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STUDY OF ANTIMICROBIAL ACTIVITY OF SEAWEED

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ABSTRACT

In this study *Gracilaria edilis*, *sargassam* and *Padina gymnospora* from Raameshwaram sea, Tamil Nadu were collected, identified and tested against various pathogenic bacteria. In Antibacterial assay Acetone shows the maximum number of activity against the Salmonella typi (32mm) length of inhibtions occurred and Acetone shows the minimum activity against *Klebsiella pneumonia* (18mm) of the inhibition level. Under the Antifungal assay Acetone shows the maximum activity against the *penicillium.sp*, (10mm) of the inhibition. Acetone shows the moderate activity against the *Aspergillus niger* (5mm) of inhibition and the minimum activity in candida sp,. (8mm) of inhibition zone level.

Keywords: Seaweeds, Antibacterial activity and Antifungal activity

1. INTRODUCTION

Seaweeds are a marine algae is a potential source of bioactive substances. Seaweeds have been traditionally used in human and animal nutrition. Seaweeds are rich source of bioactive compounds. Important polysaccharides such as agar, alginates and carrageenans obtained from seaweeds are used in pharmaceutical as well as in the food industries. 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. Use of antimicrobial 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. The presence of nutrients, epithelial debris, and secretions makes the oral cavity a favorable habitat for a great variety of oral bacteria like Streptococci, Lactobacilli,Staphylococci, Cornybacteria, and with a great number of anaerobes, especially Seaweeds are considered as a source of bioactive compounds with cytostatic, antiviral, anti helminthic, antifungal and antibacterial activities. They have also been used to treat some diseases like cancer, arthritis etc. Seaweeds are the renewable living sources which are also used as food, feed and fertilizer in many parts of the world. They have been screened extensively to isolate life saving drugs or biologically active substances all over the world.

1.1. Herbivores

Grazer induced mechanical damage triggers the production of chemicals that acts as feeding detergents or toxins in seaweeds most of the secondary metabolites produced by seaweeds have bacteriological or the antimicrobial compounds derived from seaweeds consists of divers group of bacteriostatic properties. Phillippines as the world's largest seaweed producer by 2011. Production was hit 10 million tones.

1.2. Herbalism

Alginates are commonly used in wound dressing and production of dental moulds. Seaweeds are a source of iodine necessary for thyroid function and to prevent goiter. Tuberculosis, arthritis, colds and influenza tumors.

1.3. Fertilizers

The strong photo synthesis of algae creates a large affinity for nutrients. Such as ammonia, nitrate, phosphate, iron, copper. Reefs and lakes are naturally filtered this way. This filterable process is duplicated in man-made seaweed filters such as algae scrubbers.

Micro algae required more processing to separate it from the water than macro algae does. Macro algae are simply pulled out. Compost for landscaping or means of combating beach erosion through burial in beach dunes ingredient: - Tooth

paste, cosmetics and paints. Sulphated saccharides from both red & green algae have been known to inhibit some DNA & RNA enveloped.

1.4. Healthy risks

Rotting seaweeds sources of hydrogen sulphide. A highly toxic gas. It can cause vomiting & diarrhea.

1.5. Fucus

Brown, in intertidal zones on rocky shores. Fucus vesiculosus is the scientific name of brown seaweed. 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.

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.

2. MATERIALS AND METHODS

2.1. Collection of seaweeds

The samples of *Grasillariya edilis*, *P. gymnospora* ,Sargassam, Ulva were collected by handpicking at Raameshvaram sea. 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 plastic bags should be sterilized .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 with the use of any mixer grinder. The powdered samples were then stored in refrigerator for further use.

2.2. Preparation of samples

The dried seaweed materials were blended into a coarse powder before extraction portions of the powdered samples (3.5 g) and packed in Soxhlet apparatus and extracted successively with acetone for 8 ml of this solvents. The crude extracts were weighed and deep frozen (-20 °C) until tested.

2.3. Microbial strains

Bacterial strains used for assay were as following: *Klebsiella pneumoniae, Salmonella* sp.,While fungal strains were *Aspergillus niger, Candida albicans, Penicillium* sp. Microbial strains were obtained from the Department of biotechnology, Kongunadu Arts and Science college coimbatore. The bacterial stock cultures were maintained on Mueller Hinton Agar medium at 4 °C. Fungal cultures were maintained on Potato Dextrose Agar medium at 4°C.

2.4. Antibacterial assay

The antimicrobial activities were carried using the agar disc diffusion method Paper disc of 6 mm in diameter was prepared from Whattman No. 1 filter paper. The antibacterial assay using the agar plate method. The bacterial inoculation 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 petri dishes; 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 °C overnight. The zones of inhibition were measured after the 24 hrs incubation.

2.4.1. Antifungal assay

The same method was followed by this method by using the fungal strains. It was carried out using the agar plate method. The bacterial inoculation was grown in nutrient broth overnight and a fixed volume was inoculated into 10ml aliquots nutrient agar, mixed and then poured over a nutrient agar base in sterile petri dishes this formed the fungal lawn. The paper disc of 6 mm in diameter was soaked in 6 µl of crude extract and placed onto the lawn after it had solidified, standard antibiotic disc used for control. The plates were incubated at 37 °C overnight. The zones of inhibition were measured after the 24 hrs incubation.

3. RESULTS AND DISCUSSION

3.1. Antibacterial assay

3.1.1. Padina gymnospora

The acetone extracts showed a maximum activity against *Salmonella* sp. (32mm) of the inhibitory level and the minimum activity against klebsiella pneumonia(18mm)of inhibitory level.

3.2. Antifungal assay

3.2.1. Padina gymnospora

Acetone shows the maximum activity in penicillium.sp. (10mm) and moderate activity in penicillium sp. agaist Aspergillus niger (5mm)of inhibitory level .Acetone shows the minimum activity in Candida albicans (8mm)of inhibitory level.

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., In this study *Gracilaria edilis*, *sargassam* and *padina gymnospora* from Raameshvaram sea and, Tamil Nadu were collected, identified and tested against various pathogenic bacteria. It was found that the acetone extracts of *padina gymnospora* showed maximum activity (32 mm) against salmonella typi (Table 1) and minimum activity was shown by acetone extracts against *Klebsiella pnuemonia* (18mm). The solvent system used for the extraction played a major role in displaying the antibacterial activity. Acetone were suitable solvent for extracting the antibiotic principle. However, there are reports that indicate maximum activity in acetone extracts.

Table 1. Antibacterial activity of *Padina gymnospora* against human pathogens.

Pathogen	Acetone
Klebsiella pneumonia	18 mm
Salmonella typhi	32mm

Table 2. Antifungal activity against the *Padina gymnospora* seaweed

Pathogen	Acetone
Aspergillus niger	5mm
Candida albicans	8mm
Penicillium sp.	10mm

Hence the efficiency of acetone in the extraction of seaweeds is also doing in the antifungal activity against the *Aspergillus niger, Candida albicans*, and *penicillium.sp.,* it was found that the acetone extracts of *padina gymnospora* showed maximum activity (10mm) in penicillium sp.,and the minimum activity showed (5mm) in *Aspergillus niger*, then the moderate amount of avtivity showed (8mm) in candida albicans. Acetone was found to be the best solvent for extracting the active principles in almost all species of seaweeds. Antibacterial activities of seaweeds also varied with the species division. 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 *Chlorophyta* showed the strongest activities against the test bacteria which was in agreement with the findings of Padmakumar and Ayyakannu. It may be probably due to the tested seaweeds vertical distribution. Green algae mostly occur in the intertidal zone lower region, which may be advantage for the protection of the active compounds within the algal plant from degradation.The padina gymnospora showed minimum activity against *Klebsiella* (18 mm) when acetone was used as a solvent (Table 1) and maximum against Salmonella typhi in acetone extract.

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.

Selvi and Selvaraj, (2003) 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*., (2009) 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*., (2009) 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.

Taskin *et al.*, (2001), studied the antibacterial activity of methanolic extracts of 6

marine algae, 3 gram positive and 3 gram negative in vitro. They observed a highest inhibition activity by *Corallina officinalis* against *Enterobacter aerogenes*. Bansemir *et al.,*(2006) investigated 26 algal species for their antimicrobial activity by 3 different extracts like dichloromethane, methanol and water. Highest activity was found in dichloromethane extracts. Seenivasan. *et al.,*(2011) performed antibacterial activity studies invitro with 3 extracts namely acetone, methanol and ethanol. They observed that Ulva fasciata in selective media produced good results against E.coli. Cox *et al.,* (2000) screened the antimicrobial activity of 6 species of edible Irish seaweeds. All methanolic extracts of seaweeds inhibited food spoilage and food pathogenic bacteria tested such as Listeria monocytogenes, Salmonella abony, Enterococcus faecalis and Pseudomonas aerogenosa. They found that dried methanolic extracts of red and green seaweeds had significantly lower antimicrobial activity than brown seaweeds. Red and green seaweed extracts showed significantly high antimicrobial activity.

In the present result, the Antimicrobial activity against the *salmonella typhi* (32mm) length of the inhibtions occurred and Acetone shows the minimum activity against *Klebsiella pneumonia* (18mm) of the inhibition level. Under the Antifungal assay Acetone shows the maximum activity against the *penicillium.sp*, (10mm)of the inhibition. Acetone shows the moderate activity against the *Aspergillus niger* (5mm)of inhibition and the minimum activity in *candida sp,.(*8mm) of inhibition zone level.

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