

**Research Article****Quantification of physicochemical and identification of bioactive compounds from marine red alga *Gracilaria corticata* J. Ag.**Jenifer P.<sup>1</sup>, C. P. Balakrishnan<sup>2\*</sup>, S. Chidambaram Pillai<sup>3</sup><sup>1</sup>Research Scholar, PG and Research Department of Botany, V.O. Chidambaram College, Tuticorin- 628 008, Affiliation of Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamil Nadu, India<sup>2\*</sup>Assistant Professor, Department of Botany, Aditanar College of Arts and Science, Virapandianpatnam, Tiruchendur - 628 216, Tamil Nadu, India<sup>3</sup>Associate Professor, PG and Research Department of Botany, V.O. Chidambaram College, Tuticorin- 628 008, Tamil Nadu, India

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**Abstract**

**Objective:** The present study was aimed to investigate the quantification of physicochemical and identification of bioactive compound 1, 2 Benzenedicarboxylic acid from methanolic extract of red alga *Gracilaria corticata* J. Ag. **Material and methods:** Quantification of physicochemical was carried out according to the standard method. To separate and identify the bioactive constituent by using Column chromatography (CC), GC-MS and NMR were performed. **Results:** The present study showed that physicochemical parameters such as moisture content, ash value, extractive values and crude fibre were presence at the potential of drug quality. In GC-MS spectrum a major compound revealed that 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (RT 31.54, Peak area 23.15%). The phytochemical compounds are confirmed by NMR (<sup>1</sup>H and <sup>13</sup>C) analysis. **Conclusion:** It can be concluded that the study was characterized the bioactive constituent present in methanolic extract of this seaweed which will be useful its proper identification.

**Keywords:** *G. corticata*, physicochemical, bioactive compound, CC, GC-MS, NMR

**Introduction**

All plants produce chemical compounds as part of their normal metabolic activities. These include primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites found in a smaller range of plants, some useful compound found only in a particular genus or species. In the sea, three types of plants occur and they are phytoplanktons, seaweeds or marine macroalgae and seagrasses. Macroalgae are eukaryotic thallophytes attached on the rocky substratum and are recognized as a potential source of bioactive natural products. Seaweeds form one of the important marine living renewable resources. They are most abundant in shallow coastal areas, especially where they are exposed at low tide. Marine macro

algae are considered as an excellent source of bioactive compounds which has a broad range of biological activities are reported by (Burkholder et al., 1960; Mahasneh et al., 1995; Tuney et al., 2006; Karthikaidevi et al., 2009; Alghazeer et al., 2013; Boonchum et al., 2011).

In this present study was carried out quantification of physicochemical, identification and confirmation of bioactive compound 1,2 Benzenedicarboxylic acid by using Column chromatography, GC-MS and NMR of methanolic extract of *G. corticata* of Manapad Coast, Tamil Nadu India.

**Materials and methods****Collection and preparation of seaweed**

Marine red algae *Gracilaria corticata* J. Agardh was collected from Manapad coast of Tamil Nadu, India (8.3775°N; 78.0522°E) at low tide. Specimen was washed thoroughly in seawater to remove extraneous matter such as epiphytes and sand. After collection, fresh sample was taken into plastic jar and brought back to the laboratory immediately. The collected specimen was authenticated and

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preserved in the form of herbaria (ACBHR29). Sample was washed by tap water for several times, then gently brushed and rinsed with distilled water and then dried at room temperature. The dried seaweed powder was stored in refrigerator for further uses.

### Physicochemical analysis

The dried powdered sample was used to physicochemical analysis such as total ash, acid insoluble ash, water soluble ash value, different solvent (benzene, chloroform, petroleum ether, acetone and methanol) extractive values, and moisture content were determined as per method described Anonymous, (1996), and crude fibres were determined by Sadasivam and Manickam (1992). All determinations were performed at least in triplicate. The analyzed data were expressed as mean with standard deviation (SD).

### Separation of compounds from the extract

#### Extraction

The 10g of powder samples were subjected to extraction with methanol (40ml) (British Drug Houses (AR)) grade solvent (E. Merck India Ltd., Mumbai, India) in a Soxhlet extractor (Borosilicate Brand, The Science House, Chennai, India) for six hours and the extraction was repeated twice. The extract was then concentrated to dryness under reduced pressure and controlled temperature (40-50°C). The resultant residue was stored in a refrigerator till further use.

#### Column Chromatography (CC)

Column Chromatography is common and useful separation technique in organic chemistry. The column (30cm x 1cm) was cleaned and dried. A wad of cotton was placed on top of the glass wool. The adsorbent (silica gel 60-120 mesh Merck) was measured into the column. The side of the column was tamped to ensure even packing. The solvent was carefully poured into the column and to eliminate air packets the column was gently tamped again. The solvent was drained until the level of the solvent was to the top of the adsorbent. The extract was dissolved in the solvent and applied carefully and evenly unto the adsorbent by means of a pipette. Cotton wool was placed on the sample to prevent the sample from being disturbed. A pool of solvent was carefully added and the stopcock was opened. The eluted purified compound was collected as 10 ml fractions at a flow rate of one drop per second.

#### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The dried seaweed powder sample was extracted with methanol, using soxhlet extractor. The extract which is obtained in concentrated with rotary evaporator till dry powder was obtained. The final concentrated extract analyses by using GC-MS. GC-MS analysis was carried out on equipment Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental

conditions of GC-MS system were as follows: ZB 5-MS capillary standard non-polar column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25 $\mu$ m. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 70°C raised to 260°C at 6°C/min and injection volume was 1  $\mu$ l. A sample dissolved in methanol was run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme.

#### Identification of Components

Interpretation of mass spectrum 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, molecular weight and structure of the components of the test materials were ascertained.

#### NMR

The 2 to 40 mg quantity of purified compound from methanol extract of seaweed *G. corticata* was identified by  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra dissolved in  $\text{CDCl}_3$ , were recorded at Bruker 300 MHz spectrometer equipped with a 5 mm probe. Chemical shifts were expressed as part per million (ppm)  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR used to determine the purified compound.

#### Statistical Analysis

All experiments were carried out in triplicate (n=3). The results were presented as mean  $\pm$  SE standard error using SPSS statistics 17.0 software.

#### Results and discussion

The physicochemical parameters are mainly used in judging the purity and quality of the drug. The dried red seaweed powder *G. corticata* was carried out to the physicochemical analysis such as moisture content, total ash value, acid insoluble ash value, water soluble ash value, different solvent like petroleum ether, benzene, chloroform, acetone and methanol extractive value and crude fibre were determined is presented in table 1. The less value of moisture content could prevent microorganism growth. The moisture content of *G. corticata* was 22.9 $\pm$ 0.162%. Ash value is useful in determination authenticity and purity of sample and also these values are of important qualitative standards. Total ash, acid insoluble and water soluble ash value of *G. corticata* was 42.3 $\pm$ 1.47%, 20.53 $\pm$ 0.18% and 23.72 $\pm$ 0.02% were respectively. Then the solvent extractive value like petroleum ether, benzene, chloroform, acetone and methanol was 0.65 $\pm$ 0.01%, 0.26 $\pm$ 0.01%,

**Table 1.** The percentage weight of moisture content, ash values, different solvents soluble extractive values and crude fibre of red algae *G. corticata*

S. No.	Parameters	Percentage content
1	Moisture content	22.9 ± 0.162
2	Total ash	42.3±1.47
3	Acid insoluble ash	20.53±0.18
4	Water soluble ash	23.72±0.02
5	Petroleum ether extractive value	0.65±0.01
6	Benzene extractive value	0.26±0.01
7	Chloroform extractive value	0.64±0.01
8	Acetone extractive value	0.94±0.01
9	Methanol extractive value	9.83±0.03
10	Crude fibre (dry wt.)	33.6±1.027

All values are means of triplicate determinations ± standard deviation (SD).

**Table 2.** Major Phytocompound, their nature and their biological activity of methanol extract of red algae *G. corticata* by GC-MS analysis

RT	Name of the phytoconstituents	Peak Area %	Compound Nature	Bioactivity*
31.5	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl)ester	23.15	Plasticizer compound	Antifungal, Anti retroviral, Anti tumor, Anti diabetic, Anti cancer, Antioxidant, Antiscabies, Antiinflammatory, Potent antimicrobial agent

\*Source: Dr. Duke's: Phytochemical and Ethnobotanical Databases

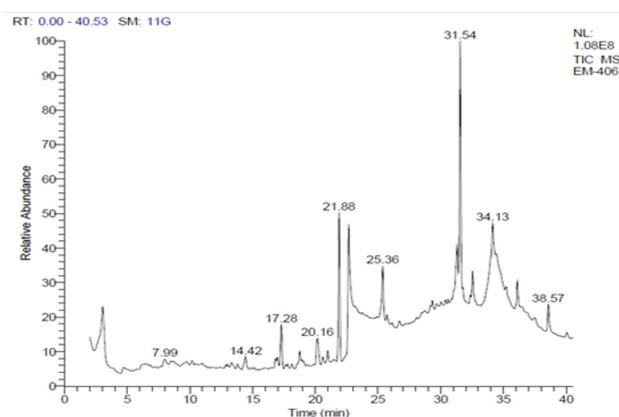
0.64±0.01%, 0.94±0.01%, and 9.83±0.03% respectively. Ash value is useful in determination authenticity and purity of sample and also these values are of important qualitative standards. Extractive value is useful for the evaluation of a crude drug as it gives idea about the present nature of chemical constituents (Lincy and Mathew, 2011). The results suggest that the powdered dried seaweed have high methanol soluble extractive value. The crude fibre content was 33.6±1.027% recorded at that can be determined the nutritive value of the seaweed. Crude fibre consists largely of cellulose, lignin and some mineral matter. The consumption of dietary fibers and plant cell walls containing such fibre components protects human organisms against a number of chronic diseases e.g., colon cancer reported by Guidel-Urbano and Goni, 2002.

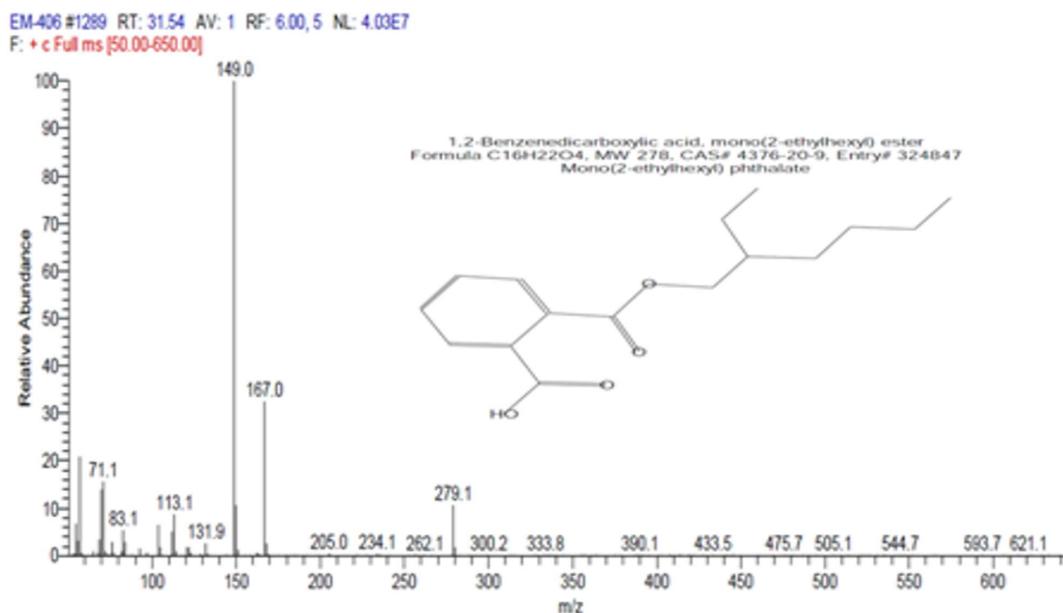
In the GC-MS analysis leads to the prediction of chemical constituent present in the methanol extract of *G. corticata* showed in (figure 1). The GC-MS analysis showed thirty peaks are obtained among these, the identification of the bioactive

compound 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester was prominent peak confirmed based on the active principles of major compound was identifying with retention time (RT), molecular formula (MF), molecular weight (MW) and peak area in percentage (%) were tabulated (Table 2). The bioactivity of the identifying phytoconstituent is based on the reference of Dr. Duke's Phytochemical and Ethno botanical Data bases by Dr. Jim Duke of the Agricultural Research Service/USDA.

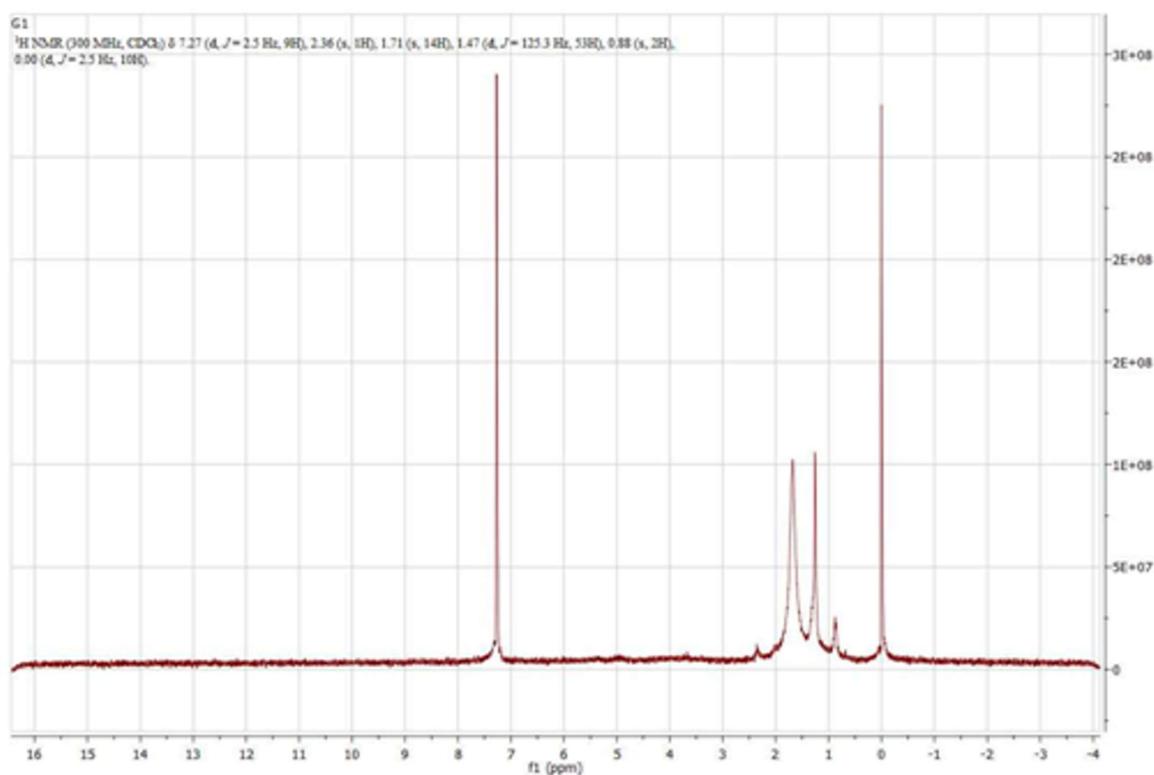
Jenifer and Balakrishnan (2015) have reported anti-pesticidal and anti-nematicidal compounds are indentified from *G. corticata* by using GC-MS profile. The results of the present study supported and supplemented the previous observations on the medicinally important plants (Akpuaka et al., 2013; Janakiraman et al., 2012; Sangeetha and Vijayalakshmi, 2011; Purushoth et al., 2013).

The proton NMR is used to find out types of hydrogen present in the compound and to find out how the hydrogen atoms are connected. The <sup>1</sup>H-NMR profiles of *G. corticata* spectral data were present in figure 3. The chemical shift values are as (400 MHz, CDC13) δ 7.27 (d, J = 2.5 Hz 9H, aromatic), δ 2.36 (s, 1H, CH<sub>3</sub>), δ 1.71 (s, 14H, allylic), δ 1.47 (d, J=125.3Hz, 53H, Alkyl), δ 0.88 (s, 2H, Alkyl). The <sup>13</sup>C-NMR profiles of *G. corticata* spectral data were present in figure 4. The chemical shift values are as (75MHz, CDC13): δ162.84 (s) (C=O, acids and esters), δ162.49 (s) (C=O, acids and esters), δ162.13 (s) (C=O, acids and esters), δ 119.42 (C=C, aromatic ring), δ104.44 (C=C, aromatic ring), δ78.89-78.04 (m) (C-O, R-CH<sub>2</sub>-OH), δ77.79-76.94 (C-O, R-CH<sub>2</sub>-OH), δ60.21 (C-O, R-CH<sub>2</sub>-OH), δ30.26 (C-C, R<sub>3</sub>-CH). Based on the spectral data, the alkyl and aromatic organic compounds presence the samples that prevailing compound was identified as 1, 2- benzene dicarboxylic acid, mono (2-ethylhexyl) ester with the molecular formula of C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>, the structure of the purified compound (figure 2).

**Figure 1.** GC-MS chromatogram of *G. corticata* in methanolic extract



**Figure 2.** Major compounds isolated from *G. corticata*. Structure of 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (C<sub>16</sub>H<sub>22</sub>O<sub>4</sub>) (RT: 31.54) by Mass spectrum



**Figure 3.** <sup>1</sup>H NMR spectra of *G. corticata* extract

## Conclusion

Results of this investigation *G. corticata* can be used as antimicrobial agent based on the Dr. Duke's Phytochemical and Ethnobotanical databases. The nature of the find bioactive compound 1, 2 Benzenedicarboxylic acid was confirmed by NMR studies and also examined physic-chemicals were agreed *G. corticata* have rich potential source. Further work of this

study will emphasize the purification of bioactive compound and its cytotoxicity analysis.

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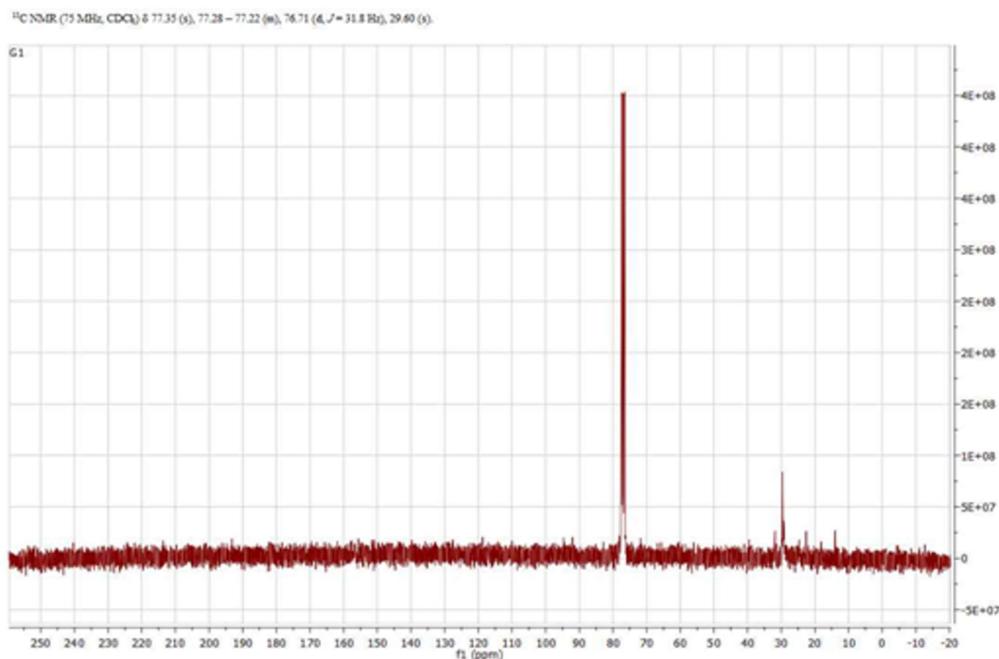


Figure 4.  $C^{13}$  NMR spectra of *G. corticata* extract

**Conflicts of interest:** Nil

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