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Research Article

Phytochemical Screening and Comparative Analysis of Antimicrobial Activity of Selected Species of Brown Seaweeds from Gulf of Mannar, Tamil Nadu, India.

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Abstract

Phytochemical analysis and antimicrobial activities are screened for hexane, chloroform, and methanol extract of five different species of seaweeds *Dictyota dumosa*, *D. dichotoma* var. *intricata*, *D. indica*, *Padina boergesenii* and *P. tetrastomatica* collected from Gulf of Mannar. The major phyto-constituent present in all the extracts includes phenols, terpenoids and cardiac glycosides. Antimicrobial activity is tested against human pathogenic bacteria like *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis,* while fungal strains include *Aspergillus niger, A. fumigatus, A. terreus, Fusarium oxysporum, and Candida albicans.* Antibacterial activity was more promising in all the extracts of seaweed than antifungal activity with the highest zone of inhibition of 17 mm shown by methanol extract of *D. dumosa* against *K. pneumonia* followed by 15 mm shown by chloroform extract of *D. indica* against *B. subtilis.*

***Keywords*:** Phaeophyceae, *Dictyota*, *Padina,* Phytochemical Analysis, Antimicrobial activity

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INTRODUCTION

Seaweeds are rich source of structurally novel and biologically active metabolites and are classified as the largest remaining reservoir of natural molecules as they are living in a very exigent, competitive, and aggressive surrounding very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. Extensive research has been carried out on seaweed with greater interest on bioactive compounds with antimicrobial qualities since in marine environment seaweeds are constantly in contact with the potentially dangerous microbes and so they naturally have evolved with chemical defense strategies against these pathogenic microorganisms.

According to the CE 258/97 regulation algae are considered as functional foods (Crespo and Yusty, 2004). In order to make still a better use of seaweed, people take

efforts to isolate some high value compounds from it. Also there is increased frequency of pathogenic bacteria resistant to traditional antibiotics has resulted in search for novel antibacterial compounds. As many components of seaweed have potential antimicrobial activity, they are now in the spotlight for natural product discovery.

Antimicrobial activity may involve complex mechanisms like the inhibition of the synthesis of cell walls and cell membranes, nucleic acid and protein, as well as the inhibition of nucleic acid metabolism. It also seems likely that substances in the extracts may act separately or synergistically to exert their effects. Antimicrobial activities of macro algae have been attributed to the presence of biologically active compounds with antimicrobial potential such as cycloeudesmol, lyengaroside A, meroditerpenoid, lanosol enol ether, diterpene, benzoic acids, halogenated sesquiterpene alcohol, eicosanoids, etc., (El Gamel, 2010). Among the three classes of seaweeds, Brown algae or Phaeophyceae is one of the most studied algal families in respect of secondary metabolites. Even though it is vastly studied, it has been classified as the largest remaining reservoir of natural molecules yet to be evaluated for drug activity. So still there is a search for novel compounds from natural source to combat the effect of synthetic compounds and its resistance towards multidrug resistant pathogen. In this context a study was undertaken to comparatively screen and analyze phytochemical constituents and antimicrobial potential of different solvent extracts of five different species of brown algae from Gulf of Mannar.

MATERIALS AND METHODS

Collection of seaweeds

Five species of brown algae belonging to the family Dictyotaceae were selected in this study. These species were collected from Gulf of Mannar, Tamil Nadu, India. The species include *Dictyota dumosa* Boergesen, *D. dichotoma* (Hudson) Lamouroux var. *intricata* (C.Agardh) Greville, *D. indica* Sonder ex Kutzing, *Padina boergesenii* Allender & Kraft and *P. tetrastomatica* Hauck. Healthy, mature and disease free seaweeds were collected and the collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles and pebbles and shells and brought to the laboratory in polyethylene bags. The samples were then thoroughly washed with fresh water, blotted and shade dried. The shade dried samples were ground to a fine powder. The powdered samples were then stored at 4ºC for further use.

Solvents and chemicals used

All chemicals were purchased from Sigma-Aldrich, Merck, Himedia and SD fine chemicals. All other reagents were of analytical grade.

Sequential extraction of seaweeds

A portion of each of the powdered samples (100 g) were packed in Soxhlet apparatus and was sequentially extracted with solvents of increasing polarity such as hexane, chloroform and methanol for 8 hours. The crude extracts obtained were concentrated using rotavapour under reduced pressure at 45°C. The collected 15 extracts were weighed, labeled and stored at 4°C for further use.

Tested microorganisms

Klebsiella pneumonia (MTCC 109), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), *and Bacillus subtilis* (MTCC 441), while fungal strains include *A. niger* (MTCC 1344), *A. fumigatus* (MTCC 9657), *A. terreus* (MTCC 1782), *Fusarium oxysporum* (MTCC 1755) and *Candida albicans* (MTCC 227).

Phytochemical screening

Test for Alkaloids

Solvent free extract (50 mg) was stirred with few ml of dilute HCL and filtered. The filtrate was tested carefully with alkaloid reagent as follows:

Mayer's test (Evans, 1997)

Mercuric Chloride, 1.358 g was dissolved in 60 ml of water and 5 g of potassium iodide was dissolved in 10 ml of water. The two solutions were mixed and made up to 100ml with water.

Procedure:

To 1.2 mL of filtrate, 0.1 ml of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicates the presence of alkaloids.

Test for Tannins (Trease and Evans, 2002)

About 0.1 g each portion was stirred with about 2 mL of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 mL of filtrate. Occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

Test for Saponins (Sofowora, 1993)

One hundred milligram of each plant extract was boiled with 1 mL of distilled water, filtered. To the filtrate, about 0.5 mL of distilled water was further added and shaken vigorously for about 5 min. Frothing which persisted on warming was taken as evidence for the presence of saponins.

Test for Flavonoids (Trease and Evans, 2002)

Alkaline reagent

One hundred milligram of each plant extracts were dissolved in 5 mL of water and filtered, to this 2 mL of 10 % aqueous sodium hydroxide was later added to produce a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Shinoda's test

In a test tube containing 0.5 mL of extract, 5-10 drops of dilute HCL and small piece of ZnCl or magnesium were added and the solution was boiled for few minutes. Appearance of reddish brown color indicated the presence of flavonoids.

Test for Terpenoids (Sofowora, 1993)

Three ml of each extract was mixed in 1 mL of chloroform and concentrated sulphuric acid (1mL) was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for Cardiac glycosides (Keller-Killan's test)

Five ml of each extract was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates deoxy-sugar characteristics of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout this layer.

Antimicrobial activity

The agar well diffusion method

The agar well diffusion method has long been used for testing antimicrobial activity, and it was used in our study (Piddock, 1990). Microbial inoculum was streaked evenly with a sterile cotton swab on Nutrient agar for bacterial culture and on Potato dextrose agar for fungal culture. Aseptically, 20 µL of different concentration of solvent extract impregnated discs (commercial antibiotic discs, Whatman) were placed on the seeded agar plate. Streptomycin and Clotrimazole (30 µg/disc) were used as positive control, whereas solvent acts as the negative control. The discs were placed on the surface of the seeded agar plates and incubated at 37 ºC for 24 hours for bacteria and 72 hours for fungi. Antimicrobial activity was indicated by clear zone of growth inhibition.

RESULTS

The phytochemical constituents of five different species of brown algae were qualitatively screened, compared and the results are summarized in Table. 1. From the present investigation, phenols, terpenoids and cardiac glycosides were the major phytochemical constituents present in all the extracts.

Table.1: Preliminary phytochemical screening of extracts.

Phytochemicals	*Dictyota dumosa*			*Dictyota dichotoma* var. *intricata*			*Dictyota indica*			*Padina boergesenii*			*Padina tetrastomatica*		
	H	C	M	H	C	M	H	C	M	H	C	M	H	C	M
Alkaloids	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-
Tannins	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+

Alkaloids were present in all the solvent extracts of *D. indica* and hexane extract of *D. dichotoma* var. *intricata* but absent in other solvent extracts. Flavonoids were present in all the extracts except hexane extract of both the *Padina* species under study. Cardiac glycosides were present in all the extracts except hexane extract of *P. boergesenii*. Saponins were present in methanol extract of *D. indica* and chloroform extract of both the species of *Padina* under study. Tannins were present in all the chloroform and methanol extract of five species of brown algae. Antimicrobial activity of different seaweed extracts were summarized in Table 2. Chloroform extract of *D. dumosa* was highly active against *K. pneumonia* with a maximum zone of inhibition of 17±2 mm, while methanol extract of *P. tetrastomatica* showed lower activity of 7.3±0.57 mm. Methanol extract of *D. indica* was active against *S. aureus* with a maximum zone of inhibition of 14.3±2.08 mm and minimum zone of inhibition of 7±0 mm was measured for hexane extract of *D. dichotoma* var. *intricata* and chloroform extract of both *D. indica* and *P. boergesenii*. Chloroform extract of *D. indica* was active against *P. aeruginosa* with a maximum zone of inhibition of 13±2 mm. Minimum zone of inhibition of 7±0 was measured for the chloroform and methanol extract of *P. boergesenii* and *P. tetrastomatica.* Hexane extract of *P. tetrastomatica* and chloroform extract of *D. indica* were active against *B. subtilis* with a zone of inhibition of 15±1 mm and 14.6±1.52 mm, respectively. Minimum zone of inhibition of 7±0 was measured for hexane extract of *D. dumosa.* Moreover the extracts are not active against all the strains of fungi used and if at all it shows only partial zone of inhibition against *C. albicans* and *A. fumigates* (Results not shown).

Table. 2: Antimicrobial activity of extracts of algae against pathogenic microorganism.

(Inhibition of growth expressed as mm diameter of inhibition zone)

Human pathogens		*Dictyota dumosa*			*Dictyota dichotoma* var. *intricate*			*Dictyota indica*			*Padina boergesenii*			*Padina tetrastomatica*		
		Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)			Inhibition zone (mm)		
Klebsiella pneumonia		H	C	M	H	C	M	H	C	M	H	C	M	H	C	M
	2	na	na	7±0	na	9±0	Na	na	na	na	na	na	7.3±0.57	7±0	na	na
	5	7±0	na	9±1	na	10±1	Na	7±0	na	na	7±0	na	7.6±0.57	11.3±0.57	na	na
	10	8±1	na	11.6± 0.57	8±0	11.6±0.57	Na	9±0	na	na	10±2	na	8±0	12±0	na	na
	20	10±1	7.6± 0.57	17±2	10±1	15±2	7.6±0.57	13±2	10±1	9±1	14±2	Na	9±1	14±1	na	7.3±0.57
	Control	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
Staphylococcus aureus	2	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
	5	7.3±0.57	na	na	7±0	7.3±0.57	Na	na	na	na	na	na	na	na	na	na
	10	8±1	na	na	7.3±0.57	8.6±0.57	Na	8.3± 0.57	7± 0	7.3± 0.57	7.3± 0.57	7± 0	7.3± 0.57	na	na	na
	20	9.6±1.52	7.3± 0.57	na	7.6± 0.57	10±1	7.6±0.57	7.6± 0.57	8±1	14.3± 2.08	8.6± 0.57	8.6±0.57	7.6±0.57	7.6±0.5	7.3± 0.5	7.3± 0.57
	Control	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
Pseudomonas aeruginosa	2	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
	5	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
	10	7.3±0.57	na	na	na	na	Na	na	na	na	na	7±0	na	na	na	na
	20	12±2	7.6±1.15	na	10.3±1.154	13±2	8±1	8±1	9.3±0.57	8±1	10.6±1.52	9±1.73	7.3±0.57	na	7.3±0.57	7±0
	Control	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
Bacillus subtilis	2	na	na	na	7.3±0.57	na	Na	8.3±0.57	7.3±0.57	na	na	na	na	12±1	na	na
	5	na	na	na	8.3±0.57	na	Na	9.6±0.57	10±1	na	na	na	na	12.6±1.15	na	na
	10	na	na	8±1	10±1	na	Na	11.6±1	12±1	na	8.6±0.57	na	na	14±1.73	na	na
	20	7±0	na	11.6±2.08	12.3±1.52		Na	13.3±1.52	14.6±1.52	na	11.6±2.08	8±1	na	15±1	na	na
	Control	na	na	na	na	na	Na	na	na	na	na	na	na	na	na	na
Escherichia coli		na														
Aspergillus niger		na														
A.fumigatus		Partial Zone of Inhibition														
A.tereus		na														
Fusarium oxysporum		na														
Candida albicans		Partial Zone of Inhibition														
Antibiotic																

DISCUSSION

Since the early days of marine natural product discovery, sessile marine organisms have dominated as the major contributing organisms of novel bioactive compounds. In our study phytochemical constituents are analyzed since phytochemicals are promoted for the prevention and treatment of many health conditions with little or no side effects. Results from the present investigation reveals that phenols, terpenoids and cardiac glycosides were the major phytochemical constituents present in all the seaweed extracts. Phenols are synthesized via the shikimic acid pathway and are known to have anti-fungal and antimicrobial effects. So phenol content of all the seaweed extracts contribute to its antimicrobial activity. Alkaloids were present in all the solvent extracts of *D. indica* and hexane extract of *D. dichotoma* var. *intricata* but absent in other solvent extracts. As it is known that alkaloids were found to be involved in defense function especially have anti-herbivore defense function in plants also contribute to the antimicrobial activity of seaweed extracts.

Flavonoids were present in all the extracts except hexane extract of *Padina* under study. Flavonoids are also an important antimicrobial agent found in marine and terrestrial plants. Flavonoids especially isoflavonones have antibacterial activity against gram positive bacteria than gram negative bacteria. Isoflavanones, isoflavons, and isoflavonones are also reported to have activity against fungal pathogens (Dakora, 1995). Cardiac glycosides were present in all the extracts except hexane extract of *P. boergesenii*. Saponins were present in methanol extract of *D. indica* and chloroform extract of both the species of *Padina* in this study. Saponins are important as it prevents disease invasion of plants by parasitic fungi and this property may be responsible for antimicrobial activity of seaweed extracts. Tannins were present in all the chloroform and methanol extract of five species of brown algae. Tannins have astringent property and anti-herbivore defense function in plants. They are also recommended for a wide range of treatments (Zhu et al., 1997).

The results obtained from the present screening revealed that the strongest antibacterial activity was exhibited by all the tested seaweed extracts than the antifungal activity. Results from our study revealed that hexane extract of *P. tetrastomatica* and chloroform extract of *D. indica* were active against *B. subtilis* with a zone of inhibition of 15±1 mm and 14.6±1.52 mm, respectively. This in agreement with the previous report that methanol extract of *P. gymnospora* had strong activity against *B. subtilis* (26.33±1.86) (Manivannan *et al*., 2011). Hexane extract of *P. tetrastomatica* and chloroform extract of *D. indica* are thus equally efficient in controlling the growth of *B. subtilis.*

Chloroform extract of *D. dumosa* was highly active against *K. pneumonia* with a maximum zone of inhibition of 17±2 mm. Methanol extract of *D. indica* was active against *S. aureus* with a maximum zone of inhibition of 14.3±2.08 mm. Al-Saif *et al*., 2014 reported that ethanol and chloroform extract of *D. ciliolate* were active against *K. pneumonia*, *S. aureus* and *E. coli.* Earlier report from us (Rachel et al., 2014) revealed the antioxidant potential of different solvent extracts of the above seaweeds. As the seaweed extracts are not active against the normal microbial flora it can also be used as antioxidant based preservative. Earlier reports from Al-Saif *et al.* (2014) concluded that extracts prepared with chloroform showed relatively higher inhibitory activities against *P. aeruginosa*. The results obtained from our study also revealed that chloroform extract of *D. indica* was active against *P. aeruginosa* with a maximum zone of inhibition of 13±2 mm.

Antifungal activity in all the extracts were found to be less or no activity was seen against tested fungal strains i.e., extracts are not active against all the strains of fungi used in our study and if at all it showed only partial zone of inhibition against *C. albicans* and *A. fumigates* (Results not given). In conclusion the results of the present investigation on selected species of marine algae indicated scope for deriving antibacterial compounds.

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