeds of Gujarat Coast. Seaweeds of India. 2009. pp. 198.
12. Sahoo D. Common Seaweeds of India. IK International Pvt Ltd; 2010.
13. Stiger-Pouvreau V, Jégou C, Cérantola S, Guérard F, Lann K Le. Phlorotannins in sargassaceae species from brittany (France): Interesting molecules for ecophysiological and valorisation purposes [Internet]. Vol. 71, Advances in Botanical Research. Elsevier; 2014. pp. 379–411. http://dx.doi.org/10.1016/B978-0-12-408062-1.00013-5
14. Harborne JB. Phytochemical Methods : A Guide to Modern Techniques of Plant Analysis. second ed., Chapman and Hall, New York, USA. Chapmer and Hall. 1984. pp. 1–214
15. Le Lann K, Surget G, Couteau C, Coiffard L, Cérantola S, Gaillard F, et al. Sunscreen, antioxidant, and bactericide capacities of phlorotannins from the brown macroalga *Halidrys siliquosa*. J Appl Phycol. 2016; 28(6): 3547–3559. http://dx.doi.org/10.1007/s10811-016-0853-0
16. Hari V, Jothieswari D, Maheswaramma KS. Total Phenolic, Flavonoid Content , and Antioxidant Activity of *Justicia tranquebariensis* LF and *Cycas Beddomei* Dyer . Leaves. Int J Pharm Sci Drug Res. 2022; 14(1): 48–53.
17. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci Technol. 1995; 28(1): 25–30.
18. Anthony KP, Saleh MA. Free radical scavenging and antioxidant activities of silymarin components. Antioxidants. 2013; 2(4): 398–407.
19. R VK, Kumar S, Shashidhara S, Anitha S, Manjula M. Comparison of the Antioxidant Capacity of an Important Hepatoprotective Plants. Int J Pharm Sci Drug Res. 2011; 3(1): 48–51.
20. Re R, Nicoletta P, Anna P, Ananth P, Min Y, Catherine R-E. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26(9-10): 1231–7.
21. Alian Désiré A, Ascension NM, Foe N, Florentine C, Steve VO, Christine FM, et al. Antibacterial and Antioxidant Activities of Ethanolic Leaves Extracts of *Dissotis multiflora triana* (Melastomataceae). Int J Pharm Sci Drug Res. 2016 ;8(01): 50–56. https://ijpsdr.com/index.php/ijpsdr/article/view/480
22. Patel A, Patel A, Patel NM. Determination of polyphenols and free radical scavenging activity of *Tephrosia purpurea* linn leaves (Leguminosae). Pharmacognosy Res. 2010; 2(3): 152–158.
23. Chakraborthy GS. Antioxidant Activity of the Successive Extracts of Aesculus indica Leaves. Int J Pharm Sci Drug Res. 2009; 1(2): 121–123.
24. Wang HMD, Li XC, Lee DJ, Chang JS. Potential biomedical applications of marine algae. Bioresour Technol. 2017; 244(5):1407–1415. http://dx.doi.org/10.1016/j.biortech.2017.05.198
25. Raj GA, Chandrasekaran M, Jegan S, Venkatesalu V, Chandrasekaran M. Phytochemical Analysis, Antibacterial and Antifungal Activities of Different Crude Extracts of Marine Red Alga *Gracilaria corticata* From The Gulf of Mannar South Coast, Navaladi, South India. Int J Pharm Sci Drug Res. 2017; 9(2): 55–63. https://ijpsdr.com/index.php/ijpsdr/article/view/507
26. Arulkumar A, Rosemary T, Paramasivam S, Rajendran RB. Phytochemical composition, in vitro antioxidant, antibacterial potential and GC-MS analysis of red seaweeds (*Gracilaria corticata* and *Gracilaria edulis*) from Palk Bay, India. Biocatal Agric Biotechnol. 2018; 15: 63–71. https://doi.org/10.1016/j.bcab.2018.05.008
27. Narasimhan MK, Pavithra SK, Krishnan V, Chandrasekaran M. In vitro analysis of antioxidant, antimicrobial and antiproliferative activity of *Enteromorpha antenna*, *Enteromorpha linza* and *Gracilaria corticata* extracts. Jundishapur J Nat Pharm Prod. 2013; 8(4): 151–9. http://www.ncbi.nlm.nih.gov/pubmed/24624206%0A
28. Ashwini S, Shantaram M. Antioxidant capacity of extracts of red seaweed gracilaria corticata (J.Agardh) J.Agardh. Pharmanest. 2017; 8(3): 13–8.
29. Anis M, Yasmeen A, Baig SG, Ahmed S, Rasheed M, Hasan MM. Phycochemical and pharmacological studies on *Ulva fasciata* Delile. Pak J Pharm Sci. 2018; 31(3): 875–83.
30. Abirami RG, Kowsalya S. Quantification and Correlation Study on Derived Phenols and Antioxidant Activity of Seaweeds from Gulf of Mannar. J Herbs, Spices Med Plants. 2017; 23(1): 9–17. http://dx.doi.org/10.1080/10496475.2016.1240132
31. Divya, S. R and Chandramohan A. Phytochemical Analysis and Screening of Total flavonoid, Tannin and Phenolic Contents In *Gracilaria edulis* and *Hypnea valentiae*. Int J Adv Res. 2013; 3(5): 875–8.
32. Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. 2009; 116(1): 240–8.http://dx.doi.org/10.1016/j.foodchem.2009.02.041
33. García V, Uribe E, Vega-Gálvez A, Delporte C, Valenzuela-Barra G, López J, et al. Health-promoting activities of edible seaweed extracts from chilean coasts: Assessment of antioxidant, anti-diabetic, anti-inflammatory and antimicrobial potential. Rev Chil Nutr. 2020; 47(5): 792–800.
34. Carmona-Hernandez JC, Taborda-Ocampo G, González-Correa CH. Folin-Ciocalteu Reaction Alternatives for Higher Polyphenol Quantitation in Colombian Passion Fruits. Int J Food Sci. 2021; 2021: 1–10.
35. Bedoux G, Hardouin K, Burlot AS, Bourgougnon N. Bioactive components from seaweeds: Cosmetic applications and future development. Vol. 71, Advances in Botanical Research. Elsevier; 2014. pp. 345–378. http://dx.doi.org/10.1016/B978-0-12-408062-1.00012-3
36. Vijayavel K, Martinez JA. In vitro antioxidant and antimicrobial activities of two Hawaiian marine Limu: *Ulva fasciata* (Chlorophyta) and *Gracilaria salicornia* (Rhodophyta). J Med Food. 2010; 13(6): 1494–9.
37. Rabecca R, Doss A. Evaluation of antioxidant activity of *Hypnea valentiae* (Red algae), Gulf of Manner, Rameshwaram, Tamil Nadu. Jounral Adv Sci Res. 2021; 12(3): 195–200. http://www.sciensage.info/journal/1359303580JASR_3006121.pdf
38. Sreejamole KL, Greeshma PM. Antioxidant and brine shrimp cytotoxic activities of ethanolic extract of red alga *Gracilaria corticata* (J. Agardh) J. Agardh. Indian J Nat Prod Resour. 2013; 4(3): 233–7.
39. Gager L, Lalegerie F, Connan S, Stiger-Pouvreau V. Marine algal derived phenolic compounds and their biological activities for medicinal and cosmetic applications. In: Recent Advances in Micro and Macroalgal Processing: Food and Health Perspectives. 2021. pp. 278–334.

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