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Original Article

Potential Antioxidant Activity of Kedondong Leaves (*Spondias dulcis* Forst.) Using DPPH Method (1,1-Diphenyl-2- Pikril Hydrazil)

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Abstract

Background: Antioxidants are substances that can reduce free radicals to protect the body's biological systems from adverse effects arising from processes or reactions that cause excess oxidants. Kedondong leaves (Spondias dulcis Forst.) contain flavonoids, tannins, and alkaloids, which have the potential to act as antioxidants. **Objective:** To determine the antioxidant activity of ethyl acetate and 95% ethanol extracts from kedondong leaves. **Methods:** The antioxidant activity was tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, a free radical stable in an aqueous solution. Each extract was tested for its antioxidant activity with a comparison compound, vitamin C, using a UV-Vis spectrophotometer. The results of the antioxidant activity test revealed the IC50 (inhibitory concentration) value, namely the concentration of antioxidant compounds capable of inhibiting DPPH free radical activity by 50%. **Result:** The ethyl acetate extract has weak antioxidant activity with an IC50 value of 194.123 ppm, the 95% ethanol extract has very weak antioxidant activity with an IC50 value of 553.3694 ppm, and vitamin C, as a comparison, has very strong antioxidant activity with an IC50 value of 4.7805 ppm. **Conclusion:** Kedondong leaves have potential antioxidant activity but are very small.

Keywords:- *Antioxidant, IC50, DPPH, Kedondong Leaves (Spondias dulcis* Forst*),*

Introduction

Free radicals are molecules that have unstable and reactive properties, because they have one or more unpaired electrons. Free radicals can attack vulnerable compounds such as, lipids and proteins, which will eventually cause harmful diseases (Pratama & Busman, 2020). Free radicals can be generated by the body's metabolism, which is an internal factor. Besides that, it is also produced by external factors such as cigarette smoke, ultraviolet irradiation results, radical trigger substances in food, and other pollutants (Fakriah *et al.*, 2019). Several degenerative diseases are closely related to free radicals, including cancer, heart and blood vessel disease, senile dementia, cataracts, and decreased cognitive function (Fransiscus *et al.,* 2016). *Antioxidants* are compounds that are useful in helping to overcome oxidative damage caused by free radicals or reactive oxygen compounds (Saefudin *et al.,* 2013). *Antioxidants* can provide endogenous protection and exogenous oxidative stress by capturing free radicals (Lai-Cheong & McGrath, 2017). The effects of free radicals in the body will be neutralized by antioxidants formed by the body itself and supplements from outside through food, drink, or drugs, such as carotenoids, vitamins C, E, and others (Anggraini *et al.,* 2012). One source of antioxidants on earth is derived from plants. Research related to antioxidants is continuously being developed to obtain potential antioxidants, even at the formulation stage, to obtain a pharmaceutical product (Haryono *et al.,* 2021)

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One of the plants that has the potential to be used as a source of antioxidants is the kedondong plant. The community often uses the kedondong plant as an alternative medicine to treat various diseases (Suparman *et a.l,* 2013). These plants include fruit or garden plants found in almost all tropical areas (Delina & Arina, 2022). According to Islam *et al.,* (2013), on the results of secondary metabolite content tests that have been carried out on kedondong leaves, there are chemical compounds, including alkaloids, saponins, flavonoids, terpenoids, steroids, and tannins. Plants contain secondary metabolites that have the potential to be antioxidants, including alkaloids, flavonoids, phenolic compounds, steroids, and terpenoids. Antioxidant compounds from plants can be obtained by extraction using solvents. Differences in the polarity of the solvents result in differences in the amount and type of secondary metabolites obtained (Huliselan *et al.,* 2015). Based on Islamic research (2013), it is known that kedondong leaf extract has the highest antioxidant activity: dichloromethane with an IC50 5.00 µg/ml, followed by methanol with an IC50 5.37 µg/ml and chloroform with an IC50 8.96 µg/ml. However, no research has been conducted on the variation of solvents in kedondong leaf extract on antioxidant activity using 95% ethanol and ethyl acetate solvents. The DPPH method has been used globally in antioxidant activity testing of natural compounds because it only needs a few samples, a simple instrument, and fast processing. Antioxidant compounds from this sample were given to the hydroxyl group through the DPPH radical, so the DPPH radical that was unstable before because of the unpaired electron became stable (Purwaningsih, 2012). Based on this, a study was conducted to determine the antioxidant activity of ethyl acetate and 95% ethanol extracts from kedondong leaves.

Material and Method

Tools

The tools used include an analytical balance (Ohaus), glassware, a blender, a glass jar, a porcelain cup, mesh sieve number 60, a dropper pipette, a micropipette, a blue tip, a water bath, a spatula, a volumetric flask, a cuvette, and a UV spectrophotometer UV-Vis Shimadzu UV-1800.

Materials

The materials used include kedondong leaves, ethyl acetate (technical), 95% ethanol, DPPH solution, vitamin C, and distilled water.

Research design

Testing the antioxidant activity of kedondong leaves was carried out through several research stages, including preparing materials, making extracts using ethyl acetate and 95% ethanol using the maceration method, and testing the antioxidant activity of kedondong leaves using the DPPH method.

Plant Determination

The determination of other plants was carried out at the Laboratory of Plant Anatomy and Systematics, Faculty of Mathematics and Natural Sciences, University of Mulawarman Samarinda.

Sample collection

Samples in the form of kedondong leaves were taken in the Tenggarong area of Kutai Kartanegara. The samples were kedondong leaves that were not too young or too old between rows 3–8 from the top of the leaf.

Making Simplicia

The collected kedondong leaves are washed thoroughly with running water, drained, and spread on paper until the water is absorbed. The leaves are dried by aerating in the open air and protected from direct sunlight until dry. The dried leaf of Simplicia was then powdered using a blender and sifted using mesh number 60. Then the powder was stored in a glass container.

Extraction (Maceration)

Kedondong leaf powder weighed as much as 100 g, was soaked with 500 ml of ethyl acetate, stirred, and then left for five days. The macerate obtained is filtered using filter paper and then put into a bottle. Then the dregs left behind were macerated with 500 ml of ethyl acetate, stirred again, allowed

to stand for two days, and filtered. Remaceration is needed to replace a saturated solution with a new solvent so that all plant chemical compounds can be extracted optimally. The liquid extract obtained was evaporated over a water bath until a thick extract was obtained. The same treatment was carried out for maceration with a 95% ethanol solvent.

Antioxidant Activity Test with DPPH Method

Each ethyl acetate extract of kedondong leaves with a concentration of 50, 75, 100, 125, and 150 ppm was pipetted as much as 1 ml, then added 2 ml of 40 ppm DPPH solution, incubated for 30 minutes, and then measured the absorbance of each solution at a wavelength obtained using 1 ml of 95% ethanol blank and 2 ml of DPPH solution. Each of the ethanol extracts of kedondong leaves with concentrations of 100, 200, 300, 400, and 500 ppm was pipetted into as much as 1 ml, then added to 2 ml of a 40 ppm DPPH solution and incubated for 30 minutes. The absorbance of each solution was measured at different wavelengths and obtained using 1 ml of a 95% ethanol blank and 2 ml of a DPPH solution.

Data analysis

All data from the results of determining the absorption curve from the blank solution, the 40 ppm DPPH solution curve plus the vitamin C series solution and the 40 ppm DPPH absorption curve plus the ethyl acetate and ethanol extract series solutions of kedondong leaves were collected. A calculation graph was made to display the results that have been obtained. The data obtained is quantitative data, which is analyzed descriptively, and presented in the form of narration, tables and graphs.

Results and Discussion

The results of plant determination carried out in Faculty of Mathematics and Natural Sciences, University of Mulawarman Samarinda is kedondong plant with the name Spondias dulcis Forst, synonymous with Spondias Cytherea Sonn, family Anacardiaceae. Plant determination is the process of knowing for sure and convincing researchers that the plants used are correct so there are no errors when using samples. The technique used is to match the morphological characteristics with the database (Rezaldi *et al.*, 2022).

Table 1. Classification of *Durio kutejensis*

Classification	***Durio kutejensis***
Kingdom	Plantae
Subkingdom	Tracheobinota
Super Division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Ordo	Sapindales
Family	Anacardiaceae
Genus	Spondias
Spesies	*Spondias Dulcis Forst*

Kedondong leaves were collected from as much as 3 kg of fresh leaves. The collected leaves were sorted by wet sorting, namely separating the leaves from the soil, gravel-damaged plant parts, and other plant parts not used in the study. After wet sorting, the leaves are washed thoroughly with running water and then spread on paper until the water is absorbed. The leaves are dried by aerating in the open air and protected from direct sunlight until dry. The drying process is carried out for eight days. The dried Simplicia was carried out by dry sorting, separating the remaining impurities, then pulverising with a blender and then sifting through a 60 mesh sieve, and 230.32 g of fine powder was obtained. Sieving with mesh number 60 aims to obtain a finer and more homogeneous powder. The smaller the pollination size of the simplicia, increases the surface area of the simplicia and homogenizes the size of the powder particles, so that the extraction process is more effective and efficient (Diniatik, 2015). The refined simplicia powder was then weighed as much as 1 g each in two dishes to determine the moisture content of the simplicia powder. The results of determining the water

content of the kedondong leaf simplicia powder were 10% and 8%. According to Malangngi *et al.*, (2012), determining the water content helps determine the resistance of a material in storage. It is the best way to handle material to avoid microbial activity's influence. According to Pasaribu *et al*, (2012), the moisture content of a simplicia powder should not exceed 10%, and if it exceeds 10%, it will be a good medium for fungal growth.

Table 2. Data on the yield of *Spondias dulcis* Forst leaf extract.

Extract Name	Extract Weight (gram)	Yield (%)
Ethyl acetate extract	9.29	9.29
95% ethanol extract	4.91	4.91

Based on table 2, it is known that the ethyl acetate extract of kedondong leaves has a higher extract weight and yield. This shows that the chemical components contained in kedondong leaves are more soluble in semi-polar ethyl acetate solvent than the solubility of chemical components from kedondong leaves in polar 95% ethanol solvent. A quantitative antioxidant activity test was carried out using a UV-Vis spectrophotometer. This quantitative test was carried out to determine the absorbance of DPPH after adding the extract. The mechanism for capturing DPPH radicals by antioxidant compounds is through the donation of hydrogen atoms, causing a change in the colour of DPPH from purple to yellow (Nastiti *et al.*, 2021). In this study, DPPH was used at a concentration of 40 ppm. 40 ppm DPPH can achieve maximum absorption at the DPPH wavelength of 517 nm. This maximum wavelength provides the maximum absorption of the test solution and gives the most significant sensitivity (Tulandi, 2015). The results of determining the maximum wavelength (λmax) using a UV-Vis spectrophotometer show that the maximum absorption of DPPH is at a wavelength of 521.4 nm. If a compound has activity as an antioxidant, there will be a decrease in the absorbance value of DPPH at a wavelength of 521.4 nm. The decrease in DPPH absorbance was measured against the absorbance of the control, namely the absorbance of DPPH in 95% ethanol without adding test material (extract). The decrease in DPPH absorbance was indicated by the color degradation of DPPH, which was directly proportional to the concentration of the added extract. The higher the concentration added, the color degradation from a deep purple will become lighter (faded) to yellowish. From the DPPH absorbance value obtained, the percentage value of DPPH radical inhibition (% inhibition) can be determined. From the value of % inhibition, the value of IC50 (inhibitory concentration) can be determined. The IC50 value is a number that indicates the concentration of the extract (ppm), which can inhibit the oxidation process by 50%. The smaller the IC50 value means, the higher the antioxidant activity (Fauziah *et al.*, 2017).

Table 3. IC_{50} (inhibitory concentration) calculation results

Component	IC_{50} (ppm)
Vitamin C	4.7805
Ethyl acetate extract	194.123
95% ethanol extract	553.3694

Based on the calculation results in table 3, it was found that the semi-polar ethyl acetate extract had a higher antioxidant activity with an IC_{50} value of 194.123 ppm compared to the polar 95% ethanol extract with an IC50 value of 553.3694 ppm but had higher antioxidant activity. Lower than vitamin C, which has an IC50 of 4.7805 ppm. The IC50 value indicates the value of the antioxidant activity (Syamsul *et al.*, 2022). Factors that might influence the low antioxidant activity found in kedondong leaves is the amount of active substance contained in kedondong leaves with a very small concentration (Tenda *et al.*, 2019). The 95% ethanol extract has lower antioxidant activity than the ethyl acetate extract. This is because the 95% ethanol solvent has more polar properties than ethyl acetate, so it can extract polar compounds. The compounds contained in the extract are a combination of polar, semi-polar, and non-polar compounds. When the extract was macerated with 95% ethanol, the extracted compounds were polar, whereas when the extract was macerated with ethyl acetate solvent, the extracted compounds were semi-polar. This shows that extraction using

solvents with different polarities will produce different phenolic components, so the antioxidant properties of each compound obtained from the extraction are also different. The IC_{50} value in semi-polar solvents is smaller than the IC_{50} value in polar solvents (Tensiska *et al.*, 2020). This is in line with Rumayati *et al.*, (2014) study where antioxidant activity is directly proportional to total phenolics, so the higher the phenolic content in a material, the higher its activity as an antioxidant. It can be analyzed that the semi-polar compounds in kedondong leaves are more concentrated in ethyl acetate solvent because they have the same polarity. Flavonoid compounds can donate hydrogen atoms so that the DPPH radical can be reduced to a more stable form. Glycoside compounds have an antioxidant effect by forming hydroperoxides as secondary antioxidants, thereby inhibiting the formation of lipid peroxides (Syarif *et al.*, 2015). Tannin compounds can stabilize free radicals by completing the lack of electrons possessed by free radicals. Alkaloid compounds act as antioxidants by donating H atoms to free radicals. The concentration level in the extract also determines the inhibition of free radicals (Nurviana, 2020). The greater the concentration of the extract, the greater the inhibition indicated by the formation of yellow colour and the greater the antioxidant activity. The concentrations used for the ethyl acetate extract were 50, 75, 100, 125 and 150 ppm. In this concentration range, colour degradation from purple to yellow indicates antioxidant activity. In the ethanol extract, new colour degradation occurred at 100, 200, 300, 400 and 500 ppm concentrations. This indicates that the 95% ethanol extract has lower antioxidant activity than the ethyl acetate extract. The ethyl acetate extract has a slightly higher effectiveness effect than the 95% ethanol extract as an antioxidant against DPPH radicals. The effectiveness of antioxidants in ethyl acetate extract in neutralizing free radicals is related to the semi-polar nature of ethyl acetate so that many chemical components with the same polarity dissolve. Although the antioxidant activity of the ethyl acetate extract is weak and the ethanol extract of kedondong leaves is very weak, they can still have potential as antioxidants.

Conclusion

Based on the results of the research above, it can be concluded that Kedondong leaves have potential antioxidant activity but are very small.

Conflict of Interest

The authors have no conflicts of interest to declare with regard to the content of this article.

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