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Antibacterial potential of white crystalline solid from red algae *Porteiria hornemanii* against the plant pathogenic bacteria

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This study was investigated by analyzing the potent bioactive compounds in the chloroform extract of *Porteiria hornemanii* using gas chromatography–mass spectrometry (GC-MS) analysis. The crude 50 µg/ml of white crystalline solid were tested *in vitro* for their antibacterial effect against *Xanthomonas axonopodis* pv. *citri* and *Xanthomonas axonopodis* pv. m*alvacearum* using paper disc diffusion technique showing 10.00 mm and 12.00 zone inhibition respectively, whereas streptomycin sulphate showed 15.00 mm zone and inhibition. The white crystalline solid mainly contained 1,2–Benzenedicarboxylic acid, diisoctyl ester (C_{24} H_{38} O_4) (48.21%) and 1,2–Benzenedicarboxylic acid, bis (2-methylpropyl) ester (C_6 H_{22} O_4) (19.67%).

Key words: Gas chromatography–mass spectrometry (GC-MS), *Porteiria hornemanii,* white crystalline solid, seaweeds.

INTRODUCTION

Intensive application of synthetic pesticides in agriculture caused damage to the ecological state of the agricultural system (Abetz and Young, 1983). Pesticides of biological origin are generally less toxic, affect only the target pest and closely related organisms and are effective in very small quantities which decompose quickly. Published literature reports on the diverse bioactivities of seaweeds, but the antibacterial efficacy of seaweeds against plant pathogens are comparatively a new concept and a few attempts have been made in this regard (Kumar et al., 2008; Kulik, 1995; Arunkumar et al., 2000, 2005; 2010; Arunkumar and Sivakumar, 2012; Manimala and Rengasamy, 1993). Harder (1917) was the first to observe antimicrobial substance in seaweeds. Then until 1970s no large scale screening of antimicrobial activity was carried out (Welch, 1962; Hornsey and Hide, 1974; Henriquez et al., 1979). The Seaweeds are bestowed with varied source of bioactive natural products that exhibits antimicrobial properties against plant pathogens (Arunkumar et al., 2012; Kulik, 1995; Ara et al., 1998; Kumar et al., 2008).

Seaweeds have been identified as a rich source of bioactive compounds (Arunkumar et al., 2010). Seaweeds constituting an important renewable marine resource occur generally on the rocky substratum in the intertidal and subtidal regions of the coastal waters. Ocean has been recognized as a storehouse of fine chemicals.

Several works have been undertaken on crude and purified compounds obtained from seaweeds for

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evaluating their bioactive potential (Faulkner, 1992). A promising strategy for the replacement of chemical pesticides has been the implementations of chemical pesticides and biological control. The recent development in the commercialization of biological control products has accelerated this approach (Fravel et al., 2003). Algae are one of the chief biological agents that have been studied for the control of fungi plant pathogens (Hewedy et al., 2000; Abdel-Kader, 1997). Hence the present investigation was carried out to determine the possible phyto components from *Porteiria hornemanii* and to analyze the potent bioactive white crystalline solid by GC-MS as the first report among the seaweeds.

MATERIALS AND METHODS

Collection of seaweed

About 1 kg of live, healthy and disease free matured seaweed of red alga *P. hornemanii* occurring along the coast of Pamban near Rameswaram, Gulf of Manner, Tamilnadu, India collected during the post–monsoon season (February) in the year 2005 in spring tide was washed thoroughly in seaweeds followed by tap water to remove extraneous materials and sand particles. Identification of the seaweed was carried out at CMFRI, Mandapam, Tamilnadu.

Seaweeds were collected during post monsoon season because Arunkumar and SivaKumar (2012) reported that this season maximum antibacterial activity are found in seaweeds followed by those collected during monsoon, pre–monsoon and seaweeds followed by those collected during monsoon, pre–monsoon and the summer season. The plant pathogenic bacteria *Xanthomonas axonopodies pv.citri* and *Xanthmonas axonopodies* pv. *malvacearum* causing canker in citrus and angular spot in cotton, respectively used in the present were isolated from the parts of the plant.

Extraction of seaweed

Shade dried algae was pulverized and ground as fine powder. The powdered 100 g of algal sample was extracted with 500 ml chloroform: methanol (1:1v/v) in air tight 1 L Erlenmeyer conical flask at room temperature in dark for 1 month and shaken at intervals daily. Extract was filtered through Whatmann No.1 filter paper. 50 mg of anhydrous $MgSO_4$ was added and shook vigorously for 5 min continuously.

Isolation and crystallization

Then the crude extract was filtered using Whatmann No.1 filter paper and kept for evaporation of the solvent under aseptic dark condition in the laboratory without any disturbance for 2 weeks.

During the course of the 2 weeks time colorless crystals starts forming in the bottom of the flask with thick reddish viscous mass. The thick reddish viscous mass was decanted and 16 mg of white solid crystals were obtained and stored at 0°C until for bioassay and GC-MS study.

Antibacterial activity of crystalline compound

A crystal weighing 1 mg dissolved in chloroform was used for bioassay. The antibacterial activity was conducted through agar disc diffusion technique.

Antibacterial assay

Antibacterial activity was determined against the selected plant pathogens using paper disk assay (Potato textrose agar) method (El-Masry et al., 2000). Control disk also maintained for each extract by impregnate respective organic solvent alone. Nutrient Agar (NA) plates (90 mm) were prepared and overnight broth culture (1.2×108 cfu / ml) of test pathogens were inoculated uniformly. Triplicates were maintained for each test pathogen. The plates were incubated at 37°C for 48 h. The zone of inhibition was measured and expressed in mm in diameter. Streptomycin sulphate was used in bioassay study for comparison.

GC-MS analysis

GC-MS analysis on chloroform extract of red algae *P. honemannii*, a white crystalline solid sample was carried out in Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India. GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument was used, employing the following conditions: Column Elite – 5MS fused silica capillary column (30 × 0.25 mm 1 D × 1 µ Mdf, composed if 100% Dimethyl polysiloxne), Operating in electron impact mode at 70 ev, helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 µl was employed (Split ratio of 20:1) injector temperature 250°, ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°/min, to 200°C, then 5°C / min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. Total GC running time is 36 min. The white crystalline solid was dissolved in chloroform and analyzed in GC–MS for different components.

Identification of components

Interpretation of GC–MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, mole under weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

GC–MS: White crystalline components in chloroform extract of *P. hornemanii* by GC–MS report

The presence of chemical components in chloroform extracts of *P. hornemannii* is tabulated (Table 1) and represented by graphical method. The GC–MS analysis resulted in the identification of a total six components in *P. hornemannii*. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are found. The prevailing compound was 1,2 – Benzene dicarboxyllic acid, diisoctyl ester (48.21%) and 1,2 – Benzene dicarboxylic acid, bis (2 methyl propyl) ester (19.67%). The zone of inhibition

Table 1. Antibacterial activity of crystalline solid of red algae *Porteria hornemanii*.

Bacteria	Extract (µg/µl)	Disc diffusion inhibition zone (mm)
Gram negative *Xanthomonas campestries* pv.*citri*	50	12.00
Xanthomonas campestries pv. *malvacearum*	50	10.00
Streptomycin sulphate (positive control)	50	15.00

Table 2. Phyto components in chloroform extract of white crystalline solid from red algae *Porteria hornemannii* by GC–MS.

S/No	RT	Name the compound	Molecular formula	MW	Peak area %
1.	9.55	Octadecane, 3 – ethyl 5 (2 – ethyl butyl)	$C_{26}H_5O_4$	366	0.30
2.	15.70	1,2 – Benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}O_4$	278	19.67
3.	17.24	N – Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.36
4.	25.51	Tert – Hexadecanethiol	$C_{16}H_{34}S$	258	9.68
5.	26.08	1,2 – Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	48.21
6	26.96	Heptacosane	$C_{27}H_{56}$	380	15.78

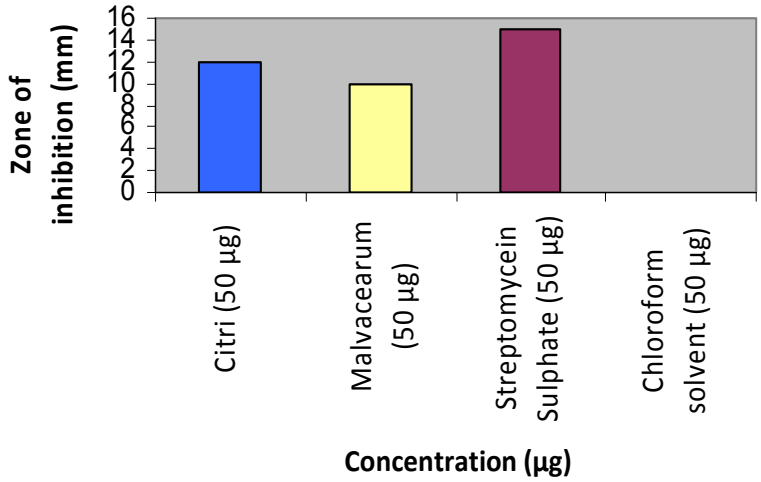


Figure 1. Antibacterial activity of white crystalline solid extract of *Porteria hornemanii* against two plant pathogens.

shown in Figure 1, GC–MS spectrum of chemical compounds shown in Figure 2 and the corresponding chemical shift peaks of the spectrum were shown, whereas the chemical structure is shown in Figure 3.

The chloroform extract, the white crystalline solid of 50 µg/ml showed inhibitory effect on *X. campesteries pv. citri* with inhibition zone of 10.0 mm and *X. campesteries pv. malvacearum* with inhibition zone of 12.00 mm (Table 2). Standard Streptomycin sulphate of 50 µg/ml showed 15.0 mm zone of inhibition. No inhibitory effect was showed by chloroform. This is the first report in the red algae. Marine algae are a rich source of novel bioactive compounds which may find several applications (Aziz et al., 2003; Delattre et al., 2005; Chandia and Mastsuhiro, 2008). Paulert et al. (2007) also found that methanol extracts have *in vitro* activity against the plant pathogenic bacteria *Erwinia carotovora* and *X. cam*pestris as well as human dermatophyte fungus (*Trichrphton mentagrophytes*).

The thin layer chromatography purified fraction of green seaweed *Cladophora glomerata* was subjected to GC-MS analysis of the chemical constituents against gram negative human pathogenic bacteria (Yuvaraj et al., 2011). The GC–MS analysis revealed the presence of hydrocarbons, fatty acids and cholesterol. Olecic acid (1 g. 58%) and n – hexandecanonic (acid 24.73%) and

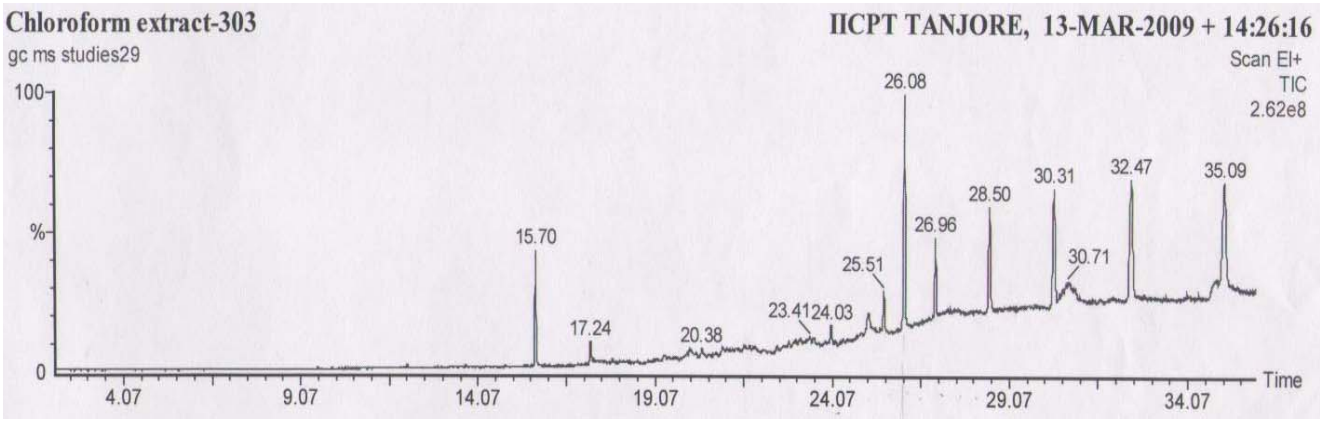
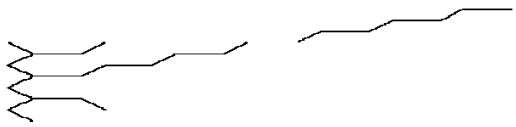
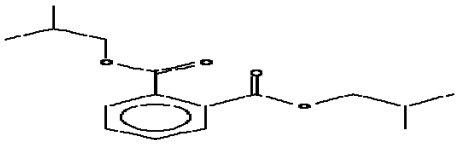


Figure 2. GC-MS analysis spectrum of the photochemical white crystalline solid from the chloroform extract of the red algae *Porteria hornemannii*.

Octadecane, 3 – ethyl 5 (2 – ethyl butyl)

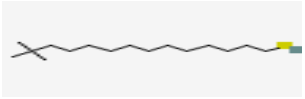


1,2 – Benzene dicarboxylic acid, bis (2-methyl propyl) ester

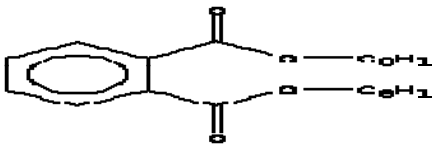


N – Hexadecanoic acid

Tert – Hexadecanethiol



1,2 – Benzenedicarboxylic acid, diisooctyl ester



Heptacosane

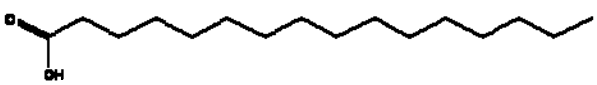


Figure 3. Structures of the white crystalline solid compound.

aromatic dicarboxylic acid were the major components of *Acanthophora spricifera* (Zakaria et al., 2011). Benzenedicarboxylic acid bis (2-ethylhexyl) phthalate has been isolated from a marine alga, *Sargassum weightii*, and apart from its plasticizing ability it was also found to have antibacterial effect on a number of bacteria (Sastry and Rao, 1995).

Conclusion

From the present study, the extraction of major phyto components was observed in chloroform extract of crystalline solid as 1,2 Benzendicarboxylic acid diisooctyl ester (48.21%) of rT 26.08 and 1,2 Benzen dicarboxylic acid, bis (2-methyl propyl) ester (19.37%) rT (15.70), Heptacosane (15.78) rT 26.96 and the components showed potent antibacterial activity against the plant pathogenic bacteria for bio control. This is the frist phytochemical analysis of volatile components performed by GC-MS reported for *P. hornemannii*.

In this study, the crude crystalline compounds and its antimicrobial mechanisms were known and thus further research should be made to identify the single active compound, which would be helpful to the agricultural society.

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Conflict of Interests

The authors have not declared any conflict of interests

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