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Original Research Article

Antibacterial activity of *Kappaphycus alvarezii* and *Ulva lactuca* extracts against human pathogenic bacteria

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A B S T R A C T

Seaweeds are one of the important living resources of the marine and have given valuable bioactive compounds. In their wide applications are distinguished in pharmacological researches, because of their antimicrobial and antioxidative properties. In this study focused on the antibacterial activity of seaweeds (*Kappaphycus alvarezii* and *Ulva lactuca*) against six human pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*). The selected seaweeds were extracted by five different types of solvents, namely Acetone, Chloroform, Ethanol, Ethyl acetate and Methanol. The antibacterial activity was done by agar well diffusion method. Here, the best activity was recorded in ethanol extracts, which shows the inhibition zones against all the pathogens tested. The *K. alvarezii* extracted by ethanol exhibited high antibacterial activity of 13mm against *Bacillus subtilis*. The results of the present study confirmed that selected seaweeds were having significant capacity for to control the pathogenic organism.

Introduction

Seaweeds are important sources of marine ecosystem having various biological activities (Bouhlal *et al.,* 2011; Kayalvizhi *et al.,* 2012). Seaweeds are macroscopic algae are one of nature's most biologically active resources, as they possess a wealth of bioactive compounds and they used to found attached to the bottom in relatively shallow coastal waters. They grow in the intertidal, shallow and deep sea areas up to 180 meter depth and also in estuaries and back waters on the solid substrate such as rocks, dead corals, pebbles, shells and other plant materials. They form one of the important living resources grouped under three divisions namely, Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae) depending on their nutrient and chemical composition. They are abundant on hard substrates and commonly extending to depths of 30 - 40 m (Anantharaman, 2002).

Ulva lactuca Linnaeus, a green alga (Division Chlorophyta), it is shows a

cosmopolitan distribution, occurring on rocks and on other algae in the littoral and sublittoral zones of coastal areas (Awad, 2000). The genus Ulva has great potentiality as a commercial product because of its fruitful taste and varied chemical composition and quality (Selvin et al., 2004; Hardy et al., 2006).

Kappaphycus alvarezzi, a red alga (Division Rhodophyta), for various nutritional products including antioxidant for use as heath food or neutraceutical suplement. *Kappaphycus alvarezzi* has nutritive and antioxidant property; different parts of the thalli are also known to differ in their antimicrobial potential (Fayaz *et al.,* 2005).

Micro organisms are responsible for the high rate of mortality in human and animals. Some microbes are sociable but most of them very pathogenic, like that *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* are causes the diseases like food borne gastroenteritis, urinary tract infections and upper respi-ratory complications (Jawetz *et al*., 1985; Leven, 1987). *Proteus mirabilis* has the ability to produce high levels of urease and it also found in the kidney stones (Rauprich *et al*., 1996).

Antibiotics are used to control the pathogenic organism, but microbes were more resistance to antibiotics has necessitated development of new alternatives (Smith *et al*., 1994). Nowadays this problem creates need for screening the new antibacterial agents from available sources and seaweed is one of them (Subba Rangaiah *et al*., 2010). Traditionally, seaweeds have been used in the treatment of various infectious diseases. Many substances obtained from seaweeds have been used for decades in medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties (Gorban *et al*., 2003).

Screening of the antimicrobial compounds from seaweeds is vital and increasing demand of therapeutic drugs in treatment of pathogenic infection (Prasad *et al*., 2013). Previously many researchers are reported in the antibacterial (Kandhasamy and Arunachalam, 2008; Adaikalaraj *et al*., 2012) antifungal (Amina *et al*., 2007) antiviral (Mayer, 2002; Ghosh *et al*., 2004) and anticancer (Luis *et al*., 2010; Keivan *et al*., 2010) activities of seaweeds.

In this present study was focused on to investigate the antibacterial activity of *Kappaphycus alvarezii* and *Ulva lactuca* seaweeds against six human pathogenic organisms both of gram positive and gram negative bacteria.

Materials and Methods

Sample collection

The seaweeds *Kappaphycus alvarezii* and *Ulva lactuca* were collected from Thoothukudi seashore, Tamil Nadu, India. The collected seaweed samples were washed with seawater and then in fresh water and extraneous matters were removed. After that they were brought into the laboratory in sterile plastic bags. The samples were rinsed with fresh water and sterile distilled water, shade dried, cut into small pieces and powdered in a lab mixer grinder. The powdered samples were then stored in freezer for further study.

Test organisms

Extracts were tested against six bacterial stains (Gram +ve: *Bacillus subtilis, Staphylococcus aureus, Lactobacillus*

acidophilus and Gram -ve: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis*). The test pathogens were obtained from the Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.

Extract Preparation from seaweed powder

The powdered sample of 5g was extracted in soxhlet apparatus using acetone, chloroform, ethanol, ethyl acetate and methanol (200 ml) as solvents for 8h at a temperature maintain not more than the boiling point of the solvent. The extracts were filtered using Whatman No.1 filter paper and kept it under Hot air oven (40^0C) for the solvent evaporation. The residues obtained were stored in a freezer at -20^0C.

Determination of Antibacterial assay

The antimicrobial activity was carried out by using agar well diffusion method. The solvents like acetone, chloroform, ethanol, ethyl acetate and methanol were used to collect the seaweed extract and were tested against the human pathogens at four dose levels (40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml). Overnight grown bacterial culture was transferred to sterile Petri plate with Mueller Hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with sterile spreader to create a lawn. About 5 wells of 6mm diameter were made in each plate with the help of a sterile cork borer. Among the five, four wells were placed with the different concentration of the extracts using sterile pipettes and remaining one well was mentioned as control had with solvent alone. The Petri dishes were prepared and incubated for 18-24hrs at 37°C and the zone of inhibition around the well was measured in nearest millimeter. Each experimental result was determined by the average of triplicates.

Results and Discussion

The antibacterial activity of the selected seaweeds *Kappaphycus alvarezii* and *Ulva lactuca* were extracted with five different solvents like acetone, chloroform, ethanol, ethyl acetate and methanol and determined the activity against six pathogenic organisms both Gram positive *(Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus* and Gram negative *Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis)* bacteria by agar well diffusion method were tabulated in the Table 1. The highest activity 7mm in low concentration (40µg/ml) was seen in *Kappaphycus alvarezii* extracted by methanol against *Proteus mirabilis*.

In *Kappaphycus alvarezii* extracted by ethanol and methanol were found to be most activity against all the tested pathogens, but the same time there was no activity recorded in chloroform extracts. Both seaweeds were exhibit activity against the entire organism, when ethanol was used as a solvent. In our study also confirmed that, antibacterial activity varied according to the solvent extract and test organism. Here, it was observed that ethanol and methanol were the best organic solvent for extracting the effective antibacterial material from the species used in this experiment.

The highest activity 13mm based on high concentration (100µg/ml) recorded in *Kappaphycus alvarezii* extracted by ethanol against *Bacillus subtilis* and the lowest activity 3mm against *E. coli* ethyl acetate as a solvent. In the antibacterial

Table.1 Antibacterial activity of *Kappaphycus alvarezii* and *Ulva lactuca* extracts against human pathogens in different concentrations

Name of the pathogens	Concentration µg/ml	Diameter zone of inhibition in mm									
		Acetone extract		Chloroform extract		Ethanol extract		Ethyl acetate extract		Methanol extract	
		K	U	K	U	K	U	K	U	K	U
Bacillus subtilis	40	–	–	–	–	6	–	–	–	2	–
	60	–	–	–	–	9	6	–	–	3	–
	80	6	7	–	–	10	6	–	–	3	–
	100	9	11	–	–	13	7	–	6	4	–
Staphylococcus aureus	40	–	–	–	–	–	–	–	–	–	–
	60	–	–	–	–	–	–	6	–	–	–
	80	–	–	–	–	–	–	6	–	7	–
	100	10	–	–	–	6	6	7	7	9	–
Lactobacillus acidophilus	40	–	–	–	–	–	–	–	–	–	–
	60	–	–	–	–	–	–	–	–	6	–
	80	–	–	–	6	7	6	–	–	8	–
	100	–	–	–	6	9	8	–	6	10	6
Escherichia coli	40	–	–	–	–	4	–	–	–	6	–
	60	7	–	–	–	7	7	–	–	7	–
	80	9	–	–	–	8	7	–	–	7	–
	100	11	6	–	–	10	8	3	8	8	9
Pseudomonas aeruginosa	40	–	–	–	–	–	–	–	–	–	–
	60	–	–	–	–	6	–	–	–	–	–
	80	–	–	–	–	8	–	–	–	6	–
	100	7	6	–	7	10	6	–	–	8	–
Proteus mirabilis	40	–	–	–	–	–	–	–	–	7	–
	60	–	–	–	–	–	–	8	–	7	–
	80	–	–	–	–	9	8	10	–	8	–
	100	–	–	–	–	9	11	12	–	10	6

activity, *K. alvarezii* was more effective than *U. lactuca.* Fayaz *et al*., reported that *Kappaphycus alvarezzi* has nutritive and antioxidant property; different parts of the thalli are also known to differ in their antimicrobial potential.

The antibacterial activity demonstrated by water extract provides the scientific bases for the use of water extracts in traditional treatment of diseases. There are also reports in literature that methanol is a better solvent for consistent extraction of antimicrobial substances for medicinal plants (Elloff, 1998). This may be attributed to two reason, firstly, the nature and potentiality of biologically active components (alkaloids, steroids, flavonoids, essential oils biterpenoids), which could be enhanced in the presence

of methanol. Secondly, the stronger extraction capacity of methanol could have produced greater number or amount of active constituents responsible for antibacterial activity (Jeyachandran *et al.*, 2010). This is also proved in our study in which methanol extracts exhibited the highest antibacterial activity against *E. coli* compared to other extracts.

The results of the present study confirmed that definitely a significant compound is containing in the selected seaweeds, so further identification and purification needed to the future research.

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