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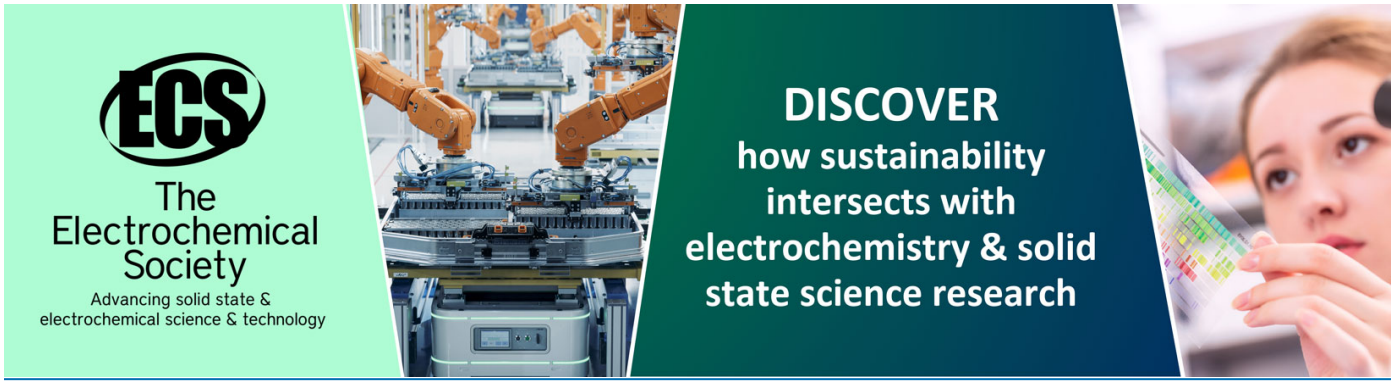

Phytochemical screening and total lipid content of marine macroalgae from Binuangeun beach

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Abstract. Marine macroalgae has potent applications for food, pharmaceutical and cosmetic. Several species of green and brown macroalgae from Binuangeun beach have been collected and analyzed by phytochemical test and total lipid content. Phytochemical screening has been carried out to discover bioactive compound in macroalgae. The lipid of algae contains fatty acid, oxylipins, and sterol, which has nutritional dan chemo-taxonomic properties. The results show the presence of bioactive compounds in green and brown macroalgae such as flavonoids, saponins and steroid. The analysis of total lipid content reveals that macroalgae of brown (*Turbinaria sp.*) and green (*Tydemania sp.*) species recorded the total lipid content, 5.69% and 5.87%, respectively.

Keywords: marine macroalgae, phytochemical screening, total lipid analysis

1. Introduction

Marine macroalgae are plant-like organisms that generally live in coastal areas. Indonesia is a maritime country with the marine area of approximately 3,1 million km^2 and has many diverse marine biodiversities as well as macroalgae. Therefore, macroalgae are possibly the most abundant biota in Indonesian coastlines [1]

The phytochemicals from marine macroalgae are potential resources for food, pharmaceutical and cosmetic applications [2]. It is known that macroalgae are the source bioactive natural products of amino acids, terpenoids, tannins, steroids, phenolic compounds, fatty acids and many more [3]. The phytochemical screening is considered effective in discovering bioactive compound of macroalgae. By knowing the bioactive content, might open new opportunities for utilization of the local macroalgae.

The aim of this study was to carry out preliminary phytochemical screening and to determine the total lipid content of macroalgae from Binuangeun beach.

2. Experimental details

2.1. Material

Fresh macroalgae were collected in July 2018 from Binuangeun Beach. Binuangeun beach is located in Banten (West Java) about 127 km from south-west of Jakarta.

2.2. Phytochemical screening

Phytochemical screening conducted qualitatively to determine the presence of alkaloids, phenolic compound (flavonoids, tannins and saponins), steroids, triterpenoids and quinones. The phytochemical test was conducted based on standard methods [4] and used fresh material to eliminate spoilage of material due to drying process [5]. It was reported that drying at 25°C could reduce 49% in the total phenol and 51% in total flavonoid content [6]. The sample was kept below 10ºC and was analyzed as quickly as possible after harvesting.

2.2.1. Test for alkaloids. About 1 g sample was ground with few drops of NH_3, then was added 5 mL chloroform. Chloroform fraction was filtered and acidified with 10 drops of H_2SO_4 2M. The acid fraction then separated into 3 parts. Each part then added with Mayer or Wagner or Dragendroff's reagent. The presence of alkaloids is indicated by the formation of cream precipitate by Mayer's reagent, brown colored precipitate by Wagner's reagent, and red-orange precipitate by Dragendroff's reagent. Tapak dara leaf was used as standard.

2.2.2. Test for phenolic (flavonoids, tannins and saponins). As much as 5 g of ground sample was added water and heated for 5 minutes, then was filtered. For flavonoids screening, the filtrate was added magnesium powder, mixture of hydrochloric acid and ethanol (1:1) and amyl alcohol. An orange coloration in amyl alcohol layer indicates the presence of flavonoids. For tannins, the filtrate was added with 3 drops of $FeCl_3$ 10% (w/v). A dark green coloration indicates the presence of tannins. For saponins, the filtrate was shaken vigorously and observed for a stable persistent froth.

2.2.3. Test for steroids and triterpenoids. One gram of sample was added hot ethanol and was filtered. The filtrate evaporated until dry and then homogenized with 1 mL of diethyl ether. One drop of concentrated sulphuric acid and 1 drop of acetic anhydride was then added and observed for the formation of two layers. Green or blue colour at upper side indicates a positive test for steroids and red or purple colour below indicates a positive test for triterpenoids.

2.2.4. Test for quinone. One gram of sample was boiled with methanol and was filtered. Then, three drops of NaOH 10% was added to the filtrate. The presence of quinone was indicated by red solution.

2.3. Total Lipid Content

Total lipid content was determined using Benchtop NMR analyzer – MQC (Oxford Instruments, Abingdon, England). Dried and ground macroalgae were dried at 70 °C for 1 hour before each measurement.

3. Results and Discussion

According to the presence of specific pigments, macroalgae can be classified into green algae (Chlorophyceae), brown algae (Phaeophyceae) and red algae (Rhodophyceae). The identification of macroalgae can be observed by the feature of their colour, length, width dan thickness of the thallus; branching pattern; shape of erect thallus; shape of holdfast; presence of gas bladders and tissue anatomy [7]. There are 13 macroalgae species that been identified from Binuangeun beach (Figure 1), 4 species are belonging to brown algae, 6 species are green algae and 3 species are red algae.

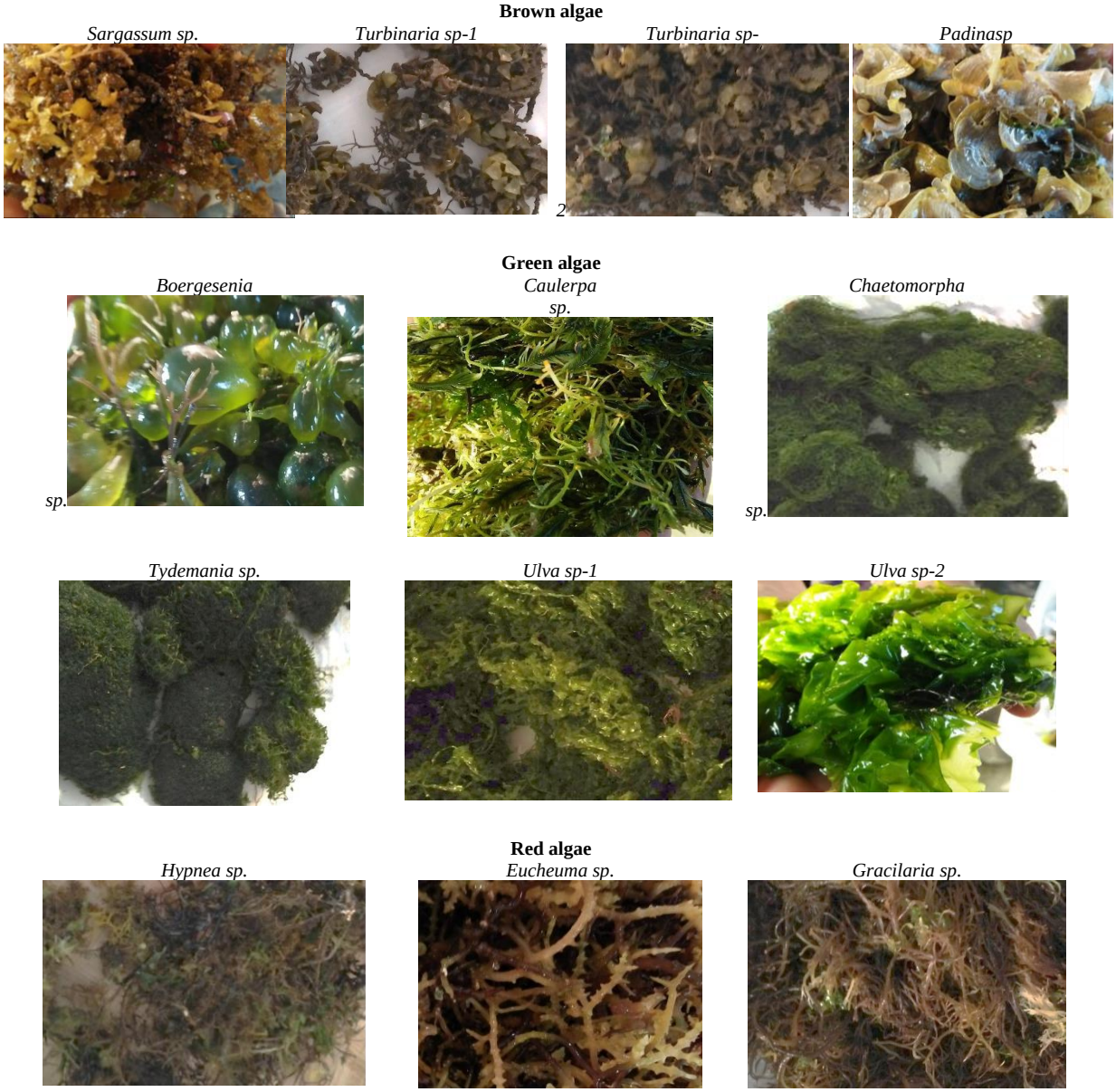

Figure 1. Macroalgae collected from Binuangeun beach

3.1. Phytochemical screening

Overall, phytochemical screening of Binuangeun'smacroalgae (Tabel 1) revealed the presence of bioactive compounds i.e *flavonoid*, *saponins* and *steroid*. *Flavonoids* are the major active nutraceutical ingredients, as is typical for phenolic compounds, they can act as antioxidants and metal chelators. They also have long been perceived to have anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities [8]. *Saponins* are important in human diets to control plasma cholesterol and to reduce the risk of heart disease. Recently known that saponins have beneficial effects in humans which is posses hypocholesterolemic, immunostimulatory and anti-carcinogenic properties, but in some cases saponins can cause hemolytic and membranolytic to human and animal [9] (therefore the consumption of saponins is limited to 100 until 200 mg/kg body weight per-day [10]). *Steroid* derivatives produced by algae known as phytosterols. These sterols are cholesterol-like compounds and undergo alkylation at C-24 which is different from animal sterols

[11]. Phytosterols have received much attention in the last few years because of their cholesterol-lowering properties [12].

Table 1. Phytochemical screening of macroalgae

Group	Species	Phytochemical Compounds						
		Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Triterpenoids	Quinone
Brown Algae	*Sargassum sp.*	—	+	—	+	+	—	—
	Turbinaria sp.-1	—	—	—	—	+	—	—
	Turbinaria sp.	—	—	—	—	+	—	—
	Padina sp.	—	—	—	—	+	—	—
Green Algae	*Boergesenia sp.*	—	+	—	—	+	—	—
	Chaetomorpha sp.	—	+	—	—	+	—	—
	Tydemania sp.	—	—	—	—	+	—	—
	Caulerpa sp.	—	—	—	—	+	—	—
	Ulva sp-1	—	—	—	—	+	—	—
	Ulva sp-2	—	—	—	+	+	—	—
Red Algae	*Hypnea sp.*	—	—	—	+	+	—	—
	Eucheuma sp.	—	—	—	+	+	—	—
	Gracilaria sp.	—	—	—	+	+	—	—

Note : (+) indicate positive result
(-) indicate negative result

Phytochemical analysis showed that all of macroalgae contain steroids and few of them have flavonoids and saponins compound. Brown algae (*Sargassum sp.*) and green alga (*Boergesenia sp.* and *Chaetomorpha sp.*) are known has flavonoids compound. Moreover, all of red algae are detected have saponins compound but only 1 species of brown algae and 1 species of green algae contains it.

Ruslin et al. studied brown algae (*Sargassum sp. and Padina sp.*) taken from the Punaga Ocean, Takalar, South Sulawesi [13]. They found that *Padina sp.* has more flavonoids content compared to *Sargassum sp*. The total flavonoids levels in *Sargassum sp.* are 1.428% while in *Padina sp.* are 2.357%. Another screening, Sari et al. tested red alga (*Eucheumaspinosum*) from south Bangka waters [14] have flavonoids, alkaloids and triterpenoids. In the contrary result, Prasetyaningsih and Rahardjo[15] conducted the phytochemical screening of brown algae (*Sargassum sp.*)and red algae *(Ulvasp)* from Wediombo beach, Gunung Kidul district, and showed that both contains saponins but none of them showed alkaloids, flavonoids and tannins content. Similar negative testing results also found in brown alga from Madura East Java [16]. From here we can conclude that phytochemical composition of macroalgae is depend on the growth location of the macroalgae. There were significant differences in chemical composition in some species of macroalgae due to environmental factor such as water temperature, salinity, light and nutrients [17]. Also, there have been reported correlation between plant phytochemicals and environmental factors, for example high light levels can increase total flavonoids synthesis [18]. Additionally, flavonoids compound consisting a complex structure with several functional groups that have different levels of solubility. For instance, solubility of quercetin and rutin in acetone significantly different which are respectively 80 mmol/L and 13.5 mmol/L [19]. This may causing a small amount of the content is undetectable, even though generally flavonoids dissolve in semi-polar to polar solvents.

In other ways, saponins content in plants is dynamic and responding to many external factors including biotic stimuli such as herbivorous attack or pathogenic infection. Saponins is synthesized and accumulated by macroalgae or plant regarded as part of their integrated defense mechanisms [20].

3.2. Total Lipid Content

From the measurement, the total lipid content of the macroalge varied from 0.32% to 5.87% dry weight (Figure 2). This result is similar with other studies which are most of macroalgae have lipid content below 5%. Even though lipid in macroalgae very small, the lipid fraction contains several bioactive components such as fucoxanthin (Fx), polyphenol and omega-3 polyunsaturated fatty acids (n-3 PUFA) [21].

Turbinaria sp. (brown algae) and *Tydemania sp.* (green alga) showed the highest lipid content, respectively 5.69% and 5.87%, while *Padina sp.* (brown algae) recorded the lowest content lipid. Generally, brown algae have the highest total lipid content, followed by green and red algae [22].

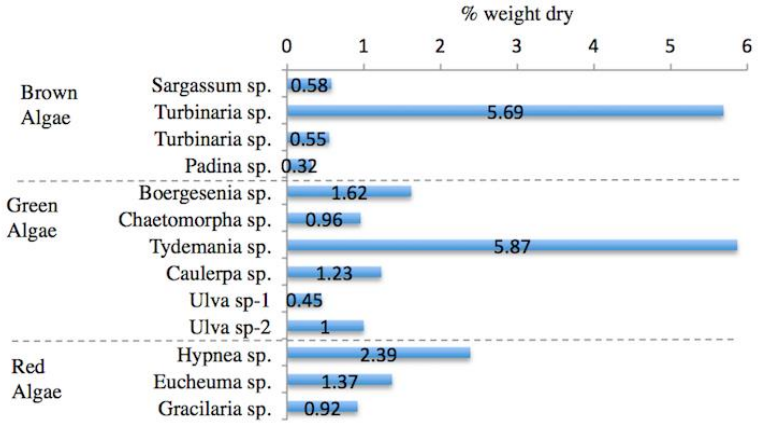

Figure 2. Total lipid content of the macroalgae

4. Conclusion

The phytochemical screening is valuable for the determination of bioactive compounds in macroalgae. Brown and green macroalgae from Binuangeun beach contains flavonoid, saponins and steroids which is useful as pharmaceutical material. The total lipid analysis in selected macroalgae is successful with respect to fatty acid and sterol.

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