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Bio-potency of *Dictyota ciliata* J. Agardh

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PEER REVIEW

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Comments

It is a promising field; the authors have ventured and have exposed the antibacterial, cytotoxicity, larvicidal potentials of *D. ciliata* J. Agardh. The authors have also brought out the importance of research in seaweeds, as the seaweeds stand as the major reservoir of newer drugs, which can be exploited and used by drug and pharmaceutical industries.
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ABSTRACT

Objective: To reveal the antibacterial, cytotoxicity, larvicidal potentials of *Dictyota ciliata* J. Agardh (*D. ciliata*).
Methods: Phytochemical, antibacterial screening, cytotoxicity and larvicidal properties of the *D. ciliata* extracts were carried out according to standard methods. Cytotoxicity and larvicidal potentials were analyzed by means of computerized probit analysis program.
Results: The phytochemical screening showed the presence of steroids, alkaloids, phenolic groups, cardiac glycosides, flavonoids and tannins in the crude extracts of *D. ciliata*. The methanolic extracts of *D. ciliata* showed the highest metabolites presence compared to other tested extracts. The antibacterial activity illustrated that the acetone extracts of *D. ciliata* exhibited the highest zone of inhibition [(10±0.2) mm] against *Morganella morganii*. Highest larval mortality (50%) was observed in the crude methanolic extracts of *D. ciliata* against *Culex quinquefasciatus* (LC_{50}=202.82 mg/L and LC_{90} value is 488.52 mg/L). The result of brine shrimp lethality bioassay showed highest cytotoxicity in methanolic extracts of *D. ciliata* with LC_{50} and LC_{90} values at 340.14 mg/L and 555.58 mg/L respectively.
Conclusions: The results of the present phytochemical analysis and biological assays will help the manufacturers for identification and selection of raw materials for drug production.

KEYWORDS
Dictyota ciliata, Larval mortality, *Morengilla morrgani*, *Culex quinquefasciatus*, Brine shrimp

1. Introduction

Naturally derived plant products with medicinal value play an important role in health care system, both in human and animals. This is evident with the growing interests in their utilization on a global perspective in the treatment of different ailments[1]. The marine environment representing approximately half of the global biodiversity is an enormous resource for natural compounds[2]. Seaweeds are marine macro algae and primitive type of plants growing abundantly in the shallow waters of sea, estuaries and backwaters which provide an excellent source of bioactive compounds such as carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals[3]. Biological compounds extracted from seaweeds were proven to have potential medicinal activities such as antibacterial, antiviral[4,5], antitumour[6], antifungal, antiprotozoal[7], antioxidant, mosquito and larva control[8,9].

Many of the secondary metabolites produced by the marine red algae are well known for their cytotoxic property. The brine shrimp cytotoxicity assay was considered as a convenient probe for preliminary assessment of toxicity,

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detection of fungal toxins, heavy metals, pesticides and cytotoxicity testing of dental materials[10]. The brine shrimp assay is applicable for the isolation of biogenic compounds from plant extracts[11]. For many centuries, phytochemicals have been used traditionally to fend off vector and pest species of insects[12]. Today mosquito plays a predominant role for the transmission of dengue, malaria, yellow fever, filariasis and other several diseases which are the greatest health problems in the world[13]. According to WHO report annually more than 700 million people suffer from mosquito-borne diseases[14]. The application of phyto-products is considered to be one of the counter measures to control pests and vectors[8].

Recently, there is a growing interest on the discovery of natural phytochemicals which are generally safer than synthetic chemicals[15]. Over 2 400 natural products have been isolated from seaweeds (mainly from the divisions Rhodophyta, Phaeophyta, and Chlorophyta). As an example of Phaeophyta's broad chemical diversity, members of the genus *Dictyota* produce an amazing array of complex terpenoids and acetogenins, possibly making it the world's most chemically complex genus. *Dictyota ciliata* J. Agardh (*D. ciliata*) are found in tropical and subtropical regions throughout the world and are an extremely rich source of secondary metabolites with therapeutic features[16]. With this knowledge, the present study was intended to examine the phytochemical constituents and their antibacterial, cytotoxicity, larvicidal potentials of *D. ciliata* J. Agardh.

2. Materials and methods

2.1. Sample collection and preparation

Healthy specimens of *D. ciliata* J. Agardh was collected from Rasthacaud coastal waters, Kanyakumari District, Tamil Nadu, India. The collected samples were washed thoroughly with clean seawater to remove extraneous materials, placed in plastic bags and brought to the laboratory. The seaweeds were blotted using blotting paper and spread out at room temperature in shade until the required weight was achieved. They were then ground to fine powder using tissue blender. The powdered samples were then stored for further analysis.

2.2. Extraction of crude compounds

The dried and powdered plant materials (10 g) were successively extracted with 60 mL of petroleum ether, chloroform, acetone, methanolic and aqueous by using cold extraction method. The sample was kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through filter paper and the filtrate was collected (crude extracts).

2.3. Preliminary phytochemical screening

The different extracts of *D. ciliata* were tested for the presence or absence of steroids, terpenoids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, cardiac glycosides, sterols and aminoacids. Phytochemical screening of the extracts was carried out according to the standard method[17].

2.4. Agar diffusion method

The agar diffusion test was used to measure the antibacterial effect of the seaweed extracts on the selected bacteria *viz.*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus flexneri* and *Morengilla morrganii*[18]. The selected bacterial pathogens were cultured in Muller-Hinton broth at 30 °C. Wells of size 6 mm were made on sterile nutrient agar plates using gel puncture. The plates were inoculated with the bacterial broth, using a micropipette. A concentration of 100 mg/mL of *D. ciliata* crude extract was poured in to each well and incubated for 24 h-28 h at 35 °C. The positive control was maintained with tetracyclin and the negative control was maintained using the solvents.

2.5. Larvicidal activity

2.5.1. Collection and maintenance of mosquitoes

Culex quinquefasciatus (*Cx. quinquefasciatus*) (IV instar) were collected from in and around Tirunelveli district (sewage), Tamil Nadu, India, with the help of 'O' type brush. These larvae were brought to the laboratory and transferred to 18 cm×13 cm×4 cm size enamel trays containing 500 mL of water maintained in the laboratory.

2.5.2. Test for larvicidal activity

Cx. quinquefasciatus was maintained at (27±2) °C, 75%-85% relative humidity and 14 L: 10 D photoperiod cycles. Fourth instar larvae of *Cx. quinquefasciatus* were transferred in 250 mL glass beaker containing desired plant extracts concentration such as 50, 100, 150, 200 and 250 mg/L. Five replicates for each concentration were set up. A volume of 2 mL acetone in 200 mL tap water was taken as control solution. The control mortality was corrected by Abbott's formula[19], and LC_{50}, LC_{90} regression equation and 95% confidence limit of lower (LCL) and upper confidence limits (UCL) were calculated by using probit analysis[20].

2.6. Cytotoxic activity

2.6.1. Preparation of sample

A weight of 25 mg of dried methanolic extract of *D. ciliata*

was taken in 80 mL beaker and 500 μL dimethylsulfoxide was added to it, finally the volume (5 mL) was adjusted by 4.5 mL methanol. The concentration of this solution was 5 μg/μL.

2.6.2. Hatching of brine shrimp

Artificial Sea water (38 g NaCl/1 000 mL tap water) was taken in small tank and shrimp eggs were added to one side of the divided tank and the side was covered. The shrimps were allowed for 48 h to hatch and mature as nauplii. During this period constant oxygen supply, temperature (around 37 °C) and light supply was maintained. The hatched shrimps were taken for bioassay.

2.6.3. Application of test sample to the test tube containing brine shrimp nauplii

Thirteen clean test tubes were taken and separated by 10 mL in each test tube. Tweenty five were for the samples in five different concentrations (five test tubes for each concentration) and 5 tubes for control. With the help of a Pasteur pipette 10 living shrimps were dropped into each test tube[21]. Dried methanolic extract of *D. ciliata* was taken in different concentrations (2.5, 5.0, 7.5, 10.0 and 12.5 mg/10 mL) to the sample tubes.

2.6.4. Preparation of control group

Control group was added in cytotoxic activity to validate the test method and result was obtained due to the cytotoxic activity of the test agent. Hence, 50 μL of dimethylsulfoxide was added to control tubes containing 5 mL of mother solution and 10 shrimp nauplii as control groups. No extract was added to prepare control solution.

2.6.5. Counting of nauplii

After 24 h, the tubes were inspected using a magnifying glass and the number of survived nauplii in each tube was counted and the LC_{50}, 95% confidence limit, LC_{90} and *Chi* square values were calculated.

3. Results

3.1. Qualitative analysis

Phytochemical screening of various extracts of *D. ciliata* revealed the presence of different secondary metabolites namely steroids, phenolic groups, cardiac glycosides, flavonoids and tannins with varied degree were illustrated in Table 1.

3.2. Screening for antibacterial activity

The crude extracts of *D. ciliata* showed a significant antimicrobial activity against all four pathogenic bacteria tested. The acetone extracts of *D. ciliata* exhibited the highest zone of inhibition [(10.0±0.2) mm] against *Morengilla morrganii* followed by *Staphylococcus flexneri* [(9.0±0.4) mm]. Methanolic and chloroform extract of *D. ciliata* displayed the moderate zone of inhibition *Escherichia coli* [(6.0±0.3) mm] and *Pseudomonas aeruginosa* [(5.0±0.4) mm]. The chloroform extracts of *D. ciliata*, petroleum ether and aqueous extracts of *D. ciliata* failed to show inhibitory response against the examined pathogens.

Table 1
Preliminary phytochemical analysis on *D. ciliata*.

Compounds	Petroleum ether	Chloroform	Acetone	Methanol	Aqueous
Steroids	−	−	+	+	−
Alkaloids	−	−	−	+	+
Phenolic groups	+	−	−	+	+
Cardiac glycosides	−	+	+	+	−
Flavonoids	+	−	−	+	−
Saponins	−	−	−	+	−
Tannins	−	−	−	+	+
Amino acids	−	−	−	−	−
Terpenoids	−	−	−	+	−

+: Presence; −: Absence.

3.3. Larvicidal activity

The larvicidal activity of methanolic extract of *D. ciliata* against the fourth instar larvae *Cx. quinquefasciatus* were tested with various concentrations ranged from 50−250 mg/L and their results are presented in Table 2. Methanolic extract of *D. ciliata* showed moderate level of larvicidal effect after 24 h. Highest larval mortality in terms of lethal concentrations for 50% mortality was observed in the crude methanolic extracts of *D. ciliata* against *Cx. quinquefasciatus* (LC_{50}=202.82 mg/L and LC_{90} value of 488.52 mg/L) were analyzed by means of computerized probit analysis program. The lower confidence limit was 168.06 and upper confidence limit was 289.96, *df*=0.236.

Table 2
Larvicidal activity of *D. ciliata* against *Cx. quinquefasciatus*.

Concentration of extracts	% of Mortality
50	25
100	32
150	40
200	50

3.4. Cytotoxic activity

The brine shrimp lethality bioassay was carried out with various concentrations of methanolic extract of *D. ciliata*. The result of brine shrimp lethality bioassay confimred that methanolic extracts of *D. ciliata* is pharmacologically active. The methanolic extracts of *D. ciliata* showed highest toxicity having an LC_{50} and LC_{90} values at 340.14 mg/L and 555.58 mg/L respectively (Table 3). The lower confidence limit was 278.37 and upper confidence limit was 509.40, *df*=1.355. The inhibitory effect of the extract might be due to the toxic

compounds present in the active fraction that possess ovicidal and larvicidal properties.

Table 3
Cytotoxic activity of *D. ciliata* against *Cx. quinquefasciatus*.

Concentration of extracts	% of Mortality
50	3
100	3
150	6
200	12
250	14

4. Discussion

The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of new active substances in the field of the development of bioactive products. More than 150 000 macro algae or seaweed species are found in oceans of the globe, rich in secondary metabolites includes alkaloids, glycosides, flavonoids, saponins, tannins, steroids, related active metabolites, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry[22]. The result of the present study revealed the phyto-constituents presence in various extracts of *D. ciliata*. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications[23].

Flavonoids, the major group of phenolic compounds reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids ability of scavenging hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals highlights many of the flavonoid health-promoting functions in organism, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA[24]. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Flavonoids, on the other hand, are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anti-cancer activity[8]. The phytoconstituents of phenolic groups are one of the largest and most obiquitous groups of seaweed metabolites[25]. They possess biological properties such as antiapoptosis, antiaging, anticarcinogenic, antiinflammation, anti atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[26]. Steroidal compounds are of important interest in pharmacy due to relationship with such compounds which possess antimicrobial activities on some bacterial isolates as sex hormones[27].

Over the last few decades, many studies on plant extracts against mosquito larvae have been conducted around the world. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae[28,29]. Numerous secondary metabolites with complex structures and different bioactivity spectra have been characterized from algae and recognized as a virtually untapped reservoir of novel drug leads[30]. Many of these metabolites have toxicological; pharmacological and therefore the marine derived algal products may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, biodegradable and are easily available around the world. The result of the present study revealed larvicidal potentials and cytotoxicity properties of *D. ciliata* is the best source of cytotoxic secondary metabolites. The results of the present study also suggest that the *D. ciliata* might be utilized for the development of anticancer drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Biological compounds extracted from seaweeds have been proven with potential medicinal activities such as antibacterial, antiviral, antitumour, antifungal, antiprotozoal, antioxidant, mosquito and larva control. The present study also suggest that the *D. ciliata* might be utilized for the development of anticancer drugs.

Research frontiers

The present study about the antibacterial, cytotoxicity, larvicidal potentials of *D. ciliata* J. Agardh is a very important work and cam lead to many new drug discoveries. The present study could also help in the discovery of natural phytochemicals which are generally safer than synthetic chemicals.

Related reports

Over 2 400 natural products have been isolated from seaweeds (mainly from the divisions Rhodophyta, Phaeophyta, and Chlorophyta). As an example of Phaeophyta's broad chemical diversity, members of the genus *Dictyota* produce an amazing array of complex terpenoids and acetogenins, possibly making it the world's most chemically complex genus.

Innovations and breakthroughs

The present study was intended to examine the phytochemical constituents and their antibacterial, cytotoxicity, larvicidal potentials of *D. ciliata* J. Agardh. The result also reveals the larvicidal potentials and cytotoxicity properties of *D. ciliata* and this plant as the best source of cytotoxic secondary metabolites.

Applications

The results also suggest that *D. ciliata* might be utilized for the development of anticancer drugs.

Peer review

It is a promising field; the authors have ventured and have exposed the antibacterial, cytotoxicity, larvicidal potentials of *D. ciliata* J. Agardh. The authors have also brought out the importance of research in seaweeds, as the seaweeds stand as the major reservoir of newer drugs, which can be exploited and used by drug and pharmaceutical industries.

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