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Potential of Marine Seaweeds for Bioactive Compounds: a Comprehensive Analysis of *Padina australis* Biomass

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Abstract
Seaweeds are a potential source for the extraction of bioactive compounds, which are beneficial to human health. Therefore, the aim of the present study was to comprehensively characterize *Padina australis* GEEL-18 biomass collected from Luhuitou small east China sea. The identification, biochemical composition, physicochemical, and spectroscopic analyses were performed. The presence of high volatile solid (>70 %) suggested a large number of secondary metabolites in *P. australis* GEEL-18 biomass. The major elements in the biomass were carbon (28.96%) with 53.50% of carbohydrates. The essential functional groups (such as amino, hydroxyl, carboxyl, and sulfate groups) were observed in Fourier transform infrared spectroscopy. The high carbohydrate content showed that *P. australis* GEEL-18 is a potential candidate for the extraction of polysaccharides (such as fucoidan, alginate, and laminarin). This study established that brown seaweed *P. australis* GEEL-18 can be used as a substrate for extraction of bioactive compounds which can further be used as food additives, antibacterial, anti-inflammatory, and drug development.

Keywords Seaweeds · Bioactive composition · *Padina australis* · Fucoidan · Alginate

Introduction

Seaweeds produce a wide variety of natural bioactive compounds with unique structures out of their strong adaptive capacity to the environment. The medical applications of seaweed have the potential for food supplements and treatment of chronic diseases due to their immunomodulatory, anti-tumor, anti-diabetes, and anti-oxidation effects (Cotas et al. 2020; Li et al. 2022; Qiu et al. 2022). Fucoidan, carrageenan, and ulvan extracted from brown, red, and green algae can be used for drug delivery (Cunha and Grenha 2016). Phytochemical compounds (such as terpenes, phenols, tannins, and alkaloids) extracted from seaweeds have the potential to prevent disease by acting on the nervous system, cardiovascular system, and immune system (Zidorn 2016). The drugs made from seaweeds can reduce the risk of side effects caused by traditional animal-derived drugs such as heparin used in anticoagulant therapy. The drugs developed from seaweeds extracts also show advantages in reducing the resistance of pathogenic bacteria to existing antibiotics and environmental pollution caused by chemosynthetic drugs. Currently, 11,000 species of seaweed, are identified which are biologically valuable for the production of bioactive compounds (Choudhary et al. 2021). The identification and characterization of more seaweed species, which are capable of producing natural bioactive compounds, are required to meet the medical applications in near future.

The brown seaweeds are characterized by high carbohydrate (such as fucoidan and alginate), low protein, and lipid contents (Garcia-Rios et al. 2012). The fucoidan is a low-cost bioactive molecule and has anti-thrombosis, anti-proliferation, anti-tumor, and antibacterial activities (Hsu and Hwang 2019). The alginate is a natural anionic polymer composed of β-d manuronic acid (M) and α-L-guluronic acid

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(G), which is present in some species of brown algae (up to 40% by dry weight) and has natural wound-healing properties (Liu et al. 2019; Rabillé et al. 2019; Rebecca 2016). The *Padina* is a fan-shaped yellow-brown alga with varying degrees of calcification, distributed in subtropical and tropical seas. The carbohydrate contents of *P. boryana* (44.79%) and *P. tetrastromatica* (59.31%) were high, which provides a baseline for the extraction of bioactive compounds from species belong to *Padina* (Ismail et al. 2017; Kokilam et al. 2013).

The yield and structure of each biochemical component in *Padina* are influenced by species, growing environment, and harvest season. *Padina* can mostly grow in any season, however its growth is relatively slow in summer, while winter is the main growth and reproduction period. The exposure to environmental contaminants (such as heavy metals) can increase the content of polysaccharides, sulfate group, uronic acid, caramel, mannose, and galactose in *P. gymnospora* (Andrade et al. 2010). The habitat such as (light availability, biotic, and abiotic factors) has also been found to influence seaweeds morphology. The seaweed species *P. tetrastromatica* (Hauck) and *P. pavonica* from different coasts showed some small differences in size and physiologic structure (Uddin et al. 2015). The choice of harvest season is a crucial step it may affect the biochemical composition of seaweeds. For instance, the biomass of *P. pavonica* harvested in July showed 12.5% of fucoidan, which was comparatively higher than the biomass obtained in March (0.09%) (Men'shova et al. 2012). Therefore, understanding *Padina's* biochemical composition is essential to interpret its biological activity and bioactive compounds potential.

In this study, the biomass of *Padina* was collected and identified based on morphology and molecular remark. This was followed by fully characterization of biocomponents (including lipids, proteins, and carbohydrates). The total solid, volatile, and elements composition (such as carbon, hydrogen, nitrogen, and sulfur) of the biomass were analyzed. Fourier transform infrared (FTIR) spectroscopy and thermogravimetric analysis (TGA) were also studied for a deep understanding of the applications of *P. australis* GEEL-18.

Materials and Methods

Seaweeds Collection

The seawater and seaweeds samples were collected in June 2021, from Luhuitou small east China sea, Jiyang District, Sanya City, Hainan Province, China (20˚N, 110˚E). The schematic diagram of biomass preparation for identification and full characterization of seaweeds is shown in Figure 1.

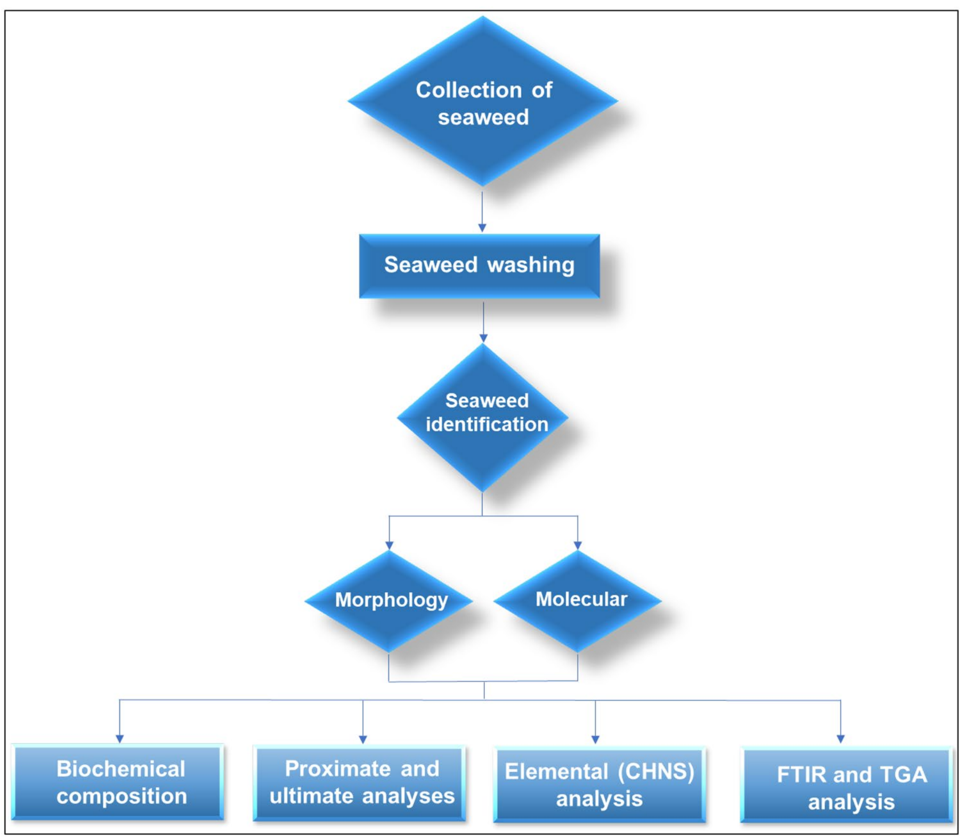

Fig. 1 The schematic diagram of biomass preparation for identification and full characterization of *P. australis* GEEL-18

The seaweed sample was washed with seawater immediately after collection to remove sediments, epiphytic plants, and small herbivores, and then transported together with seawater on ice to Green Environment and Energy Laboratory (GEEL), Lanzhou University. The discoid holdfast of seaweed was removed and rinsed repeatedly with distilled water to remove the salt. The rinsed sample was dried at room temperature for 48 h and stored in airtight plastic bags for further analysis. The analyses of the physicochemical properties were done in triplicates. The pH, conductivity, total phosphorus (TP), total nitrogen (TN), dissolved oxygen (DO), total dissolved solids (TDS), chemical oxygen demand (COD), total organic carbon (TOC), and total salt content of seawater are presented in Table 1. The physiochemical characterization of various parameters of seawater (including salt, TN, TP, DO, COD, and TDS) were performed to understand the habitat of seaweed. Such deep investigation could provide a further framework for the cultivation of seaweed cultivation on a lab and large scale.

Seaweed Identification

The preliminary identification was carried out based on morphological and physiological characteristics of the sample by comparing it with the original descriptions of the recorded species (Ni Ni et al. 2008). The final identification was carried out at the gene level using molecular-based methods. The Sanger sequencing method was used to extract DNA from samples using Ezup column plant tissue genomic DNA extraction kit (Shanghai Shenggong Bioengineering Co., LTD, China). The PCR amplification, agarose electrophoresis detection, and gel recovery were used to amplify DNA and test the purity of DNA. PCR amplification consists of three steps: denaturation of template DNA, annealing of template DNA and primer, and extension of primer. Denaturation involves heating template DNA to 94 ℃ to dissociate the double-stranded DNA into single strands. Annealing is the binding of primers 1143-510-2-F (AATTGACGGAAKGGCA) and 1637-510-2-R (CGACGGGCGGTGTGTA) to complementary sequences of single-stranded DNA cooled to 63 ℃ (dropping 0.5 ℃ per cycle). The extension of the primer is that Taq plus DNA polymerase expands the short sequence primer by binding DNA bases complementary to the template chain, and this reaction is carried out at 72 ℃. Taking 5 μL DNA solution, 1% agarose, and 1X TAE buffer solution electrophoresis (voltage 120~180 V) for DNA quality detection, there are obvious bands that meet the requirements of PCR. Gel recovery was carried out using the SanPrep column DNA gel recovery kit (Shanghai Shenggong Bioengineering Co., LTD, China). The PCR products were sequenced using the 3730 xl sequencing machine (Applied Biosystems Inc, America) (Yang et al. 2021). Subsequently, species were identified through a BLAST search of the GenBank database.

Table 1 The major physicochemical parameters for the seawater where the seaweed biomass was collected

Parameters	Values
pH	7.42
Conductivity (μs cm^{-1})	721.33±5.25
Total phosphorus (mg L^{-1})	0.07±0.01
Total nitrogen (mg L^{-1})	1.86±0.19
Dissolved oxygen (mg L^{-1})	3.23±0.21
Total dissolved solids (g L^{-1})	0.50±0.05
Chemical oxygen consumption (g L^{-1})	2.03±0.23
Total organic carbon (g L^{-1})	11.48±1.49
Total salt (g L^{-1})	36.17±0.40

Analyses of Seaweed Biocomponents

The phenol-sulfuric acid method is a rapid and sensitive method for the determination of carbohydrate content in seaweed samples (Nielsen 2010). The measured absorbance of the sample at 490_{nm} was compared with the standard curve drawn from a series of different glucose concentrations to obtain the carbohydrate concentration. The protein content was measured by the Lowry method using the bovine serum protein (BSA) standard (Waterborg 2002). 5 mL of Lowry solution and 0.5 mL of Folin-Ciocalteu reagent were added to 3 mg mL^{-1} of seaweed biomass and the absorbance was measured at 660_{nm}. The crude lipid content measurement was carried out by Bligh and Dyer method with a slight modification (Breil et al. 2017). Chloroform and methanol (1:2 v v^{-1}) were used for lipid extraction. The extracted lipid was washed with 5% sodium chloride and then placed in the oven (50 ℃) to dry and weigh.

Characteristics of Seaweed Biomass

Proximate and Ultimate Analyses

The total solid (TS) of biomass was estimated after heating the sample in a blast drying oven (DHG-9000, Shanghai Yiheng Scientific Instrument Co., LTD) at 105 ℃ for 24 h, and the lost weight was considered as volatile moisture. The sample was further heated at 550 ℃ for 2.5 h in the Leco TGA 701 instrument (America), where the lost weight represented a volatile solid (VS) and the remaining material was ash. The relative proportions of carbon, hydrogen, nitrogen, and sulfur in the seaweed sample were determined using a CHNS element analyzer (Vario EL Cube, Germany). The higher heating value (HHV) was calculated using the formula proposed by Dulong (Channiwala and Parikh 2002). The analyses were performed in triplicate and the data are expressed as mean ± standard deviation (SD). The Graphpad Prism version 8.4.3 was used for data processing and graph plotting.

Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups in biomolecules were identified by FTIR at the mid-infrared band (4000-400 cm^{-1}) to obtain the position and strength of peaks. The dried seaweed powder was scanned in transmission mode at a resolution of 4 cm^{-1} using a cadmium mercury telluride detector, and Nicolet Magna 550 (Madison, USA) was used for data collection and processing (Arif et al. 2021).

Thermogravimetric Analysis (TGA) and Differential Thermogravimetric (DTG) Analysis

The thermogravimetric analysis (TGA) uses high-resolution microbalances to accurately record the mass changes of the sample over the temperature range by heating the sample to a specified temperature in an inert gas environment. This method helps to understand the pyrolysis properties of the sample. In brief, 5 mg of sample powder was heated from 32 ℃ to 900 ℃ at a rate of 10 ℃ min^{-1} in a continuous supply of nitrogen (100 mL min^{-1}) using a thermal analyzer (Linseis Messgerate GmbH, Germany) (Bach and Chen 2017). The TGA curve represented the weight loss with increasing temperature, while differential thermogravimetric analysis (DTG) represented the degradation rate at different temperatures.

Results and Discussion

Morphological and Molecular Identification of Seaweed Species

The collected seaweed samples was observed as fan-shaped, yellow-brown, concentric bands, which appeared grayish-white. The obtained biomass was compared with the other reported seaweeds (on the bases of morphology and geographical locations). The biomass was identified preliminarily belongs to genus *Padina* (Ni Ni et al. 2010). The molecular identification confirmed the obtained biomass as *P. australis*, and its gene sequence was uploaded to the NCBI database with the accession number OL752604. The phylogenetic relationships and differentiation between *P. australis* GEEL-18 and other species of genus *Padina* are shown in Fig. 2.

Biochemical Characteristics of Potential Value-added Compounds

The biochemical composition of the *P. australis* GEEL-18 was compared with the reported seaweeds under the phylum of brown seaweeds (Table 2). The biomass of *P. australis* GEEL-18 (53.50%) showed richness in carbohydrate content, which exhibited its potential for bioactive compounds associated with polysaccharides. The biochemical components of seaweeds can be affected by the physical and chemical properties of the habitat such as air temperature, seawater temperature, salinity, and annual precipitation

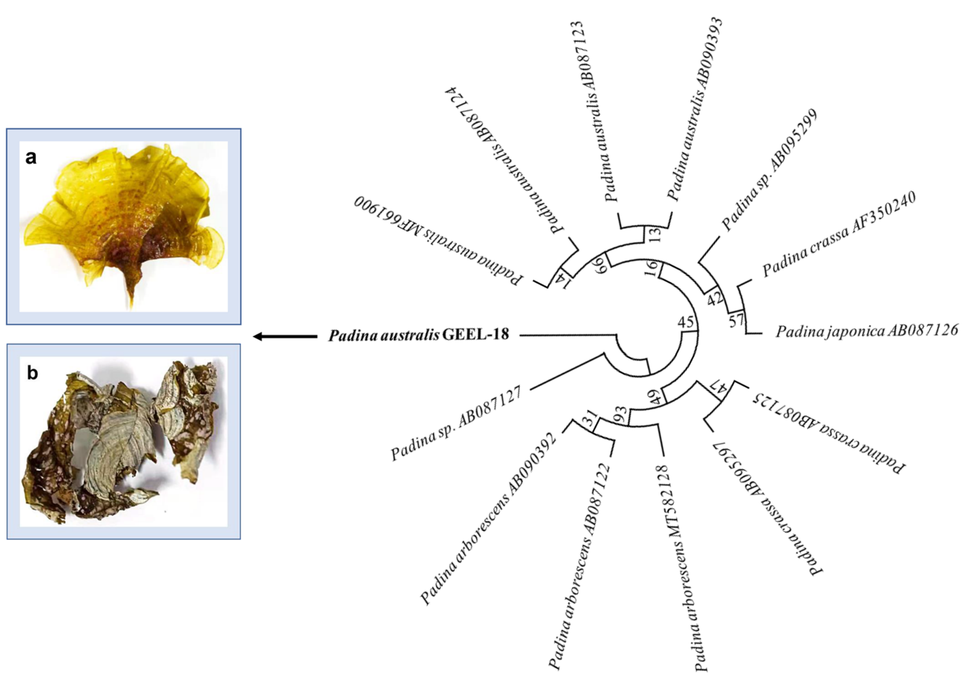

Fig. 2 The phylogenetic tree of the various seaweed species shows the relationship between *P. australis* GEEL-18 and other species of the genus *Padina*. **a** fresh seaweed and **b** dried seaweed

Table 2 The biochemical composition of *P. australis* GEEL-18 and previously reported brown seaweeds

Seaweed species	Geographical locations	Biochemical components (%)			References
		Carbohydrate	Protein	Lipid	
P. australis	Malaysia	47.91	10.21	1.95	(Jaswir et al. 2014)
P. boryana	Abu Qir Bay, Egypt	44.79	16.27	1.50	(Ismail et al. 2017)
P. tetrastromatica	Gulf of Mannar	59.30	11.39	0.55	(Kokilam et al. 2013)
P. tetrastromatica Hauck	Tamilnadu, India	5.09	18.40	1.26	(Sethi 2021)
P. gymnospora	Southeast coast of India	29.13	26.10	40.20	(Akalya et al. 2021)
P. gymnospora	Gulf of Mannar	11.81	0.57	0.002	(Shanmuganathan and Kasi 2016)
P. australis GEEL-18	Hainan Province, China	53.50	2.44	4.00	This study
Dictyota dichotoma	Madeira Archipelago, Portugal	49.76	7.22	10.00	(Nunes et al. 2020)
Halopteris scoparia	Madeira Archipelago, Portugal	29.86	5.54	3.64	(Nunes et al. 2020)
Cystoseira compressa	Madeira Archipelago, Portugal	56.55	4.05	5.61	(Nunes et al. 2020)

(Ismail et al. 2017). For example, the higher average air temperatures (>25℃) and sea water temperatures (29-32℃) in the Straits of Malacca hindered the carbohydrate accumulation of seaweed (Richardson et al. 2004). Similarly, the high annual mean sea level temperature (30 ℃) and low annual precipitation (205 mm) in the Red Sea resulted in lower carbohydrate and lipid levels of *D. dichotoma* compared to the same species grown in Madeira and Porto Santo Islands (Kasimala et al. 2020). In addition, large differences were also shown in the carbohydrate content of *P. gymnospora* (29.13% and 11.81%) and *P. tetrastromatica* (59.30% and 5.09%) collected from different sites. Polysaccharides from the genus *Padina* have been shown to possess health protection abilities. Fucoidan isolated from *P. commersonii* inhibited lipopolysaccharide-induced macrophage inflammation by blocking the TLR/NF-Kappa B signaling pathway, which is vital in pathogenesis (Sanjeewa et al. 2019). *In vivo* and *in vitro* studies showed that sulfated polysaccharides in *P. tetrastromatica* can activate PI3K/Akt/Nrf2 signaling pathway, reduce lipid peroxidation, and protect the cardiac trauma induced by isoproterenol (Lekshmi et al. 2019).

The protein content of *P. australis* GEEL-18 (2.44%) and *P. gymnospora* (0.57%) was significantly lower than that of other species of genus *Padina*. The protein accumulation in brown algae positively correlated with nitrogen element (the nitrogen-protein conversion coefficient is 6.25.), negatively correlated with temperature and salinity. Moreover, the protein content of the brown algae is also correlated with the harvesting season, higher in winter & spring, and lower in summers (Marinho-Soriano et al. 2006; Polat and Ozogul 2013). The low nitrogen (1.39%), harvest season (summer), and slightly higher salinity (36.17%) may contribute to the low protein level of *P. australis* GEEL-18. It indicates that *P. australis* growing in the Hainan Province of China is a low-protein species, which is not capable of mass production of protein and related bioactive compounds. As a result, there seem to be no reports on the biological activity of *Padina* protein or protein extract, mainly studying the biological activity of *Padina* extract and polysaccharides (Bhuyar et al. 2021; Caruana et al. 2021). The protein content of *P. australis* collected in Malaysia, *P. tetrastromatica* and *P. gymnospora* from different collection sites were significantly different, which proves that the growing environment has a significant influence on biochemical composition (Table 2). The environmental factors (such as turbidity, temperature, salinity, and nutrient level of the habitat) affect the photosynthetic rate and nutrient absorption rate of seaweeds, thereby affecting the biochemical contents (Barrow et al. 2015; Uddin et al. 2015). The clean water allows more sunlight to reach the seaweeds that promotes photosynthesis, whereas the increase of temperature and the decrease of salinity is detrimental to their growth (Baweja et al. 2016; Diehl et al. 2020).

The lipid content of *P. australis* GEEL-18 was 4.00%, which was above average among brown algae in general. The content of lipid in brown algae varied widely, ranging from 0.002% to 40.20%. The lipid content in brown algae was less affected by seasonal variation and was relatively stable. However, the types of lipid and fatty acids were related to seasonal variations (Ansari and Ghanem 2019; El Maghraby and Fakhry 2015). The phospholipids and polar lipids in *C. costata* made up the majority of total lipids in Spring and June, while neutral lipids dominate in July (Gerasimenko et al. 2010). The presence of fatty acids and flavonoid compounds (2-phenyl-4H-1-benzopyran-4-one) are reported to have antioxidant properties in *P. tetrastromatica* (Maheswari et al. 2018). The active extraction of *P. boergesenii*, which contains fatty acids and terpenoids, can inhibit the activity of α-glucosidase, thus achieving the effect of hypoglycemia and prevention of metabolic syndrome (Landa-Cansigno et al. 2020). The unsaturated fatty acids contained in lipids are essential for preventing cardiovascular diseases and maintaining the normal function of brain and nerve cells. Considering the importance of lipids and unsaturated fatty acids in medical applications is essential and more research should be done in future.

Physiochemical Analysis of Seaweed Biomass

The proximate and ultimate characterization of *P. australis* GEEL-18 provided a deep understanding of the physicochemical properties of the biomass (Fig. 3). The total solid and volatile contents of *P. australis* GEEL-18 were 92.68% and 70.83% (Fig. 3a). The volatile organic compounds represent a kind of secondary metabolites. These compounds are important mechanisms that help seaweed resist harsh environments while having a few or no side effects in treating human diseases. The high salinity (36.17 g L^{-1}) resulted in high ash content (29.17%) of *P. australis* GEEL-18 biomass. The ash formation and content in seaweeds majorly relies on the ratios of Mg/Ca, Na/K, and Cl contents (Skoglund et al. 2017; Tabassum et al. 2016).

The carbon, hydrogen, nitrogen, and sulfur elements of *P. australis* GEEL-18 were 28.96%, 3.98%, 1.39%, and 0.73%, respectively (Fig. 3b). The high C/N ratio (20.84) was consistent with the high carbohydrate and low protein levels in seaweed. The lower protein content in this study resulted in a higher C/N ratio than previous studies (6.07-11.10) (Yang et al. 2021). The contents of C, H, N, and S elements in seaweed were significantly lower than those in previous studies, leading to the decrease of the higher heating value (HHV, 3.90) (Yang et al. 2021).

FTIR Spectra Analysis of Seaweeds Biomass

The peaks of FTIR in the biomass of *P.* australis GEEL-18 were used to identify the functional groups (Fig. 4). The peak of 3427 cm^{-1} was attributed to stretching vibrations of hydroxyl (O-H) and amino (N-H) functional groups in polarity bonds, proving the existence of polysaccharides and amino acids. The weak signal at 2924 cm^{-1} was due to C–H asymmetrical stretching on -CH_2 or -CH_3, which corresponds to the aliphatic group. Similar to previous studies, there were two bands in the range of 4000-2000 cm^{-1} of FTIR spectrum (Gomez-Ordonez and Ruperez 2011). The asymmetric stretching of O-C-O of carboxylate resulted in the appearance of a weak peak at 1628 cm^{-1}, which may be related to the uronic acids in fucoidan (Mohd Fauziee et al. 2021). The intense sharp band observed at 1485 cm^{-1} is mainly related to the C-OH stretching (Drira et al. 2021). The region between 1300-400 cm^{-1} showed three characteristic absorption bands which includes, the signal at 1034 cm^{-1} related to the asymmetric stretching vibration of C-O-C of alginate and fucoidan (Drira et al. 2021; Sharma and

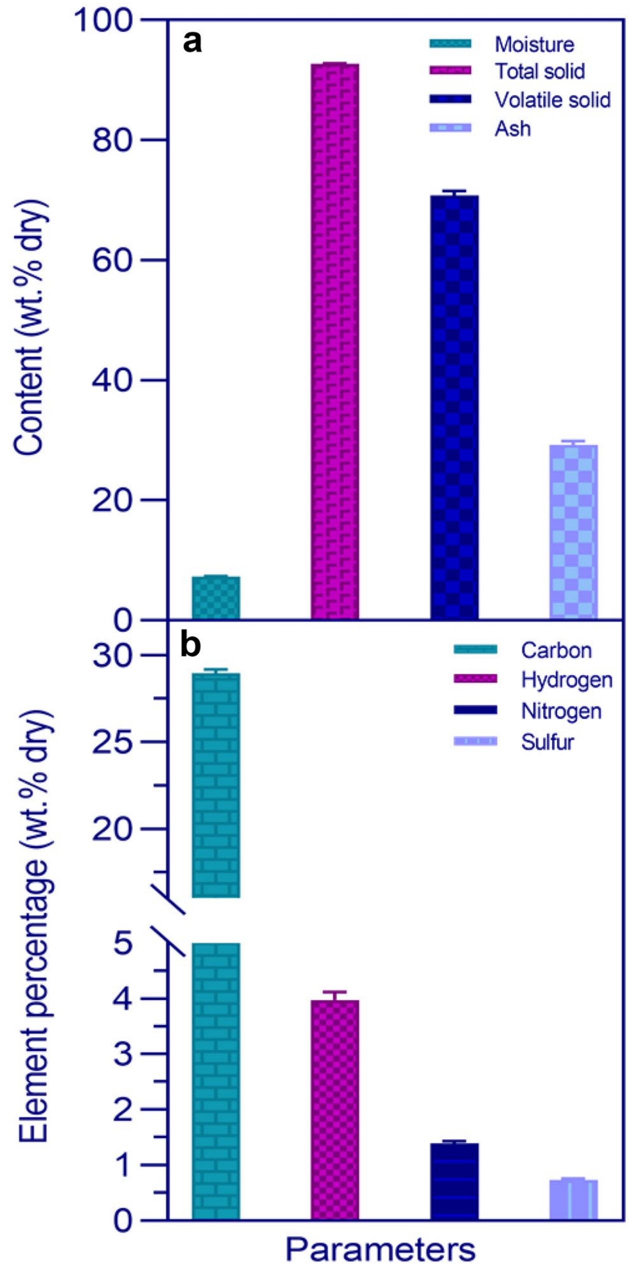

Fig. 3 The physicochemical characteristics of the biomass of *P. australis* GEEL-18 proximate analysis (**a**) and ultimate analysis (**b**)

Baskaran 2021), steep peak at 856 cm^{-1} attributed to C-O-S vibration of sulfate substituents (Synytsya et al. 2010), and peak at 713 cm^{-1} was caused by C-O-C bending vibrations in glycosidic linkages (Vanavil et al. 2020). A weak absorption peak at 1257 cm^{-1} was assigned to the S=O stretching band of the sulfate groups, which is a typical feature of fucoidan and sulfated polysaccharides in brown seaweeds (Gomez-Ordonez and Ruperez 2011). The band at 877 cm^{-1} was caused by the C1-H deformation vibration of β-mannuronic

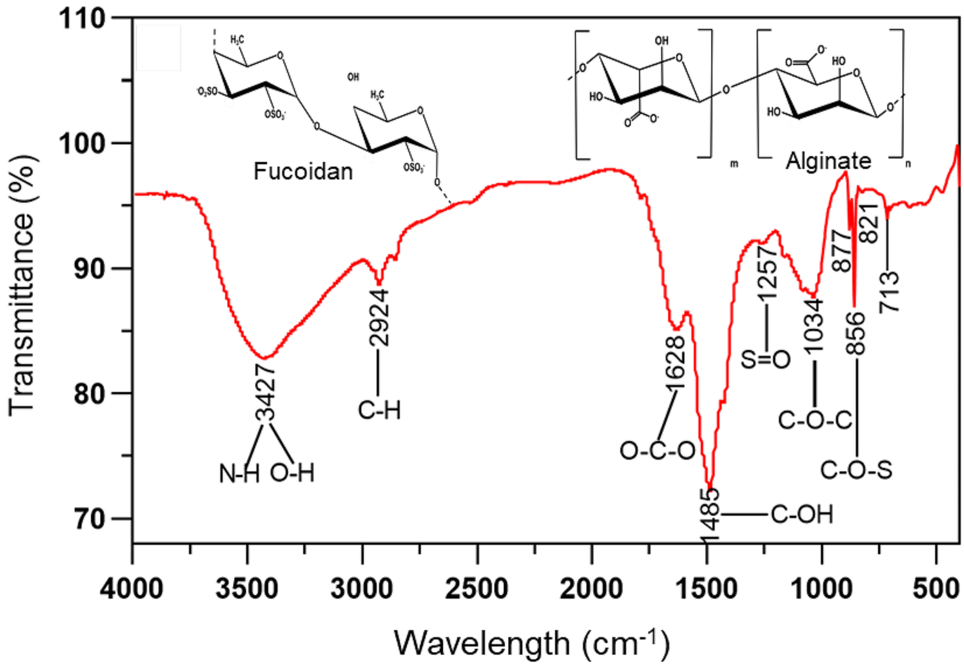

Fig. 4 Fourier transform infrared spectroscopy (FTIR) indicated the distribution of functional groups of *P. australis* GEEL-18 by position and shape of the peaks

acid residues, and the band at 821 cm^{-1} represented mannuronic acid residues (Gomez-Ordonez and Ruperez 2011). Therefore, alginate and fucoidan are the main polysaccharides found in seaweed.

TGA Analysis of Seaweeds Biomass

TGA and differential thermogravimetric analysis (DTG) curves of *P. australis* GEEL-18 monitored under nitrogen atmosphere are depicted (Fig. 5). The pyrolysis behavior can be interpreted in four stages. The weight loss (14.3%) of the

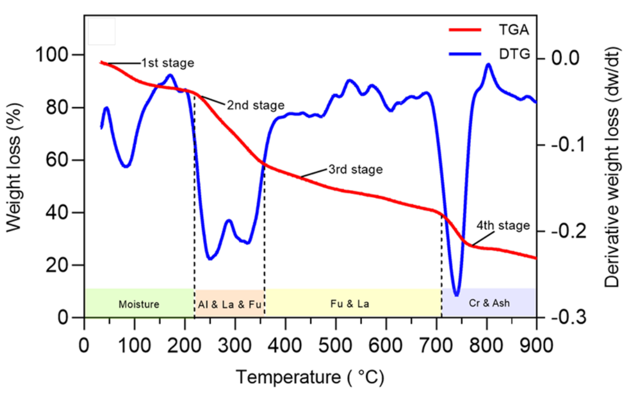

Fig. 5 Thermogravimetric analysis (TGA) and differential thermogravimetric (DTG) analysis of *P. australis* GEEL-18 demonstrated the pyrolytic behavior of biocomponents. Abbreviations: Al (alginate), La (laminarin), Fu (fucoidan), Cr (carbonized residues)

sample at the initial stage between 35 ℃ and 216 ℃ was associated with the release of free water and bound water in alginate, indicating that alginate is an important fraction of polysaccharides (Faidi et al. 2020). The TGA curve declined rapidly in the second stage (216 ℃-354 ℃). At the same time, combined with the DTG curve observed in the second stage of the two huge peaks, it is caused by the thermal degradation of the polymer in alginate (Saravana et al. 2018). Simultaneously the partial degradation of laminarin was observed (Anastasakis et al. 2011). Compared with the second stage, the pyrolysis rate of the sample in the third stage (354 ℃-704 ℃) was relatively slow. This stage was the second stage of the degradation of laminarin. The fucoidan was involved in the evolution of volatile matters in the second and third stages (Anastasakis et al. 2011).

The final stage, which began at 704 ℃ and continued to nearly 900 ℃, was the degradation process of carbonized residues, which may be phosphate, sulfate, and carbonate in polysaccharides (Vasantharaja et al. 2019). The TGA analysis proved the presence of laminarin as well as the presence of fucoidan and alginate in the sample polysaccharides. TGA can help to understand the main substances present in species and provide a general direction for subsequent research. As potential producers of bioactive compounds, the products made from seaweed ingredients are widely used in medicine, cosmetics, biofuels, prevention and control of animal and plant pathogens, food additives, and the chemical industry (Fig. 6).

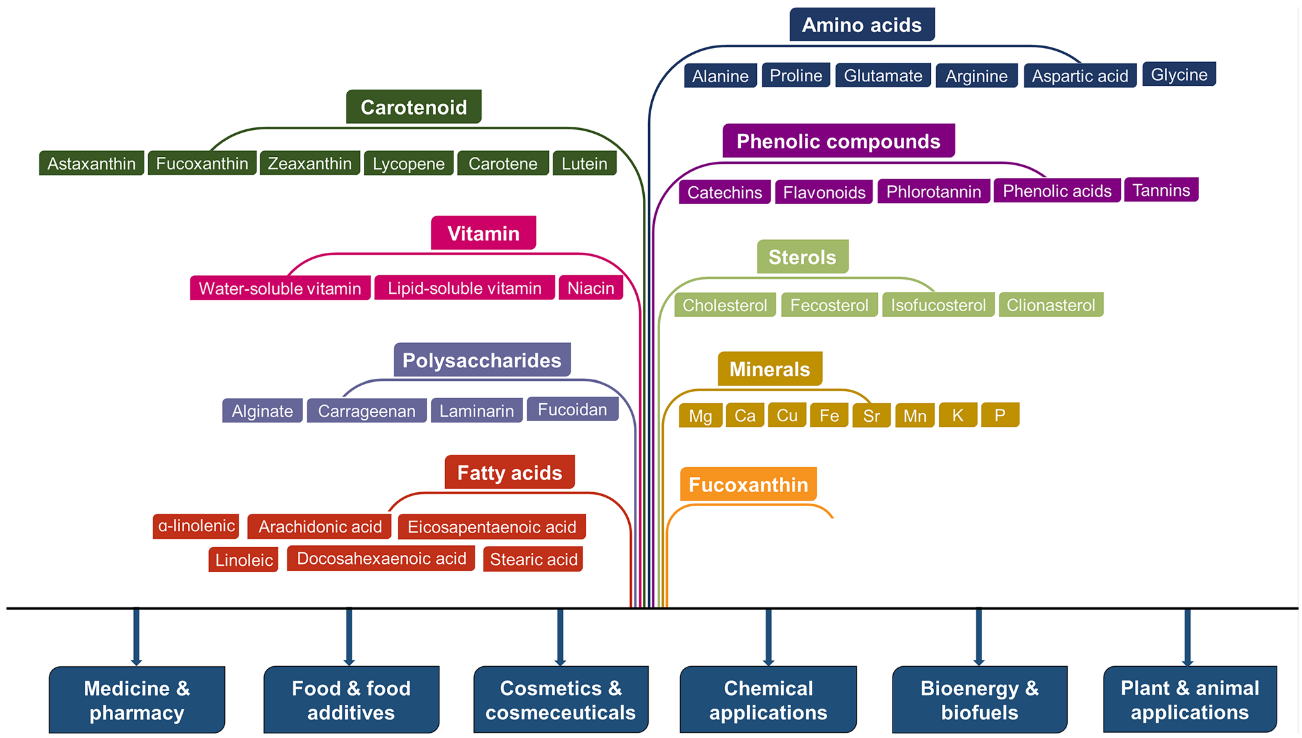

Fig. 6 Seaweeds are a potential source of bioactive compounds with a wide range of other applications (such as nutritional, medical, therapeutic, cosmetics, and bioenergy)

Conclusions

The bioactive potential of *P. australis* GEEL-18 was identified based on biochemical composition and physicochemical properties. The high carbohydrate and volatile solid confirmed that the seaweed in this study is a potential source of bioactive compounds for treating diseases and human nutritional supplements. The lipids can be a natural source of essential unsaturated fatty acids for humans. The polysaccharides (such as fucoidan, alginate, and laminarin), amino acids, and fatty acids have great potential in the medical and pharmaceutical fields. Considering its nutritional value and potential bioactive compounds, *P. australis* GEEL-18 can be used in the production of medicines and health care products to prevent/treat diseases.

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Author's Contribution Yang Yang: Visualization, Investigation, Methodology, Data curation, Formal analysis, Writing, Original Draft. Yang Qi: Investigation, Data curation, Formal analysis, Writing-Review & Editing. Adel I. Alalawy: Visualization, Formal analysis, Data Curation, Validation, Project administration. Ghena M. Mohammed: Visualization, Formal analysis. Fahad M. Almasoudi: Visualization, Formal analysis. El-Sayed Salama: Conceptualization, Supervision, Resources, Data Curation, Validation, Writing-Review & Editing, Funding acquisition, Project administration.

Data Availability The datasets presented in this study can be found in online repositories. The name of the strain and accession number is available in the below link: https://www.ncbi.nlm.nih.gov/nuccore/OL752604.

Declarations

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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