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ORIGINAL RESEARCH ARTICLE

Assessment of phytochemicals from marine algae *Ulva fasciata* and *Dictyota dichotoma* with antioxidant and antimicrobial potential

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ABSTRACT

The marine ecosystem is a rich source of novel secondary metabolites with significant biomedical applications. Seaweeds are considered as the treasury of secondary metabolites with various biological activities. This study aims to analyze antioxidant and antimicrobial potential in green seaweed *Ulva fasciata* and brown seaweed *Dictyota dichotoma*. Extracts from four different solvents such as petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet apparatus were tested for the qualitative analysis of phytochemicals. Secondary metabolites were analyzed quantitatively to correlate with the antioxidant (DPPH assay) and antimicrobial potential of seaweeds. Results showed a better antioxidant activity of *U. fasciata* in its methanolic extract (89.29%) and *D. dichotoma* manifested a maximum antioxidant activity (70.1%) for its ethyl acetate extract. Structural characteristics of seaweed derived bioactive material were investigated by Fourier transform infrared spectroscopy (FTIR) and manifested the presence of alcohol and phenolic compounds. The inhibition zone formed around the crude extract reveals the antimicrobial nature of bioactive substances of seaweed extract against the pathogens. High inhibition and antioxidant activity indicate an effective drug's evolution from seaweeds against human pathogens.

Keywords: seaweed; bioactive extract; antioxidant; antimicrobial

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1. Introduction

Marine macroalgae, also known as seaweeds, are the primitive element in the marine ecosystem. Seaweeds are obscure and inexhaustible sources of diverse bioactive compounds which exhibit tremendous biological activities[1]. The compounds possessing bioactivities are excellent raw materials for the pharmaceutical, cosmetics and food industries[2–4]. Because of the increased demand for natural resources in therapeutic industries, seaweeds are getting significant attention because of their potential to produce a great variety of secondary metabolites[1,5]. The research on the phytochemical production of seaweeds is comprehensive and attractive. Apart from terrestrial plants, seaweeds are different because of their chemical composition of bioactive elements[6]. Secondary metabolites such as carotenoids, sulfated polysaccharides, proteins, peptides, vitamins and fatty acids offer a less toxic new drug development. In a short period, much research has been conducted to exploit the biomedical applications of seaweeds. The diversity in

species and cell components builds the amiable nature of the pharmaceutical industry[7].

Reactive oxygen species (ROS) are groups that have dangerous effects on the cell when accumulated. During the life cycle, ROS are produced in every stage of metabolism. Producing profuse substances, especially plant cells, can suppress the damage[8]. This oxidative damage can be treated with the production of secondary metabolites. Ample research is going on the therapeutic activity of seaweeds because of the presence of non-animal sulfated polysaccharides and other secondary metabolites. Polysaccharides of seaweeds take over antimicrobial, antioxidant and anticancer activities[9,10], which are inquiring nowadays. Oxidative stress induced by free radicals is getting more concern as it is seeded for many deadly diseases. Antioxidants can prevent oxidative stress damage and currently, seaweed resources are analyzing for antioxidant activity to resolve this issue. The escalation of human infecting pathogens is increasing the urgency of introducing advanced antimicrobial agents of natural origin. Multifunctional secondary metabolites extracted from seaweeds have shown chemical reciprocity with antimicrobial activity[11].

Ulvophyceae are the family of green seaweeds that occur in marine and brackish water ecosystems. *Ulva fasciata* come under this family and are abundant in the marine ecosystem[12]. The plenitude of this species created scope in the field of food and biomedical industries. It is considered as relevant because of the presence of ulvan, the sulfated polysaccharide with considerable medicinal value. Ulvan, polysaccharides derived from *Ulva* sp. are a promising agent for anticancer, antiviral, anticoagulant, etc.[13]. Also, the research proved the use of ulvan to improve physiological functions.

Dictyota is a genus of seaweed in the *Dictyotaceae* family. Brown seaweeds can be observed in tropical and sub-tropical seas. Foregoing studies sense that brown seaweeds are an excellent source of substances such as tannins and phenolic compounds[14]. Fucoidan is the sulfated polysaccharide produced by most of the brown seaweed. The presence of pigments, vitamins and secondary metabolites opens a wide range of biological applications such as antitumor, antiviral, anticoagulant and antioxidant activity in brown seaweeds[7,15].

Considering the above, this work aims to evaluate the in vitro antioxidant and antimicrobial activity of green algae, *Ulva fasciata* and brown algae, *Dictyota dichotoma* extracts against human pathogens to find a promising alternative source of pharmaceutical agents. In addition, we also reported on the composition of seaweed's secondary metabolites, which are responsible for biological activities.

2. Materials and methods

2.1. Seaweed collection and processing

Live samples of seaweeds were collected from the Ettikulam coast (12.0110°N, 75.2101°E) in the Kannur district of Kerala, India. Algal samples were collected by hand-picking from the intertidal zones of the collection site. Complete plants with holdfast were detached carefully. Initially, samples were washed with seawater to remove all the impurities, sand particles and epiphytes. Two specimens were picked out from the samples and transported to the laboratory. Seaweed samples were identified as *Ulva fasciata* (Chlorophyceae) and *Dictyota dichotoma* (Phaeophyceae) at the Botanical Survey of India (BSI), Southern Regional Center (Coimbatore, Tamil Nadu, India). Seaweeds were washed thoroughly; air dried at room temperature for one week. Then the dried samples were ground to powder and stored in a bottle for further studies.

2.2. Extraction of polysaccharide

The bioactive extracts from seaweeds were extracted by using the Soxhlet apparatus. Solvents used for the extraction were petroleum ether (PE), chloroform (C), ethyl acetate (EA) and methanol (M). About 30 g

of the algal sample and 500 mL of the solvent were taken for each extraction. The extract was evaporated and the final product was stored at 4 °C for further studies.

2.3. Preliminary phytochemical analysis

The obtained solvent extract was used to confirm secondary metabolites such as alkaloids, terpenoids, steroids, tannins, saponins, flavonoids, phenols, coumarins, quinines and glycosides via standard qualitative procedure[16,17].

2.4. Estimation of total phenolic content

Folin Ciocalteu's method, as described by Taga et al.[18], was followed to determine the phenolic content of seaweed extracts. First, 50 µL of the sample from the stock solution mixed with 450 µL of distilled water and then 1.25 mL of 2% Na_2CO_3 was added and allowed to stand the mixture for 2 min at room temperature. After incubation, 250 µL of 50% Folin Ciocalteu's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 40 min at room temperature in a dark condition. The absorbance was measured at 720 nm. The total phenolic content was calculated by using gallic acid as a standard and expressed the value in term of mg gallic acid equivalent (GAE) per 100 g of fresh tissue.

2.5. Estimating antioxidant activity

DPPH free radical scavenging assay

The antioxidant activities of two seaweed samples were examined by the 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging technique[5,19]. For the study, the control solution was prepared with 150 µL DPPH solutions (4.3 mg dissolved in 3.3 mL methanol) added to 3 mL methanol. The absorbance was taken immediately at 517 nm and used as a control. Test samples of different volumes (50,100,150 and 200 µL) were mixed with methanol and made up to 3 mL. Further, 150 µL DPPH solution was added to each sample. The mixture was vortexed and kept at room temperature for 5 min in a dark condition. Absorbance was taken at 517 nm spectrophotometrically using methanol as a blank. The percentage of DPPH free radicals scavenging activity was calculated with the following formula.

$$\text{DPPH radical scavenging effect \%} = 1 - [(\text{A sample} - \text{A blank})/\text{A control}] \times 100 \quad (1)$$

where, A sample is the absorbance of sample, A blank is the absorbance of blank and A control is the absorbance of control.

2.6. Microbial isolates preparation

Microbial isolates including one fungus *Candida albicans* and two bacteria *Staphylococcus aureus* and *Bacillus subtilis* were available in the laboratory used for the study. *C. albicans* is a secondary pathogen for HIV infection. *B. subtilis* is a gram-positive strain that can resist heat and produce dormant spores[20]. *S. aureus* is a strain that can produce multiple diseases such as skin diseases, bacteremia, infective endocarditis, etc.[21]. All the strains were preserved on a Nutrient agar medium (MHA-Himedia) at 4 °C.

Antimicrobial assay

Antimicrobial activity was performed in vitro using the well-diffusion technique on the agar plate. Microbial isolates were spread on Muller Hinton agar plates and kept at room temperature to absorb microbial inoculums. Wells were made on the plate using a 6 mm diameter sterile cork borer. Each well was loaded with 100 µL of the appropriate seaweed extracts and one well was left as control. Plates with bacterial strains were incubated at 37 °C and plates with fungal strains were incubated at 28 °C for 18–24 h. Tests were conducted in triplicate. The antimicrobial activity was analyzed by measuring the inhibition zone around each well. Mean diameter values were also calculated[5,22].

2.7. FTIR analysis

The functional groups of the extracts were determined by using Fourier transform infrared spectroscopy (JASCO (FT/IR-4100 type A)). It was examined in the range between 4000 and 400 cm^{-1}. About 1 mg of extract was mixed with KBr as a 0.5–1 mm thick film and used for the analysis[23–25].

2.8. Statistical analysis

All the experiments were carried out in triplicates and data were reported as mean ± standard deviation. The linear correlation coefficient and correlation analysis were calculated using MS Office Excel 2010.

3. Results and discussion

Bioactive substances obtained from seaweeds can be used to replace harmful synthetic substances and this study aimed to reveal the properties of two marine macroalgae in the field of pharmacology. *Ulva fasciata* is seaweed that abundantly occurs on the southern coasts of India with numerous biomedical properties such as anticancer, antimicrobial and antioxidant[13]. *Dictyota dichotoma* was also studied by the scientific community to explain its characteristics as a good source of pharmaceutical agent. **Figure 1** shows the macroscopic picture of two different seaweed thallus. Macroalgae differ in their constituents responsible for biomedical activity[25] based on the change in habitat, climatic conditions and species. Ettikulam coast is a poorly explored coastal region with considerable seaweed resources. Hence the existence of phytochemical constituents is correlated with the collection site; the seaweeds on this coast need to be studied well. The presence of secondary metabolites is the basis for the development of biological activity[16]. For example, a study by Shaibi et al.[17] on *Turbinaria ornata* listed the presence of alkaloids, saponins and fixed oils that exhibits various activities such as antioxidant and wound healing.

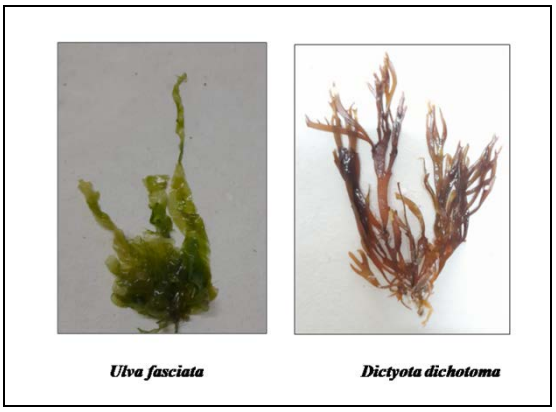

Figure 1. Macroscopic images of seaweed samples collected from Ettikulam coast, Kerala.

3.1. Extraction of polysaccharide

Among the different solvents tested, methanolic extraction was given the maximum amount of extracts for both seaweeds. In this experiment, *U. fasciata* and *D. dichotoma* (**Table 1**) was found to be 13.35% and 8.97%, respectively. This percentage yield of extracts was similar to the previous study by Alves et al.[26] reported in green seaweed (10%–20%). Venkatesan et al.[27] studied the bioactive extract from brown seaweed and finalized a production at 8%. The chemical nature of the sample and the properties of organic solvents were influenced the difference in percentage of extracts. Seaweeds, under stress conditions can produce a more complex form of secondary metabolites[28].

Table 1. Extract percentage of *U. fasciata* and *D. dichotoma*.

Solvent	Percentage yield (%)	
	U. fasciata	***D. dichotoma***
Petroleum ether	0.91	0.17
Chloroform	0.53	0.98
Ethyl acetate	0.13	0.27
Methanol	13.35	8.97

3.2. Preliminary phytochemical analysis

Qualitative analysis of primary phytochemicals in the two extracts was analyzed and tabulated (**Table 2**). Presence of secondary metabolites such as alkaloids, terpenoids, tannins, flavonoids and phenols were recorded in both seaweed species by using four different solvents. Saponins were absent in both seaweed samples. Glycosides have existed in the methanolic extract of *U. fasciata* and ethyl acetate extract of *D. dichotoma*.

Table 2. Qualitative analysis of primary phytochemicals.

Name of the Compound	*U. fasciata*				*D. dichotoma*			
	PE	**C**	**EA**	**M**	**PE**	**C**	**EA**	**M**
Alkaloids	+	−	+	+	+	−	+	+
Terpenoids	−		+	−	−	+	−	+
Steroids	+	−	−	−	+	−	−	−
Tannins	+	−	+	+	+	+	+	+
Saponins	−	−	−	−	−	−	−	−
Flavonoids	+	−	+	+	+	−	−	+
Phenols	−	+	+	+	+	+	−	+
Coumarins	−	−	−	−	−	−	+	−
Quinines	−	−	−	−	−	+	−	−
Glycosides	−	−	−	+	−		+	−

3.3. Estimation of total phenolic content

The Folin-Ciocalteu method was used for the estimation of phenolic content (**Table 3**). Among the various solvents tested, both *D. dichotoma* and *U. fasciata* had a higher phenolic content only in methanolic extract when compared to other solvents.

Table 3. Total phenolic content of *U. fasciata* and *D. dichotoma*.

Solvent	Total phenolic content (mg/g GAE)	
	U. fasciata	***D. dichotoma***
Petroleum ether	29.66 ± 0.152	26.69 ± 0.358
Chloroform	33.56 ± 1.40	16.43 ± 0.57
Ethyl acetate	50.06 ± 5.62	37.08 ± 0.25
Methanol	72.94 ± 1.16	75.99 ± 0.259

Certain plants contain phytochemicals that have extraordinary potential to prevent diseases. Phenolic acids are one of the major constituents that superintends antioxidant activity[29]. Comparative analyses on the phenolic content of various seaweeds are listed in **Table 4**. A study by Mole and Sabale[30] proved that the

phenolic content was higher in methanolic extracts than in ethanolic extracts of seaweeds. They also analyzed that the green algal methanolic extract contains more phenolic content and a positive correlation between the total phenolic content and antioxidant capacities. In this study, both *U. fasciata* and *D. dichotoma* have a high phenolic content with the methanolic extract. Phenolic compounds help to scavenge the reactive oxygen species (ROS) that may cause serious health issues in humans.

Table 4. Total phenolic content of *U. fasciata* and *D. dichotoma* compared to other seaweed.

Name of the seaweed	TPC (mg GAE/g extract)	Reference
Ulva conglobata Kjellman	35.6 ± 1.5	[31]
Chaetomorpha antennina (Bory.) Kützing	23.9 ± 1.2	-
Sargassum vachellianum Greville	61.9 ± 1.1	
Pachydictyon coriaceum	54.0 ± 2.2	
Colpomenia sinuosa	69.4 ± 1.7	
Fucus spiralis	397.23 ± 0.02	[32]
U. compressa	2.780 ± 0.002	-
U. fasciata	72.94 ± 1.16	Present study
D. dichotoma	75.99 ± 0.259	Present study

3.4. DPPH free radical scavenging assay

DPPH scavenging assay was used to determine the antioxidant activity. The percentage of DPPH radical scavenging activity of *U. fasciata* summarized in **Figure 2**. Samples was revealed an increment in activity which is dose-dependent. The methanolic extract shows the highest radical scavenging activity (89.29%), followed by ethyl acetate (71.36%) > chloroform (62.4%) > petroleum ether (36.08%) extracts of *U. fasciata*. However, the *D. dichotoma* (**Figure 3**), the highest scavenging activity was obtained from ethyl acetate extract (70.1%), followed by methanol (40.67%) > petroleum ether (34.63%) > chloroform (28.81%). This study explained the ability of crude seaweed extract to fight against the free radicals. Of the examined seaweeds, the methanolic extract of *U. fasciata* was the most potent scavenger than *D. dichotoma.*

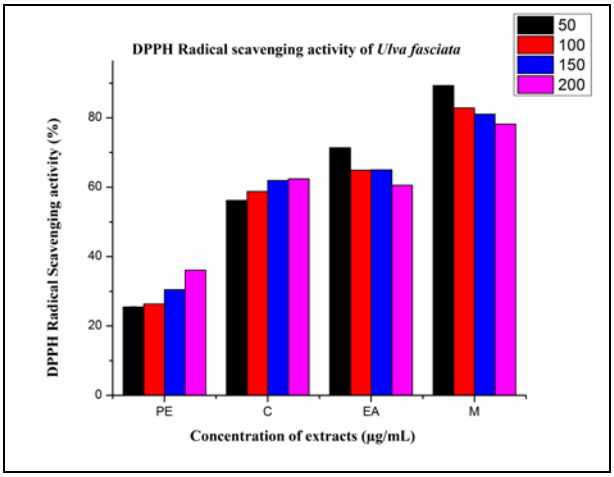

Figure 2. DPPH radical scavenging activity of crude extracts of *U. fasciata* at different concentrations; 50, 100, 150, 200 µg/mL. Values are expressed as means of triplicate determinations with ± standard deviation.

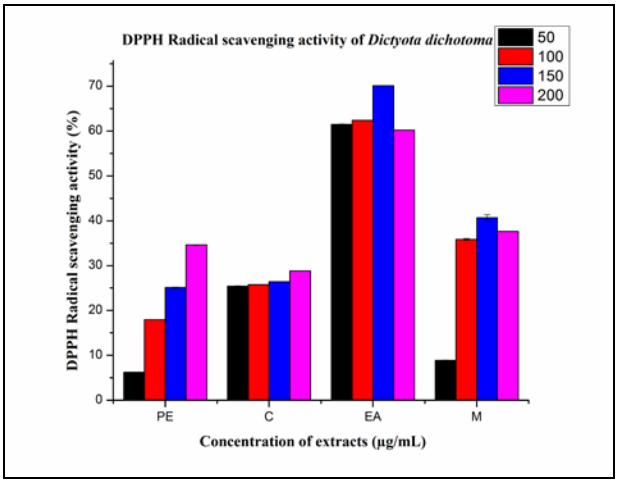

Figure 3. DPPH radical scavenging activity of crude extracts of *D. dichotoma* at different concentrations; 50, 100, 150, 200 µg/mL. Values are expressed as means of triplicate determinations with ± standard deviation.

Various metabolic or cellular processes build highly reactive oxygen species with free electrons[33]. Oxidative stress generated by free radicals was the primary reason for cellular disintegration, leading to various human diseases. Antioxidants have the property to scavenge free radicals and reduce the risk of infections. Moreover, natural antioxidants are nontoxic and potential agents for biological activities[34].

The current study analyzed the antioxidant activity of seaweed samples and disclosed the antioxidant effects in a concentration-dependent manner[35]. DPPH scavenging activity is rapid, in vitro, spectrophotometric method to detect the antioxidant activity based on electron transfer, with wide acceptance. DPPH is considered as nitrogen-centered free radical. In this method, electron transfer within the experiment system produces a violet colour with solvent and reduces it to yellow. This study explained the ability of crude seaweed extract to fight against free radicals. Of the examined seaweeds, the methanolic extract of *U. fasciata* was the most potent scavenger than *D. dichotoma*.

3.5. FTIR analysis

Macro algal cells contain various substances responsible for biological activities. Major secondary metabolites define the characteristics of seaweed extracts. Vital chemical constituents present in both algal cells were observed by FTIR analysis. IR spectra of the seaweeds were obtained between the wave numbers 4000 to 400 cm^{-1}. The resultant spectra of *U. fasciata* (**Table 5**) show the characteristic peaks at 3407.60 cm^{-1} indicating the presence of the functional group of alcohols and phenols. A prominent peak on 2906.19 cm^{-1} (**Figure 4**) indicates the C–H stretching vibration of alkanes. The characteristic solid peaks obtained at 1625.69 cm^{-1} and 1405.85 cm^{-1} indicated the presence of amides and aromatics, respectively. Peak positions at 1093.44 cm^{-1} and 827.31 cm^{-1} were assigned to primary and secondary amines, respectively.

Table 5. Interpretation of functional groups of seaweed sample using FTIR.

Peak position (wave number cm^{-1})	Bond	Functional groups
U. fasciata		
3407.60	O–H stretch, H-bonded	Alcohols, phenols
2906.19	C–H stretch	Alkanes
1625.69	C=O stretch	Amides
1405.85	C–C stretch	Aromatics
1093.44	C–N stretch	Aliphatic amines
827.31	N–H wag	Primary, secondary amines

Table 5. (Continued).

Peak position (wave number cm^{-1})	Bond	Functional groups
D. dichotoma		
3405.67	O–H stretch, H-bonded	Alcohols, phenols
2940.91	C–H stretch	Alkanes
1625.69	C=O stretch	Amides
1440.56	C–C stretch	Aromatics
1124.29	C–N stretch	Aliphatic amines
875.52	N–H wag	Primary, secondary amines
624.82	C–Br stretch	Alkyl halides

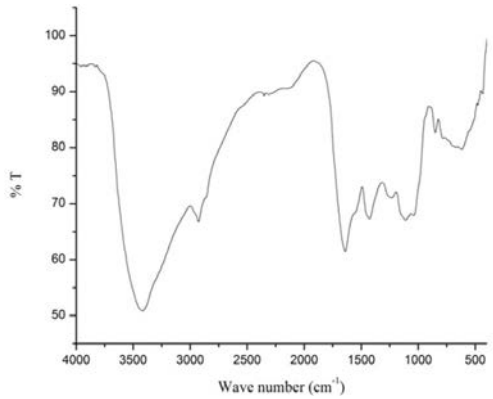

Figure 4. FTIR spectrum of *U. fasciata*.

For the *D. dichotoma* sample, the characteristic peak at 3405.67 cm^{-1} (**Figure 5**) shows the O–H stretching and H-bonding of alcohols and phenols. A peak at 2940.91 cm^{-1} represents the presence of alkanes and 1625.69 cm^{-1} shows the presence of amides. The transmission peak near 1440.56 cm^{-1} specifies the C–C stretching of aromatics. Finally, the peak at 875.52 cm^{-1} was assigned to primary and secondary amines.

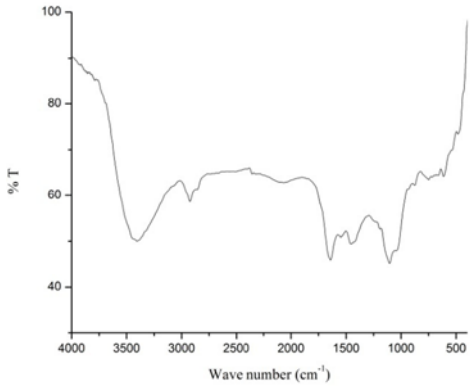

Figure 5. FTIR spectrum of *D. dichotoma*.

Many studies have explained the pharmacological importance of seaweed extracts without explaining the individual constituents and their contribution to the development of biological activity[36]. FTIR analysis of seaweed metabolites gives a clear picture of functional groups responsible for the antioxidant and other potential activities. The present investigation divulges the presence of functional groups such as alcohols and phenols. In addition, different vibrations of alkanes, amides and aromatics and alkyl halides[24,37] were also reported. This can increase the strength of the point that the hidden secondary metabolites and functional groups in plant material drive the antioxidant and antimicrobial potential.

3.6. Antimicrobial activity

Antimicrobial activity was observed in the extracts of methanolic, ethyl acetate and petroleum ether by using *U. fasciata*. The chloroform extract shows no activity towards the tested pathogens. The extract of petroleum ether and methanol exhibits a more significant inhibition zone, while ethyl acetate extract exhibits a moderate inhibition zone against the tested fungus of *C. albicans*. Further, ethyl acetate extract manifests more antimicrobial activity against *S. aureus* followed by methanolic extract and petroleum ether extract (**Table 6**). Only petroleum ether extract shows moderate activity against *B. subtilis* (**Figure 6**).

Regarding the antimicrobial activity of *D. dichotoma* (**Figure 7**), only methanolic and ethyl acetate extracts have exhibited a positive activity against the tested pathogen (**Table 7**). Methanolic extract exhibit higher activity against the bacteria, *B. subtilis* followed by ethyl acetate extract (medium inhibition zone). Methanolic extract shows moderate antimicrobial activity against *C. albicans*. The *D. dichotoma* extracts have no activity against the bacteria *S. aureus*. This prominent inhibition gives a promising indication of developing a potential drug from marine natural sources against the tested pathogen.

Table 6. Antimicrobial activity of *U. fasciata.*

Solvent	**Antimicrobial activity**		
	Inhibition zone		
	C. albicans	***S. aureus***	***B. subtilis***
Petroleum ether	+++	+	++
Chloroform	–	–	–
Ethyl acetate	++	+++	–
Methanol	+++	++	–

Data for the antimicrobial activity of *U. fasciata* extract. Inhibition zone ≥ 10 mm marked as +++; >7 mm marker as ++; =7 mm marked as +; <7 mm marked as −.

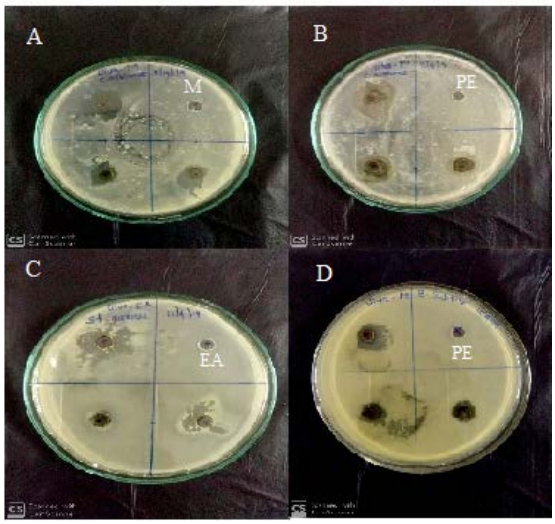

Figure 6. Inhibition zone formation: **(A)** methanolic extract *U. fasciata* against *C. albicans*; **(B)** petroleum ether extract of *U. fasciata* against *C. albicans*; **(C)** ethyl acetate extract of *U. fasciata* against *S. aureus*; **(D)** petroleum ether extract of *U. fasciata* against *B. subtilis.*

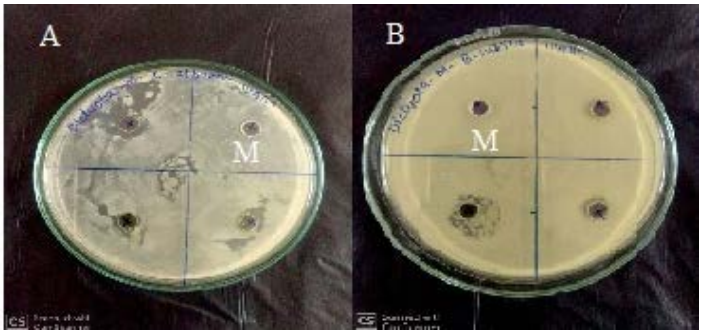

Figure 7. Inhibition zone formation: **(A)** methanolic extract of *D. dichotoma* against *C. albicans*; **(B)** methanolic extract of *D. dichotoma* against *B. subtilis*.

Table 7. Antimicrobial activity of *D. dichotoma*.

Solvent	**Antimicrobial activity**		
	Inhibition zone		
	C. albicans	***S. aureus***	***B. subtilis***
Petroleum ether		−	−
Chloroform	−	−	−
Ethyl Acetate	−	−	++
Methanol	++		+++

Data for the antimicrobial activity of *D. dichotoma* extract. Inhibition zone ≥ 10 mm marked as +++; >7 mm marker as ++; =7 mm marked as +; <7 mm marked as −.

The antimicrobial activity of seaweed is an emerging field of study because of its potential to resist the growth of pathogens[38]. Seaweed contain numerous natural compounds than terrestrial plants[39–41]. As a result of the existence of various secondary metabolites, the seaweed sample shows an antimicrobial potential against tested pathogens[42,43]. An inhibition zone was created because of the death of microorganisms caused by the reaction between microbes and seaweed extract. This clarifies the effect of the biological extract in killing bacterial or fungal strains[44,45]. Based on the measurement of the inhibition zone around the seaweed extract, it is predictable that the seaweed extracts may have therapeutic potential. This study opens the wide possibilities of algal components[46] against harmful microbes.

4. Conclusions

In conclusion, *Ulva fasciata* and *Dictyota dichotoma* were examined in terms of bioactive extracts. Bioactive extracts were obtained from the seaweeds by Soxhlet extraction method. In vitro assays were done to determine the antioxidant and antimicrobial activities of seaweed extracts. Here the samples contained a significant amount of phenolic content with redox properties that allow them to act as antioxidants. Both the seaweeds showed the highest peak of phenolic nature and other secondary metabolites responsible for the correlation of antioxidant and antimicrobial activities. The present study reveals that the extracts of *U. fasciata* and *D. dichotoma* have higher phenolic content showing high antioxidant and antimicrobial activity. Active secondary metabolites are primarily in control of all biological activities. There is a positive correlation between antioxidant and antimicrobial activity and presence of secondary metabolites. All the tested seaweeds offer a broad area of research in seaweed-derived pharmaceuticals.

Author contributions

Conceptualization, NVS and MVSP; methodology, NVS; validation, RR and SS; investigation, NVS and MVSP; writing—original draft preparation, NVS and MVSP; writing—review and editing, RR and SS; supervision, RR. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

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