

## Antibacterial Properties of *Dictyota bartayresiana* from the Gulf of Mannar - Southeast Coast of India

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**Abstract:** The antibacterial properties of chloroform, ethanol, methanol and water extracts of marine algae *Dictyota bartayresiana* J.V.Lamouroux (Phaeophyta) from mandapam coastal region of Gulf of Mannar southeast coast of India were tested against Gram - positive strains such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and Gram-negative strains *Proteus mirabilis*, *Klebsiella pneumoniae* and *Salmonella typhi*. The preliminary phytochemical analysis of extract from *Dictyota bartayresiana* showed the presence of biologically active compounds namely, alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides and tannins. Results demonstrated that methanolic extract of *Dictyota bartayresiana* exhibited antibacterial activity against both Gram- positive and Gram-negative bacteria. It was observed that the extract of *Dictyota bartayresiana* recorded maximum activity against *Staphylococcus aureus*.

**Key words:** *Dictyota bartayresiana* • Antimicrobial activity • Crude extracts

### INTRODUCTION

*Dictyota bartayresiana* J.V.Lamouroux (Class: Phaeophyceae, Order: Dictyotales, Family: Dictyotaceae) is an abundantly growing brown seaweed in coastal seashore of South India. Marine macro algae are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents [1]. In recent years, there are several reports of macro algae derived compounds that have a wide range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti inflammatory, antitumor, cytotoxic and antidiabetic [2]. At present seaweeds constitute commercially important marine renewable resources which are providing valuable thoughts for the development of new drugs against cancer, microbial infections and inflammations [3]. Marine algae have been screened broadly to isolate life saving drugs or biologically active substances all over the world [4].

Bacterial infection cause high rate of mortality in human population and aquaculture organisms. *E. coli* and *Staphylococcus aureus* cause disease like mastitis, abortion and upper respiratory complications, while

*Salmonella* sp. causes diarrhea and typhoid fever [5, 6]. Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Now days, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs [7].

Marine algae use targeted antimicrobial chemical defense strategies by eliciting secondary metabolites, which are important in ecological interactions between marine macro organisms and microorganisms [8]. The extracts of many species of marine algae inhibit the growth of Gram-positive and Gram-negative bacteria [9]. Various levels of antimicrobial activity were due to different solubility behavior of secondary metabolites which were influenced by season and geographical distribution of the species [10]. In the several species, different substances have been found in the same algae. Study on antimicrobial activity of seaweed has been reported wide in India [11].

Even though marine algae from many coastal regions of India were investigated for antimicrobial activity, majority of marine algae from Gulf of Mannar, south east coast of Tamilnadu, India were left unexplored for bioactive substances. In the present study, we

describe the antibacterial characteristics of chloroform, ethanol, methanol and water extracts of marine algae *Dictyota bartayresiana* obtained from the coast of Gulf of Mannar.

## MATERIALS AND METHODS

**Sample Collection:** In the present study, *Dictyota bartayresiana* J.V.Lamouroux macro algae was collected from mandapam coastal region (78°8'E, 9°17'N), in Gulf of Mannar, Tamilnadu South India on low tide during December 2009 and immediately brought to the laboratory in polythene bags and washed several times with seawater to remove sand, mud and attached fauna. The alga was cleaned using brush for the removal of the epiphytes with distilled water. After cleaning, algae were dried in shade at room temperature for one week. The dried algae materials were homogenized to fine powder and further subjected to extraction.

**Crude Extraction:** 500g of powdered algae sample were taken and extracted successively with different solvents in the order of their polarity chloroform, ethanol, methanol and water using soxhlet apparatus. The crude extracts were later concentrated under reduced pressure to get their corresponding residues. The algae extracts were further subjected for antimicrobial activity by agar well diffusion method.

**Microorganism Tested:** The following strains of bacteria were used: *Staphylococcus aureus* (MTCC No. 121), *Bacillus cereus* (MTCC492), *Escherichia coli* (MTCC No. 443), *Klebsiella pneumonia* (MTCC No.530), *Proteus mirabilis* (MTCC No.425) and *Salmonella typhi* (MTCC No.531) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on nutrient agar (Hi Media, India) slants at 4°C and were sub-cultured before use. The bacteria studied are clinically important ones causing several infections and it is essential to overcome them through some active therapeutic agents.

**Phytochemical Analysis:** All the extracts of *Dictyota bartayresiana* seaweed was subjected to Phytochemical analyses for the identification of various phytochemical constituents as per the standard method of Harborne [12].

**Antibacterial Assay:** In the present study, the antibacterial activity of the *Dictyota bartayresiana* was

studied by the Agar cup plate diffusion method [13]. The chloroform, ethanol, methanol and water extracts of the collected test samples were tested in three dose levels of 100, 300 and 500mg/ml. The nutrient agar medium prepared was inoculated with 18 hours old cultures of the above mentioned test organisms and were transferred into sterile 15cm diameter Petri dishes. The medium in the plates were allowed to set at room temperature for about 10 minutes and allowed to solidify in a refrigerator for about 30 minutes. 5 cups of 6mm diameter were made in each plate at equal distance. Stock solutions of the test residual extract were prepared in concentrations of 100mg/ml, 300 and 500mg/ml. 100µg/ml of each concentration were placed in the well with sterile pipettes. In each plate one well was used for control and standard. Antibiotic Chloramphenicol (100µg/ml) was used as standard and respective solvents were used as control. The Petri dishes thus prepared were incubated for 16hrs at 30°C and were later examined by measuring the zones of inhibition with the zonal scale and the results were tabulated

## RESULTS

The results of the Phytochemical screening of *Dictyota bartayresiana* extracts, confirmed the presence of cardiac glycosides, tannins, alkaloids, steroids, anthraquinones, saponins and flavonoids (data not shown).

Four different extracts of *Dictyota bartayresiana* were tested for their anti-bacterial activity against strains of Gram positive, Gram negative bacteria using agar well diffusion method. The result of antimicrobial activity against tested pathogens was tabulated in the Table 1. The crude extracts of *Dictyota bartayresiana* in different solvents exhibited diverse antibacterial activities. The methanol extract of *Dictyota bartayresiana* was most active against all tested pathogens followed by chloroform and ethanol extracts and minimum with water extracts. *Staphylococcus aureus* growth was highly inhibited (21mm at 100mg/ml, 24mm at 300mg/ml and 28mm at 500mg/ml) by the methanolic extracts of *Dictyota bartayresiana* when compared with other extracts. The antimicrobial activity of *Dictyota bartayresiana* was absorbed to be in dose dependent manner used for all four extracts. Highest antimicrobial activity was exhibited in 500 than 300 and 100mg/ml dose level against all the tested pathogens.

Table 1: Antibacterial activity of crude extracts of *Dictyota bartayresiana* J.V.Lamouroux

Test samples	Concentration Mg/ml	Pathogens used and Inhibition Zone (mm)					
		Gram positive organism			Gram negative organism		
		<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacillus cereus</i>	<i>Salmonella typhi</i>	<i>Proteus mirabilis</i>	<i>Klebsiella pneumonia</i>
Chloroform extract	100	24	2	15	12	7	13
	300	25	4	16	14	8	15
	500	28	5	17	16	11	17
Ethanol extract	100	18	7	17	17	6	10
	300	20	9	18	19	8	12
	500	22	10	21	20	10	14
Methanol extract	100	9	2	19	19	11	12
	300	11	4	21	20	13	15
	500	12	6	23	21	14	19
Water extract	100	7	1	5	7	8	13
	300	8	3	6	8	9	14
	500	9	4	7	9	11	16
Standard	25	22	25	23	21	20	

\*Values include cup borer diameter (6mm) and are mean of three replicates

## DISCUSSION

Marine algae synthesize active constituents which are used in conventional and complementary medicine. Different varieties of marine algae were reported to contain active ingredients that can cure diseases. Currently, large proportion of population prefers to use remedies of natural origin for curing illness as these claimed to produce less side effects [14]. The present study was focused on *Dictyota bartayresiana* for the presence of phytochemical constituents and antibacterial activity against Gram-positive and Gram-negative bacteria.

The evolution of antibiotic-resistant pathogenic bacterium has stimulated the search for alternative antimicrobial agents from alternative sources including sources from the ocean. The powers of marine macro algae have been realized for thousands of years and its potential as producers of pharmaceutical products have been reviewed [15].

The results of the present study clearly showed that marine algae extracts showed antibacterial activity against tested pathogenic bacterial strains including antibiotic resistant strains. The effectiveness of the active compounds present in methanolic extracts of *Dictyota bartayresiana* causes the production of growth inhibition zones that appear as clear areas surrounding the wells. Antibacterial activity may be due to active components which are present in the extracts. Among tested pathogens, the methanol extracts of *Dictyota*

*bartayresiana* showed maximum antibacterial activity against Gram-positive bacteria especially *Staphylococcus aureus*, while chloroform extraction and ethanol extraction of *Dictyota bartayresiana* showed moderate activity of all Gram-positive and Gram-negative bacteria. Water extracts of *Dictyota bartayresiana* was showed mild activity against the Gram-positive and Gram-negative pathogens. A dose dependent increase in the antimicrobial activity was observed in all the extracts of *Dictyota bartayresiana* against the tested pathogens. The present study revealed that gram-positive organisms were more susceptible to the crude extracts of *Dictyota bartayresiana* used.

Gram-positive bacteria were more effectively controlled by the extracts of algae used than Gram-negative bacteria [16]. Similar observations indicating that the more susceptibility of Gram-positive bacteria to the algal extracts is due to the differences in their cell wall structure and their composition [17]. The principle strength of the active compounds depends on the use of a suitable solvent to extract it [18].

Hornesy and Hide [19] reported that 151 species of marine algal crude extracts showed inhibitory activity against pathogenic bacteria. But variation in antibacterial activity may be due to the method of extraction, solvent used in extraction and season at which samples were collected. Previous reports on red algae exhibited high antibacterial activity [20, 21]; in contrast, brown algae (phaeophyceae), *Dictyota bartayresiana* were effective.

Results from this study revealed that the crude methanol extract of *Dictyota bartayresiana* contain certain constituents with significant antibacterial property which enables the extract to overcome the barrier in Gram-negative cell wall [13]. In addition, these results form an absolute basis for selection of the plant for additional phytochemical and pharmacological investigation.

*Dictyota bartayresiana* is currently undergoing detailed investigations with objectives of isolating biologically active molecules along with the search for novel antimicrobial agents.

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