

***In vitro* antimicrobial potentiality of some marine algae against selected phytopathogens**

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ABSTRACT

In this present study the *in vitro* antimicrobial activities of marine algae *Gracilaria corticova* (Gracilariaceae), *Gelidium pusillum* (Gelidiellaceae), *Chetomorpha annetina* (Cladophoraceae), *Ulva lactuca* (Ulvaceae) from coastline of Bay of Bengal, visakhapatnam. Soxhlet extraction method used to get the methanolic extracts. The antimicrobial activities of the organic solvent extracts on the various test microorganisms, including bacteria and fungi investigated using agar well diffusion technique. The length of inhibition zone was measured in millimeters from the edge of the well to the edge of the inhibition zone. Results of the present study confirmed the potential use of seaweed extract as a source of antimicrobial compounds.

Key words: *Ulva lactuca*, Antimicrobial activity, Macroalgae, Soxhlet extraction.

INTRODUCTION

Numerous substances were identified as antimicrobial agents from algae: chlroellin derivatives, acrylicacid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, etc (Espeche *et al.*, 1984). Many chemically unique compounds of marine origin with various biological activities have been isolated, and some of them are under investigation and are being used to develop new pharmaceuticals (Lima- Filho *et al.*, 2002). Primary or secondary metabolites produced by marine algae may be potential bioactive compounds of interest in the pharmaceutical industry (Febles *et al.*, 1995). The production of antimicrobial activities was considered to be an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites (González del Val *et al.*, 2001). A wide range of results of *in vitro* anti-fungal activities of extracts of

green algae, diatoms, and dinoflagellates have also been reported (Borowitzka and Borowitzka., 1992, Moreau *et al.*, 1988.). Similarly, to date, some microalgae screened contain and/or excrete pharmacologically active compounds.

In the present study was undertaken in order to examine the antimicrobial effects of crude methanol extracts obtained from marine algal species obtained from the coastal line of Bay of Bengal, Visakhapatnam.

MATERIAL AND METHODS

Marine algae *Gracilaria corticova* (Gracilariaceae), *Gelidium pusillum* (Gelidiellaceae), *Chetomorpha annetina* (Cladophoraceae), *Ulva lactuca* (Ulvaceae) were collected by hand from the submerged marine rocks of Visakhapatnam sea coast, Andhra Pradesh, India in low tide. Materials were identified and algal voucher specimens of were

deposited in the herbarium of the Botany Department, Andhra University, Vishakhapatnam.

Test microorganisms

The microorganisms (including fungi and bacteria) selected were *Alternaria alternata* (MTCC 2724), *Acremonium strictum* (MTCC 2599), *Aspergillus flavus* (MTCC 4633), *Aspergillus niger* (MTCC 2723), *Fusarium oxysporum* (MTCC 1755), *Macrophomina phaseolina* (MTCC), *Penicillium expansum* (MTCC 2006), *Pseudomonas syringae* (MTCC 1604), *Pseudomonas marginales* (MTCC 2758), *Rhizoctonia solani* (MTCC 4633), *Ustilago maydis* (MTCC 1474), *Xanthomonas campestris* (MTCC 2286) obtained from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi. Active cultures were generated by inoculating a loopful of culture in separate 100mL nutrient broths and incubating on a shaker at 37°C overnight. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline to obtain 5×10^5 cfu/mL.

Preparation of algal extracts

The collected algal epiphytic and extraneous matter were removed by washing first in sea water and then in fresh water. The algae were transported to the laboratory in polyethylene bags at ice temperature. Specimens were preserved in 5% formalin. The collected algae materials were chopped into small pieces and coarsely powdered in Willy mill. The coarsely powdered material weighed and extracted with chloroform, methanol and distilled water in sequential order of polarity using a soxlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2ml of solvent was used. The extracted solvents were filtered through Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuo at 40°C) using a rotary evaporator. The residue obtained were designated as crude extracts and stored in a freezer at -20°C until assayed.

The dried algae extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentration 100mg/ml of crude

extract and filtration through a 0.45µm membrane filter and stored in sterile brown bottles in a freezer at 20°C until bioassayed.

Determination of antimicrobial activity

The crude algal extracts of different species were subjected to antimicrobial assay using the agar well diffusion method of Murray *et al.*, 1995⁷ modified by Olurinola 1996⁸.

20 ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to five uniform cups/wells in each Petri dish. A drop of molten nutrient agar was used to seal the base of each cup.

The cups/wells were filled with 50µl of the extract concentration of 100 mg/ml and allow diffusing for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37°C for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media potato dextrose agar instead of nutrient agar and incubates at 25°C for 48 hours. The zones of inhibition were measured with antibiotic zone scale in mm and the experiment was carried out in duplicates.

RESULTS

The antimicrobial activity of crude methanol extracts of the four algal species summarized Table 1.

Table 1 summarizes the antimicrobial activities of zone of inhibition of methanol (9 to 13 mm) with 100 mg/ml. The variation of antimicrobial activity of our extracts might be due to distribution of antimicrobial substances, which varied from fraction to fraction of the crude extract.

DISCUSSION

G. pusillum methanolic extracts showed most active and significant against *A. niger* and *R. solani* (13 mm) than other algae and followed by

Table 1:

Microorganisms	<i>U. lactuca</i>	<i>G. corticata</i>	<i>C. annetina</i>	<i>G. pusillum</i>
<i>Alternaria alternata</i>	13	-	-	-
<i>Acremonium strictum</i>	-	9	10	12
<i>Aspergillus flavus</i>	-	10	11	-
<i>Aspergillus niger</i>	11	11	11	13
<i>Fusarium oxysporum</i>	12	13	-	-
<i>Macrophomina phaseolina</i>	-	-	-	-
<i>Penicillium expansum</i>	-	-	-	-
<i>Pseudomonas syringae</i>	9	-	-	-
<i>Pseudomonas marginales</i>	10	-	-	-
<i>Rhizoctonia solani</i>	-	-	10	13
<i>Ustilago maydis</i>	-	-	12	11
<i>Xanthomonas campestris</i>	-	9	-	-

Volume per well: 50µl; Borer size used: 6mm; extract concentration in 100 mg/ml

Zone of inhibition in mm, No zone is presented as (-)

A. strictum (12 mm) and lowest activity was against *U. maydis* (11mm). No activity against were *A. alternata*, *F. oxysporum* and etc.

U. lactuca methanolic extracts showed most active and significant against *A. alternata* (13 mm) than other algae and followed by *F. oxysporum* (12 mm) and lowest activity was against *P. syringae* (9 mm). No activities were found against *A. strictum*, *P. expansum*, *R. solani* and *U. maydis*.

G. corticata have good activity against *F. oxysporum* and low activity with *A. strictum* and *X. campestris*

C. annetina showed same activity with *A. strictum* and *R. solani* and highest activity against *U. maydis*.

C. annetina and *G. corticata* show same

activity against *A. niger* (11mm).

All three have *G. pusillum*; *U. lactuca* and *C. annetina* have no activity against *X. campestris*.

CONCLUSION

Overall, the present study provides enough data to show the potentiality of marine algae. The present study was conducted to develop newer lead for better and safer chemotherapeutic agents from algae. Finally we conclude that macroalgae from the Bay of Bengal are potential sources of bioactive compounds and should be investigated for natural antibiotics. Biochemical analysis are currently undertaken to determine the structure and nature of these compounds. Further studies are needed to identify the pure component and establish the exact mechanism of action for antibacterial and antifungal action of algal extracts.

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