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Antibacterial, cytotoxic and larvicidal potential of *Dictyota bartayresiana* Lamour

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ABSTRACT

Objective: To reveal the antibacterial, cytotoxic and larvicidal activity of *Dictyota bartayresiana* Lamour (*D. bartayresiana*).
Methods: Phytochemical analysis of various extracts of *D. bartayresiana* was performed. The antibacterial activity of *D. bartayresiana* was evaluated by agar diffusion method. The larvicidal activity of crude extract was evaluated as per the protocol described by World Health Organization. The evaluation of LC_{50} value was carried out after 24 h by probit analysis. Cytotoxicity test was carried out using the standard procedure. The LC_{50} and LC_{90} values were obtained from the best-fit line plotted concentration verses percentage lethality.
Results: Phytochemical analysis of various extracts of *D. bartayresiana* showed the presence of steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and amino acids. It was observed that the methanolic extracts of *D. bartayresiana* expressed maximum zone of inhibition against *Escherichia coli* [(9.4 ± 0.2) mm]. Crude methanolic extracts of *D. bartayresiana* showed the highest larval mortality (LC_{50} = 166.33 mg/L and LC_{90} = 265.69 mg/L) against *Culex quinquefasciatus*. Similarly, methanolic extracts of *D. bartayresiana* displayed the highest cytotoxicity with LC_{50} and LC_{90} values at 202.63 and 354.24 mg/L respectively against *Artemia salina*.
Conclusions: The present results revealed the biopotentials of *D. bartayresiana* and further investigations are needed to elucidate the active principle.

1. Introduction

Seaweeds are one of the important marine living resources and are an excellent source of vitamins (A, B, B_{12}, C, D and E), riboflavin, niacin, panthothanic acid and folic acid as well as minerals such as Ca, P, Na and K[1]. Marine organisms are emerging as good candidates of an alternate source for bioactive substances. Chemically the bioactive metabolites of marine flora include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sterols and terpenoids[2]. Marine organisms often produce antibacterial and anticancer substances as a means of maintaining relationships between epiphytic micro environments, inhibiting competing organisms and microbial pathogens[3]. There is a spurt in interest on natural compounds obtained from plants or seaweeds to investigate about their medicinal properties. The antitumor activity is one of the most important activities in marine drugs and lots of algae and their metabolites have showed potent cytotoxicity. These metabolites have played an important role in leading to new pharmaceutical compounds from algae for antitumor drugs. The brine shrimp lethality bioassay has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine shrimp. The bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity[4]. This *in vivo* lethality test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays once the active compound has been isolated[5].

Seaweeds are important natural alternatives to insecticides, as phytochemicals extracted from the whole plant using different

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solvents may act against mosquitoes as toxicant, growth regulators, repellents and ovipositional deterrent[6]. Mosquitoes being vector for many tropical and subtropical diseases act as the most important single group of insects well-known for public health importance. Despite progress in vaccine development, no effective and acceptable multivalent vaccines are currently available against mosquito-borne diseases[7]. The approach to combat these diseases largely relies on interruption of the disease transmission cycle by either destruction of the aquatic stages or by killing the adult mosquitoes using chemical insecticides[8]. The drastic effects of chemical insecticides for the control of disease vectors have received wide public apprehension and have caused many problems like insecticide resistance, resurgence of pest species, environmental pollution, toxic hazards to humans and other non-target organisms[9]. To alleviate these problems, major emphasis has been put on the use of natural plant based products as larvicides which can provide an alternate to synthetic insecticides[10].

Brown algae *Dictyota bartayresiana* Lamour (*D. bartayresiana*) of the family Phaeophyceae are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with diverse structural features[11]. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown algae *D. bartayresiana*[12]. Salvador *et al*. studied the antibacterial and antifungal activity of 82 marine macroalgae (18 of Chlorophyceae, 25 of Phaeophyceae and 39 of Rhodophyceae) to evaluate their potential for being used as natural preservatives in the cosmetic industry[13]. Ayesha *et al*. screened the *in vitro* cytotoxicity of seaweeds *viz.*, *Dictyota dichotoma* var. *velutricata*, *Dictyota hauckiana, Dictyota indica, Iyengaria stellata, Jolyna laminarioides, Melanothamnus afaqhusainii, Sargassum ilicifolium, Sargassum lanceolatum* and *Ulva fasciata* from Karachi coast on brine shrimp[14]. Khanavi *et al*. evaluated the larvicidal activity of native marine algae against main malarial vector *Anopheles stephensi*[15]. Antonisamy *et al*. explored the phytochemical constituents, UV-vis and high performance liquid chromatography spectrum profile for *D. bartayresiana*[16]. To continue the good work done in our laboratory, the present investigation was aimed to explore the antibacterial, cytotoxic and larvicidal potential of *D. bartayresiana*.

2. Materials and methods

2.1. Collection of marine algae

The specimens of *D. bartayresiana* was collected from Rasthacaud coastal waters, Kanyakumari District, Tamil Nadu, India. The collected samples were placed in plastic bags, brought to the laboratory and washed thoroughly to remove extraneous materials. The seaweeds were blotted using blotting paper and shade dried. They were then ground into fine powder using tissue blender. The powdered samples were then stored for further analysis.

2.2. Extraction of crude compounds

The powdered materials (50 g) were extracted successively with 300 mL of petroleum ether, chloroform, acetone, ethyl acetate, ethanol and distilled water in Soxhlet apparatus for 8 h. The extracts were filtered using Whatman filter paper (No. 1) and then evaporated in a rotary vacuum evaporator to yield a dark greenish mass.

2.3. Agar diffusion method

The following bacterial strains *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Shigella flexneri* (*S. flexneri*) and *Morganella morganii* (*M. morganii*) were used for the current study. The antibacterial activity of *D. bartayresiana* was evaluated by agar diffusion method[17]. The plates inoculated with different concentrations of *D. bartayresiana* extracts ranging from 25 to 100 μg/mL were used for assessment of antibacterial activity and incubated for 24-28 h at 35 ˚C. The tetracyclin (30 μg) served as positive control and the solvents (petroleum ether, acetone, chloroform and methanol) were maintained as negative control.

2.4. Larvicidal bioassay

Culex quinquefasciatus (IV instar) were collected in and around Tirunelveli District (sewage), Tamil Nadu, India, with the help of 'O' type brush. The larvae were fed on dog biscuits and yeast power in the 3:1 ratio. Mosquitoes were held at 28 ˚C, 70%-85% relative humidity, with a photo period of 14 h light, 10 h dark. The larvicidal activity of crude extract was evaluated as per the protocol described by World Health Organization. Later, fourth instar larvae (25) were placed in 249 mL of distilled water and 1 mL of methanol containing desired plant extracts at concentrations of 50, 100, 150, 200 and 250 mg/L. The beaker containing the control larvae received 1 mL of methanol. Each test was repeated for five times. The LC_{50} value was carried out after 24 h by probit analysis[18,19].

2.5. Cytotoxicity test

Cytotoxicity test was carried out using the standard procedure as described by McLaughlin[20]. Samples were prepared by dissolving in 3 mL of dimethyl sulfoxide (DMSO). From this solution, the concentrations of 50, 100, 250 and 500 mg/L were prepared by serial dilution. Each concentration was tested for cytotoxicity in triplicates. DMSO was used as negative control. Brine shrimp eggs (*Artemia salina* Leach) were hatched in a tray, filled with artificial sea water. Ten larvae of brine shrimps were transferred to each sample test-tube using disposable pipettes. The test-tubes were maintained under illumination.

Survivors were counted after 24 h and the percentage death at each concentration was determined[20]. The LC_{50} and LC_{90} values were obtained from the best-fit line plotted concentration verses percentage lethality.

3. Results

3.1. Antibacterial screening

D. bartayresiana crude extracts possessed antibacterial activities with varied frequency against the tested four pathogens (Table 1). *D. bartayresiana* methanolic extract at 100 µg/mL exhibited the highest zone of inhibition against *E. coli* [(9.4 ± 0.2) mm] followed by *D. bartayresiana* chloroform extract at 100 µg/mL [(8.6 ± 0.2) mm] against *E. coli*. *D. bartayresiana* methanolic extract at 100 µg/mL displayed maxmium percentage (75%) of efficacy (showing the antibacterial activity against three pathogens out of four pathogens) compared to other tested extracts with the maximum zone of inhibition against *E. coli* [(9.4 ± 0.2) mm], *S. flexneri* [(8.3 ± 0.4) mm] and *P. aeruginosa* [(8.7 ± 0.2) mm]. Similarly, petroleum ether extracts of *D. bartayresiana* exhibited maximum zone of inhibition against *M. morganii* [(9.3 ± 0.3) mm]. Aqueous extracts of *D. bartayresiana* failed to show inhibition against the tested pathogens.

3.2. Larvicidal bioassay

Methanolic extracts of *D. bartayresiana* showed the highest larvicidal activity with LC_{50} value of 166.33 mg/L and LC_{90} value of 265.69 mg/L against the fourth instar larvae *Culex quinquefasciatus* (Table 2).

3.3. In vivo lethality test

The *in vivo* lethality test on a simple zoological organism, such as brine shrimp nauplii (*Artemia salina*), has been used as a convenient tool for screening of bioactive natural products. Methanolic extract of *D. bartayresiana* showed different mortality rate of brine shrimp which increased proportionally with the increasing concentration of the extract. The inhibitory effect of the crude extracts might be due to the presence of the toxic compounds. The methanolic extract was found to be the most effective with LC_{50} and LC_{90} values of 202.63 and 354.249 mg/L respectively (Table 3). A negative control DMSO was used to validate the test method.

Table 2
Larvicidal activity of methanolic extracts of *D. bartayresiana*.

Concentration of extracts (mg/L)	Mortality (%)	LC_{50} (mg/L)	LCL	UCL	LC_{90} (mg/L)	χ^2 (*df*)
50	4					
100	10					
150	19	166.33	152.369	181.204	265.69	0.605
200	33					
250	44					

LCL: Lower confidence limit; UCL: Upper confidence limit.

Table 3
Cytotoxic activity of methanolic extracts of *D. bartayresiana*.

Concentration of extracts (mg/L)	Mortality (%)	LC_{50} (mg/L)	LCL	UCL	LC_{90} (mg/L)	χ^2 (*df*)
50	6					
100	10					
150	15	202.63	173.036	251.46	354.249	1.355
200	21					
250	36					

LCL: Lower confidence limit; UCL: Upper confidence limit.

4. Discussion

The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites which are not found in terrestrial environments. Thus, marine algae are one of the richest sources of known and novel bioactive compounds[21]. Bacterial infection causes high rate of mortality in human population and aquaculture organisms. *E. coli* and *P. aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications[22]. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness[23]. Selvi and Selvaraj has reported that the brown seaweeds extracts showed higher activity than the extracts of red and green seaweeds[24]. Similar to the previous observation, in the present study, the methanolic extracts of *D. bartayresiana* expressed maximum zone of inhibition against *E. coli* [(9.4 ± 0.2) mm].

Brine shrimp cytotoxicity test has been used as a bioassay for a variety of toxic substances and it can be extrapolated for cell line toxicity and antitumor activity. Cytotoxic property of plant material is due to the presence of antitumor compounds[25]. Many of the secondary metabolites biosynthesized by the marine plants are well known for their cytotoxic property. Gedara *et al*. isolated hydroazulene diterpenes from the petroleum ether fraction of alcoholic extract of brown alga *Dictyota dichotoma* that showed moderate cytotoxic activity[26]. Similar to that, the methanolic extract of *D. bartayresiana* was found to be the most effective with LC_{50} and

Table 1
Bio-efficacy of various extracts of *D. bartayresiana* against pathogens (mm).

Pathogens	Petroleum ether (µg/mL)				Chloroform (µg/mL)				Methanol (µg/mL)				Acetone (µg/mL)				Tetracycline (µg)
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100	30
E. coli	-	-	-	-	-	2.1 ± 0.3	4.7 ± 0.6	8.6 ± 0.2	2.1 ± 0.4	3.8 ± 0.3	6.1 ± 0.5	9.4 ± 0.2	0.9 ± 0.2	1.4 ± 0.4	3.1 ± 0.4	4.2 ± 0.2	3.9 ± 0.3
S. flexneri	-	-	-	-	-	1.8 ± 0.4	2.8 ± 0.5	5.4 ± 0.3	2.4 ± 0.3	4.3 ± 0.6	5.9 ± 0.7	8.3 ± 0.4	-	-	-	-	3.1 ± 0.5
M. morganii	-	-	3.1 ± 0.4	9.3 ± 0.3	-	-	-	-	-	-	-	-	-	-	-	-	2.5 ± 0.4
P. aeruginosa	-	-	-	-	-	1.9 ± 0.7	4.2 ± 0.3	7.1 ± 0.3	1.9 ± 0.6	3.1 ± 0.4	4.9 ± 0.6	8.7 ± 0.2	1.3 ± 0.4	2.4 ± 0.6	3.2 ± 0.4	5.4 ± 0.2	4.2 ± 0.3

–: Zone of inhibition was not detected.

LC_{90} values of 202.63 and 354.249 mg/L respectively. Mosquitoes are the vector for large number of human pathogens than any other groups of arthropods[27]. Their uncontrollable breeding is posing a serious threat to the modern humanity. Every year more than 500 million people become severely affected with malaria. Many plant derived natural compounds were tested for mosquito control[28]. Early report predicted that marine plant extracts contain promising larvicidal activity[29]. Similarly, in the present study potential mosquito control principle was also revealed from *D. bartayresiana* and can be used for the development of biocontrol agents.

It can be concluded that *D. bartayresiana* was found to be used as a broad spectrum antibacterial, larvicidal and cytotoxic agent after extensive investigation. Further investigations are needed to elucidate the active ingredients of the extract responsible for various pharmacological properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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