



## Chemical characteristics and phytochemicals of the brown alga *Sargassum filipendulla* from kelanit waters of south east Maluku

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### ABSTRACT

Brown algae are leading commodities with high nutrients, and potential bioactive compounds. Furthermore, the seaweed contains carbohydrates, proteins, ash, water, vitamins and macro and microminerals, edible by humans. This study aims to determine the chemical and phytochemical characteristics of *S. filipendulla* brown algae in the Kelanit waters of Southeast Maluku. In addition, this research carried out the proximate analysis, mineral content test, and phytochemicals of ethanol extract. The results showed the chemical composition of *S. filipendulla* comprised 21.61%, 24.79%, 0.19%, 2.31%, and 52.20% of water, ash, fat, protein, and carbohydrate content respectively. Furthermore, the mineral level consisted of 21.52 mg/g, 0.50 mg/g, 21.53 mg/g and 27.60 mg/g of magnesium (mg), Iron (Fe), Sodium (Na), and Potassium (K) correspondingly. Also, the phytochemical analysis showed the presence of phenol, tannins, flavonoids, saponins, and steroid compounds. Therefore, the active compound production of brown algae *S. filipendulla* is estimated to be alternative disease prevention in Indonesian aquaculture.

### INTRODUCTION

Brown algae are nutrient-rich leading commodities, containing potential bioactive compounds and used in pharmaceutical and cosmetics industries. However, this seaweed is not optimally utilized due to limited scientific studies relating to the inherent potentials. The organism resides in coral reef habitats and is distributed in Kelanit waters of Southeast Maluku, Indonesia, although considered a waste by coastal communities. Furthermore, the types include *Padina*, *Sargassum*, and *Turbinaria*. The *Sargassum* sp consist of 150 species, and lives between subtidal and intertidal zones of tropical and subtropical regions.

Furthermore, the population distribution and structure are influenced by water movement, temperature, tides, and substrate types (**Wouthuyzen *et al.*, 2016 ; Gazali *et al.*, (2018).**

The brown algae contain carbohydrates, proteins, ash, water, vitamins (vitamins B1, B2, B6, B16, C and niacin), and macro and microminerals comprising potassium (K), sodium (Na), magnesium (Mg), phosphate (P), iodine (I), and iron (Fe) (**Syad *et al.*, 2013 ; Erniati *et al.*, 2016**). Furthermore, while sea algae possess high mineral and edible content, brown algae encompass secondary metabolites including alkaloids, glycosides, tannins and steroids, with health benefits and wide use in the medical and pharmaceutical industry. Also, phenolic and flavonoids compounds with inhibitory activities towards LDL oxidation, Angiotensin Converting Enzyme (ACE),  $\alpha$ -amylase, and  $\alpha$ -glucosidase (**Bono *et al.*, 2014 ; Jeeva *et al.*, 2012 ; Nagappan *et al.*, 2017**) are contained in brown algae, to provide therapeutic effects and protection against several degenerative diseases including cancer (**Padua *et al.*, (2015)**). Additional contents comprise carotenoid, laminarian, alginate, fukoidan, and phlorotannin, being antioxidant sources for fighting free radicals in the human body (**Nursid *et al.*, 2013; Firdaus 2013**).

Research on *Sargassum* showed *S. plagyophyllum* is obtained in Serang Banten waters, and contains alkaloid, steroid, flavonoid, saponin, tannin, with vitamin C levels of 212.95 mg / kg. In addition, *S. echinocarpum* extracts containing 80% ethanol are potential nutraceutical with IC50 value of 66.155, and consists of tannins, polyphenols, saponins, glycosides, and steroids. (**Firdaus *et al.*, 2012; Firdaus 2013 ; Dolorasa *et al.*, 2017**). Also research by **Suraiya *et al.*, (2018)** *S. japonica* extract fermented by *Monascus* spp revealed increased phenolic content, and is useful as food or antidiabetic diet. However, there are no reported research on the characteristics of brown algae *S. filipendulla* from Kelanit waters in Southeast Maluku, and investigation is expected to provide useful information of beneficial value to the community. Therefore, this study aims to determine the chemical and phytochemical physiognomies of brown algae *S. filipendulla* in Southeast Maluku.

## MATERIAL AND METHODS

### Sample preparation and morphological identification features

The *S. filipendulla* samples were obtained from Kelanit waters of Southeast Maluku Regency, air dried for  $\pm$  14 days under supervision and cut into small pieces before being mashed with a blender to become a simplicia powder. Furthermore, the powders were soaked in 20 liters of 96% ethanol (maceration) for 1x24 hours, and filtered. The sample identification of brown algae was carried out microscopically by observing morphological characteristics and species by **Lanyom (1986)**.

### Characterization of algae

The proximate analysis of *S. filipendulla* characteristics AOAC (2005) involved water, protein, fat, ash, carbohydrates and crude fiber content test, while analyzed minerals were Mg, Fe, Na and K by AOAC (2005). Also, 1 gm sample and 5 ml HNO<sub>3</sub> were combined and left for 60 minutes at room temperature, before heated for four hours at 120<sup>0</sup>C. Subsequently, the sample was cooled for 12 hours, combined with 0.4 ml of H<sub>2</sub>SO<sub>4</sub> and placed on the hot plate until concentrated ( $\pm$  1 hour), prior to insertion of 2-3 HCl and HNO<sub>3</sub> drops. Furthermore, the heating was continued until the color changed from yellowish brown to light yellow, and carried on for additional  $\pm$  10-15 minutes. The sample was removed to cool, and 2 ml and 0.6 ml of distilled water and HCl respectively were added. The destruction results were analyzed using AAS (*Atomic Absorption Spectrophotometer*).

### Phytochemical Analysis

Phytochemical tests were preliminarily used to qualitatively determine the active compounds present in brown algae *S. filipendulla* samples, comprising phenol, tannin, flavonoid, saponin, triterpenoid, steroid and alkaloid. Moreover, these compounds are secondary metabolites and Herborne (1987) analysis method was employed.

The phenolic content was tested by Yangthong *et al.* (2009) technique of dissolving 10 mg of *S. filipendulla* extract with 2 ml of ethanol PA 95%, and adding 5 ml aquadest and 5% FeCl<sub>3</sub> reagent. Also, tannin is identified by combination of 500 mg of simplicia and 50 ml aquadest, to boil for 15 minutes. The presence of the compound is signified by a greenish black color after 1% FeCl<sub>3</sub> is included to 5 ml filtrate in the test tube. In addition, test for flavonoid is indicated by result of pink or purple color within 2-3 minutes, after 1 ml evaporated solution is dissolved with 2 ml ethanol PA 95%, and added to 0,5 g Mg and 0,5M HCl. Similarly, saponin test is carried out by inserting 10 ml hot water into a test tube with 1 g weighed simplicia powder, and shaking for 10 seconds after coolness. Thus, the compound is in existence where a stable white foam is formed for 10 minutes, and remains after 1 drop of HCl 2N is included.

The test for triterpenoid and steroid were performed by adding 1 g simplicia powder to 20 ml ether, and macerated for 2 hours. Subsequently, 3 drops filtrate is added to H<sub>2</sub>SO<sub>4</sub> + CH<sub>3</sub>COOH reagent in the watch glass and the formation of red or green signifies steroid and triterpenoid respectively. Also, alkaloid is tested by combination of 500 g, 1 ml and 9 ml of weighed simplicia powder, HCl 2N and water correspondingly to heat for 2 minutes, cool and strain. The compound is indicated by a chocolate result or white or yellow methanol-soluble clot deposition, after 3 ml filtrate is added to 2 drops of Dragendorff or Meyer reagent respectively.

## RESULTS

### 1. Characteristics of the brown alga *S. filipendula*

The result of primary algae metabolism is the chemical composition (proximate). In addition, while *Sargassum* sp is used as vegetables and animal feed in coastal communities, present information on macroalgae bioprospection from Southeast Maluku waters remain minimal. The results of the content test for *S. filipendula* showed water, ash, fat, protein and carbohydrate by 21.61%, 24.79%, 0.19%, 2.31%, and 52.20% respectively (*by difference*). Table 1 shows the proximate analysis of brown algae *S. filipendula* from Kelanit waters of Southeast Maluku.

**Table 1. Proximate Analysis of the brown algae *S. filipendula***

No	Test Parameters	Content (%)
1	Water content	21,61±0.18
2	Ash content	24,79±0,2
3	Fat content	0,19±0.08
4	Protein content	2,31±0.38
5	Carbohydrate content	52,20±0.16

Table 2 shows the chemical composition of brown algae.

**Table 2. Chemical Composition of Brown Algae**

Component	<i>S.polycystum</i>	<i>P.minor</i>	<i>S.oligocystum</i>	<i>P.tetrastomatoca</i>
Water	17.69±0,03	22.31±0.18	9.4	16.40
Ash	24.51±0,13	30.53±0.11	13.08	27.0
Fat	0.50±0.11	0.52±0.01	0.46	1.14
Protein	3.65±0.00	4.78±0.04	5.64	10.50
Carbohydrate	53.66±0.21	41.88±0.25	71.42	44.96
Coarse Fiber	6.52±0.65	3.81±0.35	6.49	23.96

Source: \*Maraguri *et al.*, (2016), \*\*Felix and Brindo., (2014)

Table 3 shows the results of analysis of brown algae mineral levels in several studies. The composition comprises 21.52 mg/g, 0.50 mg/g, 21.53 mg/g and 27.60 mg/g of magnesium (mg), Iron (Fe), Sodium (Na), and Potassium (K) respectively. Furthermore, the minerals are necessary nutrients for the body, in addition to carbohydrates, fats, proteins, and vitamins.

Table 3. Mineral Composition of The Brown Algae

Mineral	<i>S.polycystum</i> (mg/g)	<i>P.minor</i> (mg/g)	<i>S.polycystum</i> (g)	<i>P.pavonica</i> (mg/g)
Mg	8.89±0,02	22.41±0.21	0.09	-
Fe	0.50±0,00	1,00±0.01	211.34	249.87
K	32.71±0.24	26.90±0,24	8.35	2.970.37
Na	22.69±0,35	22.23±0,22	1.75	926.97
Ca	18.06±0,04	32.91±0.01	0.03	3.1867.86
Na:K	0.69	0.83	-	-

Source : \*Nazni and Renuga., (2015), \*\*Tabarsa *et al.*, (2012)

## 2. Phytochemical Content

Table 4 shows the phytochemical analysis result of crude ethanol extract *S. filipendulla*, indicating the presence of phenolic compounds, tannins, flavonoids, saponins, steroids. According to Daisy *et al.*, (2016) the secondary metabolite is rich in alkaloid, glycoside, flavonoids, saponins, tannins, phenolics, steroids and triterpenoids, known to demonstrate antibacterial activity.

Table 4. Phytochemical Algae Brown *S. filipendulla*

No	Secondary metabolites	Test Method	Ethanol Extract
1.	Phenolic	Reactor FeCl <sub>3</sub> 5 %	+
2.	Tanin	Reactor FeCl <sub>3</sub> 1 %	+
3.	Flavonoids	a. Reactor HCl concentrated + Mg	-
		b. Reactor H <sub>2</sub> SO <sub>4</sub> 2N	+
		c. Reactor NaOH 10 %	+
4.	Saponin	Heated up	+
5.	Triterpenoid	Reactor H <sub>2</sub> SO <sub>4</sub> concentrated + CH <sub>3</sub> COOH	-
	Steroids	Anhydrous	+
6.	Alkaloids	a. Reactor Dragendorff	-
		b. Reactor Meyer	-

Description:

(+) = Exist

(-) = Not exist

## DISCUSSION

The water content for *S. filipendulla* was determined as 21.61%, and the value obtained is useful to determine material durability. This is also applied to estimate the best storage condition for samples, in order to prevent fungal activity (microbes). Several studies have reported the water level of *Sargassum* Sp. Where 13.02% was observed in *S. subrepandum* brown algae obtained from red sea waters, and 13.37% in *P. tetrastomatica* **Osman et al. (2011)**. A research by **Manteu et al. (2018)** stipulated 17.69% for brown algae *S. polycystum* from Gorontalo waters, while **Gazali et al. (2018)** reported 10.54% in *Sargassum* sp from Aceh waters. Research by **Diachanty et al. (2017)** the moisture content of *S. hystrix* var, fluitans was 14.33%, while 26.25% was recorded for *S. polycystum* These fluctuating water levels in brown algae are strongly influenced by the drying process, the respective characteristics, and water location.

The ash content of *S. filipendulla* is 24.79%, and is the second highest value after carbohydrate. According to **Diachantry et al. (2017)** this phenomenon is attributed to the mineral nutrient absorption, besides being a form of adaptation to environmental conditions containing various minerals in high concentrations. In addition, variations were related to the amount of inorganic compounds and salts. **Vijay et al. (2017)** reported high ash content (45.04%) in brown algae, while **Manteu et al. (2018)** estimated 24.0% in *S. polycystum* obtained from Gorontalo waters. Also, 52.74% was recognized in the *Sargassum* sp from Aceh waters (**Gazali et al., 2018**). The salts and other minerals attached to marine macroalgae, including Na, Ca, K and Mg have a directly proportional relationship with high ash content (**Yuniarti et al., 2013 ; Yulius et al., 2016**), (**Nassaruddin et al., (2016)**).

The fat content of brown algae *S. filipendulla* was low, at 0.19%. According to **Garcia et al., (2016)** macroalgae in the tropics possess a much lower fat content than species in the sub-tropics. Furthermore, several studies have identified a range of 0.50% to 0.79% (**Manteu et al., 2018 ; Gazali et al., 2018**). **Diachantry et al., (2017)** reported 0.23% in *S. polycystum*, while marine macroalgae contained very insignificant amounts **Gazali et al., (2018)**.

The protein content in *S. filipendulla* is 2.31%. This is formed from several amino acids bound by peptides (**Ratarana-arporn and Chirapart, 2006**). The variation in value across different macroalgae was due to the amino acid content contained therein. **Burtin (2006)** stated lower protein content in brown algae (5-15%) compared to the red and green types (10-30%). However, 3.65% was reported in some related studies *S. polycystum* (**Manteu et al., 2018; Diachantry et al., 2017**).

The calculation result of carbohydrate content (*by difference*) showed a composition of 52.20% in *S. filipendulla*. These compounds are indicated as the main component in macroalgae, consisting of D and L-galactose, 3,6-anhydrogalactose, sulfate esters, sugar alcohols and inositol. (**Diharmi et al., 2011 ; Vijay et al., 2017**) reported on the presence

of fucoidan, laminarian, cellulose and alginate in brown algae. Furthermore, marine macroalgae generally store food reserves in the form of carbohydrates, especially polysaccharides, comprising 23.77% of *Sargassum* Sp. According to **Ma'aruf et al., (2013)** generally, carbohydrates have a significant relationship with crude fiber, which increases following the excessive clumping of polysaccharides in algal cells/marine.

The analysis of magnesium (mg) contained in *S. filipendulla* from Kelanit waters of Southeast Maluku amounted to 21.52 mg/g. This element is a cofactor for enzymes involved in important biochemical pathways within the human body. Moreover, an iron (Fe) level of 0.50 mg/g was recorded, with a general dry weight of 0.1-0.2%, while Sodium (Na) content was 21.53 mg/g (**Winarno 1990 ; Gazali et al., 2018**). According to **Herliatika et al., (2017 ; Mantau et al., 2018)** conditions of high seaweed (K) content potentially results in low (Na), as observed with Potassium (K) at 27.60 mg/g, while the brown algae *S. Polycystum* from Gorontalo waters contain Sodium 22.69 and Potassium 26.90 mg/g. Furthermore, the mineral contents varied according to seaweed species, geographical location, harvest age, wave exposure, seasonal, environmental and physiological factors, types and methods of processing and mineralization.

Phytochemical analysis of *S. filipendulla* extracts showed the presence of phenolic compounds, tannins, flavonoids, saponins, steroids found in crude ethanol extracts. Specifically, the saponins tend to possess strong glycoside bonds, estimated to be responsible for the polarity, alongside triterpena glycosides and sterols, detectable from 90 genera identified in plants. In addition, glycosides are a complex between reducing sugars (glycons) and non-sugars (aglycones), hence numerous saponins have up to five sugar units, attached to a common component, known as glucuronic acid. This compound presence in plants is indicated by foam formation during the extraction of plants or while concentrating extracts (**Harborne, 1987 ; Guedes et al., 2012**),

Steroid compounds were recognized in the extracts with ethanol and n-hexane solvents. **Alamsyah et al., (2014)** reported on the mechanism of bacterial inhibition, comprising damage to cell membranes, and is characterized by an increase in permeability, thus resulting in leakage, followed by the release of intercellular materials. These are complex molecules known to dissolve in fat with four rings joined together. The outcome of **Bhat et al, (2009)** showed the dominant steroids in algae to be generally antibacterial.

Flavonoids are a group of secondary metabolites synthesized from pyruvic acid through amino acid metabolism (**Bhat et al., 2009; Harborne, 1987**). These are phenol compounds characterized by the ability to change color following the addition of bases or ammonics. In addition, flavonoids are mostly present in plants, bound to sugar as glycosides and in mixed form, but rarely as a single compound. The content variation amongst algae is due to several influencing factors, including geographical location, season, physiological

and environmental differences, algal types, and extraction conditions (**Illing et al., 2017 ; Mantu et al., 2018; Gazali et al., 2018**).

**Harborne (1988)**, natural Phenol compounds tend to dissolve easily in water because of the general relationship with sugar as glycosides, and are sited in numerous vacuola cells. In addition, there have been reports on the structural characteristics, with flavonoid as the largest class. The role of phenols is also well documented, with lignin adopted as a building material for cell walls, while anthocyanin serves as a pigment, although the role of compounds belonging to other groups is a conjecture. **Santoso et al., (2004)** spotted a polyphenolic component in several seaweeds originating from various regions of Indonesia, and were assessed to effectively inhibit oxidation. Furthermore, other studies reported on the usefulness in human health, especially as an antioxidant, and very little information is known about the content in seaweed.

**Mann (1987)** evaluated three main compounds to be the secondary metabolic precursors, including first, sikimat acid as an antecedent for many aromatic compounds, encompassing aromatic amino, cinnamic, and amino acids. Second, polyphenols mainly form alkaloid and antibiotic peptides, comprising penicillin, cephalosporins, and third, acetate as prazates implicated in the development of poliasetilin, prostaglandin, macroscopic antibiotics, polyphenols, and terpenes, isoprenoid, steroids and caratenoid. Meanwhile, classifications based on the core structure and chemical properties consist of the following groups: alkaloid, flavonoid, quinones, tannins, saponins, steroids and triterpenoids.

The active compounds contained in macroalgae are responsible for the bacteria and viruse inhibition potentials. Therefore, the yield from Brown algae *Sargassum* sp. is expected to serve as an alternative for disease prevention in aquaculture. According to **Patra et al., (2008)**. the methanol extract showed strong antioxidant activity. Also, there have been reports on the antibacterial properties against gram-positive and gram-negative bacteria, including *Bacillus subtilis*, *Esherichia coli*, *Syaphylococcus aureus*, *Vibrio sp* and *Pseudomonas sp*.

The study by **Izzati's (2007)** reported on the possibility of developing *Sargassum* sp. for double cultivation with tiger shrimp, due to the extract activity against *Vibrio harveyii* and *Vibrio parahaemolyticus* species. **Hayashi et al., (2008)** showed the effectiveness of Fukoidan isolated from brown algae as antiviral and antioxidant agent. The administration of this active compound as a mixture with tiger shrimp feed showed antiviral activity against infections of white spot syndrome virus/WSSV, as well as increased non-specific immunity (**Wang et al., 2009 ; Pakidi et al., 2017**).



## CONCLUSION

The dominant chemical composition in brown algae *Sargassum filipendulla* obtained from Kelanit waters of Southeast Maluku include ash content (24.79%) and carbohydrate (52.20%). Furthermore, the mineral components were Mg (21.52 mg/g), Fe (0.50 mg/g), Na (21.53 mg/g) and K (27.60 mg/g). The ethanol extract contains phenolic compounds, tannins, flavonoids, saponins, and steroids. Hence, active compounds produced are expected to serve as disease prevention alternatives in aquaculture.

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