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Phytochemical, HPLC and FTIR Analysis of Methanolic Extract from *Gracilaria dura* (C Agardh) J Agardh.

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ABSTRACT

Marine algae are known to contain a wide variety of bioactive compounds. They are also rich in novel biomolecules and can be explored for the development of drugs to combat lifestyle diseases like cancer, diabetes etc. Red algae are known for their nutraceutical and functional importance. But there are a lot of limitations regarding their availability and in estimating which algal fractions are biologically active. Similarly, the mode of digestion of such compounds in human body is not yet properly traced. In this juncture, the present study was aimed to evaluate the phytochemical screening of the methanolic extract of the red alga, *Gracilaria dura*. Methanolic extract of *G. dura* showed the presence of reducing sugar, flavonoids, glycosides, tannins and terpenoids. Further, the HPLC analysis was attempted to fractionate the polyphenolics. Various phenolic acids such as of gallic acid, vanillic acid, sinapic acid, p-coumaric acid, hydroxybenzoic, phloroglucinol, catechol and cinnamic acid were identified. Subsequently, the methanolic solvent extract of *G. dura* was subjected to fourier transform infrared spectroscopy for the analysis of the functional groups. The results based on the spectral data of FTIR revealed the presence of aliphatic constituents containing alkanes, ketones, alkyl halides, hydroxyl groups etc. Thus, the observed finding envisages that methanolic extract of *G. dura* contained potential bioactive compounds which can be used for analysing the various biological activities.

Keywords: *Gracilaria dura*, Phytochemical, Methanolic extract, HPLC, FTIR

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1. INTRODUCTION

The marine ecosystem is a unique source of bioactive natural products, many of which exhibit structural features not found in terrestrial natural products. Seaweeds also have the valuable medicinal components such as antibiotics, laxatives, anticoagulants, antiulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by native people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contains significant amount of the protein, vitamins and minerals essential for the human nutrition[1]. Seaweeds contain pool of secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, steroids which have been extensively used in the drug and pharmaceutical industries[2]. The demand of marine organisms as a potential and a promising source of pharmaceutical agents has increased during recent years. Numerous pharmacological studies on marine algae have reported that the chemical compounds produced from them had a wide range of biological activities such as anti-inflammatory, anticancer, anti-HIV, antimutagenic and scavenging free radicals[3,4]. Bioactive rich marine seaweeds could potentially be exploited as functional ingredients for both human and animal health applications. Seaweeds are rich in antioxidant such as carotenoids, pigments, polyphenols, enzymes and diverse functional polysaccharide[5]. Phytochemicals are the markers for medicinal potentiality of plants. These are non-nutritive chemicals that have protected human beings from various diseases. So, phytochemical analysis of the seaweeds will be an ideal basic approach to further isolation and purification of secondary metabolite constituents and evaluation of their medicinal values. For centuries, many of the seaweed secondary metabolites have been used in traditional medicines due to their documented therapeutic potentials[6]. The components reported previously were sterols (some are fucosterol), different molecules containing vinyl and ethyl cholesterol types, cyclohexane and some sulfated polysaccharides fucoidan, neutral glucan and guluronic and mannuronic acid residues containing alginic acid with

pharmaceutical and cosmetic values[7]. Recent studies on marine macroalgae have contributed to the isolation and chemical determination of several compounds, including fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, and flavonoids. Phenolic compounds are commonly found in edible brown, green and red seaweeds, whose antioxidant properties have been correlated with their type of phenolic contents[8]. The presence of these secondary metabolites in seaweeds is highly evident of their resultant pharmaceutical potentials. Recently, Francavilla et al.[9] found that *Gracilaria* species harvested may be used as an alternative source of natural porous material with several biotechnological applications. *Gracilaria* species were used as food stuff in Japan, Hawai and Philippines. In recent years, it is getting more and more consideration, not only owing to its food value, but also for the progress of research of its potential pharmaceuticals. Further, Budarin et al.,[10] found that microwave induced pyrolysis of this macroalga produced chemical rich bio-oils which are rich in aromatics, sugars and other high value chemicals. There were several reports on antimicrobial and pharmacological activities of different solvent extracts from marine algae[11,12]. But no reports are available on intensive phytochemical studies using HPLC and FTIR studies on the marine seaweed *G. dura*. In this line, the present study was aimed to evaluate the phytochemicals, RP-HPLC analysis of phenolic acids, FT-IR analysis of methanolic extracts of the red seaweed *Gracilaria dura*.

2. MATERIALS AND METHODS

2.1 Sample collection and preparation

The marine algae *Gracilaria dura* was collected during June 2017, from the Mandapam coast (latitude 9° 17' N, longitude79° 22' E), Gulf of Mannar. The thalli were then cut into pieces, shade dried and powdered in a grinder to 40-mesh size powder. The ground samples were then kept in air-tight container and stored until for further analysis. The extraction was done by soxhlet apparatus (Methanol as solvent). The extracts was evaporated to total dryness by vacuum distillation and preserved in refrigerated condition for further analysis.

2.2 Preliminary Phytochemical Analysis

Phytochemical analysis was performed according to the standard protocol of Balachandran et al[13]. The prepared seaweed extract were subjected to preliminary phytochemical screening for the presence of reducing sugar, flavinoids, glycosides, lignin, saponins, steroids, tannins and terpenoids.

2.3 Determination of total carbohydrate

1ml of the sample was added to 5 mL of 2.5 N HCl for 3 h. It was neutralized with sodium carbonate and centrifuged. 4 mL of anthrone reagent was added. The absorbance was recorded at 630 nm.

2.4 Determination of protein

The amount of protein present in the algal extract was calculated spectrophotometrically at 670 nm after 30 min using appropriate blank[14].

2.5 Determination of total phenolic content

The amount of total phenolics in the seaweed was determined with the Folin-Ciocalteau reagent. Gallic acid was used as standard and the total phenol were expressed as mg/g gallic acid equivalents (GAE). The blue colour was measured spectrophotometrically at 765 nm and expressed as mg GAE/g of the sample.

2.6 HPLC analysis

HPLC method was performed on Shimadzu LC-10 AT VP HPLC system, equipped with LC-10AT pump, UV–VIS detector an auto injector SIL-10AT. A Hypersil -BDS C-18 column with C-18 guard column was used. An isocratic HPLC with wo LC-10 AT VP pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M10A VP, CTO-10AS VP column oven (Shimadzu), SCL-10A VP system controller (Shimadzu) and reverse phase Luna5-C18 (2) column 250 mm x 4.6 mm was used. The mobile phase consists of Potassium hydrogen phosphate and acetonitrile in a ratio of 75:25 at a flow rate of 1 ml/min which yielded a column backup pressure of 260–270 kgf/cm^2. The column temperature was maintained at 27 ºC. 20 ml of the respective sample was injected using Rheodyne syringe. The elution was carried out with gradient solvent systems with a flow rate of 1 ml /min at an ambient temperature (25–28 ºC). The sample injection volume was 20 µl whilst the wavelength of the UV–VIS detector was set at 254 nm.

2.7 FT-IR analysis

The FT-IR studies have been followed as per the method described by Diem[15]. Dried powder of different solvent extracts of algae was used for FTIR analysis. 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet. IR spectra region between 4000-400 cm-1 were recorded at room temperature on a perkin Elmer fourier transform spectrometer equipped an air cooled DTGs (deuterated triglycine sulfate) detector The frequencies for all sharp bands were accurate to 0.01 cm^{-1}.

3. RESULT AND DISCUSSION

Phytochemicals like polyphenolic groups such as flavonoids, terpenoids were growing interest because of their potent antioxidant potentiality[16]. The phytochemicals noticed in the red algae are known for their anti-inflammatory, anti-diabetic and analgesic activities and also supportive of central nervous system activity. Qualitative analysis of methanolic extract of *G. dura* shows the presence of reducing sugar, flavonoids, glycosides, tannins and terpenoids. Interestingly, the terpenoids was present in remarkable levels (Table 1).

Table 1: Qualitative analysis of phytochemicals from the methanolic solvent extract of *G. dura*

	Methanolic extract
Reducing sugar	++
Flavonoids	++
Glycosides	+
Lignin	-
Saponins	-
Steroids	-
Tannins	+
Terpenoids	+++

+++ = abundant, ++ = moderate, + = low, - = absent

The importance of alkaloids, phenols and glycosides as microbicidal against common pathogenic strains has been reported by Sharma et al[17]. Terpenoids from seaweeds displayed wide spectrum of activities such as cytotoxic, nematicidal and anti-tumour. Seaweeds have afforded to date the highest number of compounds having various biological activities within a single group of organisms. Among the red algal metabolites, dehydrothyrsiferol a triterpenoid polyether isolated from *Laurencia viridis* has exhibited promising cytotoxic activity. They show cytotoxic

effect against human estrogen receptor of breast cancer cell lines[18].

Carbohydrate content forms the major component in *G. dura* (dry weight basis) i.e., 110 mg/g DW. The protein content was 221mg /g DW. The obtained results were almost similar to previous reports in other sea weeds i.e., carbohydrate and protein content was comparable to other green and red seaweeds[16]. In addition, the phytochemical screening also showed the presence of phenols in substantial amount i.e., 1.305 ± 0.03 mg gallic acid equivalent / g dry seaweed. Previous studies reported that the total phenolic contents among the green, brown and red seaweeds displayed much variation. The green seaweeds have higher free-radical scavenging properties, followed by the red and brown seaweeds[19].

Further, the HPLC analysis was carried out to fractionate polyphenolics components present in the extract. The C18 column was used to fractionate the polyphenolic contents. From the Fig. 1, it can be interpreted that a good separation was achieved within a short period of time. For the polyphenolic standards various symmetrical, sharp and well-resolved peaks were observed. The elution order and the quantified volume of gallic, vanillic, sinapic, p-coumaric, hydroxybenzoic, phloroglucinol, catechol and cinnamic acid were 339.01, 722.34, 632.05, 84.61, 163.57, 184.32, 82.99 and 435.22 µg/g respectively (Table:2, Fig.1). Vanillic acid was found to be in higher level followed by sinapic acid, whereas coumaric acid and catechol were the lowest. Phenolic acids proved their beneficial effects in terms of antioxidant, anti-ageing and protect cardiovascular issues. Further, they help to reduce oxidative stress, lipid peroxidation, free radical generation and cholesterol-oxidation of low density lipoprotein (LDL) content[16].

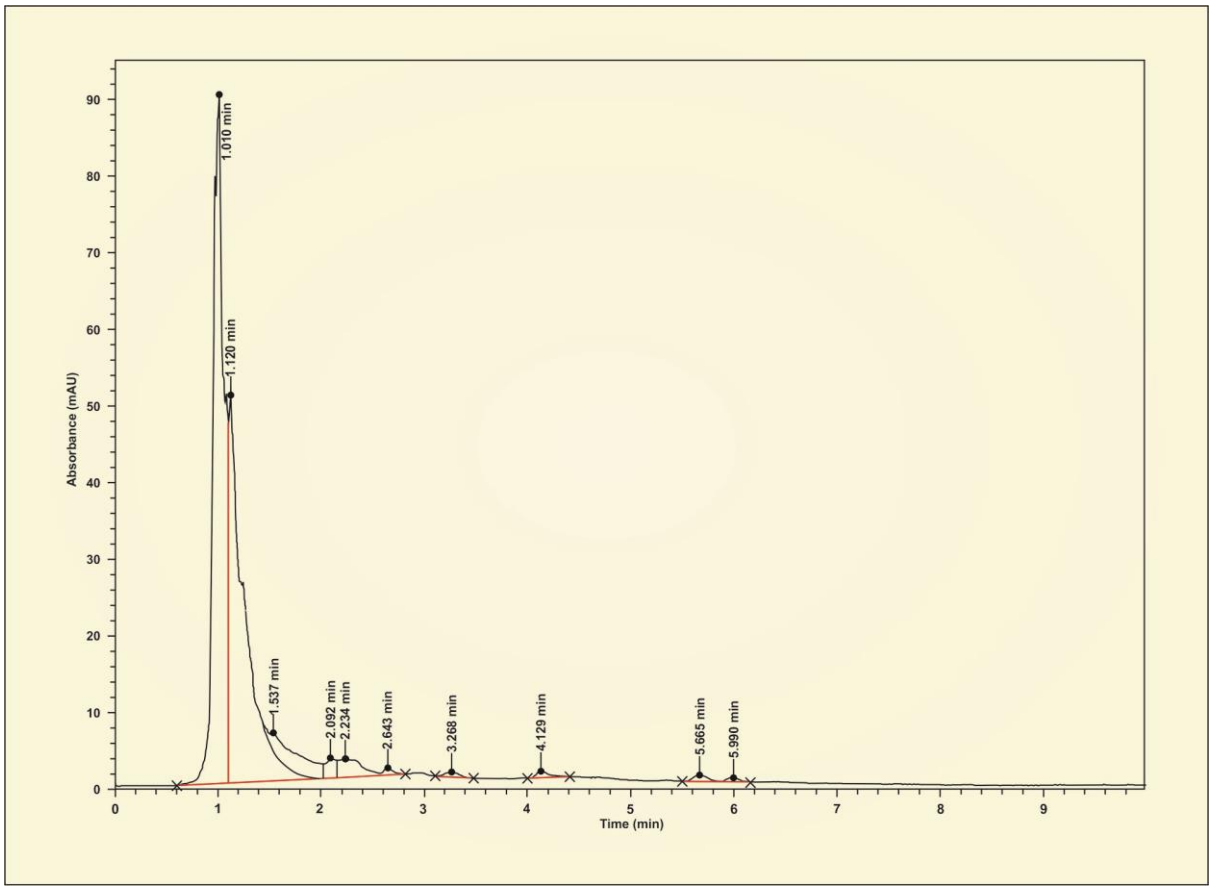

Figure 1: HPLC chromatogram of methanolic extract of *G. dura*

Table 2: RP-HPLC profile of phenolic compounds in *G. dura*

Compound	Concentrations (µg/g)
Gallic acid	339.01
Vanillic acid	722.34
Sinapic acid	632.05
p-coumaric	84.61
Hydroxybenzoic	163.57
Phloroglucinol	184.32
Catechol	82.99
Cinnamic acid	435.22

Phenolic compounds are found remarkably high in medicinal plants. They were also present in seaweeds, and have been reported to have wide range of biological activities including antioxidant[20]. The HPLC analysis of *Gracilaria corticata* and *Spirulina platensis* was correlated with the bioactive compounds vs antioxidant activity. The HPLC analysis of the algae *Amphiroa anceps* revealed that the polyphenolic compounds present in them were an effective source of antioxidants. Therefore, the seaweed extracts may have potential applications in the food industries. Nagai and Yukimoto, also reported that the phenolic compounds are one of the most effective antioxidants[21].

The FTIR analysis of the methanolic extract of *G. dura* confirmed the presence of amines1^0& 2^0, alkenes, alkanes aldehydes, ketones, carboxylic acids, aromatic amines, alkylhalides, aliphatic amines, terpenoids, anthraquiniones (Fig:2; Table: 3).

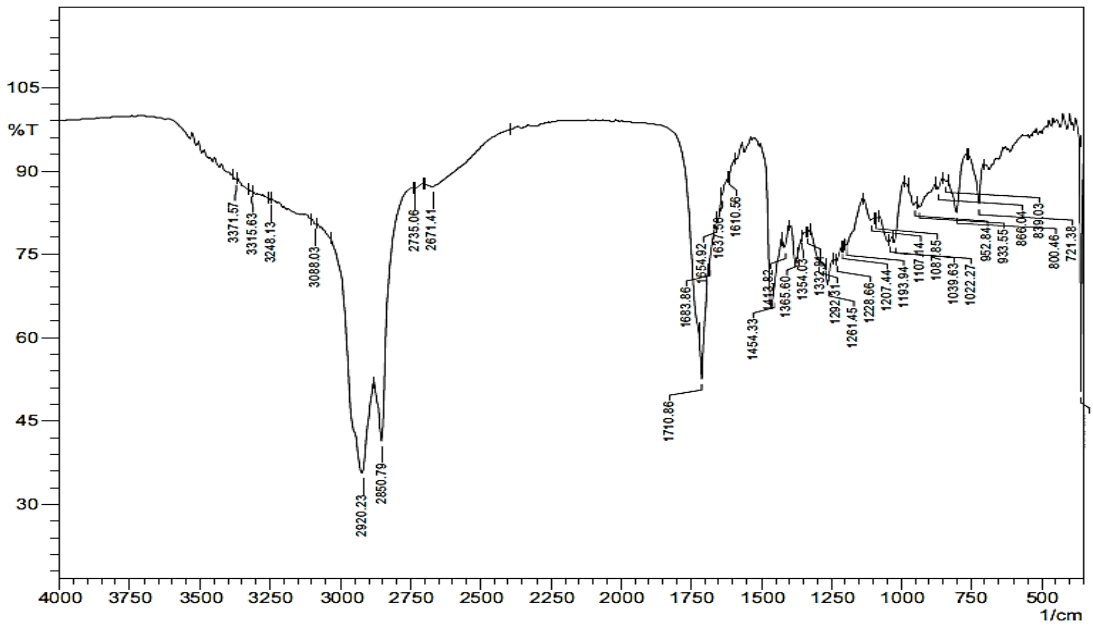

Figure 2: FTIR spectrum of methanolic extract of *G. dura*

Table 3: FT-IR profile of functional groups (cm-1) in methanolic extract of *G. dura*

Functional groups	Peaks	Bond
Amines	3371.57,3315.63,3248.13,1637.56,1610.56	N-H stretch
10& 20 amines	952.84,935.55,866.04,839.03	N-H bend,N-H wag
Alkanes	2920.23,2850.791365.60,1354.03,721.38	C-H Stretch,C-H rock
	1462.04	H-C-H bend
Alkenes	3088.03,1610.56,1637.56,1654.92	C=C-H , C=C-C symmetric stretch
Aldehydes	2850.79,2735.06,	C-H Stretch of C=O
	1710.86,1683.68,1654.92,1637.56	C=O Stretch
Ketones	1654.92,1683.68,1710.86	C=O Stretch
Carboxylic acids	2735.06,2671.41	H bonded OH stretch
	1710.86,1683.68,1654.92,935.55	C=O stretch, OH bend
Aromatic amines	1332.81,1292.31,1261.45	C-N stretch
Alkyl halides	1228.66,1207.44,1193.94	C-H wag
	866.04,839.03,800.46,721.38	C-Cl stretch
Alipatic amines	1107.14,1087.85,1039.63,1022.27	C-N stretch
Terpenoids	2850.79	C=O stretch
Anthraquinones	1261.45	C=O stretch

Similar findings were documented in brown algae like *Sargassum wightii*. Thus, the obtained data of *G. dura* corroborates with *S. wightii*[22]. Currently, FTIR spectroscopy become more popular developed method due to its ease of sample preparation and requires little samples size, and does not require the use of solvents. Most FTIR studies on the extracts of seaweeds revealed the interaction of toxic sites such as carboxyl, amino acid and hydroxyl groups. Like other biological molecules, algae show complex vibrational spectra in all bands. But metal legend stretching frequencies and properties of functional groups coordinated with be toxic centres that provide useful information. C–O stretching, NH_2 rocking, C-O and CH_2 stretching bands are metal sensitive and are shifted as the metal is changed, but NH_2 vibrations are very sensitive to the intermolecular interactions and its effect makes it difficult to discuss the strength of the metal-nitrogen bond from the frequency shift[23]. Similar FTIR studies on the fresh water algae *Spirulina* powder also confirmed the presence of several functional groups that causes improvement of the health status and in normalizing

the atrazine toxicity[23, 24]. The results of the FTIR spectrum confirmed the polyphenols and are potential antioxidants. Free radical scavenging effect of *Morinda citrifolia* fruit extract was correlated with the functional groups[25]. The presence of the bioactive compounds in the plant is significant for their medicinal properties. Phenol and other organic constituents in *Spilanthus acmella* which is an acutely threatened medicinal plant was also revealed by the FTIR data [26]. Numerous studies have showed that there are many research utilizing the FTIR for the analysis of bioactive compounds. These include research in food technology, pharmaceutical and medicinal. For example, the medicinal herb, *Clitoria ternatae*, revealed the presence of bioactive compounds such as alcohols, phenols, primary, secondary amines, carboxylic acids and nitro compounds and was validated by the FTIR spectra[27]. These phytochemicals noticed in leaves and flowers of this plant can be utilized as an alternative source of natural antioxidants.

4. CONCLUSION

The phytochemical investigation suggest that the marine red alga *G. dura* contains important phytochemicals like reducing sugar, flavonoids, glycosides, tannins and terpenoid, which may contribute to its biological activities. The present results clearly indicate that *G. dura* methanolic extract possess significant phenolic acids and therefore capable of antioxidant activities like synthetic drug ascorbate. FT-IR spectroscopy of the methanolic solvent extract of *G. dura* reveals diverse functional groups and there by confirmed the possible potential antioxidant activity and free radical scavenging properties. Future perspectives include isolation, purification of lead molecule and to justify its biological potentialities.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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