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ANALYSIS OF FLAVONOID LEVELS AND TOTAL PHENOLICS FROM BUNGUR LEAF EXTRACT (*Lagerstroemia speciosa*) AGAINST ANTIOXIDANT ACTIVITY

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Abstract

The levels of flavonoids and total phenolics contained in the methanol, 70% ethanol, and ethyl acetate extracts of Bungur leaves (*Lagerstroemia speciosa*) will be reported in this study. In addition, the antioxidant activity of each extract was also tested. The Bungur leaves (*Lagerstroemia speciosa* (L) pers) is a plant used empirically as medicine. The preparation and preparation of the extract was carried out by the maceration method, which used three different variations of solvents, including methanol, 70% ethanol, and ethyl acetate. The type of coating and the polarity of the coating significantly affect the antioxidant activity obtained. The phytochemical screening test was carried out to determine the content of chemical compounds in plant extracts. In contrast, the control of flavonoid levels and total phenolic content was carried out using the colorimetric method. Measurement of antioxidant activity levels was carried out using the DPPH test. The qualitative phytochemical screening test results of the methanol, 70% ethanol, and ethyl acetate extracts had the same secondary metabolite content. Still, the highest total flavonoid and phenolic content were found in the methanol extract of Bungur leaves, 9.06% and 16.42%. The antioxidant activity test of the methanol extract of Bungur leaves also showed the best inhibition results, as indicated by the IC50 value of 5.76 ppm. This can strengthen the total phenol and flavonoid levels correlated with antioxidant activity.

Keywords: antioxidant, bungur leaf extract, IC50, flavonoid

Introduction

Antioxidants are essential compounds in the body for maintaining human health. Antioxidants can be an antidote to free radicals formed in the body due to unhealthy environments and lifestyles.[1] Antioxidants can also neutralize free radicals by donating electrons to hydrogen atoms. Oxidative stress can be reduced due to the high content of bioactive compounds that act as antioxidant activity in the body; the body will produce antioxidant compounds such as SOD (Superoxide Dismutase), Gpx (Guttation peroxidase), and catalase which play a role in protecting the vascular endothelium from free radical attacks.[2] Free radicals are compounds or molecules that are stable in cells that are unpaired and are reactive in attacking and binding to electron

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Molecules around them. Electrons bound to ionic free radicals will have less harmful effects. Free radicals can have a hazardous impact, such as damaging the function of cells and tissues, cardiovascular disease, cancer, chronic respiratory disease, diabetes, and even kidney disease. This is due to free radicals that bind with covalent bonds.[3]

The human body has an endogenous defense system against free radical attacks primarily through normal cellular metabolic events and inflammation. The number of free radicals can increase, which results in stress factors, radiation, cigarette smoke, and environmental pollution, causing the body's existing defense system insufficient. Hence, the body needs additional antioxidants from the outside to protect against free radical attacks.[4] *Lagerstroemia speciosa* (L.) Pers. is a plant of the *Lythraceae* tribe, which is widely found in Indonesia. This plant is known as a flower. L. speciosa has secondary metabolites such as triterpenes, tannins, ellagic acid, glycosides, and flavonoids. Flavonoids in Bungur are thought to have antioxidant activity due to the presence of phenolic hydroxy groups, which can counteract free radicals.[5]

Various methods can test the antioxidant activity of the leaf Bungur extract, one of which is using the DPPH (1,2-diphenyl-2-picrylhdrazly) method. The DPPH method is a simple, fast, easy method for screening the radical scavenging activity of several compounds; effective, practical, and a fast-processing process, stable in polar solvents.[6] The solvent type and the solvent's degree of polarity are also very influential, where a solvent tends to dissolve compounds with the same level of polarity. The phenol and flavonoid components have antioxidant activity by reducing free radicals depending on the number of hydro groups acting on their molecular structure.[7] So, the need for consideration in determining the solvent in testing the antioxidant activity.

Based on previous research conducted by Sandhiutami et al., [8] leaf decoction of *L. speciosa* has antioxidant activity with an IC50 of 57.60 mg/mL and can reduce mouse sugar levels.[8] Research conducted by Roni et al.[9] has successfully isolated 5,3',4' trihydroxy flavonol compounds of the flavonoid group from *L. speciosa* leaves and ethanol extract of bungur fruit.[9] Anticancer screening has been done using this method with a Lethal concentration (LC50) value of 60 g/ml and an LC90 of 100 g/mL. Brine Shrimp Lethality Test (BSLT).[10] However, until now, there has been no research on differences in extraction solvents on flavonoid content and total phenol.

Method

Tools

Rotary evaporator (Buchi), spectrophotometer (Shimadzu), and other chemical glassware (Pyrex).

Materials

The simplicia used was the leaves of *Lagerstroemia speciosa* (L.) Pers. obtained from the Research Institute for Spices and Medicinal Plants (Balittro). Chemical reagents in the form of ethanol (Merck), methanol (Merck), and ethyl acetate (Merck).

L. speciosa Leaf Extract Production

The bungur leaf Simplicia was extracted using maceration. The collected Bungur leaf samples were cleaned and sun-dried by air drying. Then, it is ground into a powder and extracted using three distinct solvents: ethanol, methanol, and ethyl acetate. The maceration results were concentrated using a rotary evaporator and evaporated in a water bath to achieve a thick extract.[11]

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Qualitative Identification of Secondary Metabolites

A qualitative examination of the extract was conducted to observe several secondary metabolites: alkaloids, tannins, saponins, terpenoids, and flavonoids.

To identify alkaloids, 2 mL of the extract solution is evaporated in a porcelain cup until a residue is formed. The residue is dissolved in 5 mL of 10% diluted ammonia, to which 2 mL of chloroform is added and filtered. 2N HCl was added to the filtrate, and the mixture was agitated to produce two layers. The 2N HCl layer and the chloroform layer are the two layers present. Using a 3 mL pipette, the layer containing HCl 2 N was removed and then split among three test tubes. The first tube is used as a blank. Three drops of Dragendroff reagent were applied to the second test tube; if an orange precipitate appeared, yellow to brown indicated the presence of alkaloids. In the third tube, three drops of Mayer's reagent show the presence of alkaloids if a white-to-yellowish precipitate appears.[12]

To identify tannins, a 2 mL extract solution was reacted with a 10% iron (III) chloride solution; if the answer turned dark blue, black, blue, or greenish-black, tannin components were present.[12]

In a test tube, 2 mL of the sample is dissolved in 10 mL of distilled water for the saponin test. The filtrate in the test tube was forcefully shaken for about 30 seconds after cooling. If the reaction temperature is equal to room temperature, the reaction will halt or enter a rest phase. The foam production indicated the presence of saponins with a minimum height of 1 cm that lasted for at least 10 minutes after adding one drop of diluted hydrochloric acid.[12]

A 2 mL (w/v) sample was extracted with ether and then evaporated to dryness for the triterpenoid test. On the residue, the Lieberman-Burchard reagent is deposited. The production of a purple hue suggests triterpenoid group chemicals, while the formation of a green-blue color shows steroid group compounds.[12]

Three 1 mL test tubes containing the extract of the bungur leaves were supplied to detect flavonoids. After adding ten drops of H2SO4-N to each tube, violent shaking ensued. The first tube received Dragendorff reagent, whereas the second one received Wagner. Positive results are obtained when the first tube (with the addition of Dragendorff reagent) gives a red precipitate, and the second tube (with the addition of Wagner reagent) produces brownish sediment.[13]

Quantitative Identification of Secondary Metabolites

A 600 µL extract (ethanol, methanol, and ethyl alcohol) of *L. speciosa* was added to 5 mL of distilled water and 0.5 mL of Folinciocalteu (1:10 in distilled water). The solution was allowed to stand for 3 minutes, and 2 mL of 20% Na_2CO_3 was added. The microplate was then filled with 200 µL of the mixed solution. Then incubation was carried out for 45 minutes, and the absorbance value was measured at 756 nm. The total phenol content was expressed as mg gallic acid equivalent/g dry weight.

Quantitative analysis of total flavonoid levels: 600 µL (ethanol, methanol, and ethyl acetate) *L. speciosa* added 1.5 mL of ethanol, 100 µL of 10% AlCl3, 100 µL of 1 M sodium acetate, and 2.8 mL of distilled water. Then 200 µL of the solution mixture was put into a microplate and allowed to stand for 30 minutes at room temperature, and then the absorbance was measured at 417 nm. The total flavonoid content in the extract was expressed as mg quercetin/g dry weight.[14]

Antioxidant Activity Test by DPPH Method

Several sample solutions were made with a concentration of 1.25, 2.50, 5, and 10 ppm. The sample was pipetted and then added with DPPH solution (200 ppm) in a ratio of 1:4 into the microplate and then homogenized. The mixture was incubated for 30 minutes at 37ºC. The absorbance was measured using a microplate reader at a wavelength of 517 nm.

Result

Figure 1. Fresh bungur leaf

Figure 2. Extraction process by maceration

Table 1. Sample Quality Check Simplicia

Sample Name	Test Type	Test result	Testing Method
Bungur leaf simplicia	Water content	6.41	Gravimetry
	Ash level	4.97	Gravimetry

Table 2. Percent (%) Extract Yield

Sample Name	Test Type	% Yield	Testing Method
Bungur leaf simplicia	Methanol extract	10.05	Maceration
	70% ethanol extract	12.63	Maceration
	Ethyl Acetate Extract	2.56	Maceration

Table 3. Qualitative Identification of Secondary Metabolites

Secondary Metabolites	Bungur Leaf Extract with Solvent		
	Methanol	Ethanol 70%	Ethyl Acetate
Alkaloids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Phenolic	+	+	+
Flavonoids	+	+	+
Triterpenoids	+	+	+

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Table 3. (Extension)

Secondary Metabolites	Bungur Leaf Extract with Solvent		
	Methanol	Ethanol 70%	Ethyl Acetate
Steroids	+	+	+
Glycoside	+	+	+

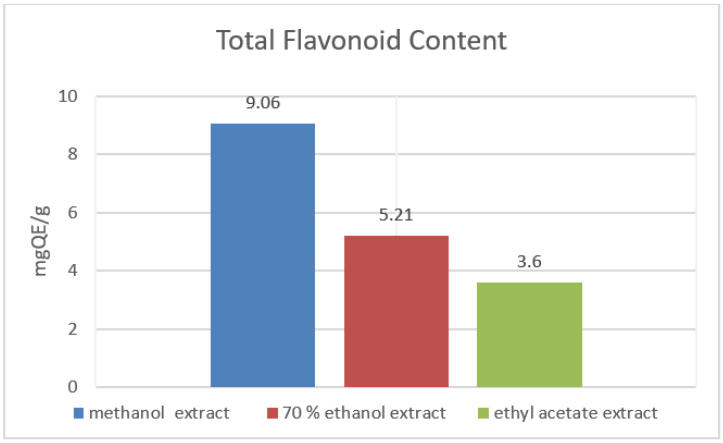

Figure 3. Bungur leaf extract flavonoid content

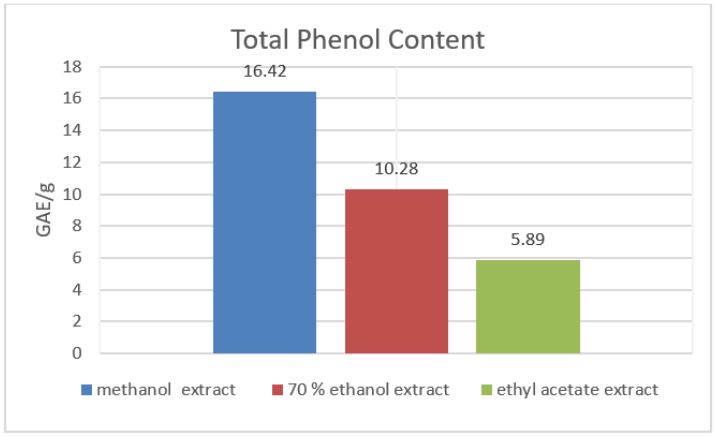

Figure 4. Total phenol content of bungur leaf extract

Table 4. Antioxidant Activity Test Result

Sample	IC50 value	Testing Method
Methanol extract	5.76	
70% ethanol extract	8.74	DPPH
Ethyl Acetate Extract	19.47	

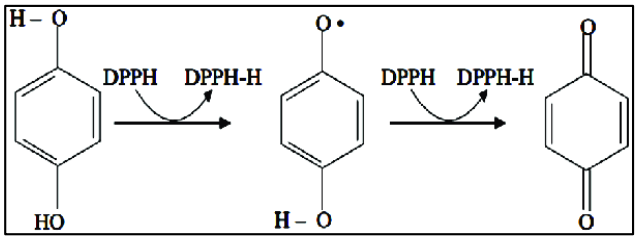

Figure 5. The reaction of phenolic compounds with DPPH[15]

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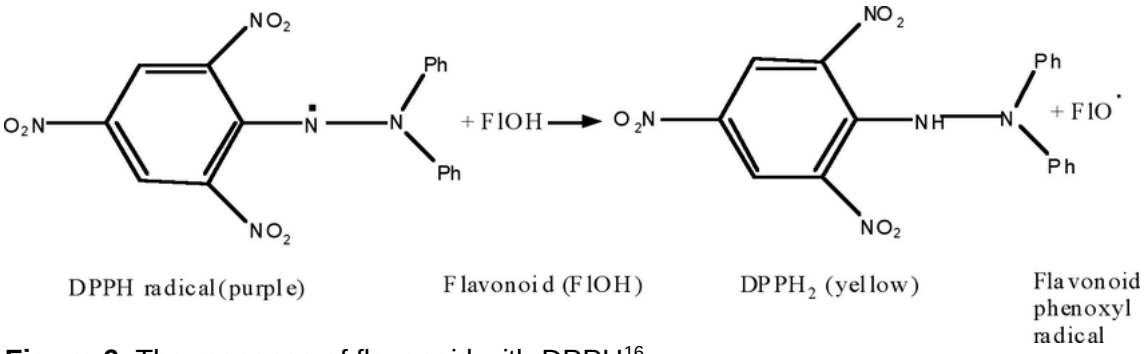

Figure 6. The response of flavonoid with DPPH[16]

Discussion

Simplisia Collection

Fresh Bungur leaf (*L. speciosa*) was obtained from Balittro (Figure. 1). Then, the leaf dried in the sun. Simplicia quality inspection can be done by checking the water content and ash content in Simplicia. The results of the simplicia leaf extract (Table 1) are to the requirements in the Indonesian herbal pharmacopeia, water content is not more than 10%, and ash content is not more than 7.7%.[17]

Determining water content is intended to determine the maximum limit of water content contained in the material. Determination of water content is carried out because it is related to the purity and the presence of contaminants in the simplicia. Therefore, removing water content up to a predetermined amount can help extend the durability of Simplicia materials during the storage process. The water content contained in Simplicia should have a percentage of less than 10.00%. The occurrence of enzymatic processes and damage caused by microbes can occur if the water content in simplicia is more than 10.00%.[18] The need for information related to the estimated internal and external mineral content from the initial process to the formation. Simplicia resulted in the determination of the total ash content. Entire ash cThe entire is related to organic and inorganic minerals obtained internally and externally.[19]

Extraction

Extracts were made by the maceration method. Extraction is intended to attract the active substances contained in simplicity. The solvents used were methanol, 70% ethanol, and ethyl acetate. The principle of selection of dissolution in the extraction method is to adjust the polarity of the solvent with the desired bioactive component properties extracted. According to such a principle, like dissolves, a solvent will tend to dissolve compounds with the same degree of polarity. This research uses different dissolutions to determine the levels of secondary metabolites with the resulting activity test. After maceration, the liquid extract was concentrated using a rotary evaporator until a thick extract was obtained and the % yield was calculated (Table 2) (Figure. 2).

Based on the Pharmacopoeia Herbal regiment extract, they obtained at least 9.2%.[20] differs from the test results because the solvents used are different. The difference in polarity in the type of solvent can affect the yield produced. Organic solvBased on their dielectric constant, organic solvents can be divided into polar and non-polar results of Bungur leaf extract using 70% ethanol as a solvent indicates that 70% ethanoate l solvent used in Bungur leaves is balanced compounds better than

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dissolving methanol or ethyl acetate. The benefit of ethanol as a solvent is that it is a universal solvent that can attract most plant-based chemical compounds.[22]

Qualitative Identification of Secondary Metabolites

A phytochemical screening can identify the secondary metabolite content of methanol, ethanol, and ethyl acetate extracts of Bungur leaves, including screening for alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoid, SDS, and steroids. The phytochemical screening results show that methanol extract, ethanol, and ethyl acetate contain the same secondary metabolites (Table 3).

Total Flavonoid Content (TFC)

Flavonoids are plant-derived compounds with antioxidant, anticancer, anti-inflammatory, and antibacterial properties. The antioxidant effect of flavonoids is one of their most critical bioactive properties.[23] Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was conducted to determine the total flavonoid levels contained in Bungur leaves (*Lagerstroemia speciosa* (L.) Pers). The goal of real flavonoid content determination is to determine the number of flavonoids present in extract samples, which are expressed as quercetin equivalents or Quercetin Equivalent (QE) using AlCl3 reagent, which is based on the formation of a stable yellow complex between AlCl3 and flavonol compounds.[24] Quercetin was used as a standard solution because quercetin is a flavonoid of the flavonol group with a keto group at C-4 and a hydroxyl group on neighboring C-3 or C-5 atoms of flavones and flavonols.[25] Complex formation was carried out with a maximum wavelength of 415 nm for standard quercetin, characterized by the formation of a yellow color. The increased absorbance indicates the high value of the flavonoid concentration in the catechol group. Figure 3 shows the results of calculating the total flavonoids of Bungur leaf extract.

Based on the results, the UV-Vis Spectrophotometry technique was used to determine the total flavonoid content in bungur extract. Total flavonoid content using methanol solvent was 9.06%, ethanol 70% solvent was 5.21%, and ethyl acetate was 3.65%. The results showed that methanol efficiently extracted flavonoids in Bungur leaves. The level of the polarity of the methanol solvent indicated compatibility with the flavonoid components in Bungur leaves, resulting in extracts with the most significant quantities of flavonoids. Methanol has a dielectric constant value of 33, which shows that methanol is a polar solution.[26] Methanol is the most efficient solvent for extraction due to the better dissolving of flavonoid compounds in medicinal plants due to interactions (hydrogen bonds) between the polar sites of antioxidant molecules and the solvent.[27] Plant flavonoid content varies according to genotype, growing environmental conditions, growth stage, postharvest handling, and storage conditions. These factors can affect the total flavonoid concentration and flavonoid composition in plants.[28]

Total Phenol Content (TPC)

Total phenol content analysis determines the amount of phenol in Bungur leaf extract samples. Total phenol content was determined using UV-Vis spectrophotometry and the Folin-Ciocalteu.[29] Folin-Ciocalteu (FC) reagent was used to analyze the total phenolic content in curd leaf extract based on its reduction ability.[30] The reduction results were indicated by changing the FC reagent from yellow to blue for the phenolic

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compounds contained in Bungur leaves. The absorbance value was measured after the blue color reduction was obtained using a UV-Vis spectrophotometer with a maximum wavelength of 750 nm. This study used gallic acid to determine the total phenolic compound in the sample as a standard solution. Gallic acid is used as a standard solution because it is a natural and stable phenol and is relatively inexpensive. Gallic acid is included in phenolic compounds, hydroxybenzoic acid derivatives, classified as simple phenolic acids. Gallic acid has become the standard for the availability of stable and pure substances.[31] The results of determining the total phenol content can be seen in Figure 4.

The results showed that total phenolic content (TPC) using methanol solvent was 16.42%, ethanol 70% solvent was 10.28%, and ethyl acetate 5.89%. According to the TPC values, Bungur leaf methanol extract has the highest phenolic content because methanol can dissolve compounds from polar to nonpolar. It might result from the enhanced solubility of nonphenolic compounds in organic solutions due to the presence of water molecules. It might also be due to the high solubility of phenolic compounds in methanol.[32] But the highest phenolic compound solubility is not always found in polar extracts but depends on the structure of the phenolic compounds.[33]

The increase in the polarity of the solvent affects the test results of total phenolic and total flavonoid content, where the growth will influence an increase in the polarity of the solvent. Based on the test results, it can be concluded that the most polar solvent, namely methanol, produces the highest total phenols and flavonoids.

Antioxidant Activity Level

Several techniques exist for evaluating antioxidant activity. Several approaches exist for assessing antioxidative activity. The DPPH (2,2-diphenyl-1-picrylhydrazyl) technique is one of them. The DPPH technique is based on the presence of hydrogen atoms from antioxidant chemicals that bind to free electrons in radical compounds, resulting in the transformation of free radicals (diphenylpicrylhydrazyl) into non-radical molecules (diphenylpicrylhydrazine).[34] The DPPH technique was selected because of its simplicity, ease of use, speed, sensitivity, and modest sample need. In assessing the antioxidant activity, the IC50 parameter was employed, which is the sample concentration required to capture 50% of the DPPH radical;[35] the lower the IC50 value, the higher the antioxidant activity.[36]

Increasing the polarity of phenolic compounds can affect the ability of the phenolic extract of Bungur leaves to overcome DPPH radicals. One of the criteria for quotes with the best ability to fight DPPH radicals is having a C50 value. The methanol extract is the most potent since it has the lowest IC50 value (Table 4), indicating the highest potential to scavenge the DPPH radical. The methanol extract had the highest concentration of polar phenolic chemicals compared to the ethanol and ethyl acetate extracts. The phenolic compounds in the methanol extract of the crepe can donate more hydrogen atoms to decrease DPPH radicals than the phenolic compounds in ethanol and ethyl acetate because the more polar phenolic compounds have a more significant amount of hydroxyl substituents.[37] This is also supported by data on total flavonoids, where there is an increase in total flavonoids followed by an increase in their activity as a radical scavenger of DPPH.

The study was conducted by testing the antioxidants in the extract of Bungur leaf using the DPPH test method and found that the extract of Bungur Leaf affected the

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inhibitory ability of antioxidants and contained flavonoids. Based on the research conducted by dissolving Bungur leaves with three solvents to make the extract, namely methanol, ethanol, and ethyl acetate, it was found that the extract with methanol as a solvent had the best ability among the three solvents in inhibiting antioxidants and had the best levels of flavonoids and total phenol. Based on previous research conducted by Rochman and Ratnadewi[38], the total phenolic content increased, followed by an increase in the polarity of the solvent, where the extract with ethanol solvent showed the best results. This could occur due to the ability of methanol to form hydrogen bonds, interaction dipoles, and van der Waals.[38]

As polyphenolic compounds, flavonoids can be antioxidants by capturing free radicals by forming less reactive flavonoid-phenoxy radicals. The potential of flavonoid compounds to scavenge free radicals is explained by their ability to donate a hydrogen atom from their hydroxyl groups; scavenge, ing free radicals is a screening reaction. This reaction produces a phenoxy flavonoid radical whanging nance structure by redistributing unpaired electrons to the aromatic core. Thus, the flavonoid phenoxy radical shows much lower reactivity and will react further to form a non-reactive compound, possibly by terminating the radicals.[39] So, the higher the levels of phenols and flavonoids, the higher the antioxidant activity.

Flavonoids offer a variety of medical effects, such as anticancer, antioxidant, anti-inflammatory, and antiviral activities.[40] Previous studies have shown antioxidant activity and total phenolic content in Lagerstroemia speciosa L. parsley seeds extract. It has been reported that the antioxidant activity produced is closely related to the entire phenolic content in the parsley seeds. The IC50 value of the methanol extract of the bungur seed was 9.63 ± 0.20 g/mL, and the total phenolic content was 325 ± 0.1 g GAE/mg extract. The results of determining the levels of polyphenols in the bark of Bungur stems were 50.69 ± 6.14 mg TAE/g. The presence of these phenolic compounds contributed to the antioxidant activity of the bark of Bungur. In addition to having antioxidant activity, the bark of the Bungur stem also has alpha-glucosidase inhibitory activity, with an IC50 value for ethanol extract of 92.96 g/mL.[41]The content of phenols and flavonoids acts as antioxidants because they can donate hydrogen atoms from hydroxyl to radical compounds so that they can turn out to be more stable.

Conclusion

The methanol extract of bungur leaf has the highest flavonoid and total phenolic content (9.06% and 16.42%) and the best antioxidant inhibitory activity (IC50 value of 5.76 ppm) compared to ethanol and ethyl acetate extracts of bungur leaf. The ability of the phenolic extract of Bungur leaves to reduce DPPH radicals increases with the increase in the polarity of the phenolic compounds.

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