**Analysis of Flavonoid Levels and Total Phenolics from Bungur Leaf Extract (*Lagerstroemia speciosa*) Against Antioxidant Activity**

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**Abstract**

This research aimed to determine the levels of flavonoids and total phenolic contained in the extract of the leaves of Bungur (Lagerstroemia speciosa) on antioxidant activity. The preparation and preparation of the extract were carried out by the maceration method, which uses 3 different solvent variations, including methanol, ethanol, and ethyl acetate solvents. Using the colorimetric method, a phytochemical screening test was carried out to determine flavonoid levels and total phenolic levels. In contrast, the determination of antioxidant activity levels was carried out using the DPPH test. The qualitative phytochemical screening test results of methanol extract, 70% ethanol, and ethyl acetate all three have the same secondary metabolite content. Still, the highest flavonoid and total phenolic content are found in the methanol extract of Bungur leave by 9.06% and 16.42%, respectively. 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 levels of total phenols and flavonoids correlated with antioxidant activity.

**Key words:** Antioxidant; Flavonoid; Bungur Leaf Extract; IC50.

**Analisis Kadar Flavonoid dan Total Fenolik dari Ekstrak Daun Bungur (*Lagerstroemia speciosa*) Terhadap Aktivitas Antioksidan**

**Abstrak**

Penelitian ini bertujuan untuk mengetahui kadar flavonoid dan total fenolik yang terkandung dalam ekstrak daun bungur (Lagerstroemia speciosa) terhadap aktivitas antioksidan. Penyiapan dan pembuatan ekstrak dilakukan dengan metode maserasi, yang menggunakan 3 variasi pelarut yang berbeda, antara lain pelarut metanol, etanol, dan etil asetat. Uji skrining fitokimia dilakukan sebagai penentu kadar flavonoid dan kadar fenolik total dengan metode kolorimetri. Sedangkan penentuan kadar aktivitas antioksidan dilakukan dengan menggunakan uji DPPH. Hasil uji skrining fitokimia kualitatif ekstrak metanol, etanol 70%, dan etil asetat ketiganya memiliki kandungan metabolit sekunder yang sama. Namun, kandungan flavonoid dan fenolik total tertinggi terdapat pada ekstrak metanol daun bungur sebesar 9,06% dan 16,42%. Uji aktivitas antioksidan ekstrak metanol daun Bungur juga menunjukkan hasil penghambatan yang paling baik, yang ditunjukkan dengan nilai IC50 sebesar 5,76 ppm. Hal ini dapat memperkuat bahwa kadar total fenol dan flavonoid berkorelasi dengan aktivitas antioksidan.

**Kata kunci:** Antioksidan, Flavonoid, Ekstrak daun bungur, IC50

**Introduction**

Degenerative diseases are diseases that arise due to a decline in the function of body cells from their normal functions (1). Cancer is one of the degenerative diseases that cause many deaths. Cancer is a disease characterized by abnormal cell growth and can attack any part of the body or organ. Based on data from the World Health Organization (WHO), Cancer is one of the leading causes of death worldwide, with nearly 10 million deaths in 2020 (2). Projected increases in cancer indicate a 50% increase in cases over the next 2 decades (3). Prostate, breast, lung, stomach, colorectal, and non-melanoma skin malignancies are the cancers that spread the most rapidly worldwide, however, there are one hundred other forms of cancer that impact people (4). Endogenous factors and exogenous factors are the cause of cancer. Endogenous factors include biological aging, genes, and certain gene products, hormones, and enzymes. At the same time, exogenous factors can be in the form of bad lifestyle, radiation, chemical carcinogens, viruses, and free radicals (Wu et al., 2018). Physiological processes need free radicals to help the process using electron transfer. An excess number of free radicals can cause oxidative stress, causing an imbalance in the number of free radicals and intracellular antioxidants. The human body produces enzymatic (endogenous) antioxidants. Still, suppose the number of free radicals in the body is more. In that case, the endogenous antioxidants cannot control the number of free radicals, resulting in oxidative stress (6,7).

Oxidative stress is essential in the pathogenesis of chronic diseases such as cardiovascular diseases, diabetes, neurodegenerative diseases, and cancer(8). The process of treating cancer has proven to be quite challenging. Traditional treatment techniques include radiotherapy, chemotherapy, and surgery (9). Treatment efforts with this method still have disadvantages, including related side effects. Therefore, people are still looking for solutions related to cancer treatment with high effectiveness and small side effects (10). One of the efforts that can be made is to develop herbal medicines to treat cancer. Natural or herbal medicine therapy is widely used to use active compounds derived from nature. *Lagerstroemia speciosa* (L.) Pers. is a plant of the Lythraceae tribe that is widely found in Indonesia (10). This plant is known as bungur. *L. speciosa* has secondary metabolites such as triterpenes, tannins, ellagic acid, glycosides, and flavonoids. The flavonoids in the bungur are thought to have antioxidant activity properties due to the presence of phenolic hydroxy groups that can ward off free radicals. The presence of this antioxidant activity in the bungur plant can inhibit the initiation process of carcinogenesis by inhibiting the activity of carcinogens (11).

Based on previous research conducted by Sandhiutami et al. (2018), leaf decoction of *L. speciosa* has antioxidant activity with an IC50 of 57.60 mg/mL and can reduce sugar levels in mice (12). Research conducted by Roni et al. (2019) has successfully isolated 5,3',4' trihydroxy flavonol compounds of the flavonoid group from *L. speciosa* leaves and ethanol extract of bungur fruit (13). Anticancer screening has been carried out 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) (14). However, until now, there has been no research on differences in extraction solvents on flavonoid content and total phenol and

**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).

Method

* 1. *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, namely ethanol, methanol, and ethyl acetate. To achieve a thick extract, the maceration results were concentrated using a rotary evaporator and evaporated in a water bath (15).

* 1. *Qualitative Identification of Secondary Metabolites*

A qualitative examination of the extract was carried out 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 (16).

To identify tannins, a 2 mL extract solution was reacted with a 10% iron (III) chloride solution; if the solution turned dark blue, black, blue, or greenish-black, tannin components were present (16).

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 production of foam indicated the presence of saponins with a minimum height of 1 cm that lasted for at least 10 minutes after the addition of 1 drop of diluted hydrochloric acid (16).

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 (16).

For the detection of flavonoids, three 1 mL test tubes containing the extract of the bungur leaves were supplied. After adding ten drops of H2SO4-N to each tube, violent shaking ensued. The first tube received Dragendorff reagent, whereas the second tube received Wagner reagent. 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 (17).

* 1. Quantitative Identification of Secondary Metabolites

A total of 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% Na2CO3 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 (Syafitri et al., 2014).

* 1. 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**

A close up of a plant

Description automatically generated with low confidence

**Figure 1.** Bungur Leaf Simplicia



**Figure 2.** Extraction Process by Maceration

**Tabel I.** Sample Quality Check

|  |  |  |  |
| --- | --- | --- | --- |
| Sample Name | Test Type | Test result | Testing Method |
| Bungur Leaf Simplicia | Water content | 6.41 | Gravimetry |
| Ash Level | 4.97 | Gravimetry |

**Tabel II.** 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 |

**Tabel III.** Qualitative Identification of Secondary Methabolites

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

Chart, bar chart

Description automatically generated

**Figure 3.** Bungur Leaf Extract Flavonoid Content

Chart, bar chart

Description automatically generated

**Figure 4.** Total Phenol Content of Bungur Leaf Extract

**Tabel IV.** Antioxidant Activity Test Result

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

**Discussion**

1. Simplisia Collection

Fresh Bungur leaf Simplicia (*L. speciosa*) was obtained from Balittro (Figure. 1). Then the simplicia is 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 I) 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% (18).

The determination of 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 be useful for extending the durabilitySimpliciaicia 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%.(19). The need for information related to the estimated internal and external mineral content from the initial process to the formation. Simpliciaicia resulted in the determination of the total ash content. Total ash content is related to organic and inorganic minerals obtained internally and externally (20).

1. 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. After maceration, the liquid extract was concentrated using a rotary evaporator until a thick extract was obtained and the % yield was calculated (Table II) (Figure. 2).

Based on the Pharmacopoeia Herbal regiment extract obtained not less than 9.2% (21). This is different 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 solvents can be divided into polar and non-polar, based on their dielectric constant (22). The high yield of Bungur leaf extract using 70% ethanol as a solvent indicates that 70% ethanothe l solvent used in Bungur leaves is balanced compounds better than 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 (23).

1. 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 III).

1. Quantitative Identification of Secondary Metabolites
   1. Total Flavonoid Content (TFC)

Flavonoids are plant-derived compounds with antioxidant, anticancer, anti-inflammatory, and antibacterial properties, among others. The antioxidant effect of flavonoids is one of their most important bioactive properties (24). Quantitative analysis of total flavonoid compounds using UV-Vis spectrophotometry was carried out to determine the total flavonoid levels contained in Bungur leaves (Lagerstroemia speciosa (L.) Pers). The goal of total flavonoid content determination is to determine the number of flavonoids present in extract samples, which is 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 (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 greatest 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).

* 1. *Total Phenol Content (TPC)*

Total phenol content analysis is used to determine 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 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. The results of determining the total phenol content can be seen in Figure 4.

Based on the results, total phenolic content (TPC) using methanol solvent was 16.42%, ethanol 70% solvent was 10.28%, and ethyl acetate was 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 (31). But the highest phenolic compound solubility is not always found in polar extracts but depends on the structure of the phenolic compounds (32).

The increase in the polarity of the solvent affects the test results of total phenolic and total flavonoid content, where the increase 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.

1. *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) (33). The DPPH technique was selected because to its simplicity, ease of use, speed, and sensitivity, as well as its 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(34); the lower the IC50 value, the higher the antioxidant activity (35).

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 extracts that have the best ability to fight DPPH radicals is to have the smallest IC50 value. The methanol extract is the most potent when compared to the other two extracts since it has the lowest IC50 value (Table IV), which indicates that it has 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 greater amount of hydroxyl substituents (36). 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 was able to affect the 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 (2016) it was found that 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 (37).

Flavonoids offer a variety of medical effects, such as anticancer, antioxidant, anti-inflammatory, and antiviral activities (38). Previous studies have shown antioxidant activity and total phenolic content in the extract of *Lagerstroemia speciosa* L. parsley seeds. It has been reported that the antioxidant activity produced is closely related to the total 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 (39). 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**

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