

Antibacterial activity of *Helimedea macroloba* extract against infectious pathogens Decne,1841

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Abstract

The green seaweed *Helimedea macroloba* Decne,1841 was collected from Gulf of Mannar, Tuticorin coast. In this study, the selected seaweed were tested for probable antimicrobial activity through agar well diffusion method and the extracts were prepared by five different solvents viz., Acetone, Chloroform, Ethanol, Ethyl acetate and methanol; screened against six infectious pathogens (Gram +ve: *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus* and Gram -ve: *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*). The experimental results shows that the highest antibacterial activity 16mm (100µl) was shown by Ethyl acetate extract against *Proteus mirabilis* and the lowest activity were seen in Chloroform extract 4mm (100µl) against *Pseudomonas aeruginosa*. The experiment concludes that Ethanol as a solvent only shows good activity against all the tested pathogens.

Keywords: Seaweed, *Helimedea macroloba*, Antimicrobial activity, Agar well diffusion method

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1. Introduction

Seaweeds are rich and varied source of bioactive natural products and have been traditionally used in human and animal nutrition. Seaweeds can be classified into three broad groups based on pigmentation: brown, red and green. Botanists refer to these broad groups as Phaeophyceae, Rhodophyceae and Chlorophyceae, respectively. Important polysaccharides such as agar, alginates and carrageenans are from seaweeds used in pharmaceutical as well as in the food industries (Bocanegra *et al.*, 2009). They also contain sterols, terpenoids, polysaccharides, peptides, proteins, vitamins, acrylic acid, terpenes, chlorophyllides, phenols, heterocyclic compounds, halo-genated ketones and alkanes and cyclic polysulphides, moreover some of them are under investigation to protect life-style related diseases (Taskin *et al.*, 2007 and Priyadharshini *et al.*, 2011). 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 antimitotic activities (Bhosale *et al.*, 2002 and Okai *et al.*, 1997). Recently, infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem. 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 reemerging infectious diseases (Westh *et al.*, 2004). The use of anti microbial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produce. These limitations demand for improved pharmacokinetic properties which necessitates continued research for the search of new antimicrobial compounds for the development of drugs (Kayalvizhi *et al.*, 2012). As a consequence of an increasing demand for biodiversity in the screening programs seeking therapeutic drugs from natural product, there is now a greater interest in marine organism, especially algae (Ganthikumar *et al.*, 2012). Hence the present study the antimicrobial activity of seaweed of *Helimeda macroloba* using different solvents was investigated.

2. Materials and Methods

2.1 Sample collection

The seaweed *Helimeda macroloba* Decne, 1841 was collected from collected from Gulf of Mannar Tuticorin coast. 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 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.

2.2 Test organisms

Extracts were tested against six bacterial stains (Gram +ve: *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus* and Gram -ve: *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*). The test pathogens were obtained from the Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.

2.3 Preparation of seaweed extract

The powdered sample (5g) was extracted in soxhlet apparatus using acetone, chloroform, ethanol, ethyl acetate and methanol (250 ml) as solvents for 8h at 60°C. The extracts were filtered using Whatman No.1 filter paper and kept it under Hot air oven (40°C) for the solvent evaporation. The residues obtained were stored in a freezer at -20°C.

2.4 Antimicrobial activity

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 infectious pathogens at different concentration levels like 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml. The 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

Table-1: The antibacterial activity of the selected seaweed *Helimeda macroloba*

Test samples	Concentration (µg/ml)	Zone of inhibition in mm					
		S.a	B.s	L.a	P.a	E.c	P.m
Acetone extract	40	—	—	—	—	—	—
	60	—	—	—	—	—	—
	80	—	—	7	—	—	—
	100	—	—	8	8	—	6
Chloroform extract	40	—	—	—	—	—	—
	60	—	—	—	—	—	—
	80	—	—	—	—	—	—
	100	—	—	7	4	—	—
Ethanol extract	40	—	—	—	—	—	—
	60	—	—	7	—	—	—
	80	7	—	9	6	8	—
	100	9	8	12	9	11	10
Ethyl acetate extract	40	—	—	7	—	—	—
	60	—	—	8	—	—	—
	80	—	8	9	—	—	13
	100	—	10	10	—	—	16
Methanol extract	40	—	—	—	—	—	—
	60	—	—	—	—	—	—
	80	—	—	—	—	—	—
	100	7	—	8	—	—	—

Gram +ve: *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus*

Gram -ve: *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis*.

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.

3. Results and Discussion

The antibacterial activity of the selected seaweed *Helimeda macroloba* was extracted by five different solvents represented in the Table 1. The highest activity (7mm) in low concentration (40µg/ml) was seen in ethyl acetate as a solvent against *L. acidophilus*. The same concentration there were no activity recorded. In 60µg/ml concentration the highest inhibition shown by ethyl acetate (8mm) and lowest inhibition (7mm) against *L. acidophilus*. The remaining five organisms

were also resistant to the same concentration. The maximum activity (13mm) at 80µg/ml concentration level when ethyl acetate used as a solvent against *Proteus mirabilis*, followed by the minimum activity (6mm) recorded in ethanol extract against *P. aeruginosa*. In 100µg/ml concentration have been shows the maximum activities, especially ethanol solvent forms good activity against all the tested pathogens. But the highest inhibition zone 16mm of ethyl acetate extract against *Proteus mirabilis* and the lowest inhibition zone 4mm of chloroform extract against *P. aeruginosa* were recorded. From the present study we concluded that the extracts of *Helimeda macroloba* have high antibacterial activity.

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