Phytochemical Screening, Total Phenolic Content and Antioxidant Activity of Tropical Brown Macroalgae *Padina pavonica* Hauck from Kabung Island, West Kalimantan

Mega Sari Juane Sofiana, Ikha Safitri, Shifa Helena, Warsidah*.
Marine Science Department, Faculty of Mathematics and Natural Sciences, Tanjungpura University, Indonesia
Jl. Prof. Dr. H. Hadari Nawawi, Pontianak, Kalimantan Barat, 78124
Corresponding author : warsidah@fmipa.untan.ac.id

ABSTRAK

Marine macroalgae are potential sources as carbohydrate alternative, food additive, and bioactive compounds with antioxidant activity. The biological activity is strongly influenced by the type and phenolic compounds concentration. Tropical brown algae Padina pavonica Hauck was found with a large abundance in Kabung Island, West Kalimantan. The objective of this study was to determine the phytochemical screening, total phenolic content, and antioxidant activity of P. pavonica Hauck extract. The phytochemical screening and determination of total phenolic compounds were carried out using two different solvants with ethanol and ethyl acetate. Whereas antioxidant activity was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenger method with UV-vis spectrophotometer. The qualitative analysis of phytocemical constituents revealed the presence of alkaloids, steroids, flavonoids, phenols, and saponins. Furthermore, for total phenolic compounds, the ethanolic extract had a higher content (20.34 mgGAE/g) dry sample than ethyl acetate extract (7 mgGAE/g). The ethanolic extract also had a higher of potential antioxidant activity which IC50 was 144.47 ppm compared to ethyl acetate extract (IC50 was 191.98 ppm).

***Keywords** : phytochemical; phenol; antioxidant; Padina pavonic.*

INTRODUCTION

Free radicals cause some damage to the body that disrupt cellular metabolism, as well as capable altering in lipids, proteins, and DNA (Young and Woodside, 2001) as the major target. Lipids are susceptible to free radical resulting in lipid peroxidation. Free radical can damage the protein and DNA. Protein damaged can result in oxidative protein denaturation and DNA damaged leading to mutagenesis and carcinogenesis (Sivanandham, 2011). It has been implicated in various kind of human diseases such as inflammation of the joints, cell damage, rheumatoid arthritis, and aging (McDonald *et al*., 2001; Jang *et al*., 2007; Abdolghaffari, 2010; Rohman *et al*., 2010).

Reactive Oxygen Species (ROS) has been implicated in the development of several diseases and the formation of ROS is a common process during cellular metabolism. The most important reactive oxygen species are hydroxyl radical, superoxide anion radical, hydrogen peroxide, hypochloride radical, and singlet oxygen (Buyukokurodlu *et al*., 2001; Chitra and Pillai, 2002). There are several antioxidant defense mechanisms in plants including seaweed (Matsukawa *et al*., 1997). Antioxidants play potential preventive role to protect the body from damage associated with oxidative stress by free radicals (Ozsoy *et al*., 2008). This substances can also inhibit the oxidation of lipids and other biomolecules by blocking the initiation step and scavenging various free radicals in order to detoxify the organism (Kumaran and Karunakaran, 2006).

Marine macroalgae have been reported as renewable sources in marine environment, rich of important bioactive compounds (Smit, 2004) that are potential to be developed. They are able to produce a great different kinds of secondary metabolites (Vimala *et al*., 2015) with several biological activities (Wijesekara and Kim, 2010; Wijesekara *et al*., 2010; Wijesekara *et al*., 2011) including antioxidant (Yuan *et al*., 2005). Moreover, marine macroalgae are useful to human in health care and extensively utilized in various fields such as medicine (Melka, 2009; Haryani *et al*., 2014), drug and pharmaceutical industry (Eluvakkal *et al*., 2007). *Padina pavonica* Hauck belonging to Phaeophyceae is one of brown macroalgae due to the pigment of fucoxantin (Kuncoro, 2004). They are widely distributed in tropical waters and abundantly grow in Kabung Island waters. *P. pavonica* contains alkaloids, flavonoids, triterpenoids, saponins, phenolhydroquinones and tannins (Wijaya, 2014). The present study revealed the phytochemical screening, total phenolic compounds and antioxidant activity of tropical brown algae *P. Pavonica* Hauck from Kabung Island, West Kalimantan.

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MATERIALS AND METHODS

Samples Collection and Identification

Samples of *P. pavonica* Hauck were collected from Kabung Island, West Kalimantan, Indonesia. Samples identification and extraction were carried out in Laboratory of Marine Science, Faculty of Mathematics and Natural Sciences, Tanjungpura University. Whereas the total phenolic compounds and antioxidant activity were conducted in Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences, Tanjungpura University.

Samples Preparation

Samples of *P. pavoniva* Hauck were cleaned, then dried at room temperature for three days to reduce water content. Furthermore, samples were roughly chopped, followed by maceration extraction process using two different solvents ethanol and ethyl acetate. Each extract was filtered with Whatman paper No. 1. Then, the ethanol and ethyl acetate extracts of *P. pavonica* were thus concentrated using a rotary evaporator, then they are prepared for further analysis.

Phytochemical Screening

Phytochemical screening of ethanol and ethyl acetate extracts of *P. pavonica* was carried out using the method reported by Senguttuvan *et al*. (2014), Sofiana *et al*. (2020) and Masriani *et al*. (2020), including the examination of alkaloids, steroids, phenols, flavonoids, and saponins. This analysis was based on a qualitative test between samples with specific reagents for each chemical component which was characterized by the occurrence of color changes, formation of deposits and foam on the surface of the samples. The alkaloid test was carried out by dissolving the extract of *P. pavonica* samples with a few drops of 2N sulfuric acid, then tested using Dragendorff and Meyer reagents. A positive result was obtained by the formation of a red to

orange precipitate with Dragendorff and a yellowish white precipitate with Meyer reagent. The steroid test was conducted by dissolving the extract in 2 mL of chloroform in a test tube, then adding of ten drops of anhydrous acetic and three drop of concentrated sulfuric acid. A positive result was obtained by the formation of red solution for the first time then turns to blue and green. The phenol test was carried out by extracting *P. pavonica* samples in 20 mL of 70% etanol, the resulting extract was 1 mL thus added with 2 drops of 5% $FeCl_3$ solution. the formation of a green or blue green color indicated a positive reaction. The flavonoid test was done by adding 0,1 mg of magnesium powder and 0,4 mL of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with the equal volume) and 4 mL of alcohol, then the mixture was shaken. A positive reaction was shown by the formation of a red, yellow or orange color on the amyl alcohol layer. The saponin test was conducted by shaking the extract of *P. pavonica* with hot water. The formation of a foam was stable for 5 minutes and did not disappear in the addition of 1 drop of HCl 2N indicating the presence of saponins.

The Determination of Total Phenolic Contents

The determination of total phenolic content was done by the method refers to Yangthong *et al.* (2009), Sharma *et al.* (2011) and Santoso *et al.* (2012) using Folin-Ciocalteau reagent. A total of 5 mg of ethanol and ethyl acetate extract from *P. pavonica* were dissolved in 2 mL of 96% ethanol, then added 5 mL of distilled water and 0.5 mL of Folin-Ciocalteau reagent 50%. Furthermore, they were incubated for 5 minutes, then added 1 mL of 5% Na_2CO_3. The solution was homogenized thus incubated in the dark condition for one hour. The resulting absorption was measured by a UV-Vis spectrophotometer at a wavelength of 725 nm with three replicates. Gallic acid was used as a standard with a concentration series of 0 ppm, 5 ppm, 15 ppm and 20 ppm. The gallic acid calibration curve was used to determine the levels of phenolic compounds contained in the samples through the regression equation and expressed in units mg equivalent of gallic acid per gram extract (mg GAE / g extract) following the formula :

$$C = C_1 \text{ x } (V/M)$$

where C is total phenolic compound (mg GAE/g extract), C_1 is gallic acid concentration (mg/L), V is extract volume (L), and M is extract weight (mg).

Antioxidant Activity Test

The antioxidant test was determined according to the method of Banerjee *et al.* (2005). Test was started with the preparation of concentration series were 100, 200, 300, and 400 ppm. Thus, preparation of 0.1 mM DPPH reagent was carried out by dissolving 0,002 g of DPPH in 50 mL of 95% ethanol. Each sample was added with 3 mL (1:3 v/v) DPPH solution. Furthermore, samples and DPPH were mixed using vortex for 1 minute and incubated for 30 minutes. The absorbance was measured using a Spectrophotometer U-1240 Shimadzu MiniUV at a wavelength of 517 nm, and methanol was used as blank solution. The percentage of inhibition was calculated by the following formula:

$$\%\ inhibition = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}}\ x\ 100\%$$

The inhibitory concentration (IC_{50}) curve is made with the % inhibition value as the Y axis and the concentration series as the X axis. The inhibitory concentration (IC_{50}) value is obtained at 50% inhibition, by entering the value in the linear regression equation obtained from the standard curve.

RESULTS AND DISCUSSIONS

Macroalgae are chlorophyll plants that live attaching to the aquatic substrate using holdfast. They are widely distributed in tropical waters and abundantly grow in tidal areas with clear water and suitable substrate conditions (Kadi, 2006). *P. pavonica* is a brown macroalgae belonging to the Phaeophyceae (Chandini *et al.*, 2008), which is an importantt source of bioactive compounds such as carotenoids, dietary fiber, proteins, vitamins, essential fatty acids and minerals (Holt, 2008). The study on brown macroalgae is recently increasing, especially on conjugated fatty acids and fucoxanthin pigments which have physiological effects in the treatment of tumors and cancer-related problems (Kumar *et al.*, 2013) as well as antioxidant (Peng *et al.*, 2011), antiobesity (Maeda *et al.*, 2008; Beppu *et al.*, 2012), and anti-inflammatory (Mise and Yasumoto, 2011). The known chemical content in *P. australis* are fucoxanthin (0.6368 mg/g wet weight) and carotenoid pigments including β-carotene, diadinoxanthin, diatoxanthin, fucoxanthin, chlorophyll a and c (Limantara and Heriyanto, 2010).

The qualitative analysis of phytocemical constituents of *P. pavonica* from Kabung Island waters revealed the presence of alkaloids, steroids, flavonoids, phenols, and saponins (**Table 1**). The presence of flavonoids and phenols in ethyl acetate extract showed a weak intensity compared to the ethanol extract. It was due to these two chemical compounds were more soluble in ethanol solvent. The results of the phytochemical screening are the initial instructions in carrying out the biological activity tests.

Table 1. Phytocemical analysis of etanol and ethyl acetate extract of *P. pavonica*

Constituents	Chemical reagents	Etanol extract	Ethyl acetate extract
Alkaloids	Dragendorff Meyer	+	+
Steroids	Liebermann-Burchard	+	+
Flavonoids	Magnesium and amyl alcohol powder	+	+
Phenols	$FeCl_3$	+	+
Saponins	Forth test	+	+

Notation: + = present; - = absent

Preliminary phytochemical screening of methanolic extract from *P. pavonica* contains unsaturated sterols and/or triterpenoides, flavonoids, carbohydrates or glycosides, proteins and/ or amino acids, tannins and coumarin (Al-Enazi *et al.*, 2018). Acetone extract of *P. pavonica* contain phenolic, flavonoid and tannin (Bernardini *et al.*, 2018). Phytochemical analysis of ethanol extract from *P. pavonica* showed presence of alkaloid, phenols, flavonoids, carbohydrates, glycosides, steroid and terpenoids (Ismail *et al.*, 2019).

Total Phenolic Content

Macroalgae contain phenolic compounds that have potential as antioxidants by inhibiting enzymes involved in radical generation (Kurniawati *et al.*, 2016) and their content varies between species (Chakraborty *et al.*, 2013[a]). Phenolic compounds can protect the macroalgae from adverse environmental conditions (Bernardini *et al.*, 2018)

The total phenolic contents of ethanolic extract had a higher content (20.34 mgGAE/g) dry sample than ethyl acetate extract (7 mgGAE/g). The phenolic content of *P. pavonica* with different solvents has been reported. The total phenolic contents of the acetone extract *P. pavonica* from French Polynesia was 27

mg per g of extracts (Bernardini *et al*., 2018). Caf *et al*. (2015) reported *Padina pavonica* have total phenolic compounds 0.96 mg per g of the aqueous extract and 1.76 mg per g of the methanolic extract. Methanol and dichloromethane extract of *P. pavonica* from Portugal were 44.61 and 10.48 mg per g, respectively (Pinteus *et al*., 2017). The phenolic contents in an extract will increase according to the polarity of the solvent (Ganesan *et al*., 2008; Bangol *et al*., 2014).

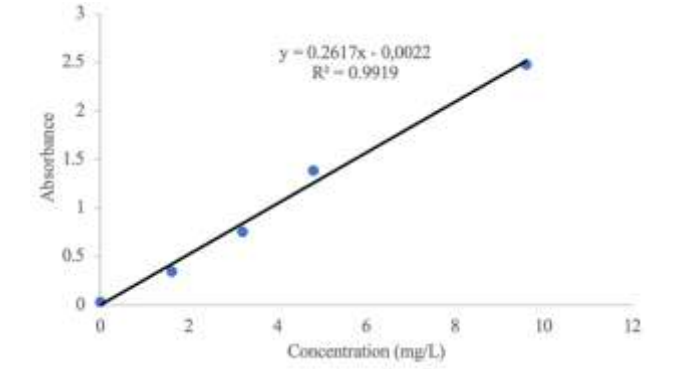

Figure 1. Total phenolic content for standard gallic acid

Antioxidant Activity

DPPH (1,1-diphenyl-2- picrylhydrazyl) is a free radical, stable and can react by delocalizing free electrons to make the molecule becomes relatively more stable. Antioxidant activity is the ability of a compound to inhibit oxidation reactions and could be expressed by the percentage of inhibition. The effectiveness of an extract in counteracting exposure to DPPH free radicals is called IC_{50} (50% inhibitory concentration) (Chakraborty and Joseph, 2016).

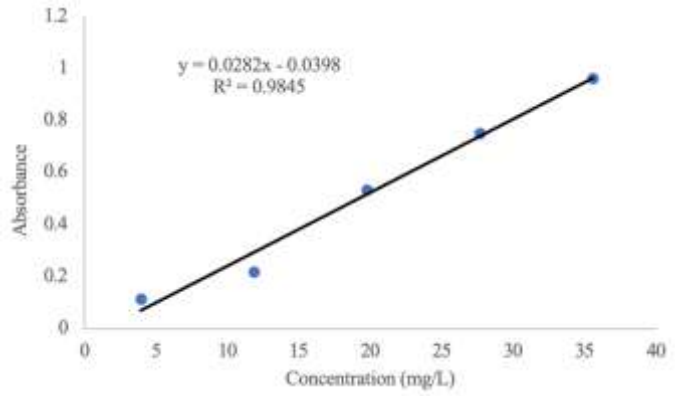

Figure 2. Standard curve of DPPH

The result of ethanolic extract of *P. pavonica* showed a higher of potential antioxidant activity which IC_{50} was 144.47 ppm compared to ethyl acetate extract (IC_{50} was 191.98 ppm) (Figure 3 and Figure 4). It is due to the higher phenolic contents of ethanolic extract. Some studies reported the antioxidant activities of the macroalgae are associated with the phenolic content (Shanura Fernando et al., 2018; Arunkumar *et al*., 2020). The IC_{50} value of the ethanol extract is classified as an antioxidant with moderate potential and the IC_{50} value of the ethyl acetate extract is classified as a weak antioxidant. The IC_{50} value for ascorbic acid as a positive control was 6.33 ppm. Antioxidant activity could be classified based on IC_{50} value as very strong antioxidant (less than 50 µg/mL), strong antioxidant (50-100 µg/mL), moderate antioxidant (101-150 µg/mL) and weak antioxidant (150-200 µg/mL) (Molyneux 2004).

Antioxidant activity of *P. pavonica* have been reported. Ethanolic extract of *P. pavonica* showed antioxidant activity with IC_{50} value 5.59 mg/mL (Al-Enazi *et al*., 2018). Arunkumar *et al*. (2020) described 1 mg/mL polysaccharide of *P. pavonica*

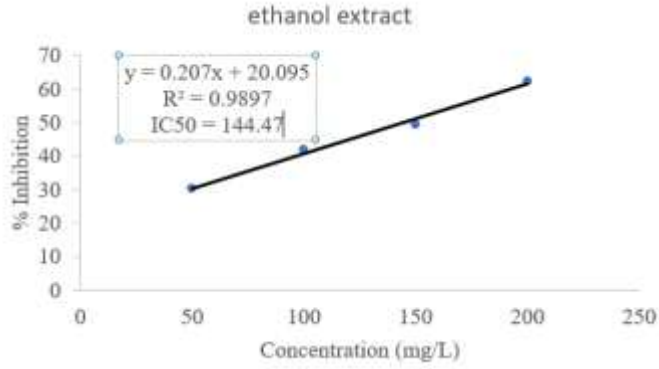

Figure 3. Antioxidant activity of ethanol extract of *P. pavonica*

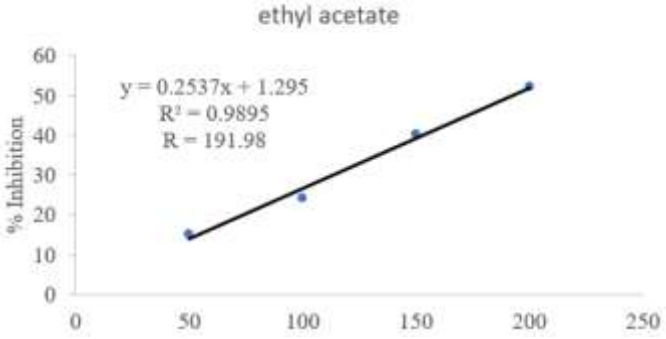

Figure 4. Antioxidant activity of the ethyl acetate extract of *P. pavonica*

showed concentration dependent manner with maximum scavenging activity of 63%. Khaled *et al*. (2012) reported that ethyl acetate extract of *P. pavonica* showed the antioxidant activity (42.5%). On addition, some studies reported the antioxidant activities of the seaweeds are associated with the phenolic algal content (Shanura Fernando et al., 2018; Arunkumar *et al*., 2020)

CONCLUSION

The qualitative analysis of phytocemical constituents of *P. pavonica* revealed the presence of alkaloids, steroids, flavonoids, phenols, and saponins. For total phenolic compounds, the ethanolic extract had a higher content (20.34 mgGAE/g) dry sample than ethyl acetate extract (7 mgGAE/g). The ethanolic extract also had a higher of potential antioxidant activity which IC_{50} was 144.47 ppm compared to ethyl acetate extract ($IC5_0$ was 191.98 ppm).

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