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The Antibacterial Activity of Some Marine Algae from South East Coast of India

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Abstract: In vitro the antibacterial activity of the Acetone, Methanol and Ethanol extracts of three marine algae from Ennore beach near Chennai (coast of Tamil Nadu) were tested. The test organism includes 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 marine algae which were tested for antibacterial activity belongs to green algae. Among all the marine algae *Ulva fasciata, Enteromorpha intestinalis* and *Chaetomorpha aerea* were collected and tested *in vitro Ulva fasciata* and *Chaetomorpha aerea* have exhibited average result. *Escherichia coli* in all the solvents have shown significant results for the seaweed *Ulva fasciata* 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. The result exhibited when antibacterial activity of the algal extracts was done on selective media for confirmation test was compared with the MHA plates. Thus the organic (80% Ethanolic, Methanolic and Acetone) extract of green algae has ability to inhibit the growth of the Gram positive and Gram negative bacteria. The coast of Tamil Nadu is vastly endowed with many algae possessing antibacterial compounds in them.

Key words:

INTRODUCTION

Algae are group of marine plants attaching global attention in terms of research work and commercial exploitation. The seaweed has touched new horizons like marine pharmacology, bioremediation, seaweed tissue culture etc. Algae are primitive nonflowering 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. They grow on rocks, dead corals, stones, pebbles, solid substance and on other plants. It is estimated that the total standing crop of algae in intertidal and shallow water is 91339 tones (wet wt) consisting of 6000 tones of agar yielding algae. Marine algae diversity of Tamil Nadu is next only to Gujarat coast in India. A total number of 1263 taxa belonging to 8 classes are reported to occur in Tamil Nadu. These algae are distributed under 432 genera belonging to 115 families under 38 orders. All the taxa are arranged following the classification proposed by Paul C. Silva and Sybil Parker [1]. This extraction of the antimicrobial compounds from algae was described by Gonzalez Del Val *et al*., [2].

Identification of geranylgeraniol derived diterpens from brown algae has been reported by Culioli *et al*., [3]. These compounds are more active but their cultivation on microbial growth has not been studied. High molecular weight antibacterial substance from sea water was determined by Saz *et al*., [4]. The antimicrobial activities of crude extract from marine algae *Cyctoseira* were evaluated on bacteria, yeasts and fungi. Daoudi *et al*., [5] have isolated acyclic diterpens and sterols from genera *Bifurcaria* and *Bifurcariopsis.* Algae contain a rich and largely entrapped source of a vast assortment of biologically active substance [6,7]. In 1981, Rao, K.S. Parekh reported the antimicrobial activity of Indian algae extract. The extract showed antibacterial activity throughout the year with a definite peak of activity between October and January. Attempts at screening of algae have been carried out mainly on human pathogenic viruses, bacteria and fungi [8]. The red algae

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Sphaerococcus coronopifolius was shown to have antibacterial activity [9], green algae *Ulva lactuca* shown to possess an anti inflammatory compound [10]. Vlachos *et al.,* [11] investigated the antimicrobial activity of a few southern African algae and identified a few candidate species for antibiotic research.

Using seven species of bacteria as test organism, crude extract of about forty two species of marine algae collected from Saurashtra coast were screened for their antibacterial activity. It was reported that pigments presented in marine algae were not responsible for antibacterial activity. Some green and red algae such as *Ulva* species, phorphyra, victnamiensis and centroces contain very rich proteins [12]. The antibacterial substance extracted from *Enteromorpha* effected complete inhibition of growth of *tubercle bacilli* [13] examined extracts of 25 number of 147 species of algae comprising 42 species of green algae, 31 species of brown algae, 69 species of red algae and 5 species of blue green algae occurred in the Gulf of Mannar islands. In Dr. K.R. Sridhar and N.Vidyavathi [14] reported the literature on the bioactivity of algae against bacteria, fungi, viruses and protozoa. The algae metabolites which are antimicrobial in nature is briefly reported including details on kind of algae, location, period of collection, chemical nature and their activity against micro organism. Wahidulla *et al.,* [15] reported that Ethanolic extracts of Indian marine algae belonging to red algae, green algae and brown algae were tested for anti-semiliki forest (SFV), Ranikhet disease (RDV) and Vaccinia (VV) viruses. Out of 31 sample seaweeds 17 appeared biologically active against the test organisms.

In 1981, the antimicrobial and antiviral activities of the thirteen algae from Eastern Sicily were tested for antimicrobial activity by Siamopoulou and Roussis the extracts were tested against *Bacillus subtilis* and for antiviral activity against Tobacco Mosaic Virus and exhibited the best antimicrobial activity and anti viral activity among all the species tested. Srinivasa Rao and Parekh [16] showed that crude extracts of Indian seaweeds were active only against gram positive bacteria. Ethanol extracts from 53 Southern Africa marine algae from the division Chlorophyta (green), Pheophyaceae (brown) and Rhodophyta (red). Vlachos *et al.,* [11] reported that Phaeophyta scored highest antibacterial activity among all other species. Similar results were reported by Caccamese and Azzolina [17] and Pesando and Caram [18] for screening studies on seaweeds of Mediterranean and Eastern Sicily coast respectively.

In Chile, fouling organisms common on ropes seeded with *Gracilaria* include diatoms and green algae of the genera *Ulva* and *Enteromorpha* [19]. Around two decades earlier around (1985) lipid extracts of 24 red and brown algae, mainly from Eastern Sicily (Southern Italy coast) were tested for antimicrobial activity against four micro organisms. Some of the extracts showed activity against bacteria, while none was active against yeast and fungus. It was observed that the greatest incidence of active species occur in the family Dictyotaceae and Rhodomelacea [20]. In Mahasneh *et al.,* [21] reported antibiotic resistant bacteria. The antibiotic activity of six species of marine algae (Rhodophyta, Phaeophyta and Chlorophyta) against multi-antibiotic resistant (MAR) bacteria was investigated. The study shows that various degrees of activity were present in 18 out of the 24 algal extracts. The highest activity was for Rhodophyta (diameter of zone of inhibition ranged from 10-22 mm) while the lowest was Chlorophyta (diameter of zone of inhibition ranged from 8-12 mm). The importance of these results on public health was discussed.

In Sastry and Rao [22] isolated antibacterial substances from marine algae using three different solvent viz benzene, chloroform, methanol successively to obtain crude extracts from five different alga. The extracts were tested against both gram positive and gram negative bacterial strains for the anti bacterial activity. The chloroform extracts exhibited the greatest antibacterial activity. All the algal sampled, *Sargassum, Padina, Gracilaria, Acanthophora, Halimeda* were collected from the Mandapam coast on the south east coast of India at latitude 9 degree 45`north and longitude 79 degree 0`east during the winter season i.e.in October annually (1988, 1989, 1990).

In K. Arun Kumar and R.Rengasamy [23] evaluated antibacterial potential of seaweeds occurring along the coast of Tamil Nadu. Eleven seaweeds were collected from seven different sites in Muttukadu, Chennai and six regions along the coast of Tamil Nadu. Unsaponified fractions of red and green seaweeds exhibited maximum antibacterial activity followed by either extracts, methanol extract of brown algae showed the best result. They concluded that seaweeds collected from backwaters of Muttukadu possessed a higher antibacterial activity than the coastal waters. In April 2000 research on the pharmacology of marine chemicals was reported by Mayer and Lehmann; it involved antibacterial. antifungal, antituberculosis and antiviral activity from marine seaweeds. In Antonio *et al.,* [24] performed screening of antimicrobial activities in red, green and brown macro

algae from Canary Islands, Spain. Extracts from 44 species of marine algae were screened for the production of antibacterial and antifungal compounds against a panel of gram positive and gram negative bacteria, Mycobacteria, yeasts and fungi. Total of 28 algal species displayed antibacterial activity; of which 6 showed antifungal activity.

Muhammad Afzal Rizvi and Mustafa Shameel [25] reported that some species of marine benthic algae belonging to Chlorophyceae, Phaeophyceae, Rhodophyceae collected from different coastal areas of Karachi (Pakistan) were investigated for their antibacterial, antifungal, phytotoxic and insecticidal activities. Brown seaweeds showed greater antibacterial activity than the green and red ones. *Botryocladia leptopoda* exhibited the greatest antifungal activity and the highest phytotoxic activity (95%) was displayed by *Enteromorpha intestinalis* at 100 microgram per liter concentration.

Jehan *et al.,* [26] reported that chloroform and methanol fractions of an ethanol extract of *Spatoglossum asperum* showed antifungal activity against the highly destructive plant pathogen, *Macrophomina phaseolina* while the n-hexane fraction showed activity against *Rhizoctonia solani*. N-Hexane and methanol fraction also showed nematicidal activity against the plant parasitic nematode, *Meloidogyne javaniea.* In April Yolanda Freile-Pelegrin and Juan Luis Morales [27] reported antibacterial activity in marine algae from the coast of Yucatan in Mexico. Ethanolic and lipid-soluble extraction from 21 marine algae species (10 Chlorophyta, 2 Phaeophyta and 9 Rhodophyta) from the coast of Mexico were evaluated for antibacterial activity against pathogenic micro organism (four gram positive, five gram negative and one fungus). All species with antibacterial activity were active against the gram positive bacteria (*Bacillus subtilis, Streptococcus faecalis* and *Micrococcus luteus)* and most of the algal species exhibited activity against *B.subtilis* (89% in ethanolic soluble extracts and 94% in lipid-soluble extracts). Significant results have been found in the extracts of *Ceramium nitens* as because no antibacterial activity has been found in previous research on this genus. Recently, Febles *et al.,* [28] studied the antibacterial and antifungal activity of a number of brown algae and green algae seaweeds collected from the littoral of Tenerife (Canary Islands). Three different solvents: n-Hexane, Ethyl acetate and Methanol have been used to obtain extracts from the Phaeophyta; *Sargassum desfontainesii, C.agardh, Halopteris scorpia* and *Stypopodium zonale* from Chlorophyta *Ulva rigida* and *Codium intertextum*. The activity of the extracts was tested using gram positive and gram negative bacteria and Yeasts. The methanol extract showed most antibacterial activity, all extracts were mainly active gram positive bacteria while fungi tested proved to be resistant to the extracts of brown algae seaweeds.

Sebastian *et al.,* [29] systematically screened extracts from marine plants for antimicrobial effects against marine pathogen and saprophytes. Extracts from 49 marine algae collected in the tropical Atlantic were screened for microbial activity against both fungi and bacteria. The extracts inhibited microbial growth to 77%. Thus this result demonstrated that microbial activities are prevalent among extracts from marine algae, suggesting that antimicrobial chemicals defenses are widespread among marine algae. Inci *et al.,* [30] reported antimicrobial activities of extracts of marine algae from the coast of Urla in Turkey. Methanol, acetone, diethyl ether and ethanol extracts of 11 seaweeds species were tested in vitro for their antimicrobial activities against *Candida* species, both gram positive bacteria and gram negative bacteria. Diethyl ether was the best solution for extracting the effective antimicrobial materials from the algae species used in this experiment. *Dictyota* exhibited most effective result in ethanol solution.

The present study was carried out with three marine algae *Chaetomorpha aerea* (green algae) belonging to the family Cladophoraceae, *Enteromorpha intestinalis* and *Ulva fasciata* (green algae) belonging to the family Ulvaceae collected from Chennai coast, Ennore Beach, Tamil Nadu. The objectives are (i) To prepare the marine algal extract in various organic solvents of different densities like Acetone, Ethanol and Methanol (ii) To test the antibacterial activity of the crude extract of marine algae against a range of gram positive and gram negative bacterial strains (iv) To compare the zone of inhibition exhibited by the algal extracts with the standard antibiotics discs.

MATERIALS AND METHODS

Collection of Marine Algae Samples: For screening of antibacterial activity of marine algae the study area considered was the coast around Chennai, Ennore beach Tamil Nadu. Chennai is the large commercial and industrial area and it is located on the Coromandel Coast of Bay of Bengal. Live and healthy marine algae were collected from the coast of Ennore beach in the month of January. The three collected algae samples were identified by algal experts. The collected algae were rinsed with water to remove epiphytes and necrotic parts. Again samples were

thoroughly rinsed 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.

Preparation of Extracts: The algae after drying were weighed and then chopped. The chopped samples were finely powered using a clean motor and pestle or any mechanical methods. The finely powered samples were weighed and 5 grams of samples were 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.

Test Microorganisms Used: Pure cultures of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae* were used as the test microorganism for antibacterial testing.

Preparation of Inoculum: 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°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 Test: Antibacterial activity was assayed using the agar well diffusion test technique. Muller Hinton Agar Medium (MHA) was prepared, the pH is maintained at 7.4 and then sterilized by autoclaving at 121C and 15 lbs pressure for 15 minutes. 20 ml of the sterilized media was poured into sterilized Petri dish and allowed to solidify at room temperature. A sterile cotton swab is used for spreading the test microorganism from the 24 hours inoculated broth evenly on the MHA plates. Similarly swabbing was done separately for each test microorganism on the MHA plates and left for few minutes to allow complete absorption of the inoculum. In each of these plates 5mm diameter wells were made at the centre using an appropriate size sterilized cork borer.

Different concentration of each algal extract was added to the respective wells on the MHA plates. Concentration ranging from 100 micro liters, 150 micro liters and 200 micro liters respectively were placed in the wells and allowed to diffuse at room temperature for 30 minutes. No extracts was added in the control MHA plates which is used for comparing the obtained result from any contamination. The extract loaded plates were kept for incubation at 37°C for 24 hours. After incubation, a clear zone was observed around the well which was evidence of the presence of antibacterial active compounds in the algal extracts. Diameters of the zone of inhibition were measured in millimeters (including the diameter of the well).

For comparing the antibacterial activity of the isolated marine algae extracts with the therapeutic action of a number of known broad spectrum antibiotics Antibiotic Disc Diffusion Test was done. 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.

MHA was prepared and sterilized. After sterilization 20ml of the sterilized media was poured into the sterile Petri dishes and allowed to solidify at room temperature. Using a sterile cotton swab the test microorganism from the liquid 24 hours inoculated nutrient broth was spread evenly on each MHA plates. Using a sterile forceps each of the antibiotic disc of 10 micro mg was on the MHA plates and then kept for incubation at 37°C for 24 hours. Control MHA plates without any test microorganism was maintained.

The diameter of the zone of inhibition was measured in millimeters. The zone exhibited by the algal extracts was compared to the inhibition zones produced by the standard antibiotics. Diameter less than 10 mm measurement was interpreted as trace activity extracts and diameter between 11-25 mm was interpreted as active and samples exhibiting zone of inhibition with diameter more than 26 mm is expressed as highly active and can be used as an antibiotic. Standardized values for diameters of the inhibition halo, expressed in mm, produced by the microorganism against known antibiotics are listed in the literature by [31].

RESULT AND DISCUSION

Among all the three marine algae samples collected and the three seaweed extracts were tested against a range of microorganisms (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumoniae)* for the presence of the antibacterial activity, *Ulva fasciata* and *Chaetomorpha aerea* has shown good result. But the other algae sample Enteromorpha did not show good result in organic solvent namely methanol, ethanol and acetone.

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

Marine algae	*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)
Enteromorpha intestinalis	Average Result	Poor Result	Average Result	Good Result
Ulva fasciata	Good Result	Average Result	Average Result	Average Result
Chaetomorpha aerea	Average Result	Poor Result	Good Result	Average Result

Table: 2 showing zone of inhibition (in mm) of acetone, ethanol and methanol extract for green algae *Enteromorpha intestinalis* in MHA media

Concentration of *Enteromorpha* extract (in microlitre)	*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)		
	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	-	-	-

Table 3: Showing the zone of inhibition (in mm) of acetone, ethanol and methanol extracts for green algae *Enteromorpha intestinalis* in selective media

Concentration of *Enteromorpha* extract (in microlitre)	*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)		
	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 4: Showing the zone of inhibition (in mm) of acetone, ethanol and methanol extracts for green algae *Ulva fasciata* in MHA media

Concentration of *Ulva* extract (in microlitre)	*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)		
	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met
100	-	16	17	13	13	-	8	8	14	-	-	-
150	-	-	18	15	15	-	11	10	-	-	-	-
200	-	-	20	15	17	-	15	12	-	-	-	-

Table 5: Showing the zone of inhibition (in mm) of acetone, ethanol and methanol extracts for green algae *Ulva fasciata* in selective media

Concentration of *Ulva* extract (in microlitre)	*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)		
	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met	Act	Eth	Met
100	18	17	23	-	-	15	-	-	-	-	11	15
150	23	29	30	-	-	15	-	22	16	-	13	21
200	25	35	39	-	12	16	-	30	20	-	-	25

Table 6: Showing the zone of inhibition (in mm) of acetone, ethanol and methanol extracts for green algae *Chaetomorpha aerea* in MHA media

Concentration of *Ulva* extract (in microlitre)	*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)		
	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	-

Table 7: Showing the zone of inhibition (in mm) of acetone, ethanol and methanol extracts for green algae *Chaetomorpha aerea* in selective media

Concentration of *Ulva* extract (in microlitre)	*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)		
	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 8: Showing the 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 9: Showing the 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 10: Showing the 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 11: Showing the 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 12: Showing the 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 13: Showing the 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 14: Showing the 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 15: Showing the 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: Act - Acetone extract
Below 10mm - least active Eth - Ethanol extract
Between 11-25mm - moderately active Met - Methanol extract
Above 26mm - very active

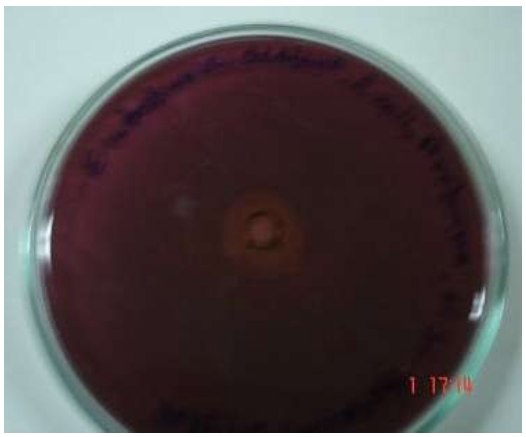
(a)

(b)

Fig 1: Zone of inhibition of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*
(A): Zone of inhibition of Ethanol extract in Kings B Media
(B): Zone of inhibition of Methanol extract in Mac Conkey Media

In the present study, it is observed that methanol and ethanol were the best organic solution for extracting 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 extract of Enteromorpha intestinalis in the ethanol extract, *Ulva fasciata* in the ethanol extract, *Chaetomorpha* in methanol extract and *Chaetomorpha aerea* in acetone extract. All these zone of inhibition was shown by *Pseudomonas aeruginosa.* Among all the test organism,

(a)



(b)

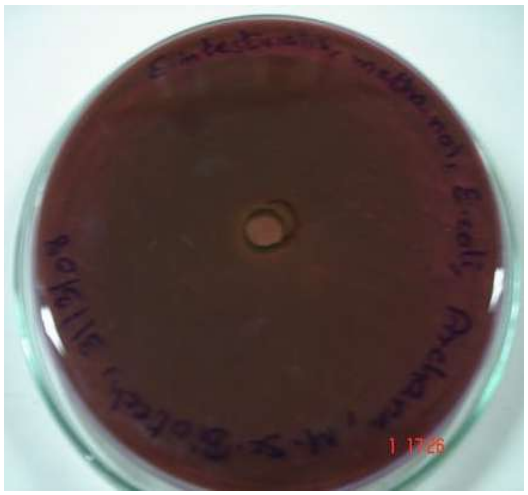


Fig. 2: Zone of inhibition of *Escherichia coli*
(A): Zone of inhibition of Acetone extract in EMB Media
(B): Zone of inhibition of Methanol extract in EMB Media

Pseudomonas aeruginosa and *Escherichia coli* has shown average result. *Escherichia coli* have shown very good result in all the three concentration in all the three organic solvents of algal extract *Ulva fasciata.*

The marine algae samples belonging to all family: Ulvaceae, Cladophoraceae were tested and both have shown average result. The percentage of antibacterial activity observed for *Ulva fasciata* was highest in all three algal groups studied. The ethanol, methanol, acetone extract of *Enteromorpha intestinalis*, *Ulva fasciata* in *Klebsiella pneumoniae*, *Chaetomorpha aerea* in *E.coli* have hardly exhibited any 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 test bacterial strains *Pseudomonas aeruginosa* and Escherichia *coli* were noted as the best Halo zone producer. The green algal extracts were not effective against gram positive test organism.

Of the three marine algae species (*Chaetomorpha aerea, Enteromorpha intestinalis, Ulva fasciata)* all species of green algae has shown some activity against bacteria. Good activity was been shown by green algae *Enteromorpha intestinalis* in all extracts (acetone, 80% ethanol, methanol) against *Klebsiella pneumoniae.* Significant activity was shown by *Ulva fasciata* in all three extracts against *Escherichia coli*, poor activity was shown by *Chaetomorpha aerea* in all three extracts of *Escherichia coli*, acetone and ethanol extract of *Pseudomonas aeruginosa* and acetone extract of *Klebsiella pneumoniae*. No activity was found in all three extracts of algae *Enteromorpha intestinalis* and *Ulva fasciata* in MHA media against *Klebsiella pneumoniae*, but good activity were shown in selective media of these two species. *Enteromorpha intestinalis* in selective media showed poor activity in all three extract against *Escherichia coli, Pseudomonas aeruginosa ,and Staphylococcus aureus.*

Chaetomorpha aerea in selective media does not show any activity in all three extracts against *Escherichia coli*, acetone and ethanol extract against *Pseudomonas aeruginosa,* acetone extract against *Klebsiella pneumoniae*. From this experiment it has been found that *Ulva fasciata* has highest antibacterial activity than the other two species of green algae.

Extracts of marine algae (*Ulva fasciata*) was reported to exhibit antibacterial activity [32,33]. In June 2007, it was reported that the marine algae *Ulva fasciata* collected from intertidal zone at Rocky Bay on the east coast of South Africa and methanol extract was tested for antibacterial activity. The two metabolites of marine algae *Ulva fasciata* and *Hypnea musciformis* collected from south east and south west coast of India were tested for biotoxicity potentials. Both species showed antibacterial activity but the green algae *Ulva fasciata* has exhibited broad spectrum antibacterial activity [34,35,36].

De Campos *et al.,* [37] and Padmini Sreenivasa Rao *et al.,* [38] compared the antibacterial activity of 37 marine algae in which green algae exhibited minimum activity and it was exhibited in this study. Also most of the earlier studies on the antibacterial activity of marine algae (*Ulva fasciata*) have low activity against gram positive bacteria *Staphylococcus aureus* which was observed in this study. *Enteromorpha intestinalis* and *Ulva fasciata* in all three extracts in MHA media does not show any activity against *Klebsiella pneumoniae* but both species have shown activity against *Klebsiella pneumoniae* in selective media. Similarly *Chaetomorpha aerea* in all three extracts in MHA media has shown activity against *Escherichia coli* but does not show any activity in selective media against *Escherichia coli.*

Thus it is noted that the green algae contains antibacterial activity. Acetone solution was least effective, 80% ethanol solution was effective while methanol was most effective organic solution. The effectiveness of various organic solvents used was: Methanol > Ethanol > Acetone. Marine algae collected in the coast of Tamil Nadu has shown antibacterial activity and the present observation were consistent with earlier results reported.

CONCLUSION

In conclusion the results shown by the marine green algae *Ulva fasciata* in the Muller Hinton Agar media and the selective media were observed to be almost same thus proving the presence of antibacterial activity in the marine algae. Earlier, many studies were reported on the presence of antibacterial activity of marine algae collected from the coastal area of Tamil Nadu. The percentage of zone of inhibition for the marine green algae Ulva *fasciata* was found to be the highest in this present study. The entire test microorganism has shown different percentage of resistance towards the algal extracts in different organic solvents. This supports the result obtained in this present study and proved that the marine algae contains biologically active compounds which is effective in resisting the growth of the pathogenic bacteria both gram positive and gram negative bacteria. The coastal area of Tamil Nadu is bestowed with a large number of pharmaceutically useful seaweeds which can be studied for the invention of drugs for many serious diseases like cancer, tumors, AIDS and many human degenerative diseases. Thus, marine algae are gaining more and more importance in the pharmaceutical industries all over the world.

REFERENCES

1. Paul, C. Silva and Sybil Parker, eds 1982. Chlorophyceae Synopsis and Classification of Living Organisms, McGraw-Hill, New York, USA, ISBN 0-07-079031-0, 1: 133-164.

2. González del Val, A., G. Platas, A. Basilio, A. Cabello, J. Gorrochategui, I. Suay, F. Vicente, E. Portillo, M. Jiménez del Rio, R.G. Garcia and F. Peláez, 2001. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. Microbial, 4: 35-40.
3. Culioli, G., M. Daoudi, A. Ortalo-Magne, R. Valls and L. Piovetti, 2001. S-(12) Hydroxygeranylgeraniol - derived diterpenes from the brown algae *Bifurcaria difurcata.* Phytochemistry, 57(4): 529-535.
4. Saz, A.K., S. Watson, S.R. Brown and D.L. Lowery, 1963. Antimicrobial activity of marine waters. I. Macromolecular nature of antistaphylococcal factor. Limnol. Oceanogr, 8: 63-67.
5. Daoudi, M., S. Bakkas, G. Culioli, A. Ortalo-Magne, H. Piovetti and M.D. Guiry, 2001. Acyclic diterpens and sterols from genera *Bifurcaria* and *Bifurcariopsis (*Cystoseiraceae, Phaeophyceae). Biochmical Systematic and Ecology, 29: 973-978.
7. Radmer, J.R. and B.C. Parker, 1994. Commercial applications of algae: opportunities and constraints. J. Appl. Phycol., 6: 93-98.
8. Glombitza, K.W. and L. Klapperich, 1985. Antibiotics from algae. XXXIV. Cleavage of the high-molecular-weight methylated Phlorotannins fraction from the brown alga Pelvetia canaliculata. Bot. Mar, 28: 139-144.
9. Donia, M. and H.T. Hamann, 2003. Marine natural products and their potential applications as anti-infective agents. The Lancet, 3: 338-348.
10. Faulkner, D.J., 2002. Marine natural products. Natural Products Reports, 19: 1-48.
11. Vlachos, V., A.T. Critchley and A. Von Holy, 1997. Antimicrobial activity of extracts from selected Southern African marine macroalgae. South African J. Sci., 93: 328-332.
12. Chennubhotla, V.S.K., B.S. Ramachandru, P. Kaladharan and S.K. Dharmaraj, 1987. Seaweed resources of Kerala coast. Seminar on Fisheries Research and Development in kerala. Trivandrum (Abstract).
13. Naqui, S.W.A., S.Y. Solimabi, L. Kamat, C.G. Fernandes, Y.K. Reddy Nahmias and B.N. Dhawan, 1981. Screening of some marine plants from the Indian coast for biological activity Botanica Marina, 24: 51.
14. Sridhar, K.R. and N. Vidyavathi, 1991. Antimicrobial Activity of Seaweeds. Acta hydrochimica et Hydrobiologica, 19(5): 455-496.
15. Wahidulla, S., S.Y. Kamat, L. D'Souza, C.G. Naik, Vijaya Ambiye, D.S. Bhakuni, A.K. Goel, H.S. Garg and R.C. Srimal, 1992. Bioactivity of Marine organisms VI. Antiviral evaluation of marine algal extracts from the Indian coasts. Bot. Mar., 35: 161.
16. Srinivasa Rao, P. and K.S. Parekh, 1981. Antimicrobial activity of Indian seaweed extracts. Bot. Mar., 24: 577-582.
17. Caccamese, S. and R. Azzolina, 1979. Screening for antimicrobial activities in marine algae from eastern Sicily. Planta Medica, 37: 333-339.
18. Pesanado, D. and B. Caram, 1984. Screening of marine algae from French Mediterranean coast for antibacterial and antifungal activity. Bot. Mar., 27: 381-386.
19. Alveal, K., H. Romo, C. Werlinger and E.C. Oliveira, 1997. Mass cultivation of the agar-producing alga Gracilaria chilensis (Rhodophyta) from spores. Aquaculture, 148: 77-83.
20. Caccamese, R. Azzolina, G. Furnari and M. Cormaci Grasso, 1988. Antibacterial and antiviral activities of extracts from Mediterranean algae. Botanica marina **Vol xx m pp. 285- 288.**
21. Mahasneh, I., M. Jamal, M. Kashashneh, M. Zibdeh, 1995. Antibiotic activity of marine algae against multiantibiotic resistant bacteria. Microbios, 83: 23-26.
22. Sastry, V.M.V.S. and G.R.K. Rao, 1994. Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. Bot. Mar., 37: 357-360.
23. Arun kumar, K. and R. Rengasamy, 2000. Evaluation of Antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the Plant Pathogenic Bacterium *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye . *Botanica marina*, 43(5): 409-415.
24. Antonio Gonzalez del Val., Gonzalo Platas, Angela Basilio, Angeles Cabello, Julian Gorrochategui, Immaculada Suay, Francisca Vicente, Eduardo portillo, Miguel Jimenez del Rio, Guillermo Garcia Reina and Fernando pelaez, 2001. screening of antimicrobial activities in red, green and brown macro algae from Gran canaria (Canary Islands, Spain). Int Microbiol., 4: 35-40.
25. Muhammad Afzal Rizvi and Mustafa Shameel, 2005. Pharmaceutical Biology of Seaweeds from the Karachi Coast of Pakistan. Pharmaceutical Biol., 43(2): 97-107.

26. Jehan Ara., Viqar Sultana, Rashida Qasim, Syed Ehteshamul-Haque and Viqar Uddin Ahmad, 2005. Biological activity of *Spatoglossum asperum*: a brown alga Phytotherapy Res., 19(7): 618-623.
27. Yolanda Freile-Pelegrin and Juan Luis Morales, 2004. Antibacterial activity in marine algae from the coast of Yucatan, Mexico. Botanica Marina, Walter de Gruyter, 47: 140-146.
28. Febles, C.I., A. Arias, M.C. Gil-Rodriguez, A. Hardisson and A. Sierra Lopez, 1995. *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (Spanish). Anuario dal Instituto de Estudios Canarios., 34: 181-192.
29. Sebastian Engel, P. Melany Puglisi, R. Paul Jensen and William Fenical, 2006. Antimicrobial activities of extracts from tropical Atlantic marine plants against marine pathogens and saprophytes Marine Biol., 149: 991-1002.
30. Inci Tuney., Bilge Hilal. Cadirci, Dilek. Unal and Atakan Sukatar, 2006. Antimicrobial Activities of the Extracts of Marine Algae from the Coast of Urla (Izmir, Turkey). Turk J. Biol, 30: 171-175.
31. Álvarez Benito, Ma. V., 1990. *Manual de técnicas en microbiolo gía clínica*. Asociación Española de Farmacéuticos Analistas San Sebastian.
32. Siddhanta, A.K., K.H. Mody, B.K. Ramavat, V.D. Chauhan, H.S. Garg, A.K. Goel, M. Jinandra Doss, M.N. Srivastava, G.K. Patnaik and V.P. Kamboj, 1997. Bioactivity of marine organisms: Part VIII- Screening of some marine flora of Western coast of India. Indian Journal Experimental Biol., 35: 638-643.
33. Sachithananthan, K and A. Sivapalan, 1975. Antibacterial properties of some marine algae of Sri Lanka. Bulletin of Fisheries Research Station, Sri Lanka., 26: 5-9.
34. Elena, S.F., R. Sanjuán, A.V. Bordería and P.E. Turner 2001. Transmission bottlenecks and the evolution of fitness in rapidly evolving RNA viruses. Infect. Gen. Evol., 1: 41-48.
35. Okai, Y., K. Higashi-Okai, S. lshizaka, U. Yamashita, 1997. Enhancing effect of polysaccharides from an edible brown alga, *H!jikia fusiforme* (Hijiki), on release of *tumor necrosis* factor from macrophages of endotoxin-nonresponder C3H/HeJ mice. Nutrition and Cancer, 27: 74 79.
36. Premila, J.C., N.S. Raviraja and K.R. Sridhar, 1996. "Antimicrobial Activity of Some Marine Algae of Southwest Coast of India," Indian J. Mar. Sci., 26(2): 201-205.
37. De Campos-Takaki, M.L. Koenong and G.C. Perira, 1988. Screening of marine algae from Brazilian northeastern coast for antimicrobial activity. Bot. Mar., 31: 375-377.
38. Padmini Srinivasa Rao, P., P.S. Rao and S.M. Karmarkar, 1986. Antibacterial substances from brown algae. II. Efficiency of solvents in the evaluation of antibacterial substance from Sargassumjohnstonii. Setchell et Gardener. Bot, Mar., 29: 503-507.
00. Mayer, A.M.S. and V.K.B. Lehmann, 2000. Marine pharmacology in 1998: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, antiplatelet, antiprotozoal and antiviral activities; with actions on the cardiovascular, endocrine, immune and nervous systems; and other miscellaneous mechanisms of action, Pharmacologist, 42: 62-69.
00. Vidyavathi, N. and K.R. Sridhar, 1991. Seasonal and geographical variations in the antimicrobial activity of seaweeds from the Mangalore coast in India. Bot. Mar., 34: 279-284.
00. Plaza, M., A. Cifuentes and E. Ibanez, 2008. In the search of new functional food ingredients from algae and microalgae. Trends Food Science Technol., 19: 31-39.