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RESEARCH ARTICLE

Bioactive compounds from *Caulerpa racemosa* as a potent larvicidal and antibacterial agent

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Abstract **Marine algae are rich sources of bioactive compounds capable of harboring secondary metabolites which are structurally and biologically active. In our study, the methanolic extract of marine algae *Caulerpa racemosa* (green algae) was employed to determine the antibacterial and larvicidal activity. The antibacterial activity showed effective inhibition against five pathogenic bacteria. A significant zone size of 16 mm was observed for *Pseudomonas aeruginosa*. The methanolic extract of *Caulerpa racemosa* showed effective larvicidal activity against *Culex tritaeniorhynchus* and the histopathological studies revealed the rupture in mid gut of larvae. The bioactive compounds in the crude extract were further identified as 2-(-3-bromo-1-adamantyl) acetic acid methyl ester and Chola-5, 22- dien-3-ol by GC-MS. Hence the bioactive compounds obtained from the methanolic extracts could be used for the bactericidal and larvicidal activity which will overcome the harmful impact of synthetic insecticide on environment.**

Keywords marine algae, GC-MS, antibacterial, larvicidal, bioactive compounds

Introduction

Seaweed is a term encompassing macroscopic, multicellular and benthic marine algae. They are grown in shallow and deep sea areas. Seaweeds are a renewable source of the marine environment (Varier et al., 2013). There are three different types of marine algae *Rhodophyceae, Phaeophycea* and *Chlorophyceae* commonly known as red, brown and green marine algae respectively (Vinodhkumar et al., 2013). Marine algae are polyphyletic members, distributed in many continents across the coastal regions (Karthikaidevi et al., 2009). They are potential sources of secondary metabolites (Luis Gomez and Soria Mercado, 2010) and are also good source of proteins, polysaccharides and fibers (Pandian et al., 2011). Marine algae also possess the capacity to synthesis antibacterial, antifungal and antiviral compounds against various pathogens (Deig et al., 1974).

There are several bioactive compounds which are produced by seaweeds and they also possess the ability to prevent the disease caused by some gram negative and gram positive pathogenic bacteria (Kolanjinathan et al., 2009). These bioactive metabolites are responsible for ecological pressures like competition, predation, deterrence and reproduction (Luis Gomez and Soria Mercado, 2010). Marine algae are used as food source and also in medication for the treatment of various diseases (Kim et al., 2007). Marine algae have been recognized as potential source of antibiotics like cyclic polysulphides and halogenates of seaweeds which are effective against pathogenic microorganisms (Varier et al., 2013).

Seaweed extract was manufactured commercially for agriculture purpose which constitutes to the enhancement of growth in specific organs of the plants like roots and leaves. It has been used as fodder supplement to increase the animal nutrition and productivity (Craigie, 2011). They also have significance for their potential in the production of natural antioxidants like ascorbate and glutathione which increase the shelf life of food (Khaled et al., 2012). Seaweeds are the only source of photochemical compounds like agar, carrageenan and algin, which has application in various industries such as food, confectionary, textiles, pharmaceuticals, dairy and paper industries mostly as gelling, stabilizing and thickening agents (Aruna et al., 2010).

Synthetic chemicals have been used to control insect for a last few decades due to its low price (Khattab et al., 2012). These synthetic insecticides are non target specific and have led to detrimental effects on the environment (Nazar et al., 2009). Mosquitoes are blood sucking insects capable of transmitting the etiological agents that causes malaria,

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dengue, yellow fever, Japanese encephalitis, and filariasis (kumar et al., 2012). Mosquitos have developed resistance for these insecticides hence the effectiveness of these chemical compounds for the control of vectors has been decreased (Poonguzhali and Josmin Laali Nisha, 2012). Therefore development of a bio-based insecticide has been a major area of research.

In our current study, *Caulerpa racemosa* was evaluated for its antibacterial and larvicidal activity using various concentrations of methanolic extract and the bioactive compound existing in the crude extract was identified by GC-MS.

Materials and methods

Sample collection

Seaweeds were collected from Gulf of Mannar (Rameshwaram), India (Latitude: 9°16′48″N; Longitude: 79°18′E) by hand picking and were washed with sea water before transferring onto sterile polyethylene bags. The samples were transported to the laboratory and were processed immediately. The seaweeds were washed with distilled water to remove the epiphytes and debris (Rizvi, 2010). The cleaned samples were shade dried and powdered (Mhadhebbia et al., 2012).

Preparation of algal extract

The *Caulerpa racemosa* was shade dried and ground into powder (Mhadhebbia et al., 2012) which weighed about 100 g and was soaked in 500 mL of methanol in a 1000 mL Erlenmeyer flask for 72 h at shaking conditions. The extract was filtered and stored under −20°C. The extract was concentrated by evaporation and the crude extract was used for antibacterial and larvicidal assay (Kandhasamy and Arunachalam, 2008).

The percentage of the bioactive compound extracted was calculated by using the formula:

$$\begin{aligned}&\%\text{ of extraction}\\&\quad=(\text{weight of extract after evaporation of solvent}\\&\qquad/\text{weight of dried plant material})\times 100.\end{aligned}$$

Phytochemical analysis of extract

The methanolic extract of *Caulerpa racemosa* was tested for the phytochemical compositions like alkaloids, saponins, phlobatannins, terpenoids and quinones (Subhathra and Poonguzhali, 2013).

Antibacterial assay

The bacterial strains *Escherichia coli* MTCC No. 9721, *Staphylococcus aureus* MTCC No. 3160, *Pseudomonas aeruginosa* MTCC No. 10462, *Salmonella typhi* MTCC No. 8587 and *Bacillus subtilis* NCIM No. 2547 was obtained from the Laboratory of School of Biosciences and Technology, VIT University, India. They were sub-cultured and maintained in glycerol stocks and slants. Seed culture of all the bacterial strains were inoculated onto Muller Hinton agar medium (MHA). Wells were made and 100 μL of methanolic extract of *Caulerpa racemosa* was added to the well at a concentration of 350 mg/L. A well contained only solvent was considered as negative control and ampicillin (100 mg/L) as positive control. These plates were incubated at 37°C. The inhibition zone was measured after overnight incubation (Varier et al., 2013).

Larvicidal activity

The larvicidal activity of methanolic extract of seaweed *Caulerpa racemosa* was conducted in batches of 20 early 4th instar larvae of *Culex tritaeniorhynchus*. The test larvae were treated with 1mL of extract was added in a beaker containing 99 mL distilled water. The concentrations were ranging from 80–400 mg/L. After treatment, behavior of the larvae were observed and recorded at different intervals. After 24 h of treatment larvae were considered to be dead when they did not show the movement and the LC_{50} of the larvicidal activity was calculated using probit software (Manilal et al., 2011). Hispathological studies were performed with the treated and untreated larvae (Almehmadi, 2011).

GC-MS analysis of crude extract of methanol

The methanolic extract of *Caulerpa racemosa* was analyzed for the presence of bioactive compounds using Perkin Elmer GC model (30 m × 0.25 mm × 0.25 μm) Clarus 680 (Mass spectrometer Clarus 600 EI). The Clarus 680 GC used purified helium as the carrier gas, at a constant flow rate of 1 mL/min. One microliter of samples were injected and oven temperature was programmed from 60°C to 300°C for 2 min at the rate of 10°C/min and then isothermally held for 6 min till the end of the analysis.

Results

Seaweed extract

The percentage of *Caulerpa racemosa* extract obtained was 12.36 which were used for further studies.

Phytochemical analysis

The methanolic extract of *Caulerpa racemosa* showed positive for alkaloids, saponins and terpenoids (Table 1).

Table 1 Phytochemical screening of *Caulerpa racemosa*

Phytochemical test	Methanol extract of *Caulerpa racemosa*
Alkaloids	+
Saponins	+
Phlobatanins	−
Terpenoids	+
Quinones	−
Flavanoids	−
Tannins	−

Antibacterial activity

Methanolic extract of *Caulerpa racemosa* exhibited antibacterial activity against the tested pathogens and a maximum zone size of 16mm was observed for *Pseudomonas aeruginosa* followed by *Escherichia coli* (12 mm) and *Salmonella typhi* (12 mm) and the extract showed the minimum activity against pathogens like *Bacillius subtilis* (10 mm) and *Staphylococcus aureus* (8 mm) (Fig. 1).

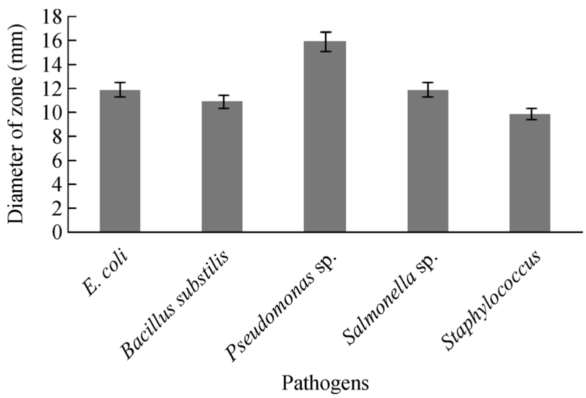

Figure 1 Antibacterial activity of methanolic extracts of *Caulerpa racemosa.*

Larvicidal activity of extract

The larvicidal activity of methanolic extract of *Caulerpa racemosa* against *Culex tritaeniorhynchus* larvae was performed under laboratory conditions. The mortality rate of *Culex tritaeniorhynchus* larvae after treating with methanolic extract was evaluated and the Lethal Concentration of larval mortality is represented in (Table 2). The lethal concentration required for the 50% mortality of *Culex tritaeniorhynchus* larvae after 24 h of incubation was calculated to be 63.324 mg/L and the 95% confidence limit was found to be LCL = 0.048 and UCL = 120.804.

Histopathological studies

The cross sections of abdominal regions of untreated and treated 4th instar larvae of *Culex tritaeniorhynchus* is shown

Table 2 Effect of methanol extract of *Caulerpa racemosa* against mosquito larvae

Extract	LC_{50} (mg/mL)	95% confidence limits	
		LCL	UCL
Methanol	63.324	0.048	120.804

LC_{50}: lethal concentration, concentration required to kill 50 of the test populations respectively; LCL: 95% of lower confidence limit; UCL: 95% of upper confidence limit.

in Figs. 2 and 3 respectively. The larvae were treated with methanolic extract of *Caulerpa racemosa* at LC_{50} 63.32 mg/L. The treated larva showed disintegration of the mid-gut epithelium and also showed the disarrangement of columnar cells.

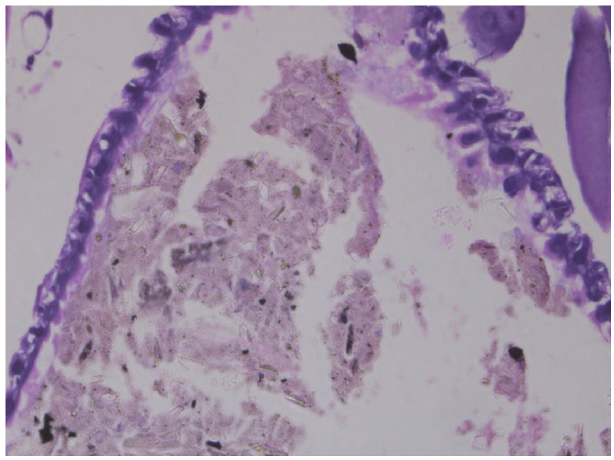

Figure 2 Longitudinal section of untreated *Culex tritaeniorhynchus* larva.

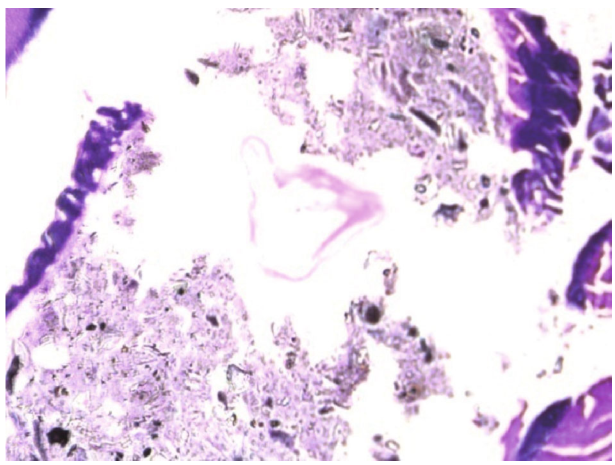

Figure 3 Longitudinal section of treated *Culex tritaeniorhynchus* larva.

GC MS analysis of methanol crude extract of *Caulerpa racemosa*

The bioactive compounds present in the methanolic extract of *Caulerpa racemosa* was detected by GC-MS analysis. The mass spectrum equipped with a data system in combination with gas chromatography was used for the chemical analysis. The peaks were observed as presented in (Fig. 4). The analysis of the crude extract revealed the presence of major constituents like methyl 3-bromo-1-adamantaneacetate and Chola-5, 22-Dien-3-Ol, 3 Beta.

Discussion

Seaweeds are proven source for several bioactive compounds. In the present study the methanolic extract of *Caulerpa racemosa* was used for the extraction of bioactive compounds since methanol has high extraction capability (Jebasingh et

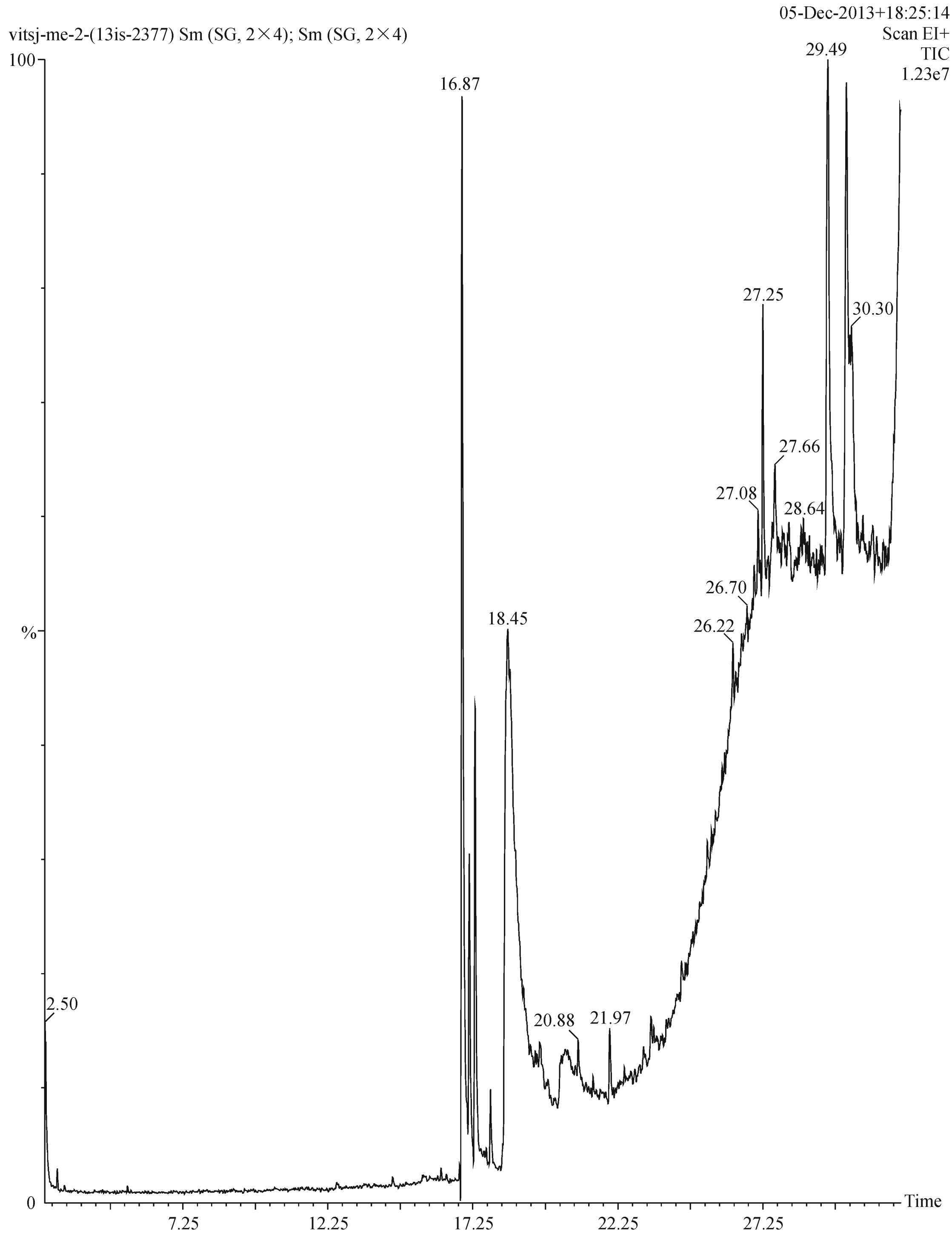

Figure 4 Chromatogram of *Caulerpa racemosa* extracts using methanol.

al., 2011). The crude extract showed positive for alkaloids, saponins and terpenoids. It is reported that these compounds possess therapeutical applications (Güven et al., 2010). Saponins can be used as dietary supplements because of its specific physical, chemical and biologic activities. Saponins also possess antimicrobial, anti-inflammatory and hemolytic effect (Jeeva et al., 2012). Terpenoids have been reported for its aromatic qualities and can also be used in herbal remedies due to its antimicrobial and other pharmaceutical (Egwaikhide et al., 2007).

The antibacterial activity of the seaweeds may vary due to habitat, seasonal collection of seaweeds, different growth stage etc. (Karthikaidevi et al., 2009). *Caulerpa racemosa* are active members of green algae incomparison with other groups of algae screened for their antibacterial activity (Kandhasamy and Arunachalam, 2008). Several studies were conducted on *Caulerpa racemosa* for its antibacterial activity using various solvents and methanolic extract of *Caulerpa racemosa* showed the maximum activity against different pathogenic organisms (Jebasingh et al., 2011). The crude methanolic extract of *Caulerpa racemosa* was active against all the tested pathogens and it showed maximum activity against *Pseudomonas aeruginosa* in comparison with the other pathogens. Inhibition was absent in the negative control whereas in the positive control (ampicillin) zone of inhibition was observed against the tested pathogens. The methanolic extract of *Caulerpa racemosa* showed considerable size of zone of inhibition than that of the positive control. Hence the bioactive compounds involved in the inhibition were further identified by GC MS.

The control of adult mosquito is an unsuccessful strategy, as the adult stage occurs beside human inhabitation and they can easily overcome remedial measures (Service, 1983). The mortality of larvae may be due to the activity of bioactive compounds produced from *Caulerpa racemosa* extract. Saponins can also serve as natural larvicidal compound (Chapagain et al., 2008). The mortality of the larvae could be due to the presence of saponins which was previously reported (Waller and Yamasaki, 1997) and the compounds identified in the present study has been discussed in the GC MS results.

The mid-gut of the control (untreated) larva of *Culex tritaeniorhynchus* consisted of a unicellular layer (epithelium) resting upon the basement membrane. The epithelium consists of columnar cells and it has striated border. The larva treated with methanolic extract of *Caulerpa racemosa* showed disintegration of the mid-gut epithelium and also showed the disarrangement of columnar cells. In the previous study (Almehmadi, 2011) the bioactive compound present in the extract had shown competent activity on the columnar cells. The disintegration of epithelium may be due to the bioactive compound present in the *Caulerpa racemosa* extract.

The crude methanolic extract of seaweeds on the basis of spectral data by GC MS analysis showed the presence of mixture of volatile compounds, esters, and other acids. Methyl formation could be a result of alkylation of hydrocarbons with methanol which acts as bioactive compound (Yuvaraj et al., 2011). In the previous studies by Jung et al., (2002), it has been reported that the compound like caulerpynyne was detected in the *Caulerpa racemosa* and *Caulerpa prolifera* by GC MS analysis which has ability to perform wound activated reaction and another study stated that compounds like caulerpin, caulerpynyne, phytol, 10-keto- 3, 7, 11- trimethyldodecanoic acid and unsaturated compounds as novel effective mosquitocidal compounds (Alarif et al., 2010). In our current study we have reported for the first time on the various compounds present in *Caulerpa racemosa* that has effective inhibition against pathogenic bacteria and larvae of *Culex tritaeniorhynchus*. The compounds in the crude methanolic extract were methyl 3-bromo-1-adamantaneacetate and Chola-5, 22-Dien-3-Ol, 3 Beta are existing in *Caulerpa racemosa* as a bioactive compounds which could be effectively used for therapeutical purposes. The identified compounds has been synthesized chemically in the laboratory for therapeutical application but this is the first time report on its presence as a bioactive compound from seaweed.

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Compliance with ethics guidelines

Sowmya Rachannanavar Nagaraj and Jabez William Dsborne declare that they have no conflict of interest.

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