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Antioxidant Activity Test of *Kersen* Leaf Extract (*Muntingia calabura* L.) as A Source of Natural Compounds

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Abstract. *Antioxidants play an important role in warding off free radicals that can cause various degenerative diseases. Kersen leaves (Muntingia calabura L.) are known to contain bioactive compounds that have the potential to be natural antioxidants. This study aims to evaluate the antioxidant activity of kersen leaf extract with the DPPH free radical reduction method using UV-Vis spectrophotometry. The results showed that kersen leaf extract had very strong antioxidant activity with an IC_{50} value of 7.2 µg/mL, although it was still lower than vitamin C as a comparison standard which had an IC_{50} of 2.8 µg/mL. This antioxidant activity is thought to come from the content of flavonoids, phenolics, saponins, and tannins that play a role in the free radical scavenging mechanism. These results suggest that kersen leaf extract has the potential to be developed as a natural source of antioxidants in various fields, including the pharmaceutical, cosmetic, and food industries. However, further research is needed to optimize extraction methods, formulations, and bioavailability of extracts so that they can be used more widely and effectively.*

Keywords: *antioxidants, DPPH, kersen leaf, IC_{50}, free radicals*

1. Introduction

Free radicals are molecules or atoms that have unpaired electrons, so they are highly reactive and can cause oxidative damage to cells and tissues. This damage contributes to the development of various degenerative diseases, such as cancer, cardiovascular disease, and premature aging. Antioxidants are compounds that are able to neutralize free radicals by donating hydrogen electrons or atoms, thereby preventing or reducing damage caused by free radicals.

One of the commonly used methods to measure antioxidant activity is the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The method is based on the ability of antioxidant compounds to dampen free radicals of DPPH, which is characterized by a color change from purple to pale yellow, which can be measured spectrophotometrically at a wavelength of 517 nm. The IC_{50} (Inhibitory Concentration 50%) value is used to express the effectiveness of antioxidants, which is the concentration needed to inhibit 50% of free radical activity. The smaller the IC_{50} value, the higher the antioxidant activity of a compound.

Antioxidants are compounds that can neutralize free radical problems that are able to break oxidative chain reactions caused by free radicals by giving electrons to free radical molecules (Mutammimah et al., 2022). Basically, the human body already contains natural antioxidants in the form of enzymes such as superoxidase dismutase, catalase, and glutathione peroxidase (Maharani et al., 2021). Flavonoids are the main compounds that act as antioxidants that have benefits as an antidote to free radicals.

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Flavonoids have high antioxidant activity because they have hydroxyl groups that are substituted by the ortho and para against the -OH and -OR groups. Antioxidant activity in flavonoids can prevent damage from chemical reactions involving free radicals (Husna et al., 2022).

As a result of excessive exposure to free radicals that enter the human body, the human body needs exogenous antioxidants such as vitamins A, C, E, flavonoids, isoflavones, anthocyanins, flavones, catechins, and isocatechins, which are antioxidants that can be obtained by the body through supplements and foods such as fruits and vegetables which are natural alternatives that can be used as a source of antioxidants that can help the body reduce damage oxidative due to free radicals (Rumyaan et al., 2022; Dewi et al., 2020). The kersen plant is one of the plants with natural antioxidant content that is easy to find in the surrounding environment. Empirically, people use the kersen plant as a traditional medicine as a medicine for cough, jaundice, and gout. Kersen leaves contain bioactive compounds such as flavonoids, triterpenes, saponins, tannins, and steroids.

The kersen plant (*Muntingia calabura L.*), which grows wildly in Aceh Province, particularly in the city of Banda Aceh (Andalia et al., 2023), kersen plant (*Muntingia calabura L.*) is a plant that is often found in tropical areas and has long been used in traditional medicine. Various parts of this plant, such as leaves, fruits, and stem bark, are known to contain bioactive compounds that have the potential to act as antioxidants. Previous research has shown that kersen leaf extract contains flavonoids, phenolics, and saponins that act as antioxidants. Phytochemical tests on kersen leaf ethanol extract showed positive results for phenolic compounds, flavonoids, and saponins (Sami, et al., 2017). The antioxidant activity of kersen leaf extract has been tested using the DPPH method. The results showed that the ethanol extract of kersen leaves had an IC_{50} value of 6.8249 µg/mL, which is included in the category of very strong antioxidants (Sami, et al., 2017). Another study reported that kersen leaf ethanol extract has an IC_{50} value of 9.01 µg/mL, also included in the very strong category of Rumyaan, et al., (2022).

Based on these findings, this study aims to test the antioxidant activity of kersen leaf extract as a source of natural compounds that have the potential to neutralize free radicals. Thus, it is hoped that kersen leaf extract can be further developed as a source of natural antioxidants that are beneficial to health.

2. Method

This research was conducted at the Research Laboratory of FMIPA Chemistry, Universitas Syiah Kuala. This research was carried out for ± 5 days. The tools used in this study are a set of extraction process tools, namely 1 set of *rotary evaporator* units (Hidolph Laborota 4003 Control), several glassware commonly used in organic chemistry laboratories. Columns, pencils, rulers, *chumbers*, statics, clamps and sprayers. The ingredients are DPPH, ascorbic acid, n-hexane, ethyl acetate, methanol, chloroform, ammonia, hydrochloric acid, NaOH, gelatin, reagents for antioxidant tests and material preparation including material collection, plant determination, and material processing. The characterization of simplicia includes the determination of total ash content, the determination of acid insoluble ash content, the determination of water insoluble ash content, and the determination of ethanolcc soluble juice content. The extract is then concentrated using a rotary vaporator so that a thick extract is obtained that can still be poured.

Samples of kersen plant leaves are cleaned and dried. The sample was smoothed,

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extracted in stages by maceration method using a solvent (methanol) of 2.5 L for 3 × 24 hours. Then the maceration results are filtered and filtrates and residues are produced. The filtrate obtained is concentrated with *a rotary evaporator* so that a concentrated extract is produced. The concentrated extract (methanol) was tested for phytochemical tests and tested for antioxidant activity and FITR. The concentrated extract is isolated and purified by chromatography method. The concentrated extract was separated from the compound components by using column chromatography using the silent phase of G-60 gel silica by emulsion gradient with solvent as the motion phase.

The phytochemical procedures carried out are as follows (Harborne, 1987): Qualitative testing of antioxidant activity was carried out by coagulation on KLT where the comparator used was vitamin C, then DPPH 0.2% in methanol was used as a spot finder. The presence of antioxidant activity is visually indicated by yellow spots or against a purple background on the plates, which stabilize for 30 minutes. Quantitative testing of antioxidant activity with DPPH attenuation method. using UV-ray visible light spectrophotometry through mixing DPPH solution with test solution in a ratio of 1:1, the volume was then measured at a wavelength of 517 nm after incubation for 30 minutes at room temperature. The total phenol content of the extract was determined using the Folin-Ciocalteu method, galic acid was used as the standard. The result is expressed as milligrams of GAE (gallic acid equivalent) in 100 grams of simplisia. The determination of total Flavonoid levels is carried out by the method Chang, quercetin used as a standard result is expressed as g QE/100 g. Determination of total carotenoid levels by dissolving the sample with nheksana, beta carotene is used as the standard. Total carotenoid levels are presented as a percentage of total beta-carotene equivalents per 100 g of extract (g BE/100g).

Sample extracts are prepared at different concentrations in methanol solvents in small vials. DPPH with a certain concentration is added to each concentration of test extract as much as 1 mL. The same DPPH solution in methanol is used for the positive control of ascorbic acid at different concentrations. After incubation for 30 min in a dark room at room temperature, the absorbance of the solution was read with a spectrophotometer at a wavelength of 517 nm. The percent inhibition is calculated using the following formula:

$$\text{DPPH free radical inhibition capacity \%} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

The IC50 value is defined as the sample concentration in mg of dry matter sample per Liter required to inhibit 50% of DPPH free radicals.

3. Results and Discussions

Test of Methanol Antioxidant Activity of Kersen Leaves (Muntingia calabura L)

This qualitative preliminary test of antioxidant activity aims to determine whether there are active compounds in the extract that have antioxidant activity in reducing *free radicals 1,1-diphenyl-2picrylhydrazyl* (DPPH). The following is a graph of the antioxidant test of kersen leaf extract (*Muntingia calabura. L*)

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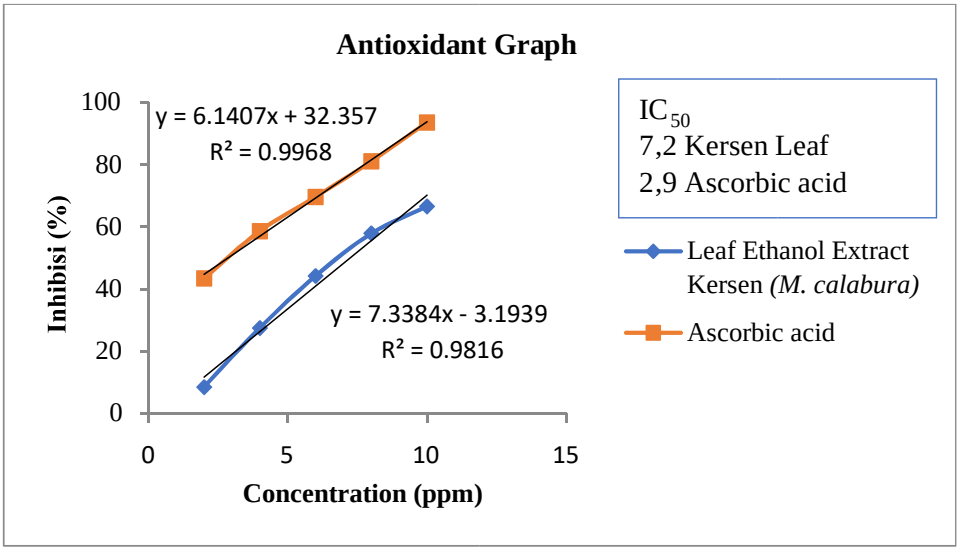

Figure 1. Graph of antioxidant test results of kersen leaf extract

The Figure 1 shown shows a graph of the results of the antioxidant activity test of kersen leaf extract (*Muntingia calabura L.)* compared to vitamin C as a comparison standard. The horizontal axis (X) on the graph represents the concentration of the extract in µg/mL, while the vertical axis (Y) indicates the percentage of free radical attenuation of DPPH. Based on the curve shown, kersen leaf extract showed an increase in antioxidant activity as concentration increased. However, when compared to vitamin C, the slope of the kersen leaf extract curve is sloping, which indicates that vitamin C has a higher effectiveness in warding off free radicals at lower concentrations.

From the measurement results, the IC_{50} value of kersen leaf extract was 7.2 µg/mL, while vitamin C as a standard had an IC_{50} value of 2.8 µg/mL. A smaller IC_{50} value indicates that a compound has higher antioxidant activity. Based on the classification of antioxidant activity according to Blois (1958), kersen leaf extract is included in the very strong category because it has an IC_{50} value that is much smaller than 50 µg/mL. These results are in line with previous research, as reported by Widjaya et al. (2019), which found that kersen leaf ethanol extract has high antioxidant activity with a low IC_{50} value. Another study by Pusparida et al. (2023) showed that the extraction method had an effect on the IC_{50} value obtained. With the maceration method, the IC_{50} value of kersen leaf extract was recorded at 3.629 ppm, while the ultrasonic method produced a higher value, indicating that the extraction method played a role in determining the effectiveness of the active compound as an antioxidant.

The high antioxidant activity of kersen leaf extract is associated with the content of its bioactive compounds, such as flavonoids, phenolics, saponins, and tannins. Flavonoids have the ability to donate hydrogen electrons or atoms to neutralize free radicals, while phenolic compounds with active hydroxyl groups (-OH) are able to dampen cellular oxidation (Puspitasari & Wulandari, 2017). In addition, tannins and saponins also act as free radical scavengers that can increase the body's natural antioxidant capacity (Mutammimah et al., 2022). The content of this active compound makes kersen leaf extract potentially developed as a natural source of antioxidants.

Based on the results of this study, kersen leaf extract has great potential to be used in various industrial applications. In the pharmaceutical industry, this extract can be developed as an antioxidant supplement that plays a role in reducing the risk of

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degenerative diseases caused by oxidative stress. In the cosmetics industry, the antioxidant content in kersen leaf extract can be used to prevent premature aging due to exposure to free radicals. In addition, kersen leaf extract also has the potential to be used as a natural preservative in the food industry due to its ability to inhibit oxidation that causes product damage. Pambudi et al. (2021) stated that in order to be widely applied, further research is needed on the formulation and stability of kersen leaf extract so that its use is more effective and efficient in various industries.

Overall, this study shows that kersen leaf extract has very strong antioxidant activity with low IC_{50} values. Although its activity is still below vitamin C, this extract still has great potential as a natural source of antioxidants that can be used in various fields, such as health, cosmetics, and food. Further studies are needed to explore the best formulations and improve the bioavailability of active compounds in kersen leaf extract to optimize its benefits.

4. Conclusions

Based on the results of the study, kersen leaf extract (*Muntingia calabura* L.) is proven to have very strong antioxidant activity with an IC_{50} value of 7.2 µg/mL, although it is still lower than vitamin C which has an IC_{50} of 2.8 µg/mL. The antioxidant activity of kersen leaf extract is supported by the content of bioactive compounds such as flavonoids, phenolics, saponins, and tannins, which play a role in warding off free radicals through the DPPH damping mechanism. These results suggest that kersen leaf extract has the potential to be developed as a natural source of antioxidants that can be used in a variety of fields, including health, pharmaceuticals, cosmetics, and the food industry. Although the results of this study show the great potential of kersen leaf extract, further studies are still needed to optimize the extraction method that can improve the effectiveness of its active compounds. In addition, research on the stability, formulation, and bioavailability of kersen leaf extract in pharmaceutical and food applications also needs to be carried out so that its benefits can be utilized to the maximum. With further development, kersen leaf extract can be a natural alternative as a source of antioxidants that are useful and have high economic value.

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Proceeding of ICMR 7(2), 329-334 DOI: https://doi.org/10.32672/picmr.v7i2.3098

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