ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 1, January 2014

In-Vitro antibacterial activity of *Kappaphycus alvarezii* extracts collected from Mandapam Coast, Rameswaram, Tamil Nadu

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Abstract: The antibacterial activity of acetone and methanol extracts of marine red alga *Kappaphycus alvarezii* (red algae) collected from Mandapam Coast (Gulf of Mannar) was screened invitro for their antibacterial activity against four human bacterial pathogens Viz.; *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis* and *Vibrio cholerae* using well diffusion method. In this study it was suggested that the methanol and acetone extracts were the best medium for the extraction of the effective antibacterial material from the red alga *Kappaphycus alvarezii*. It was observed that the methanolic extract recorded maximum activity against *Staphylococcus aureus* and *Proteus mirabilis*, whereas the acetone extract exhibited antibacterial effect against *Bacillus subtilis* and *Staphylococcus aureus*. The maximum inhibition zone (11mm) was noted in the methanolic extract of *Kappaphycus alvarezii* against *Proteus mirabilis*.

Keywords: Antibacterial activity, *Kappaphycus alvarezii*, seaweeds, well diffusion method

I. INTRODUCTION

Seaweeds are the marine algae which have been widely used as sea vegetables, cattle fodder, herbal medicines and fertilizers in Asia, Pacific and mediterranean regions for centuries. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. Seaweeds have been used as food stuff in the Asia diet for centuries as it contains carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals which are highly essential for human nutrition [1]. Seaweeds have recently received significant attention for their potential as natural antioxidants. Most of the compounds of marine algae show antibacterial activities against different bacteria[2]. Many metabolites isolated from marine algae have been shown to possess bioactive efforts [3]. Seaweeds are known to contain large amounts of polysaccharides especially storage polysaccharides. They contain incomparable wealth of mineral elements, macro elements and trace elements. The green and red algae contain higher protein content and it is good source of vitamins from group B.

Kappaphycus alvarezii is one of the red seaweed come under the class Rhodophyceae. It is economically important species that has been extensively cultivated in more than 20 countries for the source of carrageenan. The red seaweed *Kappaphycus alvarezii* has been previously reported for its antioxidant potential and free radical scavenging activity [4] and in- vitro antiproliferative activity in cancer cell lines [6]. In the present investigation the antibacterial activity of different solvent extracts were investigated against a range of gram positive and gram negative bacterial strains.

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II. MATERIALS AND METHODS

Seaweed material:

The marine red algae *Kappaphycus alvarezii* was collected from the seacoast of Mandapam, Rameswaram, Tamilnadu, India. Healthy and well grown algae were collected from the rocks which are submerged under water during low tides by handpicking. The alga samples were washed thoroughly with sterile water to remove unwanted shells, mud, debris, epiphytes and other marine organisms. The collected red algae *Kappaphycus alvarezii* was identified by using seaweeds manual named "seaweeds – A field manual" and verified from Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai-25, Tamil Nadu, India.

Preparation of seaweed Extracts:

The seaweed *K. alvarezii* extract was prepared as indicated in previous studies with slight modifications [4]. The pulverized moisture free sample (25.0 g) was extracted with 200 ml of methanol and acetone as solvent for several times. Filtrate was condensed in a rotary evaporator at 40 rpm to remove excess solvent and stored as such. This solvent extract was used to determine antibacterial activity of selected human pathogenic bacteria.

Test Microorganisms:

Pure cultures of *Bacillus subtilis, Staphylococcus aureus, Proteus mirabilis* and *Vibrio cholerae* 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 hrs to allow microorganism to grow till the log phase.

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 121°C 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 hrs 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 of *Kappaphycus alvarezii* was added to the respective wells on the MHA plates. Concentration ranging from 25 µL, 50 µL, 75 µL and 100 µL/mL 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. Streptomycin was used as positive control. The extract loaded plates were kept for incubation at 37°C for 24 hrs. After incubation a clear zone was observed around the well which was evidence of the presence of antibacterial active compounds in the algal extract *Kappaphycus alvarezii.* Diameters of the zone of inhibition were measured in millimeters (including the diameter of the well).

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III. RESULTS AND DISCUSSION

The antibacterial activity of different solvent extracts (methanol and acetone) against four different human pathogens (*Bacillus subtilis, Staphylococcus aureus, Proteus mirabilis, Vibrio cholerae*) at various concentrations showed the zone of inhibition at diverse level. The results were presented in table 1 and 2 respectively. The zone of inhibition was measured in millimeters (mm). The methanolic extract of *Kappaphycus alvarezii* exhibited the highest and broadest antibacterial activity against *Proteus mirabilis* (11 mm). It has no activity against the pathogens like *Vibrio Cholerae* and *Bacillus subtilis*. The maximum inhibition zone (10 mm) was observed in acetone extract (100 µL/mL per well) of *Kappaphycus alvarezii* against *Bacillus subtilis* but no activity was observed against the pathogens *Vibrio Cholerae and Proteus mirabilis.* The antibacterial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plant and also the experimental methods adopted for the investigation. The acetone extract (75 µL and 100 µL) of *Kappaphycus alvarezii* extract showed the inhibition zone (5 mm) against *Staphylococcus aureus* and the minimum inhibition zone (1 mm) was observed in the methanol extract (50 µL/mL per well) of *Kappaphycus alvarezii* against *Staphylococcus aureus*. It was reported earlier the antibacterial activity of commercially important seaweeds namely *Ulva lactuca, Padina gymnospora, Sargassum wightii* and *Gracilaria edulis* against bacterial pathogens *Staphylococcus aureus, Vibrio cholera, Shigella dysentriae, Shigella bodii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The maximum activity (8.8 mm) was recorded from the extract of *Gracilaria edulis* against *Staphylococcus aureus* and minimum (1.2 mm) by *Ulva lactuca* against *Pseudomonas aeruginosa* [7]. Antibacterial activity of nine species of seaweeds belonging to brown, green and red algae revealed that red and brown seaweeds had greater antibacterial activity than green algae [8]. However some extracts were unable to exhibit antibacterial activity against tested bacterial strains may have some kind of resistance mechanisms. There have been reports on significant antimicrobial activities of marine macro algae by several researchers which confirm our findings [9, 10, 11].

Table – I. Zone of Inhibition (mm) of Microorganisms by Methanol extract of *Kappaphycus alvarezii*

S.No	Volume of extract (in µL/mL)	Plate 1 *Bacillus subtilis*	Plate 2 *Staphylococcus aureus*	Plate 3 *Proteus mirabilis*	Plate 4 *Vibrio cholerae*
1	25	-	-	7.5mm	-
2	50	-	1mm	7.5mm	-
3	75	-	2mm	11mm	-
4	100	-	2mm	10mm	-
5	Antibiotic Penicillin	10 mm	8 mm	7.5mm	9 mm
6	Control(Methanol)	0.20	0.25	0.20	0.1

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Table –II. Zone of Inhibition (mm) of Microorganisms by Acetone extract of *Kappaphycus alvarezii*

S.N o	Volume of extract (in µL/mL)	Plate 1 *Bacillus subtilis*	Plate 2 *Staphylococcus aureus*	Plate 3 *P. mirabilis*	Plate 4 *Vibrio cholerae*
1	25	4mm	-	-	-
2	50	5mm	2mm	-	-
3	75	8mm	5mm	-	-
4	100	9mm	5mm	-	-
5	Antibiotic Penicillin	10mm	7 mm	8 mm	8.5 mm
6	Control(Acetone)	0.25	0.15	0.25	0.15

IV. CONCLUSION

From the present investigation, it was suggested that a particular solvent is required to extract a suitable antibacterial substances from red algae *Kappaphycus alvarezii.* The percentage of bacterial growth inhibition can be improved when this particular solvent extract is used for further screening. Hence, isolation of active compounds from red seaweed *Kappaphycus alvarezii* is highly needed for detailed investigation for further pharmacological evaluation.

ACKNOWLEDGEMENT: We acknowledge Mr. K.Vijayakumar for lab Assistance.

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