Phytochemical and Gas Chromatography-mass spectrometry Analysis of ethanol Extracts of *Gracilaria edulis*

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Abstract

Seaweeds are potential renewable resources in the marine environment. Marine algae have been gaining importance as sources of pharmacologically active constituents possessing antioxidant, antiproliferative, antimutagenic, antidiabetic, anticoagulant, antibacterial and antitumor activities. The present study was performed to investigate the phytochemical constituents of *Gracilaria edulis*. Phytochemical screening of the various extracts was carried out according to the standard methods. To identify the functional constituents present in the effective fractions, the GC- MS analysis were performed. *Gracilaria edulis* showed the steroid, triterpenoid, flavanoid, coumarin, quinone, saponin, tannin, acid, phenol, and alkaloid were determined in various extracts (hexane, ethylacetate, aqueous and ethanol). Based on the qualitative analysis, ethanol extract of *Gracilaria edulis* possessed maximum quantity of phytoconstiuents. The algal extraction was mostly done with polar solvents. More yields were depending upon the solvent type which dissolves more of a particular. Hence, the ethanolic extraction of *Gracilaria edulis* were contains more yields followed by other solvents. GC – MS analysis of ethanol extract of effective fractions of *Gracilaria edulis* showed several bioactive compounds namely Phytol, N-Hexadecanoic Acid, Oleic Acid, Eicosanoic Acid, Pentadecanoic Acid, Cholesta-8, 24-Dien-3-Ol, Methyl-, (3.Beta. 4. Alpha.), Hexanoic Acid, Hexadecyl Ester, Stigmasteryl Tosylate and 1-Hexyl-2-Nitrocyclohexane. The results of the present study confirmed that *Gracilaria edulis* may be rich sources of phytoconstituents which can be isolated and further screened for various biological activities.

Keywords

Gracilaria edulis, phytochemistry, GC- MS analysis

1. Introduction

Algae are a type of marine organism that lives in the water. They are ecologically and physiologically significant components of the marine ecosystem. In many regions of the world, marine microalgae are renewable living resources that are also used as food, feed, and fertilizer (Kolanjinathan, 2014). The seaweeds are a rich source of various natural products and find an important place in the pharmaceutical, cosmetics and drug development industries. The seaweeds possess the unique feature that promotes their survival in the salty marine ecosystem (Morais et al., 2021; Martins et al., 2014). Proteins, minerals, carbohydrates, vitamins, polysaccharides, amino acids, terpenoids, phlorotannins, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids, acrylic acid and steroids are all abundant in them (Sanjeewa et al., 2016; Wijesinghe and Jeon, 2012). Seaweeds are a major source of bioactive secondary metabolites, and they are one of the most promising. The bioactive compounds isolated from seaweeds are used as medicine as well as food all over the world since 13,000 years. Marine algae are able to biosynthesize secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between organisms including chemical defenses. In the last few decades, their finding has increased and their discovery is essential for mankind. Marine macroalgae are considered as an excellent source of bioactive compounds which has a broad range of biological activities including antibacterial, antiviral, antifungal, anticoagulant, antitumor and anti-inflammatory activities (Khalid et al., 2018; Barzkar et al., 2019). In this line, the present work was aimed to analysis of profiles of the primary and secondary phytochemicals of *Gracilaria edulis.* Studies on *Gracilaria edulis* and their phytochemicals, bioactive compounds and medicinal properties are less revealed when compared to other species of

Gracilaria. Therefore, this research work illuminates on the various bioactive compounds and their properties from GC-MS analysis of *Gracilaria edulis*.

2. Materials and Methods

Sample collection and extraction methods

Gracilaria edulis, a marine brown alga, was collected from the Mandapam coastal water's intertidal zone and transported to the laboratory in plastic bags holding water to avoid evaporation. The sample was then extensively cleaned with sea water to remove any foreign contaminants. The samples were dried and ground in an electric mixer at 37°C. The coarse powder was steeped for 24 hours in hexane, ethyl acetate, ethanol, and water, after which the extract was separated using filter paper. A rotary evaporator was used to concentrate and dry the extract. The material was subjected to a conventional technique for quantitative phytochemical analysis.

Qualitative analysis of different solvents extracts of *Gracilaria edulis*

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various solvent such as hexane, ethylacetate, aqueous and ethanol, *Gracilaria edulis*. Visible colour change or precipitate formation was taken into consideration for the presence (+) or absence (–) of particular active constituents (Harborne, 1998).

(a) Analysis of Tannin

2ml of the plant extract and 1ml of 10 % of ferric chloride was added. Brownish blue or black colour formation indicates the presence of tannins.

(b) Analysis of Saponin

2 ml of distilled water was mixed with 2 ml plant extract in a test tube and it was mixed vigorously and the foam appearance showed the presence of saponins.

(c) Analysis of Quinones

2 ml of plant extract and 1ml of concentrated sulphuric acid (H2SO4) was added. Red colour indicates the presence of Quinones.

(d) Analysis of flavonoids

2 ml of 2% NaOH mixture was mixed with 2ml of plant extract. Formation of yellow colour indicates the presence of flavonoids.

(e) Analysis of alkaloids

2 ml of Plant extract was added with 2ml of concentrated Hydrochloric acid (HC1) and few drops Mayer's reagent. Presence of green colour or white precipitate indicates the presence of alkaloids.

(f) Analysis of terpenoids

2 ml of chloroform was added with the 2 ml plant extract and evaporated on the water path and then boiled with 1 ml of H2SO4 concentrated. Development of red brown colour at the interface shows the presence of Terpenoids.

(g) Analysis of phenol

2 ml of the plant extract, 2ml of distilled water followed by few drops of 5 % ferric chloride was added. Formation of blue /green colour indicates the presence of phenols.

(h) Analysis of steroid

2 ml of chloroform and concentrated H2SO4 were added with the 2 ml of plant extract Red colour layer appeared that indicated the presence of steroids

(i) Analysis of Coumarins

1ml of plant extract, 1ml of 10% NaOH was added. Development of yellow colour shows the presence of coumarins.

Quantification of ethanolic extracts of *Gracilaria edulis*

Total phenolic content

The amount of total phenolic content in the ethanolic extracts of *Gracilaria edulis* was determined using Folin– Ciocalteau reagent (singleton *et al*., 1999)

Total flavonoids content

The amount of total flavonoids content in the ethanolic extracts of *Gracilaria edulis* was determined by the aluminium chloride colorimetric method (Chelladurai and Chinnachamy, 2018).

Total Alkaloids content

Quantitative estimation of alkaloids in the ethanolic extracts of *Gracilaria edulis* was carried out following the method of Edeoga *et al*., (2005)

Total Saponin content

Determination of saponin in the ethanolic extracts of *Gracilaria edulis* was carried out following the method as described by Obadoni and Ochuko (2001).

Total tannin content

Quntitaive Analysis of tannin content in the ethanolic extracts of *Gracilaria edulis* was carried out following the method as described by Ejikeme *et al*. (2014)

Separation of fractions of potential plant material utilizing column and TLC (Thin layer chromatography)

Around 40 g of selected plant extracts were diluted in ethanol and blended thoroughly with silica gel (100-200 mesh). Separation of column chromatographic was executed utilizing glass columns packed with silica gel of 200g (100-200 mesh). Plant extracts combined with

silica gel (100-200 mesh) was formed into a slurry utilizing chloroform and packed. Then the top layer was covered using cotton. Then the column was eluted with solvents in the order of chloroform, ethyl acetate, and ethanol of increasing polarity. Elutes of plant extracts were collected in conical flasks at regular time intervals. Elutes of ten microlitres were spotted on aluminum plate pre-coated with 60 F254 silica gel of 0.2 mm thickness and progress in different solvent systems. Identical fractions were selected using TLC. Fractions with identical TLC bands were blended together and dehydrated in vacuum dessicator.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the effective fractions of ethanolic extracts of *Gracilaria edulis*

GC-MS examination was assayed to determine the phytocomponents in the plant extracts. GC-MS observation of the fractions was analysed utilizing Shimadzu (GC-MS-QP 2010) and GC-MS (Gas chromatograph incorporate with a mass spectrometer) furnished with Elite - 1 bonded silica capillary tube (Film thickness: 0.25 µm, Diameter: 0.25 mm, Length: 30.0 m, formed of 100 % Dimethylpolysiloxane). For GC-MS discovery, an electron ionization vitality system with energy ionization of 70 eV was utilized. Helium gas (99.999 %) was utilized as the transporter gas at a consistent stream pace of 1.51 ml/min and an infusion volume of 1 µl was used (break ratio: 10), Ion-source temperature 200 °C; Injector temperature 240 °C. The broiler temperature was modified from 70 °C (isothermal for 3 min), with an expansion of 300 °C for 10 min. Mass spectra were taken at 70 eV; a scan break of 0.5 second with scan range of 40 – 1000 m/z. whole GC processing time was 35 min. The relative rate measure of every component was determined by contrasting its average peak zone with the absolute regions. Programming adjusted to deal with mass spectra and chromatograms was a GC-MS arrangement ver. 2.53.

Identification of Phytocomponents

Explanation of mass–spectrum GC–MS was organized utilizing the database of WILEY8 and NIST08 (National Institute Standard and Technology). The spectrum range of the unidentified components was correlated with the spectrum of identified components saved in the library. The name, structure and atomic weight, of the parts of the test materials were confirmed.

3. Results and Discussion

There are many studies were reported on the presence of different phytochemical compounds of marine brown algae (Akremi *et al*., 2017; Hakim and Patel, 2020). The present study reveals that the secondary metabolites such as steroid, triterpenoid, flavanoid, coumarin, quinone, saponin, tannin, acid, phenol, and alkaloid were determined in various extracts (hexane, ethylacetate, aqueous and ethanol) of *Gracilaria edulis* (Table 1). *Gracilaria edulis* were proved to be a good source of bioactive components namely flavonoids, alkaloids, tannins, titerpenoids teroids , saponin, and phenols compounds. Based on the qualitative analysis, ethanol extract of *Gracilaria edulis* possessed maximum quantity of phytoconstiuents. Hence quantitative analysis of ethanol extract of *Gracilaria edulis* was studied (Table 2; Fig.1). Ethanol extract of *Gracilaria edulis* revealed the quantity of Steroid (40.26 ± 0.15), Triterpenoid (42.16 ± 0.22), Flavanoid (37.08 ± 0.13), Alkaloid (42.03 ± 0.11) and Tannin (49.12 ± 0.13). The algal extraction was mostly done with polar solvents. More yields were depending upon the solvent type which dissolves more of a particular compound (Samarakoon *et al*., 2019). Hence, the ethanolic extraction of *Gracilaria edulis* were contains more yields followed by other solvents. The composition of tannin, alkaloid and triterpenoid are rich in *Gracilaria edulis* when compared to other components.

Flavonoids have been shown to have antioxidant, free radical scavenging, antileukemic, v asodilator, and antibacterial effects, and have been shown to help improve blood circulation in Al zheimer's patients' brains (Sharma *et al*., 2016). Plants, especially seaweeds contain phenolic chemicals, which have been reported to have a wide range of biological functions. Phenols are structural and allelopathic components involved in a variety of processes, including enzyme activation, food absorption, synthesis of protein, and photosynthesis (Robbins, 2003). Saponins have a variety of therapeutic qualities, including hypocholesterolemic, anticarcinogenic, anti-inflammatory, antimicrobial, and antioxidant capabilities. Saponins have a unique residue called 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4 one (DDMP), which allows them to scavenge superoxides by creating hydroperoxide intermediates that protect biomolecular structures (Yadav and Baquer, 2014; Yoshiki etal., 1998; Yoshiki *et al*., 2001). Terpenoids have been found to be beneficial in the prevention and treatment of a variety of disorders, including cancer. Antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, anti-inflammatory, and immunomodulatory activities have also been identified in terpenoids (Paduch *et al*., 2007; Ajikumar *et al*., 2008). Quinones are a class of chemicals that have long been employed in pharmacopoeia to treat malaria and, more recently, tumours. They are rich in anti-inflammatory, antimicrobial, and immunomodulatory properties (Okigbo *et al*., 2009; Gurib - Fakim, 2006). Alkaloids are commonly found to have antimicrobial, cytotoxic and antiplasmodic properties (Guven, 2020). Tannins are used in medicine as mild antiseptics in treatment of diarrhea and to check small hemorrhages. Tannins attach to proline-rich proteins, preventing them from being synthesised. Tannin-based treatments are used as antihelmintic, antioxidant, antibacterial, and antiviral agents, as well as for cancer treatment (Akbar, 2020; Fraga-Corral *et al*., 2021). Steroids isolated from marine algae have

medicinal value. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal (Doughari, 2012; Kim and Van, 2011). In the present study ethanol extract of *Gracilaria edulis* showed the various secondary metabolites and possesses biological activities. Similarly, Priyadharshini *et al*. (2015) reported that seaweeds were an excellent source of secondary metabolites that exhibited various biological activities. These secondary metabolites have long been used in preparation of drug industry and the pharmaceutical sector. They're also vital for algae's survival in their environment since they regulate algal development, block or kill many bacterial strains, inhibit major viral enzymes, and eliminate some pathogenic protozoans (Strik *et al*., 2007).

GC – MS analysis of ethanol extract of effective fractions of *Gracilaria edulis* showed several bioactive compounds namely Phytol, N-Hexadecanoic Acid, Oleic Acid, Eicosanoic Acid, Pentadecanoic Acid, Cholesta-8, 24-Dien-3-Ol, Methyl-, (3.Beta. 4. Alpha.), Hexanoic Acid, Hexadecyl Ester, Stigmasteryl Tosylate and 1-Hexyl-2-Nitrocyclohexane (Table 3). Fatty acids like n-hexadecanoic acid, eicosanoic acid, N-Hexadecanoic Acid, Oleic Acid, Eicosanoic Acid, Pentadecanoic Acid, that have reported to possess high antibacterial, antifungal and antioxidant properties (Sermakkani and Thangapandian, 2012; Elizabeth and Arumugam, 2014). Methyl esters such as n-hexadecanoic acid (Palmitic acid), eicosanoic acid (Arachidic acid), oleic acid and pentadecanoic acid have shown potential to inhibit various bacterial pathogens and polymorphic fungal species. Thus in the present study Gracilaria *edulis* showed the various biological activities. The identification of new sources of therapeutically and industrially relevant

chemicals requires phytochemical screening of the *Gracilaria edulis*. It is important to initiate an urgent step for the screening of *Gracilaria edulis* for secondary metabolites.

4. Conclusion

The present study revealed that *Gracilaria edulis* contained significant amount of primary and secondary phytochemical constituents which may contribute to its biological activities. The pharmacological properties require further studies of these active phytoconstituents by implementing various techniques of compound isolation and identification.

5. References

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Table 1 Qualitative analysis of different extracts of *Gracilaria edulis*

S. No	Phytochemicals	Hexane	Ethyl acetate	Aqueous	Ethanol
1	Steroid	-	+	+	++
2	Triterpenoid	-	+	+	++
3	Flavanoid	-	+	++	++
4	Coumarin	-	-	-	-
5	Quinone	+	+	+	++
6	Saponin	-	-	+	+
7	Tannin	+	+	++	++
8	Acid	+	+	++	++
9	Phenol	-	-	-	-
10	Alkaloid	+	+	+	++

Table 2 Quantitative analysis of different extracts of *Gracilaria edulis*

S. No	Phytochemicals	Ethanol extract
1	Steroid	40.26 ± 0.15
2	Triterpenoid	42.16 ± 0.22
3	Flavanoid	37.08 ± 0.13
4	Alkaloid	42.03 ± 0.11
5	Tannin	49.12 ± 0.13

Table 3 GC- MS analysis of ethanol extract of effective fractions of *Gracilaria edulis*

S.No	Name of the compound	Molecular weight g/mol	Chemical Formula
1	Phytol	296.5	$C_{20}H_{40}O$
2	N-Hexadecanoic Acid	256.42	$C_{16}H_{32}O_2$
3	Oleic Acid	282.5	$C_{18}H_{34}O_2$
4	Eicosanoic Acid	312.5	$C_{20}H_{40}O_2$
5	Pentadecanoic Acid	242.4	$C_{15}H_{30}O_2$
6	Cholesta-8, 24-Dien-3-Ol	426.7	$C_{29}H_{46}O_2$
7	Methyl-, (3.Beta. 4. Alpha.)	387.5	$C_{24}H_{21}NO_2S$
8	Hexanoic Acid, Hexadecyl Ester	340.6	$C_{22}H_{44}O_2$
9	Stigmasteryl Tosylate	566.9	$C_{36}H_{54}O_3S$
10	1-Hexyl-2-Nitrocyclohexane	213.32	$C_{12}H_{23}NO_2$

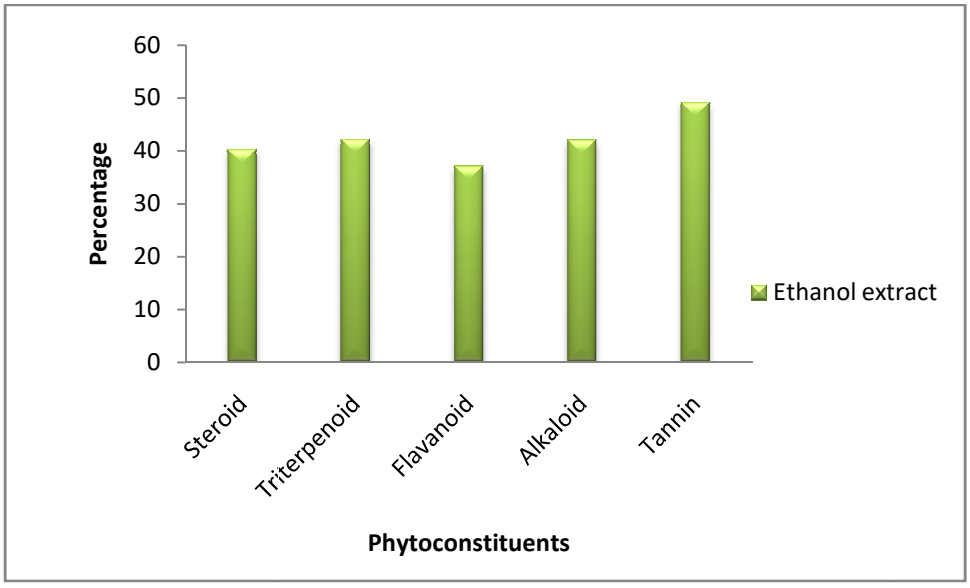

Fig. 1 Quantitative analysis of different extracts of *Gracilaria edulis*