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A STUDY ON THE ANTIBACTERIAL ACTIVITY OF THE SEAWEED *ENTEROMORPHA INTESTINALIS*

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ABSTRACT

The marine environment covers more than 70% of the world's surface. Marine algae constitute one of the largest producers of biomass in the marine environment. Marine algae also known as seaweeds attract global attention in terms of research work and commercial exploitation. Seaweeds have touched new horizons like marine pharmacology, bioremediation, seaweed tissue culture etc. The antibacterial activity of the Acetone, Methanol and Ethanol extracts of the marine algae *Enteromorpha intestinalis* from Ennore Beach near Chennai (coast of Tamil Nadu) were tested in vitro against a panel of Gram positive and Gram negative bacterial strains. The test organism included Gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and Gram positive bacteria such as *Staphylococcus aureus*. In this study, agar well diffusion test technique was followed. The result exhibited antibacterial activity of the algal extracts against both Gram positive and Gram negative bacteria on selective media.

KEYWORDS: Marine Environment, Seaweeds, *Enteromorpha intestinalis*, Antibacterial Activity and Agar Diffusion Test.

INTRODUCTION

Seaweeds are primitive non-flowering plants without true stems and leaves. They form important marine living resources. They are abundant in intertidal, shallow, coastal estuaries

and backwaters and flourish wherever the substratum is available (Arun Kumar.K and Rengasamy.R, 2000). They grow on rocks, dead corals, stones, pebbles, solid substance and on other plants. On the basis of pigmentation present on them the algae are classified into red (Rhodophyta), brown (Phaeophyta) and green (Chlorophyta). Seaweeds are the only source of phytochemical substances like agar, alginate and carrageenan (Caccamese *et.al.*, 1981). They are used in various industries such as food confectionary, textile, pharmaceutical, diary and paper mostly as gelling, stabilizing and thickening agents. Apart from these biochemicals, other products such as mannitol, fucoidin are also obtained from seaweeds. Seaweeds contain many trace elements like minerals, proteins, iodine, bromine, vitamins and many bioactive substances. Seaweed contain 60 trace elements in concentration much higher than in terrestrial plants (Hellio *et.al.*, 2000). It is estimated that the total standing crop of seaweeds in intertidal and shallow water is 91339 tonnes (wet wt) consisting of 6000 tonnes of agar yielding seaweeds. To determine the active components in the extracts of the most abundant algae species several works on marine algae has been carried out (Mautner *et.al.*, 1953 and Mithlesh *et.al.*, 1998).

Test Organisms

Escherichia coli is a facultative anaerobic and gram negative rod. It is an facultative anaerobe which belong to family Enterobacteriaceae. Antibiotics which are used to treat *Escherichia coli* infection include amoxicillin, ciprofloxacin and aminoglycosides. Modified *Escherichia coli* have been used in vaccine development, bioremediation and production of immobilized enzymes. *Escherichia coli* has been an integral part of first experiments conducted to understand Phage Genetics (Padmakumar K and Ayyakannu K, 1997).

Staphylococcus aureus is a spherical bacterium living on the skin or in the nose of a person. *Staphylococcus aureus* is more aggressive and likely to be antibiotic resistant. Some strains of *Staphylococcus aureus* cause food poisoning in humans and mastitis in cows. *Staphylococcus aureus* is capable of secreting many toxins (Premnathan *et.al.*, 1992).

Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. It is an opportunistic human pathogen. *Pseudomonas aeruginosa* is identified by its pearlescent appearance and grape-like odor in vitro. Although this organism is classified as an aerobic organism, it is considered by many as a facultative anaerobe as it is adapted to proliferate in condition of partial or total oxygen depletion. One of the most worrisome

characteristics of *P.aeruginosa* consists in its low antibiotic susceptibility (Pillai CGS, 1996 & Sreenivasa Rao, P. and K.S.Parekh, 1981).

Klebsiella pneumonia is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin and intestine. *Klebsiella pneumoniae* tends to affects people with underlying diseases, such as alcoholism, diabetes and chronic lung disease. Community-acquired pneumonia caused by *Klebsiella pneumoniae* may be called Friedlanders Pneumonia, after Carl Frierlandler (Sreenivasa Rao P. and Karmakar S.M, 1988).

MATERIALS AND METHODOLOGY

In the present antibacterial study the marine algae *Enteromorpha intestinalis* was collected from the coast around Ennore Beach in Chennai, Tamil Nadu. The collected algae sample was identified by algal experts and were rinsed with water to remove epiphytes and necrotic parts. It was then rinsed again with sterile water to remove any associated debris. The algae after rinsing were dried carefully in shade under room temperature for 10 days and then immediately subjected to extraction. The algae after drying were weighed and then chopped and finely powered using a clean motor and pistle. The finely powered sample was weighed and 5 grams of sample was dissolved in various organic solvents, such as 80% Ethanol, Methanol and Acetone. It was kept for 48 hours at room temperature and mixed at regular intervals. After 48 hours the sample dissolved in each solvent was filtered using Whatman filter paper to separate the filtrate for further use in antimicrobial testing of algal samples. Pure cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae* were used as the test microorganism for antibacterial testing. Each bacterial strain was maintained in a nutrient agar slant. Slant of all the four microorganisms were prepared in nutrient agar media at a pH-7.2, and kept for incubation at 37^{o}C for 24 hours. A nutrient agar slant without any bacterial strain was maintained as control. From the 24 hours incubated nutrient agar slant of each test organism a loop full of the microorganism was inoculated in nutrient broth at pH-7.4 so as to activate the bacterial strains used as test organisms. The broths were kept for incubation at 37^{o}C for 24 hours so that the microorganism can grow till the log phase. A nutrient broth was maintained as a control without inoculating the test organisms. Antibacterial activity was assayed using the agar well diffusion test technique. For comparing the antibacterial activity of the isolated seaweed

extracts with the therapeutic action of a number of known broad spectrum antibiotics, Antibiotic Disc Diffusion Test was done.

Standard antibiotics disc which were used are as follows

- ✓ Nalidaxic Acid N30-30mcg/disc
- ✓ Oxicillin O10-10 mcg/disc
- ✓ Bacitracin B10-10Units/disc
- ✓ Streptomycin S10-10mcg/disc
- ✓ Erythromycin E10-10 mcg/disc
- ✓ Chloramphenicol C10-10mcg/disc

Confirmation Test

Screening of the algal extract was done for testing the antibacterial activity against the test microorganisms. It is done by allowing test organisms to grow in the respective selective media. In this confirmation test, agar well diffusion technique was done to obtain a sure result exhibiting the antibacterial activity of the seaweed extracts.

RESULTS

The seaweed sample was collected and the extract was tested against a range of microorganisms (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae)* for the presence of the antibacterial activity. In the present study, it is observed that methanol and ethanol were the best organic solution for the effective antibacterial material from the algae species used in this experiment. The result exhibited by acetone was less than that exhibited by ethanol and methanol. The best halo-zone produced was in the ethanol extract of *Entermorpha intestinalis*. The ethanol, methanol, acetone extract of *Enteromorpha intestinalis* in *E.coli* exhibited less zone of inhibition. The experiment showed that the gram positive bacterial strain used as test organism was less effective compared to the gram negative bacterial strains. Among all the three gram negative bacterial strains, *Pseudomonas aeruginosa* and *Escherichia coli* were noted as the best Halo zone producers **(Tables 1 – 10)**.

Table 1: Zone of inhibition (in mm) of acetone, ethanol and methanol extract for green algae *Enteromorpha intestinalis* in MHA media.

Concentration of *Enteromorpha* extract	*E.coli* (zone of inhibition in mm)			*P.aeruginosa* (zone of inhibition in mm)			*S.aureus* (zone of inhibition in mm)			*K.pneumoniae* (zone of inhibition in mm)		
(in microlitre)	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met
100	12	10	10	-	14	-	-	-	11	-	-	-
150	-	11	-	-	15	-	-	-	17	-	-	-
200	-	15	23	-	20	11	-	-	19	-	-	-

Act - Acetone extract, Eth - Ethanol extract, Met – Methanol extract.

Table 2: Zone of inhibition (in mm) of acetone, ethanol and methanol extract for green algae *Enteromorpha intestinalis* in Selective media.

Concentration of *Enteromorpha* extract	*E.coli* (zone of inhibition in mm)			*P.aeruginosa* (zone of inhibition in mm)			*S.aureus* (zone of inhibition in mm)			*K.pneumoniae* (zone of inhibition in mm)		
(in microlitre)	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met
100	-	-	-	-	-	-	-	-	14	15	11	11
150	-	-	-	-	-	-	-	-	-	18	15	12
200	20	-	-	-	-	-	-	-	18	22	17	23

Table 3: Zone of inhibition for *Escherichia coli* in Disc Diffusion Test in MHA media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	26
Gentamycin G10	16
Ampicillin A10	19
Chloramphenicol C30	21
Bacitracin B10	10
Oxacillin Ox1	-

Table 4: Zone of inhibition for *Escherichia coli* in Disc Diffusion Test in Selective media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	23
Gentamycin G10	15
Ampicillin A10	23
Chloramphenicol C30	22
Bacitracin B10	8
Oxacillin Ox1	-

Table 5: Zone of inhibition for *Pseudomonas aeruginosa* in Disc Diffusion Test in MHA media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	6
Gentamycin G10	18
Ampicillin A10	-
Chloramphenicol C30	12
Bacitracin B10	-
Oxacillin Ox1	-

Table 6: Zone of inhibition for *Pseudomonas aeruginosa* in Disc Diffusion Test in Selective media.

Names of the Antibiotic discs used(mcg/disc)	Zone of inhibition(in mm)
Nalidixic Acid N30	5
Gentamycin G10	15
Ampicillin A10	-
Chloramphenicol C30	21
Bacitracin B10	9
Oxacillin Ox1	3

Table 7: Zone of inhibition for *Staphylococcus aureus* in Disc Diffusion Test in MHA media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	19
Gentamycin G10	25
Ampicillin A10	11
Chloramphenicol C30	20
Bacitracin B10	7
Oxacillin Ox1	-

Table 8: Zone of inhibition for *Staphylococcus aureus* in Disc Diffusion Test in Selective media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	22
Gentamycin G10	21
Ampicillin A10	35
Chloramphenicol C30	30
Bacitracin B10	15
Oxacillin Ox1	20

Table 9: Zone of inhibition for *Klebsiella pneumoniae* in Disc Diffusion Test in MHA media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	15
Gentamycin G10	9
Ampicillin A10	8
Chloramphenicol C30	20
Bacitracin B10	-
Oxacillin Ox1	-

Table 10: Zone of inhibition for *Klebsiella pneumoniae* in Disc Diffusion Test in Selective media.

Names of the Antibiotic discs used (mcg/disc)	Zone of inhibition (in mm)
Nalidixic Acid N30	20
Gentamycin G10	18
Ampicillin A10	-
Chloramphenicol C30	25
Bacitracin B10	-
Oxacillin Ox1	-

Zone of inhibition

Below 10mm – least active

Between 11-25mm – active

Above 26mm – very active

CONCLUSION

The marine algae *Enteromorpha intestinalis* was collected and tested for antibacterial activity. 80% Ethanol and Methanol were the best organic solution for extracting the effective antibacterial materials from the algae species used in this experiment. *Klebsiella pneumoniae* did not show any zone in any of the organic solvent in MHA media but showed good zone in selective media for seaweed *Enteromorpha intestinalis. Pseudomonas aeruginosa* and *Staphylococcus_aureus* did not show any zone in any solvent for the seaweed *Enteromorpha intestinalis* in selective media. The zone of inhibition was compared with the zone of inhibition produced by the standard antibiotic discs in the Antibiotic Disc Diffusion Test. In the antibiotic disc diffusion test the best result was seen in *Escherichia coli* and *Staphylococcus aureus* plates. The zone of inhibition was 26mm and above 26mm in Ampicillin, Nalidixic acid and Chloramphenicol indicates very high antibacterial activity whereas the zone of inhibition of Bacitracin disc of *Escherichia coli*, Oxacillin disc, Nalidixic acid disc of *Pseudomonas aeruginosa*, Gentamycin disc, Ampicillin disc of *Klebsiella*

pneumoniae were 10 mm and less than 10 mm indicates their least activity against the antibiotic. The coast of Tamil Nadu is vastly endowed with many such algae which possess antibacterial compounds in them. It is high time we explore and discover their potential and use them in pharmacological studies for the betterment of human society.

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