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RESEARCH ARTICLE

GC- MS analysis and antibacterial potential of white crystalline solid from red algae Portieria hornemannii against the plant pathogenic bacteria Xanthomnas axonopodis pv. citri (Hasse) Vauterin et al. and Xanthomonas campestris pv. malvacearum (smith 1901) dye 1978b

*** S.R.Sivakumar**

Assistant Professor Department of Plant Science Bharathidasan University Trichy – 620024 Tamilnadu India.

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****Corresponding Author***

S.R.Sivakumar
E:mail:srsiva1976@gmail.com

Abstract

The investigation was carried out to determine the possible phyto components from the chloroform: methanol (1:1v/v) extracts of Portieria hornemanii. This study was investigated by analyzing the potent bioactive compounds in the chloroform extract of P.hornemanii using GC-MS analysis. The crude 50µg/ml of white crystalline solid were tested invitro for their antibacterial effect against Xanthomonas axonopodis pv. citri & Xanthomonas campestris pv.malvalearum using paper disc diffusion technique showing 10.00 mm & 12.00 zone inhibition respective, Whereas Streptomycin Sulphate showed 15.00 mm zone & inhibition.

The analysis revealed that P.hornemanii a white crystalline solid mainly 1,2 – benzene dicarboxylic acid, diisoctyl ester (C_{24} H_{38} O_4) (48.21%) and 1,2 – Benzene dicarboxylic acid, bis (2-methyl propyl) ester (C_6 H_{22} O_4) (19.67%). The antibacterial potential of the compound has to be isolated for further structural elucidation and for bio control use to agricultural community.

Introduction:

Novel antimicrobials and other cell inhibitory compounds are examples of natural bioactive compounds which have gained increased interest. The search for bioactive natural products started in the scientific world many years ago and logically went on mostly on land, but late in the 20th century, the oceanic world started to raise interest and has already revealed many novelties. This chapter gives a broad overview of bioactivity in nature; placing special emphasis on exploiting bioactive compounds of marine origin. The concept of biological control has received widespread attention during the last few years. Therefore, the main objective of this work was to look for active substances that could be used as antibacterial agents for plant pathogens.

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.2005., Arunkumar et al.2001., Arunkumar and. Sivakumar, 2012., Arunkumar et al. 2010., 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 betowed with varied source of bioactive natural products that exhibiting antimicrobial properties against plant pathogens (Arunkumar et, 2005, Arunkumar

and Rengasamy 2000 a b; Ara et al; 2005; Arunkumar and Sivakumar 2012; Sivakumar and Arunkumar 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 sub tidal 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 evaluating their bioactive potential (Faulkner, 1992).A promising strategy for the replacement of chemical pesticides has been the implementations of chemical pesticides have been the implementation of bio logical 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 Portieria hornemanii and to analyze the potent bioactive white crystalline solid by GC-MS as the first report among the seaweeds.

Materials and methods

Seaweed Collection site:

The Gulf of Mannar biosphere reserve comprises of 20 islands located in a chain between Tuticorin (8^0 48^0 9E) and Rameswaram (9^0 14^1 N 79^0 14 E) on the South East coast of India. Portieria hornemanii were collected from depths, in very clear water deep lagoons, where the floor is purely sandy and white or grayish in color with good penetration of sun – light during the year 2005.

Collection of Seaweed:

About 1kg of live, healthy and disease free matured seaweed of red alga Portieria hornemanii occurring along the coast of Pamban near Rameswaram, Gulf of Mannar, Tamilnadu, India collected during the post – monsoon season (January, February & March) in the year 2005 in spring tide was washed thoroughly in seaweeds followed by tap water to remove extraneous materials and sand particles. The algae were immediately air – dried under shade at room temperature for 3 days. A preliminary identification of collected seaweed was made on the spot. In the laboratory further identification was carried out at CMFRI, Mandapam, Tamilnadu and herbarium, material was prepared and some specimens were preserved in 4% formalin in seawater.

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

Extraction of Seaweed:

Shade dried algae was pulverized and ground as fine powder. The powdered 100gms of algal sample was extracted with 500ml chloroform: methanol (1:1v/v) in air tight 1 liter 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. 50mg of anhydrous $MgSO_4$ was added and shook vigorously for 5 minutes continuously.

Isolation & 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 yellowish viscous mass. The thick yellowish viscous mass was decanted and 16 mg of white solid crystals were obtained and stored at 0^0c until for bioassay and GC - MS Study.

GC - MS analysis:

GC - MS analysis on chloroform extract of red algae Portieria 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 (30mm x 0.25mm 1D x 1µ Mdf, composed if 100% Dimethyl polysiloxne), Operating in electron impact mode at 70ev, helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 2 µl was employed (Split ratio of 20:1) injector temperature 250^O , ion-source temperature 280^OC .The oven

temperature was programmed from 110^0C (isothermal for 2min), with an increase of 10^0 / min, to 200^0C, then 5^0C / min to 280^0C, ending with a 9 min isothermal at 280^0 C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45-450 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.

Antibacterial activity of crystalline compound:

A crystal weighing 1mg 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 (PDA) method [El-Masry et al.2000]. Whatman No.1 filter paper disk of 6mm diameter absorbent disks was cut and sterilized by autoclaving. Tested compounds were dissolved in chloroform and 50µL of each (50 µg disk-1) was pipetted onto a sterile antibiotic filter disc and placed onto NA medium. The sterile disk was impregnated with different solvent extracts (50µl/per disk). 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 using sterile cotton swab. The impregnated disks were placed on the plates using sterile forceps suitably spaced at equal distance. Triplicates were maintained for each test pathogen. The plates were incubated at 37°C for 48h. The zone of inhibition was measured and expressed in mm in diameter.

Efficacy of isolated compound against Streptomycin Sulphate:

The Efficacy of pure white solid crystal isolated from the red alga Portieria hornemanii was compared with the commercial antibiotics used to control the two bacterial pathogens using agar disc diffusion technique invitro. Each 50 µg of isolated white crystalline solid and antibiotics were loaded on the disc separately was used for this study.

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 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 shown in Fig.1,GC – MS spectrum of chemical compounds shown in Fig.2 and the corresponding chemical shift peaks of the spectrum were shown in Tabble:1, Where as the chemical structure in shown in Fig 3.

The antimicrobial activities of the chloroform extract were evaluated using disc diffusion technique against 2 Gram negative plant pathogenic potential strains. The chloroform extract showed antibacterial activity against gram – negative bacteria (X. axonopodies pv.citri and X. campestris pv. malvacearum) as seen in Table. 2. The white crystalline solid of 50 µg / ml showed inhibitory effect on X. axonopodies pv. citri with inhibition zone of 10.0 mm & X. campesteries pv. malvacearum with inhibition zone of 12.00mm (Table 2). Standard Strepto mycin sulphate of 50 µg / ml showed 15.0mm zone of inhibition. No inhibitory effect was showed by chloroform on bacteria tested (Control). This is the first report in the red algae. Marine algae are a rich source of novel bioactive compounds which may find several applications in aquiculture (Aziz et al. 2003; Delattre et al. 2005; Chandia and Mastsuhiro 2008). Paulert et al (2007) also found that methanol extracts have invitro activity against the plant pathogenic bacteria Erwinia carotovora and Xanthomonas campestris as well as human dermatophyte fungus (Trichrphton mentagrophytes).

The Thin layer chromatography purified fraction of green seaweed Cladophora glomerata subjected to GC - MS analyze of the chemical constituents. against gram negative human pathogenic bacteria (Yuvaraj et at. 2011). The GC – MS analysis revealed the presence of hydrocarbons, fatting acids and cholesterol. Olecic acid (1g.58%)

and n – hexandecanonic (acid 24.73%) and aromatic dicarboxylic acid were the major components of Acanthophora Spricifera (Zakaria et al. 2011). Heptadecane and hexadeeance was reported as the common hydrocarbons found in seaweeds (Sukatar et al. 2006).Most of the hydrocarborn detected have been documented to exhibit inhibitory effect on P. aeruginosa (Silva and kutluca 2005; Wagh et al 2007). In the majority of prior studies, bacterial growth inhibiting activities of different macro algal extracts were investigated on human pathogens (Dubber & Harder 2008).In the present study, 88% of the macro algae displayed antibacterial activity against the two test strains. The antibiotic effect was observed only against 2 bacterial stains from the standard set and less than 80% of inhibition. Although these studies clearly showed that macro algae commonly contain active metabolites with antibacterial properties. (Jormalainer & Honkanen 2008). 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).

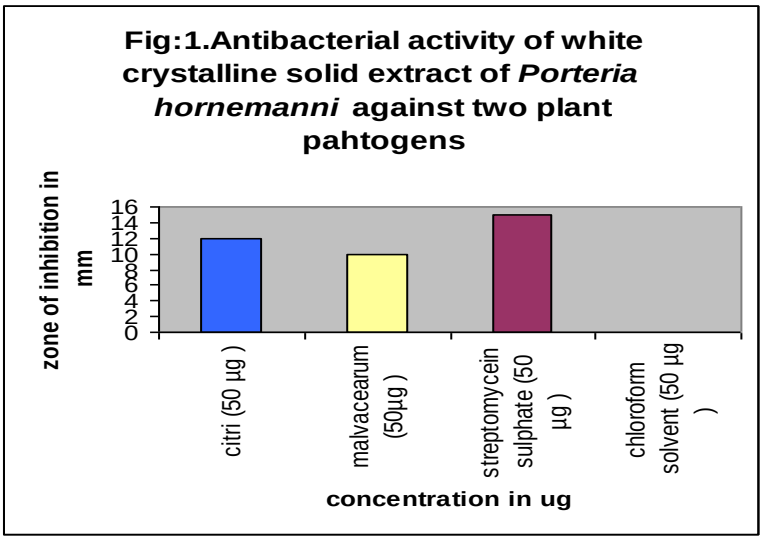

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Fig: 2Fig: 2.GC-MS analysis spectrum of the photochemical white crystalline solid from the chloroform extract of the red algae Portieria hornemannii.

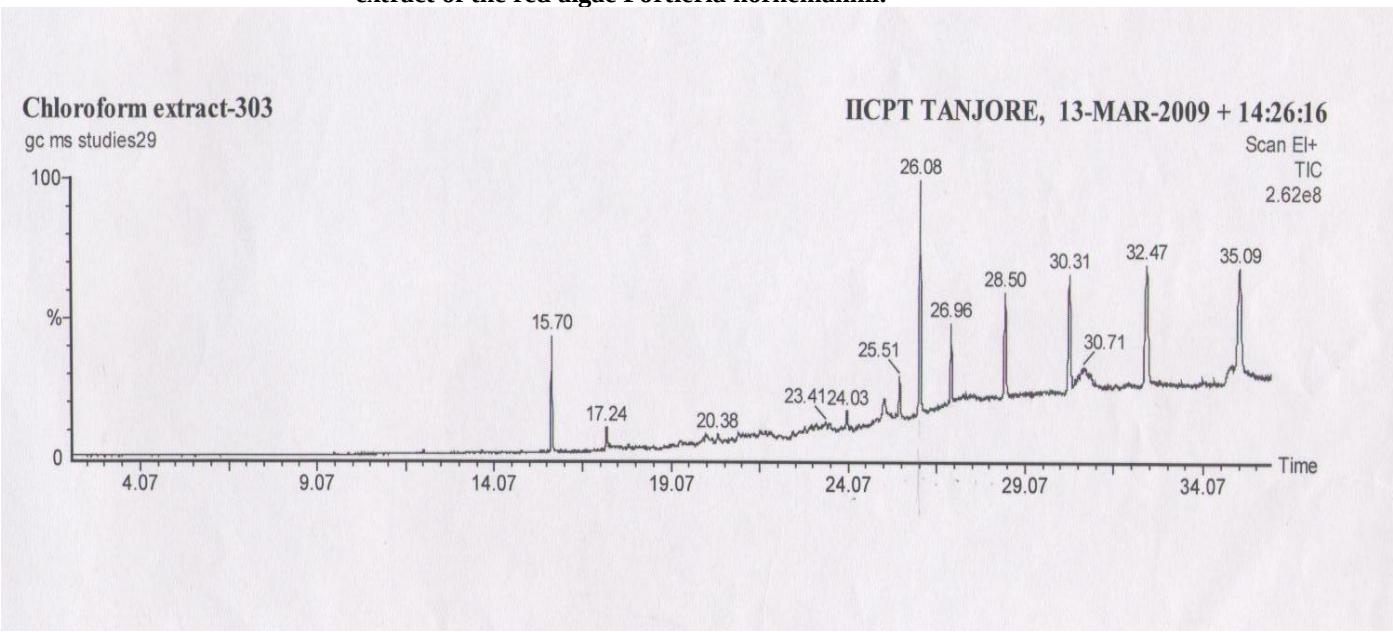
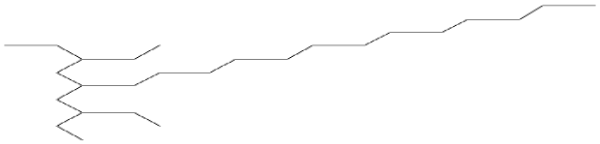


Table :1 Phyto components in chloroform extract of white crystalline solid from red algae *Portieria hornemannii* by GC – MS

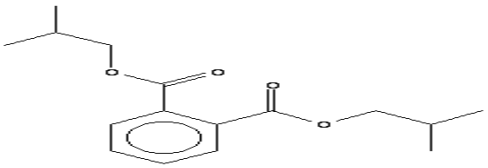
S. No	RT	Name the Compound	Molecular Formula	MW	Peak Area %
1.	9.55	Octadecane, 3 – ethyl 5 (2 – ethyl butyl)	$C_{26}H_5{}^0 4$	366	0.30
2.	15.70	1,2 – Benzene dicarboxylic acid, bis (2-methyl propyl) ester	$C_{16}H_{22}o_4$	278	19.67
3.	17.24	N – Hexadecanoic acid	$C_{16}H_{32}o_2$	256	6.36
4.	25.51	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

Fig:3. Structures of the white crystalline solid compound

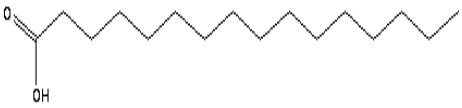
Octadecane, 3 – ethyl 5 (2 – ethyl butyl)



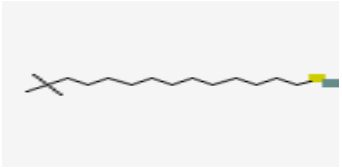
1,2 – Benzene dicarboxylic acid, bis (2-methyl propyl) ester



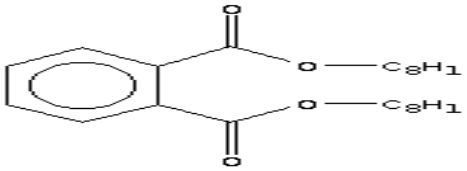
N – Hexadecanoic acid



Tert – Hexadecanethiol



1,2 – Benzenedicarboxylic acid, diisooctyl ester



Heptacosane

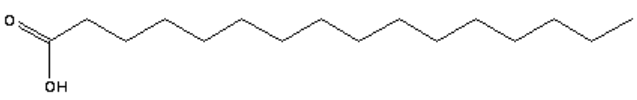


Table: 2 Antibacterial activity of crystalline solid of red algae Portieria hornemanii.

Bacteria	Extract µg/µL	Disc diffusion Inhibition Zone (mm)
Gram negative *Xanthomonas* axonopodies pv.*citri*	50 µg	12.00 mm
Xanthomonas campestries pv. *malvacearum*	50 µg	10.00 mm
Streptomycin sulphate (positive control)	50 µg	15.00 mm
Chloroform solvent (Negative control)	50 µg	NIL

Conclusion:

Form the present study it is concluded that the extraction of major phyto componets was observed in chloroform extract of crystalline solid was 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 phyto chemical analysis of volatile components performed by GC-MS reported for *Portieria 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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