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Comparison of Antioxidant Activity Tests Using the DPPH, ABTS, and FRAP Methods on Ethanol Extracts of Cherry Leaves (Muntingia calabura L.) and Total Flavonoids

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Abstract

Pollution from sources such as vehicle exhaust and cigarette smoke can increase the production of free radicals in the body, leading to oxidative stress that contributes to the development of various degenerative diseases, including cardiovascular disorders, diabetes, and cancer. Antioxidants play a crucial role in neutralizing free radicals by donating electrons and thereby preventing cellular damage. Natural antioxidants from plants are considered safer alternatives to synthetic compounds. Kersen leaves (Muntingia calabura L.) have been traditionally used in herbal medicine and are reported to contain flavonoids, a class of bioactive compounds with strong antioxidant properties. This study aimed to determine the total flavonoid content and to compare the antioxidant activity of ethanol extract of kersen leaves using three different in vitro assays, namely DPPH, ABTS, and FRAP methods. The extraction was carried out by maceration using 96% ethanol, and the analysis was conducted with UV-Vis spectrophotometry, employing quercetin as the standard reference compound. The results revealed that the total flavonoid content of the ethanol extract was 47.205 mgQE/g. The IC_{50} values obtained were 31.825 µg/mL for DPPH, 56.901 µg/mL for ABTS, and 22.079 µg/mL for FRAP, indicating very strong antioxidant activity across all methods tested. Among the assays, FRAP demonstrated the highest sensitivity in detecting antioxidant capacity. In conclusion, the ethanol extract of kersen leaves exhibits potent antioxidant potential, and the choice of analytical method plays a significant role in evaluating antioxidant activity.

Keywords: *Kersen Leaves, Total Flavonoid, Antioxidant, DPPH, ABTS, FRAP*

INTRODUCTION

Pollution from sources like vehicle exhaust and cigarette smoke has become a significant concern in modern society. This environmental degradation, often exacerbated by technological advancements and human activities such as forest fires, leads to the increased formation of free radicals in the body (Harningsih & Wimpy, 2018; Chen et al., 2023). Free radicals are highly unstable molecules with unpaired electrons that can damage crucial biomolecules like lipids, proteins, and DNA by stealing electrons from them (Theafelicia & Narsito Wulan, 2023). This process, known as oxidative stress, is a key factor in the development of various degenerative diseases, including heart disease, diabetes, and neurodegenerative disorders, while also accelerating the aging process (Lobo et al., 2024). To counteract this damage, the body relies on antioxidants, compounds that can neutralize free radicals by donating an electron, thereby preventing cellular damage and maintaining overall health (Theafelicia & Narsito Wulan, 2023; Aryanti et al., 2021).

Fortunately, nature provides a rich source of these beneficial compounds. One such plant is the kersen tree (*Muntingia calabura L.*), which is remarkably adaptable and thrives in various environments, from roadsides to water channels (Puspitasari & Syam, 2017). The leaves of this plant are particularly noted for their high content of natural antioxidants, including flavonoids, saponins, polyphenols, and tannins (Puspitasari & Syam, 2017; Calarabura et al., 2024). These compounds give kersen leaves the potential to serve as an effective natural remedy for mitigating the effects of oxidative stress.

Despite the known antioxidant properties of kersen leaves, a significant challenge in antioxidant research is the lack of a single, standardized method for their evaluation. Different testing methods can yield varying results because they assess different mechanisms of antioxidant action (Aryanti et al., 2021).

For example, the DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assays measure the ability of a compound to scavenge free radicals, while the FRAP (Ferric Reducing Antioxidant Power) assay evaluates its capacity to reduce ferric ions (Fe3+) to ferrous ions (Fe2+) (Theafelicia & Narsito Wulan, 2023; Li et al., 2022). The varying principles of these methods mean that a substance's antioxidant capacity can be perceived differently depending on the assay used, which can complicate the comparison of results across studies. Previous research by Rumyaan et al. (2022) focused solely on the DPPH method and found that kersen leaves possess very strong antioxidant activity with an IC50 value of 9.01 ppm, but this does not provide a complete picture of the leaves' antioxidant potential across different mechanisms. Therefore, a comparative analysis is essential to understand the full scope of their activity.

This research aims to address these existing gaps by conducting a comprehensive analysis of the antioxidant activity of kersen leaf ethanol extract. The study will first quantify the total flavonoid content of the extract. Furthermore, it seeks to determine if there are significant differences in the results obtained from three widely-used methods: DPPH, ABTS, and FRAP, to identify which method is most sensitive for this particular extract. The findings will provide a more complete and nuanced understanding of the antioxidant efficacy of kersen leaves. This research is highly relevant as it contributes to the body of knowledge on natural antioxidants, potentially paving the way for the development of new health products. The novelty of this study lies in its direct comparison of multiple antioxidant assays on the same kersen leaf extract, which is crucial for determining the most reliable method for future research and practical applications.

RESEARCH METHODS

This study is a comparative research that aims to evaluate the effectiveness and sensitivity of three different antioxidant testing methods: DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and FRAP (Ferric Reducing Antioxidant Power) on the ethanol extract of kersen leaves (*Muntingia calabura L.*) (Sugiyono, 2019; Calarabura et al., 2024). The research was conducted at the Natural Products Laboratory of the Pharmacy Department, Duta Bangsa University, Surakarta, Indonesia, from March to June 2025. The study's independent variable is the antioxidant testing method, while the dependent variable is the antioxidant activity, measured by the ability of the extract to neutralize free radicals (Hikmah, 2020; Cresswell, 2014). The controlled variable is the kersen leaf ethanol extract itself, which serves as the object of the comparative analysis.

Population, Sample, and Research Instruments

The population for this study is the kersen plant (*Muntingia calabura*), and the sample consists of fresh kersen leaves collected from Jl. Mantung, Dusun II, Manang, Grogol District, Sukoharjo Regency, Central Java (Puspitasari & Syam, 2017). The leaves were carefully selected to ensure freshness, and all impurities were removed before processing. The instruments used in this research include analytical balances, a UV-Vis spectrophotometer, a rotary evaporator, and various laboratory glassware and equipment necessary for extraction, purification, and analysis. The main chemical reagents were 96% ethanol, DPPH, ABTS, FRAP reagents (including TPTZ, sodium acetate, and FeCl3), and quercetin as the standard reference compound (Theafelicia & Narsito Wulan, 2023; Amin et al., 2022). All chemicals used were of analytical grade to ensure the accuracy and reliability of the results.

Research Procedures

The research protocol began with plant determination at the Center for Research and Development of Medicinal and Traditional Plants in Tawangmangu to verify the plant species (*Muntingia calabura L.*). Following this, the collected fresh kersen leaves were cleaned, air-dried for 4-5 days, and then ground into a fine powder (Mukhriani, 2019). The dried powder was subjected to simplisia standardization tests, including organoleptic analysis, loss on drying, water content, and total ash content, to ensure the quality and purity of the raw material (Najib et al., 2017; Emzir, 2018).

Subsequently, the extraction of the leaves was performed using the maceration method with 96% ethanol. The powdered leaves were soaked in ethanol for 3x24 hours with occasional stirring to maximize the extraction of active compounds. The resulting liquid extract was then concentrated using a rotary evaporator to obtain a viscous extract (Sudaryono, 2022). This crude extract was further standardized by measuring its water content, total ash, and loss on drying to ensure consistency.

A phytochemical screening was then conducted to identify the presence of key compounds, including alkaloids, flavonoids, saponins, steroids, and tannins, using standard colorimetric tests (Musman, 2017; Vonna et al., 2021). The total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the standard (Purnamasari et al., 2022). Absorbance was measured at a maximum wavelength using a UV-Vis spectrophotometer, and the concentration was calculated from a quercetin standard curve.

Finally, the antioxidant activity of the kersen leaf extract was tested using three different methods: DPPH, ABTS, and FRAP. For each method, specific reagents were prepared, and the maximum wavelength and optimal reaction time were determined. A series of different concentrations of the kersen leaf extract were prepared, and their absorbance was measured and compared to that of a quercetin standard curve (Theafelicia & Narsito Wulan, 2023; Amin et al., 2022). The IC50 value for each method was calculated using linear regression analysis, where a lower IC50 indicates stronger antioxidant activity (Rumyaan et al., 2022; Chairina et al., 2023). The final data were analyzed descriptively by comparing the IC50 values obtained from the three methods to determine which assay was most effective in assessing the antioxidant potential of the kersen leaf extract.

RESULTS AND DISCUSSION

RESULT

Determination and Preparation of Kersen Leaf Samples

The sample used in this study is kersen leaves which have another name (Muntingia Calabura L). This study aims to determine the total flavonoid and antioxidant levels contained by different methods, namely DPPH, ABTS and also FRAP in kersen leaves (Muntingia Calabura L). The collection of kersen leaves was taken from Jl. Mantung, Dusun II, Manang, District. Grogol, Sukoharjo Regency, Central Java.

The determination of kersen leaves (Muntingia Calabura L) was carried out at the Technical Implementation Unit (UPT) of Traditional Health Services (Yankestrad) Sardjito Tawangmangu. The purpose of the determination is to find out the authenticity or truth of the sample. Based on the results of the determination, it is confirmed that the plant used in this study is indeed kersen leaves (Muntingia Calabura L).

A total of 3000 grams of kersen leaves (Muntingia Calabura L). that has been collected is then washed using running water to clean the dirt or residues that are still attached to the kersen leaves. The kersen leaves (Muntingia Calabura L) that have been cleaned are then sorted wet. After the wet sorting process, the separation of dirt or foreign objects attached to the kersen leaves is carried out. Next, kersen leaves are cut into pieces to facilitate the drying process of simplicia. The drying process is then carried out by drying using sunlight with a black cloth covered for 3 days. After drying, a weight of 1300 grams was obtained and the amount of randemen obtained was 43.33%.

Table 1. Weight Simplification

Bobot Basah (g)	Bobot Kering (g)	Randemen (%)
3000 gram	1300 gram	43,33 %

Kersen leaves (Muntingia Calabura L) that have undergone a drying process are then dried sorted with the aim of separating dirt or foreign objects that are still left from the previous wet sorting stage. Next, the kersen leaves are crushed using a blender until they form a powder. This pollination process aims to reduce the particle size so as to increase the surface area of contact between the simplicia and the solvent, which can ultimately speed up and maximize the extraction process. The resulting powder is then sifted using a 40-mesh sieve. From a total of 1300 grams of dried simplisia, 500 grams of powder was obtained with a yield of 44.23%.

Table 2. Weight Simplisia Powder

Bobot Basah (g)	Bobot Kering (g)	Randemen (%)

1300 gram	500 gram	44,23 %

Standardization of Kersen Leaf Simplicia (Muntingia Calabura L)

Standardization is a process of determining the characteristics of materials based on certain parameters in order to obtain a uniform level of quality. This process is carried out on the extract using two types of parameters, namely specific parameters and non-specific parameters. Specific parameters include identification, organoleptic characteristics, content of water-soluble chemical compounds and ethanol, and chemical composition. Meanwhile, non-specific parameters include drying shrinkage, moisture content, ash content, heavy metal content, and type weight (Najib *et al.*, 2017).

1. **Uji Organoleptik**

In this study, the organoleptic test of kersen leaf simplicia powder (Muntingia Calabura L) was carried out by visual observation related to color, aroma, shape and taste (*Dewicitra, 2020*, n.d.). The results of the characterization of kersen leaf simplicia powder (Muntingia Calabura L) can be seen in the following table 5

Table 3 Results of Organoleptic Test Determination of Kersen Leaf Simplicia (Muntingia Calabura L)

Parameter Pengamatan	Hasil
Warna	Hijau Tua
Bentuk	Serbuk Kasar
Aroma	Bau Khas Lemah
Rasa	Pahit

Based on the observations, the color of kersen leaf simplicia powder is dark green. This color shows that the drying and pollination process successfully maintains the original color characterization of kersen leaves, despite slight changes due to processing. M. calabura leaf simplicia powder has a coarse powder in the form of a coarse powder. The aroma of kersen leaves has a weak characteristic aroma and has a bitter taste according to the description of simplicia listed in the journal (Vonna *et al*., 2021).

2. **Drying Shrinkage Test**

Weighing 2 grams of simplicia and extracts from each kersen leaf test sample were placed in an empty cross that had previously been preheated for 30 minutes at 105°C in oven, then weighed the weight of the empty cross after cooling. Then put the cross containing the sample in an open state along with the lid in the oven, which is first weighed by the weight of the cross containing the sample. Heating is carried out at 105°C for 1 hour. Then the crux is cooled in a desiccant to room temperature and weighed after heating to find out the shrinkage weight of the dryer (Misgiati, 2022).

Table 4. Simplisia Drying Shrinkage

Berat Sampel	Berat Kurs Kosong	Berat Sampel Sebelum Pemanasan	Berat Sampel Setelah Pemanasan	Nilai %
2	43,78	45,78	45,63	7,5%
2	44,17	46,17	46,05	6%
2	45,56	47,56	47,39	8,5%

Hasil susut uji pengeringan metode kering oven yaitu 1,4 %, dari metode ini menghasilkan susut pengeringan yang baik sesuai yang ditetapkan BPOM, yaitu lebih kecil dari pada 10% (Sinaga *et al*., 2021).

3. Determination of Simplisia Water Content

Moisture content was determined on kersen leaf powder (Muntingia Calabura L) using the Moisture Balance tool. Based on the results of the study, the moisture content in leaf simplicia is still within the permissible limit. Referring to the literature, the moisture content of simplicia should be no more than 10%. The goal is to prevent fungal growth which can occur if the moisture content is too high if there is fungal growth that will cause a decrease in biological activity during the storage process of simplicia powder (Wandira *et al*., 2023). The results of determining the moisture content of kersen leaf simplicia can be seen in table 4.

Table 4. Water Content of Simplisia

Berat Sampel	Replikasi 1	Replikasi 2	Replikasi 3	Rata – Rata
2	6,24%	6,30%	6,44%	6,32%

Hasil yang diperoleh simplisia daun kersen (Muntingia Calabura L) pada tabel diatas didapat sesuai dan stabil dengan penetapan persyaratan kadar air yang ditetapkan untuk maintaining good simplicia quality, which is less than 10% (Wandira *et al.,* 2023).

4. Simplisia Powder Ash Powder Test

The determination of total ash content for kersen leaf simplicia was carried out with the aim of providing an overview of the internal and external mineral content contained in the extract (Vonna *et al*., 2021). The results of the simplicia powder ash content test with 3 replication obtained an average of 2.5 %

Table 5. Ash Content of Simplisia

Berat Sampel	Berat Krus kosong	Berat Krus + Abu	Nilai %
2	44,39	44,45	3%
2	44,45	44,50	2,5%
2	44,51	44,55	2%

The results of the simplicia powder ash content test resulting from the three replications produced a requirement value in accordance with the standard total ash content parameters with an average of 2.8 % of the three replications (Evifania *et al.*, 2020).

Extract Making Process

The manufacture of kersen leaf extract (Muntingia Calabura L) is carried out by maceration method with 96% ethanol solvent as much as 500 grams of kersen leaf powder (Muntingia Calabura L) extracted in a ratio of 1:10 with ethanol as much as 5000 ml. The maceration method was chosen because it has a simple procedure, does not use high temperatures that may damage chemical compounds that have antioxidant activity in kersen leaves (Muntingia Calabura L). The choice of 96% ethanol is due to its properties that are able to dissolve almost all substances, both polar, semipolar and nonpolar and can also optimally attract flavonoid and phenolic compounds (Ramadhani *et al*., 2020). Maceration is carried out for 3x24 hours by occasionally stirring to attract the active substances contained in kersen leaves (Muntingia Calabura L). After 3x24 hours of filtering, the maceration filtration residue is then remacerated using 96% ethanol as much as 1000 ml for 1x24 hours, the results of the remaceration are filtered and combined with the results of the initial maceration and concentrated with a rotary evaporator at a temperature of 50 °C and heated using a waterbath to obtain a thick extract. The result of the weight of simplicia powder, which was 500 grams, was obtained with a weight of 38.77 grams of thick extract and the randemen result obtained was 7.75%.

Table 6. Randemen Extract

Bobot Serbuk (g)	Bobot Ekstrak (g)	Randemen (%)
500 gram	38,77 gram	7,75 %

Then the standardization of the extracts was carried out which included a drying shrinkage test, an ash content test and a moisture content test.

1. **Extract Drying Shrinkage Test**

Weighing 2 grams of kersen leaf test sample extract was carried out in an empty cross that had previously been preheated for 30 minutes at a temperature of 105°C in the oven, then weighed the weight of the empty cross after cooling. Then put the cross containing the sample in an open state along with the lid in the oven, which is first weighed by the weight of the cross containing the sample. Heating is carried out at 105°C for 1 hour. Then the crux is cooled in a desiccant to room temperature and weighed after heating to find out the shrinkage weight of the dryer (Dewi *et al.*, 2017).

Table 7. Shrinkage Drying Extract

Berat Sampel	Berat Kurs Kosong	Berat Sampel Sebelum Pemanasan	Berat Sampel Setelah Pemanasan	Nilai %
2	45,65	47,65	47,52	6,5%
2	44,29	46,29	46,14	7,5%
2	43,86	45,86	45,74	6%

From the results of the drying shrinkage test, an average of 6.6% was obtained, which resulted in a good drying shrinkage as set by BPOM, which was smaller than 10% (Sinaga *et al*., 2021).

2. **Extract Ash Level Test**

The determination of ash content for kersen leaf extract is carried out with the aim of providing an overview of the internal and external mineral content contained in the extract. This determination is carried out by squeezing the extract at a temperature of 600°C where the organic compounds in the extract will be destroyed and evaporated, so that only mineral and inorganic elements are left in the extract (Ulfah *et al*., 2020).

Table 8. Extract Ash Level Test

Berat Sampel	Berat Krus kosong	Berat Krus + Abu	Nilai %
2	44,30	44,36	3%
2	45,52	45,60	4%
2	44,85	45,79	3%

From the results of the test of the ash content of the extract, an average of 6.32% was obtained, which resulted in a good requirement value of less than 10% (Sinaga *et al*., 2021).

3. **Extract Moisture Content Test**

The determination of the moisture content of simplicia and extracts is carried out with the aim of determining the range of water content contained in the extract. The moisture content of simplicia is the percentage of the amount of water contained in the simplicia, where the high water content allows for fungal growth. The growth of these fungi can reduce the quality of simplicia and can affect the content of compounds contained in simplicia. In

addition, the water content can also affect the quality of the extract. The higher the water content in an extract, it will facilitate the growth of fungi in the extract and can reduce the biological activity of the extract.

Table 9. Extract Moisture Content Test

Berat Sampel	Replikasi 1	Replikasi 2	Replikasi 3	Rata – Rata
2	5,64%	5,78%	6,07%	5,83%

From the results of the moisture content test, kersen leaf extract was obtained an average of 5.83%, which produced a good value and in accordance with the requirements, which was less than 10% (Sinaga *et al*., 2021).

Identification of the Chemical Compound Content of Kersen Leaf (Muntingia Calabura L)

The purpose of identifying chemical compounds in the sample is to find out the type of compounds found in fennel stem extract (Foeniculum vulgare Mill). The process of identifying or screening phytochemicals is carried out to detect the presence of secondary metabolites in the extract. Secondary metabolites analyzed qualitatively include flavonoids, alkaloids, saponins, terpenoids, and tannins. Phytochemical screening can be carried out by 2 methods, namely the test tube test method (color complex) and the KLT test (thin-layer chromatography).

1. **Test Test Tube**

Phytochemical screening is the first step in the study of natural chemical compounds from plants, which aims to find out the types of compounds contained in the plants being studied. This screening process is carried out by observing color changes as a result of the reaction between the compounds in the plant extract and certain reagents. A factor that strongly determines the success of phytochemical screening is the selection of appropriate solvents as well as the extraction methods used (Dewi *et al.*, 2017).

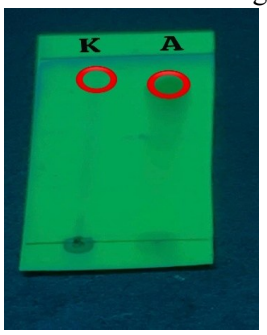
Table 10. Phytochemical Screening

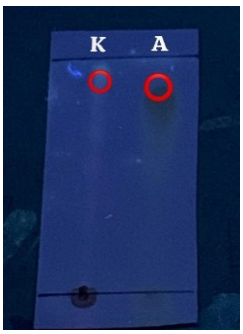
Senyawa	Preaksi	Hasil pengamatan	Kesimpulan
Flavonoid	Mg + HCI p	Terjadi perubahan warna kuning	**(+)**
Alkaloid	Mayer	Terdapat endapan putih	**(+)**
	Wagner	Terdapat endapan coklat	**(+)**
	Dragondraff	Terdapat endapan jingga	**(+)**
Saponin	Air Suling	Terbentuk busa	**(+)**
Steroid	Liberman Burchard	Terdapat warna hijau	**(+)**
Tanin	$FeCI_3$ 5%	Terdapat warna hijau kehitaman	**(+)**

The results of the tubular test on phytochemical analysis of kersen leaf extract (Muntingia Calabura L) showed that kersen leaves contained phytochemical compounds in the form of alkaloids, flavonoids, tannins, saponins and steroids.

2. KLT (Thin Layer Chromatography) Test

The next phytochemical screening test is using the thin layer chromatography (KLT) method. In the KLT test, the phase of motion used is n – hexane and ethyl acetate with a ratio of 5:1. The results can be seen in the image below

(a). 

(b). 

Information:
Ekstrak Ethanol Daun Kersen *(Muntingia Calabura L).*
: Kuersetin
: Observed under 254 nm light.
: Observed under 366 nm light.

The extract was dissolved with 96% p.a. ethanol, then pushed along the plate using a micro pipette and a comparator used in thin layer chromatography (KLT), namely quercetin. The results of the KLT were winded and examined under UV light at 254 nm and 366 nm light and at observation at 254 nm and 366 nm of quercetin light produced yellow *(Zebua et al.*, 2019). In the quercetin standard, the Rf value was 0.87. Based on research (Hasanah *et al*., 2024) which states that the comparative Rf value of the quercetin used as a comparison has an Rf value range of 0.69-0.81.

On observation of the extract under 254 nm UV light, dark stains appear on the KLT plate. This shows that the compounds contained in the sample have the ability to absorb UV rays, which is characteristic of compounds with a conjugation system such as aromatic compounds, including flavonoids. However, at this stage the existence of flavonoids cannot be confirmed because tests with specific reagents have not been carried out. and observed under 366 nm UV light, bright or fluorescent stains appear on the plate with a spot distance of 2.2 cm from the point of origin and a solvent distance of 4.0 cm. The calculation of the Rf (Retardation factor) value showed a result of 0.55, which indicates the compound has a moderate polarity. Bright stains visible on 366 nm rays indicate that the compound reacts positively with the reagent, which signals the presence of flavonoids in the sample. Therefore, these results indicate that the extracts tested contain flavonoid compounds (Pramiastuti *et al*., 2020).**Determination of Total Flavonoid Levels**

The determination of the total flavonoid level was carried out by measuring the absorbance of kersen leaf samples (Muntingia Calabura L) using UV-Vis spectrophotometry. Ethanol extract of kersen leaves (Muntingia Calabura L) is reacted with aluminum chloride (AlCl3) and sodium acetate, which produces a yellow-colored solution. In determining flavonoid levels, the standard compound used is quercetin, because it belongs to the flavonol group, which is a type of flavonoid (Yanti & Vera, 2019). The stage of determining flavonoid levels from determining wavelength, *operating time*, and making a quercetin standard curve can be done first before determining flavonoids in kersen leaf ethanol extract (Muntingia Calabura L). Determination of wavelengths in the range of 400 – 600 nm. From the determination of the wavelength, it was obtained in the range of 436 nm with an absorbance of 0.538. The wavelength result can be seen in figure 1.

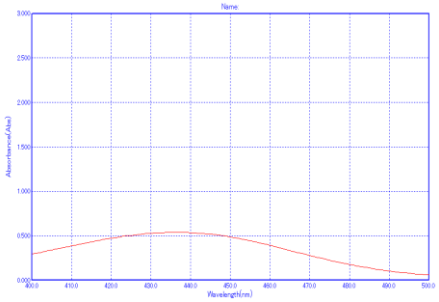


Figure 1. Flavonoid Wavelength

The maximum wavelength of the quercetin that has been obtained is then used to determine *the operating time*. The determination *of operating time* is carried out to determine the most stable time to take measurements of compounds based on the absorbance value obtained. The total query operating time data can be seen in table 11.

Tabel 11. Operating Time Kuersetin

No.	Sample Name	Wavelength(nm)	Abs
1	0	436,0	0,379
2	1	436,0	0,384
3	2	436,0	0,376
4	3	436,0	0,382
5	4	436,0	0,377
6	5	436,0	0,394
7	6	436,0	0,376
8	7	436,0	0,376
9	8	436,0	0,376
10	9	436,0	0,380
11	10	436,0	0,395
12	11	436,0	0,380
13	12	436,0	0,395
14	13	436,0	0,375
15	14	436,0	0,670
16	15	436,0	0,379
17	16	436,0	0,379
18	17	436,0	0,379

Based on the results of the determination of the operating time for quercetin, it was obtained that the absorbance value showed stability in the 15 to 17 minute time range. Absorbance was then measured at the 15th minute, with results showing a stable absorbance value of 0.379. The determination of the quercetin standard curve was obtained by a linear regression equation between the concentration of

quercetin (x-axis) and absorbance (axis) obtained the equation y = 0.0052x + 0.2732 with a value of correlation coefficient (r) = 0.9916.

Table 12. Quercetin Comparator Absorbance Measurement

Konsentrasi	Abs			Rata-Rata
	I	II	III	
10	0,335	0,347	0,339	0,340
20	0,365	0,379	0,355	0,366
30	0,451	0,429	0,434	0,438
40	0,475	0,489	0,483	0,482
50	0,538	0,528	0,531	0,532

The results of the Standard Curve can be seen in figure 2.

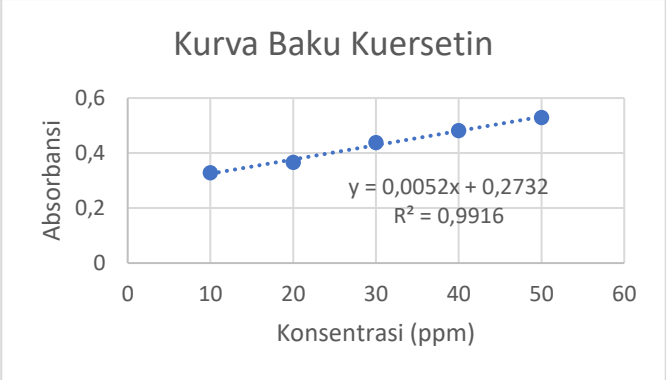


Figure 2. Quercetin Raw Curve

Table 13. Measurement of Absorbance of KFT Extract

Sampel	Abs	TFC	Rata- rata	SD
Replikasi 1	**0,522**	**47,846**	**47,205**	**0,675**
Replikasi 2	**0,519**	**47,269**		
Replikasi 3	**0,515**	**46,500**		

Flavonoid levels were determined on ethanol extract of kersen leaves (Muntingia Calabura L) in the calculation of total flavonoid levels from three replications had a KFT value, replication 1 obtained a KFT value of 47.846 mgQE/g, replication 2 obtained a KFT value of 47.269 mgQE/g and in replication 3 obtained a KFT value of 46.500 and had an average of 47.205 mgQE/g. These results show that kersen leaf ethanol extract contains a fairly high amount of flavonoids. Flavonoids are one of the groups of phenolic compounds that are known to have good antioxidant activity.

Antioxidant Activity Test DPPH Method

The antioxidant activity of kersen leaf ethanol extract (Muntingia Calabura L) with the DPPH method is based on the ability of kersen leaf ethanol extract in reducing or capturing DPPH radicals. The ability of kersen leaf ethanol extract and quartetin comparator can be seen from the reduced purple intensity of the DPPH solution that has been added in the sample and comparator (Abdul *et al*., 2020). Before testing the antioxidant activity on the sample (Muntingia Calabura L), the wavelength was determined measured in the wavelength range of 400 – 600 nm. The results can be seen in figure 8.

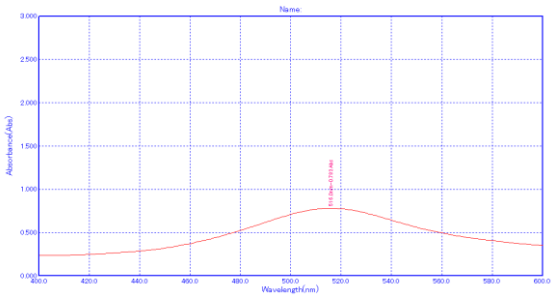


Figure 3. DPPH Wavelength

Based on the graph above, the wavelength results can be seen that the DPPH wavelength obtained in the wavelength range is 516.0 nm with an absorbance value of 0.782. After obtaining the wavelength, it is then used to determine *the operating time*. The determination *of operating time* is carried out to determine the most stable time to take measurements of compounds based on the absorbance value obtained. The active time graph based on the measurement results of the quercetin comparator solution shows that the absorbance begins to stabilize at between 18 minutes with an absorbance of 0.157. The results *of operating time* can be seen in table 14.

Table 4. *Operating Time* DPPH

Waktu	Abs
0	0,186
1	0,176
2	0,175
3	0,170
4	3,000
4	0,171
5	0,169
6	0,170
7	0,167
8	0,165
9	0,164
10	0,005
10	0,163
11	0,162
12	0,161
13	0,161
14	0,159
15	0,000
15	0,161
16	0,204
17	0,157
18	0,157
19	0,157

In this study quercetin was used as a positive control because quercetin has strong antioxidant properties, quercetin was chosen as a comparator because quercetin is a derivative of flavonoid compounds, which has a structural approach between flavonoid molecules in the sample, it is the reason why quercetin was chosen as a comparator. The results of the antioxidant activity test on quercetin as a comparison and kersen leaf extract (Muntingia Calabura L) can be seen in table 18

Table 15. Quercetin Comparator Values

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
10	0,345	0,339	0,348	0,344	53,45	
20	0,324	0,326	0,321	0,323	56,292	
30	0,261	0,263	0,267	0,263	64,411	5,310
40	0,204	0,207	0,211	0,207	71,989	
50	0,194	0,191	0,193	0,192	74,018	

Based on the table above, the results of the antioxidant activity test on quercetin as a comparison show that quercetin has antioxidant activity with 5.310 µg/mL which means it has a very strong category because it is <50 ppm.

The results of the antioxidant activity test carried out on kersen leaf extract (Muntingia Calabura L) obtained an IC50 value of 31.825 µg/mL which is categorized as very strong. And the results can be seen in table 16.

Table 16. IC50 Extract Value

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
10	0,579	0,544	0,543	0,555	24,898	
20	0,457	0,46	0,459	0,458	38,024	
30	0,356	0,364	0,366	0,362	51,014	31,825
40	0,267	0,275	0,289	0,277	62,516	
50	0,264	0,265	0,268	0,265	64,14	

Based on the test results, quercetin as a comparator showed an IC_{50} value of 5.310 µg/mL, which is classified as a very strong antioxidant activity because it is well below the threshold of <50 µg/mL (ppm). This value is in accordance with the profile of quercetin as a pure flavonoid compound which is known to have high antioxidant potential.

Meanwhile, ethanol extract of kersen leaves (*Muntingia calabura* L.) showed an IC_{50} value of 31.825 µg/mL. Although this value is higher than pure quercetin, it is still in the category of very strong antioxidant activity, given that the IC_{50} range for that category is <50 µg/mL.

These results suggest that kersen leaf extract has excellent antioxidant potential, although not as potent as pure quercetin.

ABTS Method Antioxidant Activity Test

Ethanol extract of kersen leaves (Muntingia Calabura L) was tested for antioxidant activity by the ABTS method. The ABTS method can be used in both water-based and organic systems, and has a faster reaction time and can work over a wide range of Ph (Theafelicia & Narsito Wulan, 2023). Before testing the antioxidant activity on the sample, the wavelength was determined. Wavelengths are measured in the wavelength range of 600 – 800 nm. The results can be seen in figure 4

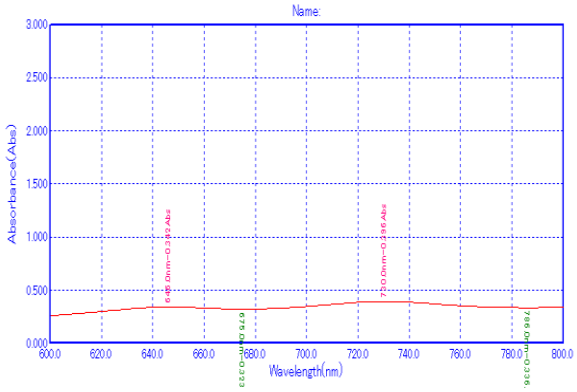


Figure 4. ABTS Wavelength

Based on the graph of wavelength results, it can be seen that the ABTS wavelength obtained is in the range of 730.0 nm with an absorbance value of 0.396. After obtaining the wavelength, then

determining the operating time, the purpose is to carry out the operating time to find out the time needed for the comparison solution in this study using a quercetin comparator. The active time graph based on the measurement results of the quercetin comparator solution shows that the absorbance begins to stabilize between 5 minutes, namely at an absorbance of 0.274 nm. The results of operating time can be seen in table 17.

Tabel 17. Operating Time ABTS

Time(s)	Abs
0,0	0,314
1,0	0,302
2,0	0,293
3,0	0,284
4,0	0,281
5,0	0,274
6,0	0,274
7,0	0,259

` From the results A wavelength of 730 nm was used to measure the quercetin absorbent to determine the IC50 value. The results of the antioxidant activity test on quercetin as a comparison and ethanol extract of kersen leaf (Muntingia Calabura L) can be seen in the table below:

Table 18. Quercetin Comparator Values

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
10	0,306	0,312	0,301	0,306	54,599	
20	0,287	0,211	0,297	0,265	60,682	
30	0,206	0,217	0,129	0,184	72,7	16,013
40	0,113	0,121	0,115	0,116	82,789	
50	0,118	0,123	0,132	0,124	81,602	

Based on the table above, the results of the antioxidant activity test on quercetin as a comparison show that quercetin has antioxidant activity with 16.013 µg/mL which means it is categorized as very strong because it is <50 ppm.

The results of the antioxidant activity test carried out on kersen leaf extract (Muntingia Calabura L) obtained an IC50 value of 56.901 µg/mL which means strong. The results can be seen in the table below.

Table 19. IC50 Value of Kersen Leaf Ethanol Extract

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
10	0,519	0,516	0,518	0,517	23,293	
20	0,484	0,486	0,485	0,501	25,667	
30	0,455	0,454	0,457	0,47	30,267	56,901
40	0,363	0,368	0,362	0,409	39,317	
50	0,34	0,345	0,342	0,353	47,626	

Based on the results obtained, quercetin as a comparator showed an IC_{50} value of 16.013 µg/mL, which falls into the very strong category (<50 µg/mL). These results are in line with the properties of quercetin as a pure antioxidant compound that is highly active in capturing radicals.

Meanwhile, ethanol extract of kersen leaves (Muntingia calabura L.) showed an IC_{50} value of 56.901 µg/mL, which is in the strong category. Although its activity is lower than that of pure quercetin, these results still show that kersen leaf extract has the potential to be an effective source of antioxidants.

Antioxidant Activity Test FRAP Metho

Ethanol extract of kersen leaf (Muntingia Calabura L) was tested for antioxidant activity by the FRAP method. The principle of the FRAP test is the electron transfer reaction from the antioxidant to the Fe3+- TPTZ compound. Fe3+-TPTZ compounds themselves represent oxidizing compounds that may be present in the body and can damage cells (Maryam *et al*., 2016). Before testing the antioxidant activity on the sample (Muntingia Calabura L), a wavelength was determined measured at a wavelength of 400 - 600 nm. The results can be seen in figure 5.

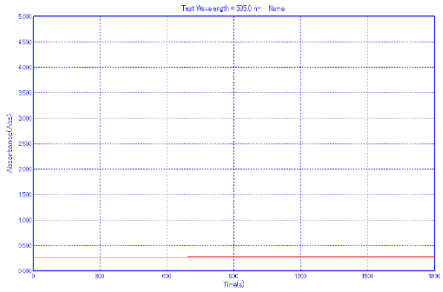


Figure 5. FRAP Wavelength

Based on the graph of wavelength results, it can be seen that the FRAP wavelength obtained is in the range of 595.0 nm with an absorbance value of 0.363. After obtaining the wavelength, then determining the operating time, the purpose is to carry out the operating time to find out the time needed for the comparison solution in this study using a quercetin comparator. The active time graph based on the measurement results of the quercetin comparator solution shows that the absorbance begins to stabilize between 20 minutes, namely at an absorbance of 0.276 nm. The results of operating time can be seen in table 20.

Tabel 20. Operating Time FRAP

No.	Time(s)	Abs	Trans(%T)	Energy
19	1080,0	0,276	53,0	14475
20	1140,0	0,275	53,1	14484
21	1200,0	0,276	53,0	14469
22	1260,0	0,276	53,0	14473
23	1320,0	0,276	53,0	14455

From the results A wavelength of 595 nm was used to measure the quercetin absorbent to determine the IC50 value. The results of the antioxidant activity test on quercetin as a comparison and ethanol extract of kersen leaf (Muntingia Calabura L) can be seen in the table below:

Table 21. Quercetin Comparator Values

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
5	0,34	0,345	0,347	0,344	54,376	
10	0,326	0,329	0,331	0,328	56,498	
15	0,256	0,26	0,263	0,259	65,649	1,764
20	0,219	0,215	0,212	0,215	71,485	
25	0,195	0,198	0,192	0,195	74,137	

Based on the table above, the results of the antioxidant activity test on quercetin as a comparison show that quercetin has antioxidant activity with an IC50 value of 1.764 µg/mL which means it is categorized as very strong because it is <50 ppm.

The results of the antioxidant activity test carried out on kersen leaf extract (Muntingia Calabura L) obtained an IC50 value of 22.079 µg/mL which means it is very strong. The results can be seen in the table below:

Table 22. IC50 Extract Value

Konsentrasi (ppm)	Abs			Rata-Rata	% Inhibisi	IC50
	I	II	III			
5	0,534	0,537	0,54	0,537	28,779	
10	0,519	0,524	0,527	0,523	30,636	
15	0,432	0,437	0,439	0,436	42,175	22,709
20	0,421	0,425	0,427	0,424	43,766	
25	0,342	0,347	0,339	0,342	54,641	

In this study, the three methods in measuring antioxidant activity have different IC50 values produced by these three methods are influenced by the characteristics of each test. The DPPH method is selective against lipophilic compounds, whereas ABTS can detect polar and non-polar compounds, and FRAP assesses the strength of electron reduction without directly involving free radicals. Consistent results showed that kersen leaf ethanol extract had very strong antioxidant activity in all test methods, demonstrating its multi-mechanism ability as an antioxidant (Hidayah *et al.*, 2024).

CONCLUSION

The primary conclusion of this study indicates that ethanol extract of cherry leaves (Muntingia calabura L.) exhibits very strong antioxidant activity as demonstrated by the results of DPPH, ABTS, and FRAP assays. The IC50 values obtained were below 50 µg/mL, with the highest sensitivity observed in the FRAP method. Additionally, the total flavonoid content in the extract was found to be 47.205 mgQE/g, which supports the antioxidant potential attributable to phenolic compounds. These findings suggest that cherry leaves have significant potential as a natural source of antioxidants capable of counteracting free radicals that contribute to degenerative diseases and aging.

However, the results of this research have limitations, including the sampling being restricted to a specific location and using a single extraction method along with specific analytical techniques. Future studies are recommended to include samples from various locations, explore different extraction methods, and conduct more comprehensive antioxidant evaluations such as in vivo testing and detailed structural analysis of active compounds. The practical implication of this research is that cherry leaves could be developed into natural health products, supplements, or ingredients in the pharmaceutical and cosmetic industries that require safe and effective natural antioxidants.

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