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Antimicrobial potential of selected brown seaweeds from Vedalai coastal waters, Gulf of Mannar

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ABSTRACT

Objective: To evaluate the antimicrobial activity of *Turbinaria conoides* (*T. conoides*), *Padina gymnospora* (*P. gymnospora*) and *Sargassum tenerrimum* against human bacterial and fungal pathogens. **Methods:** The antimicrobial activities of the extracts against various organisms were tested by using disc diffusion method. **Results:** The methanol extract showed the better result than the other extracts. Whereas, the strong antibacterial inhibition was noted in methanol extracts of *P. gymnospora* against *Bacillus subtilus* (26.33±1.86) and the mild inhibition of ethanol extracts from *T. conoides* against *Klebsiella pneumoniae* (2.33±0.51). Acetone extraction of *P. gymnospora* had strong antifungal inhibition against *Cryptococcus neoformans* (23.00±1.78), and acetone extract of *T. conoides* had mild inhibition against *Aspergillus niger* (3.00±0.89). **Conclusions:** The seven different solvent extracts of seaweeds used in the present study have shown significant bacterial action. Further, a detailed study on the principle compound in the seaweeds which is responsible for antimicrobial activity is still needed and it can be achieved by using advanced separation techniques.

1. Introduction

Commercially available varieties of marine macro algae are commonly refered to as seaweeds. Macro algae can be classified as green algae (chlorophyta), brown algae (phaeophyta) and red algae (rhodophyta), depending on their nutrient and chemical composition. Red and brown algae are mainly used as human food sources. Seaweeds serve as an important source of bioactive natural substances. Seaweeds have been used as food stuff in the Asia diet for centuries as it contains carotenoids, dietary fibres, proteins, essential fatty acids, vitamins and minerals. Marine algae are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan, not for health aspects. Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some valuable medicinal components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals, which are essential nutrition for human[1]. The nutrient composition of seaweed varies and is affected by the species, geographic areas, and seasons of the year and temperature of the water. Seaweeds have recently been received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial activities[2,3]. Many metabolites isolated from marine algae have bioactive efforts[4-6]. Among different compounds with functional properties, antioxidants are the most widely studied. Moreover, the important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers. Marine algae serve as important resources for bioactive natural products[7,8]. Brazilian red algae have been found to have phenolic substances. Oxidative stress is an important factor in the pathological genesis, from cancer to cardiovascular and degenerative disease[9,10].

Mohamed Fayaz *et al*[1] suggested the utility of various nutritional products from *Kappaphycus alvarezii* including antioxidant used as health food or nutraceutical

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supplement. Different parts of the thalli are also known to differ in their antimicrobial potential. Extracts prepared from fresh seaweed samples are reported to show negligible antimicrobial activity as compared with that obtained from dried seaweeds. The levels of antimicrobial activities of marine algae have showed seasonal and geographical variation. However, information is lacking on the seasonal and geographic variations in the specific metabolites of marine algae with potential antimicrobial activity, especially the marine algae in South India. The coastal region of Tamilnadu, South India produces a rich vegetation of marine algae. Many studies have reported a great diversity in the macro algal community of the marine algal vegetation in the region. Among the macro algae in the region, the brown algae *Padina* sp. and one red algae *Kappaphycus* sp. grow in abundance as dominant communities in the shores of Kanyakumari and Ramanthapuram Districts of Tamilnadu State, India.

Seaweeds or marine algae are primitive non-flowering plants without true root stem and leaves. They include one of the commercially important marine renewable prosperity. They comprise different vitamins, minerals, trace elements, proteins, iodine, bromine and bioactive substances. Seaweeds are admirable sources of medicine. To date, there are quite a lot of reports on antibacterial activity of solvent extracts from marine algae. However, there are very few reports pertaining to antifungal activity of crude solvent extracts from the seaweeds representing *Phaeophyceae* and *Rhodophyceae*[11]. Many scientists also reported antimicrobial activities in marine algae[12-14].

Several compounds from the ocean show pharmacological activities and bioactive compounds, primarily for treating deadly diseases like cancer, Acquired Immuno Deficiency Syndrome (AIDS), arthritis, *etc*., while some compounds have been used to treat inflammation, *etc*. Marine algae are widely distributed in the coastal regions of many continents. Several works have been carried out on the extracts from marine algae. Extracts of marine algae were reported to exhibit antibacterial activity[15,16]. Sreenivasa Rao and Parekh[17] showed that crude extracts of Indian seaweeds are active only against gram positive bacteria. Antimicrobial activities against bacteria and fungi were reported by Hellio *et al*[18]. Hence, this study was to investigate the antimicrobial activities of brown marine macro algae [*Turbinaria conoides* (*T. conoides*), *Padina gymnospora* (*P. gymnospora*) and *Sargassum tenerrimum* (*S. tenerrimum*)] collected from Vedalai coastal waters (Gulf of Mannar Coast).

2. Materials and methods

2.1. Collection of samples

The samples of *T. conoides* Kutz, *P. gymnospora* (Kutz) Vicker, and *S. tenerrimum* J.Ag. were collected by handpicking at Vedalai coastal waters (Gulf of Mannar Coast, Lat 8° 35′- 9° 25′ N; Long 78° 08′- 79° 30′ E). The collected samples were cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in plastic bags. The samples were then thoroughly washed with freshwater, blotted and spread out at room temperature for drying. Shade dried samples were grounded to fine powder. The powdered samples were then stored in refrigerator for further use.

2.2. Preparation of extracts

The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples (5 g) and packed in Soxhlet apparatus and extracted successively with methanol, acetone, petroleum ether, ethanol, ethyl acetate, chloroform, diethyl ether for 10 h[19]. The crude extracts were weighed and deep frozen (-20 ℃) until tested.

2.3. Microbial strains

Bacterial strains used for assay were as following: *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enteroccoci* sp., *Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp., *Shewanella* sp., *Vibrio fluvialis and V. splendidus, Enterococcus faecalis, Vibrio cholerae, Shigella flexneri, Staphylococcus epidermitis, Aeromonas liquefaciens* and *Bacillus subtilus*.

While fungal strains were *Aspergillus niger, Candida albicans, Penicillium* sp. *Aspergillus tetreus, Candida glabrata, Cryptococcus neoformans*. Microbial strains were obtained from the Department of Medical Microbiology, Rajah Muthaiah Medical College, Annamalai University, Annamalai Nagar. The bacterial stock cultures were maintained on Mueller Hinton Agar medium at 4 ℃. Fungal cultures were maintained on Potato Dextrose Agar medium at 4 ℃.

2.4. Antibacterial and antifungal assay

The antimicrobial activities were carried using the aga diffusion method[19]. Paper disc of 6 mm in diameter was prepared from Whatman No. 1 filter paper. The antibacterial assay using gram +ve and gram -ve bacteria, was carried out using the agar plate method. The bacterial inocula was grown in nutrient broth overnight and a fixed volume was inoculated into 10 mL aliquots nutrient agar, mixed and then poured over a nutrient agar base in sterile petridishes; this formed the bacterial lawn. Initially both paper discs and well were used for testing the crude extracts. The paper disc of 6 mm in diameter was soaked in 6 μL of crude extract and placed onto the bacterial lawn after it had solidified, standard antibiotic disc used for control. The plates were incubated at 37 ℃ overnight. The zones of inhibition were

measured after the 24 h incubation.

3. Results

3.1. Antibacterial assay

3.1.1. Turbinaria conoides

The methanol extract showed maximum activity against *Entercococci* sp. (16.00±0.89), and minimum activity against *Shewanella* sp. (3.00±0.89). Chloroform extracts showed a maximum activity against *Enterococcus facaelis* (11.66±1.36) and minimum activity against *Aeromonas liquefaciens* (2.66±0.51). The ethanol extract showed a maximum activity against *Staphylococcus aureus* (2.33±0.51), and minimum activity against *Klebsiella pneumonia* (15.66±1.36). The diethyl ether extract displayed maximum activity against *Shewanella* sp. (11.00±0.89) and minimum activity against *Escherichia coli*, *Staphylococcus aureus*(4.00±0.89), *Shigella flexneri*(4.00±0.89) and *Aeromonas liquefaciens* (4.00±0.89). The petroleum ether extract had the maximum activity against *Aeromonas liquefaciens* (12.66±1.86), and minimum activity against *Escherichia coli* (2.33±0.51). Ethyl acetate extract had the maximum activity against *Staphylococcus aureus* (11.00±0.89), and minimum activity against *Salmonella* sp. (3.33±0.51). Acetone extract had the maximum activity against *Bacillus subtilus* (16.00±3.22) and minimum activity against *Salmonella* sp.(2.33±0.51). The antibiotic disc revealed maximum activity noted against *Escherichia coli* (16.66±1.86), and the minimum activity against *Shewanella* sp. (4.00±0.89) (Table 1).

Table 1
Antibacterial activity of *T. conoides* against human pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl acetate	Acetone	Antibiotic disc
Klebsiella pneumoniae	7.33±0.51	11.00±0.89	2.33±0.51	10.00±0.89	9.33±0.51	9.33±0.51	6.33±0.51	10.33±1.03
Escherchia coli	8.00±0.89	4.33±0.51	8.00±0.89	4.00±0.89	2.33±0.51	6.00±0.89	7.60±0.51	16.66±1.86
Staphylococcus aureus	3.66±0.51	6.00±0.89	15.66±1.36	4.00±0.89	6.33±0.51	11.00±0.89	5.00±0.89	16.33±0.51
Enterococci sp.	16.00±0.89	7.00±0.89	9.00±0.89	7.00±0.89	6.00±0.89	6.00±0.89	8.00±0.89	14.66±2.87
Proteus sp.	8.00±0.89	8.66±1.36	8.00±0.89	6.33±0.51	8.33±1.36	3.66±0.51	13.00±0.89	13.00±0.89
Streptococcus sp.	4.00±0.89	7.33±0.51	6.00±0.89	5.33±0.51	5.00±0.89	4.00±0.89	16.00±0.89	15.00±0.89
Pseudomonas aeruginosa	7.00±0.89	4.00±0.89	7.00±0.89	7.33±1.36	3.00±0.89	6.33±0.51	8.00±0.89	14.33±1.36
Vibrio parahaemolyticus	7.00±0.89	5.66±0.51	6.00±0.89	5.33±0.51	6.33±0.51	4.00±0.89	4.33±0.51	13.00±0.89
Salmonella sp.	3.00±0.89	8.33±0.51	4.33±0.51	11.00±0.89	8.33±0.51	3.33±0.51	2.33±0.51	14.00±0.89
Shewanella sp.	4.33±0.51	5.00±0.89	8.33±0.51	8.00±0.89	3.00±0.89	3.66±0.51	4.33±0.51	4.00±0.89
Vibrio fluvialis	6.00±0.89	5.00±0.89	6.33±0.51	6.33±0.51	4.66±0.51	7.00±0.89	8.66±0.51	7.00±0.89
Vibrio splendidus	9.33±0.51	7.33±0.51	6.66±0.51	5.00±0.89	5.00±0.89	4.33±0.51	4.66±0.51	15.66±1.86
Enterococci faecalis	5.33±0.51	11.66±1.36	6.66±0.51	6.33±0.51	6.66±0.51	7.66±0.51	6.00±0.89	13.33±0.51
Vibrio cholerae	4.33±0.51	5.33±0.51	6.33±0.51	5.00±0.89	8.00±0.89	10.66±1.36	7.00±0.89	14.00±1.54
Shigella flexneri	4.00±0.89	6.00±0.89	7.66±0.51	4.00±0.89	7.00±0.89	7.00±0.89	8.00±0.89	13.33±1.36
Staphylococcus epidermitis	5.33±0.51	8.33±0.51	3.00±0.89	5.00±0.89	8.66±0.51	9.33±0.51	8.00±0.89	14.00±0.89
Aeromonas liquefaciens	4.66±0.51	2.66±0.51	4.00±0.89	4.00±0.89	12.66±1.86	6.00±0.89	4.66±0.51	15.00±1.78
Bacillus subtilus	4.33±0.51	7.33±0.51	7.00±0.89	6.33±0.51	6.33±0.51	7.00±0.89	16.00±3.22	16.66±1.36

DEE: Diethyl ether; PE: Petroleum ether.

Table 2
Antibacterial activity of *P. gymnospora* against human pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl acetate	Acetone	Antibiotic disc
Klebsiella pneumoniae	16.66±0.51	16.66±1.86	16.66±1.03	16.66±1.36	16.66±0.51	16.66±1.36	16.00±1.78	18.00±0.89
Escherchia coli	8.00±0.89	11.66±1.03	11.33±1.03	17.33±1.36	12.66±0.51	13.00±0.89	12.00±0.89	14.66±1.86
Staphylococcus aureus	11.66±0.51	11.00±0.89	12.00±0.89	16.00±0.89	11.66±0.51	10.00±1.78	14.66±1.86	15.66±1.86
Enterococci sp.	5.33±0.51	7.33±0.51	12.00±0.89	11.66±1.36	12.33±0.51	12.66±1.03	12.33±0.31	13.66±1.36
Proteus sp.	14.33±1.03	14.66±1.03	8.00±0.89	6.66±0.51	3.00±0.89	5.00±0.89	6.66±0.51	14.33±0.51
Streptococcus sp.	17.00±1.54	14.00±0.89	8.00±0.89	15.33±2.06	14.66±0.51	20.00±0.89	7.33±0.51	16.66±0.51
Pseudomonas aeruginosa	15.33±1.36	13.66±1.36	9.66±1.03	7.33±0.51	11.66±0.51	4.66±0.51	16.66±1.36	16.66±1.03
Vibrio parahaemolyticus	17.00±0.89	11.00±0.51	7.00±0.89	21.00±1.78	18.00±0.51	19.33±1.36	14.66±1.86	13.66±1.86
Salmonella sp.	18.00±0.51	23.66±1.86	14.33±1.86	7.66±0.51	7.33±0.51	11.33±0.51	15.33±2.06	17.00±0.89
Shewanella sp.	11.33±0.51	11.66±1.03	11.66±0.51	17.66±1.36	17.00±0.89	15.00±1.54	18.00±1.78	17.33±0.21
Vibrio fluvialis	7.66±0.51	8.33±0.51	10.66±136	17.00±1.54	16.66±1.03	16.66±1.03	19.00±1.78	12.00±1.54
Vibrio splendidus	8.33±0.51	8.33±0.89	17.00±0.89	8.66±0.51	7.66±0.51	7.33±0.51	–	–
Enterococci faecalis	13.33±1.03	12.66±0.51	12.33±1.36	12.00±0.89	14.00±1.78	18.33±0.51	8.33±0.51	13.00±0.89
Vibrio cholerae	20.66±1.86	8.00±0.89	15.66±1.86	16.33±1.36	14.66±0.51	16.33±2.03	15.66±2.87	19.00±0.89
Shigella flexneri	17.00±1.78	14.33±2.87	11.00±1.78	17.66±0.51	13.66±1.36	18.00±0.89	16.33±1.36	17.00±2.36
Staphylococcus epidermitis	21.00±1.78	11.33±1.86	14.66±1.03	15.66±2.87	3.00±0.89	21.00±1.78	17.00±0.89	16.66±0.51
Aeromonas liquefaciens	16.00±1.78	15.33±1.36	14.33±1.86	11.33±1.86	7.66±0.51	17.33±1.86	17.66±1.03	17.33±1.36
Bacillus subtilus	26.33±1.86	15.66±1.36	7.66±1.03	13.00±0.89	17.00±0.89	17.66±0.51	15.66±2.87	16.66±1.36

DEE: Diethyl ether; PE: Petroleum ether.

Table 3
Antibacterial activity of *S. tenerrimum* against human pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl acetate	Acetone	Antibiotic disc
Klebsiella pneumoniae	11.33±0.51	17.00±0.89	14.66±1.03	5.66±0.51	7.00±0.89	18.00±0.89	17.33±0.51	13.66±1.86
Escherchia coli	12.33±1.36	13.00±2.36	13.66±2.58	13.00±2.36	12.33±1.36	14.66±2.73	17.00±1.54	16.00±1.54
Staphylococcus aureus	10.33±1.86	15.66±2.73	20.33±1.03	8.00±0.89	14.33±2.87	21.00±1.78	11.33±1.86	13.00±1.54
Enterococci sp.	13.66±1.36	16.66±1.36	10.66±1.36	12.66±1.86	7.66±0.51	17.66±0.51	16.00±2.36	16.00±2.36
Proteus sp.	8.66±0.51	16.66±1.86	10.66±1.36	12.00±0.89	19.00±1.54	17.00±2.36	16.33±1.03	14.33±1.36
Streptococcus sp.	15.66±2.87	11.66±0.51	11.33±1.86	11.66±0.51	3.00±0.89	7.33±0.51	12.33±0.51	11.00±0.89
Pseudomonas aeruginosa	11.66±0.51	8.66±0.51	8.33±0.51	10.00±1.54	13.33±1.86	11.33±1.36	12.0±0.89	13.00±1.54
Vibrio parahaemolyticus	10.33±0.51	8.33±0.51	7.66±0.51	8.66±0.51	8.00±0.89	8.66±0.51	8.66±0.51	11.66±0.51
Salmonella sp.	11.00±2.36	8.00±0.89	12.33±1.36	11.33±1.86	8.00±0.89	13.66±1.36	11.66±0.51	14.00±1.78
Shewanella sp.	5.66±0.51	8.00±0.89	13.00±2.36	11.66±1.36	4.00±0.89	8.01±0.89	8.00±0.89	13.33±2.06
Vibrio fluvialis	11.00±1.78	13.00±2.36	12.66±2.06	10.66±2.25	12.66±1.36	11.33±0.51	15.66±3.14	12.00±0.84
Vibrio splendidus	10.00±1.54	11.33±1.86	8.33±0.51	8.00±0.89	13.00±1.54	5.66±1.03	19.00±1.54	17.00±0.89
Enterococci faecalis	13.66±1.86	13.33±1.36	13.00±1.78	12.66±1.03	15.00±3.57	14.00±2.68	21.33±1.36	14.00±3.22
Vibrio cholerae	14.66±2.73	18.66±1.86	11.00±1.78	12.00±0.89	12.00±2.58	12.33±1.36	14.00±2.68	14.33±1.03
Shigella flexneri	15.66±2.58	13.66±1.36	11.66±2.06	18.00±0.89	17.00±1.03	19.33±1.03	18.66±3.66	20.66±2.25
Staphylococcus epidermitis	13.33±1.86	18.66±2.25	15.33±2.25	16.66±1.36	20.00±2.36	16.00±0.89	19.33±1.36	20.66±2.25
Aeromonas liquefaciens	17.33±1.86	20.66±3.14	20.33±2.06	16.00±1.78	18.33±3.72	15.33±2.06	16.00±2.36	17.66±2.87
Bacillus subtilus	13.66±2.73	19.33±3.14	16.33±2.06	19.33±2.87	18.00±2.68	21.33±3.14	19.33±3.14	18.00±2.36

DEE: Diethyl ether; PE: Petroleum ether.

Table 4
Antifungal activity of *T. conoides* against fungal pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl acetate	Acetone	Antibiotic disc
Aspergillus niger	4.66±0.51	4.00±0.89	5.33±0.51	11.00±0.89	7.00±0.89	4.33±0.89	3.00±0.89	14.00±3.09
Candida albicans	18.00±2.68	13.00±2.68	18.33±2.25	14.33±2.87	15.33±2.06	18.33±2.25	11.33±2.73	13.00±0.89
Penicillium sp.	18.00±2.68	13.00±2.68	18.33±2.25	14.33±2.89	15.33±2.06	18.33±2.25	11.33±2.73	13.00±0.89
Aspergillus flavus	14.66±2.73	15.66±2.58	14.00±3.09	14.33±0.51	15.66±2.87	11.66±2.25	12.66±1.86	20.66±3.14
Aspergillus tetreus	17.00±4.09	17.00±5.86	14.66±2.73	15.33±2.06	15.66±2.87	11.66±2.25	12.33±1.36	13.33±2.25
Candida glabrata	8.00±0.89	14.66±2.73	14.66±1.86	16.33±3.72	13.33±2.25	14.33±2.87	11.33±2.73	14.33±2.87
Cryptococcus neoformans	13.33±1.26	16.66±2.58	16.66±2.58	13.00±1.54	12.66±0.51	15.66±2.36	13.00±1.78	12.66±1.03

DEE: Diethyl ether; PE: Petroleum ether.

Table 5
Antifungal activity of *P. gymnospora* against fungal pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl acetate	Acetone	Antibiotic disc
Aspergillus niger	13.66±1.36	16.33±2.58	16.66±2.25	13.00±1.54	12.66±0.51	15.00±2.36	13.00±1.78	12.66±1.03
Candida albicans	14.33±2.87	13.66±3.38	13.66±1.36	14.33±2.25	14.66±2.25	11.66±1.03	14.66±1.86	13.33±0.51
Penicillium sp.	16.33±1.86	14.66±2.58	14.33±2.25	12.33±0.51	16.00±2.36	14.00±1.78	13.33±0.51	13.00±0.89
Aspergillus flavus	12.00±1.78	14.66±2.73	18.66±2.25	19.00±3.22	14.33±2.87	16.33±2.25	15.66±2.58	15.33±2.89
Aspergillus tetreus	18.33±3.09	13.66±4.03	14.66±3.88	16.00±3.22	15.66±2.58	14.66±3.38	17.66±4.58	11.66±1.36
Candida glabrata	14.00±3.09	13.66±4.03	14.66±3.38	16.00±3.22	16.66±2.58	14.66±3.38	17.66±4.58	11.66±1.36
Cryptococcus neoformans	20.00±1.54	16.00±2.36	12.66±1.86	15.33±1.86	15.66±1.86	15.00±2.68	23.00±1.78	15.66±2.73

DEE: Diethyl ether; PE: Petroleum ether.

Table 6
Antifungal activity of *S. tenerrimum* against fungal pathogens.

Pathogens	Methanol	Chloroform	Ethanol	DEE	PE	Ethyl Acetate	Acetone	Antibiotic Disc
Aspergillus niger	5.66±0.51	13.00±1.54	5.66±0.51	13.00±1.54	12.33±1.36	16.33±2.73	11.66±0.51	12.66±1.03
Candida albicans	15.00±1.78	7.66±0.51	13.33±2.87	13.00±0.89	13.00±0.89	6.66±0.51	14.66±2.58	21.33±1.36
Penicillium sp.	13.66±1.36	4.66±0.51	18.66±1.36	14.66±2.73	16.66±2.87	11.66±0.51	13.00±1.54	18.66±2.25
Aspergillus flavus	12.33±2.25	14.66±2.73	12.66±1.36	16.66±2.06	16.33±2.25	10.66±1.36	13.66±1.36	10.66±1.86
Aspergillus tetreus	11.66±2.25	10.66±1.36	13.33±1.36	18.00±3.22	15.00±2.36	14.00±2.36	15.00±2.36	15.66±2.58
Candida glabrata	12.33±0.51	12.66±1.86	13.66±2.25	14.66±2.25	16.33±1.86	14.00±0.89	17.00±4.09	18.66±4.03
Cryptococcus neoformans	14.00±2.36	10.33±2.06	14.00±0.89	12.00±2.36	15.33±2.87	13.00±1.78	16.00±1.78	13.00±2.68

DEE: Diethyl ether; PE: Petroleum ether.

3.1.2. Padina gymnospora

The methanol extracts had maximum activity against *Bacillus subtilus* (26.33±1.86), and minimum activity against *Enterococci* sp. (5.33±0.51). The chloroform extracts showed a maximum activity against *Salmonella* sp. (23.66±1.86), and minimum activity against *Enterococci* sp. (7.33±0.51). The ethanol extract showed a maximum activity against *Vibrio splendidus* (17.00±0.89), and minimum activity against *Vibrio parahaemolyticus* (7.00±0.89). The diethyl ether extraction

had maximum activity against *Vibrio parahaemolyticus* (21.00±1.78), and minimum activity against *Pseudomonas aeruginosa* (7.33±0.51). The petroleum ether extract showed a maximum activity against *Vibrio parahaemolyticus* (18.00 ±0.51), and minimum against *Proteus* sp. (3.00±0.89) and *Staphylococcus epidermitis* (3.00±0.89). Ethyl acetate extract showed maximum activity against *Staphylococcus epidermitis* (21.00±1.78), and minimum activity against *Pseudomonas aeruginosa* (4.66±0.51). Acetone extract pointed out maximum activity against *Vibrio fluvialis* (19.00 ±1.78), and minimum activity against *Proteus* sp. (6.66±0.51). The antibiotic disc displayed the maximum activity against *Vibrio cholerae* (19.00±0.89) and minimum against *Vibrio fluvialis* (12.00±1.54) (Table 2).

3.1.3. Sargassum tenerrimum

The methanol extract showed a maximum activity against *Aeromonas liquefaciens* (17.33±1.86), and the minimum activity against *Shewanella* sp. (5.66±0.51). The chloroform extracts showed maximum activity against *Aeromonas liquefaciens* (20.66±3.14), and a minimum activity against *Shewanella* sp., and *Salmonella* sp. (8.00±0.89). Ethanol extract had maximum activity against *Aeromonas liquefaciens* (20.33±2.06), and a minimum activity against *Vibrio parahaemolyticus* (7.66±0.51). The diethyl ether extraction showed a maximum activity against *Bacillus subtilus* (19.33±2.87) and minimum activity against *Klebsiella pneumoniae* (5.66±0.51). The petroleum ether extract showed maximum activity against *Staphylococcus epidermitis* (20.00 ±2.36), and minimum against *Streptococcus* sp. (3.00±0.89). The ethyl acetate extract had maximum activity against *Bacillus subtilus* (21.33±3.14) and minimum activity against *Vibrio splendidus* (5.66±1.03). Acetone extracts showed the maximum activity against *Enterococci faecalis* (21.33±1.36), and minimum activity against *Shewanella* sp. (8.00±0.89). The antibiotic disc displayed the maximum activity against *Staphylococcus epidermitis* (20.66±2.25) and *Shigella flexneri* (20.66±2.25), and the minimum activity against *Streptococcus* sp. (11.0±0.89) (Table 3).

3.2. Antifungal activity

3.2.1. Turbinaria conoides

The methanol extract showed maximum activity against *Candida albicans* (18.00±2.68) and *Penicillium* sp. (18.00± 2.68), and minimum activity against *Aspergillus niger* (4.66± 0.51). Chloroform extract showed a maximum activity against *Aspergillus tetreus* (17.00±5.86) and minimum activity against *Aspergillus niger* (4.00±0.89). The ethanol extracts showed a maximum activity against *Candida albicans* (18.33±2.25), and *Penicillium* sp. (18.33±2.25), and a minimum activity against *Aspergillus niger* (5.33±0.51). Diethyl ether extract showed maximum activity against *Candida glabrata* (16.33±3.72), and the minimum activity against *Aspergillus niger* (11.00± 0.89). Petroleum ether extract showed a maximum activity against *Aspergillus flavus* (15.66±2.87) and *Aspergillus tetreus* (15.66±2.87), and the minimum activity against *Aspergillus niger* (7.00±0.89). Ethyl acetate extract showed a maximum activity against penicillium sp., and *Candida albicans* (18.33 ±2.25) the minimum activity against *Aspergillus niger* (4.33 ±0.89). Acetone extract showed maximum activity against *Cryptococcus neoformans* (13.00±1.78), and minimum activity against *Aspergillus niger* (3.00±0.89). The antibiotic disc showed maximum activity against *Aspergillus flavus* (20.66±3.14), and a maximum activity against *Cryptococcus neoformans* (12.66±1.03) (Table 4).

3.2.2. Padina gymnospora

The methanol extract recorded maximum activity against *Cryptococcus neoformans* (20.00±1.54) and a minimum activity against *Asprgillus niger* (12.00±1.78). The chloroform extract showed maximum activity against *Aspergillus tetreus* (16.33±2.58) and the minimum activity against *Candida albicans* (13.66±3.38). The ethanol extract showed maximum activity against *Aspergillus flavus* (18.66±2.25), and a minimum activity against *Cryptococcus neoformans* (12.66 ±1.86). The diethyl ether extract showed maximum activity against *Aspergillus flavus* (19.00±3.22) , and a minimum activity against *Penicillium* sp. (12.33±0.51). Petroleum ether extract showed maximum activity against *Candida glabrata* (16.66±2.58) and the minimum activity against *Aspergillus niger* (12.66±0.51). Ethyl acetate extract showed the maximum activity against *Aspergillus flavus* (16.33± 2.25), and minimum activity against *Candida albicans* (11.66 ±1.03). Acetone extract had a maximum activity against *Cryptococcus neoformans* (23.00±1.78) and minimum activity against *Aspergillus niger* (13.00±1.78). The antibiotic disc showed maximum activity against *Cryptococcus neoformans* (15.66±2.73), and a minimum activity against *Aspergillus tetreus* (11.66±1.36) and *Candida glabrata* (11.66±1.36) (Table 5).

3.2.3. Sargassum tenerrimum

The methanol extract showed a maximum activity against *Candida albicans* (15.00±1.78), and minimum activity against *Aspergillus niger* (5.66±0.51). The chloroform extract had maximum activity against *Aspergillus flavus* (14.66±2.73), and a minimum activity against *Penicillium* sp. (4.66±0.51). Ethanol extract had a maximum activity against *Penicillium* sp. (18.66±1.36), and minimum activity against *Aspergillus niger* (5.66±0.51). The diethyl ether extract showed maximum activity against *Aspergillus tetreus* (18.00±3.22), and minimum activity against *Cryptococcus neoformans* (12.00± 2.36). Petroleum ether extract showed a maximum activity against *Penicillium* sp. (16.66±2.87) and minimum activity against *Aspergillus niger* (12.33±1.36). The ethyl acetate extract had the maximum activity against *Aspergillus niger* (16.33±2.73) and minimum activity against *Candida albicans* (6.66±0.51).The antibiotic disc showed the maximum activity against *Candida albicans* (21.33±1.36) and the minimum activity against *Aspergillus flavus* (10.66±1.86) (Table 6).

4. Discussion

The antimicrobial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant, experimental methods, *etc*. Although a variety of solvents have been employed in screening seaweeds for antimicrobial activity, it is still uncertain what kinds of solvent is the most effective and suitable for extraction of seaweeds. A few workers tried using different solvents for screening the antimicrobial activity of seaweeds and made comparisons. Martinez-Nadal *et al*[20] mentioned that benzene and diethyl ether were suitable solvents for extracting the antibiotic principle. Parekh *et al*[21] reported that extracts obtained with acetone, ethyl alcohol and ether showed higher antibacterial activity than that of extracts obtained with chloroform. Rosell and Srivastava[22] found similar antibacterial activity when they screened brown algae from Canada with acetone, chloroform, ethyl- ether, methanol and acetic acid.

De compos- Takaki *et al*[23] found the chlorophyceae off Brazilian coast had high antibacterial activity. Based on these results, it is suggested that due to the presence of high water content in fresh samples the antibiotic principle is diluted leading to dissipated activity, while dried samples accumulated or concentrated the antibiotic principle. They also indicate that degradation and active metabolites may not occur during shade drying. Sastry and Rao[24] carried out a successive extraction using benzene, chloroform and methanol, and reported the chloroform extract exhibited the strongest activity. It can be seen from the above reports that the efficiency of chloroform in the extraction of seaweeds remains uncertain.

The results from the present screening revealed that the strongest antibacterial activity was exhibited by the methanol extract and the least by the chloroform and petroleum ether. In some species (*Gelidium amansii*) the inhibitory activity was only observed in the extract obtained with one kind of solvent but not in extracts obtained in other solvents, which may suggest that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the percentage of inhibitory activity will go up when several solvents are used in the screening.

Antibacterial activities of seaweeds also varied with the species division. Rao and Padmakumar *et al*[25,26] reported that the species of *Rhodophyta* showed the highest antibacterial activity, whereas Caccamese, Azzolina[27], Pesando and Caram[28] found that the highest antibacterial activity was exhibited by the species of *Phaeophyta*. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to definite conclusion. In the present study, the species of *Phaeophyta* showed the strongest activities against the test bacteria and fungi, which was in agreement with the findings of Padmakumar and Ayyakannu[26]. It maybe probably due to the tested seaweeds vertical distribution. The active compounds in the species causing the strong antibacterial activities remains to be identified. Hornsey and Hide[29] used acetone as a solvent for extracting antimicrobial compounds from British marine algae. Selvi *et al*[30] screened around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *Bacillus subtilis* and *Staphylococcus* sp. were highly susceptible to most of the algal extracts. In the present investigation the ethanol extract showed less activity against *Staphylococcus* sp.

Thirumaran *et al*[31] reported that antibacterial activity of marine macro alga *Dictyota dichotoma* from Gulf of Mannar coast, the maximum activity was noted in diethyl ether extracts against *Salmonella paratyphii*. Thirumaran *et al*[32] screened the antimicrobial activity of *Hydroclathrus clathratus* using methanol extracts along the Gulf of Mannar Coast and reported that *Pseudomonas aeruginosa* were more susceptible than the other extracts. In the present study, seaweed of *Padina gymnospora* shows promising results against antibacterial pathogens. This finding supports that of Naqvi *et al*[33] who demonstrated that some marine plants showed antibacterial activity against three bacterial strains. It has been reported the activity of marine algae against animal moulds *Aspergillus niger*. Thus, earlier results are dissimilar to the present study, in which well antifungal activity was observed in all the extracts against almost all the fungal pathogens. The present study showed minimum activity against *Aspergillus niger*.

Padmakumar and Ayyakkannu[26] screened 80 species of marine algae for antifungal activities but did not find a single algal extract active against *Aspergillus flavus*. Present investigation, on the other hand, showed that extract of the seaweed is effective against *Aspergillus flavus*. Thus, this study is entirely different from earlier workers on this aspect. The dried extracts have less effect on bacteria in comparison to the fresh extracts. This result can be related to volatile antimicrobial compounds in the sample such as hydrogen peroxide, terpenoid and bromo ether compounds[34,35]. Another reason might be the loss of active materials that may be present in alga, like volatile fatty acids, during the drying process. Another significant result of the present study was that the acetone extracts of all algal species showed antibacterial activity. The antibacterial activities of the extracts from 26 algal species were prepared by dichloromethane, methanol and water[25].

The seven different solvent extracts of seaweeds used in the present study showed significant bacterial action. The interesting information is that the product is in the form of natural good for health and fails to cause side effects. From these preliminary investigations the algal members of both the estuaries merit need further investigation. a detailed study on the principle compound in the seaweeds which is responsible for antimicrobial activity is still needed.

Conflict of interest statement

We declare that we have no conflict of interest.

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