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*Original Research Article*

# LC-HRMS Profiling, Total Flavonoid and Phenolic Content of Brown Algae Originating from the Southern Coast of Indonesia

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ABSTRACT

Brown algae (*Sargassum polycystum*) are widely distributed in Indonesian marine ecosystems. Despite their abundance, pharmaceutical exploration remains limited compared to red and green algae. This study aimed to profile metabolites and evaluate the total phenolic content (TPC) and total flavonoid content (TFC) of brown algae collected from Indonesia's southern coast using liquid chromatography-high-resolution mass spectrometry (LC-HRMS). Powdered samples of brown algae were extracted separately by maceration using three different polarity-based solvents: ethanol, ethyl acetate, and n-hexane. The extracts were analysed for TPC and TFC using Folin ciocalteu and aluminium chloride colorimetric assays, respectively. The metabolites profile of the extracts was analysed using LC-HRMS. Ethyl acetate extract exhibited the highest TPC (48.570 ± 0.008 mg GAE/g), while n-Hexane extract showed the highest TFC (85.271 ± 0.017 mg QE/g). LC-HRMS profiling identified 232-328 metabolites across the extracts, with n-hexane yielding the greatest chemical diversity. Key bioactive compounds identified included Xestoaminol C (highest in ethanol extract at 5.57%), Ceramides (13.46% of total content in ethanol extract), and Fucoxanthin (exclusive to ethanol extract, 0.52%). These metabolites are known for their anticancer, neuroprotective, antioxidant and antimicrobial properties. Heatmap analysis of the top 20 metabolites revealed solvent-dependent clustering patterns, highlighting the influence of extraction methods on metabolites recovery. The findings underscore the pharmaceutical and nutraceutical potential of Indonesian brown algae and demonstrate the invaluable role of LC-HRMS for marine natural product research.

***Keywords:*** Brown Algae, Liquid Chromatography-High Resolution Mass Spectrometry, Flavonoid, Phenolic Content.

## Introduction

Brown algae or brown seaweed (*Sargassum polycystum*) are among the macroalgae that are known for their nutritional value and beneficial non-nutritional components, especially phenolic and flavonoid compounds with bioactive properties.[1] Polar molecules in macroalgae such as pigments and phenolic compounds have been shown to have potential as antimicrobials.[2] In Indonesia, macroalgae have become one of the sources of income for coastal communities. However, macroalgae cultivation is dominated by red algae (*Eucheuma cottonii*), while the cultivation of brown algae is limited. Despite their potential as raw material in the pharmaceutical and cosmetic industries, the research on brown algae in Indonesia is still very limited.

Brown algae contain a variety of phenolic and flavonoid compounds, especially phlorotannins. Phlorotannins are unique polyphenol compounds found only in brown algae and have are potent antioxidants. Other compounds found in brown algae are fucoidan and fucoxanthin, which are known to have antioxidant and antimicrobial activities.[3,4]

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Previous studies have found that the ethanol extract of brown algae from Bangladesh had antibacterial activity against *Escherichia coli*.[5] In addition, brown algae from the Red Sea have shown antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*.[6] The antibacterial activity of Indonesian brown algae has also been demonstrated.[7] Furthermore, the methanol extract of brown algae from Bangladesh have been shown to have high total phenol content (95.34 ± 1.21 mg GAE/g), and total flavonoid content (51.48 ± 0.92 mg QE/g).[5] Phenolic compounds have demonstrated antimicrobial activity due to their ability to denature proteins and damage cytoplasmic membrane, leading to leakage of intracellular components, loss of cell function, and eventual death of the microorganisms.[8]

Untargeted metabolomics has emerged as a powerful analytical approach for evaluating dynamic changes in plant metabolites profile, serving as a primary technique for the comprehensive identification and quantification of complex natural products.[9,10] The integration of liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) has become particularly valuable in this field due to its exceptional sensitivity, superior mass accuracy, and enhanced selectivity in detecting diverse phytochemical constituents.[11,12]

Despite these advantages, the interpretation of complex metabolite datasets and their biological correlations presents significant analytical challenges, typically necessitating advanced chemometric approaches for meaningful data interpretation.[12] When properly implemented, this analytical strategy offers several key benefits such as comprehensive metabolic profiling of bioactive constituents, comparative analysis of samples across different extraction methodologies, and solvent-dependent characterisation of phytochemical composition.[13]

Beyond structure elucidation, LC-HRMS demonstrates significant advantages for natural product research, including ultrahigh sensitivity with detection limits in the picogram range, high-throughput, and capacity for simultaneous quantification of multiple compound classes.

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The technique has proven particularly valuable for specialized workflows targeting flavonoid and phenolic compounds screening, making it an indispensable tool for modern phytochemical analysis.[14] This comprehensive analytical approach has transformed natural product research by delivering detailed metabolite profiling while maintaining the throughput required for large-scale investigations. To our knowledge, this is the first study to provide a comprehensive LC-HRMS-based metabolite profiling of *Sargassum polycystum* from the southern coast of Indonesia, comparing multiple extraction solvents to highlight solvent-dependent variations.

## Materials and Methods

*Reagents and Chemicals*

Ethanol, ethylacetate, *n*-hexane, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride, acetic acid, quercetin, MS-grade water with 0.1% formic acid, MS-grade acetonitrile with 0.1% formic acid and HPLC-grade methanol were products of Merck, Germany.

*Algae collection and identification*

Brown algae (*Sargassum polycystum*) were collected on March 15, 2025 from the coastal waters of Pangandaran, West Java Province, Indonesia. The geographical coordinates of the collection site are 7°42'22.5"S 108°39'50.3"E. Identification was performed by Mr. Joko Kusmoro, Padjadjaran University, Bandung, Indonesia. A voucher specimen with voucher number 113/HB/03/2025 was deposited at the Herbarium Jatinangor, Plant Taxonomy Laboratory, Padjadjaran University, Bandung, Indonesia.

*Sample preparation and extraction*

The sample preparation followed established methodologies from previous studies.[15] The brown algae were thoroughly rinsed under running water to remove impurities, followed by sun-drying. Subsequently, the samples were further dried in an oven at 40°C for 24 h to achieve maximal moisture reduction. The completely dried samples were then pulverised using a blender to obtain a fine powder, which was stored in airtight containers until extraction. For extraction, the brown algae were subjected to maceration using three different polarity-based solvents: ethanol, ethyl acetate, and n-hexane (1:10 w/v ratio; 100 g sample in 1 L solvent), with each extraction lasting 3×24 h. The resulting extracts were concentrated under reduced pressure to obtain viscous crude extracts.[16]

*Determination of total phenolic content (TPC)*

The TPC analysis was conducted following a previously described method with minor modifications.[17] A gallic acid standard solution was prepared in a concentration series ranging from 20 to 50 ppm at 5 ppm intervals. The reaction mixture consisted of 0.5 mL gallic acid solution, 2.5 mL Folin-Ciocalteu reagent, and 2 mL of 7.5% (w/v) sodium carbonate solution. The sample was prepared by dissolving 10 mg of each extract in 5 mL of methanol. Subsequently, 0.5 mL of this solution was reacted with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution (7.5% w/v). After 35 min of incubation in the dark at room temperature (25 ± 2°C), absorbance was measured at 760 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). A standard calibration curve was generated by plotting absorbance values against gallic acid concentrations. The TPC was expressed as gallic acid equivalents (GAE) in milligrams per gram of extract (mg GAE/100 g extract). The measurements were performed in triplicate.

*Determination of total flavonoid content (TFC)*

The TFC analysis was conducted following the method described by a previous study.[18] The diluted sample solution was prepared by dissolving 10 mg of the extract in 5 mL of methanol. The colorimetric reaction was initiated by mixing 0.5 mL of the diluted sample solution with 1 mL of 2% (w/v) aluminium chloride solution, followed by the addition of 0.1 mL of 1 M potassium acetate solution and 1.5 mL of 96% ethanol. The reaction mixture was thoroughly vortexed and allowed to stand for 30 min at ambient temperature (25 ± 2°C) in the dark to facilitate complete chromophore formation. Absorbance measurements were performed in triplicate at 415 nm using a UV-Vis spectrophotometer, with ethanol serving as the blank. The mean absorbance values obtained from triplicate measurements were correlated against a pre-established quercetin standard calibration curve ($y = ax + b$, $R^2 > 0.995$) to determine the flavonoid content. The results were expressed as quercetin equivalents (QE) in milligrams per gram of extract (mg QE/g extract).

*Identification of metabolites by LC-HRMS*

The LC-HRMS analytical procedure was conducted following established methods from previous studies with appropriate modifications to optimise performance for brown algae metabolites.[19–21] The chemical constituents of brown algae extract were characterised using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) employing a Thermo Scientific™ Vanquish™ Horizon UHPLC system (Germering, Germany) coupled with a Thermo Scientific™ Orbitrap™ Exploris 240 mass spectrometer (Bremen, Germany). The mobile phase consisted of two eluents: Eluent A (water with 0.1% formic acid) and Eluent B (acetonitrile with 0.1% formic acid). Chromatographic separation was performed on a Thermo Scientific™ Accucore™ Phenyl-Hexyl analytical column (100 mm × 2.1 mm ID, 2.6 µm particle size; Lithuania) maintained at 40°C. The analysis employed a gradient elution program at a flow rate of 0.3 mL/min over 25 minutes, with an injection volume of 5 µL. The mobile phase composition began at 5% B, increased linearly to 90% B over 16 min, maintained at 90% B for 4 min, then returned to initial conditions (5% B) for column re-equilibration. For sample preparation, 10 mg of extract was dissolved in 1 mL of methanol, homogenised by vortex mixing, and filtered through a 0.2 µm nylon membrane filter. Aliquots (5 µL) were injected into the HRMS system. Mass spectrometric data acquisition continued until completion, after which the results were processed for compound identification and analysis.

*Statistical analysis*

The experimental results for total phenolic and flavonoid contents were presented as mean values ± standard deviation (SD). Chromatographic and spectral data were processed using Thermo Scientific Compound Discoverer 3.3 software (San Jose, CA, USA). The data processing workflow consisted of multiple stages: chromatogram generation, noise reduction, molecular feature detection, and compound identification. Compound identification was achieved through a multi-reference approach combining mzCloud high-resolution mass spectral library matching, ChemSpider database queries and Custom mass list searches.

## Results and Discussion

*TPC and TFC of brown algae extracts*

The TPC of the brown algae extracts exhibited significant variation depending on the extraction solvent, with values ranging from 15.818 to 48.570 mg GAE/g (Table 1). Ethyl acetate extract demonstrated the highest phenolic content (48.570 ± 0.42 mg GAE/g), while ethanol extraction produced the lowest phenolic recovery (15.818 mg GAE/g). In contrast, the TFC followed a different distribution pattern, with values ranging from 69.116 to 85.271 mg QE/g. The n-Hexane extract showed the highest flavonoid content (85.271 mg QE/g), whereas the ethanol extract again yielded the lowest flavonoid content (69.116 ± 0.47 mg QE/g). This inverse relationship between phenolic and flavonoid extraction efficiencies suggests differential solvent selectivity for various classes of secondary metabolites in brown algae.

Compared with previous studies, the phenolic content obtained from ethyl acetate extract in this study was lower than that reported in Bangladesh samples (95.34 mg GAE/g),[5] but consistent with other Indonesian *Sargassum* reports. The flavonoid levels observed (up to 85.27 mg QE/g) were notably higher than those from Bangladesh species, highlighting the high phytochemical content of Indonesian brown algae. These differences underscore the impact of geography and solvent selection on metabolites recovery.

Numerous studies have demonstrated that critical parameters including solvent selection, pH, extraction method, temperature, and processing duration significantly influence phenolic compound yields.[22–24]

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**Table 1:** Total phenolic and total flavonoid contents of *brown algae* extracts

| Sample | TPC (mg GAE/g) | TFC (mg QE/g) |
|---|---|---|
| EtOH Extract | 15.818 ± 0.002 | 69.16 ± 0.055 |
| EtOAc Extract | 48.570 ± 0.008 | 81.320 ± 0.009 |
| n-Hexane Extract | 20.286 ± 0.018 | 85.271 ± 0.017 |

Among various solvents, ethyl acetate has been identified as particularly effective for maximising the extraction efficiency of both phenolic and flavonoid compounds from brown algae. For comprehensive metabolic profiling, contemporary research recommends employing a multi-solvent approach encompassing polar (ethanol), semi-polar (ethyl acetate), and non-polar (n-hexane) solvents to ensure broad-spectrum metabolites recovery.[25]

*Identification of brown algae metabolites*

The chemical constituents of brown algae extracts were comprehensively characterised through Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). The Total Ion Chromatogram (TIC) obtained from LC-HRMS analysis displays a continuous plot of ion signal intensity versus retention time throughout the analytical run. This chromatographic representation incorporates all ions detected within the predefined mass-to-charge (m/z) range of the HRMS instrument configuration. The TIC serves as a fundamental diagnostic tool that provides a global profile of sample composition while enabling preliminary detection of major chromatographic peaks potentially corresponding to specific chemical constituents. As a primary output of HRMS analysis, the TIC offers valuable information about the overall complexity and chemical diversity of the analysed sample. The chromatogram's peak distribution patterns reflect the relative abundance of various components present in the sample, with retention characteristics indicative of compound polarity and molecular properties. This comprehensive detection approach facilitates initial compound screening and guides subsequent targeted analyses through extracted ion chromatograms (EICs) or MS/MS fragmentation experiments for more specific compound identification and verification. The use of TIC extends to quality control assessment of the analytical process, enabling evaluation of system performance and detection sensitivity throughout the acquisition period.[26] The TIC of the brown algae extract is presented in Figure 1.

In HRMS analysis, the Max Area parameter refers to the maximum integrated peak area value derived from the curve representing specific ions in the mass spectrum. This peak area serves as a quantitative measure of the total ion count detected over a defined time interval, which correlates with the relative abundance of the corresponding compound in the analysed sample. The magnitude of the peak area is directly proportional to the concentration of the molecular species generating the ion signal, making it a crucial parameter for both qualitative identification and quantitative assessment in mass spectrometric analysis.[27] The data collected from the detector were used to generate the TIC, which plots ion signal intensity against elution time. In the chemical composition analysis of brown algae, 232 compounds were identified in the ethanol extract, 265 in the ethyl acetate extract, and 328 in the n-hexane extract. The top 20 compounds in each of the ethanol, ethyl acetate, and n-hexane extracts of brown algae that were successfully identified using LC-HRMS are presented in Tables 2, 3, and 4, respectively.

Figure 2 presents a heatmap analysis of LC-HRMS data showing the metabolites profiles of brown algae extracts obtained using three different solvent systems: ethanol, ethyl acetate, and n-hexane. The colour gradient (ranging from blue to red) represents the relative abundance of detected metabolites, with clustering patterns revealing distinct chemical compositions characteristic of each extract type.

**Table 2:** Top 20 compounds identified in the ethanol extract of brown algae

| No. | Compound Name | Molecular Formula | Percent Area (%) |
|---|---|---|---|
| 1 | Xestoaminol C | $C_{14}H_{31}NO$ | 5.567 |
| 2 | Isoheptadecasphinganine | $C_{17}H_{37}NO_2$ | 4.745 |
| 3 | Cocamidopropyl betaine | $C_{19}H_{38}N_2O_3$ | 4.116 |
| 4 | 2-Amino-1,3,4-octadecanetriol | $C_{18}H_{39}NO_3$ | 4.092 |
| 5 | (Nitroimino)dimethanol | $C_2H_6N_2O_4$ | 4.026 |
| 6 | Cer(d18:0/14:0) | $C_{32}H_{65}NO_3$ | 3.859 |
| 7 | Diethylene glycol n-butyl ether | $C_8H_{18}O_3$ | 3.825 |
| 8 | Dichloroacetic acid | $C_2H_2Cl_2O_2$ | 3.806 |
| 9 | Tetradecanedioicacid | $C_{14}H_{26}O_4$ | 3.759 |
| 10 | Cer(d18:0/17:0) | $C_{35}H_{71}NO_3$ | 2.999 |
| 11 | Lauramidopropyl dimethylamine | $C_{17}H_{36}N_2O$ | 2.957 |
| 12 | Cer(d18:0/16:0) | $C_{34}H_{69}NO_3$ | 2.682 |
| 13 | Cer(d18:0/15:0) | $C_{33}H_{67}NO_3$ | 2.177 |
| 14 | (4E)-2-Oxohexenoicacid | $C_6H_8O_3$ | 1.788 |
| 15 | Cyclooctene | $C_8H_{14}$ | 1.472 |
| 16 | Ecdysone 22-palmitoleate | $C_{43}H_{72}O_7$ | 1.418 |
| 17 | (R)-4-Dehydropantoate | $C_6H_{10}O_4$ | 1.253 |
| 18 | (24R_24'R)-Fucosterolepoxide | $C_{29}H_{48}O_2$ | 1.248 |
| 19 | Muscone | $C_{16}H_{30}O$ | 1.237 |
| 20 | Trilaurylamine | $C_{36}H_{75}N$ | 1.232 |

This comparative visualisation highlights solvent-dependent variations in secondary metabolite recovery, demonstrating the influence of polarity on phytochemical extraction efficiency from marine macroalgae.

The heatmap visualisation of the top 20 metabolites identified in positive ion mode reveals distinct distribution patterns across the three brown algae extracts (ethanol, ethyl acetate, and n-hexane). By applying hierarchical clustering and z-score normalisation, the relative abundance of each metabolite is clearly differentiated from high (red) to low (blue) intensity. Several metabolites, such as Tetradecanedioic acid and (4E)-2-Oxohexenoic acid, exhibited consistently high relative abundance in both hexane and ethyl acetate extracts, suggesting their affinity toward non-polar to semi-polar solvents. This may be attributed to their lipophilic properties, making them more soluble in these organic solvents. In contrast, compounds such as Xestoaminol C and Isoheptadecasphinganine showed significantly higher abundance in the ethanol extract, indicating their more polar nature and better extraction efficiency in hydrophilic conditions.

The clustering pattern also reflects solvent-dependent extraction profiles, where certain metabolites cluster strongly with specific solvents. This supports the idea that extraction solvent polarity plays a critical role in shaping the chemical composition of the extracts.

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**Table 3:** Top 20 compounds identified in the ethyl acetate extract of brown algae

| No. | Compound Name | Molecular Formula | Percent Area (%) |
|---|---|---|---|
| 1 | 3-Acetoxy-eicosanoic acid | $C_{22}H_{42}O_4$ | 55.585 |
| 2 | Tetradecanedioicacid | $C_{14}H_{26}O_4$ | 5.335 |
| 3 | Cyclo[D-Leu-Leu-Leu-Leu-Leu-Leu] | $C_{36}H_{66}N_6O_6$ | 3.927 |
| 4 | (4E)-2-Oxohexenoicacid | $C_6H_8O_3$ | 2.679 |
| 5 | 6,10,15,19,23-Pentamethyl-tetracosa-1,2,6,10,14,18,22-heptaene | $C_{29}H_{46}$ | 2.135 |
| 6 | (R)-4-Dehydropantoate | $C_6H_{10}O_4$ | 1.858 |
| 7 | Dihydroxyethyl Lauramine Oxide | $C_{16}H_{35}NO_3$ | 1.492 |
| 8 | Xestoaminol C | $C_{14}H_{31}NO$ | 1.279 |
| 9 | (Nitroimino)dimethanol | $C_2H_6N_2O_4$ | 1.114 |
| 10 | Cocamidopropyl betaine | $C_{19}H_{38}N_2O_3$ | 1.047 |
| 11 | Isoheptadecasphinganine | $C_{17}H_{37}NO_2$ | 1.045 |
| 12 | Cer(d18:0/14:0) | $C_{32}H_{65}NO_3$ | 0.932 |
| 13 | Diethylene glycol n-butyl ether | $C_8H_{18}O_3$ | 0.889 |
| 14 | Lauramidopropyl dimethylamine | $C_{17}H_{36}N_2O$ | 0.797 |
| 15 | Nebrosteroid M | $C_{29}H_{50}O_3$ | 0.796 |
| 16 | Adonirubin | $C_{40}H_{52}O_3$ | 0.709 |
| 17 | Cer(d18:0/17:0) | $C_{35}H_{71}NO_3$ | 0.667 |
| 18 | Cer(d18:0/16:0) | $C_{34}H_{69}NO_3$ | 0.613 |
| 19 | N-{(1S)-3-[4-(3-Isopropyl-5-methyl-4H-1,2,4-triazol-4-yl)-1-piperidinyl]-1-phenylbutyl}-4-morpholinecarboxamide | $C_{26}H_{40}N_6O_2$ | 0.601 |
| 20 | 4,4-Dimethyl-5alpha-cholesta-8,14,24-trien-3beta-ol | $C_{29}H_{46}O$ | 0.597 |

Furthermore, the distribution of metabolites across samples underscores the chemical diversity of brown algae and highlights the potential for targeted metabolite isolation depending on the desired biological activity. For instance, highly abundant lipophilic compounds in hexane or ethyl acetate extract may be more suitable for applications involving membrane permeability or lipid-targeted bioactivities. In summary, the heatmap analysis not only simplifies the interpretation of complex metabolomic data but also provides a strategic guide for selecting appropriate extraction methods based on metabolite polarity and abundance.

The results demonstrate significant variations in chemical composition among the different solvent extracts, with the n-hexane fraction exhibiting the highest chemical diversity. This comprehensive metabolite profiling provides valuable insights into the phytochemical complexity of brown algae and serves as a foundation for further bioactivity studies of these marine-derived compounds

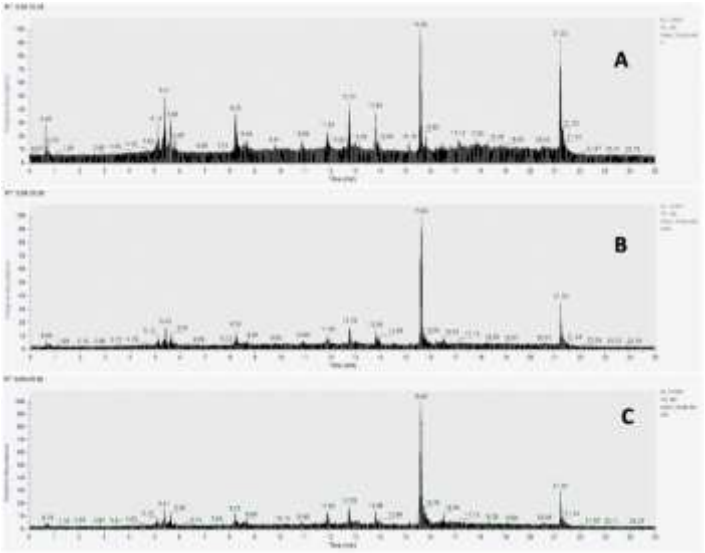


**Figure 1:** LC-HRMS total ion chromatograms of brown algae extracts obtained using different solvents: **(A)** ethanol extract, **(B)** ethyl acetate extract, and **(C)** n-hexane extract

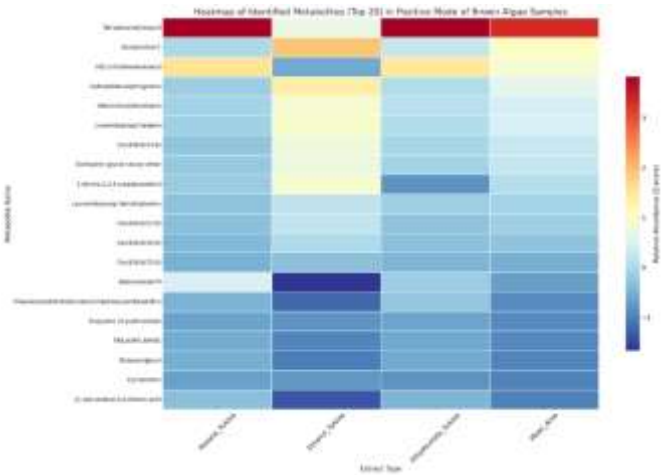

**Figure 2:** Heatmap of identified metabolites (top 20) in positive modes of group samples. Their distribution and relative abundance are high (3) to low (-1) in each brown algae extract

*Potential bioactive compounds*

*Xestoaminol C*

Xestoaminol C or 1-deoxytetradecasphinganine is a tetradecasphinganine type sphingoid in which the terminal hydroxy group has been replaced by a hydrogen. It has a role as a metabolite and also an amino alcohol.[28] Xestoaminol C was detected in all three brown algal extracts, with the highest abundance observed in the ethanol extract. The mass spectrum of Xestoaminol C in the ethanolic extract is presented in Figure 3. Xestoaminol C was identified as a novel-reverse transcriptase inhibitor and found to be less potent than spisulosine. This anti-cancer agent inhibits cell proliferation in several cancer cell lines, such as glioblastoma, leukaemia, and kidney.[29]

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**Table 4:** Top 20 compounds identified in the n-hexane extract of brown algae

| No. | Compound Name | Molecular Formula | Percent Area (%) |
|---|---|---|---|
| 1 | 3-Acetoxy-eicosanoic acid | $C_{22}H_{42}O_4$ | 56.621 |
| 2 | Tetradecanedioicacid | $C_{14}H_{26}O_4$ | 5.606 |
| 3 | 6,10,15,19,23-Pentamethyl-tetracosa-1,2,6,10,14,18,22-heptaene | $C_{29}H_{46}$ | 3.868 |
| 4 | (4E)-2-Oxohexenoicacid | $C_6H_8O_3$ | 2.833 |
| 5 | Cyclo[D-Leu-Leu-Leu-Leu-Leu-Leu] | $C_{36}H_{66}N_6O_6$ | 2.628 |
| 6 | Nebrosteroid M | $C_{29}H_{50}O_3$ | 1.469 |
| 7 | Dihydroxyethyl Lauramine Oxide | $C_{16}H_{35}NO_3$ | 1.117 |
| 8 | Xestoaminol C | $C_{14}H_{31}NO$ | 0.903 |
| 9 | 10Z,13Z,16Z,19Z,22Z,25Z-octacosahexaenoic acid | $C_{28}H_{44}O_2$ | 0.826 |
| 10 | (Nitroimino)dimethanol | $C_2H_6N_2O_4$ | 0.801 |
| 11 | cocamidopropyl betaine | $C_{19}H_{38}N_2O_3$ | 0.774 |
| 12 | Isoheptadecasphinganine | $C_{17}H_{37}NO_2$ | 0.705 |
| 13 | 2-Amino-1,3,4-octadecanetriol | $C_{18}H_{39}NO_3$ | 0.681 |
| 14 | Phytosphingosine | $C_{43}H_{54}N_4O_2$ | 0.676 |
| 15 | Diethylene glycol n-butyl ether | $C_8H_{18}O_3$ | 0.631 |
| 16 | Cer(d18:0/14:0) | $C_{32}H_{65}NO_3$ | 0.582 |
| 17 | 1-Nitrosopyrrolidine (NPYR) | $C_4H_8N_2O$ | 0.569 |
| 18 | Tricosanedioic acid | $C_{23}H_{44}O_4$ | 0.561 |
| 19 | Lauramidopropyl dimethylamine | $C_{17}H_{36}N_2O$ | 0.545 |
| 20 | 11-oxo-undeca-5,8-dienoic acid | $C_{11}H_{16}O_3$ | 0.511 |

*Ceramide*

Ceramides were identified as major constituents of the brown algae extracts, with the highest abundance (13.46% of total ceramide content based on LC-HRMS peak area percentage) observed in the ethanol extract. The predominant ceramide groups, listed in descending order of abundance, were Cer(d18:0/14:0), Cer(d18:0/17:0), Cer(d18:0/16:0) and Cer(d18:0/15:0). Figure 4 illustrates the mass spectrum of Cer(d18:0/14:0).

Ceramides serve as the fundamental structural framework for all sphingolipids, including both glycosphingolipids and phosphosphingolipids.[30] Marine-derived ceramides show potential as anticholinesterase candidates for Alzheimer's disease prevention.[31] Ceramide functions as a potent tumour suppressor lipid that simultaneously induces apoptotic pathways and activates autophagic responses while inhibiting cellular proliferation. As a key apoptotic mediator, ceramide executes programmed cell death in response to TNF-α3 and various proapoptotic stimuli.[32] Marine-derived ceramides have demonstrated significant efficacy in enhancing skin barrier function.[33]

*Fucoxanthin*

Fucoxanthin, a marine-derived carotenoid predominantly found in brown algae and diatoms, represents over 10% of naturally occurring carotenoids and is characterised by its distinct orange pigmentation.[34]

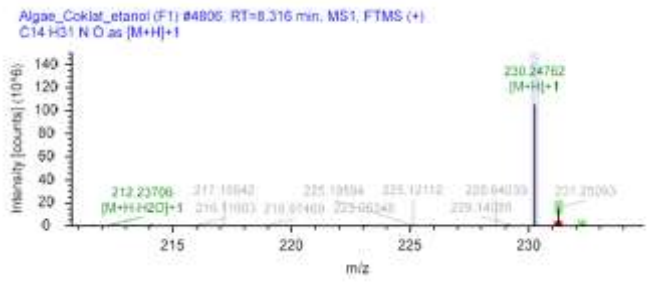


**Figure 3:** Mass spectrum of Xestoaminol C

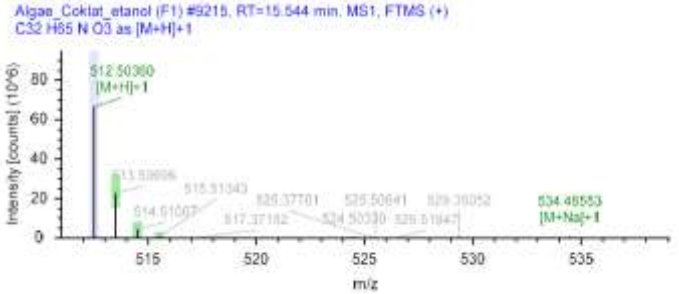


**Figure 4:** Mass spectrum of Cer(d18:0/14:0)

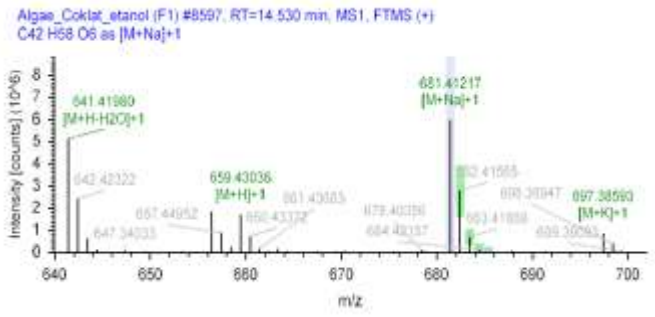


**Figure 5:** Mass spectrum of Fucoxanthin

Fucoxanthin was exclusively detected in the ethanol extract of brown algae at a low concentration (0.52% relative abundance). Figure 5 presents the mass spectrum of fucoxanthin from the ethanol extract.
Fucoxanthin has been scientifically demonstrated to exhibit multiple bioactive properties. These include potent antioxidant activity, significant anti-inflammatory effects, notable anti-breast cancer properties, body weight reduction capability, therapeutic potential for skin disorders, and promising antibacterial potential.[35–40]

## Conclusion

This study successfully characterised the phytochemical composition of brown algae *Sargassum polycystum* from the southern coast of Indonesia using LC-HRMS. Ethyl acetate extract showed the highest phenolic content, while n-hexane extract contained the highest flavonoid content. LC-HRMS analysis identified 232-328 distinct compounds depending on the solvent, with key bioactive compounds including xestoaminol C, ceramides, and fucoxanthin demonstrating potential as anticancer, neuroprotective, and antioxidant agents. These findings highlight the potential of Indonesian brown algae as a valuable source of bioactive compounds for pharmaceutical and cosmetic applications. Future research should focus on bioassay-guided isolation of key metabolites such as Xestoaminol C, ceramides, and fucoxanthin, as well as *in vivo* studies to validate their pharmacological activities.

## Conflict of Interest

The authors declare no conflicts of interest.

## Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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## References

1. El-Gammal MI, Abou-Dobara MI, Ibrahim HAH, Abdulhafith SA, Okbah MA. Polyphenols in Selected Marine Algae and Aromatic Herbs with Antimicrobial Properties: A Comparative Study. Egypt J Aqua Res. 2024; 50(1):71–77. doi:10.1016/j.ejar.2023.12.006.
2. Delgado A, Gonçalves S, Romano A. Mediterranean Diet: The Role of Phenolic Compounds from Aromatic Plant Foods. Foods. 2023; 12(4):840. doi:10.3390/foods12040840.
3. Murdiono WE, Razak NAA, Halmi MIE, Yong JWH, Mahmud K. Optimization of Aqueous Extraction of Phenolic Compounds and Bioactive Profiles from Brown (*Sargassum polycystum*) and Red (*Kappaphycus alvarezii*) Seaweeds Using the Response Surface Method. Algal Res. 2025; 86:103924. doi:10.1016/j.algal.2025.103924.
4. Sobuj MKA, Shemul MS, Islam MdS, Islam MdA, Mely SS, Ayon MH. Qualitative and Quantitative Phytochemical Analysis of Brown Seaweed *Sargassum polycystum* Collected from Bangladesh with Its Antioxidant Activity Determination. Food Chem Adv. 2024; 4:100565. doi:10.1016/j.focha.2023.100565.
5. Dip MdRR, Sobuj MKA, Islam MdS, Akter A, Hasan MdM, Tasnim N. Phytochemicals, Antioxidant and Antibacterial Activity of Crude Extract of *Sargassum polycystum* Collected from Bangladesh. Food Hum. 2024; 2:100278. doi:10.1016/j.foohum.2024.100278.
6. Alreshidi M, Badraoui R, Adnan M, Patel M, Alotaibi A, Saeed M. Phytochemical Profiling, Antibacterial, and Antibiofilm Activities of *Sargassum* sp. (Brown Algae) from the Red Sea: ADMET Prediction and Molecular Docking Analysis. Algal Res. 2023; 69:102912. doi:10.1016/j.algal.2022.102912.
7. Meinita MDN, Yulia R, Nursid M, Nurulita NA, Harwanto D, Riviani R. Morpho-Anatomical Characteristics, Phytochemical and Antibacterial Potential of *Sargassum polycystum* Collected Along the Southern Coast of Java, Indonesia. Biodiversitas. 2024; 25(8):2669-2681. doi:10.13057/biodiv/d250840.
8. Susanti S, Sundari RS, Sarwatiningsih Y, Yuliawati S, Kurniawan R, Mardianingrum R. The Effect of Ultrasound-Assisted Extraction Solvent on Antimicrobial Activity of Gadung Tuber (*Dioscorea hispida* Dennst.). J Pharmacopolium. 2020; 3(3):144–151. doi:10.36465/jop.v3i3.654.
9. Beniddir MA, Kang K Bin, Genta-Jouve G, Huber F, Rogers S, van der Hooft JJJ. Advances in Decomposing Complex Metabolite Mixtures Using Substructure- and Network-Based Computational Metabolomics Approaches. Nat Prod Rep. 2021; 38(11):1967–1993. doi:10.1039/D1NP00023C.
10. Ningsih R, Rafi M, Tjahjoleksono A, Bintang M, Megia R. Ripe Pulp Metabolite Profiling of Ten Indonesian Dessert Banana Cultivars Using UHPLC-Q-Orbitrap HRMS. Eur Food Res Technol. 2021; 247(11):2821–2830. doi:10.1007/s00217-021-03834-7.
11. Aziz Z, Yuliana ND, Simanjuntak P, Rafi M, Mulatsari E, Abdillah S. Investigation of Yacon Leaves (*Smallanthus sonchifolius*) for alpha-Glucosidase Inhibitors Using Metabolomics and In Silico Approach. Plant Foods Hum Nutr. 2021; 76(4):487–493. doi:10.1007/s11130-021-00926-3.
12. Umar AH, Ratnadewi D, Rafi M, Sulistyaningsih YC. Untargeted Metabolomics Analysis Using FTIR and UHPLC-Q-Orbitrap HRMS of Two *Curculigo* Species and Evaluation of Their Antioxidant and alpha-Glucosidase Inhibitory Activities. Metabolites. 2021; 11(1):42. doi:10.3390/metabo11010042.
13. Sinan KI, Saftić L, Peršurić Ž, Pavelić SK, Etienne OK, Picot-Allain MCN. A Comparative Study of the Chemical Composition, Biological and Multivariate Analysis of *Crotalaria retusa* L. Stem Barks, Fruits, and Flowers Obtained via Different Extraction Protocols. S Afr J Bot. 2020; 128:101–108. doi:10.1016/j.sajb.2019.10.019.
14. Aryal B, Adhikari B, Aryal N, Bhattarai BR, Khadayat K, Parajuli N. LC-HRMS Profiling and Antidiabetic, Antioxidant, and Antibacterial Activities of *Acacia catechu* (L.f.) Willd. Biomed Res Int. 2021; 2021(1):7588711. doi:10.1155/2021/7588711.
15. Alallam B, Abdulameed HT, Lim V. Unbiased Metabolomic and Chemometric Profiles of Three *Sargassum polycystum* Extracts Using GCMS and LCMS/MS: Content Analysis,

Correlation Analysis and Molecular Docking. Food Chem. 2025; 470:142666. doi:10.1016/j.foodchem.2024.142666.
16. Irianto I, Agustien N, Ni WT, Astuti A, Kokom K, Lailatul Q, Chaidir C, Ariyanti S, Rika W, Dwila NR, Nicky RP. From Sea to Solution: A Review of Green Extraction Approaches for Unlocking the Potential of Brown Algae. S Afr J Chem Eng. 2024; 48:1–21. doi:10.1016/j.sajce.2024.01.001.
17. Susanti S, Sundari RS, Rizkuloh LR, Mardianingrum R. Effect of Solvents Extraction on Total Phenolic Content and Antioxidant Activity of Gadung Extract (*Dioscorea hispida* Dennst.). Biopropal Ind. 2021; 12(1):43-49. doi:10.36974/jbi.v12i1.6482.
18. Septiani G, Susanti S, Sucitra F. Effect of Different Extraction Method on Total Flavonoid Contents of *Sansevieria trifasciata* P. Leaves Extract. J Farm Galenika (Galenika J Pharm) (e-J). 2021; 7(2):143–150. doi:10.22487/j24428744.2021.v7.i2.15573.
19. Supriadi A, Ridhowati S, Saputra D, Wulandari, Lestari SD. Untargeted Metabolomics Profiling for the Geographical Authentication of Traditional Pempek Using High-Resolution Orbitrap Mass Spectrometry. Food Chem Adv. 2025; 6:100914. doi:10.1016/j.focha.2025.100914.
20. Kusuma J, Analianasari, Wahyudi A, Abdullah MK, Hasan AZ, Asrowardi I. Diversity of the Non-Targeted Metabolomic Data Across Various Varieties of Cloves (*Syzygium* spp.). Data Brief. 2025; 58:111237. doi:10.1016/j.dib.2024.111237.
21. Rivaldi M, Frediansyah A, Aziz SAA, Nugroho AP. Active Biomonitoring of Stream Ecosystems: Untargeted Metabolomic and Proteomic Responses and Free Radical Scavenging Activities in Mussels. Ecotoxicology. 2025; 34(3):425–443. doi:10.1007/s10646-024-02846-9.
22. Dea Firdaus M, Artanti N, Hanafi M, Rosmalena R. Phytochemical Constituents and In vitro Antidiabetic and Antioxidant Properties of Various Extracts of Kenikir (*Cosmos caudatus*) Leaves. Pharmacogn J. 2021; 13(4):890–895. doi:10.5530/pj.2021.13.114.
23. Rahimmalek M, Afshari M, Sarfaraz D, Miroliaei M. Using HPLC and Multivariate Analyses to Investigate Variations in the Polyphenolic Compounds as well as Antioxidant and Antiglycative Activities of Some Lamiaceae Species Native to Iran. Ind Crops Prod. 2020; 154:112640. doi:10.1016/j.indcrop.2020.112640.
24. Yusoff NAH, Rukayadi Y, Abas F, Khatib A, Hassan M. Antimicrobial Stability of *Cosmos caudatus* Extract at Varies pH and Temperature, and Compounds Identification for Application as Food Sanitiser. Food Res. 2021; 5(3):83–91. doi:10.26656/fr.2017.5(3).710.
25. Saw NMMT, Suwanchaikasem P, Zuniga-Montanez R, Qiu G, Marzinelli EM, Wuertz S. Influence of Extraction Solvent on Nontargeted Metabolomics Analysis of Enrichment Reactor Cultures Performing Enhanced Biological Phosphorus Removal (EBPR). Metabolites. 2021; 11(5):269. doi:10.3390/metabo11050269.
26. Wiedmaier-Czerny N, Vetter W. LC-Orbitrap-HRMS Method for Analysis of Traces of Triacylglycerols Featuring Furan Fatty Acids. Anal Bioanal Chem. 2023; 415(5):875–885. doi:10.1007/s00216-022-04480-y.
27. Tamoradi T, Kiasat AR, Veisi H, Nobakht V, Karmakar B. RSM Process Optimization of Biodiesel Production from Rapeseed Oil and Waste Corn Oil in the Presence of Green and Novel Catalyst. Sci Rep. 2022; 12(1):19652. doi:10.1038/s41598-022-20538-4.
28. PubChem. Xestoaminol C. National Institutes of Health. 2025. CID 14756407. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/14756407.
29. Steven AN, Bachuri K, Mat Hussin NSS, Mohd Noh NI. Anticancer Agents and Genetic Identification of *Pterospermum*, an Indigenous Plant of Sarawak, Malaysia. J Taibah Univ Sci. 2024; 18(1):2325677. doi:10.1080/16583655.2024.2325677.
30. Uchida Y, Park K. Ceramides in Skin Health and Disease: An Update. Am J Clin Dermatol. 2021; 22(6):853–866. doi:10.1007/s40257-021-00619-2.
31. Abdelhameed R, Elgawish MS, Mira A, Ibrahim AK, Ahmed SA, Shimizu K. Anti-Choline Esterase Activity of Ceramides from the Red Sea Marine Sponge *Mycale euplectellioides*. RSC Adv. 2016; 6(24):20422–20430. doi:10.1039/C5RA26424C.
32. Wang X, Zhao M, Xia G, Shi H, Li C, Shen X. A Review of Sphingolipids from Marine Sources and Their Analytical Method, Metabolic Process, and Essential Roles in Human Health. Food Front. 2024; 5(5):2015–2042. doi:10.1002/fft2.450.
33. Sugawara T. Sphingolipids as Functional Food Components: Benefits in Skin Improvement and Disease Prevention. J Agric Food Chem. 2022; 70(31):9597–9609. doi:10.1021/acs.jafc.2c01731.
34. Mohibbullah Md, Haque MdN, Sohag AAM, Hossain MdT, Zahan MdS, Uddin MdJ. A Systematic Review on Marine Algae-Derived Fucoxanthin: An Update of Pharmacological Insights. Mar Drugs. 2022; 20(5):279. doi:10.3390/md20050279.
35. Li Y, Kim MB, Park YK, Lee JY. Fucoxanthin Metabolites Exert Anti-Fibrogenic and Antioxidant Effects in Hepatic Stellate Cells. J Agric Food Res. 2021; 6:100245. doi:10.1016/j.jafr.2021.100245.
36. Yang YP, Tong QY, Zheng SH, Zhou MD, Zeng YM, Zhou TT. Anti-Inflammatory Effect of Fucoxanthin on Dextran Sulfate Sodium-Induced Colitis in Mice. Nat Prod Res. 2020; 34(12):1791–1795. doi:10.1080/14786419.2018.1528593.
37. Wang J, Ma Y, Yang J, Jin L, Gao Z, Xue L. Fucoxanthin Inhibits Tumour-Related Lymphangiogenesis and Growth of Breast Cancer. J Cell Mol Med. 2019; 23(3):2219–2229. doi:10.1111/jcmm.14151.
38. Hitoe S and Shimoda H. Seaweed Fucoxanthin Supplementation Improves Obesity Parameters in Mild Obese Japanese Subjects. Funct Foods Health Dis. 2017; 7(4):246-262. doi:10.31989/ffhd.v7i4.333.
39. Rodríguez-Luna A, Ávila-Román J, González-Rodríguez ML, Cózar MJ, Rabasco AM, Motilva V. Fucoxanthin-Containing Cream Prevents Epidermal Hyperplasia and UVB-Induced Skin Erythema in Mice. Mar Drugs. 2018; 16(10):378. doi:10.3390/md16100378.
40. Karpiński TM and Adamczak A. Fucoxanthin—An Antibacterial Carotenoid. Antioxidants. 2019; 8(8):239. doi:10.3390/antiox8080239.