

Antioxidant Activity and Gas Chromatographic-Mass Spectrometric Analysis of Extracts of the Marine Algae, *Caulerpa peltata* and *Padina gymnospora*

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Murugan and Iyer: Antioxidant Activity and GC-MS Analysis of Marine Algae

The results of our previous investigations on extracts of selected marine algae showed that *Caulerpa peltata* and *Padina gymnospora* had more promising antiproliferative and antioxidant activities than *Gelidiella acerosa* and *Sargassum wightii*. Based on these results, the more active chloroform extract of *C. peltata* and ethyl acetate extract of *P. gymnospora* were further analyzed for their constituents by using gas chromatography in tandem with mass spectrometry. The GC-MS analysis (GC % peak area given in parentheses) showed that fucosterol (12.45%) and L-(+)-ascorbic acid 2,6-dihexadecanoate (8.13%) were the major compounds present in *P. gymnospora* ethyl acetate extract. On the other hand, *C. peltata* chloroform extract had 1-heptacosanol (10.52%), hexacosanol acetate (9.28%), tetradecyl ester of chloroacetic acid (7.22%), Z,Z-6,28-heptatriacontadien-2-one (6.77%) and 10,13-dimethyl-methyl ester of tetradecanoic acid (5.34%) as major compounds. Also described in the report are the beta-carotene bleaching inhibitory and total reducing activities of the chloroform and ethyl acetate extracts of *C. peltata* and *P. gymnospora*, respectively, relative to the other three extracts (aqueous, methanol, chloroform or ethyl acetate) of the two algae.

Key words: GC-MS, antiproliferative, marine algae, *Caulerpa peltata*, *Padina gymnospora*

In recent times, marine algae have been gaining importance as sources of pharmacologically active constituents possessing antioxidant, antiproliferative, antimutagenic, antidiabetic, anticoagulant, antibacterial and antitumor activities^[1,2]. Exploration for bioactive compounds led to the screening of selected marine algae from the Tamil Nadu coast for antiproliferative

and antioxidant activities^[3]. The results of that study indicated that the chloroform (C) extract of *Caulerpa peltata* J.V. Lamouroux (green alga belonging to Chlorophyta) and ethyl acetate (E) extract of *Padina gymnospora* (Kützinger) Sonder (brown alga belonging to Heterokontophyta) showed significant antiproliferative (in four different human cancer cell lines) and antioxidant (DPPH radical-scavenging and ferrous ion-chelating) activities. GC-MS is an easy and rapid method for analyzing the different constituents

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present in an unknown sample. This method has also been employed in the identification of compounds in crude extracts of plants and other natural sources. Based on the results of both the previous^[3] and the current investigations, the C extract of *C. peltata* and E extract of *P. gymnospora* were subjected to GC-MS analysis. Lipid peroxidation is mediated by reactive oxygen species (ROS) causing cellular damage, eventually leading to carcinogenesis. β -Carotene bleaching (BCB) inhibition involves a coupled auto-oxidation of linoleic acid- β -carotene to form peroxy radicals and bleaching of the yellow colour of β -carotene. Also, the overall reducing/antioxidant capacity of the extracts was determined with the total reducing activity (TRA) assay. Hence, the BCB inhibition and TRA assays were performed for the C and E extracts of *C. peltata* and *P. gymnospora*, respectively, along with other three extracts.

Caulerpa peltata and *Padina gymnospora* were collected between Mar-Jul 2011, from the Mandapam coast of Tamil Nadu, and were authenticated (Central Marine Fisheries Research Institute, Mandapam). As reported earlier by Murugan and Iyer, 2013, washed, shade-dried and powdered algae were separately extracted with four different solvents such as methanol, chloroform, ethyl acetate and distilled water to give M, C, E and A extracts, which were further used for the assays^[3].

Based on the higher antiproliferative activity in four different cell lines and antioxidant activities obtained from the previous study, the C extract of *C. peltata* and E extract of *P. gymnospora* were subjected to GC-MS analysis. GC-MS analysis of the crude extracts was carried out by using Perkin Elmer-Claruss

680 model with the specific column Elite 5MS ((30 m \times 0.25 mm ID and 250 μ m film thickness, 95% dimethylpolysiloxane) operating in an electron impact mode at 70 eV with helium as a carrier gas at a flow rate of 1 ml/min. The injection volume was 1 μ l (split ratio 10:1); temperature was set at 250 $^{\circ}$. The ion source and transfer temperatures were 230 $^{\circ}$. The oven temperature was set from 60 to 300 $^{\circ}$ with a 10 $^{\circ}$ increase per min, ending with a 6 min hold at 300 $^{\circ}$ and a total run time of 32 min. Mass spectra were recorded with fragments ranging from 50 to 600 Da. The spectrum obtained after GC-MS analysis was interpreted and compared with the National Institute Standard and Technology (NIST) compounds library^[4].

The C extract of *C. peltata* and E extract of *P. gymnospora* were examined for BCB inhibition and TRA, and compared with their other three extracts (M, C, E or A). The assays were performed according to the previously reported method^[5]. All the chemicals and solvents used for the assays were obtained from SRL, Mumbai.

All the assays were performed at least three times (n=3) and the results have been expressed as mean \pm standard error of mean (SEM). The assays were compared by using one way analysis of variance (ANOVA), followed by Tukey's post-hoc test. $P < 0.05$ indicates statistical significance. All statistical analyses were performed using GraphPad Prism 5 software.

The chromatograms of the GC-MS analysis of *C. peltata* C extract and *P. gymnospora* E extract are shown in figs. 1a and b. When the peaks obtained were compared with those in the NIST libraries, eight compounds were identified in the *C. peltata*

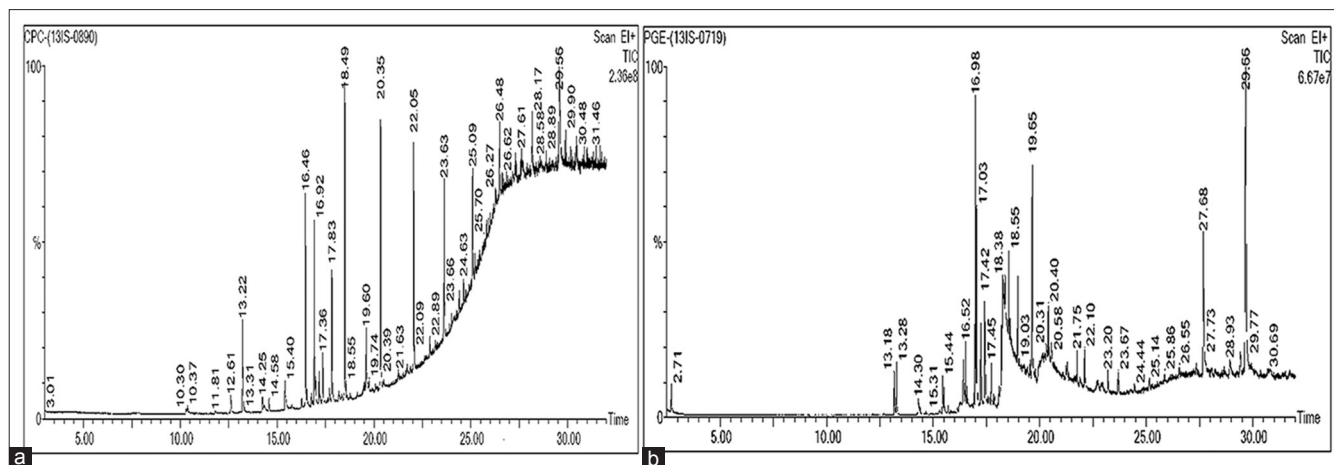


Fig. 1: Chromatograms of the GC-MS analysis. Chromatograms of the GC-MS analysis of (a) *C. peltata* chloroform extract and (b) *P. gymnospora* ethyl acetate extract.

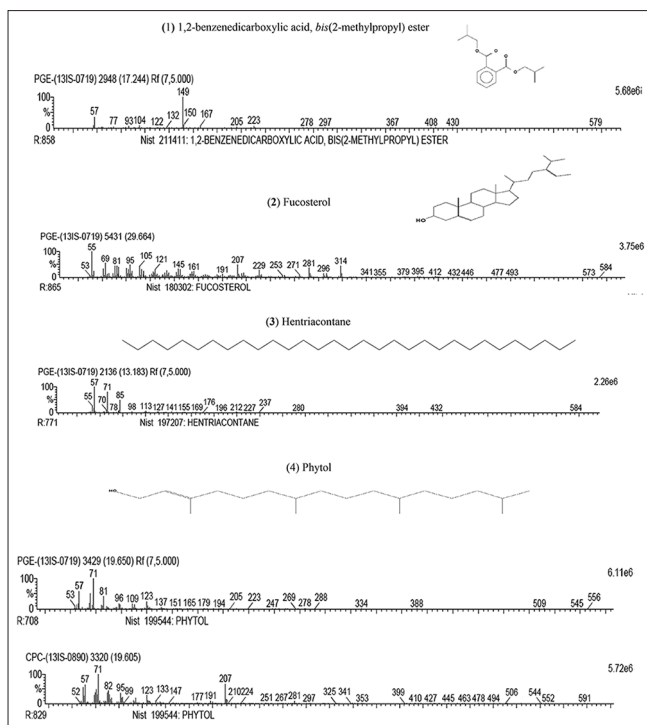


Fig. 2: Mass spectra and structures of the compounds identified from *C. peltata* and *P. gymnospora*.

Mass spectra and structures of the discussed compounds identified from *C. peltata* chloroform extract and *P. gymnospora* ethyl acetate extract.

C extract and *P. gymnospora* E extract was found to contain 16 compounds. These compounds were mostly alcohols, esters and long chain hydrocarbons. Fig. 2 displays structures for compounds (1-4) which have been reported earlier in literature in other marine algae and are discussed below. Compounds (1-3) are present in *P. gymnospora* ethyl acetate extract and (4) is present in both extracts. Compound (1), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (fig. 2), was found to be present in *P. gymnospora* E extract. This compound was also reported in GC-MS analysis of a hexane extract of the antimicrobial red marine alga, *Acanthophora spicifera* (Vahl) Borgesen^[6].

The GC-MS profile of the E extract of the brown alga, *P. gymnospora*, showed the presence of (2) fucosterol (fig. 2) with the highest peak area of 12.45%. Fucosterol has been reported to be a major sterol in brown algae, which is consistent with the results of the present study^[7]. Literature reports are available on several biological activities on fucosterol, including antioxidant, cytotoxic, antidiabetic and acetylcholinesterase (ACE) inhibitory activities. A fucosterol isolated from the hexane fraction of the brown marine alga, *Sargassum angustifolium*

C. Agardh was found to be cytotoxic against T47D (breast ductal carcinoma) and HT29 (colon carcinoma) cell lines^[8]. Similarly, antioxidant and antidiabetic activities of fucosterol isolated from another brown marine alga, *Pelvetia siliquosa* Tseng and Chang, have been reported^[9,10]. In 1995, Al Easa *et al.*, reported the presence of fucosterol in *P. gymnospora* collected from Qatar coast^[11]. Therefore, it could be hypothesized that the antioxidant and antiproliferative activities of the E extract of *P. gymnospora* (collected from the Tamil Nadu coast), observed from the earlier study by Murugan and Iyer, 2013, could be due to the presence of this major component, fucosterol.

A hydrocarbon compound, (3) hentriacontane (fig. 2), which was found in *P. gymnospora* E extract in this study, has already been reported to be a constituent of Phaeophyceae (brown algae)^[12]. In an earlier study, the straight chain hydrocarbon, hentriacontane, was found to be present in the Japanese fermented soy food-“*Natto*” when analyzed by gas chromatography, which was responsible for antitumor activity^[13].

The compound (4) phytol was found in both *C. peltata* C extract and *P. gymnospora* E extract (fig. 2). Phytol, a diterpene alcohol, is a constituent of chlorophyll and is used as a precursor in manufacturing vitamins E and K. It was reported that phytol metabolites activate transcription factors and nuclear receptors^[14]. In 2013, a study conducted by Silva *et al.*, phytol was found to inhibit inflammatory response by reducing cytokine production and oxidative stress^[15]. Xiao *et al.* described the extraction of phytol from a nonaqueous two-phase solvent system of two edible marine algae, namely, *Undaria pinnatifida* (Harvey) Suringar and *Sargassum fusiforme* (Harvey) Setchell^[16].

Tables 1 and 2 show the list of compounds present in *C. peltata* C extract and *P. gymnospora* E extract, respectively, apart from the four compounds discussed above and were found to be present in natural sources (fungi and higher plants) other than marine algae. Some of these compounds are reported to have biological activities including antiinflammatory, antioxidant and antibacterial.

Results for the BCB inhibition and TRA assays have been shown for all four extracts of both *C. peltata* and *P. gymnospora* (data for *G. acerosa* and *S. wightii* have not been shown). In both BCB inhibition and TRA assays, the C extract of *C. peltata* showed higher

TABLE 1: COMPOUNDS IDENTIFIED BY GC-MS IN *C. PELTATA* CHLOROFORM EXTRACT AND THEIR NATURAL OCCURRENCE

| RT (min) | Compound | Mol. formula | Mol. weight | Peak area (%) | Sources | Activity |
|----------|--|---|-------------|---------------|--|--|
| 13.22 | 3,5-bis (1,1-dimethylethyl)-phenol | C ₁₄ H ₂₂ O | 206 | 4.27 | <i>Monochaetia kansesis</i> ^[17] | Antiinflammatory ^[18] |
| 15.41 | cis-2,4-dimethylthiane, S, S-dioxide | C ₇ H ₁₄ O ₂ S | 162 | 2.54 | <i>Ixora coccinea</i> | Antioxidant* ^[19] |
| 16.47 | Chloroacetic acid, tetradecyl ester | C ₁₆ H ₃₁ O ₂ Cl | 290 | 7.22 | <i>Ixora coccinea</i> | Antioxidant* ^[19] |
| 16.92 | Z,Z-6,28-heptatriactontadien-2-one | C ₃₇ H ₇₀ O | 530 | 6.77 | <i>Mukia maderaspatana</i> | Vasodilatory ^[20] |
| 17.83 | Tetradecanoic acid 10,13-dimethyl-, methyl ester | C ₁₇ H ₃₄ O ₂ | 270 | 5.34 | <i>Actinodaphne maderaspatana</i> ^[21] | Not found |
| 18.49 | 1-heptacosanol | C ₂₇ H ₅₆ O | 396 | 10.52 | <i>Eupatorium odoratum</i> <i>Buddleja crispa</i> | Antibacterial*, Antioxidant* ^[22] Nematicidal* ^[23] |
| 20.34 | Hexacosanol, acetate | C ₂₈ H ₅₆ O ₂ | 424 | 9.28 | <i>Solanecio manii</i> | Antimicrobial ^[24] |

*Activity is related to the source or its extract and not the compound; RT: retention time

TABLE 2: COMPOUNDS IDENTIFIED BY GC-MS IN *P. GYMNOSPORA* ETHYL ACETATE EXTRACT AND THEIR NATURAL OCCURRENCE

| RT (min) | Compound | Mol. formula | Mol. weight | Peak area (%) | Sources | Activity |
|----------|--|---|-------------|---------------|---|---|
| 13.29 | 2,6-bis (1,1-dimethylethyl) phenol | C ₁₄ H ₂₂ O | 206 | 1.14 | <i>Monochaetia kansesis</i> ^[17] | Antiinflammatory ^[18] |
| 15.45 | 3-n-hexylthiolane, S, S-dioxide | C ₁₀ H ₂₀ O ₂ S | 204 | 0.79 | <i>Hibiscus rosa-sinensis</i> | Antioxidant* ^[25] |
| 16.53 | Chloroacetic acid, tetradecyl ester | C ₁₆ H ₃₁ O ₂ Cl | 290 | 1.03 | <i>Ixora coccinea</i> | Antioxidant* ^[19] |
| 16.98 | Z,Z-6, 28-heptatriactontadien-2-one | C ₃₇ H ₇₀ O | 530 | 5.10 | <i>Mukia maderaspatana</i> | Vasodilatory ^[20] |
| 17.04 | 3-methyl-2-(2-oxopropyl) furan | C ₈ H ₁₀ O ₂ | 138 | 3.21 | <i>Mallotus tetracoccus</i> | Antipyretic*, Anti-inflammatory* ^[26] Hepatoprotective* ^[27] |
| 18.27 | L-(+)-ascorbic acid 2,6-dihexadecanoate | C ₃₈ H ₆₈ O ₈ | 652 | 8.13 | <i>Alstomia boonei Nelumbo nucifera</i> | Antioxidant ^[28] |
| 18.55 | Hexacosanol, acetate | C ₂₈ H ₅₆ O ₂ | 424 | 4.43 | <i>Solanecio manii</i> | Antimicrobial ^[24] |
| 18.97 | 5,8,11,14- eicosatetraenoic acid, methyl ester, (all Z)- | C ₂₁ H ₃₄ O ₂ | 318 | 2.68 | <i>Michelia champaca</i> | Antimicrobial*, Antioxidant*, Anticancer* ^[29] |
| 21.75 | Tetrahydro-6-nonyl-2H pyran-2-one, | C ₁₄ H ₂₆ O ₂ | 226 | 0.69 | <i>Parkia Speciosa</i> | Antidiabetic*, Antibacterail*, Mitogenic* ^[30] |
| 22.10 | Dotriacontyl pentafluoropropionate | C ₃₅ H ₆₅ O ₂ F ₅ | 612 | 0.61 | <i>Schizophylum commune</i> | Cytotoxic* ^[31] |
| 27.68 | 3-methoxy- (3.beta)- cholest-5-ene | C ₂₈ H ₄₈ O | 400 | 4.77 | <i>Orthosiphon tomentosus</i> ^[32] <i>Kalanchoe pinnata</i> ^[33] | Not found |
| 29.42 | 9,10-dihydro-9,10-diethyl anthracene | C ₁₈ H ₂₀ | 236 | 1.06 | <i>Cassia sp.</i> | Antioxidant ^[34] |

*Activity is related to the source or its extract and not the compound; RT: retention time

activity ($P < 0.05$) than the other three extracts (E, M and A). Among the extracts of *P. gymnospora*, the M extract showed the highest activity ($P < 0.05$), followed by C and E extracts for the BCB inhibition assay, whereas the A extract showed the highest activity ($P < 0.05$) for TRA. The M, C, E extracts of *P. gymnospora* showed almost similar TRA values (fig. 3a-b). The overall results suggest that these compounds (discussed above and in Table 1) present in the C extract of *C. peltata* might have been responsible for the observed BCB inhibition and TRA activities, whereas the higher TRA of the A extract of *P. gymnospora* could be attributed to the presence of higher amounts of reducing sugars and polysaccharides. However, although the BCB inhibition of the M extract of *P. gymnospora* was higher than that of its other extracts, the E extract was subjected to GC-MS analysis based on its greater activity in the previous study^[3].

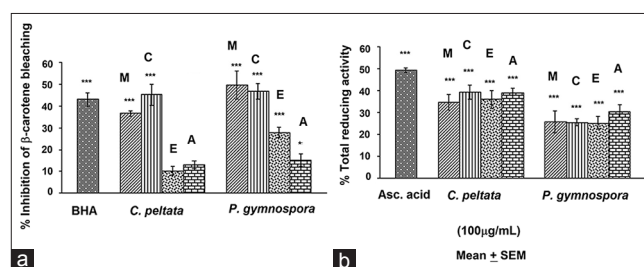


Fig. 3: Antioxidant activity of *C. peltata* and *P. gymnospora* extracts. Antioxidant activity of *C. peltata* and *P. gymnospora* extracts (a) % β-carotene bleaching inhibition (b) % total reducing activity. M- methanol, C- chloroform, E- ethyl acetate, A- aqueous extracts. Data have been represented as mean ± SEM. Asterisks indicate level of significance ($P < 0.05$).

The present study was carried out to identify the compounds present in *C. peltata* C and *P. gymnospora* E extracts by using GC-MS, and to compare the BCB inhibition and TRA of these extracts with the other solvent extracts of the two algae (M, C, E or A).

This is the first report of these compounds present in the C and E extracts of *C. peltata* and *P. gymnospora* collected from the Tamil Nadu coast.

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