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Research Article

A STUDY ON THE ANTIBACTERIAL PROPERTY OF THE SEAWEED CHAETOMORPHA AEREA

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A R T I C L E I N F O

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A B S T R A C T

Marine macroalgae, or seaweeds, are plant – like organisms that generally live attached to rock or other hard substrata in coastal areas. They belong to three different groups, empirically distinguished since the mid – nineteenth century on the basis of thallus color. Seaweeds are far more complex organisms than generally realized. Many have specialized tissues and growth forms. They may have very complicated sex, with many of them producing sex pheromones and with many different types of sex organs. Seaweed has a unique ability to absorb concentrated amounts of iodine from the ocean. Seaweed also contains an amino acid called tyrosine, which is used alongside iodine to make two key hormones that help the thyroid gland do its job properly. The protein present in some seaweed, such as Spirulina and Chlorella, contain all essential amino acids. This means seaweed can help ensure us to get full range of aminoacids. Seaweed are also a good source of omega 3 fats and vitamin B12.In addition to containing the antioxidant vitamin A, C and E, seaweed boasts a wide variety of beneficial plant compounds, including flavonoids and carotenoids. These have been shown to protect our body 's cells from free radical damage. Seaweed contains a lot of fibre , which does not contain any calories. The fibre in seaweed may slow stomach emptying too. This helps us feel fuller for longer and can delay hunger pangs. The present study forma a foundational study in exploring the Antibacterial property of the seaweed *Chaetomorpha aerea* paves way for its potential contribution to the field of Pharmacogenomics.

INTRODUCTION

Seaweeds need salty or brackish water, sunlight and a surface to attach themselves to. Because of these factors they are generally found in the littoral zone (this includes the intertidal zone but generally extends out much further).They are usually found on rocks rather than on sand or shingle shores. Seaweeds are a source for marine animals such as sea urchins and fishes, and are the base of some marine food webs. They also provide shelter and a home for numerous fishes, invertebrates, birds and mammals. Seaweeds are a food source for humans especially in East Asia, as it is most commonly associated with Japanese food. Seaweeds are also used to make a number of food additives such as alginates and carrageenan which is used in cooking and baking as a vegetarian alternative to gelatin. Many seaweed are used as medicine. Alginates are used in wound dressing and in the production of dental moulds and agar in Microbiology to help grow bacterial cultures. Seaweeds are ingredients in toothpaste, cosmetics and paints and are used in industrial products such as paper coatings, adhesives, dyes, gels, explosives and many more. Many of the oil and natural gas we use today are formed from seaweed which partially decompose on the sea floor many million years ago. *Chaetomorpha aerea* is a species of green algae of the family Cladophoraceae. *Chaetomorpha aerea* and *Chaetomorpha linum* are considered conspecific by some authors [1] while others do not accept this synonymy. *Chaetomorpha areae* is the attached form [2]. Algae of this genus are made up of macroscopic filaments of cylindrical cells [3]. The genus is characterized by its unbranched filaments, making it distinctive ; its closest relatives are branching species of the genus *Cladophora* [4]. These algae are popular with aquarium hobbyists. Dumping of aquarium specimens into waterways has led to the establishment of non-native *Chaetomorpha* populations, which degrades ecosystems when the algae become invasive species. Biologists recommend boiling, microwaving, freezing, or dessicating aquarium *Chaetomorpha* before disposing of it to avoid inadvertent releases [5]. *C.aerea* is used for human food and as a feed for milkfish. *C.antennina* is eaten as a salad in Malaysia, *C.crassa* can be used to make a gelatin like sweetmeat [6]. The three *Chaetomorpha* species form important constituents of the thick mats of mixed filamentous green and blue – green algae, covered by many periphyton algae found in fish ponds.

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Test Organisms

Escherichia coli is the most prevalent infecting organism in the family of gram – negative bacteria known as Enterobacteriaceae [7]. The *E.coli* that are responsible for the numerous reports of contaminated foods and beverages are those that produce Shiga toxin, so called because the toxin is virtually identical to that produced by *Shigella type 1* [8]. Approximately half of the children who suffer require dialysis, and at least 5 % of those who survive have long term renal impairment. While somewhat rare, serious injury to the pancreas, resulting in death or development of dialysis also occurs [9].

Staphylococcus aureus : (grape – like clusters) when viewed through a microscope, has a large, round, golden yellow colonies, often with hemolysis, when grown on blood agar plates. *S.aureus* reproduces asexually by binary fission. Complete separation of the daughter cells is mediated by *S.aureus* autolysin and in its absence or targeted inhibition, the daughter cells remain attached to one another and appear as clusters [10]. Aminoglycosides antibiotics, such as kanamycin, gentamycin, streptomycin were once effective against staphylococcal infections until strains evolved mechanisms to inhibit the aminoglycosides action which occurs via pronated amine.

Pseudomonas aeruginosa : is a facultative anaerobe ,as it is well adapted to proliferate in conditions of partial or total oxygen depletion. This organism can achieve anaerobic growth with nitrate or nitrite as a terminal electron acceptor. When oxygen nitrate or nitrite are absent, it is able to ferment arginine and pyruvate by substrate – level phosphorylation [11]. *P.aeruginosa* growth within the human body can be asymptomatic until the bacteria form a biofilm, which overwhelms the immune system .These biofilms are found in the lungs of people with cystic fibrosis and primary ciliary dyskinensia and can prove fatal.

Klebsiella pneumonia: is a lactose fermenting, facultative anaerobic bacterium and appears as a mucoid lactose fermenter on MacConkey agar. *Klebsiella* species have become important pathogens in nosocomial infections. As a free living diazotroph, its nitrogen –fixation system has been much studied and is of agricultural interest, as *K. pneumoniae* has been demonstrated to increase crop yield in agricultural conditions. *K. pneumoniae* bacteria can be spread through person-to-person contact (for example, contaminated hands of healthcare personnel, or other people via patient to patient) or, less commonly, by contamination of the environment; the role of transmission directly from the environment to patients is controversial and requires further investigation [12].

MATERIALS AND METHODOLOGY

In the present antibacterial study the marine algae *Chaetomorpha aerea* was collected from the coast around Ennore Beach in Chennai, Tamil Nadu. The collected algae sample was identified by algal experts and was 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 AND DISCUSSION

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. The result exhibited by acetone was less than that exhibited by ethanol and methanol. The best halo-zone was produced in the ethanol extract of *Chaetomorpha aerea*. The percentage of antibacterial activity observed for *Chaetomorpha aerea* was good. 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, *Staphylococcus aureus* was noted as the best Halo zone producers (**Tables 1 – 10**).

Table 1 Activity of extract of the marine algae against the test bacterial strains.

Marine algae	E.coli (zone of	P.aeruginosa (zone of	S.aureus (zone of	K.pneumoniae (zone of

	inhibition in mm)	inhibition in mm)	inhibition in mm)	inhibition in mm)
Chaetomorpha aerea	Average Result	Poor Result	Good Result	Average Result

Table 2 Zone of inhibition (in mm) of acetone, ethanol and methanol extract for green algae *Chaetomorpha aerea* in MHA media

Concentration of *Chaetomorpha* 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	18	13	14	15	-	-	-	10	11	-	-	-
150	-	18	19	17	-	-	-	12	15	-	-	-
200	-	19	23	18	-	13	16	13	18	-	15	-

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

Table 3 Zone of inhibition (in mm) of acetone, ethanol and methanol extract for green algae in *Chaetomorpha aerea* in Selective Media

Concentrationof *Chaetomorpha* 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	-	-	-	-	-	29	-	10	14	-	-	13
150	-	-	-	-	-	30	13	10	17	-	-	15
200	-	-	-	-	-	34	15	13	19	-	11	20

Table 4 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 5 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 6 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 7 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 8 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 9 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 10 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 11 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

Seaweeds are used in many maritime countries as a source of food, for industrial applications and as a fertiliser. The major utilisation of these plants as food is in Asia, particularly Japan, Korea and China, where seaweed cultivation has become a major industry. In most western countries, food and animal consumption is restricted and there has not been any major pressure to develop seaweed cultivation techniques. Industrial utilisation is at present largely confined to extraction for phycocolloids and, to a much lesser extent, certain fine biochemicals. The present uses of seaweeds at present are as human foods, cosmetics, fertilisers, and for the extraction of industrial gums and chemicals. They have the potential to be used as a source of long- and short-chain chemicals with medicinal and industrial uses. Marine algae may also be used as energy-collectors and potentially useful substances may be extracted by fermentation and pyrolysis. The present research findings can thus form a foundational study to explore the potential contribution of this seaweed to the field of Pharmacogenomics.

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