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GAS CHROMATOGRAPHY- MASS SPECTROMETRY ANALYSIS OF *GRACILARIA CORTICATA* (RED SEAWEED) ETHANOLIC EXTRACT AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

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Abstract:
The chemical composition and antioxidant properties of *Gracilaria corticata* ethanolic extract were examined. Six components were identified in the extract by gas chromatography-mass spectrometry and Hexadecanoic acid and its ethyl ester accounted for 52.38% of total identified components. Furthermore, we assessed the extract antimicrobial activity using different bacterial and fungal strains. Ethanolic extract of *Gracilaria corticata* was found effective against bacteria *Bacillus subtilis* and Fungi *Aspergillus oryzae* moderate effect. The study reveals the bioactive potential of *Gracilaria corticata* and its bioactive potential for the production of valuable therapeutics and other related compounds of economic viability and social accessibility.

Keywords: *Gracilaria corticata*, antimicrobial activity, bacteria, fungi, Hexadecanoic acid, bioactive potential, GC-MS, *Bacillus subtilis*, *Aspergillus oryzae*.

Introduction

Marine algae represent one of the major living resources acquired from ocean. Seaweeds are marine macroalgae used in food, pharmaceuticals, textile industries and various other fields (Ruggieri, 1976; Chen, 1977). Traditionally, seaweeds have been used for treating arthritis, constipation, nervous disorders, rheumatism, colds and skin irritation (Yaychuck, 2006). Production of inhibitory substances from seaweeds was noted in 1917 (Harder and Oppermann, 1953). Since then numerous studies have been carried out to detect and extract antimicrobial compounds from marine algae of all the three groups- green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) (Biard *et al.*, 1980).

Material and Methods

Expermental

Collection area- Mature thalii of *Gracilaria corticata* was collected during low tide from the submerged marine rocks at Kunakeshwar [164'0.120"N latitude and 7328'0.120"E longitude] in Sindhudurg district of Maharashtra [India] and brought into laboratory. The algal samples were cleaned with fresh seawater and then in distilled water to remove epiphytes, suspended matter and sand particles. The material was air dried in shade and after complete drying it was ground to form a fine powder using a mechanical grinder. The powdered samples were stored in airtight containers in dark at room temperature. Ten grams of dry algal powder were extracted in 100ml of an organic solvent for 24 hours using an orbital shaker and the extract was filtered through Buchner's funnel using Whatman No.1 filter paper. The filtrate was condensed to half of the original volume (50ml) and stored in a glass vial until used (Yuvraj *et al.*, 2011). Extraction of algal samples was done using Ethanol.

Antimicrobial activity: Antibacterial activity: To study the antibacterial activity by agar well diffusion method described by Murray *et al.* (1995) was used. The bacterial cultures (*Echerischia coli, Proteus vulgaris, Bacillus subtilis, Salmonella typhi and Staphylococcus aureus*) were inoculated on the surface of solid medium and wells of eight mm diameter were prepared with the help of a sterilized cork borer. Each well was filled with 0.2 ml of an algal extract. Respective solvent was used as a negative control while antibiotic ampicilin was used as a positive control for comparative efficacy. The plates were incubated at 37^{0}C for 24 h. Zone of inhibition around the well was measured and recorded in each plate after 24 h.

Antifungal activity: Sensitivity of fungal strains to algal extracts was analyzed using food poisoning method described by Dekker and Glelink (1979). Czapek Dox Agar medium plates were prepared by mixing one ml algal extract with autoclaved Czapek Dox Agar in a 30ml marked beaker to make final volume of 30ml. The contents were mixed well and poured into a sterile petriplate. Discs of 8mm of actively growing margins in the plates of eight days old fungal culture (*Aspergillus oryzae, Rhizopus artocarpi and Fusarium oxysporum*) were placed inverted on the agar surface of plates at the center. The control was maintained without algal extract. Plates were incubated at 25±2^{0}C in an incubator, linear growth was measured after 72h and percent inhibition was calculated.

GCMS analysis of seaweeds

Seaweed extracts were analyzed by gas chromatography and mass spectrometry for the quantitative determination of phytochemicals.

GC-MS analysis was carried out using Shimadzu Make QP-2010 with non polar 60 M RTX 5MS Column. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40^0C and held for 3 min and the final temperature of the oven was 480^0C with rate at 10^0C [min.sup.-1]. A two µL sample was injected with splitless mode. Mass spectrum was recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min. .

Identification of components

Identification of mass spectrum was done using data base of NIST (National Institute of Standards and Technology). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The retention time, molecular weight, molecular formula and percent amount of individual compounds were recorded.

Result and Discussion

1. Antibacterial activity: Extract of *G. corticata* was more active against *Bacillus subtilis and Salmonella typhi* producing inhibition zones of 17.56mm and 15.50 mm respectively. Antibacterial index was >60% for *B. subtilis* in this extract. Several red seaweed species have shown antibacterial activity against a variety of gram positive and gram negative bacteria (Sultana *et al.*, 1990; Usmani *et al.*, 1991; Ali *et al.*, 2000). Chloroform extract of *Gracilaria verrucosa* showed highest inhibition against *Salmonella paratyphi.*
2. Antifungal activity: Extract of *G. corticata* inhibited the mycelial growth of *A. oryzae* (19.20mm). There are reports of antifungal activity against *Aspergillus niger* and *Aspergillus tetreus* by the extracts of red seaweeds such as *Corollina officinalis* (ethanol), *Ceramium cilatum* (ethanol) and *Gracilaria folifera* (Chloroform) (Erturk and Tas, 2011; Kayalvizhi *et al.*, 2012). Rangaiah *et al.* (2010) have reported maximum activity against *Rhizopus stolonifer* in crude extracts (methanol, chloroform, ethanol, aqueous) of *G. corticata*. In the present study *A. oryzae* was found significantly inhibited by *G. corticata*.
3. GC-MS analysis: The GC-MS analysis of crude ethanolic extract of *Gracilaria corticata* showed the presence of mixture of compounds. A total of 6 peaks were observed with different retention times as presented in fig.3. The molecular formula and molecular weight for the compounds identified given in Table 3. The GC-MS analysis of the crude extract revealed that the main phyco-constituent was n-Hexadecanoic acid ethyl ester (52.38), n-Hexadecanoic acid (22.41) may be involved in biological activity. Seaweeds exhibit high level potential bioactivity.

Oleic acid (14.58%) and n hexadecanoic acid (24.73%) were the major compounds detected in *Acanthophora spicifera* (Zakaria *et al.*, 2011). GC-MS analysis of crude extract of *Hypnea musciformis* revealed n hexadecanoic acid and tetradecanoic acid as main constituents (Balamurugan *et al.*, 2013).

Table 1. Antibacterial activity of ethanolic extract of *Gracilaria corticata*.

Bacterial Strains	Inhibition Zone (mm)
E. coli	14.30±0.30
P. vulgaris	13.56±0.49
B. subtilis	17.56±0.49
S. typhi	15.50±0.50
S. aureus	13.50±0.50

Table 2. Antifungal activity of ethanolic extract of *Gracilaria corticata*.

Fungal Strains	Growth zone (mm)
A. oryzae	19.20 ±0.20
R. artocarpi	19.36 ±0.32
F. oxysporum	26.50 ±0.43

Table 3: Phytochemical composition of ethanolic extract of *Gracilaria corticata* by GC-MS analysis

Sr. No	RT	Phytochemical compounds	Molecular formula	Molecular weight	% composition
1	7.551	Propane,1,1,3-triethoxy	$C_9H_{20}O_3$	176	6.87
2	19.710	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	22.41
3	20.034	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	52.38
4	21.717	9-octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	6.71
5	21.767	Ethyl 9-octadecenoate	$C_{20}H_{38}O_2$	310	6.14
6	21.911	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	5.48

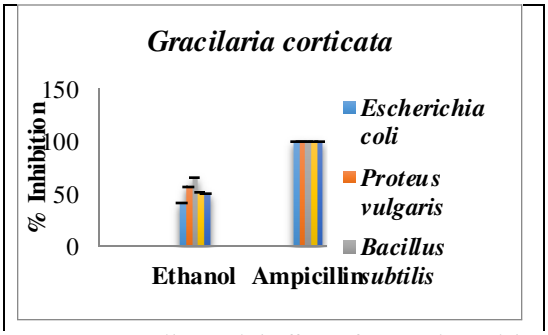

Figure 1 Antibacterial effect of *G. corticta* with Ampicillin

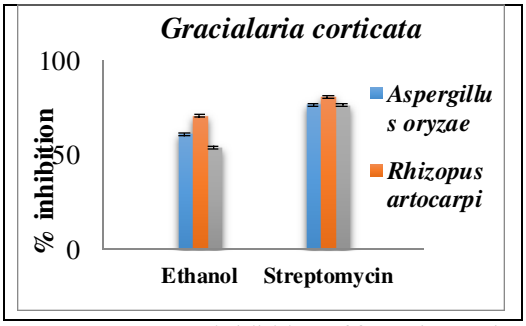

Figure 2 Percent inhibition of fungal growth by *G. corticata*

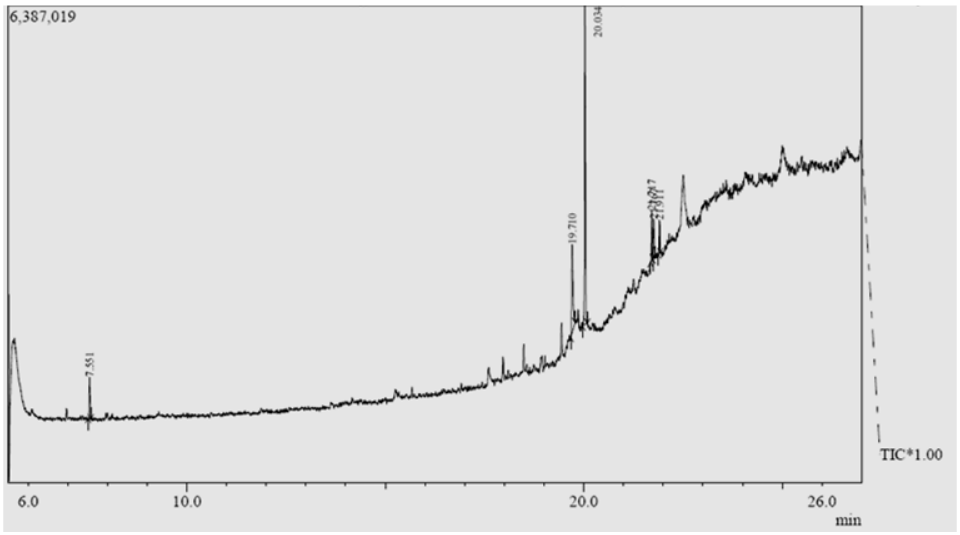

Figure 3: GC-MS chromatogram of the crude extract of *Gracilaria corticata.*

Conclusion:

The ethanolic extract of *G. corticata* showed effective inhibitory activity against bacteria *B. subtilis* and fungi *A. oryzae*. This study shown that ethanolic extract of *G. corticata*contains useful bioactive principles, with strong antimicrobial activities. Thus, they may be explored for the development of therapeutic agents.

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