Journal of Coastal Life Medicine

Journal homepage: www.jclmm.com

Original article doi:10.12980/JCLM.3.201514J26

Phytochemical analysis and antifungal activity of selected seaweeds from Okha coast, Gujarat, India

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ARTICLE INFO

Article history:
Received 21 Mar 2014
Received in revised form 22 Mar, 2nd revised form 2 Apr 2014
Accepted 3 Jan 2015
Available online 12 Jun 2015

Keywords:
Antifungal activity
Biochemical compounds
Marine seaweeds
Okha coast
Gujarat

ABSTRACT

Objective: To deal with the assessment of the chemical composition of carbohydrate, protein, phenol, flavanoid, chlorophyll, and carotenoid and antifungal activity of various marine seaweeds collected from Okha coast, Gujarat during September, 2013.
Methods: Biochemical compounds of selected seaweeds were quantified and antifungal activity of these species belonging to red, green, and brown seaweeds was explored and the seaweeds were extracted in acetone, ethanol and chloroform.
Results: The carbohydrate content was highest in *Cystoseira indica* Mairh, protein was highest in *Gracilaria corticata* J. Agardh and phenol content was highest in *Padina boergesenii*; flavanoid content was found greater in *Cystoseira indica*, chlorophyll content was found greater in *Monostroma latissimum* Wittrock and carotenoid content was more in *Dictyopteris acrostichoides* Bornet. The highest inhibiting effect was noted for *Sargassum tenerrimum* J. Agardh and *Turbinaria ornata* J. Agardh belonging to brown algae, against *Aspergillus niger* and *Penicillium janthinellum* in chloroform extracts and ethanolic extracts, which caused opportunistic infection of HIV-infected person, lung disease, aspergillosis, and otomycosis (fungal ear infections).
Conclusions: The study reveals that the seaweeds contain high amount of biochemical constituents. Besides, the crude extracts of the seaweeds showed promising activity against the tested fungal pathogens. Therefore, seaweeds collected from Okha coast, Gujarat region are biochemical compounds with potential capacity which make them useful for screening natural products for pharmaceutical industry.

1. Introduction

Marine seaweeds comprise thousands of species and they represent a considerable part of the littoral biomass. According to their nutrient value and chemical composition, they are classified as red (Rhodophyta), brown (Phaeophyta), and green seaweeds (Chlorophyta)[1]. Many seaweed species are used in the industry, principally for the extraction of phycocolloids and as a source of pharmaceutical substances. Also, they are used as herbal medicine, fertilizer, fungicides, and herbicides and for the direct use in human nutrition, too[2-4]. Seaweeds are known as a highly nutritive food containing vitamin, protein, mineral, fiber contents, and essential fatty acids[5]. Seaweeds are the only source of phytochemicals, namely, agar·agar, carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents.

Parekh *et al.* studied the chemical compositions of 27 species of green seaweeds of Saurashtra coast that are the raw material for many industries[6]. Dinesh *et al.* studied the nutritive properties of 20 species of seaweeds from Gulf of Mannar[7]. The seaweeds are also known to contain bioactive products that display antibacterial, antiviral and antifungal properties[8]. Seaweeds are exposed to seasonal variations of abiotic factors that influence their metabolic responses (photosynthesis and growth rates) and levels of proximate constituents[9]. Seasonal variations on the chemical composition and nutritive value have been reported in common marine seaweeds from different parts of the world[10-12].

A large number of algal extract products have been found to have antimicrobial activity. Seaweeds represent a potential source of antimicrobial substances due to their diverse secondary metabolites with antiviral, antibacterial, and antifungal activities[13-15]. Zovko *et al.* studied antifungal activity against fungal strains

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Foundation Project: Supported by University Grant Commission (UGC-F No. 42-415), New Delhi.

of *Candida albicans* with a high activity of algal extracts[16]. Gao *et al.* showed that a few extracts of marine algae have not only an antifungal activity but toxicity towards cancer cells[17]. Several extractable compounds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and, therefore, responsible for the antibiotic activity of some seaweeds[18,19]. Some commonly occurring marine algae *Caulerpa scalpelliformis, Ulva lactuca, Padina tetrastromatica, Stoecchospermum marginatum* and *Acanthophora spicifera* have been collected from the coast of Tuticorin, Tamilnadu and evaluated for antifungal and antibacterial activity by using four solvents such as petroleum ether, chloroform, methanol and benzene by Jothibai *et al.*[20]. Many marine algae were screened for their antimicrobial activity by Reichelt and Borowitzka and Salvador *et al.* who studied antimicrobial activities of 82 marine algae[21,22]. Bansemir *et al.* have investigated the antibacterial activities of the extracts from 26 algal species prepared by dichloromethane, methanol and water against five fish-pathogenic bacteria[23]. Therefore, the present paper aims to analyze variations of the levels of proximate constituents like protein, carbohydrate, phenol, flavanoid, chlorophyll and carotenoid and antifungal activity of the seaweeds.

2. Materials and methods

2.1. Study area

Okha coast, situated at 22°28' N and 69°05' E in the north of Gulf of Kutch on the north-westernmost part of Saurashtra in Gujarat (Figure 1), is one of the most important places of interest for algal growth in India. This coast being the north of Gulf of Kutch experiences strong water currents all the year round compared to other parts of the country. The coast is characterized by rocks made up of tertiary formations alternating with patches of sand deposits, making the area more hospitable for the growth of all types of marine algae throughout the year.

2.2. Sampling

The seaweeds samples were collected during September, 2013, picked with hand and immediately washed with seawater to remove the foreign particles, sand particles and epiphytes. Then they were kept in an ice box and immediately transported to the laboratory and washed thoroughly with tap water to remove the salt on the surface of the samples. After that, the species were identified by method of Jha *et al.*[24]. They were spread on blotting paper to remove excess water. The air dried samples were placed in an oven at 50 ˚C and water content was calculated. Then the samples were pulverized in the grinder and sieved through a screen with an aperture of 0.5 mm. Then, the powdered material was kept in airtight plastic bottles at room temperature until further analysis.

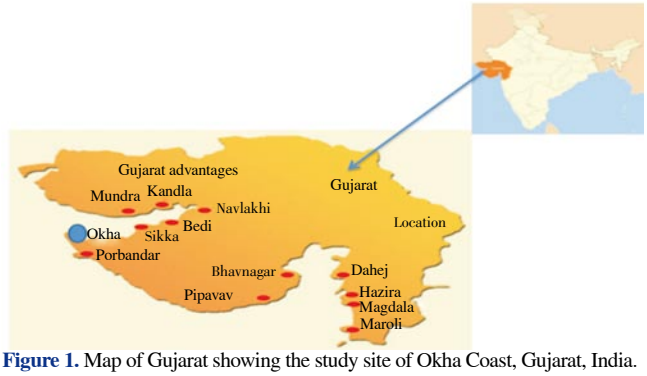

Figure 1. Map of Gujarat showing the study site of Okha Coast, Gujarat, India.

The total carbohydrate content was estimated by anthrone method[25], and protein was quantified by Biurette method[26]. Total phenolic assay was determined by using Folin Ciocalteu assay[27]. Total flavanoid content was measured by the aluminum chloride calorimetric assay[28]. The amount of chlorophyll-*a* present in the alga was estimated by method of Arnon[29]. The amount of carotenoid was determined by method of Parsons and Strickland[30].

2.3. Extraction

The collected samples were air-dried and coarsely powdered. The powdered form of seaweeds was subjected to step wise extraction using acetone, chloroform and ethanol by soxhlation process. The three different extraction solvents were used according to the order of their polarity as different compounds get extracted in different solvents. The crude extracts were concentrated under reduced pressure to get their corresponding residues. The seaweed extracts were further subjected for antifungal activity by agar cup plate method[31]. For comparing antifungal activity of selected seaweeds with known broad spectrum antifungal drug, three standard antifungal disc of fluconazole (10 µg), ketoconazole (10 µg) and amphotericin b (20 µg) were used for antifungal assay, and

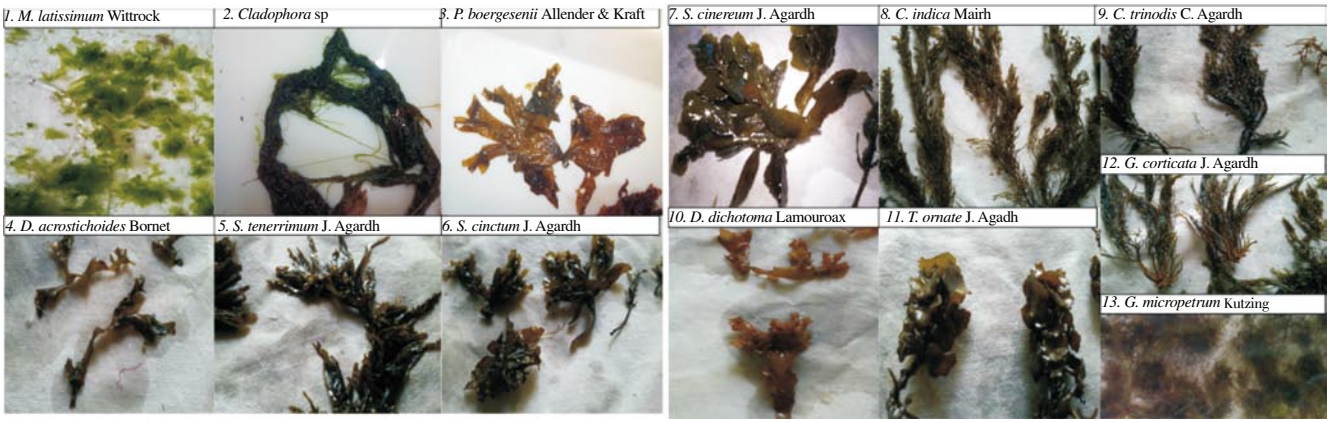

Figure 2. Identified seaweeds collected from the site.

chloroform (100 μL) and ethanol (100 μL) were used as control. Each selected seaweeds *Sargassum tenerrimum* J. Agardh (*S. tenerrimum*), *Turbinaria ornate* J. Agardh (*T. ornate*), *Cladophora* sp, *Dictyopteris acrostichoides* Bornet (*D. acrostichoides*), *Sargassum cinereum* J. Agardh (*S. cinereum*), *Cystoseira indica* Mairh (*C. indica*), *Cystoseira trinodis* C. Agardh (*C. trinodis*), *Gelidium micropterum* Kutzing (*G. micropterum*) extract were subjected to two fungal species *Aspergillus niger* (*A. niger*) (NCBI accession number, KC545848) and *Penicillium janthinellum (P. janthinellum)* (NCBI No. KC545842) used for antifungal assay.

3. Results

Thirteen seaweeds were identified based on their morphological criteria mentioned in Figure 2. Thirteen species of seaweeds belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae were collected from Okha coast, Gujarat (Table 1) and their percent class wise distribution is represented in Figure 3.

Table 1
List of the seaweeds belonging to different classes, collected from Okha coast, Gujarat during September, 2013.

Class	Species
Chlorophyta	*M. latissimum* Wittrock, *Cladophora* sp.
Phaeophyta	*P. boergesenii* Allender & Kraft, *D. acrostichoides*, *S. tenerrimum*, *S. cinctum* J. Agardh, *S. cinereum*, *C. indica*, *C. trinodis*, *D. dichotoma* Lamouroax, *T. ornate*
Rhodophyta	*G. corticata* J. Agardh, *G. micropterum*

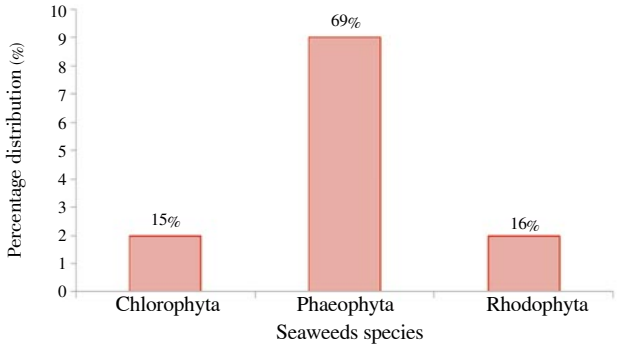

Figure 3. Percentage distribution of seaweeds at selected site.

3.1. Biochemical analysis

Biochemical analysis of carbohydrate, protein, total phenol, flavanoid, chlorophyll *a* and carotenoide content of seaweeds are presented in Figures 4-9.

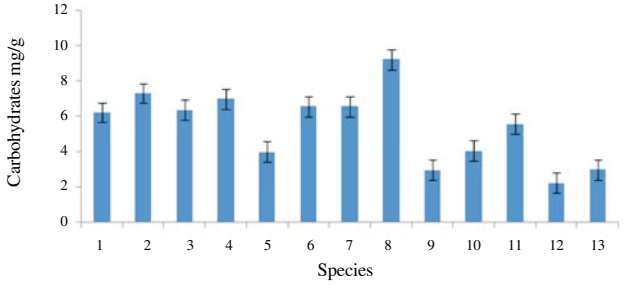

Figure 4. Carbohydrate content of different seaweeds collected from Okha coast.
1. *M. latissimum*; 2. *Cladophora* sp; 3. *P. boergesenii*; 4. *D. acrostichoides*; 5. *S. tenerrimum*; 6. *S. cinctum*; 7. *S. cinereum*; 8. *C. indica*; 9. *C. trinodis*; 10. *D.dichotoma*; 11. *T. ornate*; 12. *G. corticata*; 13. *G. micropetrum.*

The carbohydrate content varied from (2.247±0.200) to (9.219 ±0.300) mg/g; maximum carbohydrate was recorded in *C. indica* [(9.21±0.40) mg/g] followed by *Cladophora* sp. [(7.3±0.4) mg/g] and *D. acrostichoides* [(6.99±0.20) mg/g]. Moreover, the minimum carbohydrate was observed in *Gracilaria corticata (G. corticata)* [(2.247±0.200) mg/g] followed by *C. trinodis* [(2.96±0.20) mg/g] and *G. micropterum* [(2.98±0.40) mg/g] (Figure 4).

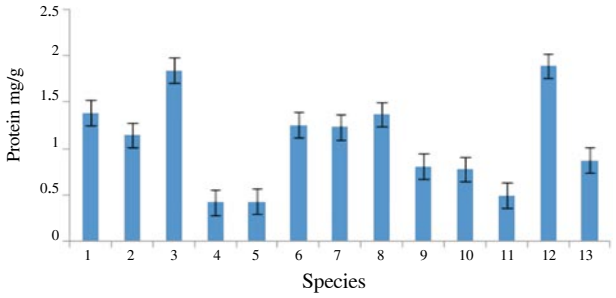

Figure 5. Protein content of different seaweeds collected from Okha coast.
1. *M. latissimum*; 2. *Cladophora* sp; 3. *P. boergesenii*; 4. *D. acrostichoides*; 5. *S. tenerrimum*; 6. *S. cinctum*; 7. *S. cinereum*; 8. *C. indica*; 9. *C. trinodis*; 10. *D. dichotoma*; 11. *T. ornate*; 12. *G. corticata*; 13. *G. micropetrum.*

The protein concentration of seaweeds ranged from (0.429±0.020) to (1.888 7±0.300 0) mg/g; highest protein was registered in *G. corticata* [(1.888 7±0.400 0) mg/g] followed by *Padina boergesenii* (*P. boergesenii*) [(1.839 2±0.400 0) mg/g], *Monostroma latissimum* (*M. latissimum*) [(1.384±0.200) mg/g], *C. indica* [(1.367±0.100) mg/g] and *Sargassum cinctum* (*S. cinctum*) [(1.250 7±0.200 0) mg/g]. Whereas the lowest protein content was recorded from *S. tenerrimum* [(0.429±0.020) mg/g] followed by *D. acrostichoides* [(0.423 5± 0.020 0) mg/g], *T. ornate* [(0.493 9±0.010 0) mg/g] and *Dictyota dichotoma* (*D. dichotoma)* [(0.777 7±0.020 0) mg/g] (Figure 5).

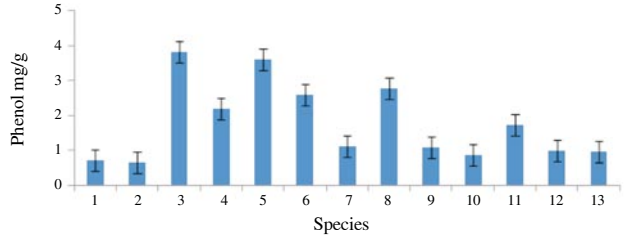

Figure 6. Phenol content of different seaweeds collected from Okha coast.
1. *M. latissimum*, 2. *Cladophora* sp, 3. *P. boergesenii*, 4. *D. acrostichoides*, 5. *S. tenerrimum*, 6. *S. cinctum*, 7. *S. cinereum*, 8. *C. indica*, 9. *C. trinodis*, 10. *D. dichotoma*, 11. *T. ornate*, 12. *G. corticata*, 13. *G. micropetrum.*

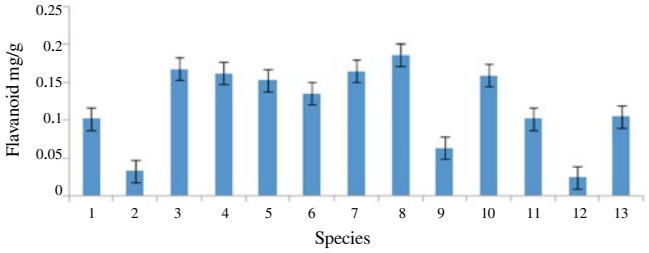

Figure 7. Flavanoid content of different seaweeds collected from Okha coast.
1. *M. latissimum*; 2. *Cladophora* sp; 3. *P. boergesenii*; 4. *D. acrostichoides*; 5. *S. tenerrimum*; 6. *S. cinctum*; 7. *S. cinereum*; 8. *C. indica*; 9. *C. trinodis*; 10. *D. dichotoma*; 11. *T. ornate*; 12. *G. corticata*; 13. *G. micropetrum.*

The phenol content of seaweeds fluctuated from (0.658±0.020) to (3.808±0.500) mg/g; maximum phenol was encountered in *P. boergesenii* [(3.808±0.500) mg/g] followed by *S. tenerrimum* [(3.598

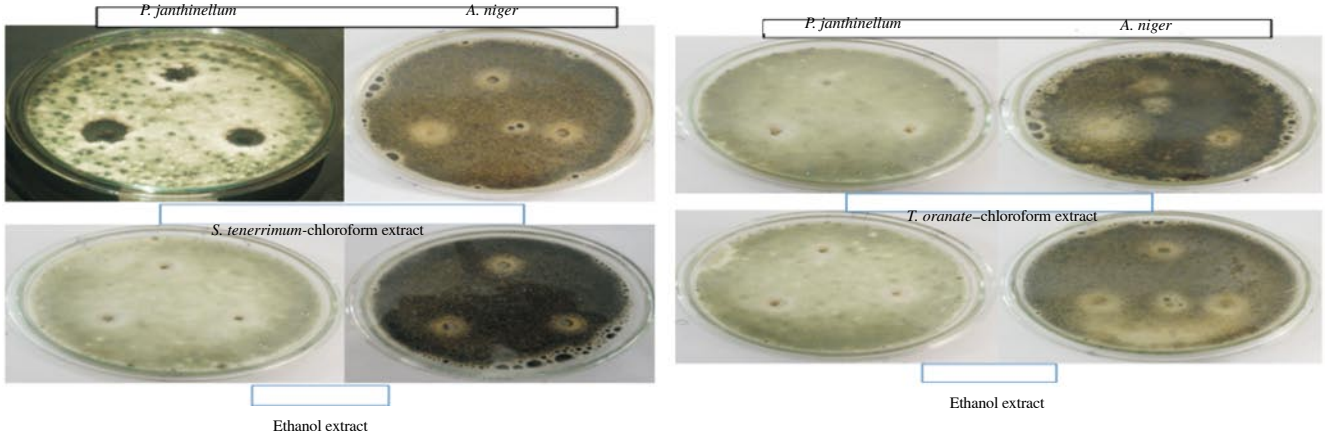

Figure 10. Antifungal assay of *S. tenerrimum* and *T. oranate.*

±0.400) mg/g], *S. cinereum* [(2.765±0.400) mg/g] and *S. cinctum* [(2.576±0.200) mg/g]. However, the minimum phenol was noticed in *Cladophora* sp. [(0.658±0.060) mg/g] followed by *M. latissimum* [(0.707±0.080) mg/g], *D. dichotoma* [(0.882±0.030) mg/g] and *G. micropterum* [(0.959±0.010) mg/g] (Figure 6).

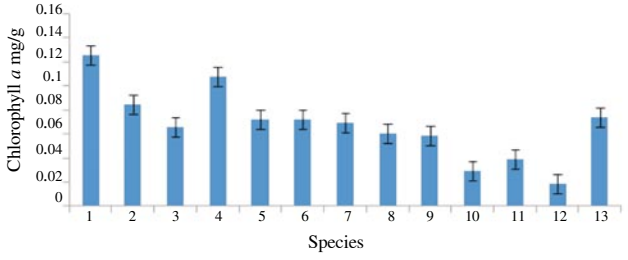

Figure 8. Chlorophyll a content of different seaweeds collected from Okha coast.
1. *M. latissimum*; 2. *Cladophora* sp; 3. *P. boergesenii*; 4. *D. acrostichoides*; 5. *S. tenerrimum*; 6. *S. cinctum*; 7. *S. cinereum*; 8. *C. indica*; 9. *C. trinodis*; 10. *D. dichotoma*; 11. *T. ornate*; 12. *G. corticata*;-- 13. *G. micropetrum.*

The maximum flavanoid concentration fluctuated from (0.024± 0.002) to (0.186±0.030) mg/g; higher content was recorded in *C. indica* [(0.186±0.030) mg/g] followed by *P. boergesenii* [(0.168± 0.020) mg/g] and *S. cinereum* [(0.165±0.020) mg/g]. However the lower content was observed in *G. corticata* [(0.024±0.002) mg/g] followed by *Cladophora* sp. [(0.033±0.002) mg/g] and *C. trinodis* [(0.063±0.001) mg/g] (Figure 7). The chlorophyll *a* concentration ranged from (0.019±0.001) to (0.125 8±0.020 0) mg/g; maximum concentration was found in *M. latissimum* [(0.125 8±0.020 0) mg/10 mL], followed by *D. acrostichoides* [(0.107 8±0.020 0) mg/10 mL] and *Cladophora* sp. [(0.084 5±0.001 0) mg/10 mL]. The minimum content was observed in *G. corticata* [(0.019±0.001) mg/10 mL], followed by *D. dichotoma* [(0.029 4±0.002 0) mg/10 mL] and *T. ornate* [(0.039±0.001) mg/10 mL] (Figure 8).

The carotenoid concentration fluctuated from (0.042±0.001) to (0.161±0.010) mg/g; the content was greater in *D. acrostichoides* [(0.161±0.010) mg/g] followed by *P. boergesenii* [(0.138±0.010) mg/g] and *G. micropterum* [(0.124±0.010) mg/g]. The lower concentration was found in *D. dichotoma* [(0.042±0.001) mg/g] followed by *T. ornate* [(0.057±0.002) mg/g] and *G. corticata* [(0.074 ±0.002) mg/g] (Figure 9).

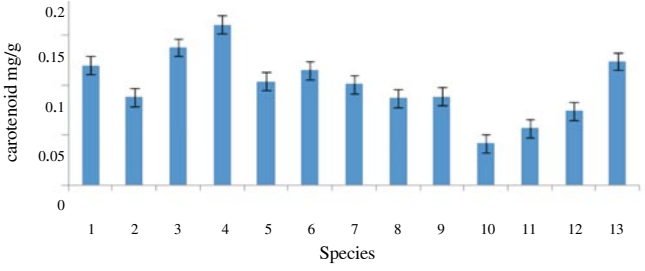

Figure 9. Carotenoid content of different seaweeds collected from Okha coast.
1. *M. latissimum*, 2. *Cladophora* sp, 3. *P. boergesenii*, 4. *D. acrostichoides*, 5. *S. tenerrimum*, 6. *S. cinctum*, 7. *S. cinereum*, 8. *C. indica*, 9. *C. trinodis*, 10. *D. dichotoma*, 11. *T. ornate*, 12. *G. corticata*, 13. *G. micropetrum*.

3.2. Antifungal assay

Different extracts of eight seaweed species were tested for their antifungal activity against two strains *A. niger* and *P. jenthinellum*, by cup plate method. The chloroform extracts of *S. tenerrimum* and *T. ornate*, showed considerable antifungal activity. *S. cinereum* and *Cladophora* sp. also shown the resistance against tested organism.

Table 2
Antifungal activity of two species.

Test organisms	Inhibition zone (mm)												
	Chloroform extract of *S. tenerrimum*		Ethanol extract of *S. tenerrimum*		Chloroform extract of *T. ornate*		Ethanol extract of *T. ornate*		Standard			Control	
	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	Fluconazole 10 µg	Ketoconazole 10 µg	Amphotericin B 20 µg	Chloroform 100 µL	Ethanol 100 µL
A. niger	19	20	9	12	11	13	10	14	10	17	18	8	8
P. jenthinellum	12	14	13	15	13	16	10	11	12	20	19	9	9

S. tenerrimum and *T. ornate* shows high resistance against selected pathogenic fungus and the resistance were higher in Chloroform and Ethanolic extract of these two seaweeds then standard antifungal drug. (Fluconazole, Ketoconazole, Amphotericin B).

The results of antifungal activity against tested pathogens were tabulated in the Table 2 for the crude extractions of *S. tenerrimum* and *T. ornate* for antifungal activity. Table 3 represented zone diameter of other species which shown the moderate and low activity against tested pathogen.

The zone of inhibition of seaweeds extract with high activity for two different pathogen was depicted in Figure 10.

Table 3

Antifungal activity of seaweeds with moderate and low activity.

Seaweed species		Inhibition zone diameter (mm)			
		A. niger		*P. janthinellum*	
		50 µL	100 µL	50 µL	100 µL
Cladophora sp.	CE	12	12	11	13
	AE	7	9	NA	7
	EE	6	9	7	7
D. acrostichoides	CE	19	20	8	9
	AE	NA	NA	NA	NA
	EE	8	12	13	15
S. cinereum	CE	NA	6	9	11
	AE	NA	8	NA	NA
	EE	7	7	4	5
C. indica	CE	NA	NA	12	12
	AE	7	9	NA	7
	EE	5	9	6	5
C. trinodis	CE	7	7	13	16
	AE	7	8	7	8
	EE	10	11	5	6
G. micropterum	CE	11	13	9	10
	AE	NA	NA	NA	7
	EE	8	7	5	6

CE: chloroform extract; AE: acetone extract; EE: ethanol extract; NA: no activity.

4. Discussion

The results of the phytochemical analysis and antifungal screening revealed the presence of high amount of biochemical compounds and antifungal substances in seaweeds studied. From the study, maximum carbohydrate was recorded in *C. indica* belonging to Phaeophyceae and some seaweeds with high carbohydrate contents are the Chlorophyceae members. Similarly, Chakraborthy and Santra recorded higher carbohydrate in the green seaweeds like *Ulva lactuca* (35.27%) and *Encephalitozoon intestinalis* (30.58%) [32]. Kaliaperumal *et al.* also reported similar kind of results that the green seaweeds have high carbohydrate than the red and brown seaweeds[33]. Investigations of Pise and Sabale revealed that the maximum carbohydrate was recorded in *Sargassum* a brown alga and minimum was found in *Gracilaria*, a member of Rhodophyceae which is corroborated with the present investigations[34].

In the present study the highest protein content was encountered in the brown alga *P. boergesenii* and red algae *G. corticata* other than the green alga. Similarly Dinesh *et al.* recorded highest protein content in brown alga *T. ornata* from Gulf of Mannar region and Anitha *et al.* recorded maximum protein in the brown alga *T. conoides* and minimum in *G. corticata* from the same Mandapam coast[7,35]. Besides, Selvi *et al.* reported more protein content in red alga *Hypnea valentiae* whereas Mairh *et al.* reported 22.22% of crude protein in *Ulva fasciata*[36,37] .

The amount of total phenol and flavanoid was higher in the brown seaweeds *P. boergesenii* and *C. indica*, respectively. Marry and Vimalabai screened four brown seaweeds from Tuticorin coast for their phenol content and reported highest value in *Padina tetrastromatica*[38]. Pedersen reported that the phenol content increased with the increasing age of the tissue andincreasing salinity[39]. The highest total chlorophyll was recorded in the green alga *M. latissimum* and minimum in the red alga *G. corticata*. Similarly Muthuraman and Ranganathan reported maximum chlorophyll in the green alga *Caulerpa scalpelliformis* among the 12 seaweeds tested which included Phaeophyceae and Rhodophyceae member also[40]. The highest carotenoid content was recorded in the brown seaweed *D. acrostichoides*, similarly Muthuraman and Ranganathan reported maximum carotenoid content in the brown seaweed[40].

The study evaluated the activity of different species of seaweeds from the Okha coast against pathogenic fungus. As for the tests with pathogenic fungus, the extracts showed differences in their activity depending on the solvent used in the extraction. The brown seaweeds show high antifungal activity as compare to red and green algae. The chloroform and ethanol extract of *S. tenerrimum* and *T. ornate* showed highest antifungal activity against tested pathogenic organism than other seaweeds whereas *S. cinereum*, and *Cladophora sp.* shown moderate activity against tested pathogens. The acetone extract of seaweeds showed minimum activity against both organisms. In the present study, the species of Phaeophyta showed the strongest activities against fungi, which was in agreement with the findings of Padmakumar and Ayyakkannu[41]. The brown seaweeds contain high amount of flavanoid and phenolic compound could be the reason for antifungal activity, Cowan *et al.*[42], further confirmed the greater amounts of phenolic compound in brown algae in the present investigation.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The author would like to thanks University Grant Commission, New Delhi for financial support.

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