

Original Research Article

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MAA-Palythine Compound of Three Red (Macro) Algae: *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis* of Gulf of Mannar, Southeast Coast of India

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ABSTRACT

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Gulf of Mannar is a unique marine habitat with diverse of macroalgae. They have a rich source of nutrients, biologically active UV absorbing compounds like Mycosporine like Amino acids (MAAs). In this study, red algae *Gelidiella acerosa*, *Acanthophora spicifera* and *Hypnea musciformis* collected from Pudumadam, Gulf of Mannar, southeast coast of India were used for isolation of MAAs. The UV-Vis spectrophotometer analysis of methanol extract of *G. acerosa*, *A. spicifera* and *H. musciformis* showed an absorption peak at λ_{max} between 309–360nm. Further UV-absorbing compounds were identified using RP-HPLC analysis and the compound identified in all three species of red algae is MAA-Palythine type. This serves as an important role in photoprotectant in the marine environment.

Introduction

Mycosporine-like amino acids (MAAs), a family of UV-absorbing compounds have recently much consideration because of the photoprotective molecules against UVR in the aquatic organisms (Rastogi *et al.*, 2010; Hartmann *et al.*, 2015). MAAs are small (244–374 Da) and water soluble UV-absorbing molecules in the wavelength range between 310–365 nm (Oren and Cimerman, 2007). These were occurring in marine, freshwater and terrestrial organisms *i.e.*, cyanobacteria, algae, metazoans etc., (Favre Bonvin *et al.*, 1976; Karentz, 2001; Shick and Dunlap, 2002; Sinha *et al.*, 2007; Hartman *et*

al., 2015). The first description and ‘baptizing’ of Mycosporine refers to *sporulating* terrestrial fungi (Leach, 1965; Whitehead *et al.*, 2001). MAAs are a family of intracellular compounds biosynthesized by shikimic acid pathway for the synthesis of aromatic amino acids involved in the protection of aquatic organisms against solar radiation (Singh *et al.*, 2010; Bhatia *et al.*, 2011).

More than twenty different MAAs are successfully identified and characterized from various organisms (Whitehead and Hedges,

2002; Korbee *et al.*, 2010). Organisms exposed to intense solar radiation have been developed a certain mechanisms such as avoidance, repair and protection by synthesizing or accumulating a series of photo-protective compounds such as MAAs, scytonemin, carotenoids and certain other compounds to counteract the toxicity of UV (mainly UVB) radiation (Sommaruga *et al.*, 2006; Sinha *et al.*, 2007; Singh *et al.*, 2008; Klisch and Hader, 2008; Fleming and Castenholz, 2007).

Macroalgae play an important role in the marine ecosystems. They can provide nutrients, agricultural products, pharmaceutical lead compounds, potential bio-fuels and sun screen biomolecules (Gao and McKinley, 1994; Oreon and Cimerman, 2007). Macroalgae have a well-developed mechanism against Ultra Violet Radiation (UVR) damaging effects (Drollet *et al.*, 1997; Rastogi *et al.*, 2010). Macroalgae show more resistance against UV than terrestrial plants (Yuan *et al.*, 2008; Talarico and Maranzana, 2000; Gao and McKinley, 1994; Oreon and Cimerman, 2007).

A known mechanism of protection involves the synthesis of UV absorbing compounds. UVACs have absorption spectra at λ_{max} between 310–360 nm (Nakamura *et al.*, 1982). As photo-protectants, MAAs in the cells absorb the lethal doses of highly energetic UVR and then dissipating this energy in the form of harmless heat radiation to their surroundings (Oreon and Cimerman, 2007). As nature's sunscreen compounds, MAAs actively excreted and accumulated at the epidermis where they show suncreening effect. Studies on the photodegradation and photophysical characteristics have shown that MAAs are stable and effective sunscreen (Oreon and Cimerman, 2007). A product 'Helioguard 365' from red algae, *Porphyra umbilicalis* has been commercialized

successfully after a large evaluation and they are also of immense importance for human beings to effectively block thymine dimer formation by UVR *in-vitro* and to provide growth stimulation activity in human cells (Bandaranayake, 1998; Schulz and Scherer, 1999; Cockell and Knowland, 1999). The other functions of the MAAs are antioxidants, abiotic stressors, intracellular nitrogen storage etc., (Whitehead and Hedges, 2005; Kogej *et al.*, 2006; Shick *et al.*, 1992; Oreon and Cimerman, 2007), as a result, MAAs has varied ecological and therapeutic significance.

The concentration of UVACs is affected by various factors such as light, depth, temperature etc., (Karsten *et al.*, 1998; Korbee *et al.*, 2004). MAA content shows a negative correlation with water depth, but the ability for MAA synthesis in response to environmental changes is varied among algae (Hoyer *et al.*, 2001). MAAs also act as antioxidants, for example, Mycosporine-glycine shows a moderate antioxidant potential (Dunlap and Yamamoto, 1995; Oreon and Cimerman, 2007). Many macroalgae produce one or more MAAs. Most of MAAs-producing macroalgae belong to Rhodophyceae (red algae), Phaeophyceae (brown algae) and very few in Chlorophyceae (green algae). Different techniques have been involved in the identification and characterization of MAAs (*i.e.*, UV-spectrophotometer, ESI-LC/MS and HPLC analysis) and it is very challenging one (Dunlap and Chalker, 1986; Tartarotti and Sommaruga, 2002; Hartmann *et al.*, 2015). For separation of MAAs, the reversed phase HPLC has been utilized to date (Stochaj *et al.*, 1994; Volkmann and Gorbushina, 2006). Hence the MAAs from macroalgae were separated using RP-HPLC analysis.

Very few reports are available on isolation and identification of Mycosporine like amino acids in marine flora and fauna of Indian

coastal water (Bhandari and Sharma, 2006). There was an increasing amount of Mycosporine like amino acids in cyanobacteria species isolated from corals *Porites* sp., and *Phormidium corium* in Kavarathi reefs of the Lakshadweep islands exposed under UV-B treatment (Bhandari and Sharma, 2010). There was a lack of studies in isolation and identification of MAAs in marine flora and fauna of Gulf of Mannar, except a study done recently by Pandey *et al.*, (2017) and they isolated shinorine, porphyra-334 and palythine types of MAAs from both marine red macro algae, *Gelidium* sp. and *Ceramium* sp., collected from the west coast (Arabian sea) of India. Therefore the present study was aimed to isolate and separate MAA compounds in *G. acerosa*, *A. spicifera* and *H. musciformis* belonging to red algae of Gulf of Mannar, Southeast coast of India.

Materials and Methods

Collection of samples

In this study, Red algae (Rhodophyta) such as *Gelidiella acerosa* (Forsskal) (Feldmann and Hamel, 1934), *Acanthophora spicifera* (Vahl) (Borgesen 1910) and *Hypnea musciformis* (Wulfen) (Lamouroux 1813) were collected during March-April, 2012 from the rocky shore at low tide region in Pudumadam (Long N 9°16.313 Lat E 79°00.073), Gulf of Mannar, Southeast coast of India. The collected samples were immediately rinsed with water to remove all kinds of epiphytes and other impurities. The cleaned samples were immediately kept in sterilized Ziploc bags and transferred to a laboratory for further study.

MAAs extraction

The samples were allowed to shade dry (up to 7 days) and individually made powder form. The powdered sample of each species (5 to 10 gm each) was individually suspended in

100% methanol and kept in horizontal shaker at 25°C for 24 hours. The methanol layer was filtered using Whatman No.1 filter paper. The filtered sample was individually centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was collected in a separate flask. The residue was extracted at least 2 to 3 times until the solution as colourless. At each centrifugation, the supernatant was pooled and kept separately. Then the extract was concentrated using a rotary vacuum evaporator (Super Fit: PMTC-3040) at 40°C. The final concentrated crude extract was individually stored in sterile air tight bottles and kept in a refrigerator until use.

UV-Vis Spectrophotometer Analysis

For the spectral analysis, 1mL of crude extract was individually dissolved in methanol (1:9, v/v). The individual aliquot was read in the absorbance range between 200 to 600 nm at regular interval in double beam UV-Vis spectrophotometer (JASCO-V550). The raw spectral data were analysed by UV-resolution software (Origin Pro 6, Origin Lab Corp., USA).

RP-HPLC analysis

For isolation and identification of MAAs from the crude extract, the analysis was carried out using an HPLC system (PR-8 Column, VIVA C8-5 micrometer 250×46mm) equipped with a diode-array detector using isocratic reverse-phase HPLC.

An aliquot of 20µl of the extract was injected into a C8-column protected with RP8 guard column. During analysis, the samples in the auto sampler were kept at 15°C (SIL 20AHT) while the column was maintained at 20°C.

The mobile phase consisted of 20% aqueous methanol (v/v) and 0.1% acetic acid (v/v) in water at a flow rate of 0.79 mL min⁻¹. Purification peaks were monitored at 330nm.

The compound showing variable peaks were eluted and the identification of the MAA was done by comparing the absorption spectra and retention times of previously published papers.

Results and Discussion

In this study, the presence of UV absorbing compound in the methanol extract of *G. acerosa*, *A. spicifera* and *H. musciformis* collected from Pudumadam, Gulf of Mannar Coast was confirmed by UV-Visible spectral analysis and the absorption peaks were obtained in the range between 300 nm and 360 nm.

Based on this UV- spectral analysis, the MAA peak was observed at λ_{\max} 320 nm in all three algal samples (Fig. 1). Generally, MAA is abundantly present in macroalgae of tropical/polar regions (Banaszak and Lesser, 1995; Karsten *et al.*, 1998a). The MAAs were abundant in Rhodophyceae (red algae) (Xiong *et al.*, 1999).

The low concentration of (MAAs) palythine, porphyra-334, shinorine, Mycosporine-glycine, astesina-330, palythinol and palythenelusujirene like MAA compounds found in *Palmaria decipiens*, *Iridea* sp. and *Porphyra columbina* (Carreto *et al.*, 2005). Based on this statement, the present study was carried out to choosing the macroalgae possess UV absorbing compounds.

Normally the MAAs are detected in white, yellow and blue light regions. Generally the MAA- Palythine compound was exposed in blue light regions at λ_{\max} 320nm (Franklin *et al.*, 2001).

Further, the compound was identified using the spectrum obtained from RP-HPLC analysis. The absorbance peak of *G. acerosa*, *A. spicifera* and *H. musciformis* were eluted at R_t (Retention time) of 3.194 min, 3.215 min

and 3.198 min, respectively, which exhibited λ_{\max} at 320nm (Figs. 2 to 4). Based on the result of UV-Vis spectrophotometer and RP-HPLC analysis, the identified compound was MAA-palythine.

Tsujino *et al.*, (1998) isolated Palythine compound from the aqueous ethanol extract of *Chondrus yondoi* which is stable under acidic conditions and the structure was confirmed by 1D NMR and IR measurements and the absorption peak appears at 320 nm (ϵ 35,500). Three different types of MAAs such as shinorine (λ_{\max} = 333.5 nm), porphyra-334 (λ_{\max} =332.3 nm) and palythine (λ_{\max} =317.9 nm) having retention times (RT) 1.26, 2.12 and 3.64 min, respectively, from *Gelidium* sp. and shinorine (λ_{\max} =332.3 nm), porphyra-334 (λ_{\max} =333.5 nm) and palythinol (λ_{\max} =332.5 nm) with RT 1.27, 2.13 and 4.61 min, respectively, from *Ceramium* sp., collected from the West Coast (Arabian Sea) of India (Pandey *et al.*, 2017)

The lyophilized methanol extract of *Palmaria palmata* found high polar MAAs *i.e.*, palythine, shinorine, asterina-330 and porphyra and these compounds were separated and identified with the help of RP-HPLC in λ_{\max} 330 (Yuan *et al.*, 2009; Carignan and Carreto, 2013; Sung-Suk *et al.*, 2014).

Generally the lead MAAs were mostly obtained from UV- stress condition in dinoflagellate (Garcia-Pichel and Castenholz, 1993; Klisch and Hader, 2002; Rosic and Dove, 2011). Similarly, the MAAs and palythine compounds were identified in red alga *Bryocladia* sp and *Chondrus crispus* under UV-A radiation (Riegger and Robinson, 1997; Kannaujiya *et al.*, 2014). Similar type of MAA compound was also identified in some Antarctic algae, cyanobacteria (Saman Mushir and Tasneem Fatma, 2011).

Fig.1 UV-Vis spectrophotometer absorbance of Palythine compound at λ_{max} 320nm in methanol extracts of *G. acerosa*, *A. spicifera* and *H. musciformis*

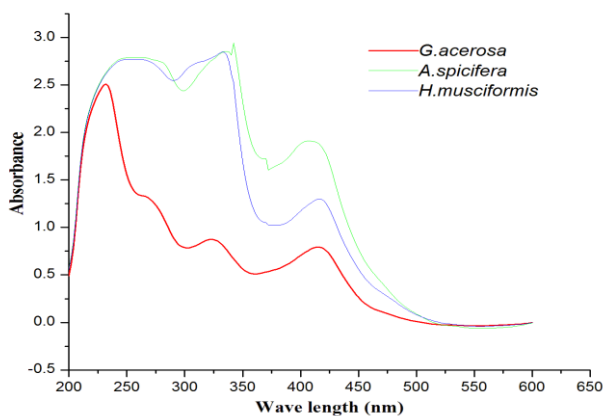


Fig.2 HPLC chromatogram of *G. acerosa* showing the peak for Palythine compound at R_t : 3.194 min

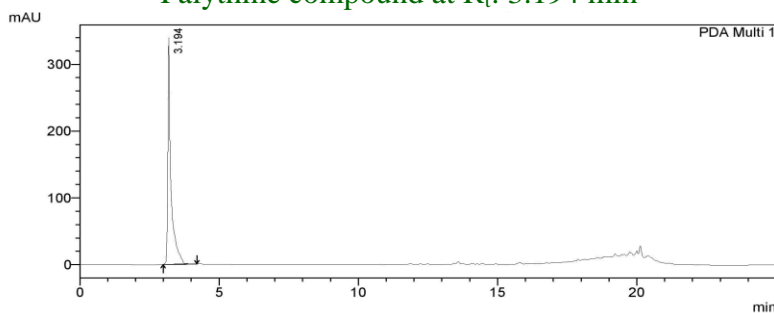


Fig.3 HPLC chromatogram of *A. spicifera* showing the peak for Palythine compound at R_t : 3.215 min

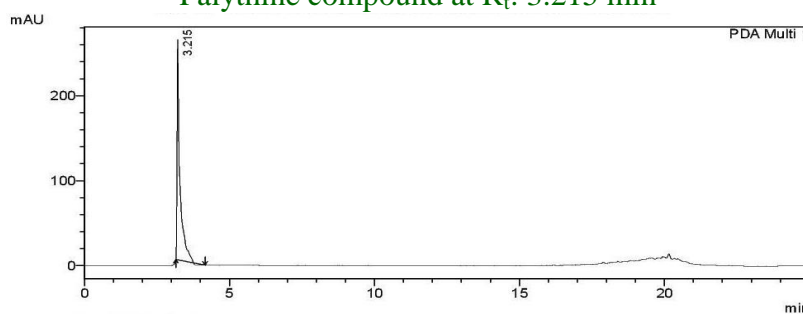
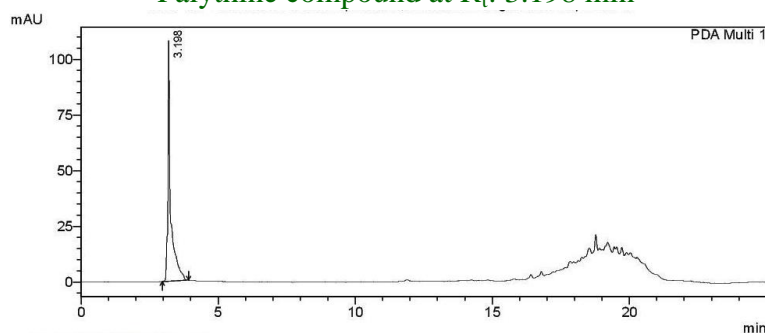


Fig.4 HPLC chromatogram of *H. musciformis* showing the peak for Palythine compound at R_t : 3.198 min



The present investigation on the isolation and identification of MAAs in the methanol extract of *G. acerosa*, *A. spicifera* and *H. musciformis* confirmed that all three species of red algae have a single type of MAA Palythine compound with absorption peak strongly showed λ_{\max} at 320nm.

The comprehensive results strongly designate that the red algae *G. acerosa*, *A. spicifera* and *H. musciformis* of Gulf of Mannar, southeast coast of India found to have a rich source of MAA-Palythine type photoprotective compound.

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Conflict of interest statement

We declare that we have no conflict of interest.

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