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Original Article

SCREENING OF PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTI-BACTERIAL ACTIVITY OF MARINE SEAWEEDS

RAJASEKAR T.*, MARY SHAMYA A., JERRINE JOSEPH

Centre for Drug Discovery and Development, Col. Dr. Jeppiaar Research, Park, Sathyabama Institute of Science and Technology (Deemed University), Chennai 600119

***Email: microraja09@gmail.com**

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ABSTRACT

Objective: Phytochemical is naturally present in the seaweeds which biologically play a significant role. The intention of this study was designed to screen the phytochemical constituents and antimicrobial potential of selected seaweed collected from Rameshwaram and Tuticorin Southern coast of India.

Methods: The present study investigated the presence of phytochemical constituents and also total phenol, total carbohydrate and total protein quantity of the brown seaweed. *Dictyopteris delicatula*, *Padina gymnospora*, *Acanthophora spicifera*, *Portieria hornemannii* and *Ulva faciata* were extracted with solvents having different polarities like methanol, ethanol, chloroform and water and screened for the phytochemical constituents, total phenol, total carbohydrate, total protein and DPPH with standard procedure. The antibacterial activities of the seaweeds were examined by agar well diffusion method.

Results: Among the five seaweeds, *U. faciata* showed the maximum number of active constituents in the methanol extract likewise *P. gymnospora* was found to have a number of diligent compounds in ethanol extract. *A. spicifera* showed minimum compounds in ethanol as well as chloroform extract. Moreover *A. spicifera*, *P. hornemannii* have shown the superior quantity of protein and carbohydrate when compared to other species. The scavenging activity of methanol extracts at 5 mg/ml concentration *P. hornemannii* shows 18.2% and *A. spicifera* possess 17.1%. In the antibacterial activity, methanol extracts of all the seaweed showed a potential inhibitory activity against *B. cereus* and *P. aeruginosa* compared to other pathogens.

Conclusion: The crude extract of seaweed manifest preferable antimicrobial and antioxidant activities, hence in the future, it would be good if it is further taken for treatment of human diseases or as new antimicrobial agents to replace synthetic antimicrobial agents.

Keywords: Seaweed, DPPH, Protein, Carbohydrate, *Dictyopteris delicatula, Padina gymnospora, Acanthophora spicifera, Portieria hornemannii, Ulva faciata*

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INTRODUCTION

Seaweed is a common name of marine algae, a group of species from the Protista kingdom, meaning they are not plants at all, even though they may look like underwater plants. They have therapeutic and nutritive properties as innovative, inventive sources of natural products. The bioactive compound obtained from this seaweed is having a unique structure and activity [1]. Most of the seaweed we consume is as food, feed, and fertilizer in many parts of the world. They constitute a vital part of marine ecosystems. These seaweeds are reservoirs of pigments, Polyphenols, enzymes, carotenoids, diverse functional polysaccharides [2-3]. They have a good source of vitamin A, B1, B12, D, C and E. These seaweeds are potential reservoirs of bioactive compounds, which might be a potential source of nutrients [4]. Today seaweeds are the raw material for industrial production of agar, alginic acid, and carrageenan, but they continue to be widely consumed as food in Asian countries [5]. Algal phytochemical extracts are used in various industries such as food, confectionery, textile, pharmaceutical, dairy and paper, mostly as gelling, stabilizing and thickening agents. They are living, renewable resources and may also a good source of protein, polysaccharides and fibers in addition of vitamins and minerals [6-7]. A wide range of studies has described the high antioxidant capacity of a range of edible seaweeds. This capacity is endowed by the presence of sulfated polysaccharides, Polyphenolic compounds and antioxidant enzymes. Oxidative stress may play a key role in the development of cancers and cardiovascular disease. Brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulfur-nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides, proteins, halogenated ketone, alkanes, and cyclic polysulfides are generally present in the seaweed as photochemical compounds [8]. These stunning medicinal properties comprise antibacterial, antifungal, antiprotozoal, antifertility, antiviral, anticancer and antimalarial activities [9-11]. Even hundreds of biologically active compounds are discovered till now. But the unique therapeutic compounds are needed for the upcoming diseases and drug-resistant microorganisms [12]. The phytochemical research approach is considered effective in discovering bioactive profile of the marine algae of therapeutic importance. The present study investigated the presence of phytochemical constituents and also total phenol, total carbohydrate and total protein quantity of the brown seaweed *D. delicatula, P. gymnospora* red seaweed *A. specifera, P. hornemannii* and green seaweed *Ulva faciata.*

MATERIALS AND METHODS

Chemical required

Alcoholic sodium hydroxide, distilled water, concentrated sulphuric acid, 1% aqueous iron chloride, acetic anhydride, sulphuric acid, glacial acetic acid, chloroform, sodium hydroxide, hydrochloric acid, ammonia, Bradford reagent, aqueous ferric chloride, copper sulphate.

Sample collection

The seaweeds were collected from Mandapam, Rameshwaram and Tuticorin Southern coast of India. It is authenticated by Dr. Anantharaman, Associate Professor, CAS Marine Biology, Parangipettai and Annamalai University. The collected sample was washed well with seawater to remove all the extraneous impurities such as epithets, sand particles, pebbles, and shells and brought to the laboratory in plastic bags aseptically in ice boxes. The samples were then thoroughly washed with tap water followed by distilled water. Washed seaweeds were blotted on the blotting paper, shade

dried at ambient temperature and the samples were grounded into a fine powder using tissue blender. The powdered samples were then stored in the refrigerator for further use. The classifications of seaweed are shown in table 1.

Table 1: Classification seaweeds

Seaweed	Kingdom	Order	Family	Genus	Species
Brown Seaweed 	Chromista	*Dictyotales*	*Dictyotaceae*	*Dictyopteris* *Padina*	*D. delicatula,* *P. gymnospora*
Red Seaweed 	*Plantae*	*Ceramiales*	*Rhodomelaceae*	*Acanthophora* *Portieria*	*A. spicifera* *P. hornemannii*
Green Seaweed 	*Plantae*	*Ulvales*	*Ulvaceae*	*Ulva*	*U. fasciata*

Preparation of seaweed extracts

Ten grams of powdered samples were packed in Soxhlet apparatus and extracted with (1:10) solvents like methanol, chloroform, and ethanol and aqueous for 8 h, and the filtrate was collected (crude extracts) and stored in the refrigerator until further use.

Phytochemical analysis

To detect the presence of following biomolecules by standard qualitative phytochemical procedures [13-14]. Test for alkaloids using Mayer's reagent, for tannins using ferric chloride, flavonoids using ammonia, for glycosides with chloroform and 10% ammonium solution [15]. Saponins test done by distilled water with vigorous shaking [16], the presence of terpenoid confirmed with chloroform and sulfuric acid [17]. Phlobatanin, steroids, cardiac glycoside, phenols, quinones, carbohydrate, protein, betacyanin, and coumarin are also tested with standard procedures.

Antimicrobial activity test

The antimicrobial activity extracts tested against five pathogenic bacteria *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Staphylococcus aureus* (obtained from the Medical Microbiology Laboratory, Periyar University, Salem, Tamil Nadu) by the Agar well diffusion method. Briefly, the Medium was prepared using Muller Hinton Agar Medium (HiMedia) in distilled water and autoclaved. The autoclaved medium was mixed well and poured onto 100 mm Petri plates (25-30 ml/plate) while still molten. The Muller Hinton medium was seeded with the 24hr culture of bacterial strains. Wells were cut, and 100 µl of the methanol extract stock (10 mg/ml) was added. The plates were then incubated at 37 °C for 12 to 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well [18]. The experiments were implemented in triplicates, and the mean±values were calculated and tabulated [19].

Antioxidant activity–DPPH method

Free radical scavenging inhibition assay it is used to determine the antioxidant capacity of the methanol extract of seaweed [20]. DPPH was prepared in ethanol and it has to be prepared freshly and used. Add 1 ml of methanol extract of seaweed sample in this 1 ml of DPPH solution were added it was incubated for about 30 min in a dark condition and the decrease in absorbance was monitored at 517 nm in UV-VIS spectrophotometer (UV-Shimadzu UV 1800). Ascorbic acid is used as a standard. The percentage of inhibition was calculated as

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank})x100}{Abs_{control}}\right]$$

Total phenol content

According to Wang *et al.* 2013 [21] total phenol content has been determined. 1 ml of seaweed extract was mixed with 1 ml of diluted 1:10 Folin-Ciocalteau reagent and 1 ml 20%, w/v sodium bicarbonate were added. And the solutions are kept at room temperature for about 30 min and the reading has been taken at 735 NM by UV-VIS spectrophotometer (UV3100PC, VWR International). Results were expressed as mg Phloroglucinol equivalents (PGE)/gdb.

Sulfate content

It involves acid hydrolysis of the sample followed by the determination of inorganic sulfate by turbid metric method [22]. After acid hydrolysis 0.2 ml of sample with 3.8 ml of 3% Trichloroacetic acid into this adds 1 ml of barium chloride-gelatin reagent was added and mixed well. Then it was incubated at room temperature for about 15 min, and the OD was measured at 360 NM by using a spectrophotometer. Potassium sulfate was used as a standard. Percentage of Sulphate content present in the sample was calculated by using this formula,

$$\text{Sulfate content of the sample (\%)} = \frac{\text{OD of the sample}}{\text{OD of the standard}} \times \text{Concentration of the std} \times \frac{100}{\text{Weight of the sample}}$$

Galactose content

It was determined by a colorimetric method using Anthrone reagent Yaphe *et al.* 1960 [23] and galactose was used as a standard. Anthrone reagent was prepared by dissolving 200 mg of reagent in 100 ml of 83.6 % sulfuric acid and stored at 4 °C for further use. In this 1 ml of sample mixed with Anthrone reagents (10 ml) and heated in a boiling water bath for about 11 min. The tube was then cooled and kept in an ice bath. The absorbance is taken at 630 nm.

Statistical analysis

All the outcome was summarized by mean±SD and statistical significance was estimated by one-way analysis of variance (ANOVA) using SPSS (version 16) program followed by LSD.

RESULTS AND DISCUSSION

Phytochemical

Seaweed contributes to its efficacy as nutraceutical and traditional medicine based on the presence of their chemical components. Some factors like climatic condition, season, species, subspecies, harvest and the method used for extraction of compounds will devastate the chemical compositions of the extract [24]. Seaweeds are primitive non-

flowering plants without roots, stems, and leaves. They contain different vitamins, minerals, trace elements, proteins and bioactive substances [25]. In our present study, five different extract methanol and aqueous extract exhibited the highest number of compounds whereas ethanol and chloroform are having the least possible number of phytochemical compounds. The presence of photochemical in different solvents Methanol (table 2), Aqueous (table 3), Ethanol (table 4) and chloroform (table 5). An alkaloid, flavonoids are having various medicinal properties and detected in the extracts are compounds that have been documented to possess a variety of medicinal properties and health-promoting effects. Alkaloids were absent in all the selected seaweeds; Phenols found to be plentiful as reported by Wang *et al.* 2013 [21]. The preliminary qualitative test shows the presence of tannins, betacyanin, flavonoid, terpenoid, betacyanin, carbohydrate, protein and phenol in methanol extract in most of the seaweed. Glycoside, cardiac glycoside, and quinone are present in some seaweeds of ethanol extract. Saponin is present in *D. delicatula,* and it is used for their ammonia emissions in animal feeding. Gomathi and Sheba reported that the presence of carbohydrates, protein, Glycosides, Anthraquinones, and Alkaloids in the methanol extract of *U. reticulate* [26].

Table 2: Phytochemical content of underexploited seaweeds in methanol extract

Phytochemical	**Methanol extract**				
	P. gymnospora	***D. delicatula***	***A. spicifera***	***U. faciata***	***P. hornemannii***
Alkaloid	-	-	-	-	-
Saponins	+	+	-	+	+
Tannins	+	+	+	+	+
Phlobatannins	+	+	-	-	+
Flavonoids	+	+	+	+	+
Steroid	+	+	+	+	-
Terpenoids	+	+	+	+	+
Glycoside	-	-	+	-	
Cardiac Glycosides	-	-	+	+	-
Phenolics	+	+	-	+	-
Quainones	-	+	+	-	+
Carbohydrate	+	+	+	+	+
Protein	+	+	+	+	+
Betacyanin	-	+	-	+	+
Cumarin	-	-	-	-	-

n=3;+indicates presence; -indicates absence

Table 3: Phytochemical content of underexploited seaweeds in the aqueous extract

Phytochemical	**Aqueous extract**				
	P. gymnospora	***D. delicatula***	***A. spicifera***	***U. faciata***	***P. hornemannii***
Alkaloid	-	-	-	-	-
Saponins	-	+	-	-	-
Tannins	+	-	+	+	-
Phlobatannins	-	-	-	-	-
Flavonoids	+	-	+	+	+
Steroid	+	+	+	-	-
Terpenoids	+	-	-	+	+
Glycoside	+	-	+	-	-
Cardiac Glycosides	+	-	+	-	-
Phenolics	+	-	+	-	-
Quainone	+	+	-	-	+
Carbohydrate	-	-	+	+	-
Protein	+	+	+	+	+
Betacyanin	-	-	-	+	-
Cumarin	-	+	-	-	-

n=3;+indicates presence; -indicates absence

Table 4: Phytochemical content of underexploited seaweeds in Ethanol extract

Phytochemical	**Ethanol extract**				
	P. gymnospora	***D. delicatula***	***A. spicifera***	***U. faciata***	***P. hornemannii***
Alkaloid	-	-	-		-
Saponins	-	+	-	-	+
Tannins	+	+	+	-	+
Phlobatannins	-	-	-	-	-
Flavonoids	+	-	+	-	-
Steroid	-	-	-	-	+
Terpenoids	+	+	-	-	+
Glycoside	+	-	+	-	-
Cardiac Glycosides	+	-	+	-	-
Phenolics	+	-	-	-	-
Quainone	+	+	+	-	+
Carbohydrate	-	-	-	-	-
Protein	+	+	+	+	+
Betacyanin	-	-	+	+	-
Cumarin	-	+	-	-	-

n=3;+indicates presence; -indicates absence

Table 5: Phytochemical content of underexploited seaweeds in Chloroform extract

Phytochemical	Chloroform extract				
	P. gymnospora	*D. delicatula*	*A. spicifera*	*U. faciata*	*P. hornemannii*
Alkaloids	-	-	-	-	-
Saponins	+	+	-	-	+
Tannins	+	-	+	+	+
Phlobatannins	-	-	-	+	-
Flavonoids	+	-	+	-	+
Steroid	+	+	+	-	-
Terpenoids	+	-	-	-	+
Glycosides	+	-	+	-	-
Cardiac Glycosides	+	-	+	-	-
Phenolics	+	-	-	-	-
Quanoin	+	+	+	+	+
Carbohydrate	-	-	+	-	+
Protein	-	-	+	-	+
Betacyanin	+	-	+	+	-
Cumarin	-	-	-	+	+

n=3;+indicates presence; -indicates absence

Biochemical and antioxidant activity

The qualitative analysis of phytochemical investigation revealed the presence of carbohydrates, protein, and phenols in all selected seaweeds. Among the five seaweeds, the carbohydrate was varied from 50.6 to % of dry weight to 26% dry weight showed a high carbohydrate content than other seaweeds was reported by Roy and Anantharaman 2017 [24]. Nguyen *et al.* 2011[27] reported that high carbohydrate content (64.00% dry weight) present in *Caulerpa lentillifera*, Similarly, we have a high percentage of carbohydrate in *P. hornemannii* (51.2±0.78 %) and *A. spicifera* (45.4%). Quantitative analysis of protein content ranged from 28.5 % and 4.4 %. Dhargalkar *et al.* 1986 [28] reported that proximate biochemical composition of some seaweed from Mandapam which revealed that *P. gymnospora* contained maximum protein (17.08±0.28%) including *Enteromorpha intestinalis* (16.38±0.50%) and *Sargassum tenerimum* (12.42±0.63%). In this present study protein content of the *P. gymnospora* was 25.7±0.89 % of dry/wt. of the seaweeds. Higher protein content was found in the brown seaweed *T. ornate* and lower in the red seaweed *G. verrucosa* reported by Parthiban *et al.* 2013[29]. Whereas *A. spicifera* was showed 28.6±0.53 % dry/wt high amount of protein content. Similarly, Dinesh *et al.* 2007[30] recorded highest protein content in brown alga *Tubinaria ornate* from Gulf of Mannar region, and Selvi *et al.* 1999[31] reported more protein content in red alga *Hypnea valentiae*. The antioxidant activity seaweed extract was measured using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), hydroxyl radical scavenging; it is a less time-consuming method when compared to other methods. It is neutralized by single electron transfer (SET) or by hydrogen atom transfer (HAT). The reaction mixture color was slowly changed from purple to yellow, and it is measured by spectrophotometer [32-33]. The scavenging activity of methanol extract compared with the standard Gallic acid. At 5 mg/ml concentration *P. hornemannii* fascinate 18.2±0.45% and *A. spicifera* possess17.1±0.57% scavenging activity on DPPH. Whereas Revathi *et al.* [34] reported that *Hypneava lentiae* showed 41.22±0.65 % scavenging activity at 50 mg/ml concentration. *D. delicatula* showed a high amount of sulfate content followed by *P. hornemannii*, *A. spicifera*, *U. facita*, *P. gymonospora* [35]. Likewise, galactose, total phenol content and sulfate content of *D. delicatula* showed the best result when compared to other seaweed. The existence and percentage of biochemical and antioxidant level in the selected seaweed were depicted in fig. 1.

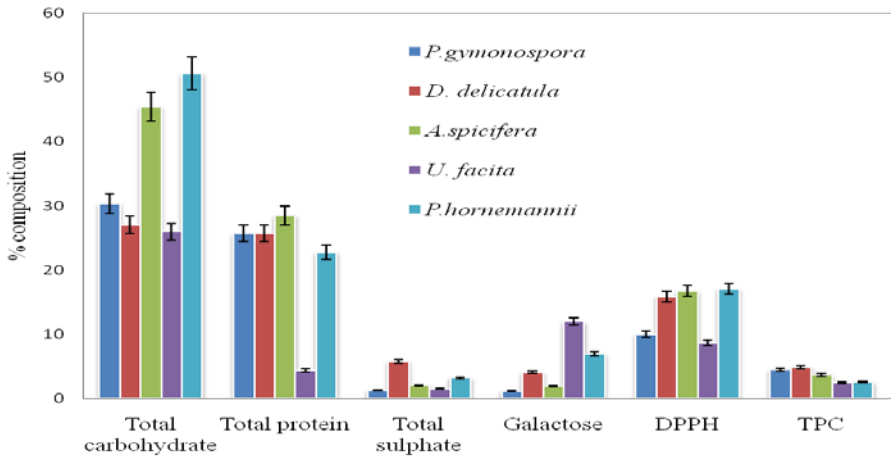

Fig. 1: Biochemical and antioxidant activity and the values are expressed as mean±SD; n=3

Antibacterial activity of seaweeds

Priyadharshini *et al.*,(2011) [36] have reported that seaweeds are an excellent source of components such as polysaccharides, tannins, flavonoids, phenolic acids, bromophenols, and carotenoids has exhibits different biological activities. Depending upon their solubility and polarity, different solvents shows the different antimicrobial activity. So chemical compounds should be extracted from different seaweeds in order to optimize their antibacterial activity by selecting the best solvent system.

The antimicrobial activity of the methanol crude seaweed extract

showed a prospective activity against most of the pathogens and it has been portrayed in fig. 2. Methanol have higher antibacterial activity than that of extracts obtained with other organic solvents (Lavany and veerappan, 2011) [37]. Devi *et al.*, 2008 [38] reported that the methanol extract of seaweeds contains phenolics, alkaloids and amino acids which may be responsible for the antimicrobial activity. Earlier reported that ethanol extract of marine seaweed *Enteropmorpha compressa*, *Cladophorosis zollingeri*, *P. gymnospora*, *S. wightii*, and *G. corticata* are active against most of the gram-positive and negative bacteria [39]. In this present study, we conclude that methanol extract showed an adequate amount of phytochemical compounds hence it is taken for antimicrobial activity testing. *P. gymonospora* exhibit maximum activity to *B. cereus* (2.4±1.57 mm) and minimum activity against *C. albicans*, *D. delicatula* showed better activity towards *P. aeruginosa* (2.0±0.28 mm) and lower activity against *S. aureus* (0.8±1.20 mm), *A. spicifera* exhibit higher activity on *B. cereus* (2.3±0.06 mm) and lower activity on *S. aureus* (0.5±1.22 mm), *Ulva facita* possess better activity towards *P. aeruginosa* (2.3±0.28 mm) and least activity against *C. albicans* (0.9±2.31 mm), P. hornemannii showed maximum activity against *B. cereus* (1.6±0.4 mm) minimum activity towards *S. aureus* (1.1±0.6 mm). Lavanya *et al.* 2017 [40] state that the least inhibition was observed in methanol and ethanol extract of seaweed. *E. coli* (3.5 mm), *P. putida* (2.5 mm), *K. pneumonia* (4.3 mm), and *S. aureus* (3.5 mm) against *Gracilaria sps*. In future, this present findings may come as a better potent antibacterial compound.

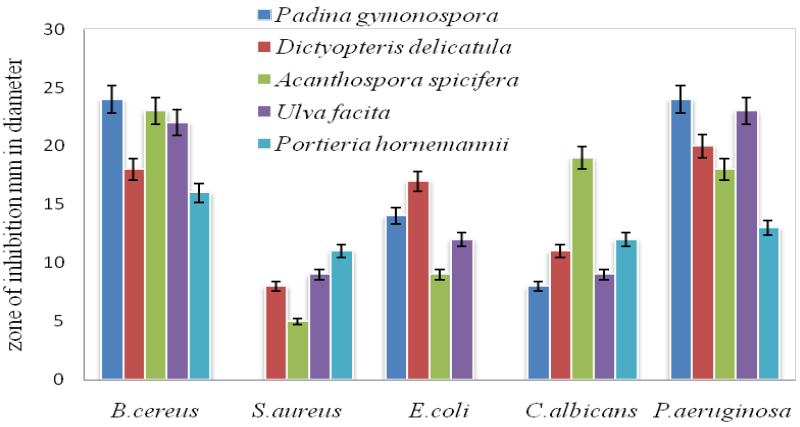

Fig. 2: Antibacterial activity of seaweeds and the values are expressed as mean±SD; n=3

CONCLUSION

The phytochemical and biochemical analysis of these seaweeds has sufficient nutrient supplements such as proteins, carbohydrates, phenol, etc. It has been taken as a feed for cattle and act as a good fertilizer for plant growth, cost-effective nutrient supplement with predominant yield. Also, seaweeds may solve the problems of deficiency of protein, carbohydrate, and mineral deficiency in human nutrition by consuming them in daily life. Further seaweed being a novel candidate in pharmaceuticals to develop a natural compound as an anticancer agent for production. Our results revealed that the marine alga has several chemical constituents of high therapeutic efficacy. Further studies are required to investigate the extracts of for potential pharmacological properties.

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AUTHORS CONTRIBUTIONS

R. T. has designed, directed the study and co-wrote the paper; M. S. performed the experiments, interpreting the results and worked on the manuscript; J. J. helped in paper writing and correction. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTERESTS

Declared none

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