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 and 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 compounds (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 10°C 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 NH₃, then was added 5 mL chloroform. Chloroform fraction was filtered and acidified with 10 drops of H₂SO₄ 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 FeCl₃ 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.
Brown algae

Sargassum sp.  Turbinaria sp-1  Turbinaria sp-2  Padina sp

Green algae

Boergesenia sp.  Caulerpa sp.  Chaetomorpha sp.

Tydemania sp.  Ulva sp-1  Ulva sp-2

Red algae

Hypnea sp.  Eucheuma sp.  Gracilaria sp.

Figure 1. Macroalgae collected from Binuangeun beach

3.1. Phytochemical screening

Overall, phytochemical screening of Binuangeun’s macroalgae (Table 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 anticarcinogenic 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
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.   |                         | —         | —          | —       | —        | +        | —            | —       |
|             | Turbinaria sp.   |                         | —         | —          | —       | —        | —        | —            | —       |
|             | Padina sp.       |                         | —         | —          | —       | —        | —        | —            | —       |
| Green Algae | Boergesenia sp.  |                         | —         | +          | —       | —        | —        | —            | —       |
|             | Chaetomorpha sp. |                         | —         | —          | —       | —        | —        | —            | —       |
|             | Tydemania sp.    |                         | —         | —          | —       | —        | —        | —            | —       |
|             | Caderpa 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 alga (Sargassum sp.) and red alga (Ulvasp) from Wediodmo beach, Gunung Kidul district, and showed that both contains saponins but none of them showed flavonoids, 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].

![Total lipid content of the macroalgae](image)

**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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### References

[1] Irianto HE, Dewi AS. Prospects of Indonesian Uncultivated Macroalgae for Anticancer Nutraceuticals. Mar Nutraceuticals Prospect Perspect 2013:169.

[2] Griffiths M, Harrison STL, Smit M, Maharajh D. Major Commercial Products from Micro- and Macroalgae BT - Algae Biotechnology: Products and Processes. In: Bux F, Chisti Y, editors., Cham: Springer International Publishing; 2016, p. 269–300. doi:10.1007/978-3-319-12334-9_14.

[3] Vinoth Kumar R, Murugesan S, Bhuvaneswari S. Phytochemical analysis of red alga Champia parvula (C. Agardh) collected from Mandapam coast of Tamil Nadu, India. Int J Adv Pharm 2015;4:15–20.

[4] Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Springer Netherlands; 1973.

[5] Culvenor CCJ, Fitzgerald JS. A field method for alkaloid screening of plants. J Pharm Sci 2018;52:303–4. doi:10.1002/jps.2600520327.

[6] Gupta S, Cox S, Abu-Ghannam N. Effect of different drying temperatures on the moisture and phytochemical constituents of edible Irish brown seaweed. LWT - Food Sci Technol 2011;44:1266–72. doi:https://doi.org/10.1016/j.lwt.2010.12.022.

[7] Rigby PR, Iken K, Shirayama Y. Sampling Biodiversity in Coastal Communities: NaGISA Protocols for Seagrass and Macroalgal Habitats. Kyoto University Press; 2007.

[8] Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. Trop J Pharm Res
2008;7:1089–99.

[9] Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y. Saponins from Edible Legumes: Chemistry, Processing, and Health Benefits. J Med Food 2004;7:67–78. doi:10.1089/109662004322984734.

[10] Diwan FH, Abdel Hassan IA, Mohammed ST. Effect of saponin on mortality and histopathological changes in mice 2000.

[11] Maschek JA, Baker BJ. The Chemistry of Algal Secondary Metabolism BT - Algal Chemical Ecology. In: Amsler CD, editor., Berlin, Heidelberg: Springer Berlin Heidelberg; 2008, p. 1–24. doi:10.1007/978-3-540-74181-7_1.

[12] Li Y-X, Kim S-K. Utilization of seaweed derived ingredients as potential antioxidants and functional ingredients in the food industry: An overview. Food Sci Biotechnol 2011;20:1461–6. doi:10.1007/s10068-011-0202-7.

[13] M R, Husain AF, As HY, Subehan S. Analysis of Total Flavonoid Levels In Brown Algae (Sargassum Sp. and Padina Sp.) as Analgesic Drug Therapy. Asian J Pharm Clin Res Vol 11 Issue 7 July 2018 2018. doi:10.22159/ajpcr.2018.v11i7.25657.

[14] Sari BL, Susanti N, Sutanto S. Skrining Fitokimia dan Aktivitas Antioksidan Fraksi Etanol Alga Merah Eucheuma spinosum. Pharm Sci Res 2017;2:59–68.

[15] Prasetyaningisih A, Rahardjo D. Keanekaragaman dan Bioaktivitas Senyawa Aktif Makroalga Pantai Widiombo Kabupaten Gunung Kidul n.d.

[16] Jannah M, Hanapi A, Fasya AG. Uji Toksisitas dan Fitokimia Ekstrak Kasar Metanol, Kloroform dan n-Heksana Alga Coklat Sargassum Vulgare dari Pantai Kapong Pamekasan Madura. ALCHEMY 2014:194–203.

[17] Lobban CS, Harrison PJ. Seaweed ecology and physiology. New York: Cambridge University Press; n.d.

[18] Tiwari BK, Brunton NP, Brennan C. Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction. Wiley; 2013.

[19] Chebil L, Humeau C, Anthoni J, Dehez F, Engasser J-M, Ghoul M. Solubility of Flavonoids in Organic Solvents. J Chem Eng Data 2007;52:1552–6. doi:10.1021/je7001094.

[20] Szakiel A, Pączkowski C, Henry M. Influence of environmental biotic factors on the content of saponins in plants. Phytochem Rev 2011;10:493–502. doi:10.1007/s11101-010-9164-2.

[21] Susanto E, Fahmi AS, Abe M, Hosokawa M, Miyashita K. Lipids, Fatty Acids, and Fucoxanthin Content from Temperate and Tropical Brown Seaweeds. Aquat Procedia 2016;7:66–75. doi:https://doi.org/10.1016/j.aqpro.2016.07.009.

[22] Gosch BJ, Magnusson M, Paul NA, Nys R. Total lipid and fatty acid composition of seaweeds for the selection of species for oil-based biofuel and bioproducts. GCB Bioenergy 2012;4:919–30. doi:10.1111/j.1757-1707.2012.01175.x.