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ANTIMICROBIAL ACTIVITY OF SEAWEEDS FROM THE GULF OF MANNAR

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ABSTRACT

Marine algae are known as a potential source of bioactive substances. In the present work, we used four seaweeds (*Sargassum wightii, Stocheospermum marginatum, Gracilaria foliifera* and *Padina boergesenii*) , extracted in four solvents (acetone, methanol, chloroform and diethyl ether) and tested for their antimicrobial activity against 12 bacterial pathogens (*Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enterococci* sp. *Proteus* sp. *Streptococcus* sp. *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp, *Shewanella* sp. *Vibrio flurialis* and *Vibrio splendidus*) and also against five fungal pathogens (*Aspergillus niger, Candida albicans, Penicilium* sp., *Aspergillus flavus* and *Aspergillus tetreus*). All the extracts, in particular brown seaweeds extracted in acetone exhibited the significant antimicrobial activity. This study established acetone extracts of brown seaweed were highly effective,against bacteria and fungi.In future the research may help to identify the bioactive compounds from the brown species.

Keywords: Marine algae, seaweeds, antimicrobial activity

INTRODUCTION

Seaweeds have been traditionally used in human and animal nutrition. Seaweeds are rich source of bioactive compounds such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins and minerals. Important polysaccharides such as agar, alginates and carrageenans obtained from seaweeds are used in pharmaceutical as well as in the food industries [1]. Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organisms and pathogens chemical defense mechanisms against herbivore; for example, grazer- induced mechanical damage triggers the production of chemicals that acts as feeding detergents or toxins in seaweeds [2].

Most of the secondary metabolites produced by seaweeds have bacteriocidal or the antimicrobial compounds derived from seaweeds consist of diverse groups of bacteriostatic properties

brominates phenols, oxygen heterocyclic; Terpenols, Sterols, Polysaccharides, dibutenolides peptides and proteins. Although most of the antibiotics found from terrestrial sources are used as therapeutic agents to treat various diseases, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value. [3, 4].The use of anti microbial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produce. These limitations demand for improved pharmacokinetic properties which necessitates continued research for the search of new antimicrobial compounds for the development of drugs. Hence the present study the antimicrobial activities of red and brown algae using different solvents were investigated.

MATERIALS AND METHODS

Sample collection and preparation

Four seaweeds species (*Sargassum wightii***,** *Stochospermum marginatum*
Padina boergesenii and *Gracilaria foliifera)* were collected from Gulf of Mannar, southeast coast of India at a latitude 9°45' N and longitude 79°0' E during the low tide.

Collected samples were washed with seawater to remove epiphytes and other marine organisms. The seaweeds were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder.

Extraction of marine algae [5]

Organic solvents (acetone, methanol, chloroform and diethyl ether) were used for extraction. Each powdered sample (5g) was soaked in about 40 ml of the solvent for three days. The resultant crude extracts were filtered and then concentrated in a rotatory evaporator at a temperature of less than 40 ^{0} C. The residual water was removed with a vacuum pump. The crude extracts were weighed and deep frozen (-20^{0}C) until testing.

Bacterial and Fungal strains used for assay

The antibacterial assay was carried out by using *Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Enterococci* sp., *Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp., *Shewanella* sp. , *Vibrio flurialis* and *Vibrio splendidus.*

The antifungal assay was carried out by using *Aspergillus niger, Candida albicans, Penicilium* sp. *Aspergillus flavus* and *Aspergillus tetreus.* The bacterial stock cultures were maintained on Muller Hinton Agar medium and fungal cultures were maintained on Saboured Dextrose Agar medium. Microbial strains were obtained from the Muthaiah Medical College, Annamalai University, Annamalai nagar.

Antibacterial Assay

The bioassay was carried out using the agar disc diffusion method [5] with paper disc of 6mm diameter, prepared from Whatman No.1 filter papers. The bacteria inoculated were grown in nutrient broth overnight and fixed volume inoculated into 10 ml aliquots of nutrient agar, mixed and then poured over a nutrient agar sterile Petri dishes. This formed the bacterial lawn. Initially both paper discs and well were used for testing the crude extracts. The paper disc of 6mm soaked in 6 microlitre of crude extract and placed on to the bacterial lawn after it had solidified, standard antibiotic discs were incubated at 37^{0} C over night. The zones of inhibition were measured after 24 hour incubation.

Antifungal assay:

Young fungal cultures were incubated for 2-3 days at room temperature and seeded on Sabouraud Dextrose Agar plates (SDA) for bioassay by agar disc diffusion method. Whatman

No.1 filter paper disc of 6mm containing the seaweed extract were placed on surface of the plates. After 72 hours at 30^0 C the plates were observed for the presence of inhibition zones.

RESULTS

ANTIBACTERIAL ACTIVITY

Sargassum wightii :

The extract obtained using acetone showed a maximum activity against pathogen like *Proteus* sp., (7mm) *Enterococci* sp.(6mm), *Staphylococcus aureus (6mm), Escherichia coli* (6mm) and minimum activity against *Salmonella* sp.,(2mm). Observation was made from methanol extract showed a maximum activity against *Enterococi* sp**.,** (11mm), *Sterptococcus* sp., (9mm), *Escherichia coli* (8mm), *Salmonella* sp., (8mm), *Shewanella* sp., (8mm), *Vibrio splendidles* (6mm) and minimum activity against *Vibrio flurialis* (3mm). The extract obtained using chloroform showed a maximum activity against pathogen like *Vibrio flurialis* (7mm), *Vibrio splendidus* (6mm), *Shewanella* sp.,(6mm), *Klebsiella pneumoniae* (6mm) and the minimum activity against *Proteus* sp.,(5mm), *Escherichia coli*(4mm), *Staphylococcus aureus*(4mm), *Vibrio parahaemolyticus*(4mm), *Salmonella* sp.,(4mm), *Enterococci* sp., *Pseudomonas aeruginosa*,(3mm) and *Streptococcus* sp., (3mm).The diethyl ether showed the maximum activity against pathogen like *Klebsiella pneumoniae* (6mm), *Vibrio parahaemolyticus*(6mm), *Vibrio splendidles* (6mm) and minimum activity with *Vibrio flurialis* (1mm) (Fig.1.).

***Stochospermum marginatum*:**

The investigation made on acetone extracts showed maximum activity against *Escherichia coli* (11mm), *Streptococcus* sp., (1 mm). Methanol extract showed maximum activity *against Klebsiella pneumoniae* (10mm), and *Escherichia coli* (10mm), V*ibrio flurialis* (3mm), *Enterococci sp.,* (3mm*), Staphylococcus aureus* (3mm) and *Pseudomonas aeruginosa* (3mm). The extracts using chloroform showed maximum activity against *Vibrio splendidus* (7mm), *Staphylococcus aureus* (3mm), and *Klebsiella pneumoniae* (3mm). Diethyl ether pointed out maximum activity against *Vibrio splendidus* (7mm) and minimum activity against , *Vibrio flurialis* (1mm) and where as no activity against *Pseudomonas aeruginosa* and *Vibrio parahaemolytics* was observed (Fig.2.).

***Gracilaria foliifera*:**

The extract obtained using acetone showed a maximum activity against pathogens like *Vibrio splendidles* (8mm), and minimum activity against *Vibrio parahaemolytics* (2mm) and *Streptococcus* sp., (2mm). The extract using methanol showed maximum activity against *Streptococcus* sp., (8mm), and showed minimum activity against pathogens like *Proteus* sp., (1mm) where as very less activity against like *Shewanella* sp., Chloroform extract showed maximum activity against *Vibrio flurialis* (8mm), *Proteus* sp., (2mm). The diethyl ether observed the maximum activity against *Staphylococcus aureus* (6mm) and showed minimum activity against *Vibrio parahaemolytics* (1mm) and where as no activity was observed against pathogen like *Klebsiella –pneumoniae* (Fig.3.)

***Padina boergesenii*:**

Acetone extract showed maximum activity against *Escherichia coli.* (7mm), *Pseudomonas aeruginosa* (7mm), *Streptococcus* sp.,(7mm), and minimum activity against *Vibrio parahaemolytics* (2mm). The methanol extract pointed out maximum activity against pathogen *Streptococcus* sp., (12mm), *Pseudomonas aeruginosa* (10mm). Minimum activity was showed against *Enterococci* sp., (2mm) and V*ibrio flurialis* (2mm).where as no activity was seen against *Echerichia coli* and *Vibrio*

splendidus. The chloroform observed the maximum activity against pathogen *Enterococci* sp., (9mm), and *Salmonella* sp., (9mm), and minimum activity against *Vibrio flurialis* (3mm).The extracts using diethyl ether showed maximum activity against *Staphylococcus aureus* (7mm) and *Vibrio parahaemolytics* (7mm) and minimum activity against *Klebsiella pneumoniae* (1mm). whereas no activity was seen *Salmonella* sp against (Fig.4.).

ANTIFUNGAL ACTIVITY

Sargassum wightii :

The investigation made on acetone extracts showed maximum activity against *Candida albicans* (7mm) and minimum activity against *Aspergillus niger* (2mm).Observation made from methanol extract showed a maximum activity against pathogen like *Aspergillus flavus* (5mm) and minimum activity against pathogen like *Penicilium* sp., (2mm).The chloroform showed maximum activity against *Aspergillus tetreus* (5mm) and minimum activity against *Aspergillus flavus* (2mm) and *Aspergillus niger* (2mm). Whereas no activity was seen against *Penicilium* sp.The extract obtained using diethyl ether showed a maximum activity against *Candida albicans* (15mm) and minimum activity against *Penicilium* sp., (1mm) (Fig.5.).

***Stochospermum marginatum*:**

Acetone extract pointed out maximum activity against *Aspergillus niger* (14mm) and minimum activity against *Aspergillus tetreus* (2mm).Methanol extract showed maximum activity against *Aspergillus niger* (7mm), and minimum activity against *Candida albicans* (2mm).The chloroform extract observed the maximum activity against *Aspergillus tetreus* (6mm) and minimum activity against *Aspergillus niger* (1mm) and *Penicilium* sp., (1mm). Where as no activity was seen against *Candida albicans*.The diethyl ether observed the maximum activity against *Penicilium* sp., (13mm) and minimum activity against *Aspergillus niger* (2mm) Fig.6.

Gracilaria foliifera:

The acetone proved the maximum activity against pathogen like *Aspergillus niger* (14mm) and minimum activity against *Aspergillus tetreus* (3mm).Observation made from methanol extract showed a maximum activity against *Aspergillus tetreus* (6mm) and the minimum activity against *Candida albicans* (2mm), *Penicilium* sp., (2mm), and *Aspergillus flavus* (2mm) respectively.The extract using diethyl ether showed maximum activity against *Candida albicans* (9mm), minimum activity against *Aspergillus tetreus* (2mm).Chloroform extract obtained maximum activity against *Aspergillus tetreus* (8mm) and minimum activity against *Aspergillus niger* (1mm) and *Candida albicans* (1mm) (Fig.7).

***Padina boergesenii*:**

Acetone dried extract obtained showed maximum activity against and minimum activity against *Candida albicans* (2mm).Methanol observed Maximum activity against *Aspergillus tetreus* (5mm) and *Candida albicans* (5mm) and showed minimum activity against *Aspergillus flavus* (3mm).Chloroform extract observed maximum activity against *Aspergillus flavus* (3mm) and minimum activity against *Penicilium* sp., (1mm) and *Aspergillus tetreus*. Whereas no activity was observed against *Candida albicans*.The diethyl ether extract proved maximum activity against *Aspergillus flavus* (12mm) and minimum activity against *Aspergillus niger* (2mm).Whereas no activity was seen against pathogen like *Penicilium* sp (Fig.8).

DISCUSSION:

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [6] with antiviral, antibacterial and antifungal activities

[7]. These seaweeds act as potential bioactive compounds of interest for pharmaceutical applications [8].

Hodgson (1984) [9] has reported antimicrobial activity of seaweeds belonging to Chlorophyta, Phaeophyta and Rhodophyta. A number of marine algae from eastern Sicily like *Dictyota dichotoma, Cystoseria elegans and Laurencia obtusa* showed antibacterial activity [10]. Lipid extract of *Zonardinia prototypes* and *Cystoseira balearica* showed strong antimicrobial activity against *Bacilas subtillis,Phoma tracheiphila* and antiviral activity against Tobaco Mosaic Virus [11]. Pesando and Caram (1984)[12] and Reichelt and Borowitzka (1984) [13] have reported that extracts from brown algae show higher degrees of anti bacterial activity rather than extracts obtained from red and green algae. Also, pholorotannins, phenolic compounds and cliterpenediol (crinitol) are reported to be produced by brown algae *Sargassum critaefolium, S. tortile, Ecklonia kurome, E. bicyclis* and *Cystoseira crinite* and also exhibit antibacterial activity [14-16]and antifungal activity [17]. The majority of the compounds isolated from marine algae are responsible for the antimicrobial activity. The compounds are [18-23], phenolic [24, 25] and lipidic is nature [26-29].

The maximum antimicrobial activity shown by brown algae in the present work conforms to the earlier work. Disc diffusion methods are extensively used to investigate the anti microbial activity of natural substances and plant extracts. In the present work, the crude extract of four different seaweeds were evaluated for antimicrobial activity against pathogenic bacteria. *Padina boergesenii* showed maximum inhibition against *Streptococcus* sp., and *Stochospermum marginatum* showed maximum inhibition against *Aspergillus niger*. *Stocheospermum marginatum* showed maximum fungal inhibition against *Aspergillus niger* whereas *Gracillaria folifera* exhibited strong antifungal activity against all the fungal strains tested.

This study showed that agar diffusion methods using different test microorganisms are valuable tool for the antimicrobial activity of seaweeds. Among the bacterial test strain, the Gram positive bacteria were more susceptible than Gram negative bacteria. This may be due to the more complex structure of cell wall of Gram negative bacteria [30, 12, 13]. In the present work the acetone extract exhibited a strong antimicrobial activity against bacteria when compared to other extracts.

There have been many reports on the screening of seaweeds for antimicrobial activity. This capability of the seaweeds can be attributed to synthesis of bioactive secondary metabolites. The complexity of antimicrobial properties in seaweeds is due to their multiple inhibitory properties. It may be due to both long term defense as well as rapid activation induced by environmental conditions. The intra specific variability in the production of secondary metabolites in seaweeds is generally related to seasonal variation; differences in the extraction protocols to recover the active metabolites and assay methods that would result in different susceptibilities of the target strains.

The genera *Sargassum* and *Gracilaria* have been extensively studied for their economic use mainly phyco collid production. But not much research work has been done in *Padina* and *Stochospermum,*which is abundantly available in Indian waters. These species may be a good candidate as a source of bioactive compounds, which deserve further investigation.

The acetone extract exhibited a strong antimicrobial activity against both gram–positive and gram–negative bacteria when compared to other solvent extracts in all the species of seaweeds. This study established acetone extracts of brown seaweed were highly effective, against

bacteria and fungi. Organic solvents always provide a higher efficiency in extracting antimicrobial activities as compared to water extraction as was all so found in the present study [31].

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Figures

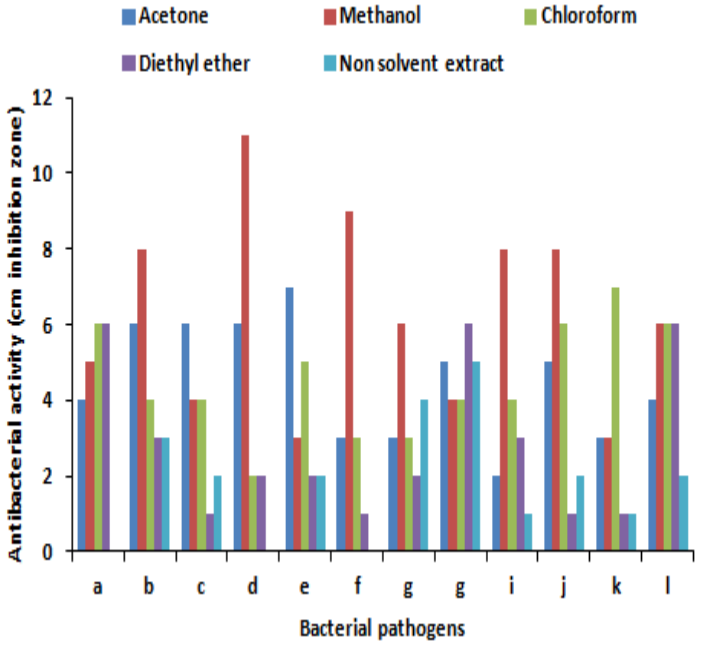

Fig.1. Antibacterial activity of *Sargassum wightii*. a-*Klebsiella pnaemoniae*, b-*Escheria coli*, c-*Staphylococus aureus*, d-*Enterococci* sp, e-*Proteus* sp, f-*Streptococus* sp, g-*Pseudomonas aeruginosa*, h-*Vibrio parahaemolyticus*, i-*Salmonella* sp, j-*Shawanella* sp, k-*Vibrio flurialis*, l-*Vibrio splendidus*

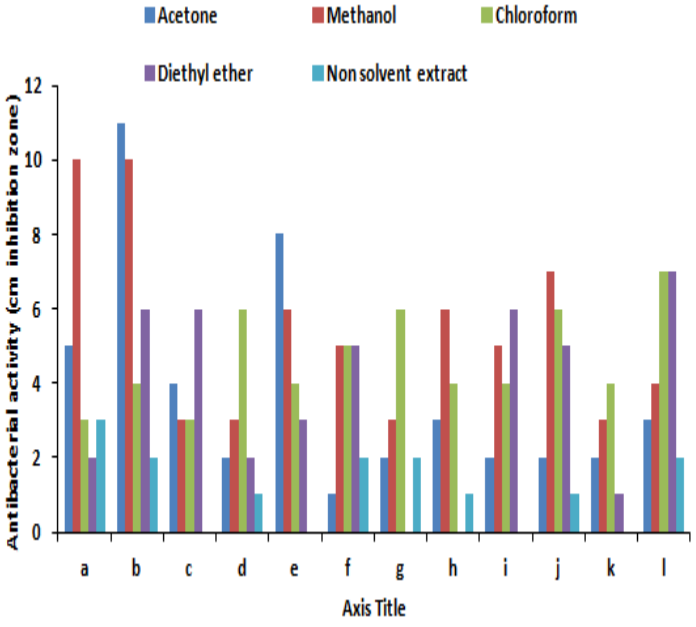

Fig.2. Antibacterial activity of *Stocheospermum marginatum* (Seaweed). a-*Klebsiella pnaemoniae*, b-*Escheria coli*, c-*Staphylococus aureus*, d-*Enterococci* sp, e-*Proteus* sp, f-*Streptococus* sp, g-*Pseudomonas*

aeruginosa, h-*Vibrio parahaemolyticus*, i-*Salmonella* sp, j-*Shawanella* sp, k-*Vibrio flurialis*, l-*Vibrio splendidus*

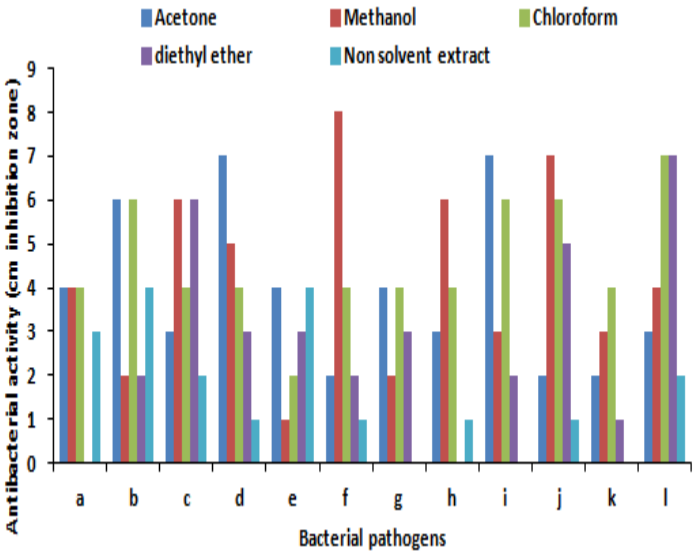

Fig.3. Antibacterial activity of *Gracilaria folifera* (Seaweed). a-*Klebsiella pnaemoniae*, b-*Escheria coli*, c-*Staphylococus aureus*, d-*Enterococci* sp, e-*Proteus* sp, f-*Streptococus* sp, g-*Pseudomonas aeruginosa*, h-*Vibrio parahaemolyticus*, i-*Salmonella* sp, j-*Shawanella* sp, k-*Vibrio flurialis*, l-*Vibrio splendidus*

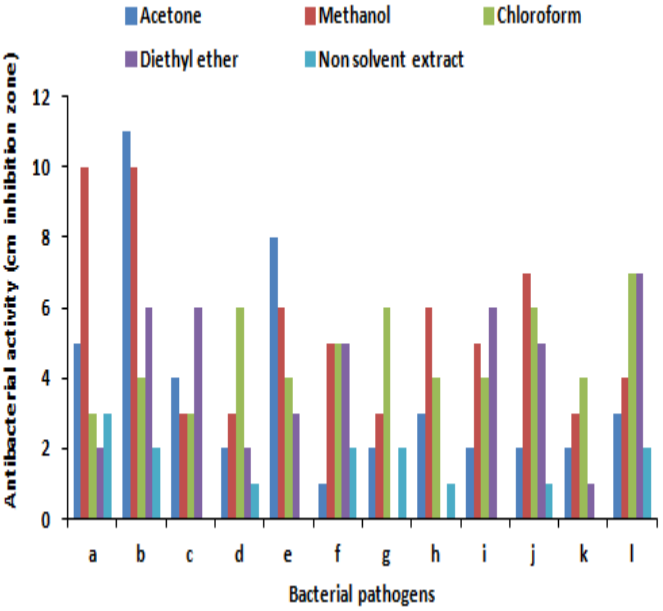

Fig.4. Antibacterial activity of *padina boergesenii* (Seaweed). a-*Klebsiella pnaemoniae*, b-*Escheria coli*, c-*Staphylococus aureus*, d-*Enterococci* sp, e-*Proteus* sp, f-*Streptococus* sp, g-*Pseudomonas aeruginosa*, h-*Vibrio parahaemolyticus*, i-*Salmonella* sp, j-*Shawanella* sp, k-*Vibrio flurialis*, l-*Vibrio splendidus*

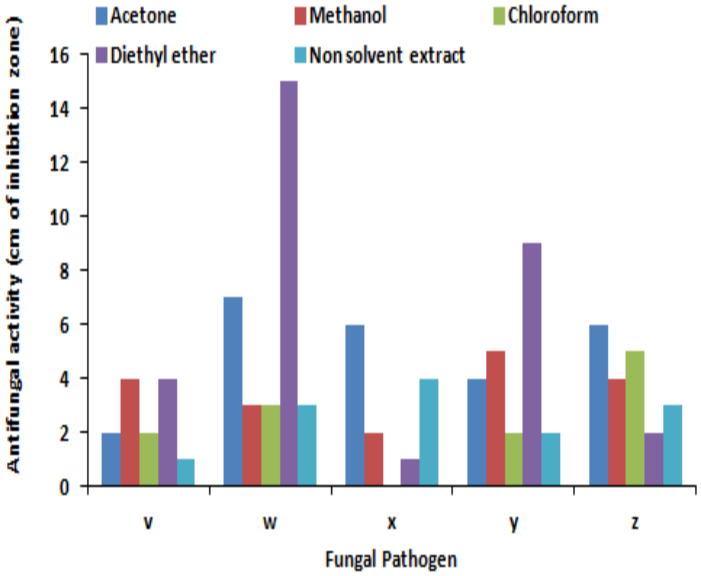

Fig.5. Antifungal activity of *Sargassum wightii* (Seaweed). a- *Aspergillus niger*, b- *Candida albicans*, c- *Penicilium* sp, d- *Aspergillus flavus*, e- *Aspergillus tetreus*

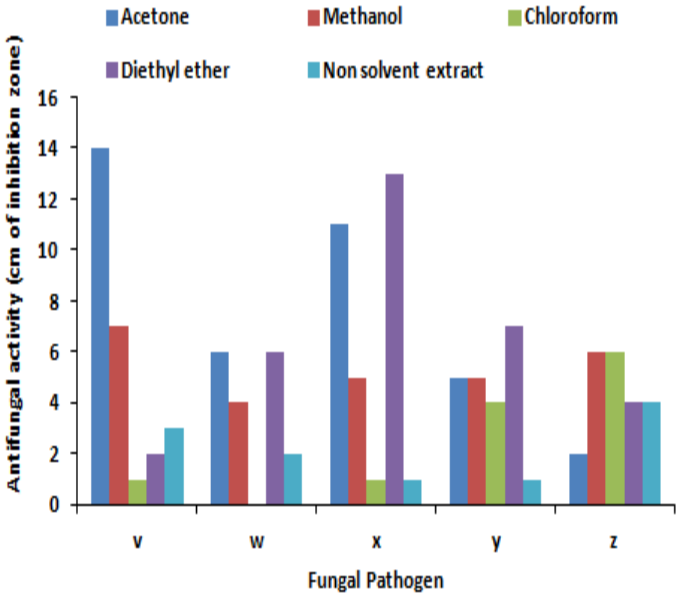

Fig.6. Antifungal activity of *Stocheospermum marginatum* (Seaweed). a- *Aspergillus niger*, b- *Candida albicans*, c- *Penicilium* sp, d- *Aspergillus flavus*, e- *Aspergillus tetreus*

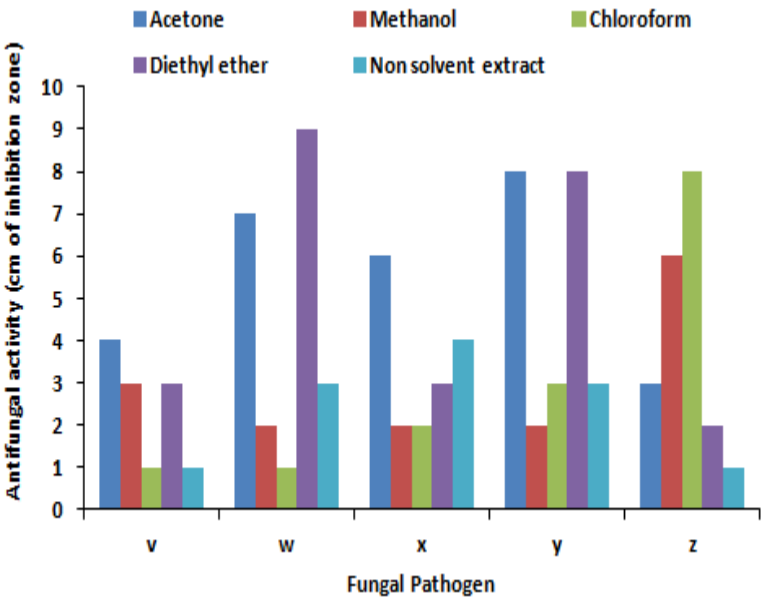

Fig.7. Antifungal activity of *Gracilaria foliifera (*Seaweed). a- *Aspergillus niger*, b- *Candida albicans*, c- *Penicilium* sp, d- *Aspergillus flavus*, e- *Aspergillus tetreus*

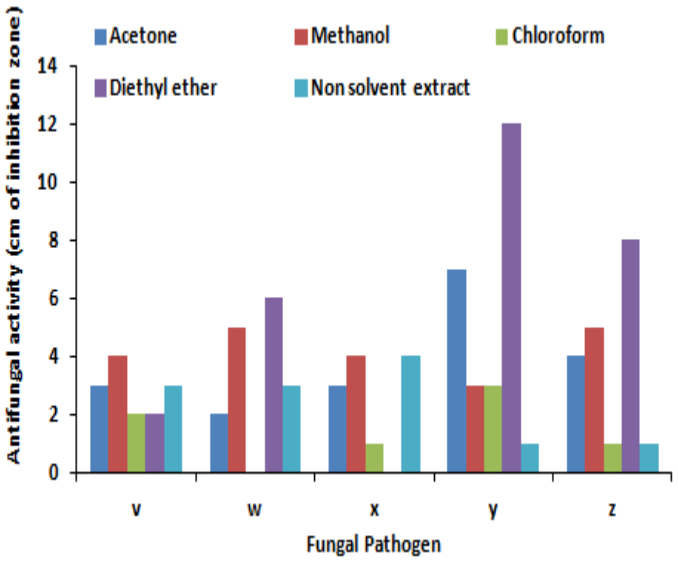

Fig.8. Antifungal activity of *padina boergesenii (*Seaweed). a- *Aspergillus niger*, b- *Candida albicans*, c- *Penicilium* sp, d- *Aspergillus flavus*, e- *Aspergillus tetreus*