



## **ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF FRESH WATER AND MARINE ALGAL EXTRACTS**

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### **Abstract:**

*Antimicrobial resistance (AMR) is the resistance of a microbe to an antimicrobial medication that is used to be effective in treating or preventing an infection caused by microbes. Aquatic environment is known to have a rich diversity of living organisms because of high competition for survival, particularly sessile aquatic organisms which lack physical means of defense, hence they produced a variety of bioactive and toxic compounds as their defense strategy. Algae have not been adequately explored for their potential as a source of bioactive substances. The present study, deals with bactericidal activity of fresh water and marine algae from Kanchipuram and Covelong beach, respectively. In vitro screening of antibacterial activity (methanolic extracts) of both fresh water and the marine water (green and red) algae were performed against Gram positive and Gram negative bacteria by well diffusion method. The zone of inhibition was measured for the crude methanolic extracts at various concentrations of 30 $\mu$ L, 60 $\mu$ L, 90 $\mu$ L and 120 $\mu$ L, respectively. The above results gave an indication of the presence of promising antimicrobial compounds in the algal species under studied. Further phytochemical studies are needed to elucidate these compounds structures and activity for use of algae as an alternative natural antibiotic against synthetic conventional antibiotics.*

**Key Words:** Algae, Antimicrobial Activity, Well Diffusion, Zone of Inhibition & Phytochemicals

### **Introduction:**

Algae are a large and diverse group of chlorophyll bearing, simple, photosynthetic, thalloid organisms largely aquatic with no differentiation of true roots, stems and leaves, belonging to the division Thallophyta (NCERT 2008). There is an increasing demand for therapeutic drugs from the vastly bio diverse natural resources (1). To survive in a competitive environment, freshwater and marine algae have developed defense strategies that result in a significant level of structural- chemical diversity, from different metabolic pathways (2, 3). A lot of active substances with antibacterial, antiviral, fungicide, enzyme inhibiting, immunosuppressive, cytotoxic and algicide activity has been isolated from algal biomass [4, 5]. The potential contribution of marine organisms to the discovery of new bioactive molecules is remarkably increasing [6]. Marine organisms are potential sources of bioactive secondary metabolites with potential for use in the development of new pharmaceutical agents and many of these substances have been demonstrated to possess interesting biological activities (7, 8). The aim of this study was to assess the antibacterial effect of aqueous and the marine methanolic crude extracts of algae against the pathogenic bacteria (Gram positive and Gram negative).

### **Materials and Methods:**

#### **Algal Collection**

Green algae (fresh water) and Red algae (marine) were collected from Kanchipuram and Covelong Beach respectively. The algal samples after collection were thoroughly washed to remove any associated debris and then gently air dried.

### **Bacterial Culture:**

Five bacterial strains (Gram positive and negative) were selected for the study. The Gram positive *Staphylococcus aureus*, and the Gram negative *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, were obtained from MTTC, IMTECH, Chandigarh, India.

### **Preparation of Algae:**

25 gms of fresh air dried algae were crushed with 50mL of methanol using motor and pestle (9). The solution was filtered through Whatman No. 1 sterile filter paper. Resulting methanolic extracts were evaporated to dryness using Rotary evaporator (Heidolph 300 LabroRota, Germany), while filtrates of aqueous extracts were dried using freeze dryer. Each dried precipitate was re-dissolved in the corresponding solvents to give 50 mg/ml extracts, and then stored at -20°C until tested

### **Determination of Minimum Inhibitory Concentration (MIC):**

Antimicrobial activity was evaluated using the agar diffusion technique in petri dishes. Briefly, 30 µl, 60 µl, 90 µl and 120 µl of each extract was loaded into the well (10, 11). Indicator microorganisms were spread on Mueller-Hinton agar plates with sterile buds and the wells were loaded with the different microliters of the extracts. After incubation for 24 h at 30 °C, a clear zone around a disc was evidence of antimicrobial activity. Diameters of the zones of inhibition were measured in millimeters. Each test was prepared in duplicate. The MICs were determined by the agar dilution method adopted by Daud and Sanchaz (2005).

### **Result and Discussion:**

Fresh water algae and the marine water algae are found to be wide spread and they have been found to have antimicrobial activity. Freshwater green algae and the marine red algae were subjected for the antimicrobial study with both the Gram positive and the Gram negative micro organisms using well diffusion method are shown in Table 1 and 2. The extracts were pipetted out on sterile condition in their respective wells with negative control in the centre. The fresh water green algal extract recorded the maximum inhibitory zone with *Staphylococcus aureus* at 120µL concentration of 30mm diameter zone of inhibition followed by 24 mm with *Salmonella typhi*. The third limit of zone (22mm) was registered by *Pseudomonas aeruginosa* followed by *Escherichia coli* with 20mm and the last zone of inhibition (6mm) was registered by *Klebsiella pneumoniae* during the period of study. The marine red algae subjected for the antimicrobial studies similar to that of the fresh water green algae, comparatively showed less inhibitory effective compared to the fresh water green algae. The red algal extract recorded the maximum inhibitory zone with *Klebsiella pneumoniae* at 120µL concentration of 25mm diameter zone of inhibition followed by 18 mm with *Pseudomonas aeruginosa*. The third limit of zone (17mm) was registered by *Staphylococcus aureus* followed by *Escherichia coli* with 15mm and the last zone of inhibition (10mm) was registered by *Salmonella typhi* during the period of study. The extracts showed significant antibacterial activity against Gram negative as well as Gram positive bacteria that have few similarities of the previous findings (12, 13, 14). Overall, antimicrobial activity of the extracts was higher in the green algae. In most cases, algal extracts showed profoundly distinct antibacterial activity by having observable inhibition with diameters ranging from 02mm to 30mm on the tested bacteria. A remarkable effect was obtained with fresh water green algae. The nutrient composition of the seaweed varies and is affected by species, geographic areas, season of the year and temperature of the water (15, 16).

**Conclusion:**

In the present investigation, our results indicate that the algal soecies collected in the current study from the Kanchipuram and Covelong beach represents the rich source of valuable medicinal compounds and their extracts exhibit a significant capacity of antibacterial activities. Further studies will be undertaken to characterize the active compounds residing in these types of algae.

Table 1: Antimicrobial Studies of Fresh Water Green Algae

Concentration/ Organisms	Zone of inhibition (cm)				
	<i>S. aureus</i>	<i>S. typhi</i>	<i>P.aeruginosa</i>	<i>K. pneumoniae</i>	<i>E.coli</i>
30µl	1.4	1.4	1.6	-	0.4
60µl	2.0	1.5	1.7	-	1.6
90µl	2.4	2.0	1.8	0.2	1.8
120µl	3.0	2.4	2.2	0.6	2.0

Table 2: Antimicrobial Studies of Marine Red Algae

Concentration/ Organisms	Zone of inhibition (cm)				
	<i>S. aureus</i>	<i>S. typhi</i>	<i>P.aeruginosa</i>	<i>K. pneumoniae</i>	<i>E.coli</i>
30µl	0.4	-	-	0.7	-
60µl	0.7	-	0.4	1.3	0.6
90µl	1.1	0.3	1.7	2.0	1.0
120µl	1.7	1.0	1.8	2.5	1.5

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