Antioxidant Activity and Bioactive Compounds of Tropical Brown Algae *Padina* sp. from Bintan Island, Indonesia

Jelita Rahma Hidayati[1*], Muhammad Syaifudien Bahry[2], Ita Karlina[1], Ervia Yudiati[3]

[1]Department of Marine Science, Faculty of Marine and Fisheries Sciences, Raja Ali Haji Maritime University
Jl. Politeknik Senggarang Tanjung Pinang, Riau Islands, Indonesia
[2]Natural Product Laboratory, Centre for Research and Services, Universitas Diponegoro
Jl. Prof. Soedharto, S.H. Tembalang, Semarang 50275. Central Java, Indonesia
[3]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University
Email: jelitarahmahidayati@umrah.ac.id

Abstract

Macroalgae is one of the marine resources that have the ability as an antioxidant. Its ability is obtained from bioactive compounds produced through secondary metabolism. One type of macroalgae that has the potential as an antioxidant is *Padina* sp. This study aims to analyze the content of bioactive compounds in *Padina* sp and determine their antioxidant activity using DPPH as free radicals. *Padina* sp. was taken from the Bintan waters and shade-dried for three days. Bioactive compounds were analyzed through phytochemical screening to determine the content of flavonoid compounds, steroids, triterpenoids, saponins, and tannins. Determination of antioxidant activity begins with measuring the maximum wavelength of DPPH at 400-800 nm and determining the incubation time of the sample and DPPH. Antioxidant activity was determined based on the value of Inhibition concentration (IC50) at a wavelength of 515.5 nm; total phenolic content was determined using gallic acid standard (725 nm); total flavonoid content was determined using quercetin standard (415 nm), chlorophyll a and carotenoids were selected to determine pigment content on the sample. The results showed that *Padina* sp. contains flavonoid compounds, steroids, and tannins. Extract of *Padina* sp. has a total phenolic content of 46.02 mg/GAE g; total flavonoid content of 35.36 mg/QE g; chlorophyll content of 9.18 mg/g; and carotenoid content of 26.46 µmol/g. Methanol extract of *Padina* sp. has an IC_{50} value of 92.17 ppm and is classified as a strong antioxidant.

Keywords: *Padina* sp., Antioxidant Activity, Phytochemical Screening, Bioactive Compounds

INTRODUCTION

Bintan Island is located in the Archipelago Region, which has a high potential for marine biological resources, one of which is macroalgae. Macroalgae have a high abundance but are still lacking in their utilization, so it is necessary to develop the potential for marine biodiversity that provides ecologically and economically valuable. Macroalgae is the main fishery commodity that produces phytocoloids such as agar (Syam *et al*., 2020), carrageenan (Nosa *et al*., 2020), and alginate (Hidayati *et al*., 2021). The highest composition of macroalgae species in Bintan Regency was found in the Phaeophyta division (brown algae), with a percentage of 85% (Prakoso *et al*., 2019). One of the abundant species is *Padina* sp., which spreads in the intertidal to subtidal zones and has high adaptability. *Padina* sp. is found in various substrates such as sand, muddy sand, sandy mud, and coral rubble (Sukmawati *et al*., 2021). According to Melsasail *et al*. (2018), *Padina* sp. can grow well on dead coral substrates and can be found at each study site because they have a high tolerance for drought at the lowest low tide.

Macroalgae such as *Padina* sp. produce organic substances through photosynthesis and produce bioactive compounds due to environmental pressures on their habitat (Malo *et al*., 2018).

*) Corresponding author
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The differences in environmental conditions (Paraeng *et al*., 2016) and species (Manteu *et al*., 2018) can impact the variation and content of bioactive compounds produced. *Padina* sp. contains flavonoid compounds, phenols, triterpenoids, tannins, saponins (Maharany *et al*., 2017), and alkaloids (Manteu *et al*., 2018) compounds. The bioactive compounds produced have the potential to be used as antibacterial (Montolalu *et al*., 2019), antifungal, anticancer (Al-Enazi *et al*., 2018), anti-inflammatory, and antioxidant (Hidayati *et al*., 2019).

Antioxidants are compounds or chemical components that prevent the formation of free radicals in the body (Zulaikhah, 2017) and inhibit damage due to oxidation. The oxidation process occurs continuously in the body due to metabolic processes and forms free radicals (Simanjuntak and Zulham, 2020). In addition, free radicals in the body also come from vehicle fumes, cigarette smoke, food, heavy metals, industry, and solar radiation (Maharani *et al*., 2021). The high number of free radicals in the body will cause oxidative stress due to an imbalance between the number of free radicals and endogenous antioxidants produced by the body (Loho *et al*., 2021). This condition can damage cells, proteins, fats, and DNA (Halliwell and Gutterigde, 2015), which causes degenerative diseases. Therefore, antioxidant compounds are needed to inhibit free radicals and prevent chain reactions.

Padina sp. is known to have antioxidant activity. *Padina australis* contains flavonoids, alkaloids, saponins, and steroids and has antioxidant activity with an IC_{50} value of 102,590 g/mL (Junopia *et al*., 2020). While, the ethanolic extract of *Padina australis* from Kabung Island, West Kalimantan, had the potential for antioxidant activity, with an IC_{50} value of 144.47 ppm. Other studies have shown that the antioxidant ability can be used to accelerate the wound healing process (Comino-Sanz *et al*., 2021). The study aims to determine the antioxidant activity and bioactive compounds in *Padina* sp. and this research is the first step toward a more comprehensive exploration of macroalgae on a broader scale.

MATERIALS AND METHODS

The *Padina* sp. sample was collected at low tide from Bintan Waters, Riau islands (Figure 1). The samples were washed using fresh water and dried up at room temperature. The *Padina* sp. sample was collected at low tide from Bintan Waters, Riau islands (Figure 1). The samples were washed using fresh water and dried up at room temperature. The dried *Padina* sp. (50 g) was extracted with methanol for 2x24 hours (Widodo *et al*., 2019), then evaporated using a rotary evaporator (Eyela N1100, Japan) at a temperature of 35 °C for 60 minutes to obtain crude extract (Bahry *et al*., 2017). Extract yield was calculated using the formula:

$$\text{Yield} = \frac{\text{Weight of Vial} + \text{Extract} - \text{Weight of Vial}}{\text{Weight of sample}}$$

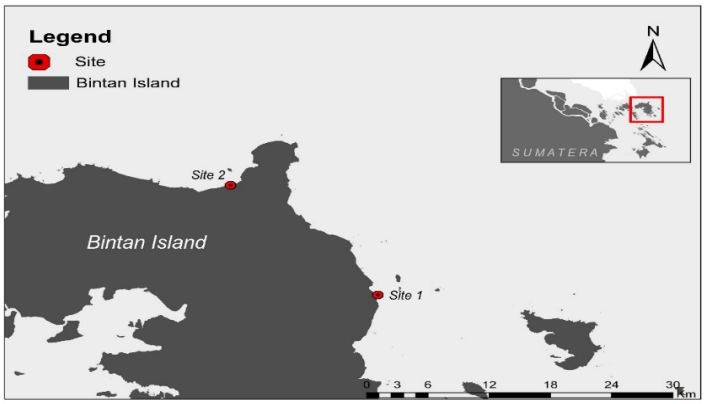

Figure 1. Map of sampling areas

Phytochemical Screening

Flavonoid

The extract was boiled with 100 mL of hot water, then filtered. 5 mL of filtrate was added with 0.05 g of Mg powder and 1 mL of HCl 37% (Merck), then shaken vigorously (Mondong *et al*., 2015). A positive test is indicated by the formation of a red, yellow, or orange color (Harborne, 1987).

Steroid and Terpenoid

Two mL of *Padina* sp. (1000 ppm) was taken, then ten drops of CH_3COOH and two drops of H_2SO_4 were added. The solution was shaken slowly and left for several minutes (Mondong *et al*., 2015). The extract contains steroids if it produces a blue or green color and triterpenoids if it produces a red or purple color (Harborne, 1987).

Saponins

Two ml extract of *Padina* sp. (1000 ppm) was made, then homogenized with 5 mL of chloroform, and allowed for 2 minutes. After that, two drops of 2 N HCl were added to the solution (Pontoh *et al*., 2019). The extract contains saponins if the foam formed remains stable for 7 minutes (Harborne, 1987).

Tannin

One ml extract of *Padina* sp. (1000 ppm) was made, then added with a gelatin solution and 5 ml of NaCl (10%) (Ikalinus *et al*., 2015). Extracts contain tannins when forming yellowish precipitates (Harborne, 1987).

Determination of Maximum Wavelength DPPH

DPPH solution with a concentration of 0.1 mM was made with ethanol as a solvent. The absorbance was observed automatically with a spectrophotometer (UV-1800, Japan) in the wavelength range of 400 - 800 nm (Hidayati *et al*., 2017). The wavelength with the maximum absorbance is used when determining the antioxidant activity.

Determination of Incubation Time

Three mL of DPPH solution was added with 1 mL of *Padina* sp extract. The solution was homogenized and put into a cuvette. The absorbance was measured in 70 minutes with an interval of 5 minutes at the maximum wavelength of DPPH. The incubation time is selected when the solution shows a stable absorbance value.

Determination of Antioxidant Activity

The determination of antioxidant activity was determined using spectrophotometric methods. *Padina* sp. has various concentrations of 50, 100, 150, 200, and 250 ppm. Each concentration was taken as much as 1 mL and added with 3 mL of 0.1 mM DPPH solution (1:3) (Mailuhu *et al*., 2017). The solution was incubated for 55 minutes in a dark room; then, the absorbance was measured at 515.5 nm. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition percentage} = \frac{(\text{Absorbance of DPPH} - \text{Absorbance of DPPH} + \text{Extract})}{\text{Absorbance of DPPH}} \text{x } 100\ \%$$

The percentage of inhibition of each concentration was then analyzed for its regression equation (y= ax ± b) to determine the IC50 value (Mailuhu *et al*., 2017).

Determination of Total Phenol Content

The total phenol content was determined using gallic acid as a standard. Gallic acid is made with concentrations of 5,10,15,20 and 25 ppm. Each concentration was taken as much as 2 mL, added 5 mL of aquadest and 0.5 mL of Folin-Ciocalteu reagent. The solution was allowed for 3 minutes, then added 1 mL of 5% Na2CO3 solution and incubated at room temperature for 1 hour (Hidayati *et al*., 2017). The absorbance was measured at a wavelength of 725 nm, and the regression equation was analyzed to calculate the sample's total phenol content.

The total phenol content was determined by taking a 2 mL extract of *Padina* sp. with a concentration of 1000 ppm. The same solution was added as in the gallic acid solution. The absorbance was measured at a wavelength of 725 nm. The total phenol content was determined based on the gallic acid regression equation and calculated by the formula (Sedjati *et al*., 2018):

$$\text{Total Phenol Content (mg/GAE g)} = \frac{\text{(Volume of Extract x Concentration of Extract x Dillution Factor)}}{\text{Weight of Extract}}$$

Determination of Total Flavonoid Content

The total flavonoid content was determined using quercetin as a standard. Quercetin was made with concentrations of 5,10,15,20, 25 ppm. Each concentration was taken as much as 1 mL, added 1 mL of 10% AlCl3 and 8 mL of 5% acetic acid, then allowed to stand for 16 minutes. The absorbance was measured at a wavelength of 415 nm (Bakti *et al*., 2017), and the regression equation was analyzed to calculate the total flavonoid content in the sample.

The total flavonoid content was determined by taking a 1 mL extract of *Padina* sp. with a concentration of 1000 ppm, and the same solution was added as in the quercetin solution. The absorbance was measured at a wavelength of 415 nm, The total content of flavonoids was determined based on the quercetin regression equation and calculated by the formula (Mukhriani *et al*., 2019):

$$\text{Total Flavonoid Content (mg/QE g)} = \frac{\text{(Volume of Extract x Concentration of Extract x Dillution Factor)}}{\text{Weight of Extract}}$$

Determination of Chlorophyll a and Carotenoid Content

Padina sp. is made with a concentration of 1000 ppm with acetone solvent. The extract was then measured at wavelengths of 645 nm, 663 nm, and 480 nm. The pigment content was calculated using the formula of Pramesti *et al*. (2017).

RESULT AND DISCUSSION

The percentage yield of extract

Extraction of *Padina* sp. was carried out by single maceration using methanol as solvent. The extraction results from 50 grams of dried *Padina* sp. showed a yield value of 6.37%, with the characteristics of the extract having a dark brown color and a liquid texture. The resulting yield is higher than Wijayanti *et al*. (2018), which is 1,2044%, whereas *Padina australis* from Poteran Island, Madura, is macerated with 96% ethanol solvent. However, Hudaifah *et al*. (2020) obtained a higher yield of *Padina australis* extract from Banyuwangi, 29.37%. Differences in the percentage yield of extracts are influenced by the extraction method (Wijaya *et al*., 2022), simplicia size, extraction time (Ardyanti *et al*., 2020), type of solvent (Hidayati *et al*., 2019), and habitat differences.

Bioactive compounds of *Padina* sp.

Phytochemical screening was carried out to determine the content of bioactive compounds in *Padina* sp. The results showed that *Padina* sp. extract contained flavonoids, steroid, and tannins compounds (Table 1).

Table 1. Bioactive Compounds of Padina sp.

Bioactive compound	Method	Result	Description
Flavonoids	Concentrated HCl + Mg Powder	+	Showed a yellow color
Terpenoids	Liebermann-Burchard Reagent	+	Showed a blue color
Triterpenoids	Liebermann-Burchard Reagent	-	Doesn't show a red color
Saponins	Foam test	-	Doesn't produce a foam
Tannin	Gelatin test	+	Formed a precipitates

The bioactive compounds obtained in this research were more varied than Nuzul *et al*. (2018), which only found the flavonoid compounds in the ethanol extract of *Padina* sp. Another study showed that the extract of *Padina* sp. contains Alkaloids, Flavonoids, Steroids, Terpenoids, Tannins, and Saponins (Wijayanti *et al*., 2020). The difference in the bioactive compound is thought to be caused by the drying process in the sample. According to Masduqi *et al*. (2014), the drying method can affect macroalgae's chemical content. During the drying process with wind or direct sunlight, it will cause activation of oxidative enzymes that cause the loss of phenolic compounds and flavonoids (Gumusay *et al*., 2015). In addition, shade-drying produces a higher water content than hot temperatures, known as case-hardening, which can block the release of water and slow the drying rate (Apsari *et al*., 2021).

Secondary metabolite compounds such as flavonoids in plants give color to leaves and flowers and protect the harm from free radicals (Procházková *et al*., 2011; Fithriani *et al*., 2015). Steroids are compounds widely found in high to low plants, generally in the form of sterols (Suryelita *et al*., 2017). According to Payghami *et al*. (2015), fucosterol is the most abundant type of steroid in the Phaeophyta division and has the potential to be used as an antioxidant (Rohim *et al*., 2019). The other bioactive compounds found in *Padina* sp. are tannins. Tannin compounds consist of two types: condensed tannins and hydrolyzed tannins (Makatamba *et al*., 2020). Tannins are polyphenolic compounds that have the potential to be used as antioxidants because they can chelate iron ions and slow down oxidation (Amarowicz, 2007; Fithriani *et al*., 2015).

Maximum DPPH Wavelength and Incubation Time

The results showed that the maximum absorbance of DPPH was obtained at a wavelength of 515.5 nm (Figure 2). This shows that at this wavelength DPPH has a high measurement sensitivity (Hidayati *et al*., 2017), so it can be used to measure free radical scavenging by extracts of *Padina* sp. The stable incubation time for the extract of *Padina* sp. and DPPH reacted was 55 minutes. Based on Figure 3. The absorbance value of the extract solution and the sample had a stable absorbance from the 50th minute to the 60th minute. This shows that in that time, the sample has reacted perfectly and is the optimal time to take measurements.

Antioxidant Activity

Antioxidant activity was determined by calculating the value of Inhibition concentration 50 (IC_{50}). The IC_{50} value indicates that the sample extract can inhibit free radicals at this concentration by as much as 50%. Based on the linear regression on the sample ($y = 0.1912x + 32.372$), the IC_{50} value of 92.17 ppm was obtained. This value indicates that the methanol extract of *Padina* sp. from Bintan waters has antioxidant activity with a strong category (Molyneux, 2004).

The results are relatively high compared to Khadijah *et al*. (2020), where *Padina* sp. obtained from Kayoa Waters, West Maluku showed weak antioxidant activity with an IC*50* value of 564.99 ppm. This could be due to the characteristics of the sampling site or different habitats. Differences in pressure in the environment affect the bioactive compounds and antioxidant activity produced by organisms (Paraeng *et al*., 2016). Different drying methods also affect the antioxidant activity of the samples. For example, drying methods using shade-dry and solar tunnel driers produced different antioxidant activities, namely 87,082 ppm (Maharany *et al*., 2017) and 1554.45 ppm (Hidayati *et al*., 2017), respectively. After a long air-drying process, antioxidant compounds lose their activity because they have poor stability and decrease their sensitivity to inhibiting the oxidation process (Ruiz-Medina *et al*., 2022).

The occurrence of a purple color change to yellow indicates the presence of free radical scavenging activity of DPPH by the sample at the maximum wavelength of DPPH. Discoloration indicated the formation of free radical compounds into more stable compounds through electron transfer (Saptari *et al*., 2019). Increasing the concentration of the extract will increase the inhibition value and antioxidant ability and indicates the number of antioxidant compounds in the sample that inhibit free radicals.

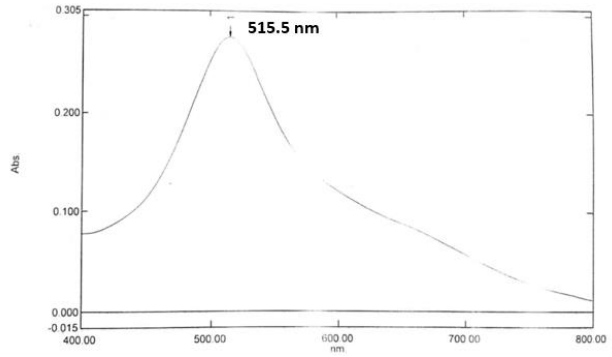

Figure 2. The spectrum pattern of DPPH in ethanol solvent

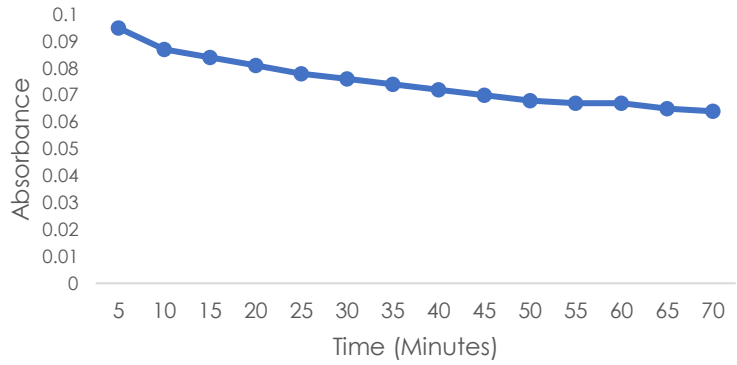

Figure 3. Incubation Time of *Padina* sp. Extract and DPPH

Total Phenol and Flavonoid Content

Polyphenol compounds act as antioxidants by donating hydrogen atoms to free radicals (Khadijah *et al*., 2021). Antioxidant ability correlated with phenol content in the sample. According to Gazali *et al*. (2018), 99% of antioxidant activity is obtained from the phenolic compound group; the other is obtained from other compounds with antioxidant activity. One of the phenolic compounds is a flavonoid. Flavonoids have polar and non-polar parts (Pangestuti *et al*., 2017), so they can dissolve in several solvents with different polarities. The total phenol content was determined using a standard solution of gallic acid (Pontoh *et al*., 2019). The total flavonoid content uses a standard quercetin solution because this compound is one of the derivatives of flavonoid compounds that have powerful antioxidant activity (Sari and Suharyanto, 2021). The linear regression equation obtained in the standard solution (Figure 4) was used to determine the total phenol and flavonoid content.

The results showed that the linear regression equation for gallic acid was y = 0.0192x + 0.0364 with R^2 = 0.9887. While the linear regression equation for quercetin was y = 0.0158x + 0.0103 with a value of R^2 = 0.9886. The value of R^2 indicates a linear relationship between the increase in concentration and absorbance (Lailatussifa and Pereira, 2022). Based on these equations, *Padina* sp. has a total phenol content of 46.02 mg/GAE g and flavonoids of 35.36 mg/QE g. The antioxidant ability is obtained from the hydroxyl group in phenolic and flavonoid compounds (Gazali *et al*., 2018). The hydroxyl group has an oxygen atom with a lone pair of electrons which is useful for inhibiting the reactivity of free radical compounds (Lantah *et al*., 2017).

Chlorophyll-a and Carotenoid Pigments

Pigments are natural dyes formed based on wavelength absorption. Chlorophyll-a and carotenoids are photosynthetic pigments in macroalgae (Pesang *et al*., 2020). Brown algae

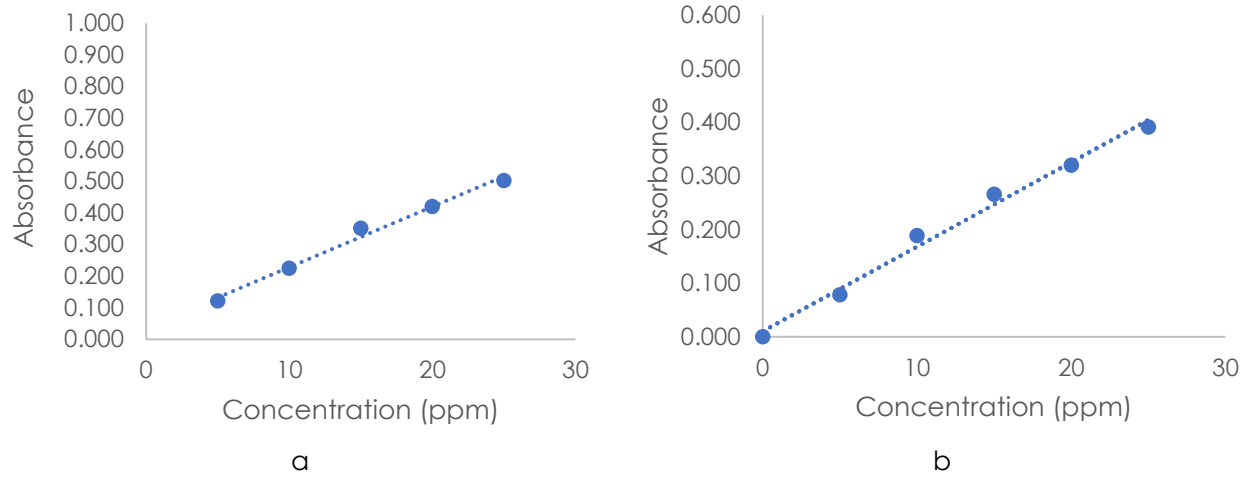

Figure 4. Standard Curve of (a) Gallic Acid; (b) Quercetin; Sample absorbance (Yellow point)

generally, contain carotenoid pigments, especially fucoxanthin (Sadvika *et al*., 2022; Nisa *et al*., 2020). The results showed that the pigment content of chlorophyll-a was 9.18 mg/g and carotenoid was 26.46 µmol/g. The results were higher than *Padina* sp. obtained from Bandengan Beach (0.39 mg/g;0.53 µmol/g) (Hidayati *et al*., 2017) and *Acanthophora muscoides* from Krakal Beach (7.72 mg/g; 28.53 µmol/g) (Pramesti *et al*., 2017). Environmental factors, algae morphology, and light intensity affect differences in pigment content in each organism (Pesang *et al*., 2020).

The carotenoid content of macroalgae is directly proportional to chlorophyll a's content because this pigment can protect against the damage of chlorophyll-a (Akmal *et al*., 2017). Chlorophyll-a is the primary pigment needed for photosynthesis, and its content is determined by the increased number of nutrients in the waters (Yudiati *et al*., 2020; Yudiati *et al*., 2021). Carotenoid compounds are known to have antioxidant abilities by donating one of their electrons to free radicals to be neutralized (Labola and Puspita, 2017). They can reduce singlet oxygen through conjugate bonds in the carbon chain. In addition, chlorophyll is also a potential compound as an antioxidant. It is shown that the absence of chlorophyll content can reduce the antioxidant ability of the sample (Sedjati *et al*., 2018).

CONCLUSION

Methanol extract of *Padina* sp. has an IC_{50} value of 92.17 ppm and is classified as a strong antioxidant. Antioxidant activity is supported by qualitatively discovering flavonoid compounds, steroids, and tannins. The extract contained 46.02 mg/GAE g of total phenols, 35.36 mg/QE g of flavonoids, 9.18 mg/g of chlorophyll-a, and 26.46 µmol/g of carotenoids.

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