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Chemical Composition, Phytochemical Constituent, and Toxicity of Methanol Extract of Brown Algae (*Padina* sp.) from Puntondo Coast, Takalar (Indonesia)

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HIGHLIGHTS

- *Padina* sp. from Puntondo coast is rich in nutrients (protein, carbohydrates, and minerals).
- *Padina* sp. has bioactive components such as alkaloids, flavonoids, steroids, and tannins.
- *Padina* sp. can be developed as a raw material source for the functional foods.

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Acronyms and abbreviations
AAS=Atomic Absorption Spectrophotometry
FTIR=Fourier-Transform Infrared Spectroscopy
GC-MS=Gas Chromatography-Mass Spectrometry

ABSTRACT

Background: Padina sp. is an algae that has potential as a functional food. This study aimed to explore the chemical and photochemical constituent in the methanolic exract of *Padina* sp.

Methods: Brown algae of *Padina* sp. from Puntondo coast, Takalar, Indonesia was prepared. The algae characterization was carried out based on the standard procedure of Association of Official Agricultural Chemists (AOAC). Toxicity of *Padina* sp. was determined with Brine Shrimp Lethality Test (BSLT).

Results: Chemical contents were 13.46% water, 38.02% ash, 12.33% protein, 1.60% fat, 20.02% fiber, and 48.06% carbohydrate. The FTIR spectrum displayed the presence of hydroxyl, carboxylic acids, aldehydes, aliphatic hydrocarbons, fatty acids, and unsaturated hydrocarbons. *Padina* sp. extract consisted of phytol compound which had 70-96% similarity with steroids, fatty acids, carboxylic acids, terpenoid, and proteins. The result of toxicity was 6344.54 ppm indicating not toxic.

Conclusion: *Padina* sp. can be used as a raw material source for functional food and pharmaceutical industry.

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Introduction

Indonesia, as a maritime country with ¾ an area in the form of oceans, has abundant natural resources, including various types of algae. Algae are a group of chlorophyll plants that consists of one or many cells and forms

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colonies. Algae contain organic materials such as polysaccharides, hormones, vitamins, as well as secondary metabolite compounds that can act as bioactive compounds. Antioxidants and antibacterial properties have been found in marine algae (Renhoran et al., 2017; Sanger et al., 2013; Shannon and Abu-Ghannam, 2016). Algae also have anti-inflammatory, anti-diabetic, anti-cancer, and the other properties (Chew et al., 2008; Kim et al., 2008). So far, the use of algae as a trade commodity or industrial raw material is still relatively small compared to the diversity of algae species in Indonesia (Kadi, 2004). The high diversity of algae species provides great opportunities for the exploration of bioactive compounds.

Biological active compounds are secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, and saponins. The content of secondary metabolite compounds in algae can be determined with an approach method that can provide information on the presence of secondary metabolite compounds. The phytochemical assay is a method that can be used to analyze the phytochemical components possessed by algae (Qin, 2020). Some algae also have pigments utilized as dyes and have many health benefits. The type of photosynthetic pigments of algae consists of chlorophyll (a, b, c), carotenoids (carotene and xanthophyll), and phycobilin (phycoerythrin and phycocyanin) (Pesang et al., 2020).

In addition, algae are admitted to be utilized in the food sector and are potential as a new source of the bioactive compound for humans, animals, plant fertility, as well as a source of synthons and biocatalysts in sustainable chemistry studies (Wells et al., 2017). Therefore, algae can be a source of functional food.

Brown algae are one of the resources that grow on the plains of coral reefs. Several types of brown algae that have been known are *Padina*, *Sargassum*, and *Turbinaria* (Wouthuyzen et al., 2015). Brown algae contain 54.3-73.8% carbohydrates, 0.3–5.9% protein, vitamins (vitamins B1, B2, B6, B16, C, and niacin), and minerals, especially calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), iodine (I), iron (Fe), and contain several bioactive components, namely phenolic compounds, natural pigments, sulfate polysaccharides, fiber, and other bioactive components that have been studied for health benefits (Qin, 2020). Santoso et al. (2013) stated that algae have a high mineral content that can be consumed by humans.

Brown algae in Indonesian waters have not been utilized by coastal communities. For example, in the waters of Puntondo, Takalar Regency in South Sulawesi Province, brown algae, especially *Padina* sp. type is still considered as ocean waste because of the lack of public knowledge about the benefits of this type of brown algae.

The chemical composition, even the phytochemicals constituent of brown algae, namely *Padina* sp., from the coastal waters of Puntondo has not been widely reported. Thus, this study aimed to explore the chemical and photochemical constituent of *Padina* sp. in a form of methanolic extract. Research on chemical composition and phytochemicals in brown algae of *Padina* sp. is expected to provide useful information, especially for coastal communities, so that brown algae can have a useful value that can benefit society.

Materials and methods

Materials

The raw material in this study is *Padina* sp., brown algae from Puntondo, Takalar regency (Indonesia) which taken at a water depth of±1 meter. The chemicals used for analysis were methanol p.a (Merck, Germany), Kjeldahl tablets (Merck, Germany), K_2SO_4 (Merck, Germany), $CuSO_4$ (Merck, Germany), concentrated H_2SO_4 (Merck, Germany), H_2O_2 (Merck, Germany), H_3BO_3 4% (Merck, Germany), NaOH 40% (Merck, Germany), HCl 0.2 N (Merck, Germany), Chloroform (Merck, Germany), NH_4OH (Merck, Germany), Mayer's witness (solution A 1.36 g $HgCl_2$ (Sigma Aldrich,US) dissolved in 60 ml of distilled water, solution B 0.5 g KI (Merck, Germany) dissolved in 10 ml distilled water, Wagner reagen (2.5 g I2, 3 Kl (Merck) and 10 ml aquades), Dragendroff reagen (solution A 0.85 g bismuth nitrate (Sigma Aldrich, US), 2 ml concentrated HCl (Merck, Germany) and 10 ml distilled water, solution B 8 g KI_2 (Merck) and 10 ml distilled water), CH_3OH 70% (Merck), Mg (Merck, Austria), $FeCl_3$ 1% (Merck, Germany), Na_2CO_3 (Merck, Germany). The tools used were Blenders (Miyako CH-501, China), glasswares (Pyrex, Japan), destructive pumpkins (Pyrex, Japan), hot plate (Favourite HP0707V2, Japan), Fourier-transform Infrared Spectroscopy (FTIR) (Shimadzu 8,501, Japan), Atomic Absorption Spectrophotometry (AAS) (Shimadzu AA 7,000, Japan), Gas Chromatography-Mass Spectrometry (GC-MS) (Shimadzu QP-2010, Japan).

Algae identification and characterization

Samples of brown algae of *Padina* sp. were obtained from Puntondo coast, Takalar Regency, washed with seawater to clean from dirt and sands. Those samples were dried by wind and grounded using a crusher machine, also sieved with a size of 60 mesh to obtain algae flour. The identification of brown algae species was carried out by observing the morphology characteristics and identifying algae species (Lanyon, 1986).

The chemical characterization of brown algae (*Padina* sp.) consisted of proximate and mineral analysis. Proximate analysis refers to the Association of Official

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Analytical Chemists (AOAC, 2005) method which analyzes moisture content, protein, fat, ash, carbohydrates, and coarse fiber. Mineral analysis of K, Ca, Fe was carried out following the AOAC method (2005) by using AAS. Five ml HNO_3 was added to 1 g sample then left for 1 h at temperature room. The sample was left 12 h in a closed container. Then, it was added with 0.4 ml H_2SO_4 and heated on a hot plate until the solution was reduced or more concentrated (±1 h). The sample then added with a mixture solution of HCl and HNO_3 as much as 2-3 drops. The sample remains placed on a hot plate and the heating continued until the mixture changed the color from brown to dark yellow and turned to light yellow. After a discoloration, heating continued for 10-15 min, then the sample was moved, then cooled and added with 2 ml distilled water and 0.6 ml HCl. The destructive result was analyzed using AAS at wavelengths of 766.5 nm for K, 422.7 nm for Ca, and 248.3 nm for Fe.

The characterization of the chemical contents of *Padina* sp. included proximate and mineral analysis, which followed AOAC (2005) standard method. The proximate analysis comprised moisture content, protein, fat, ash, carbohydrate, and coarse fiber. Meanwhile, mineral analysis consisted of K, Ca, and Fe by using AAS. A weight of 1 g sample was added with 5 ml HNO_3 and left at room temperature for 1 h. The sample was closed for 12 h and then added with 0.4 ml H_2SO_4.

Preparation of Methanol Extract

The extraction was carried out following Lee et al. (2017) method. About 50 g dried samples were placed into Erlenmeyer and added with 250 ml methanol for 72 h. The extract was evaporated using a vacuum evaporator at a temperature of 60 °C and the extract obtained was in a paste form.

Phytochemical analysis

The phytochemical analysis referred to Marimuthu and Gurumoorthi (2013) and Paul and Yuvaraj (2013) with modification. The analysis aimed to find out the active compound in *Padina* sp. This phytochemical analysis included alkaloid, flavonoid, tannin, saponin, steroid, and triterpenoid.

Toxicity test

For toxicity test, Brine Shrimp Lethality Test (BSLT) method was performed on the methanol extract *Padina* sp. based on the Meyer et al. (1982) procedure with several stages. At first, 20 mg extract was dissolved in a mixture of 200 µl Dimethyl Sulfoxide (DMSO) and 9,800 µl seawater. Hence, the concentration of the stock solution was 2,000 ppm. Then, the concentrations of 0.1, 1, 10, 100, and 1,000 ppm were varied from the stock solution. Each concentration was made three times for triple measurements. The control solution was made by using the same procedure as the procedure above without a sample. For hatching evaluation, *Artemia salina* shrimp eggs were put in a container filled with seawater. Furthermore, it was aerated under 40-60 W incandescent lamps. Then, it was left for 2 days (48 h) to be *A. salina* larvae.

Elucidation structure

The tructural analysis was carried out by using FTIR instruments in Integrated Laboratory, Department of Chemistry, Hasanuddin University and GC-MS from Department of Chemical Engineering, Politeknik Negeri Ujung Pandang.

Results

The chemical composition of *Padina* sp. referred to the proximate analysis. Proximate analysis indicated that *Padina* sp. contained water (13.64%), ash (38.02%), protein (12.33%), fat (1.6%), fiber (20.02%), and carbohydrate (48.06%). Moreover, this study presents that *Padina* sp. from Takalar contained Ca (7.61%), K (1.5%), and Fe (0.26%).

Phytochemical analysis of methanol extract in *Padina* sp. shows the presence of several active compounds in the form of alkaloids, flavonoids, steroids, phenolic compounds, and tannins (Table 1). The LC_{50} value of *Padina* sp. extract in methanol was found to be non-toxic over *A. salina* larvae. The value was 6344.54 ppm, as indicated by LC_{50}>1,000 ppm. The results explain that *Padina* sp. from Puntondo coast was non-toxic and safe to be used as a functional food, even in the other place, extract of *Padina* sp.

Meanwhile, the bioactive compounds of methanol extract were investigated by using FTIR and GC-MS. Figure 1 presents the FTIR spectrum data of methanol extract which indicated the presence of OH groups (3,412 1/cm). This OH group is suspected from C-O-H since it was also found at 1,074 1/cm and supported by the absorption at 1,095 1/cm. This absorbance indicates the presence of aliphatic groups. C-H stretch from alkanes was also found at 2,922 and 2,852 1/cm which was supported by the presence of the absorbance at 1,458 1/cm (CH_2) and 1,404 1/cm (CH_3) groups. The extract also contained a carbonyl (C=O) group that was shown by the absorbance at 1,647 1/cm. This carbonyl group was suspected to have a substitution of para amplified since there was a signal at the wavenumber of 804 1/cm. Furthermore, the absorbances at 1,739 and 1,712 1/cm indicated the presence of C=O ketone and ester groups.

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The spectrum also presents C=C olefin group that was found at 1,629 1/cm. The inorganic content and fatty acid were found at 678-383 1/cm and 2,376-2,312 1/cm, respectively.

The findings of compounds contained in methanol extract of *Padina* sp. were also determined by using GC-MS (Figure 2) while the phytochemical constituent is shown in Table 2. The active compound test of *Padina* sp. extract in methanol by using GC-MS method resulted in 25 compounds, shown in Table 2. Those compounds had the percentage of similarity ranging from 70-96%. In line with the phytochemical test results, *Padina* sp. was suspected containing several compounds, such as alkaloid, steroid, terpenoid, phenolic, and tannin.

Table 1: Phytochemical assay of extract methanol brown algae *Padina* sp.

No.	Phytochemical Assay	*Padina* sp.	Remarks
1	Alkaloid		
	a. Dragendorff	-	
	b. Mayer	+	White sediment
	c. Wagner	+	Violet ring
2	Flavonoid		
	a. Lead(II) acetate	+	Yellow sediment
	b. Mg	-	
3	Steroid	+	Green to blue color
4	Phenolic	+	Yellow-green color
5	Saponin	-	
6	Tannin	+	Dark Green

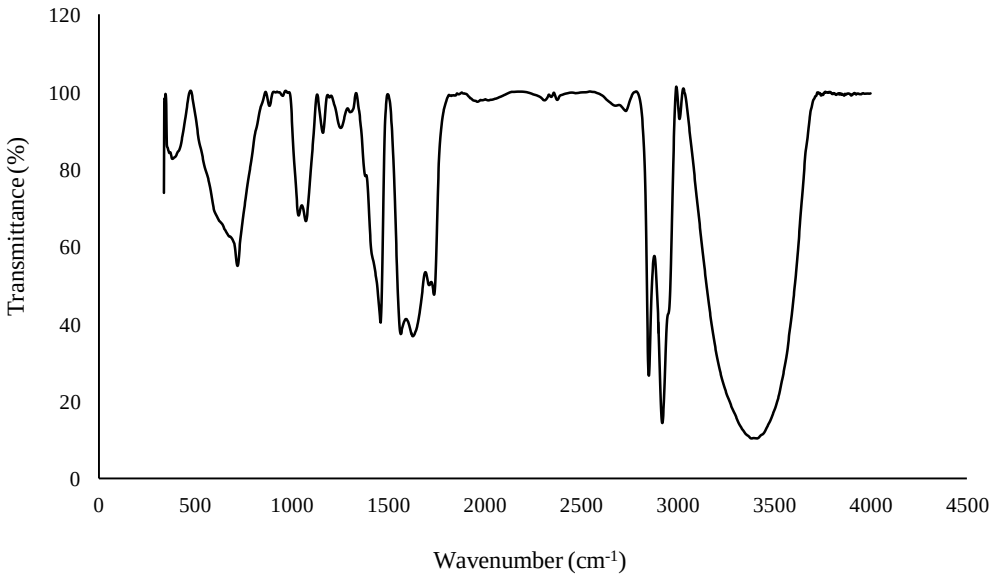


Figure 1: Fourier-transform Transform Infrared Spectroscopy (FTIR) analysis from methanol extract of *Padina* sp. from Puntondo coast, Takalar

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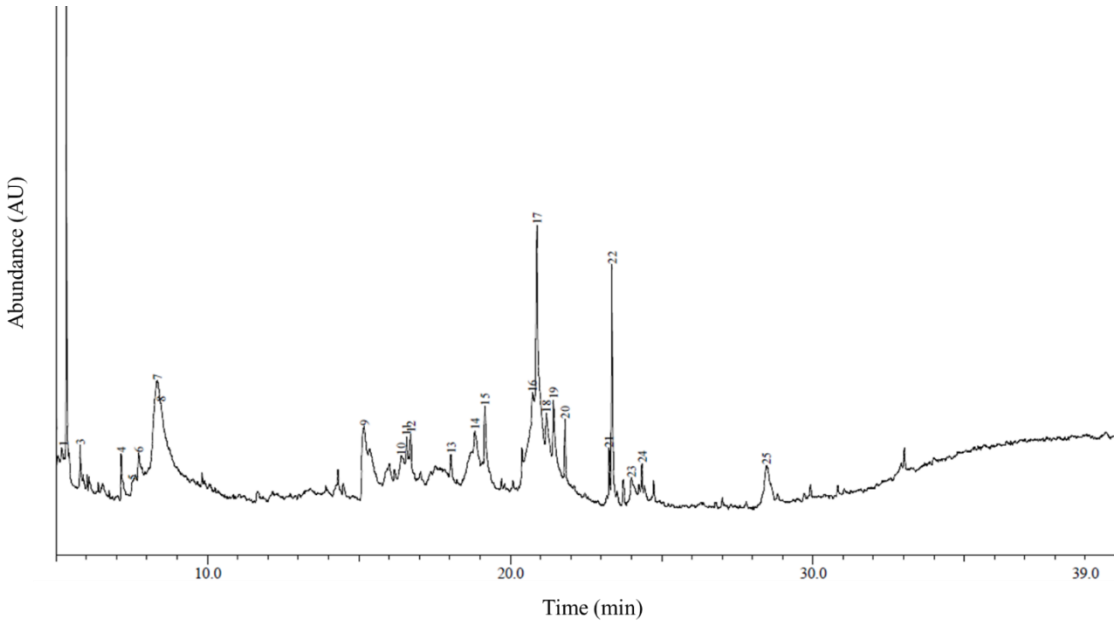


Figure 2: Chromatogram from methanol extract of *Padina* sp. from Puntondo coast, Takalar

Table 2: Constituents of methanol extract of brown algae *Padina* sp. from Puntondo coast, Takalar

Peak#	R.Time	Area%	Name	SI (%)
17	20.883	15.29	Hexadecanoic acid, methyl ester (asam lemak jenuh)	93
2	5.344	14.01	2-Propanone, 1,1-dimethoxy	85
7	8.345	12.83	Glycerin	95
22	23.362	10.08	9-Octadecenoic Acid, Methyl Ester	96
9	15.179	5.17	1-Tert-butoxy-5-trimethylsilyloxypentane	75
16	20.733	4.38	Pentadecanoic acid, 14-methyl-, methyl ester	80
19	21.425	3.77	l-(+)-Ascorbic acid 2,6-dihexadecanoate	92
15	19.169	3.23	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-Hydroxy-4,4,7a-Trimethyl	85
10	16.392	2.77	1,4-Anhydrohexitol	86
8	8.467	2.55	1-Pentene, 2,4,4-trimethyl-	80
18	21.2	2.52	1,1-Dimethyldecahydronaphthalene	81
25	28.464	2.27	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-	84
14	18.837	2.23	9-OCTADECENOIC ACID (Z)-	71
12	16.71	2.17	PROPANOIC ACID, 2-METHYL-, 1-(1,1-DIMETHYLETHYL)-2-METHYL-1,3-PROPANEDIYL EST	89
21	23.269	1.97	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	94

Discussion

Macroalgae, especially brown algae, has high carbohydrate content, such as fucoidan, laminaran, cellulose, and alginate (Dawczynski et al., 2007; Mabeau and Fleurence, 1993). The chemical composition of macroalgae varies considering several factors, such as habitat, geographical distribution, season, and concentration of nutrition in its environment, particularly in species and genus (Fleurence, 1999; Goecke et al., 2010; Mansilla and Ávila, 2011). These factors will influence the minerals provided that can be absorbed by *Padina* sp. (Goecke et al., 2012; Rupérez, 2002). This study presents that *Padina* sp. from Takalar contained Ca (7.61%), K (1.5%), and Fe (0.26%) which is in line with a study conducted by Manteu et al. (2018). The highest minerals in the brown algae of *Padina* sp. from Pohuwato waters, Gorontalo, Indonesia were Ca (10.22%), k (1.48%), and Fe (0.125%).

The minerals found in *Padina sp* significantly affect the chemical composition of this *Padina sp.*, especially ash and fiber levels. High ash content in food indicates that

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the mineral settles in a material high, as well as as a form of adaptation to conditions marine environment that contains various minerals with high concentrations (Fleurence,1999). Compared to terrestrial food sources, microalgae contained more trace elements and minerals (MacArtain et al., 2007). *Padina* sp. species are an important source of calcium carbonate and organic matter in the hallow waters of tropical and subtropical areas (Wefer, 1980).

The content found in *Padina* sp. from Puntondo coast differed from the previously reported of *Padina* sp. *Padina* sp. collected from Gorontalo, Indonesia (Manteu et al., 2018) which had lower content of ash (30.53%), fat (0.52%), protein (4.78%), and fiber (3.81%). The other study reported by Goecke et al. (2012) presents *Padina fernandeziana* collected from Chile had a greater ash content of 53.79%, with a protein composition of 6.54%, and a lower water content of 7.33%. Meanwhile, the brown algae from Kupang bay contained water content (11.21%), ash content (34.58%), protein (13.39%), and fat (2.66%).

The ash (mineral) content of marine macroalgae is commonly ranged from 8 to 40%, depending on the type of water. High ash content in algae is related to the way of the absorption of nutrient minerals, as well as a form of adaptation to environmental conditions in marine waters that contain various minerals with high concentrations. In addition, Fleurence (1999) stated that the highest protein content was obtained in winter and spring while the lowest was recorded during summer. However, in this present study, *Padina* sp. from Puntondo coast had sufficient protein levels for nutritional intake. According to Wu (2016), protein intake for human is about 0.8-3.5 g per kg body weight per day. Protein is important to note since it has a function as a building block, a regulator, and a burning agent. As a protein-building substance, it forms various new tissues for growth, replaces damaged tissue, and reproduces.

This present study exhibits the carbohydrate content of *Padina* sp. from Puntondo coast (48.06%) (Indonesia) was higher than the previous study reported by Manteu et al. (2018) and Salosso et al. (2020) from Gorontalo, Indonesa (41.88%), and Kupang, Indonesia (38.15%), respectively. It was also higher than the carbohydrate content of brown algae (30.48%) from Chile, *Padina fernandeziana* from Southeast India (14.73%) (Goecke et al., 2012). Carbohydrates which comprise monosaccharides, oligosaccharides, and polysaccharides, are considered as the critical source of energy. It has been poven that polysaccharides and oligomers contained in algae possess a broad range of biological properties, including antioxidant, anticoagulant, anti-inflammatory, anti-arthritic, immunomodulatory, and immunostimulant properties. It also has potential to be a hepatoprotective, neuroprotective, chemopreventive, anti-diabetic, and anti-obesity medication. The pharmacology effects of the algae carbohydrates are essentially determined by subjective changes in the composition of the sugar backbone, the degree of sulfation, and molecular weight. Aside, the level of carbohydrate is affected by the growth environment, age, and collection season, and the geographical location. Also, the differences in the chemical composition of carbohydrates are suspected to be associated with their biological activities (Lee et al., 2017).

The other substance that is essential for human body and can be found in *Padina* sp. from Puntondo coast is fat. Interestingly, in this study, saponin was not found. The absence of this compound was shown by the absence of foam. In contrast, Haryani et al. (2019) reported that the brown algae of *Padina australis* from Lampung (Sumatera), Indonesia, contains saponin.

Moreover, the substances contained in algae that is usually investigated is the phytochemical composition. The phytochemical composition of algae is also influenced by the habitat conditions and the type of algae. Manteu et al. (2018) reported that *Padina* in Gorontalo (Sulawesi) contained alkaloids, flavonoids, saponin, and tannin. Salosso et al. (2020) reported *Padina australis* in Kupang (East Timor) had alkaloids, flavonoids, saponin, and tannin. The different results can be caused by the different methods and solvents used to extract the components (Harborne, 1998).

In this study, the analysis of toxicity of *Padina* sp. was carried out by examining the LC_{50}. A compound can be toxic if in a short time, it can kill 50% of *A. salina* larvae. The result shows that the LC_{50} of the extract was 6344.54 ppm. Thus, it was non-toxic over *A. salina* larvae. The value higher than 1,000 ppm implies the safety of using *Padina* sp. from Putondo as a functional food. This toxicity value of *Padina* sp. can be different as reported by Haryani et al. (2019) which the LC_{50} was 177.83 ppm. The low toxicity of *Padina* sp. was supposed to be caused by the absence of saponin. Saponin is a glycoside which is a mixture of simple carbohydrates and aglycones found in various plants. Saponins can destroy blood grains or hemolysis. Hence, it is toxic to cold-blooded animals (Prihatna, 2001).

Based on the FTIR spectrum, methanol extract of *Padina* sp. contained alkaloids, flavonoid, and steroids. This positively correlated with the phytochemical results obtained. Meanwhile, the results of GC-MS confirmed the presence of terpenoid, alkaloid, steroid, and fatty acid which were showed in FTIR spectrum. Phenolic or polyphenolic compounds contained in this sample are alkaloid groups, cyanate acid derivatives, coumarin, tocopherol, and functional acids that can serve as antioxidant compounds (Marimuthu and Gurumoorthi, 2013).

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However, this study focused on the investigation and characterization of the chemical compounds in *Padina* sp. from Puntondo coast, Takalar, Indonesia by using AAS, FTIR, GC-MS, and phytochemical assay. Thus, the results can be acknowledged as a reference to utilize *Padina* sp. as a functional food.

Conclusion

Padina sp. from Puntondo coast comprises rich nutrients (protein, carbohydrates, and minerals) and has bioactive components such as alkaloids, flavonoids, steroids, tannins, and unsaturated fatty acids that can be used to be a functional food. Based on the results, further studies are necessary to advance *Padina* sp. from Puntondo coast as a raw material source for the food and pharmaceutical industry.

Author contributions

All authors designed the study; N.H.S., F.F. conducted the experimental work; N.H.S., F.F., and Y.M.S. analyzed the data; K.K., N.H.S., and S.M.T.C. wrote the manuscript. All authors read and revised the final manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

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