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Research Article

INVITRO ANTAGONISTIC ACTIVITY OF MARINE SEAWEED EXTRACTS AGAINST HUMAN PATHOGENS

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ABSTRACT

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites and are possessed with antibacterial, antiviral, cytotoxic and larvicidal potentials. Five different seaweed samples were collected from different locations at Gulf of Mannar, Southeast Coastal region, Pudhumadam, India. They were identified as *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora* and *Chaetomorpha antennina*. Sample preparation was done by obtaining aqueous extracts and solvents extracts of seaweeds for testing their antimicrobial potential against human pathogens. Solvent extracts of seaweed samples were prepared using different solvent system. The results obtained on antibiogram pattern of *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora and Chaetomorpha antennina* against bacterial and fungal test pathogens, showed that among the five seaweeds two samples namely *Sargassum wightii* and *Caulerpa sertularioides* showed wide spectrum activity and high degree of zone of inhibition against the target organisms. Fourier Transmission Infra Red Spectroscopy was used for the chemical characterization of potential seaweed samples such as *Sargassum wightii* and *Caulerpa sertularioides.* FTIR spectral data for major peaks of *Sargassum wightii* and *Caulerpa sertularioides* showed the presence of C =N stretch, C- H stretch and hydrogen bonded O –H stretch which indicated the presence of functional groups such as nitriles, aldehydes and phenols or alcoholic groups, esters and nitro groups. This study will provide scope in exploiting indigenous resources for further isolation of antimicrobial compounds.

Keywords: Marine algae, Seaweeds, Bioactivity, Antimicrobial activity, Solvent Extracts, Human pathogens, Pudhumadam.

INTRODUCTION

Marine plants have long been recognized as producers of biologically active substances. Potential activities of some marine plants like mangroves, seaweeds, seagrasses and lichens have been reported from both India and elsewhere[1]. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds, and other marine organisms. As a consequence of an increasing demand in the screening programs for therapeutic drugs from natural products, there is now a greater interest on marine organisms especially seaweeds. Seaweeds are extraordinary sustainable resources in the marine ecosystem which have been used as a source of food and feed and about 50% of the global photosynthesis is contributed from marine algae[2]. Approximately 841 species of marine algae found in both inter – tidal and deep water regions of the Indian coast[3].

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites[4]. Recent findings evidenced that seaweeds contained antibacterial[5], antiviral[6], antifungal[7], cytotoxic[7] and larvicidal potentials[8]. The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthytoxicity in lower form of animals[9].

Ethanolic extract of 8 species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad spectrum antibacterial and antifungal activities[10]. The algal extracts such as *Enteromorpha compressa, Cladophorois zoolingeri, Padina gymnospora, Sargassum wightii* and *Gracilaria corticata* were active against gram positive and gram negative bacteria. Many bioactive and pharmacologically active substances have been isolated from algae. The bioactive properties of seaweeds are generally assayed using extracts in various organic solvents.

For instance, extracts of marine algae were reported to exhibit antibacterial activity[11]. Many authors had found antibacterial activities of microalgae due to fatty acids[12]. Compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algal

solvent extracts[13,14]. Keeping the above facts in mind, the main objective of the present study is to collect different marine seaweeds from Pudhumadam region and to screen and characterize their antimicrobial activity.

MATERIALS AND METHODS

COLLECTION & IDENTIFICATION OF SEAWEEDS

Five different seaweed samples were collected during the low tidal conditions at depths of 1 to 3m from different locations of Gulf of Mannar, Southeast coastal region, Mandapam with the help of divers. To avoid air contamination, seaweed samples were collected aseptically below surface level, using sterilized wide mouthed glass bottle containers. The containers and its accessories were steam sterilized for 5 minutes at 15 lbs. They were transferred to the laboratory and were identified at Department of Marine and Coastal Studies, Madurai Kamaraj University, Tamil Nadu, India.

SAMPLE PREPARATION

Algal samples were cleaned of epiphytes and extraneous matter using sea water and necrotic parts were removed. Then they were rinsed with sterile distilled water and shade dried, cut into small pieces and powdered in a mixer grinder.

AQUEOUS EXTRACTION OF SEAWEEDS

Aqueous extracts were prepared by transferring 1 g of the different seaweed powder samples to appropriately labelled sterile wide mouthed screw-capped bottles of 50 ml volume. 4 ml of sterile deionised distilled water was added to the powdered samples which were allowed to soak for 24 – 48 hrs at room temperature. The mixtures were then centrifuged at 2000 rpm for 10 min at 4ºC.The supernatants were filtered through sterile Whatman No. 1 filter paper.

SOLVENT EXTRACTION OF SEAWEEDS

The experimental procedure was carried out with different solvent system such as methanol, dichloromethane, diethyl ether, ethyl acetate, hexane, benzene, ethanol, n-butanol, chloroform One gram of each seaweed sample was extracted with 4m1 of the solvents. The dried sample were soaked in the solvents for 24 – 48 hrs in sterile wide-mouthed screw-capped bottles of 50 ml volume and then covered with aluminium foil. The mixture was then centrifuged at 2000 rpm for 10 min at 4ºC. The supernatants were filtered through sterile Whatman No. 1 filter paper. Extracts obtained were used for screening of their antimicrobial potential[15].

SCREENING FOR ANTIBACTERIAL ACTIVITY CHARACTERIZATION OF TEST ORGANISMS

The target organisms used in the present study includes the bacterial (gram positive and gram negative) species such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris*, *Pseudomonas aeruginosa, Salmonella typhimurium, Shigella flexneri, Vibrio cholerae* and the fungal species (filamentous and non filamentous) such as *Candida albicans, Aspergillus niger.* The target organisms were obtained from Clinical Laboratory, Madurai, Tamilnadu and screened according to Bergey's manual of Bacteriology[16,17].

WELL DIFFUSION ASSAY

This assay was used for testing the antagonistic activities of crude seaweed extracts[18]. Required wells were made in the Muller Hinton agar using well cutter and 100µl of the extracts of different seaweeds were transferred into each well by taking solvent as control. The plates were incubated for 24 hrs at 30°C and examined for clear inhibition zone around the well. The assay was carried out in duplicate for all the test organisms[15].

CHEMICAL CHARACTERIZATION FTIR ANALYSIS

The seaweed extracts which possessed potential antimicrobial activities were subjected to chemical characterization using Fourier Transform Infra Red Spectroscopy (FTIR). FT-IR - 8400S, SHIMADZU model was used for the analysis and the spectrum was taken in the mid IR region of 400 – 4000 cm^{-1}. The spectrum was recorded using ATR (Attenuated Total Reflectance) technique. The sample was directly placed in the Potassium bromide crystal and the spectrum was recorded in the transmittance mode.

RESULTS

Five different seaweed samples were collected during the low tidal conditions at depths of 1 to 3m from different locations at Gulf of Mannar, southeast coastal region, Mandapam, India. The collected marine seaweed samples were identified as *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora* and *Chaetomorpha antennina* at Department of Marine and Coastal Studies, Madurai Kamaraj University, Tamil Nadu, India.

Aqueous extracts were prepared by adding sterile deionised distilled water to the powdered samples which were allowed to soak for 24 – 48 hrs at room temperature. The extraction procedure was also carried out with different solvent system such as methanol, dichloromethane, diethyl ether, ethyl acetate, hexane, benzene, ethanol, n-butanol, chloroform. Solvent extraction of seaweeds was carried out by soaking the dried samples in the solvents for 48 hrs and the extracts obtained by the above method were used for screening of their antimicrobial potential by taking solvent samples as control. Well diffusion assay was used for testing the antimicrobial activity of seaweed extracts. In this technique, bacterial (both gram positive and gram negative) and fungal organisms were used (**Table: 1 – 5**).

Fourier Transmission InfraRed Spectroscopy was used for the chemical characterization of solvent extracted potential seaweed sample (*Sargassum wightii* and *Caulerpa sertularioides* (**Table: 6; Fig: 1 and 2**). The spectrum was taken in the mid IR region of 400 – 4000 cm^{-1}. FTIR spectrum for all the experimental samples exhibited absorption bands at 3432.09 cm^{-1}, 2088.75 cm^{-1}, and 520.74 which indicates primary, secondary amides, hydroxyl groups and alkyl halides respectively. The spectrum at 1641.31 cm^{-1} showed the presence of alkenes in the extracted seaweed samples**.**

DISCUSSION

Bacterial infection causes high rate of mortality in human population. For example, *Bacillus subtilis* is responsible for causing food borne gastroenteritis. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella sp*. causes diarrhoea and typhoid fever[19,20]. *P. aeruginosa* is an important and prevalent

pathogen among burned patients capable of causing life-threatening illness[21]. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria[22]. Moreover the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives[9].

The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from sea-weeds are used in medicine and pharmacy[11]. Thus, marine algae are among the richest sources of known and novel bioactive compounds[23,24].

Given the economic potential of this seaweed and the importance of α-1,3-glucanase and chitinase as potential determinants in the resistance of plants to fungal diseases[25], the presence of these proteins in the lectin-rich fraction obtained from the red seaweed *H. musciformis* was analyzed and the activity against the human pathogen yeasts *Candida albicans* and *C.guilliermondii* was assessed.

Five different seaweed samples were collected from different locations of Gulf of Mannar, Mandapam, India and were identified as *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora* and *Chaetomorpha antennina* in the present study. Aqueous extracts and solvents extracts were prepared for testing the antimicrobial potential of the collected seaweed samples. Extract obtained was used for screening their antimicrobial potential (bacterial and fungal) by taking different solvent samples as control. Based on the results obtained for antagonistic activity, it is documented that though several solvents were utilized the following order of the solvents showed high degree of activity against test organisms i.e. Methanol, dichloromethane, benzene, carbon tetrachloride and hexane respectively. This order of activity in case of solvents was found to be revealed for the entire seaweed samples.

While inferring the broad spectrum activity of *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora* and *Chaetomorpha antennina* against bacterial and fungal test pathogens the seaweed samples predominantly were effective against gram negative organisms. In addition to that, it was documented from the present investigation among the five different seaweeds two samples namely *Sargassum wightii* and *Caulerpa sertularioides* showed wide spectrum activity and high degree of zone of inhibition against the target organisms. In aqueous extracts antagonistic activity for all test organisms was not obtained. These results were in accordance with earlier reports[26,27].

Antibacterial activities of two species of seaweeds *Caulerpa sertularioides, Grateloupia lithophila* tested against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Staphylococcus epidermidis* and *Bacillus sp*. For some species the anti bacterial activity we observed was similar to previous screening studies. We have evaluated the antimicrobial potential of from both aqueous and solvent extracts. Different solvents such as Methanol, Ethanol, Butanol, Chloroform, Acetone, Dichloromethane, water were used. Present work revealed the presence of moderate to effective antimicrobial activity in almost all the seaweed extracts[26,27,28].

Seaweed extracts are considered to be a rich source of phenolic compounds[26,29]. The large majority of these (about 60%) are terpenes, but fatty acids are also common (comprising about 20% of the metabolites), with nitrogenous compounds and compounds of mixed biosynthesis each making up only about 10%[30]. Fatty acids are isolated from micro algae that exhibited antibacterial activity[31]. Methanolic extracts of fifty-six seaweeds collected from South African coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae showed antibacterial activity.

Srinivasa Rao and Parekh[32] showed that crude extracts of Indian seaweeds were active only against Gram positive bacteria. Ethanol extracts from 56 Southern African seaweeds from the divisions Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red) scored highest antibacterial activity for Phaeophyta. Similar results were reported by Caccamese and Azzolina[33], Caccamese *et al.,*[34,35] and Pesando and Caram[36] for screening studies on seaweeds of Mediterranean and Eastern Sicily coast respectively.

Fourier Transmission Infra Red Spectroscopy was used for the chemical characterization of solvent extracted potential seaweed sample (*Sargassum wightii* and *Caulerpa sertularioides*). FTIR spectral data for major peaks of *Sargassum wightii* showed the presence of C =N stretch, C- H stretch and hydrogen bonded O –H stretch which showed the presence of nitriles, aldehydes and phenols or alcoholic groups in the crude extract. FTIR spectral data for predominant peaks of *Caulerpa sertularioides* showed the presence of C =N stretch, C- H stretch, hydrogen bonded O –H stretch, C=O stretch and N=O bend which indicated the presence of functional groups such as nitriles, aldehydes and phenols or alcoholic groups, esters and nitro groups.

The use of seaweeds in the development of pharmaceuticals, nutraceuticals, botanicals and as a source of pigments, bioactive compounds and antiviral agents is in full expansion. Ecologically and commercially seaweeds are considered important as the potential source as food supplement of 21st century because it contains proteins, lipids, polysaccharides, minerals, vitamins and enzymes. They are being used for food industry, pharmaceutical industry and other uses such as integrated aquaculture with fishes or mollusc culture and also have been tried to remove heavy metals in cleaning waste water.

CONCLUSION

Marine Macroalgae collected from the Pudhumadam of India have shown to possess a number of biological activities. In our studies *Sargassum wightii, Caulerpa racemosa, Caulerpa sertularioides, Padina gymnospora* and *Chaetomorpha*

antennina were collected and checked for their antimicrobial activity. These seaweeds are currently undergoing preliminary investigations with the objective of screening of biologically active species. Furthermore, the encouraging biological activities seen in this study showed that the Indian coastline is a potential source of variety of marine organisms worthy for further investigation. From the present study, it is initiated to explore the bioactive potential of major seaweeds infested along Gulf of Mannar as a potential source of marine bioprospecting in future. This study aims to pave way for the identification of new bioactive compounds for treating the dreadful diseases and thereby help to reduce the multidrug resistant organisms.

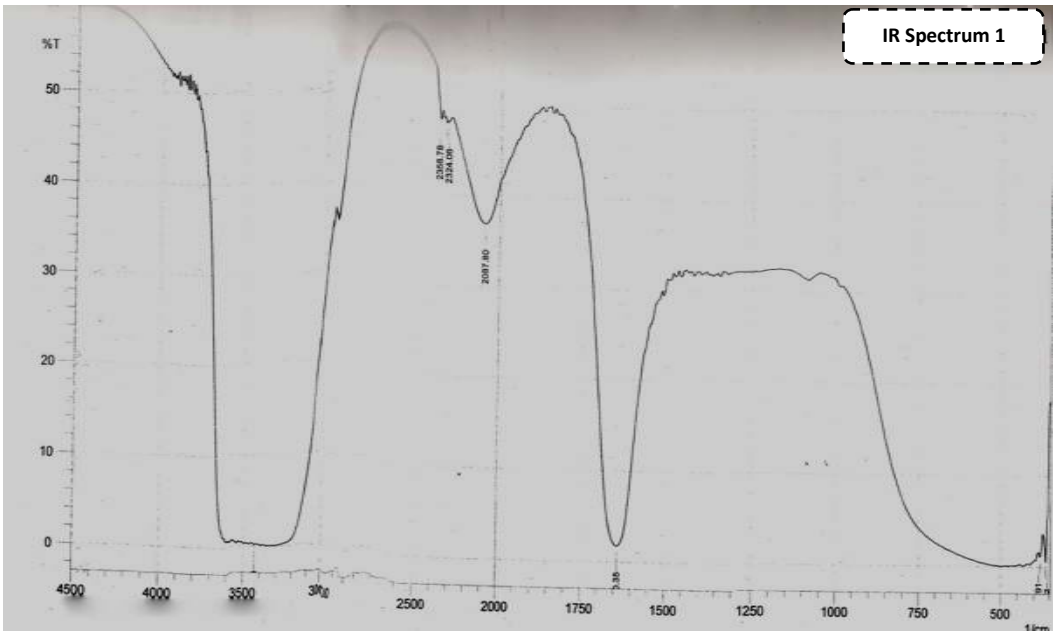

Figure 1: Infra Red Spectrum Analysis of *Sargassum wightii* Extracts

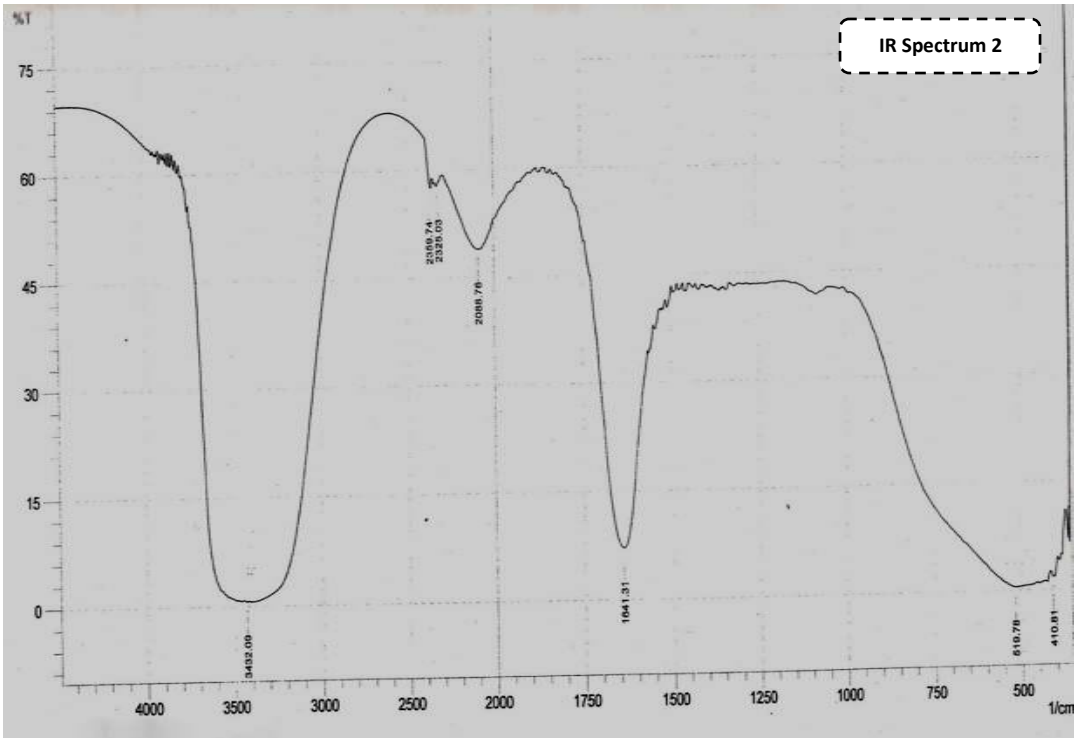

Figure 2: Infra Red Spectrum Analysis of *Caulerpa sertularioides* Extracts

Table 1: Antagonistic Activity of *Sargassum wightii* against Test Organisms

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl Acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
Gram Positive Organisms *Streptococcus mutans*	20	23	15	11	11	24	20	17	18	-
Staphylococcus aureus	21	18	15	20	19	10	11	19	24	-
Staphylococcus epidermidis	19	24	18	13	11	11	20	19	23	-
Gram Negative Organisms *Escherichia coli*	20	20	13	20	17	15	13	15	18	-
Enterobacter aerogenes	20	24	16	22	10	20	14	12	10	-
Pseudomonas aeruginosa	19	25	21	10	19	19	19	11	19	-
Proteus vulgaris	21	17	24	20	11	18	14	16	22	-
Salmonella typhimurium	21	19	10	20	19	20	13	19	14	-
Shigella flexneri	20	19	21	17	12	20	12	10	20	-
Vibrio cholerae	20	23	15	11	11	24	20	17	18	-
Fungal Organisms *Candida albicans*	20	18	16	17	12	12	20	14	15	-
Aspergillus niger	21	17	19	24	12	12	12	10	20	-

Table 2: Antagonistic Activity of C*aulerpa racemosa* against Test Organisms

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl Acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
Gram Positive Organisms *Streptococcus mutans*	20	21	19	18	21	23	24	19	26	-
Staphylococcus aureus	21	23	26	19	24	18	23	27	19	-
Staphylococcus epidermidis	19	24	27	17	20	24	26	23	19	-
Gram Negative Organisms *Escherichia coli*	24	23	23	27	19	20	12	18	13	-
Enterobacter aerogenes	23	18	24	25	26	19	20	11	16	-
Pseudomonas aeruginosa	11	23	11	19	17	18	11	20	21	-
Proteus vulgaris	20	24	21	20	19	20	19	20	24	-
Salmonella typhimurium	20	19	18	19	20	24	22	22	23	-
Shigella flexneri	19	20	18	20	24	25	21	19	18	-
Vibrio cholerae	21	20	19	18	20	17	18	20	25	-
Fungal Organisms *Candida albicans*	20	24	21	20	19	20	19	20	24	-
Aspergillus niger	20	19	18	19	20	24	22	22	23	-

Table 3: Antagonistic Activity of *Caulerpa sertularoides* against Test Organisms

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl Acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
Gram Positive Organisms *Streptococcus mutans*	20	19	18	21	19	18	21	23	24	-
Staphylococcus aureus	19	20	18	23	26	19	24	18	23	-
Staphylococcus epidermidis	21	21	20	24	27	17	20	24	26	-
Gram Negative Organisms *Escherichia coli*	20	19	19	19	19	19	19	20	12	-
Enterobacter aerogenes	18	23	21	21	21	21	26	19	20	-
Pseudomonas aeruginosa	23	24	23	23	23	23	17	18	11	-
Proteus vulgaris	19	26	20	20	20	20	19	24	18	-
Salmonella typhimurium	21	20	24	24	24	24	24	19	19	-
Shigella flexneri	21	21	23	23	23	23	19	21	21	-
Vibrio cholerae	20	19	18	20	25	19	20	23	23	-
Fungal Organisms *Candida albicans*	19	24	27	17	20	19	20	18	20	-
Aspergillus niger	20	19	18	19	20	18	17	19	18	-

Table 4: Antagonistic Activity of *Padina gymnospora* against Test Organisms

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
Gram Positive Organisms *Streptococcus mutans*	20	19	18	21	19	18	21	23	24	-
Staphylococcus aureus	19	20	18	23	26	19	24	18	23	-
Staphylococcus epidermidis	23	11	23	24	25	26	19	24	27	-
Gram Negative Organisms *Escherichia coli*	23	26	17	18	20	25	21	26	19	-
Enterobacter aerogenes	20	25	19	20	20	22	21	25	20	-
Pseudomonas aeruginosa	24	27	19	20	25	24	19	27	17	-
Proteus vulgaris	23	23	23	24	19	26	24	23	26	-
Salmonella typhimurium	18	24	18	23	27	19	20	20	25	-
Shigella flexneri	23	11	23	24	25	26	19	24	27	-
Vibrio cholerae	18	23	24	26	23	19	20	20	22	-
Fungal Organisms *Candida albicans*	20	23	20	13	17	17	19	20	19	-
Aspergillus niger	21	18	14	12	11	11	20	19	23	-

Table 5: Antagonistic Activity of *Chaetomorpha antennina* against Test Organisms

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
Gram Positive Organisms *Streptococcus mutans*	20	19	19	23	19	19	11	19	24	-
Staphylococcus aureus	20	23	20	13	17	17	19	20	19	-
Staphylococcus epidermidis	23	23	11	17	12	12	20	14	15	-
Gram Negative Organisms *Escherichia coli*	19	24	18	11	20	20	18	17	15	-
Enterobacter aerogenes	20	24	17	19	11	11	24	13	10	-
Pseudomonas aeruginosa	20	19	18	19	20	24	22	22	23	-
Proteus vulgaris	19	20	18	20	24	25	21	19	18	-
Salmonella typhimurium	21	20	19	18	20	17	18	20	25	-
Shigella flexneri	21	21	20	18	17	19	20	20	22	-
Vibrio cholerae	20	19	18	20	25	19	20	25	24	-
Fungal Organisms *Candida albicans*	20	21	19	18	21	23	24	17	19	-
Aspergillus niger	21	23	19	14	20	12	23	25	19	-

Table 6: Infra Red Spectral Data for Selected Seaweed Samples

Selected Seaweed Samples	IR Spectral Data
Sargassum wightii	3432 .09– Primary amines(Amides or Amines) 2324.06 and 2358.78 – Nitriles 2087.8 – Alkynes 1640.35 – Aliphatic amines (C= N) Below 600 – Alkyl halides
Caulerpa sertularioides	3432.09 – Primary amines (N=H) 2325.03 and 2359.74 – Nitriles 2088.76 – Primary amines 1641.31 – Aliphatic amines Below 600 – Alkyl halides (Bromo alkane or Chloroalkane)

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