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Spectroscopic Profiling of Phlorotannins of Selected Brown Algae Species from Pemba Island Using UV-Vis and FTIR Techniques

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Abstract

Phlorotannins are polyphenolic compounds widely distributed in brown algae, differing with regard to species and environmental conditions. This study investigated the total phlorotannin content (TPC) and spectroscopic characteristics of phlorotannins isolated from six brown algae species (VMBK-SC, VMBK-TO, VMBK-PBY, MSH-SC, MSH-TO, and WF-PBY). Extraction was conducted using three solvents of different polarity (methanol, ethanol, and petroleum ether). Significant differences in TPC were observed among the species and solvents ($p < 0.05$). Polar solvents (methanol and ethanol) revealed higher TPC compared to petroleum ether. In general, the TPC extracted by ethanol was 76.26 ± 0.25 mg PGE/g DW followed by methanol, 41.13 ± 0.64 mg PGE/ g DW and the rest was petroleum ether 31.61±0.21 mg PGE/ g DW. The results indicated that methanol was more effective for *Sargassum* sp. whereas ethanol showed better extraction efficiency for *Turbinaria* sp. and *Padina* sp. UV–Visible spectroscopy showed characteristic absorption peaks around 300–400 nm in all extracts, confirming the presence of phenolic compounds. FTIR analysis additionally validates these results by identifying functional groups typical of phlorotannins, including O–H, C–H, C=O, C=C, and C–O stretching vibrations. Conclusively, the present study confirms that brown seaweeds are rich sources of phlorotannins and that solvent polarity plays a significant role in extraction efficiency.

Keywords: Marine algae, metabolites, Phlorotannins, UV-Vis spectrophotometer, FTIR spectroscopy.

Introduction

Marine algae, commonly known as seaweeds are a diverse group of multicellular photosynthetic organisms widely found in marine environments around the world (Chandralega & Ramadas, 2020). They grow abundantly in shallow coastline waters, estuaries, and backwater systems, where they attach to substrates such as coral rocks, reefs, and other marine materials that support their growth (Marimuthu *et al.*, 2012). Based on their biochemical composition and photosynthetic pigments, marine algae are generally categorized into three major groups: red algae (Rhodophyta), brown algae (Phaeophyta), and green algae (Chlorophyta) (Princely & Dhanaraju, 2017; Rindi *et al*., 2011).

African coastal regions support a rich diversity of marine algae due to favorable environmental conditions and varied coastal ecosystems. Along the East African coastline, particularly in Tanzania, the Zanzibar Archipelago including Pemba Island, hosts diverse seaweed communities distributed across tropical

marine habitats (Msuya *et al*., 2022). Brown seaweeds are commonly found along the coastal waters of Pemba Island due to suitable environmental conditions, including nutrient availability, temperature, and light penetration (Msuya, 2020; Berya, 2023). However, during high tides and rough ocean conditions, these algae are often detached from their substrates by strong waves, winds, and ocean currents and subsequently washed onto shorelines. The accumulation of seaweed biomass along beaches can lead to environmental challenges, including unpleasant odors and increased organic matter in coastal sediments due to microbial decomposition. Marine algae have gained considerable attention as sources of nutritionally and pharmacologically valuable substances such as alkaloids, carotenoids, proteins, unsaturated fatty acids, polysaccharides and polyphenols (Al-Muqbali, 2023). In particular, brown algae are recognized to be rich sources of phytochemicals compared to red and green seaweeds (Gupta & Abu-Ghannam, 2011; Keskinkaya *et al.*, 2022). Phytochemicals produced by marine organisms, are generally categorized into primary and secondary metabolites. Primary metabolites are directly involved in growth and development processes (Jimenez-Lopeza *et al*., 2021) of organisms.

In contrast, secondary metabolites are organic compounds that play important ecological and physiological roles (Agostini-Costa *et al*., 2012). These metabolites, function as chemical defense mechanisms against herbivores, pests, and pathogens, as well as signaling molecules involved in symbiotic interactions (Murphy *et al.*, 2014). Additionally, secondary metabolites help organisms adapt to environmental stresses include ultraviolet (UV) radiation, salinity fluctuations, temperature changes, as well as oxidative stress (Molyneux *et al*., 2007). Among the various secondary metabolites present in brown algae, polyphenolic compounds particularly phlorotannins have attracted considerable scientific interest due to their distinctive chemical structures and diverse biological activities (Ford *et al*., 2020; Sardari *et al.*, 2021). These compounds exhibit fundamental functions, making them promising candidates for applications in food, pharmacological, and nutraceutical productions (Sameeh *et al.*, 2016). Traditionally, brown algae have been used in folk medicine for wound healing, disease prevention, and other therapeutic purposes (Mohanta *et al*., 2003; Aher *et al*., 2025). The consumption of phlorotannin-rich brown algae has been reported to have minimal adverse effects compared to synthetic alternatives (Sudha & Balasundaram, 2018).

Despite their potential, many brown algae species remain underutilized and are often regarded as marine waste due to limited awareness of their bioactive composition. This highlights the need for detailed chemical profiling to better understand their functional properties and potential industrial applications (Meng *et al*., 2021). Spectroscopic techniques provide effective tools for such analyses. UV–Vis spectroscopy is commonly used to detect phenolic substances based on their characteristic absorption in the ultraviolet region, while Fourier transform infrared (FTIR) spectroscopy enables the identification of functional groups and molecular bonding patterns within organic compounds (Ismail *et al.*, 2023). The combined use of UV–Vis and FTIR techniques therefore provides complementary information enhancing the reliability of phlorotannin identification and structural characterization. Therefore, this study aims to extract and spectroscopically profile phlorotannins from selected brown algae species collected from Pemba Island using UV–Vis and FTIR analytical techniques.

Materials and Methods

Study Area

The present study was conducted on the coasts of Kiweni Island (Mwambe Shamiani) located at (5°27'10.3"S, 39°44'14.7" E), Fundu Lagoon (Wambaa) at (5°18'29.4" S, 39°40'04.0" E) and Mbuyu

Mkavu (Vitongoji) at (5°13'20.6" S, 39°50'52.8" E) in south region of Pemba Island (Figure 1) where various types of algae include brown algae species were found abundantly in the shallow waters that making them ecologically important.

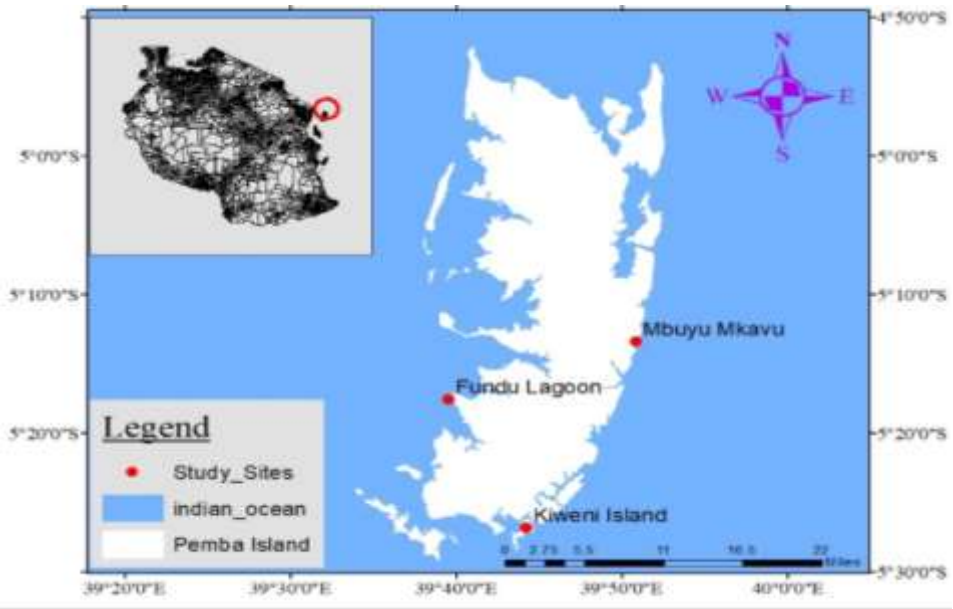

Figure 1: Sampling sites from Pemba Island

Samples collection

Brown algae samples: *Padina Boryana* (PBY), *Turbinaria ornata* (TO) and *Sargassum cristaefolium* (SC) species were collected using a random sampling approach across three sampling sites by handpicking in the shallow waters during summer, at depth between 0.1 to 0.5 meters. The collection was performed during low tide. The identification of algal species was conducted immediately after collection on the basis of morphological characteristics using A Field Guide to the Seashores of Eastern Africa and the Western Indian Ocean Islands (Richmond, 2011) as well as Algae database (Guiry & Guiry, 2018). Approximately 200 g wet weight was collected for each species at every collection point and the collected samples were quickly cleaned well with seawater to remove all the contaminants include epiphytes, sand particles, pebbles and shells. The cleaned collected samples were placed in the sterile plastic bags before kept in icebox. Then, the fresh collected seaweeds kept in icebox were transferred to the Chemistry laboratory at the State University of Zanzibar (SUZA). The whole plant was used for each sample collected. In the laboratory, samples were washed thoroughly with freshwater and spread out at room temperature for about three days drying. Sun dried algae samples were then ground using a blender to obtain a fine powder which sealed in a vial. All important information including the collection station and name of the sample were clearly labeled then the sample stored in refrigerator at -20^0 C prior to extraction.

Samples preparation

The extraction of crude extracts was performed by using Soxhlet (Gerhardt, Germany) apparatus. This technique was conducted to isolate phytochemicals or bioactive compounds (Phlorotannins) from brown algae samples (Manguntungi *et al.*, 2022). Three solvents with different polarities, include 99.8% methanol, HPLC grade (LOBA CHEMIE PVT, LTD India), 99% ethanol (WINLAB CO. LTD) and petroleum ether (LOBA CHEMIE PVT, LTD AR India, 40-60 ^{0}C) were used, to selectively target the

wide variety of chemical compounds present in sample. 5g of each dried algal powder sample contained in a thimble was placed into an extraction chamber. The chamber was then connected to a flask containing 250 mL of each solvent for 10 hours, to increase extraction efficiency (Hasibuan & Gulton, 2021). The extracts were then centrifuged with 3500 rpm for 10 min to remove solid residue of brown algae and the clear supernatant were concentrated by rotary evaporator (BUCHI R-300) to reduce the volume. The obtained crude extract of each sample was transferred into sterilize vials and then kept in a refrigerator at about -20^0 C until analysis.

Determination of total phlorotannin content

The total phlorotannin content of each sample from the extract was estimated by using dimethoxybenzaldehyde (DMBA) method as designated by Aminina *et al*. (2020). 98% of 2, 4-dimethoxybenzaldehyde (Fisher Scientific Compony) reagent and 6 g per 100 mL of hydrochloric acid was prepared in glacial acetic acid (sigma Aldrich). The reagent was prepared by mixing equal volumes of these two solutions, then was kept at room temperature before starting the assay. Phloroglucinol of 99% analytical grade from Fisher scientific company was used as a standard. Calibration curve was plotted within the standard concentration range of 2 to 20 µg/mL. Each sample from extracts was diluted with 4 mL of water as required by the operating range of the calibration curve. Each 50 µL of diluted sample from extracts was mixed with prepared DMBA solution of 250 µL before left in dark for 60 min at 18 ^{0}C. The solutions were centrifuged and the supernatant optical density (OD) was measured at 494 nm using UV-Vis spectrophotometer (PerkinElmer Lambda 465). The results obtained was determined by the regression equation of the calibration curve y = 0.0015x - 0.0012 with a regression co-efficient, R^2 = 0.9721. The total phlorotannins content was expressed as milligrams of phloroglucinol equivalents per gram of sample powder (mg PGE/g dry weight of sample) (Ismail, 2023).

Phloroglucinol standard curve

Phloroglucinol as a standard was used for the preparation of calibration curve with the serial phloroglucinol dilution of 2, 8, 14, 18 and 20µg/mL. The graph (Figure 2) was constructed from absorbance unit (AU) vertical axis against phloroglucinol standard horizontal axis, provided a linear regression.

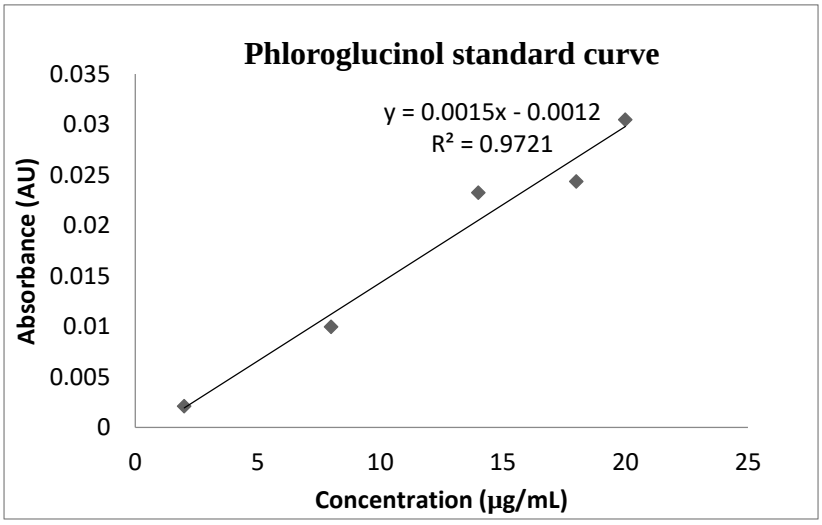

Figure 2: Phloroglucinol standard curve.

Total phlorotannin content within the samples was calculated using linear regression equation as identified from standard curve in Figure 2. The total phlorotannin content was calculated by the formula C= (C1 × V)/m, where C = total phlorotannin content in mg/g, in phloroglucinol equivalent (PGE), C1 = concentration of phloroglucinol obtained from the standard curve in mg/mL, V = volume of extract in ml and m = weight of solid sample in gram as revealed in Siddiqui *et al*. (2017).

Fourier Transform Infrared Spectroscopy for polyphenols

Infrared spectroscopy is very important instrumental tool in applied and basic sciences for the identification of different functional groups (Khan *et al*., 2018), particularly in phenolic compounds (Dev & Mukadam, 2025) such as polyphenols. This technique is based on the vibrations of atoms in a molecule (Petit & Madejova, 2013), and it can be used for a wide range of materials as a qualitative analysis (Mohamed *et al.*, 2017). In this study, Fourier Transform Infrared spectroscopy (FTIR) was performed to characterize the functional groups present in solvent algal extracts. The dried powder of methanol, ethanol and petroleum ether extracts was used for FTIR analysis. About 10 mg of dried extract powder was mixed with potassium bromide (KBr) pellet in order to prepare translucent sample discs. The measurements were taken in an automatic recording FTIR spectrophotometer in the range of about 4000 to 400cm-1 (Sumayya *et al.*, 2016).

Data Analysis

All experiments were conducted in triplicate (n = 3). Statistical analysis was done using analysis of variance (ANOVA), followed by Tukey post-hoc test, SPSS package (IBM SPSS statistics for version 20.0). Statistical significance was defined as $p < 0.05$. All data are represented as mean ± SD.

Results

Total phlorotannin content

The total phlorotannin content (TPC) of selected brown algae species extracted using methanol, ethanol, and petroleum ether is presented in Table 1 and illustrated in Figure 3. The results revealed statistically significant differences in TPC among the algal species as well as among the extraction solvents ($p < 0.05$), indicating that both species-specific characteristics and solvent properties play a crucial role in the extraction efficiency of phlorotannins. Quantitatively, methanolic extracts exhibited TPC values ranging from 0.65 ± 0.22 to 29.90 ± 0.01 mg PGE/g DW, while ethanolic extracts showed a broader and generally higher range of 0.91 ± 0.03 to 39.08 ± 0.06 mg PGE/g DW. In contrast, petroleum ether extracts consistently produced the lowest TPC values, ranging from 0.07 ± 0.00 to 14.91 ± 0.06 mg PGE/g DW. Among all the analyzed samples, VMBK-PBY extracted with ethanol exhibited the highest TPC, highlighting the high phenolic content of *Padina boryana* from Vitongoji Mbuyu Mkavu. This was followed by VMBK-SC extracted with methanol. On the other hand, the lowest TPC was recorded in MSH-SC petroleum ether extract. A clear species-dependent extraction pattern was observed across the samples. Methanol extracts produced the highest TPC values for *Sargassum cristaefolium*. In contrast, ethanol extracts were more effective for *Turbinaria ornata* and *Padina boryana*. Additionally, geographical variation appears to influence phlorotannin content. Samples from Vitongoji Mbuyu Mkavu (VMBK) generally showed higher TPC values compared to those from Mwambe Shamiani (MSH) and Wambaa Fundu Lagoon (WF). Petroleum ether extracts consistently showed lower TPC values across most species, although relatively higher values were observed in *Turbinaria ornata*.

Table 1: Total phlorotannin content of selected algal species in different solvents.

Total phlorotannin content (mg PGE/g DW)			
Algal samples	**Methanol**	**Ethanol**	**Petroleum ether**
VMBK-SC	29.90 ± 0.02	5.72 ± 0.03	0.11 ± 0.03
MSH-SC	1.54 ± 0.07	0.91 ± 0.03	0.07 ± 0.00
VMBK-TO	7.07 ± 0.10	17.34 ± 0.05	14.91 ± 0.06
MSH-TO	1.17 ± 0.03	7.87 ± 0.03	14.91 ± 0.03
VMBK-PBY	0.80 ± 0.20	39.08 ± 0.06	0.47 ± 0.03
WF-PBY	0.65 ± 0.22	5.34 ± 0.05	1.14 ± 0.06

PGE = Phloroglucinol equivalent, DW = Dry weight, VMBK = Vitongoji Mbuyu Mkavu, MSH = Mwambe Shamiani, WF = Wambaa Fundu Lagoon, SC = *Sargassum cristaefolium*, TO = *Turbinaria ornata*, PBY = *Padina boryana*. The TPC of the sample determined in triplicate (n = 3) and the values are expressed as mean ± SD.

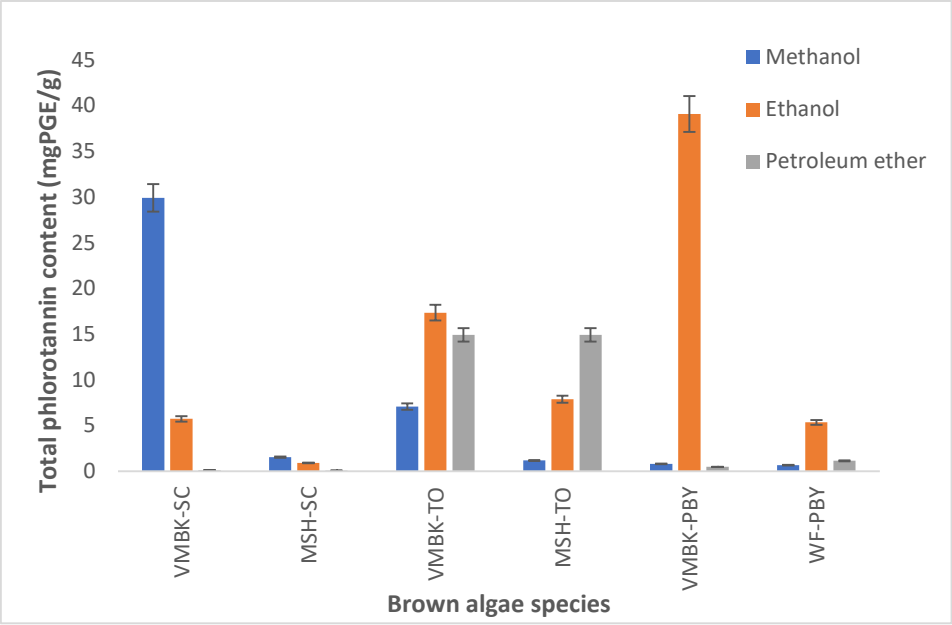

Figure 3: Bar chart showing TPC of selected algae extracted using different solvents.

Effect of solvent on TPC

The comparative effect of solvents on TPC across all samples is summarized in Table 2. Statistical analysis (ANOVA followed by Tukey post-hoc test) indicated a significant difference in TPC among the solvents ($p < 0.05$), with ethanol extracts showing the highest overall TPC. These results demonstrate the superior efficiency of polar solvents, particularly ethanol, in extracting phlorotannin compounds.

Table 2: TPC across solvents

Solvent	TPC mg PGE/g DW
Methanol	41.13±0.64
Ethanol	76.26±0.25
Petroleum ether	31.61±0.21

Spectroscopic characterization of phlorotannins

UV-Visible spectroscopy analysis

UV–Visible spectra were recorded using a spectrophotometer over the wavelength range of 200–1100 nm with a PerkinElmer Lambda 465 instrument to identify the presence of phenolic (phlorotannin) compounds in each extract, as presented in Figures 4 – 6. Distinct absorption peaks (λmax) were observed for all samples across the different solvents. For methanolic extracts, MSH-SC (M) exhibited a λmax at 356.78 nm, while MSH-TO (M) showed a slightly lower value at 354.90 nm. In contrast, VMBK-SC (M) displayed a higher λmax at 369.92 nm, representing the most pronounced shift among all methanolic samples. VMBK-TO (M) showed a λmax at 355.54 nm, while VMBK-PBY (M) exhibited a slightly higher value at 358.74 nm. The WF-PBY (M) sample recorded a λmax at 354.43 nm, representing the lowest value among methanolic extracts. Across locations, VMBK samples generally exhibited slightly higher λmax values compared to MSH and WF samples. Between species, *Sargassum cristaefolium* showed greater variation compared to Turbinaria ornata and *Padina boryana* within methanolic extracts. For ethanolic extracts, MSH-SC (E) and MSH-TO (E) exhibited λmax values of 355.75 nm and 356.25 nm, respectively, indicating very similar spectral profiles within the same sampling site. VMBK-SC (E) showed a slightly lower λmax at 350.83 nm, while VMBK-TO (E) exhibited a markedly higher λmax at 415.64 nm. Similarly, VMBK-PBY (E) recorded a λmax at 418.18 nm, representing the highest value among all analyzed samples. WF-PBY (E) showed a λmax at 353.79 nm, comparable to the MSH samples. A clear variation was observed within VMBK samples, where *Turbinaria ornata* and *Padina boryana* exhibited substantially higher λmax values compared to *Sargassum cristaefolium*. In contrast, samples from MSH and WF remained within a narrower range around 350–360 nm. Across species, *Padina boryana* and *Turbinaria ornata* demonstrated higher λmax values in ethanol compared to *Sargassum cristaefolium*. For petroleum ether extracts, MSH-SC (P) exhibited a λmax at 353.81 nm, while MSH-TO (P) showed a higher value at 361.32 nm, indicating a noticeable difference between species within the same location. VMBK-SC (P) recorded a λmax at 354.42 nm, whereas VMBK-TO (P) exhibited a slightly higher value at 356.36 nm. VMBK-PBY (P) showed a λmax at 364.16 nm, representing the highest value among petroleum ether extracts. WF-PBY (P) recorded a λmax at 356.62 nm, falling within the mid-range of the observed values. Compared to methanolic and ethanolic extracts, petroleum ether extracts showed relatively clustered λmax values across all samples. However, slight variations were still evident between species, with *Padina boryana* and *Turbinaria ornata* generally exhibiting higher λmax values than *Sargassum cristaefolium*.

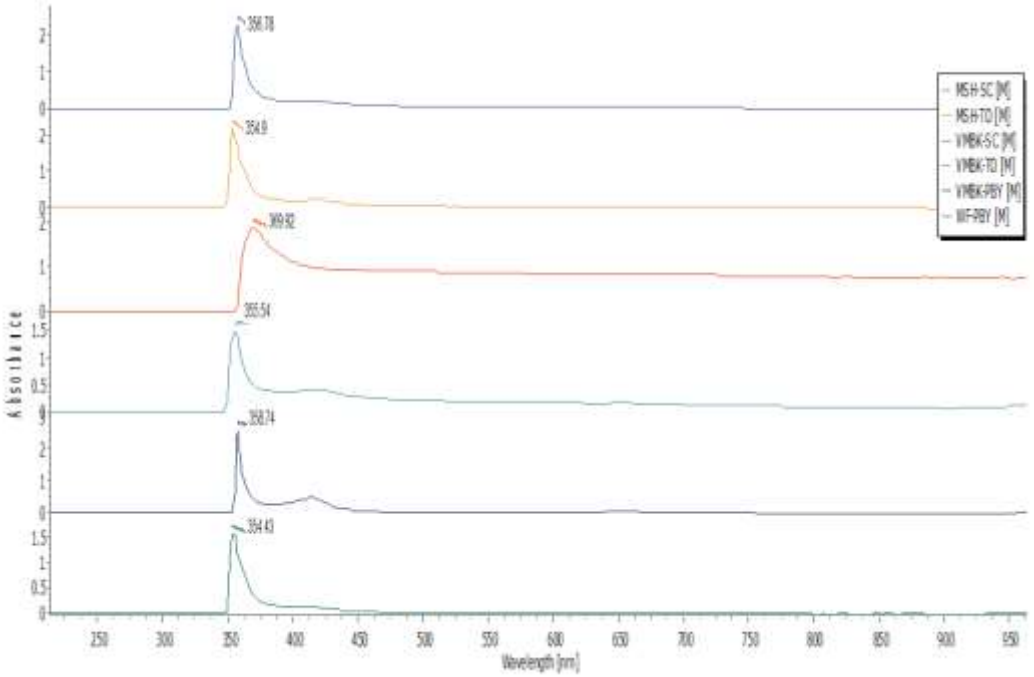

Figure 4: UV-Vis spectra of methanolic extracts.

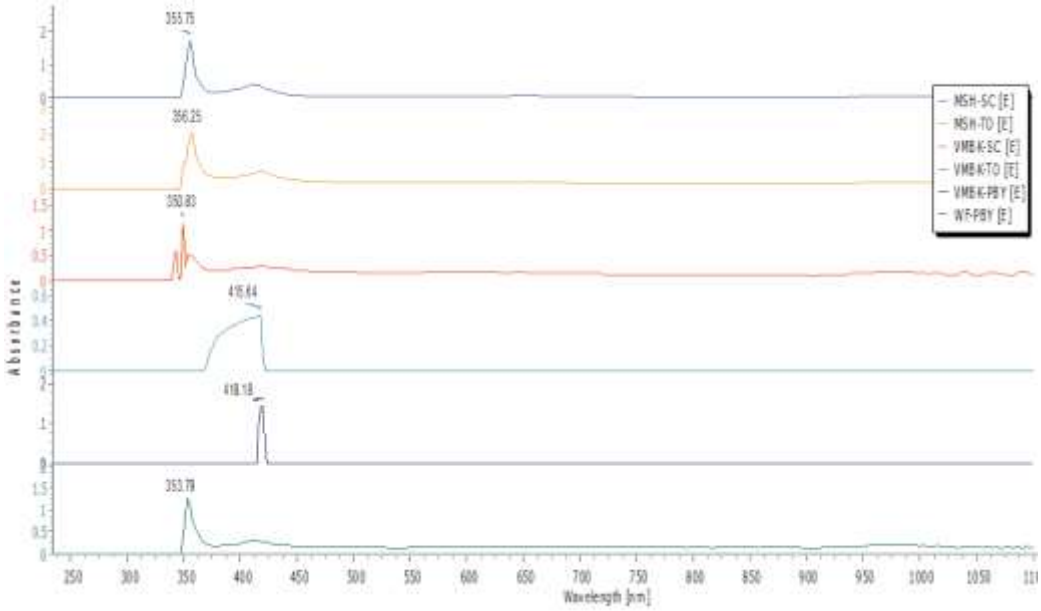

Figure 5: UV-Vis spectra of ethanolic extracts.

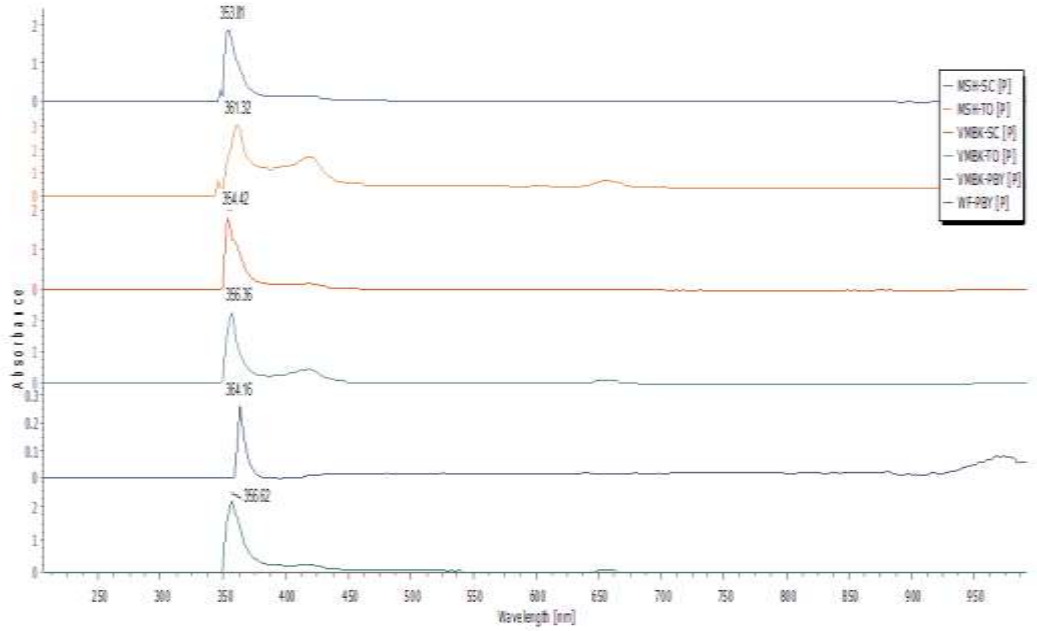

Figure 6: UV-Vis spectra of petroleum ether extracts.

FTIR spectroscopy analysis

FTIR spectra methanolic, ethanolic, and petroleum ether extracts are shown in **Figure 7-9,** while the major functional groups identified are summarized in **Tables 3-5.** Broad and strong absorption bands observed around 3200–3430 cm^{-1} in most extracts, except WF-PBY (P), indicate the presence of hydroxyl (O–H) groups associated with phenolic compounds. Vibrational bands around 2850–2940 cm^{-1} were observed in almost all extracts except VMBK-PBY (M) and VMBK-PBY (E), indicating the presence of alkyl (C–H) stretching vibrations. Peaks near 2900 cm^{-1} observed, corresponding to both aromatic and non-aromatic C–H bond vibrations. Sharp peaks associated with carbonyl (C=O) stretching vibrations were revealed around 1710–1731 cm^{-1} in all petroleum ether extracts, VMBK-TO (M) as well as VMBK-TO (E). Absorption bands observed around 1620–1650 cm^{-1} in most extracts, except petroleum ether extracts, VMBK-TO (M) and VMBK-TO (E), indicate the presence of C=C stretching vibrations. The appearance of bands between 1500 and 1600 cm^{-1} in MSH-SC (P), VMBK-TO (P), and VMBK-PBY (P) suggests the presence of N–H bending vibrations. Stretching bands around 1400 cm^{-1} recorded in all petroleum ether extracts, WF-PBY (M), VMBK-PBY (M), VMBK-SC (M), MSH-SC (M), WF-PBY (E), VMBK-PBY (E), and MSH-TO (E) correspond to O–H bending vibrations. Absorption peaks around 1372–1378 cm^{-1} observed in all petroleum ether extracts, MSH-TO (M), VMBK-SC (M), VMBK-TO (M), VMBK-SC (E), as well as VMBK-TO (E), indicated the presence of N=O bending vibrations. The peaks observed around 1120–1277 cm^{-1} in most extracts, except VMBK-PBY (M), WF-PBY (M), MSH-SC (E), VMBK-PBY (E), MSH-SC (P), and VMBK-PBY (P), correspond to C–O stretching vibrations. Similarly, peaks observed between 1019 and 1078 cm^{-1} in most extracts, except MSH-SC (P), MSH-TO (P), VMBK-TO (P), and WF-PBY (P), indicate the presence of C–O–C ether linkages. Additional bands in the region of 627–972 cm^{-1} revealed in all petroleum ether extracts, MSH-TO (M), VMBK-SC (M), VMBK-TO (M), MSH-SC (E), VMBK-SC (E), as well as VMBK-TO (E), are associated with C–H bending vibrations.

Table 3: Major bands identified in the FTIR spectrum of the methanolic extracts of selected brown algae species and their related functional groups.
W = Wave number, F. groups = Functional groups

MSH-SC	MSH-TO	VMBK-SC	VMBK-TO	VMBK-PBY	WF-PBY	F. groups	Compound
W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}		
3308.6	3275.8	3272.3	3278.0	3306.8	3293.8	O-H stretching	Phenols, carboxylic acid
2922.2	2926.4	2936.4	2922.7	-	2920.3	C-H stretching	Alkanes, alphatic hydrocarbons
-	-.	-	1717.1	-	-	C=O stretching	Esters, ketones, carboxylic acids
1638.8	1648.2	1631.0	-	1637.7	1638.0	C=C stretching	Alkenes, aromatic rings
1411.4	-	1457.6	-	1407.5	1406.1	O-H stretching	Alcohols, phenols
-	1376.5	1373.4	1374.9	-	-	N=O bending	Nitro groups
1276.6	1193.2	1260.9	1262.0	-	-	C-O stretching	Alcohols, esters
1076.3	1079.6	1019.1	1078.9	1079.5	1075.5	C-O-C stretching	Ethers, polysaccharides

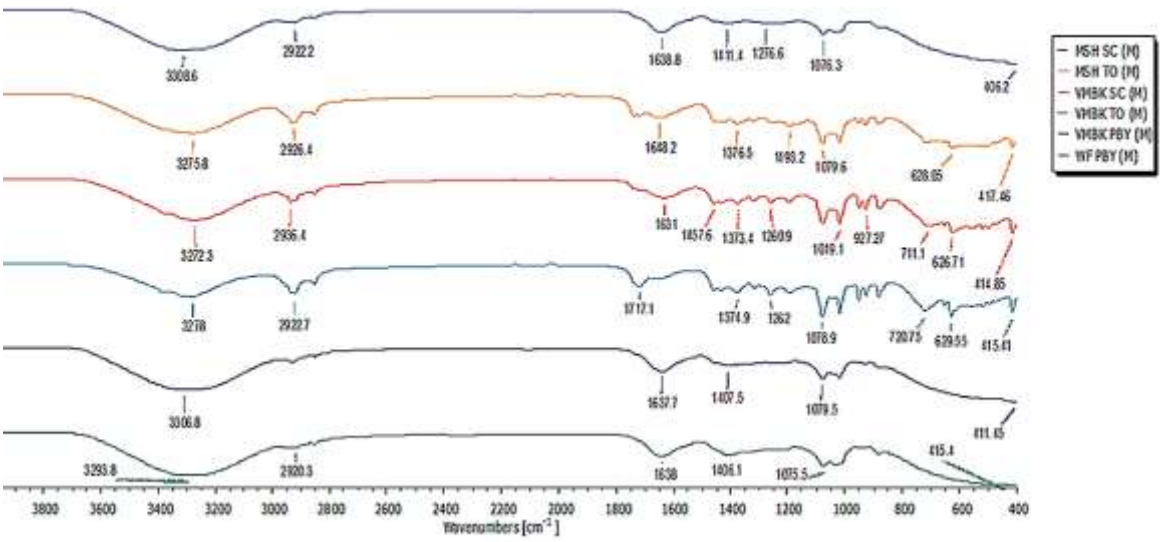

Figure 7: FTIR spectra for methanolic extracts

Table 4: Major bands identified in FTIR spectrum of the ethanolic extracts of selected brown algae species and their related functional groups.

MSH-SC	MSH-TO	VMB-SC	VMBK-TO	VMBK-PBY	WF-PBY	F. groups	Compound
W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}		
3351.8	3308.5	3274.0	3281.0	3307.8	3271.5	O-H stretching	phenols, carboxylic acid
2920.0	2927.7	2935.9	2924.3	-	-	C-H stretching	Alkanes, aliphatic chain
-	-	-	1716.8	-	-	C=O stretching	Esters, aldehydes, ketones
1643.7	1638.9	1629.0	-	1637.2	1639.0	C=C stretching	Alkenes, aromatic rings
1464.4	1411.4	1457.6	-	1404.3	1406.3	O-H bending	Alcohols, phenols
-	-	1374.8	1376.4	-	-	N=O bending	Nitro groups
-	1201.5	1194.5	1193.1	-	1226.0	C-O Stretching	Ethers
1035.6	1040.6	1078.7	1078.0	1076.7	1075.6	C-O-C stretching	Ethers

696.53	-	627.39	719.4, 629.69	-	-	C-H bending	Alkanes, aromatic rings

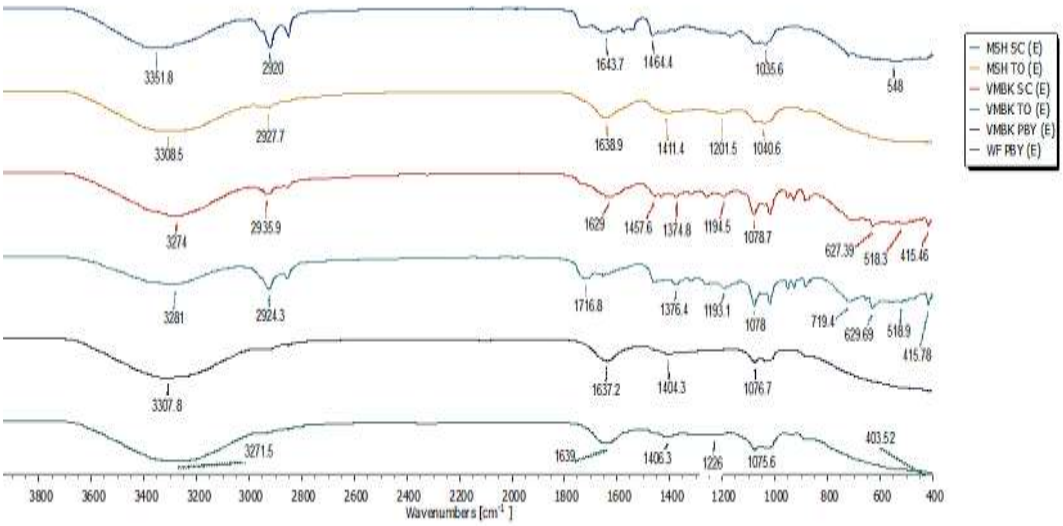

Figure 8: FTIR spectra for ethanolic extracts

Table 5: Major bands identified in the FTIR spectrum of the petroleum ether extracts of selected brown algae species and their related functional groups.

MSH-SC	MSH-TO	VMBK-SC	VMBK-TO	VMBK-PBY	WF-PBY	F. groups	Compound
W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}	W/cm^{-1}		
3424.8	3371.8	3383.9	3362.0	3417.6	-	O-H stretching	Phenols
2921.2	2924.0	2922.5, 2853.0	2920.4, 2851.6	2922.4, 2853.1	2922.0, 2852.8	C-H stretching	Alkanes
1711.5	1730.5	1711.3,	1715.1	1711.1	1712.0	C=O stretching	Esters/ Aldehydes
1540.0	-	-	1540.3	1540.7	-	N-H stretching	Amides
1463.6	1452.1	1462.5	1463.0	1463.2	1462.8	O-H Bending	Alcohols/ Phenols
1377.5	1376.2	1377.4	1377.6	1377.7	1377.5	N=O bending	Nitro groups
-	1232.5, 1169.6	1164.5	1164.8	-	1274.0	C-O stretching	Esters/ Alcohols
-	-	1038.7	-	1053.7	-	C-O-C stretching	Ethers

721.07	971.23, 892.22, 699.28	721.02	971.18, 720.98	721.21	721.27	C-H bending	Aromatic rings

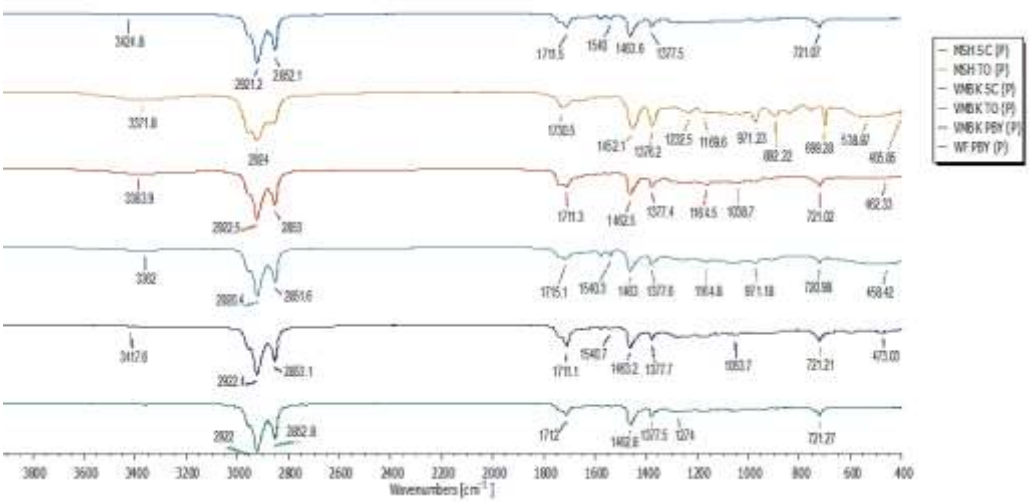

Figure 9: FTIR spectra for petroleum ether extracts

Discussion

Total phlorotannin content

Phlorotannins are the principal phenolic compounds in brown seaweeds, exhibiting diverse chemical structures and biological activities (Ismail *et al*., 2023). Their accumulation is influenced by environmental conditions, geographical location, nutrient availability, and species-specific metabolic characteristics (Khadija *et al*., 2021). In this study, two-way ANOVA revealed that both solvent type and algal species significantly affected total phlorotannin content (TPC) ($p < 0.001$), with a significant interaction effect ($p < 0.001$). This indicates that extraction efficiency is species-dependent, consistent with previous reports (Liu *et al*., 2017; Sarangi *et al*., 2023; Lee *et al*., 2024). Ethanol generally produced higher TPC values across most species, while methanol showed superior performance in specific cases, suggesting that optimal solvent selection depends on the biochemical composition of individual algae. This behavior can be explained by the polar nature of phlorotannins, which readily form hydrogen bonds with polar solvents, enhancing their solubility and extraction efficiency (Paul *et al*., 2024). In contrast, petroleum ether yielded very low TPC values in most samples, confirming the poor solubility of phlorotannins in non-polar solvents (Cassani *et al*., 2020). However, unusually high TPC values observed in some p. ether extracts likely resulted from co-extraction of non-phenolic lipophilic compounds, such as pigments and terpenoids, which may interfere with colorimetric assays. This suggests a limitation of the method and highlights the need for improved purification steps (Rajhard *et al*., 2021).

UV-Visible spectroscopy

UV–Visible spectroscopic analysis revealed distinct absorption maxima (λmax) across all extracts, confirming the presence of phenolic compounds, particularly phlorotannins. The absorption bands within ~350–420 nm are attributed to π→π* transitions of aromatic rings and conjugated systems, which are structural features of phloroglucinol-based phlorotannin polymers, supporting the TPC results (Nastasi,

2025). Methanolic extracts showed a narrow λmax range (354–370 nm), indicating relatively similar phenolic structures with limited variation in conjugation among species. Slight shifts toward higher wavelengths in VMBK samples suggest increased conjugation or substitution within the phlorotannin backbone, reflecting enhanced electron delocalization associated with environmental stress-induced polymerization (Le *et al.*, 2023). Ethanolic extracts exhibited the widest λmax variation, with peaks extending beyond 400 nm (up to 418 nm in VMBK samples). These bathochromic shifts indicate the presence of highly polymerized or structurally complex phlorotannins with extended conjugation systems. This confirms ethanol's ability to extract higher molecular weight phenolic fractions, consistent with its higher TPC values (Lohvina *et al*., 2021). In contrast, petroleum ether extracts showed limited λmax variation (353–364 nm), reflecting restricted extraction of highly polar phlorotannins. The observed peaks likely correspond to low-polarity phenolic fragments or co-extracted compounds with minimal conjugation. Species-specific trends were evident, with *Padina boryana* and *Turbinaria ornata* showing broader spectral variation than *Sargassum cristaefolium*, suggesting greater structural diversity of phenolic metabolites. This variation reflects differences in biosynthetic capacity and ecological adaptation among species (Stiger & Payri, 2004). In general, the UV–Visible results confirm that polarity of the solvent as well as species identity significantly affect the degree of conjugation and structural complexity of phlorotannins (Steevensz *et al*., 2012). The pronounced bathochromic shifts in ethanolic extracts, particularly from VMBK samples, indicate higher polymerization and structural diversity of phenolic compounds, reinforcing ethanol as the most effective solvent for extracting phlorotannins.

FTIR spectroscopy

The FTIR analysis of methanolic, ethanolic, and petroleum ether extracts revealed the presence of multiple functional groups associated with phlorotannins and other bioactive compounds in the studied brown algae. The broad bands observed at ~3200–3350 cm^{-1} across most samples indicated the presence of hydroxyl (O–H) group and H-bonded stretching, which is characteristic of polyphenolic compounds (Wongsa *et al.*, 2022), particularly phlorotannins. In the present study, methanolic and ethanolic extracts exhibited similar spectral patterns, especially in the higher frequency regions, indicating comparable classes of compounds extracted by these polar solvents. The consistent presence of O–H stretching and aromatic C=C stretching bands around 1629–1648 cm^{-1} suggests that both solvents effectively extracted phenolic compounds with aromatic structures (Wongsa *et al.*, 2022). The presence of C–O and C–O–C bands in several samples further confirms the existence of phenolic ethers and alcohol functionalities commonly found in phlorotannins (Catarino *et al*., 2022). However, slight differences were observed between the two solvents. The detection of carbonyl (C=O) stretching bands predominantly in Turbinaria ornata both in methanolic and ethanolic extracts suggests the presence of oxidized phenolic derivatives or other carbonyl-containing compounds specific to this species. Additionally, the absence of certain bands such as C–H and C–O in some *Padina boryana* samples indicates variability in chemical composition, possibly due to structural differences in phlorotannins. The fingerprint region (1500–500 cm^{-1}) showed more pronounced variation among samples, highlighting differences in molecular structure and composition across species and locations (Kassem *et al.*, 2023). VMBK samples generally exhibited a wider range of functional groups, suggesting greater chemical diversity. In contrast, petroleum ether extracts displayed a distinctly different spectral profile. The consistent presence of strong carbonyl (C=O) bands at ~1711–1730 cm^{-1} (Kamal *et al.*, 2020), across all samples suggests the extraction of non-polar or less polar compounds such as lipids. The frequent detection of nitro (N=O) and C–H bending vibrations

further supports the presence of diverse non-polar constituents. Although O–H bands were observed in most samples, their absence in WF-PBY (P) indicates minimal extraction of phenolic compounds in that sample using petroleum ether. The presence of N–H stretching bands in selected petroleum ether extracts may indicate trace amounts of protein-related compounds or nitrogen-containing metabolites (Patle *et al*., 2020). However, these were not consistently observed, suggesting that such compounds are either present in low concentrations or not efficiently extracted by non-polar solvents. Species-specific variation was clearly evident, with *Turbinaria ornata* and *Padina boryana* showing more pronounced bands in the carbonyl and nitro regions compared to *Sargassum cristaefolium*. This suggests a higher diversity of functional groups. On the other hand, *Sargassum cristaefolium* exhibited a more consistent and uniform spectral pattern, indicating a relatively simpler or more stable chemical composition.

Location-based variation was also observed, particularly in the fingerprint region. Samples from Vitongoji Mbuyu Mkavu (VMBK) showed a broader range of detectable functional groups compared to Mwambe Shamiani (MSH) and Wambaa Fundu Lagoon (WF). This supports the earlier observation that environmental conditions effect the biosynthesis and accumulation of secondary metabolites, including phlorotannins.

Generally, the FTIR results demonstrate that solvent polarity plays a crucial role in determining the type of compounds extracted (da Silva *et al*., 2025), with methanol and ethanol favoring phenolic compounds, while petroleum ether primarily extracts non-polar constituents. The combination of FTIR, UV–Visible, and TPC analyses provides strong evidence for the presence and variability of phlorotannins across species, solvents, and locations, highlighting the complex chemical nature of brown algae extracts (Ford *et al.*, 2019).

Conclusion

This study has demonstrated that the total phlorotannin content (TPC) and structural characteristics of phlorotannins in selected brown algae from Pemba coastal waters is influenced by environmental conditions and solvent polarity, with marked variability across species and sampling sites.

Samples collected from VMBK, is characterized by intense UV radiation, strong wave action, and rocky habitats, it also exhibited the highest levels of phlorotannins. This suggests that environmental stressors such as ultraviolet radiation and mechanical forces enhance phlorotannin accumulation as a protective response. In contrast, algae from MSH estuary and WF lagoon environments, which experience tidal influence, high long-term rainfall, and lower UV exposure, showed comparatively lower TPC values, indicating diminished environmental stress and limited phlorotannin synthesis. These findings demonstrate the significant influence of ecological conditions on phlorotannin variability.

Spectroscopic analyses confirmed the presence and variability of phenolic compounds in the studied samples. The results demonstrate significant structural differences and confirm the presence of phlorotannin compounds in the analyzed extracts. UV–Visible spectroscopy revealed absorbance peaks within the range of 300–420 nm, consistent with characteristic phenolic compounds, particularly phlorotannins. Methanolic extracts exhibited maximum wavelengths between 354 and 370 nm, indicating the presence of both highly polymerized and moderately conjugated phlorotannin structures. Ethanolic extracts showed similar absorption patterns, with some peaks extending toward the visible region, suggesting larger conjugated systems. In contrast, petroleum ether extracts displayed lower absorbance intensity, reflecting the limited solubility of phlorotannins in non-polar solvents.

FTIR analysis further confirmed the presence of key functional groups associated with phlorotannins, including O–H, C–O, C–O–C, C=C, and C–H vibrations. These functional group patterns provide strong evidence for the structural characteristics of phlorotannins present in the extracts. Minor nitrogen-containing peaks observed in some extracts were attributed to co-extracted proteins and other nitrogenous compounds, indicating the crude nature of the extracts.

In general, the findings confirm that the selected brown algae species are rich sources of phlorotannins and that extraction efficiency is significantly influenced by solvent polarity and environmental conditions. Polar solvents consistently extracted higher phlorotannins, highlighting the importance of solvent polarity. Ethanol generally produced the highest TPC values, while methanol, demonstrated strong extraction efficiency depending on the species. These results provide important baseline data for future research on phlorotannin purification, structural characterization, antioxidant evaluation, and potential biotechnological applications of brown algae from Tanzanian coastal ecosystems.

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References

1. Agostini-Costa, T., Viera, R., Bizzo, H., Silveira, D., & Gimenes, M. (2012). Secondary Metabolites.
2. Aher, A. A., Thitame, S. N., Viddyasagar, M., & Wabale, A. S. (2025). Bioactive Algae in Wound Healing: Natural Solutions for Modern Medicine. Journal of Pharmacy and Bioallied Sciences. 17(1, Suppl), S20-S23.
3. Al-Muqbali, T. H. M. (2023). Characterization Extracts and Agar Extracted from the Omani Seaweed “Gracilaria debilis”. Master thesis, Sultan Qaboos University.
4. Aminina, N. M., Karaulova, E. P., Vishnevskaya, T. I., Yakush, E. V., Kim, Y. K., Nam, K. H ., & Son, K. T. (2020). Characteristics of Polyphenolic Content in Brown Algae of the Pacific Coast of Russia. Molecules. 25 (17), 3909.
5. Berya, S. (2023). Seaweed in Tanzania: A Multitude of Green Potentials. https://www.digest.tz
6. Cassani, L., Gomez-Zavaglia, A., Jimenez-Lopez, C., Lourenco-Lopes, C., Prieto, M. A., & Simal-Gandara, J. (2020). Seaweed-based natural ingredients: Stability of phlorotannins during extraction, storage, passage through the gastrointestinal tract and potential incorporation into functional foods. Food Research International 137, 109676.
7. Catarino, M. D., Pires, S. M. G., Silva, S., Costa, F., Braga, S. S., Pinto, D. C. G. A., Silva, A. M. S., & Cardoso, S. M .(2022). Overview of Phlorotannins' Constituents in Fucales. Marine drugs, 20 (12), 754.
8. Chandralega, G., & Ramadas, V. (2020). Screening of Phytochemicals, Fatty Acid Composition and In-Vitro Analysis of Antioxidant Property of Green Edible Seaweed Caulerpa Lentillifera (Family: Caulerpaceae). Chandralega and Ramdas, IJPSR, 11(3), 1495-1505.
9. da Silva, L. G., da Costa, C., Batista, G., Amorim, K., Abreu, D., Pio, L., Boas, E. V., & Carvalho, E. E. (2025). Synergy between solvent polarity and composition for efficient extraction of bioactive compounds from pitaya. Food materials research, 5.

10. Dev, M., & Mukadam, M. (2025). Functional group profiling of medicinal plants using FTIR spectroscopy. World journal of Biology Pharmacy and Health Sciences, 21(01), 243-249.
11. Ford, L., Stratakos, A. Ch., Theodoridou, K., Dick, J. T. A., Sheldrake, G. N., Linton, M., Corcionivoschi, N., & Walsh, P. J. (2020). Polyphenols from Brown Seaweeds as a Potential Antimicrobial Agent in Animal Feeds, American Chemical Society, 4627-4630.
12. Ford, L., Theodoridou, K., Sheldrake, G. N., & Walsh, P. J. (2019). A critical review of analytical methods used for the chemical charecterisation and quantification of phlorotannin compounds in brown seaweeds. Phytochemical Analysis, 30, 587-599.
13. Guiry, M.D., & Guiry, G.M. (2018). AlgaeBase. National University of Ireland, Galway. Available at: http://www.algaebase.org.
14. Gupta, S., & Abu-Ghannam, N. (2011). Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods, Innovative Food Science and Emerging Technologies, 12, 600-609.
15. Hasibuan, R., & Gultom, E. (2021). The effect of method, type of solvent and extraction time towards the yield of oil on essential oil extraction from lime peel (Citrus aurantifolia). IOP conference series: materials science and engineering, 1122, 012108.
16. Ismail, M. M., El Zokm, G. M., El Sikaily, A. M., Selim, A., & Ismail, G. A. (2023). Chemodiversity and bioactivity assessment of phlorotannins from some Phaeophyta species from the Red Sea. Journal of Applied phycology: 35, 1769-1788.
17. Jimenez-Lopez, C., Pereira, A., Lourenco-Lopes, C., Garcia-Oliveira, P., Cassani, L., Fraga-Corral, M., Prieto, M. A., & Simal-Gandara, J. (2021). Main bioactive phenolic compounds in marine algae and their mechanisms of action supporting potential health benefits: Food Chemistry, (202), 341.
18. Kamal, M., Gheda, S., Abdel Hamid, A., & Mohamed, T. M. (2020). Antibacterial and antioxidant activities of Phlorotannins extracted from *Sargassum linifolium* brown alga. Delta Journal of Science, 41, 32-38.
19. Kassem, A., Abbas, L., Coutinho, O., Opara, S., Najaf, H., Kasperek, D., Pokhrel, K., Li, X., & Tiquia-Arashiro, S. (2023). Applications of Fourier Transform-Infrared spectroscopy in microbial cell biology and environmental microbiology: advances, challenges, and future perspectives. Frontiers in microbiology, 14, 1304081.
20. Keskinkaya, H. B., Deveci, E., Gunes, E., Okudan, E. S., Akkoz, C., Gumus, N. E., & Karakurt, S. (2022). Chemical composition, in vitro antimicrobial and antioxidant activities of marine macroalgae codium fragile (Suringaar) hariot.Comm.J.Biol.6(1), 94-104.
21. Khadijah, K., Soekanto, N. H., Firdaus, F., Chalid, S. M. T., & Syah, Y. M. (2021). Chemical Composition, Phytochemical Constituent, and Toxicity of Methanol Extract of Brown Algae (*Padina* sp.) from Puntondo Coast, Takalar (Indonesia). Journal of Food Quality and Hazards Control, 8, 178-185.
22. Khan, S. A., Khan, S. B., Khan, L. U., Farooq, A., Akhtar, K & Asiri, A. M. (2018). Fourier Transform Infrared Spectroscopy: Fundamentals and Application in Functional Groups and Nanomaterials Characterization. In Sharma, S. (eds) Handbook of Materials Characterization. Springer, Cham.
23. Quynh, L. N., Lien, V., Pham, V., Thinh, T., Thuy, P., Chu, V. H., Doanh, V., Le Tien, H., Hanh, C., & Thao, P. (2023). Effect of extended π-conjugation on photophysical characteristics of chalcone and cinnamylideneacetophenone. Materials Science in Semiconductor Processing, 162, 107507.

24. Lee, J., Jayakody, J., Kim, J., Jeong, J., Choi, K., Kim, T., Seo, C., Azimi, I., Hyun, J., & Ryu, B. (2024). The influence of solvent choice on the extraction of bioactive compounds from *Asteraceae*: A comparative review. Foods (Base1, Switzerland). 13 (19), 3151.
25. Liu, X., Yuan, W., & Meng, X. (2017). Extraction and quantification of phlorotannins from edible brown algae. American Society of Agricultural and Biological Engineers, 60 (1), 265-271.
26. Lohvina, H., Sándor, M., & Wink, M. (2021). Effect of Ethanol Solvents on Total Phenolic Content and Antioxidant Properties of Seed Extracts of Fenugreek (Trigonella foenum-graecum L.) Varieties and Determination of Phenolic Composition by HPLC-ESI-MS. Diversity, 14, 7.
27. Marimuthu, J., Essakimuthu, P., Janakiraman, N., Anantham, B., Tharmaraj, R. J. J. M., & Arumugam, S. (2012). 'Phytochemical characterization of brown seaweed Sargassum wightii', Asian Pacific Journal of Tropical Disease.
28. Meng, W., Mu, T., Sun, H., & Garcia-Vaquera. (2021). Phlorotannins: A review of extraction methods, structural charecteristics, bioactivities, bioavailability, and future trends. Algal Research 60, 102484.
29. Mohamed, M. A.; Jaafar, J.; Ismail, A. F.; Othman, M. H. D., & Rahman, M. A. (2017). Fourier Transform Infrared (FTIR) Spectroscopy. In Membrane Characterization, 3-29, Elsevier.
30. Mohanta, B., Chakraboty, A., Sudarshan, M., Dutta, R. K & Baruah, M. (2003). Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeuric usage. Journal of radioanalytical and nuclear chemistry 258 (1), 175-179.
31. Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., & James, L. F. (2007). Phytochemicals: the good, the bad and the ugly? Phytochemistry, 68 (22-24), 2973- 2985.
32. Msuya, F. E. (2020). Seaweed resources of Tanzania: status, potential species, challenges and development potentials, Botanica Marina, 63(4), 371-380.
33. Msuya, F. E., Bolton, J., Pascal, F., Narrain, K., Nyonje, B., Elizabeth, J., & Cottier-Cook. (2022). Seaweed farming in Africa: current status and future potential. Journal of Applied Phycology, 34, 985–1005.
34. Murphy, C., Hotchkiss, S., Worthington, J., & McKeown, S. R. (2014). The potential of seaweed as a source of drugs for use in cancer chemotherapy. Journal of Appl Phycology. 26, 64 - 2211.
35. Nastasi, J. R. (2025). Colourimetric assays for assessing polyphenolic phytonutrients with nutraceutical applications: History, guidelines, mechanisms, and critical evaluation. Nutraceuticals, 5 (4), 40.
36. Patle, T., Shrivas, K., Kurrey., Upadhyay, S., Jangde, R., & Chauhan, R. (2020). Phytochemical screening and determination of phenolics and flavonoids in Dillenia pentagyna using UV–Vis and FTIR spectroscopy. Spectrochimica Acta Part A. Molecular and Biomolecular Spectroscopy, 242, 118717.
37. Paul, P. T., Joy, J. M., Akshay, P., Rosemol, J. M., Amruth, P., Chatterjee, N. S., Mathew, S. (2024). Assessment of Total Phenolic Content and In Vitro Antioxidant Activity in Brown Seaweeds Turbinaria conoides and Padina tetrastromatica from the Mandapam Coast. Society of Fisheries Technologists (India) Fishery Technology 61: 264-272.
38. Petit, S., & Madejova, J. (2013). Fourier Transform Infrared Spectroscopy. Developments in Clay Science, 213-231.
39. Princely, S., & Dhanaraju, M. D. (2017). Assessment of Phytochemical Constituents, In Vitro Antimicrobial and Antioxidant Potential of Ulva Extracts from Vishakhapatnam Coast. Asian J Pharm Clin Res, 10 (8), 87-95.

40. Rajhard, S., Hladnik, L., Vicente, F. A., Srcic, S., Grilc, M., & Likozar, B. (2021). Solubility of Luteolin and Other Polyphenolic Compounds in Water, Nonpolar, Polar Aprotic and Protic Solvents by Applying FTIR/HPLC. Processes. 9, 1952.
41. Richmond, M. D. (2011). A field guide to the seashores of Eastern Africa and the Western Indian Ocean Islands (3rd ed.). Sida/WIOMSA.
42. Rindi, F., Soler-Vila, A., & Guiry, M.D. (2011). Taxonomy of marine macroalgae used as sources of bioactive compounds. In Marine bioactive compounds: sources, characterization and applications, 1-53. Marine Bioactive Compounds.
43. Sameeh, M. Y., Mohamed, A. A and Elazzazy, A. M. (2016). Polyphenolic contents and antimicrobial activity of different axtracts of *Padina boryana Thivy* and *Enteromorpha sp* marine algae, 6 (09), 087-092.
44. Sarangi, N. V., Saranya Prakasan, M. V., Rajkumar, R., & Srinivavasan, S. (2023). Assessmnt of phytochemicals from marine algae *Ulva fasciata* and Dictyota dichotoma with antioxidant and antimicrobial potential. Applied chemical engineering, 6.
45. Sardari, R. R. R., Prothmann, J., Gregersen, O., Turner, C & Nordberg Karlsson, E. (2021). Identification of Phlorotannins in the Brown Algae, *Saccharina latissima* and *Ascophyllum nodosum* by Ultra-High-Performance Liquid Chromatography Coupled to High-Resolution Tandem Mass Spectrometry. Molecules. 26 (1), 43.
46. Siddiqui, N., Rauf, A., Latif, A., & Mahmood, Z. (2017). Spectrophotometric determination of the total phenolic content and spectral fluorescence of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). Journal of Taibah University Medical Sciences. 12 (4), 360-363.
47. Steevensz A. J., Mackinnon, S. L., Hankinson, R., Craft, C., Connan, S., Stengel, D. B., & Melanson, J. E. (2012). Profiling phlorotannins in brown macroalgae by liquid chromatography-high resolution mass spectrometry. Phytochem Anal. S23 (5), 547-53.
48. Stiger, V., & Payri, C. (2004). Phenolic contents of two brown algae, Turbinaria ornata and Sargassum mangarevense on Tahiti (French Polynesia): Interspecific, ontogenic and spatio-temporal variations. Botanica Marina, 47, 402-409.
49. Sudha, G., & Balasundaram, A. (2018). Analysis of bioactive compounds in Padina pavonica using HPLC, UV-VIS and FTIR techniques. Journal of Pharmacognosy and Phytochemistry, 7(3), 3192-3195.
50. Sumayya, S. S., Lawarence, B. B., Babu, D. K. V., & Murugan, K. (2016). FTIR analysis and reverse phase high performance chromatographic determination of phenolic acids of *hypnea musciformis* (wulfen) j.v. lamouroux. International Journal of Pharmacy, 6(3), 97-104.
51. Wongsa, P., Phatikulrungsun, P., & Prathumthong, S. (2022). FT-IR characteristics, phenolic profiles and inhibitory potential against digestive enzymes of 25 herbal infusions. Scientific reports, 12 (1), 6631.