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Fourier Transform Infrared (FTIR) Spectroscopic Characterisation of Selected Marine Macroalgae from The Ratnagiri Coast, Maharashtra, India

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Abstract

Marine macroalgae are recognised as a rich reservoir of bioactive compounds with potential pharmaceutical and nutraceutical applications. This study characterised the biochemical profiles of *Gracilaria sp.* (Rhodophyta), *Padina sp.* (Phaeophyta), and *Caulerpa sp.* (Chlorophyta) collected from the Ratnagiri coast using Fourier Transform Infrared (FTIR) spectroscopy. Spectra revealed common functional groups including O-H/N-H stretching (3270–3285 cm^{-1}), C-H stretching (2920–2925 cm^{-1}), amide I/II (1638–1542 cm^{-1}), carboxylate (1408–1361 cm^{-1}), and polysaccharides (1240–1020 cm^{-1}). Low-frequency absorptions (595–581 cm^{-1}) indicated sulphate. Comparative analysis revealed species-specific variations in polysaccharide and sulphate-related functional groups. Species-specific traits included sulphated polysaccharides in *Gracilaria sp.* (581 cm^{-1}), alginate-like bands in *Padina sp.* (1240–1151 cm^{-1}), and glycosidic linkages in *Caulerpa sp.* (1161–907 cm^{-1}). These findings highlight biochemical diversity, supporting the potential of these macroalgae as sources of bioactive polysaccharides and protein-associated biomolecules.

Keywords: Marine macroalgae; FTIR spectroscopy; Bioactive compounds; Secondary metabolites

1. Introduction

Seaweeds are simple photosynthetic organisms lacking true roots, stems, and leaves, and have been utilised since ancient times for food, feed, agricultural fertilisation, and traditional medicinal purposes (Kolanjinathan *et al.,* 2014, Kasanah *et al.,* 2022). Based on their photosynthetic pigments, seaweeds are broadly classified into Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) (Baweja *et al.,* 2016; Samiee *et al.,* 2019; Cotas *et al.,* 2023). Marine ecosystems represent a rich reservoir of biodiversity and bioactive compounds, with seaweeds emerging as an important source of nutritionally and pharmacologically relevant metabolites. Seaweed consumption has been documented since prehistoric times, particularly in Asian countries, and has gained renewed global interest in recent decades due to the increasing demand for natural marine products and functional foods (Ferdouse *et al.,* 2018). Globally, brown algae account for approximately 59% of cultivated macroalgae, followed by red algae (~40%), while green algae contribute less than 1% of total production (Rahikainen *et al.,* 2021).

Seaweeds synthesise a wide range of primary metabolites, including proteins, lipids, and polysaccharides, and produce diverse secondary metabolites in response to environmental stressors such as ultraviolet radiation, salinity, and temperature fluctuations (Pereira, 2018; Tanna *et al.,* 2022). These secondary metabolites—comprising sulphated polysaccharides, phenolic compounds, pigments, terpenoids, and alkaloids—exhibit multiple biological activities, including antioxidant, antimicrobial, antiviral, anti-inflammatory, anticancer, anticoagulant, antidiabetic, immunomodulatory, prebiotic, and cholesterol-lowering properties (Charoensiddhi *et al.,* 2017; Carpena, 2023).

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Fourier Transform Infrared (FTIR) spectroscopy is a rapid, non-destructive technique widely employed for identifying functional groups and characterising the biochemical composition of marine macroalgae (Naumann, 2000; Socrates, 2004). It provides a characteristic molecular fingerprint, enabling effective profiling of major macromolecules without extensive extraction. Although FTIR-based studies on Indian coastal seaweeds have reported characteristic signatures such as sulphated polysaccharides in *Gracilaria* and alginate-related groups in *Padina* (Chandini *et al.,* 2008; Prasad *et al.,* 2019), spectroscopic data specific to the Ratnagiri coast of Maharashtra remain scarce despite its recognised marine biodiversity. Therefore, the present study aims to analyse and compare the FTIR functional group profiles of *Gracilaria sp., Padina sp.,* and *Caulerpa sp.* collected from the Ratnagiri coast to generate baseline biochemical data and evaluate interspecific variations relevant to their functional and biotechnological potential.

2. Materials and Methods

2.1. Study area and sample collection

Figure 1 Study area map showing sampling locations of marine macroalgae along the Ratnagiri coast, Maharashtra, India (Mirya and Phansop)

2.2. Collection of Marine Macroalgae

Marine macroalgae samples—*Gracilaria sp.* (Rhodophyta), *Padina sp.* (Phaeophyta), and *Caulerpa sp.* (Chlorophyta)—were collected from Alava Beach at Mirya and Vayangani Beach at Phansop along the Ratnagiri coast of Maharashtra, India (17°00′–17°30′ N; 73°10′–73°30′ E). Sampling was carried out during the low-tide conditions to ensure maximum exposure of intertidal macroalgae (Dhargalkar & Kavlekar, 2004). Random quadrat sampling (0.25 m^2; n = 10 per species) was employed, targeting healthy and undamaged thalli while minimising disturbance to the surrounding biota (Krebs, 2014).

Immediately after collection, the samples were thoroughly rinsed with ambient seawater to remove loosely attached debris, epiphytes, and sand particles, followed by repeated washing with sterile freshwater to eliminate residual salts, reducing salinity to below 0.5 ppt. This washing procedure is commonly adopted to prevent salt interference in downstream biochemical and spectroscopic analyses (Rao & Mantri, 2006; Chandini *et al.,* 2008).

2.3. Sample Preparation and Identification

The cleaned macroalgal samples were shade-dried at room temperature (28 ± 2 °C) until constant weight was achieved, typically within 7–10 days. The dried samples were then finely ground using a mechanical grinder to obtain a homogeneous powder with a particle size less than 300 µm. The powdered samples were stored in airtight, desiccated containers to prevent moisture absorption and degradation before analysis (Prasad *et al.,* 2019). Taxonomic identification of the macroalgae as *Gracilaria sp., Padina sp.,* and *Caulerpa sp.* was carried out based on standard morphological characteristics and taxonomic keys (Jha *et al.,* 2009). Authenticated voucher specimens were retained for future reference.

2.4. FTIR Spectral Analysis

Fourier Transform Infrared (FTIR) analysis was performed using a Bruker Tensor 27 FTIR spectrophotometer. For spectral acquisition, 2 mg of powdered sample was thoroughly mixed with 200 mg of dry, spectroscopy-grade potassium bromide (KBr) in a 1:100 ratio. The mixture was compressed into pellets (13 mm diameter, approximately 1 mm thickness) under a pressure of 10 tons for 2 minutes (Stuart, 2004; Coates, 2000). The pellet was immediately put

into the sample holder, and FT-IR spectra were recorded in the range of 500-4000 cm^{-1} using a spectrometer. Transmittance was measured to identify the absorption peaks corresponding to various molecular vibrations. A pure KBr pellet was used as a background and automatically subtracted. Spectral data were processed using OPUS software, including baseline correction and normalisation. All analyses were performed in triplicate to ensure reproducibility, and the coefficient of variation for major absorption peaks was maintained below 5% (John Peter Paul and Shri Devi, 2013).

3. Results

The FTIR spectra of *Gracilaria sp., Padina sp.,* and *Caulerpa sp.* exhibited characteristic absorption bands corresponding to polysaccharides, proteins, lipids, and sulphate-containing functional groups (Figures 2-4; Tables 1–3).

All three macroalgal species showed broad absorption bands in the range of 3274–3285 cm^{-1}, attributable to O–H and N–H stretching vibrations, indicating the presence of hydroxyl-rich polysaccharides and protein-associated functional groups. Prominent aliphatic C–H stretching vibrations were observed near 2920–2925 cm^{-1}, suggesting lipid and chlorophyll-associated hydrocarbon chains.

Protein-related functional groups were confirmed by the presence of amide I bands around 1638–1639 cm^{-1} and amide II bands in the range of 1535–1542 cm^{-1} across all species. Additional absorptions corresponding to carboxylate groups (COO^-) were observed between 1414–1361 cm^{-1}, indicating acidic polysaccharides and organic acids.

Species-specific variations were evident in the polysaccharide-dominated fingerprint region. *Gracilaria sp*. exhibited a distinct S–O stretching vibration at 581 cm^{-1}, indicative of sulphated polysaccharides typical of red algae. *Padina* sp. showed strong C–O–C stretching bands between 1240 and 1151 cm^{-1}, characteristic of alginate-type polysaccharides in brown algae, along with a sulphate-related band at 595 cm^{-1}. The FTIR spectrum of *Caulerpa sp.* was characterised by prominent C–O and C–O–C stretching vibrations between 1161 and 1022 cm^{-1}, along with a low-frequency band at 907 cm^{-1}, reflecting glycosidic linkages and sulphate or mineral-associated vibrations.

Overall, the FTIR profiles revealed both conserved biochemical features and distinct interspecific differences among the studied macroalgae.

Table 1 FTIR absorption peaks and corresponding functional group assignments of *Gracilaria sp*

Wavenumber (cm^{-1})	**Functional group**	**Interpretation**
3284.51	O–H / N–H stretching	Hydroxyl groups and protein-associated bonds
2924.50	C–H stretching	Aliphatic chains (lipids)
1638.22	Amide I (C=O stretching)	Amide (protein-associated)
1535.98	Amide II (N–H bending, C–N stretching)	Amide (protein-associated)
1408.62	COO^- symmetric stretching	Carboxylate groups
1231.56	C–O–C asymmetric stretching	Polysaccharides/ether linkages
1025.10	C–O stretching	Polysaccharides and alcohol groups
581.30	S–O stretching	Sulphated polysaccharides / mineral-associated vibrations

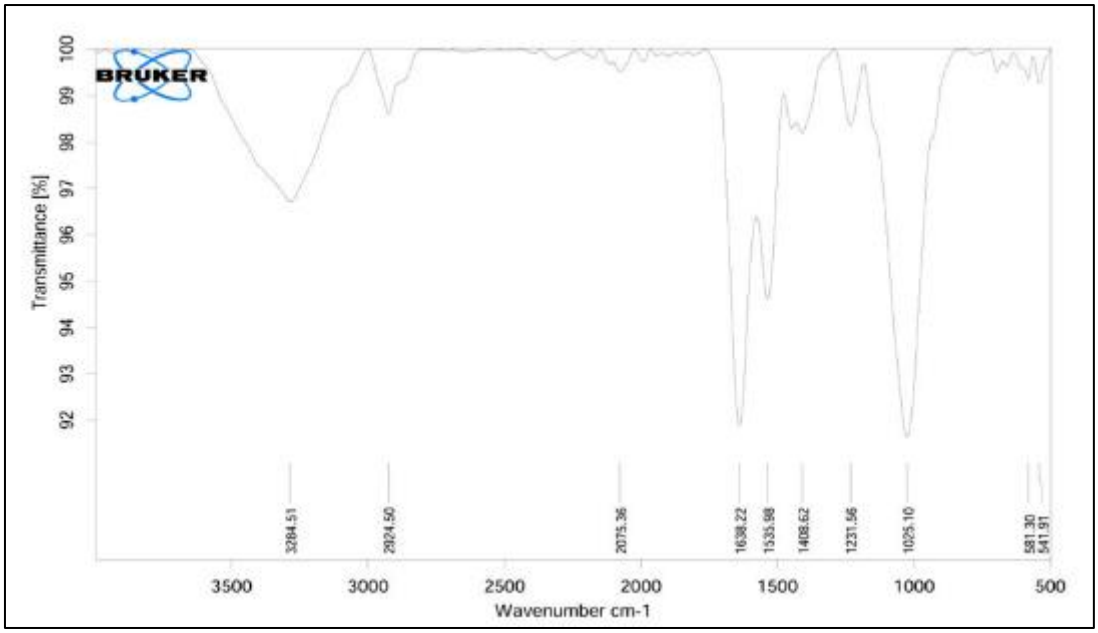

Figure 2 FTIR spectrum of *Gracilaria sp.* showing characteristic absorption bands corresponding to polysaccharides, proteins, lipids, and sulphated functional groups

Table 2 FTIR absorption peaks and corresponding functional group assignments of *Padina sp.*

Wavenumber (cm^{-1})	**Functional group**	**Interpretation**
3283.10	O–H / N–H stretching	Hydroxyl groups and protein-associated bonds
2920.43	C–H stretching	Aliphatic chains (lipids)
1639.33	Amide I (C=O stretching)	Amide (protein-associated)
1540.39	Amide II (N–H bending, C–N stretching)	Amide (protein-associated)
1362.36	O–H bending / COO^- stretching	Alcohols and carboxylate groups
1240.04	C–O–C stretching	Glycosidic linkages (alginates)
1151.05	C–O–C stretching	Sulphated polysaccharides
1021.04	C–O stretching	Polysaccharides/alcohols
595.00	S–O stretching	Sulfated polysaccharides / mineral-associated vibrations

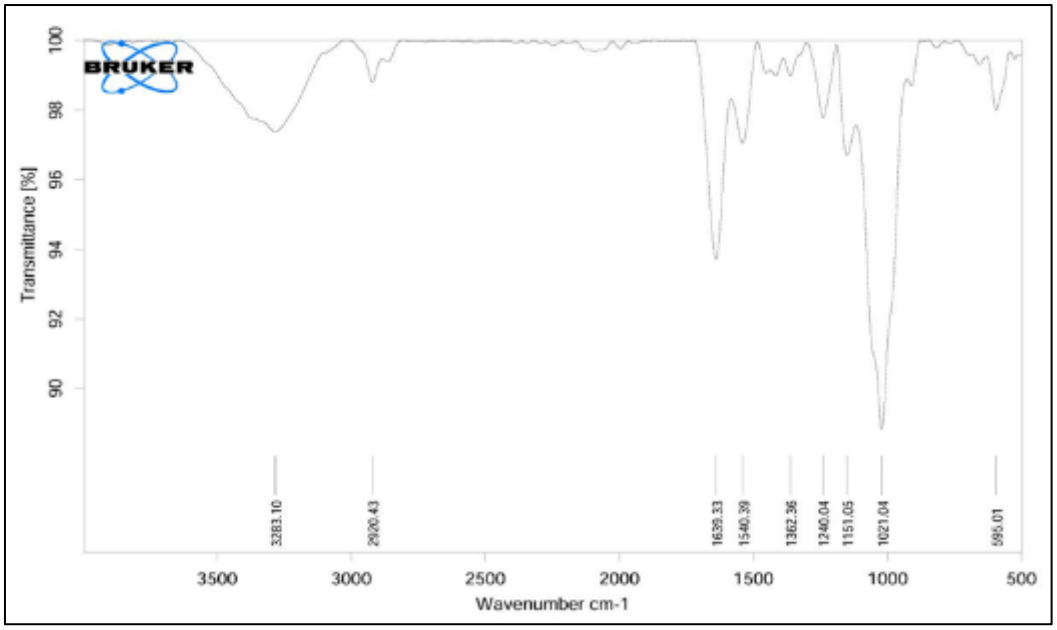

Figure 3 FTIR spectrum of *Padina* sp. showing characteristic absorption bands associated with polysaccharides, proteins, lipids, and sulphate-containing functional groups

Table 3 FTIR absorption peaks and corresponding functional group assignments of *Caulerpa sp.*

Wavenumber (cm^{-1})	**Functional group**	**Interpretation**
3274.40	O-H/N-H stretching	Alcohol
2921.71	C-H stretching	Alkanes
1639.27	Amide I (C=O stretching)	Ketones/Amide
1542.32	Amide II (N-H bend, C-N stretch)	Amide
1414.70, 1361.42	O-H bend or COO^- asymmetric	Alcohols/Carboxylates
1240.10	C-O-C	Glycosidic linkages or sulphate esters
1161.81	C-O-C	Sulphated polysaccharides
1054.14, 1022.26	C-O	Alcohols/ Ethers
907.70	S-O or mineral vibrations	Sulphates/Minerals

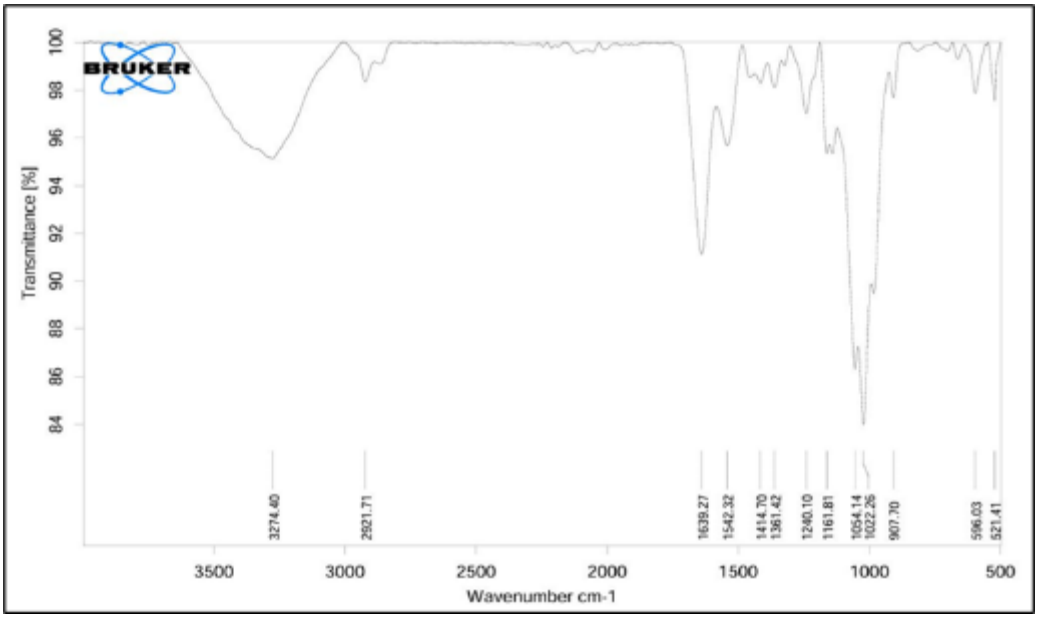

Figure 4 FTIR spectrum of *Caulerpa* sp. showing characteristic absorption bands corresponding to hydroxyl and amine groups, aliphatic C–H stretching, amide bands, carboxylate groups, and glycosidic linkages associated with polysaccharides and proteins

4. Discussion

The FTIR spectra of *Gracilaria sp., Padina sp.,* and *Caulerpa sp.* revealed both shared and species-specific functional group patterns, reflecting their taxonomic affiliations and biochemical compositions. The broad absorption bands observed at 3274–3285 cm^{-1} across all species correspond to O–H and N–H stretching vibrations, indicating the abundance of hydroxyl groups from polysaccharides and amide linkages from proteins and amino acids (Socrates, 2004). Such functional groups are fundamental components of algal cell walls and intracellular metabolic systems and are commonly reported in marine macroalgae (Pereira, 2018).

The presence of absorption bands near 2920–2925 cm^{-1}, attributed to CH_2 and CH_3 stretching vibrations, suggests aliphatic chains associated with membrane lipids and chlorophyll pigments (Socrates, 1994). These bands are consistent with photosynthetic and structural lipid components essential for algal physiology.

Protein-associated functional groups were clearly evident through amide I (1638–1639 cm^{-1}) and amide II (1535–1542 cm^{-1}) bands in all three species, confirming the presence of peptide bonds and proteinaceous constituents. Additional bands observed between 1414 and 1361 cm^{-1} correspond to carboxylate (COO^-) vibrations, indicating acidic polysaccharides and organic acid derivatives commonly found in marine algae.

Distinct interspecific differences were observed in the polysaccharide and sulphate-associated regions of the spectra. *Gracilaria* sp. exhibited a characteristic S–O stretching band at 581 cm^{-1}, which is a diagnostic marker of sulphated galactans, such as agar and carrageenan, commonly present in red algae. These sulphated polysaccharides are known for their gelling, stabilising, antiviral, and immunomodulatory properties (Ghanbarzadeh *et al.,* 2018).

In *Padina sp.,* strong absorption bands in the 1240–1151 cm^{-1} region were attributed to C–O–C stretching vibrations, confirming the dominance of alginate-rich polysaccharides, a defining biochemical feature of brown algae. The additional sulphate-related band observed at 595 cm^{-1} suggests the presence of sulphated alginates or mineral-associated sulphate groups, which have been reported in several brown algal taxa.

The FTIR spectrum of *Caulerpa sp.* was characterised by prominent absorption bands between 1161 and 1022 cm^{-1}, corresponding to C–O and C–O–C stretching vibrations indicative of glycosidic linkages and carbohydrate polymers (Paul, 2018). The low-frequency band at 907 cm^{-1} may be attributed to sulphate or mineral-associated vibrations, suggesting the presence of weakly sulphated or structurally modified polysaccharides typical of green algae (Filimon *et al.,* 2016).

The absence of very low-frequency halogen-related bands (<550 cm^{-1}) suggests limited brominated or iodinated compound accumulation in the studied samples, highlighting species- and habitat-specific biochemical variation. Overall, the FTIR fingerprints obtained in this study are consistent with previous reports from Indian and tropical coastal macroalgae and confirm the suitability of FTIR spectroscopy as a rapid and reliable tool for macroalgal biochemical characterisation (Naumann, 2000).

5. Conclusion

FTIR spectroscopic analysis *of Gracilaria sp., Padina sp.,* and *Caulerpa sp.* confirmed the presence of major functional groups associated with polysaccharides, proteins, and lipid-related components. The detection of hydroxyl, carboxylate, sulphate, and glycosidic linkages highlights the dominance of structural carbohydrates and sulphated polysaccharides, particularly in red and brown algal species. Notable interspecific variations in functional group composition reflect inherent biochemical differences among Rhodophyta, Phaeophyta, and Chlorophyta. Overall, the generated FTIR fingerprints provide reliable baseline biochemical information and demonstrate the utility of FTIR spectroscopy as a rapid and non-destructive tool for macroalgal characterisation, supporting further detailed chemical, functional, and bioactivity-oriented investigations for food, pharmaceutical, and biotechnological applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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