

Antibacterial Activity of the Extracts of Marine Red and Brown Algae

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ABSTRACT :In the marine eco system, seaweeds are directly exposed and are susceptible to ambient micro organisms such as bacteria, fungi and viruses. Seaweed species of kappaphycus (red algae) and padina (brown algae) from the coast of Tamilnadu, India were tested in vitro for their antibacterial activities against different types of bacteria using disc diffusion method. Methanol was used for inhibition of different bacterias such as pseudomonas flouresences, staphylococcus aureus, vibriochloera and proteus mirabilis in the case of red algae. In the study, it is observed that kappaphycus maximum activity against pseudomonas flouresences, staphylococcus aureus and less inhibition on vibriochloera and proteus mirabilis. Benzene, n-hexane, ethylacetate, methanol, chloroform : methanol solvents were used for inhibition of staphylococcus aureus and E-coli. It is noted that chloroform : methonal is the best solution for extracting the effective antibacterial materials from the brown algae species. The chloroform: methanol solvent further used for antibacterial activity against eleven pathogenic bacterias. It is observed from the experiments that the extract residues of algae recorded maximum activity against staphylococcus aureus with an inhibition zone compared to other bacterias. The extract residues of brown algae did not show any effect on the growth of proteus vulgaris and psedudomonoaeruginosa.[Journal of American Science 2009:5(3) 20-25] (ISSN: 1545-1003)

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1. INTRODUCTION

Commercially available varieties of marine macroalgae are commonly referred to as seaweeds. Macroalgae can be classified as red algae (rhodophyta), brown algae (phaeophyta) or green algae (chlorophyta) 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. Seaweeds have been used as food stuff in the 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. Biostimulant properties of seaweeds are explored for use in agriculture and the antimicrobial activities for the development of novel antibiotics. Seaweeds have some of the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a

significant amount of the protein, vitamins and minerals essential for the human nutrition (Fayaz et al., 2005). The nutrient composition of seaweed varies and is affected by the species, geographic areas, seasons of the year and temperature of the water. Seaweeds have recently received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show anti-bacterial activities (Vairappan et al., 2001, Vlachos et al., 1996). Many metabolites isolated from marine algae have been shown to possess bioactive effects (Oh et al., 2008, Venkateswarlu et al., 2007 and Yang et al., 2006).

Among the different compounds with functional properties, antioxidants are the most widely studied. Moreover the important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers. Marine algae serve as important resources for bioactive natural products (Illiopoulere et al., 2002; Metzger et al., 2002). Brazilian red algae have been found to have phenolic substances. Oxidative stress is an important factor in the genesis of pathology, from cancer to cardiovascular and degenerative disease (Parthasarathy et al., 2001; Croke et al., 2003). Fayaz et al. (2005) suggested the utility of

Kappaphycus alvarezzi for various nutritional products including antioxidant for use as health food or nutraceutical supplement. Different parts of the thalli are also known to differ in their antimicrobial potential. Extracts prepared from fresh seaweed samples are reported to show negligible antimicrobial activity as compared to that obtained with dried seaweeds. Seasonal and geographical variation in the levels of antimicrobial activities of marine algae has been shown by many. However, information is lacking on the seasonal and geographic variations in the specific metabolites of marine algae of antimicrobial potential, especially for the marine algae of South India. The coastal region of Tamilnadu, South India support a rich vegetation of marine algae. These studies have shown a great diversity in the macroalgal community of the marine algal vegetation of the region. Among the macro algae of the region, the brown algae padina sps and one red algae kappa sps grow in abundance as dominant communities in the shores of Kanyakumari and Ramanthapuram Districts of Tamilnadu State, India. In the present study, antibacterial activities of the extracts of red and brown algae using different solvent systems have been investigated.

2. MATERIALS AND METHODS

The marine red alga was collected from the sea coast of Rameshwaram, Tamilnadu, India and the marine brown alga was collected from the sea coast of kanyakumari and Ramanathapuram district of Tamilnadu, India. Both are used as the experimental algae to study their bioactivity. Samples were rinsed with sterile water to remove any associated debris. Sample was kept under sunshade for 7 days. After drying the sample, it was ground thoroughly to powder form. The powder was then used for the estimation of the antibacterial activity. The strains of pseudomonas fluorescences, staphylococcus aureus, vibriochloera and proteus mirabilis for red algae, vibriochloerae, staphylococcus aureus, E-coli, bacillusmegaterium, citrobactersp, enterobactersp, klebsiellapneumoniae, salmonellatyphi, shigellaflexneri, pseudomonasaeruginosa, proteusvulgaris were obtained from the University of Madras, Guindy campus, Chennai, Tamilnadu, India. The

antibacterial activity of the extract was assayed using the disc diffusion method (Bauer et al., 1996). For inoculum preparation and assay of antibacterial activity, Muller-hinton agar was used. The bacteria were sub cultured and routinely maintained on both nutrient agar and Muller-hinton agar. Antimicrobial activity was evaluated using the agar diffusion technique in petridishes. Each extract was loaded on sterile filter paper discs and air dried. Indicator microbes were spread on muller-hinton agar plates with sterile effusion the discs were placed on plates. After incubation for 24 hours at 30°C, a clear zone around a disc was evidence of antimicrobial activity. Discs loaded with the extracting agents were tested as controls (Incitunes et al 2006).

Nutrient Agar

Beef extract	3.0 g,
Peptone	5.0 g,
Nacl	5.0 g,
Agar	15.0 g,
P ^H	7.2

The above were dissolved in one liter distilled water and sterilized at 121°C for 15 minutes.

Muller Hinton Agar

The medium contained in one liter of water

Casein hydrolysate (enzymic)	17.5 g
Beef infusion	30.0 g
Soluble starch	1.5 g
Agar	20.0 g

The final P^H of the medium after sterilization at 1.1 kg/cm² to 7.4 ± 0.2 (at 25°C) (121°C) for 15 minutes was adjusted.

3.0 RESULTS AND DISCUSSION

Extracts of red and brown seaweed were tested against bacteria. The results of primary screening test of red algae (Kappa species) are summarized in Table 1. In this study, five test pathogens were considered, namely, pseudomonas fluorescence, staphylococcus aureus, vibrio cholera, Proteus mirabilis towards study of inhibition of microbial growth by methanol extract. The plates were incubated at 37°C. The zone of inhibition of assay was scored (+), if it is <2mm, double positive (++), if the zone is 2mm. The results are presented in Table 1 and Figs. 1, 2,3 and 4 respectively.

Table 1 Inhibition of Microorganisms by Methanol extract

Volume (In μ l)	Plate-1	Plate-2	Plate-3	Plate-4
	<i>Pseudomonas fluorescens</i>	<i>Staphylococcus aureus</i>	<i>Vibrio cholera</i>	<i>Proteus mirabilis</i>
25	-	+	-	-
50	+	++	-	-
75	++	+++	+	-
100	+++	+++	++	+



Fig. 1 *Pseudomonas fluorescens*



Fig. 2 *Staphylococcus aureus*



Fig. 3 *Vibrio cholera*



Fig. 4 *Proteus mirabilis*

Fiho et al. (2002) found that hexane extract of *gracilaria* species (red algae) inhibits only *bacillus subtilis* in contrast our results showed that the methanol extract of *kappa* species (red algae) inhibited the bacterias, namely, *pseudomonas fluorescens*, *staphylococcus aureus*, *vibrio cholera*, *proteus mirabilis*.

For studies on the antibacterial activity of the extracts of the experimental brown algae, the five solvent systems used, namely, n-hexane, benzene, ethyl acetate, methanol and the mixture of chloroform: methanol (2:1 v/v) were used. The bacteria *staphylococcus aureus* and *E-coli* were used as test organisms (Table 2). In this primary investigation, the algal extract

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prepared with mixture of chloroform and methanol (2:1 v/v) proved to be more effective than the other solvent systems used in inhibiting the growth of both staphylococcus aureus and E-coli on the muller-hinton agar plates. Methanolic extracts of the algae were able to exhibit only 25-30% maximum activity against test organisms (Table 2). The other solvent extract appeared to be ineffective in inhibiting the growth of staphylococcus aureus and E-coli in muller-hinton agar, based on these observations, further experiments on the antibacterial activities of the experimental algae were restricted to chloroform: methanol extracts only.

Chloroform: methanol (2:1 v/v) extracts of experimental algae were prepared as described earlier and tested at a concentration of 700 µg/disc by disc diffusion method against 11 pathogenic bacteria, namely, vibriocholerae, staphylococcus aureus, E-coli, bacillus megaterium, citrobactersp, enterobactersp, klebsiellapneumoniae, salmonellatyphi, shigellaflexneri, pseudomonasaeruginosa, proteusvulgaris. The results are presented in Table 2 and Figs 5 to 9. The extracts residues of algae recorded maximum activity against staphylococcus aureus with an inhibition zone. Bacillus megaterium, klebsiellapneumoniae, shigellaflexneri and vibriocholera were also inhibited by the extract residues of the experimental algae. 32 to 50 % of maximum activity was observed against citroacitro species, entro bacter species and E-coli. The extract residues of the brown algae did not show any effect on the growth of proteus vulgaris.

Some studies concerning the effectiveness of extraction methods highlight that the methanol extraction yields higher antibacterial activity than n-hexane and ethyl acetate whereas others report that chloroform is better than methanol and benzyne. It is clear that using organic solvent always provides a higher efficiency in extracting compounds for antibacterial activities comparative water based methods (Incituney, et al., 2006). According to our experimental results, mainly extract residues of the kappa sp. (red algae) and padina sp. (brown algae) have good antibacterial activity related compounds. The remarkable differences between our results and the results obtained in previous studies may be due to several factors. First of all, this can be

because of the intraspecific variability in the production of secondary metabolites, occasionally related to seasonal variations. Secondly, there may also be differences in the capability of the extraction protocols to recover the active metabolites and differences in the assay methods that would result in different susceptibilities of the target strains. This is an inevitable fact for all biochemical research because test materials have trace impurities (Incituney, et. al., 2006).

Table 2 Antibacterial activity of the crude solvent extracts of the brown algae

Sl. No	Solvent used for extraction	Antibacterial activity % (max. activity)	
		Staphylococcus aureus	E-coli
1	n-hexane	++ (10)	-
2	benzyne	+ (7)	++
3	Ethyl acetate	++ (10)	++
4	methanol	+++ (25)	+++
5	Chloroform: methanol (2:1 v/v)	++++ (100)	++++

- no activity ,

+ low activity (7-10 mm halo)

++ high activity (10-15 mm halo),

+++ to ++++ more activity (25-100 mm/halo)

Table 3 Antibacterial activity proved chloroform: methanol (2:1 v/v) extract residue of the experimental brown algae

Sl. No.	Test pathogen	Zone of inhibition (cms) ± s.e
1	Bacillus megaterium	4.0 ± 0.063 (64.5)
2	Staphylococcus aureus	6.2 ± 0.128 (100)
3	citroacitro species	2.0 ± 0.032 (32.5)
4	entro bacter species	3.0 ± 0.086 (48.4)
5	klebsiella pneumoniae	3.8 ± 0.032 (61.3)
6	E-coli	2.2 ± 0.063 (35.5)
7	salmonellatyphi	3.6 ± 0.10 (58.1)
8	shigellaflexneri	3.8 ± 0.063 (61.3)
9	proteus vulgaris	nil
10	psedudomonoaeruginosa	nil
11	vibriocholera	3.5 ± 0.07 (56.5)

700µg extract residue/disc was used in the

assay

Values given in parentheses indicate % maximum activity



Fig. 5 Bacillusmegaterium & Enterobacter sp.

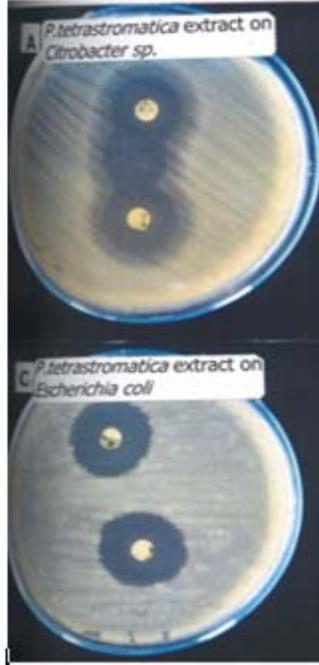


Fig. 6 Citrobactr sp. & Salmonellatyphi



Fig. 7 E-coli Staphylococcus aureus

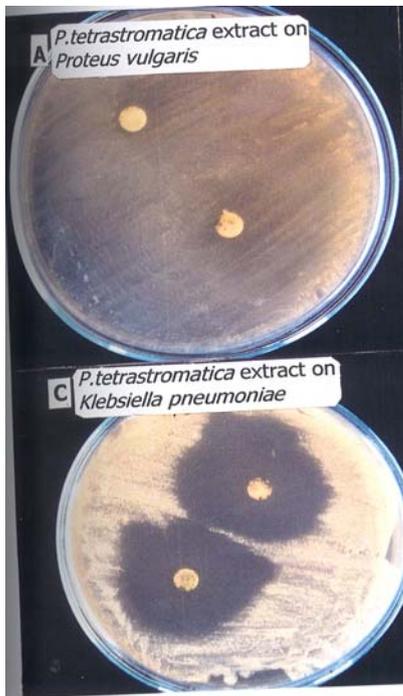


Fig. 8 Proteusvulgaris & Klebsiellapneumoniae



Fig. 9 Pseudomonasaeruginosa & Shigellaflexneri

SUMMARY AND CONCLUDING

REMARKS

Samples (red and brown algae) collected from different sites locate in the Kanyakumari and Ramanathapuram districts of Tamilnadu, India werescreened for antibacterial activity. Methanol was used for inhibition of different bacterias such as pseudomonas flouresences, staphylococcus aureus, vibriochloera and proteus mirabilis in the case of red algae. In the study, it is observed that kappaphycus maximum activity against pseudomonas flouresences, staphylococcus aureus and less inhibition on vibriochloera and proteus mirabilis. Benzene, n-hexane, ethylacetate, methanol, chloroform : methanol solvents were used for inhibition of staphylococcus aureus and E-coli. It is noted that chloroform : methonal is the best solution for extracting the effective antibacterial materials from the brown algae species. The chloroform: methanol solvent further used for antibacterial activity against eleven pathogenic bacterias. It is observed from the experiments that the extract residues of algae recorded maximum activity against staphylococcus aureus with an inhibition zone compared to other bacterias. The extract residues of brown algae did not show any effect on the growth of proteus vulgaris and psedudomonoaeruginosa. Finally, we conclude that marine macro algae (red and brown algae) from the South coast of Tamilnadu, India are potential sources of bioactive compounds and should be investigated for natural antibiotics. However, further work is required to identify these active compounds in kappaphycus sp. which is carrageeno phytic seaweed.

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