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Antioxidant of n-hexane, ethyl acetate and methanol extracts of *Padina* sp with DPPH method

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Abstract. *Padina* sp. is seaweed which has high abundance in Indonesian waters, but the utilization of bioactive compounds is not yet optimal. This research intends to test the antioxidant activity of the secondary metabolites of *Padina sp*. in South Sulawesi waters. The extraction process uses a multilevel maceration method with n-hexane, ethyl acetate and methanol solvents, then followed by concentrating/drying the sample using the evaporation method. The extract obtained was then tested for phytochemistry, determined the levels of flavonoids and phenolics, and tested for antioxidant activity using DPPH method. The results of this research indicate that the extracts of n-hexane, ethyl acetate and methanol from *Padina sp.* contains flavonoids and phenolics with levels 103.0435; 124.5109; 111.7391 mg QE/g sample and 1.1504; 7.1289; 3.6325 mg GAE/g sample, respectively. n-hexane, ethyl acetate and methanol extracts of *Padina sp.* has antioxidant activity with IC50 values 117.3622; 115.7171; 116.8350 ppm, respectively. The IC50 value of n-hexane, ethyl acetate and methanol extracts of *Padina sp.*is in the medium category for antioxidant activity.

1. Introduction

Padina sp. seaweed has an abundance of 33.33% in Indonesian waters, but its utilization is not optimal. *Padina* sp. contains secondary metabolites such as alkaloids, flavonoids, steroids, saponins, and phenolic. Phenolic was the largest secondary metabolite found in *Padina sp*. at 17% while flavonoids were 2% [1]. Phenolic and flavonoids have bioactivity as antioxidant. Antioxidant are associated with various application purposes in health and medicine [2].

Phenolic and flavonoids antioxidant compounds can be extracted using several solvents with different levels of polarity. The solvents that are usually used to extract phenolic and flavonoids in a material are n-hexane, ethyl acetate and methanol [3]. Phenolic and flavonoids antioxidant compounds can fight free radicals in the human body. These compounds work by donating hydrogen or free electron to free radicals, so that free radicals do not damage cell components and can indirectly prevent biological damage such as cancer, diabetes and other degenerative diseases [4].

The method of testing antioxidants can use the spectrophotometry method. The most widely used antioxidant test method is the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method because it is fast and has a high reproductive ability [5]. The purpose of this research was to determine the phenolic levels, flavonoid levels and antioxidant IC_{50} value of n-hexane, ethyl acetate and methanol extracts of *Padina sp*.

2. Materials and Method

2.1. Extraction

Fresh *Padina sp.* seaweed obtained from Selayar Island, South Sulawesi is washed and rinsed with distilled water until clean (5 times washing). Then dried at room temperature. The dry *Padina sp.* was

grinder to obtain *Padina sp.* powder. Then it was extracted by graded maceration method using n-hexane then ethyl acetate than methanol (5-10 times of remaceration). Then the extract was filtered and evaporated to obtain a thick/dry extract of n-hexane, ethyl acetate and methanol.

2.2. Phytochemical test

The extracts of n-hexane, ethyl acetate and methanol were tested for phytochemistry. Phytochemical tests were carried out to determine the presence of groups of secondary metabolite compounds such as alkaloids, flavonoids, steroids, saponins, and phenolic using the Harborne standard procedure [6].

2.3. Determination of total phenolic content

Determination of total phenolic content was carried out using a modified method Chanwitheesuk et al. The sample was weighed as much as 0.05 g, then dissolved in 10 mL of hot distilled water (80 °C). After that, it was allowed to stand for 10 minutes, then it was filtered so that the sample filtrate was obtained and diluted if needed. A sample of 5 mL was added with 0.25 mL of 50% follin reagent, then 0.5 mL of saturated Na_2CO_3 was added, then let stand for 30 minutes. After that, the absorbance of the mixture was measured at the maximum wavelength (743 nm) using a UV-Vis spectrophotometer. Gallic acid was used as standard while distilled water was used as blank [7]. Phenolic content is calculated using a linear equation obtained from the standard curve for gallic acid.

2.4. Determination of total flavonoid content

Determination of total flavonoid levels was carried out using a modified Singleton and Rossi method. The sample was weighed as much as 0.02 g, then dissolved in 10 mL of methanol. Then filtered so that the sample filtrate is obtained and diluted if needed. A sample of 0.5 mL was added to 3 mL of methanol, then 0.2 mL of 10% $AlCl_3$ and 0.2 mL of 1 M CH_3COOK were added. Then the volume was added to 10 mL by adding 6.1 mL of distilled water. After that, the absorbance of the mixture was measured at the maximum wavelength (434 nm) using a UV-Vis spectrophotometer. Quercetin was used as a standard while methanol was used as a blank. [8] Flavonoid levels were calculated using a linear equation obtained from the standard quercetin curve.

2.5. Antioxidant activity test

The antioxidant activity test was carried out using a modified method of Brand-Williams et al. The sample was weighed as much as 0.005 g, then dissolved in 10 mL of methanol to obtain a sample of 500 ppm. Then the sample of 500 ppm pipettes as much as 0.025; 0.05; 0.1; 0.2; and 0.4 mL into different test tubes for variations in concentrations of 2.5; 5.0; 10.0; 20.0; and 40.0 ppm, then added 1 mL of DPPH 0.4 mM. Then each mixture is sufficient to 5 mL with methanol. The mixture was allowed to stand at dark room temperature for 30 minutes. The absorbance of the mixture was measured with a spectrophotometer at a maximum wavelength (515 nm). 1 mL of DPPH + 4 mL of methanol was used as a control while methanol was used as a blank [9]. The percentage of antioxidant activity or percent inhibition is calculated using the following equation:

$$\%\,\text{antioxidant activity} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \text{ x } 100$$

The IC_{50} value is calculated using a linear equation obtained from the curve between the concentration and percent of antioxidant activity.

3. Result and Discussion

3.1. Extraction and phytochemical test

The extraction process was started by washing and rinsing the samples of *Padina sp.* use running water and distilled water to remove impurities such as sand, salt and other macro contaminants. Then the sample is dried to reduce or remove water so that the sample is easier to grind. The sample in

powder form will facilitate the extraction process because the surface area of the sample is larger so that the contact between the sample and the solvent can be maximized. The extraction method used is a graded maceration method so that the extracted compounds can be separated easily based on differences in polarity. Maceration was started using a nonpolar solvent, namely n-hexane (10 ×24 hours of remaceration). The filtrate of each n-hexane remaceration combined is then called n-hexane macerate. Then the sample dregs from n-hexane maceration were dried at room temperature, then maceration was continued using a semipolar solvent, namely ethyl acetate (10 ×24 hours of remaceration). The filtrate from each ethyl acetate remaceration is combined then called ethyl acetate macerate. Then the sample dregs from ethyl acetate maceration were dried at room temperature, then maceration was continued using a semipolar solvent that tends to be polar, namely methanol (5 ×24 hours of remaceration). The filtrate of each combined methanol remaceration is then called methanol macerate. N-hexane, ethyl acetate and methanol macerate are evaporated until thick or dry to obtain n-hexane, ethyl acetate and methanol extracts of *Padina sp*. Then the resulting yield was calculated for each extract with a total yield of about 5.51%. The yield of each extract is shown in Table 1. The least n-hexane extract yield shows that the compound is nonpolar in *Padina sp*. also the least, while the yield of ethyl acetate and methanol extracts showed that the semipolar compounds tended to be polar in *Padina sp*. also a lot more.

N-hexane, ethyl acetate and methanol extracts of *Padina sp*. each of them was tested for phytochemistry to determine the presence of groups of secondary metabolite compounds such as alkaloids, flavonoids, steroids, saponins, and phenolic. The results of the phytochemical test for each extract are shown in table 1. The alkaloid test was carried out using dragendorff reagent with positive results indicated by the formation of orange deposits. The flavonoid test was carried out using $Pb(CH_3COO)_2$ reagent with positive results indicated by the formation of white deposits. The steroid test was carried out using Liebermann-Bourchard reagent with positive results indicated by the formation of a blue-green solution. The saponin test was carried out by adding some distilled water to the extract then shaking it vigorously with a positive result indicated by the formation of foam which can last for 30 seconds. The phenolic test was carried out using $FeCl_3$ reagent with positive results indicated by the formation of green deposits [6].

Table 1. Phytochemical test results and yield of *Padina sp.*

Phytochemical test	Results		
	n-hexane	Ethyl acetate	Methanol
Alkaloids	+	+	-
Flavonoids	+	++	++
Steroids	+	+	-
Saponins	-	-	+
Phenolic	+	++	++
Yield	0.18%	0.65%	4.68%

3.2. Determination of total phenolic content

Determination of total phenolic content was carried out to determine the correlation between antioxidant activity and total phenolic content in a sample because phenolic group compounds have been widely reported to have very strong antioxidant activity [10]. The results of determining the total phenolic content of the extract of *Padina sp*. shown in figure 1. The highest total phenolic content is found in ethyl acetate extract then methanol then n-hexane because phenolic group compounds tend to

be semipolar-polar, so that more phenolic compounds are extracted in semipolar-polar solvents, namely ethyl acetate and methanol. The total phenolic content in ethyl acetate extract is higher than methanol because the maceration that is carried out is graded maceration and the solvent used first is ethyl acetate, so that most phenolic compounds are extracted in ethyl acetate solvent.

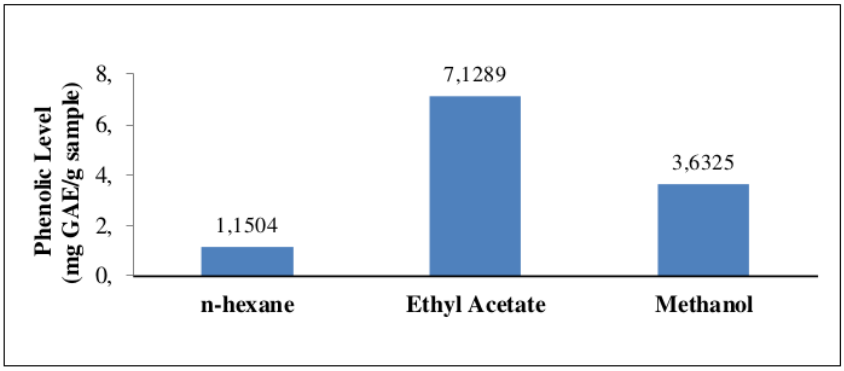

Figure 1. Total phenolic content of *Padina sp.*

3.3. Determination of total flavonoid content

Determination of total flavonoid content was carried out to determine the correlation between antioxidant activity and total flavonoids content in a sample because the flavonoid class compounds have been widely reported to have very strong antioxidant activity [10]. The results of determining the total levels of flavonoids from the extract of *Padina sp.* shown in figure 2. The highest levels of total flavonoids are found in ethyl acetate extract then methanol then n-hexane because the flavonoid class compounds tend to be semipolar-polar, so that more flavonoid compounds are extracted in semipolar-polar solvents, namely ethyl acetate and methanol. The total flavonoid content in ethyl acetate extract was higher than methanol because the maceration that was carried out was multilevel maceration and the solvent used first was ethyl acetate, so that most of the flavonoid compounds were extracted in ethyl acetate solvent.

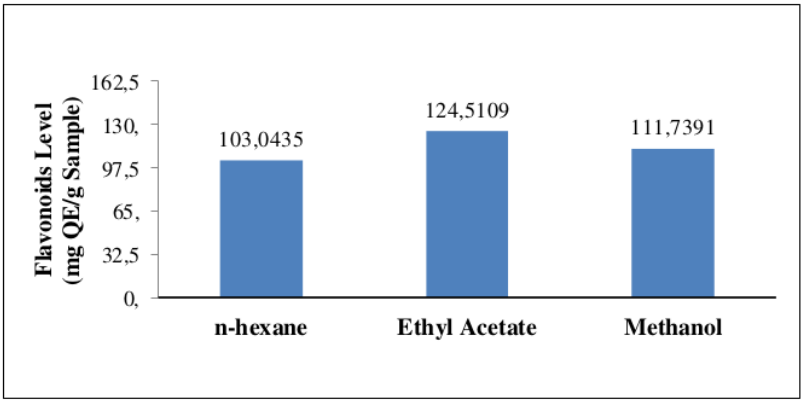

Figure 2. Total phenolic content of *Padina sp.*

3.4. Antioxidant activity test

Antioxidant activity test of extract of *Padina sp.* performed using the DPPH method. DPPH is a molecule that will accept hydrogen or free electrons from the substrate or antioxidant compound present in the sample to become a stable diamagnetic molecule. The stable non-radical form of DPPH is characterized by a simultaneous change in the color of the solution from purple to pale yellow [2]. The IC_{50} value of the antioxidant extract of *Padina sp.* shown in figure 3. The IC_{50} antioxidant value is a value that indicates the concentration of a sample extract or compound that can inhibit 50% of the activity of a free radical. The lower IC_{50} value of a sample extract or compound, the stronger its antioxidant activity. Ethyl acetate extract had the lowest IC_{50} value then methanol then n-hexane. This shows that ethyl acetate extract has better antioxidant activity than methanol than n-hexane. This is supported by phytochemical test data and data on total phenolic and flavonoid levels. Data on total phenolic and flavonoid levels showed that ethyl acetate extract had the highest levels than methanol and n-hexane. This is consistent with the results of research by Huyut et al. (2017) who stated that phenolic and flavonoid levels are directly proportional to antioxidant activity. However, the antioxidant IC_{50} value of n-hexane, ethyl acetate and methanol extracts of *Padina sp.* belongs to the same category of antioxidants, namely the moderate category of antioxidants.

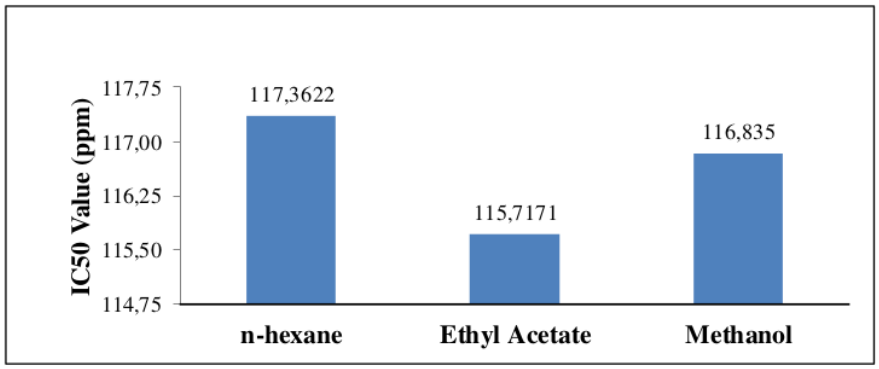

Figure 3. Antioxidant IC_{50} value of *Padina sp.* extract

4. Conclusion

The results of this research indicate that the extracts of n-hexane, ethyl acetate and methanol from *Padina sp.* contains flavonoids and phenolics with levels respectively 103.0435; 124.5109; 111.7391 mg QE/g sample and 1.1504; 7.1289; 3.6325 mg GAE/g sample. n-hexane, ethyl acetate and methanol extracts of *Padina sp.* has antioxidant activity with IC_{50} values respectively 117.3622; 115.7171; 116.8350 ppm. The IC_{50} value of n-hexane, ethyl acetate and methanol extracts of *Padina sp.* is in the medium category for antioxidant activity.

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