



An antibacterial activity of selected brown, green and red seaweeds from Manapad, Thoothukudi, Tamil Nadu, India

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ABSTRACT

Antibacterial activity of three seaweeds, namely *Padina tetrastromatica*, *Caulerpa sertularioides* and *Kappaphycus alvarezii* were screened against six bacterial pathogens (*Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*). Extracts of selected seaweeds were prepared in Acetone, Chloroform, Ethanol, Ethyl acetate and Methanol. The antibacterial activity was carried out by the agar well diffusion method. Ethanol extracts showed effective inhibition against all the bacterial pathogens used. But the highest activity (18mm) was observed by the extract of *Caulerpa sertularioides* against *Proteus mirabilis* by using Ethyl acetate as a solvent. The lowest activity was reported for the ethyl acetate extracted *Kappaphycus alvarezii* (3mm) against bacterium *E. coli*. The entire six pathogens were resistant to the chloroform extracts of *Caulerpa sertularioides* and *Kappaphycus alvarezii*.

Key Words: Antibacterial screening, Agar well diffusion method, *Padina tetrastromatica*, *Caulerpa sertularioides*, *Kappaphycus alvarezii*.



INTRODUCTION

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 [1]. They are abundant on hard substrates and commonly extending to depths of 30-40 m. About 624 species have been reported in India with a potential of 77,000 tons (wet weight) per annum. The red seaweeds contribute 27.0%, brown 0.2 % and others 72.8 %. About 206 species of algae have been reported from the mangrove environment. Seaweeds are the only source of phytochemicals namely agaragar,

carrageenan and algin, which are extensively used in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents. They are also used for human consumption, animal feed and as manure in several countries. The greatest use of agar is in association with food preparation and in the pharmaceutical industry as a laxative or as an outer cover of capsules. Being rich in minerals, vitamins, trace elements and bioactive substances, seaweeds are called medical food of the 21st century. Marine environment is unique with respect to its biological and chemical diversities and represents a source of novel antimicrobial compounds. In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimutagenic activities [2,3]. Most of the compounds of marine algae show anti-bacterial activities. Many metabolites isolated from marine algae have been shown to possess bioactive efforts [4].

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Marine environment is unique with respect to its biological and chemical diversities and represents a source of novel antimicrobial compounds [5]. Traditionally, seaweeds have been used in the treatment of various infectious diseases. Many essential substances obtained from seaweeds have been used for decades in medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties [6,7,8]. Seaweeds excessively contain several bioactive compounds possessing that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimutagenic activities [9,10,11].

Numerous bioactive compounds of marine algae with antimicrobial activity have been isolated. Some important compounds are sterols, terpenoids, polysaccharides, peptides, proteins, vitamins, acrylic acid, terpenes, chlorophyllides, phenols, heterocyclic compounds, halo-generated ketones and alkanes and cyclic polysulphides, moreover some of them are under investigation to protect life-style related diseases [11,12,13]. The aim of the present study was to determine the antibacterial activity of the selected three seaweeds *Padina tetraströmatica*, *Caulerpa sertularioides* and *Kappaphycus alvarezii* against six pathogenic bacteria.

MATERIALS AND METHODS

The seaweeds of *Padina tetraströmatica*, *Caulerpa sertularioides* and *Kappaphycus alvarezii* were collected from Manapad, Thoothukudi, Tamil nadu (India). The collected samples were thoroughly cleaned with seawater and then fresh water followed by distilled water to remove all the extraneous matter such as epiphytes, suspended matter and sand particles. The material was shade dried and cut into small pieces to form powder. 20 grams of powdered sample were extracted by soxhlet apparatus using 250 ml of acetone, chloroform, ethanol, ethyl acetate and methanol as solvents for 8h at 60°C. The extract was filtered by using Whatman no.1 filter paper and kept it under Hot air oven (40°C) for the solvent evaporation, and then 10 mg of extract was diluted with 10 ml of above mentioned solvents for further study. The antibacterial activity was done by agar well diffusion method [14]. The bacterial cultures (*Bacillus subtilis*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) were inoculated on the Mueller Hinton agar (Hi Media Laboratories Limited, Mumbai, India) medium and wells of six mm were prepared with the help of a sterile cork borer. Each well was filled

with 0.1 ml concentration of extracts. The solvents were used as a negative control. The plates were incubated at 37°C for 24 hours and diameter of zone of inhibition around the well was measured and recorded in each plate by the average of triplicates.

RESULTS AND DISCUSSION

Seaweeds are primitive non flowering plants without root, stem and leaves. They contain different vitamins, minerals, trace elements, protein, iodine, bromine and bioactive substances. Infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem nowadays. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and emerging infectious diseases [15]. 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, experimental methods etc. But variation in antibacterial activity may be due to the method of extraction and solvent used in extraction [16].

The antibacterial activity of *Padina tetraströmatica*, *Caulerpa sertularioides* and *Kappaphycus alvarezii* were tabulated in table 1. Antibacterial activities of the three species prepared in different solvents were under investigation. In *Caulerpa sertularioides* highest inhibition zone of 18 mm was noticed for ethyl acetate extract against *Proteus mirabilis*, and lowest inhibition 6 mm were recorded against *E. coli* (acetone and methanol extract, respectively) and *Proteus mirabilis* (acetone extract). Followed by *Padina tetraströmatica* shows the maximum inhibition of 15 mm was observed by the same ethyl acetate extract against *S. aureus* and the minimum zone 6 mm for acetone extract against *E. coli* and *P. aeruginosa*, and chloroform extract against *B. subtilis* and *S. aureus* respectively. In *Kappaphycus alvarezii* extract obtained by ethanol showed the maximum inhibition 13 mm against *B. subtilis* and minimum activity 3 mm against *E. coli* of ethyl acetate extract. But the same time entire six pathogens were resistant against *Caulerpa sertularioides* and *Kappaphycus alvarezii* extracted by chloroform. Variations occurred each one of the experimental results of antibacterial activity may be due to the different bioactive substances and their concentration present in the respective extract. These bacterial strains may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the

compound used may not be sufficient [17]. Previous reports have shown that gram positive bacteria were effectively controlled by the marine algal extracts as compared to gram negative bacteria [18,19]. In this present study, it was observed that the three selected seaweeds extracted by ethanol showed significant activity against all the test pathogen. It is also necessary to the separation and purification of the biological active compounds for develops new drugs to control the deadly diseases. This research finding gives further

scope to screen the chemical constituents of the extracts which will be very useful to combat the various diseases caused by pathogenic bacteria.

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Table 1: Antibacterial activity of *Padina tetrastromatica*, *Caulerpa sertularioides* and *Kappaphycus alvarezii*

Name of the pathogens	Diameter zone of inhibition in mm														
	Acetone			Chloroform			Ethanol			Ethyl acetate			Methanol		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
<i>Bacillus subtilis</i>	8	7	9	6	–	–	9	9	13	14	9	–	7	–	4
<i>Staphylococcus aureus</i>	7	7	10	6	–	–	10	11	6	15	13	7	–	8	9
<i>Lactobacillus acidophilus</i>	8	–	–	7	–	–	9	7	9	12	14	–	9	10	10
<i>Escherichia coli</i>	6	6	11	–	–	–	11	9	10	8	10	3	11	6	8
<i>Pseudomonas aeruginosa</i>	6	–	7	–	–	–	8	9	10	12	11	–	10	7	8
<i>Proteus mirabilis</i>	9	6	–	–	–	–	12	12	9	10	18	12	–	9	10

A. *Padina tetrastromatica* B. *Caulerpa sertularioides* C. *Kappaphycus alvarezii*

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