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Green-Solvent Extraction and Antioxidant Evaluation of *Muntingia calabura* L. Flowers Using the DPPH Free Radical Scavenging Method

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ABSTRACT: Kersen (*Muntingia calabura* L.) flowers are known to contain bioactive compounds such as flavonoids and tannins, which contribute to various pharmacological activities, including anticancer, antidiabetic, and antioxidant effects. Antioxidants play a crucial role in preventing or slowing oxidative damage by neutralizing free radicals. This study aimed to evaluate the antioxidant activity of aqueous extracts of *Muntingia calabura* L. flowers using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The extraction process was carried out using Microwave-Assisted Extraction (MAE) with a green solvent approach. The antioxidant activity was analyzed using a UV–Visible spectrophotometer, and the results were expressed as the half-maximal inhibitory concentration (IC_{50}), with quercetin used as a reference standard. The results showed that the *Muntingia calabura* flower extract exhibited strong antioxidant activity, with an IC_{50} value of 15.81 µg/mL, which falls within the category of very strong antioxidants (<50 µg/mL). In conclusion, the aqueous extract of *Muntingia calabura* L. flowers demonstrates significant antioxidant potential and may serve as a promising natural source of antioxidants for further development in pharmaceutical or nutraceutical applications.

KEYWORDS: Extraction; MAE; phytochemical; radical; spectrophotometry

1. INTRODUCTION

Indonesia is well known for its rich biodiversity and long-standing tradition of utilizing medicinal plants to address various health conditions. Traditional Indonesian medicine encompasses a wide range of herbal remedies derived from indigenous knowledge that has been passed down through generations. This extensive use of medicinal plants reflects the country's reliance on natural resources as sources of therapeutic agents. One plant commonly found in tropical regions such as Indonesia is *Muntingia calabura* L., locally known as kersen. This species thrives in warm climates and is widely distributed across various regions of Indonesia (Zela & Diah, 2021). The kersen plant has been reported to possess numerous pharmacological properties, including anticancer, antidiabetic, antioxidant, analgesic, and antitussive activities (Mutamimmah, 2021). Among its various parts, the flowers are frequently utilized in traditional medicine due to their potential health benefits. Previous studies have reported that kersen flowers exhibit therapeutic effects such as reducing edema, acting as antispasmodic and antidiabetic agents, and alleviating symptoms of headaches and flu (Kuchekar et al., 2021).

The biological activities of *Muntingia calabura* L. are largely attributed to its bioactive constituents, particularly flavonoids and tannins (Singh et al., 2017). Flavonoids are well known for their antioxidant properties, as they can donate electrons to neutralize free radicals and prevent oxidative stress. However, flavonoid stability can be affected by environmental factors, especially temperature, which may reduce their activity when exposed to excessive heat (Riadini et al., 2015). Previous research by Sari (2019) demonstrated that methanolic extracts of kersen flowers exhibit strong antioxidant activity, with an IC_{50} value of 9.721 µg/mL, indicating their high potential as natural antioxidants.

Antioxidants play a crucial role in protecting biological systems from oxidative damage caused by free radicals. Free radicals are highly reactive molecules with unpaired electrons that can induce cellular damage by destabilizing other molecules in the body (Tristantini et al., 2016). The antioxidant capacity of plant extracts can be evaluated using several in vitro methods, among which the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is widely used due to its simplicity, rapidity, and sensitivity. The DPPH method measures the ability of antioxidant compounds to scavenge stable free radicals. Although other methods such as ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) are also available, the DPPH method is often preferred for its practicality and suitability for low-concentration samples (Wulansari et al., 2018; Syarif et al., 2015).

In addition to selecting appropriate analytical methods, the extraction technique plays a critical role in determining the yield and quality of bioactive compounds. Various extraction methods have been developed, ranging from conventional techniques such as maceration and Soxhlet extraction to more advanced approaches like Microwave-Assisted Extraction (MAE). MAE has gained attention due to its advantages, including shorter extraction time, reduced

solvent consumption, improved energy efficiency, and enhanced extraction yield (Sari et al., 2020). Comparative studies have shown that MAE is more efficient than conventional methods, such as Soxhlet extraction (Daniswara et al., 2017).

Based on these considerations, this study aims to evaluate the antioxidant activity of *Muntingia calabura* L. flower extract using the DPPH free radical scavenging method. The extraction process was carried out using Microwave-Assisted Extraction (MAE) with water as a green solvent, chosen for its safety, low cost, and environmental friendliness (Badriyah & Farihah, 2022). This study is expected to provide scientific evidence supporting the potential of kersen flowers as a natural source of antioxidants for pharmaceutical and nutraceutical applications.

2. EXPERIMENTAL SECTION

2.1. Population and Samples

The plant material used in this study consisted of cherry blossoms (*Muntingia calabura* L.) collected from Jl. Sukaria No. 18, Makassar City, South Sulawesi Province, Indonesia (119°24′17.38″ E; 5°8′6.19″ S). The samples were harvested in the morning at approximately 08:00 WITA. Collected flowers were cleaned to remove adhering impurities and subsequently air-dried at room temperature, protected from direct sunlight, for approximately one week. After complete drying, the samples were pulverized to obtain simplisia powder (Tahir et al., 2022).

2.2. Extraction

Extraction was performed using the Microwave-Assisted Extraction (MAE) method with a solid-to-solvent ratio of 1:20 (w/v). Briefly, 1 g of powdered sample was mixed with 20 mL of distilled water as a green solvent. The mixture was subjected to microwave irradiation under optimal extraction conditions (temperature and time), followed by filtration to separate the extract from the residue. The filtrate was then concentrated through evaporation to obtain the crude extract (Mutammimah et al., 2022).

2.3. Preparation of DPPH and Blank Solution

A total of 5 mg of DPPH was weighed, then dissolved using p.a. (*pro analysis*) methanol solvent in a 10 mL volumetric flask to obtain a 500 µg/mL DPPH solution. After the volume was adjusted to the mark, the solution was stored in a dark room (Mutammimah et al., 2022). A blank solution with a concentration of 35 µg/mL was prepared by taking 0.7 mL of 500 µg/mL DPPH and then making up the volume to 10 mL. The maximum wavelength was then measured using a UV-Vis spectrophotometer (Maryam, 2019; Fawwaz et al., 2024).

2.4. Preparation of quercetin standard solution

A stock solution of 1000 µg/mL was prepared by accurately weighing 10 mg of quercetin and dissolving it in analytical-grade (p.a.) methanol, followed by thorough mixing until fully homogenized. The solution volume was then adjusted to 10 mL using a volumetric flask. Subsequently, a 100 µg/mL working solution was prepared by pipetting 1 mL of the 1000 µg/mL stock solution and diluting it to 10 mL with methanol in a 10 mL volumetric flask. From this working solution, a series of standard solutions with concentrations of 2, 4, 6, 8, and 10 µg/mL were prepared for further analysis (Asri et al., 2024).

2.5. Data Analysis

The parameters used to determine the antioxidant activity of each sample are the inhibition level and lC_{50} value. The lC_{50} value is the concentration of the extract at which 50% of the oxidation process is inhibited. The lC_{50} value is obtained from the regression equation calculated from the measurements of each test solution concentration. The smaller the lC_{50} value, the higher the antioxidant activity (Mutammimah et al., 2022). The inhibition level can be calculated using the following formula:

$$\text{Inhibition (\%)} = (\text{Abs blank} - \text{Abs sample}) / \text{Abs Blank} \times 100\%$$

Linear regression equations were used to calculate absorbance and determine the equation of the line between absorbance and quercetin concentration (Susiloningrum et al., 2021). The linear regression equation formula is as $y = a + bx$.

3. RESULTS AND DISCUSSION

In this study, the antioxidant activity of the samples was evaluated using the DPPH free radical scavenging assay. This method is widely used to assess the ability of a sample to donate hydrogen atoms or electrons to neutralize free radicals. The DPPH assay offers several advantages, including simplicity, rapid analysis, high sensitivity, and minimal sample requirement. Moreover, the DPPH radical is relatively stable compared to other radical systems, making it suitable for routine antioxidant evaluation. The underlying principle of this method involves the reduction of the purple-colored DPPH radical to a non-radical form upon reaction with antioxidant compounds, resulting in a color change to yellow. The degree of discoloration is inversely proportional to the antioxidant capacity of the sample (Devitria, 2020).

The plant material used in this study was the flower of *Muntingia calabura* L., which was dried and ground into a fine powder to enhance extraction efficiency. The extraction process was carried out using Microwave-Assisted Extraction (MAE), a green extraction technique known for its efficiency in extracting bioactive compounds in a shorter time compared to conventional methods. Methanol (p.a., pro analysis grade) was used as the extraction solvent due to its high purity and effectiveness in dissolving a wide range of compounds, including polar, semi-polar, and certain non-polar constituents. The use of methanol also minimizes interference from impurities during analysis. Following extraction, the solvent was removed using a water bath to obtain a concentrated dry extract (Zhang et al., 2018). The result of extraction is shown in **Table 1**.

Table 1. The results of the calculation of the percentage yield of cherry blossom extract (*Muntingia calabura* L.

Sample (g)	Extract (g)	Yield (%)
1.0	0.34	34

The selection of *Muntingia calabura* flower extract for antioxidant testing was based on preliminary optimization of flavonoid content using the MAE method. Among the tested samples, sample C—extracted for 1 minute at medium temperature—exhibited the highest total flavonoid content, measuring 16.708 ± 0.59 mg quercetin equivalents per gram (mgQE/g). Flavonoids are secondary metabolites belonging to the polyphenol group, well known for their antioxidant activity due to their ability to donate hydrogen atoms and neutralize free radicals. Therefore, the high flavonoid content observed in this extract is likely to contribute significantly to its antioxidant capacity.

Before testing the cherry blossom extract (*Muntingia calabura* L.), the maximum wavelength was determined in DPPH using a UV-Vis spectrophotometer. The wavelength obtained was 515 nm. The purpose of wavelength determination is to identify the wavelength at which the DPPH solution exhibits maximum absorbance on the UV-Vis spectrophotometer. The reason for measuring at the maximum wavelength is that the change in absorbance per unit concentration is greatest at the maximum wavelength, thereby achieving maximum analytical sensitivity (Sapatri et al., 2019).

The antioxidant activity of the reference standard quercetin was determined by preparing test solutions with various concentration series, each of which was added with 3 mL of DPPH 35 µg/mL in a DPPH:quercetin ratio of 3:1. The solutions were then homogenized and incubated for 30 minutes. Absorbance was measured at a wavelength of 515 nm. The measurement results are shown in **Table 2**.

Table 2. Measurement Results of Absorbance of Antioxidant Activity of Quercetin Compared to DPPH

Sample	Concentration (µg/mL)	Absorbance	Inhibition (%)	IC_{50} (µg/mL)
Quercetin	2	0.730	11.408	8.70
	4	0.642	22.088	
	6	0.562	31.796	
	8	0.455	44.781	
	10	0.355	56.917	

Table 2 shows that as the concentration of quercetin increases, the absorbance of the solution decreases. Meanwhile, as the concentration of the solution increases, the percentage of inhibition increases. This occurs because as the concentration of the solution increases, the number of antioxidants contained within it also increases. Based on the measurement data of the standard quercetin solution, the calibration curve between concentration and absorbance was obtained, yielding the linearity equation y = 5.6856x – 0.7153 with R^2 = 0.9971 and a correlation coefficient (r) of 0.9970 (**Figure 1**).

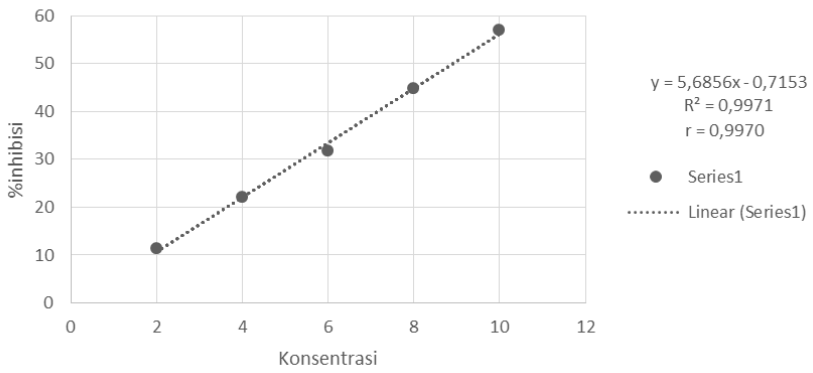

Figure 1. Quercetin standard curve

The results met the linearity requirement, with a value of r > 0.995, indicating a linear relationship between absorbance and standard solution concentration. The antioxidant activity of the reference compound quercetin was calculated to have an IC_{50} value of 8.70 µg/mL. The water extract of kersen flowers *(Muntingia calabura L.)* was further tested for its antioxidant activity using the DPPH free radical scavenging method by preparing test solutions with various concentration series. These concentration series were mixed with DPPH (3:1) and incubated for 30 minutes in the dark.

From the results of measuring the antioxidant activity of cherry blossom extract using the DPPH method, different IC_{50} values were obtained. In the calculation of the antioxidant activity of cherry blossom extract, the results of the linear regression equation graph were y = 2.5049x + 10.376, where the R2 value was 0.9982 and the r value was 0.9970. Based on the IC_{50} value category, the antioxidant activity of cherry blossom extract falls into the very strong category, with a value of 15.81 µg/mL (<50 µg/mL) as shown in **Table 3**.

Table 3. Antioxidant activity cherry blossom extract (*Muntingia calabura* L.)

Sample	Concentration (µg/mL)	Absorbance	Inhibition (%)	IC_{50} (µg/mL)
Cherry Blossom Extract	5	0.824	22.330	15.81
	10	0.824	36.773	
	15	0.824	45.146	
	20	0.824	60.316	
	25	0.824	73.180	

The IC_{50} value of cherry blossom extract is 15.81 µg/mL, while the IC_{50} value of standard quercetin is 8.70 µg/mL. The antioxidant activity of cherry blossom extract and standard quercetin are both classified as very strong antioxidants. According to Molyneux (2004), if the IC_{50} value of an extract is below 50 µg/mL, its antioxidant activity is categorized as very strong; if the IC_{50} value is between 100–150 µg/mL, its antioxidant activity is categorized as weak; and if the IC_{50} value is above 200 µg/mL, its antioxidant activity is categorized as very weak. Thus, it can be concluded that cherry blossom extract has antioxidant activity that falls into the strong category.

The IC_{50} value of quercetin is not significantly different, as quercetin has an IC_{50} value that falls into the very strong category. This is because quercetin is a single flavonoid compound belonging to the flavonol group and has been proven to have very strong antioxidant activity. Therefore, in this study, quercetin was used as a reference standard in the DPPH free radical scavenging method. Quercetin is a pure flavonoid compound that has been isolated, so its antioxidant activity is more focused and effective compared to cherry blossom extract, which still contains various other compounds that may inhibit or not contribute to antioxidant activity. Cherry blossom extract contains various bioactive compounds besides quercetin, such as alkaloids, tannins, and saponins. Some of these compounds may have lower antioxidant activity or even be pro-oxidant, which can increase the overall IC_{50} value. The higher IC_{50} value in the extract compared to quercetin indicates that although the extract has strong antioxidant activity, its effectiveness is still lower than that of the pure, isolated compound (Subandrate and Fatmawati, 2024).

4. CONCLUSION

Based on the results of this study, it can be concluded that the extract of cherry blossoms (*Muntingia calabura* L.) exhibits significant antioxidant activity, as demonstrated by its ability to scavenge DPPH free radicals. The obtained IC_{50} value of 15.81 µg/mL (<50 µg/mL) categorizes the extract as having very strong antioxidant capacity. This high level of activity is likely attributed to the presence of bioactive compounds, particularly flavonoids and other phenolic constituents, which are known for their ability to donate hydrogen atoms or electrons to neutralize free radicals. Furthermore, the use of Microwave-Assisted Extraction (MAE) with a green solvent approach may have contributed to the efficient extraction of these bioactive compounds, resulting in a more potent antioxidant extract. These findings highlight the potential of *Muntingia calabura* L. flowers as a promising natural source of antioxidants that could be further developed for pharmaceutical, nutraceutical, or functional food applications. However, further studies are recommended to isolate and characterize the specific active compounds responsible for the antioxidant activity, as well as to evaluate their mechanisms of action and effectiveness through in vivo studies.

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REFERENCES

A., Singh, R., Iye, S., Prasad, S., Deshmukh, N., Gupta, U., Zanje, A., Patil, S., & Joshi, S. Phytochemical Analysis of Muntingia calabura Extract with Antimicrobial and Antifouling Activity. 2017. Https://Doi.Org/10.25258/Phyto.V9i6.8186

Agustina, E. Testing the Antioxidant Activity of Compounds from Fig Leaf Extract (*Ficus Carica* Linn) Using Water Solvent. *Chlorophyll*, 2017; 1(1), 38–47.

Badriyah, L., & Farihah, D. A. Analysis of Red Onion Skin (*Allium Cepa* L.) Extraction Using the Maceration Method. 2022; 3(1), 30–37.

Devitria, R. Testing the Antioxidant Activity of Ciplukan Leaf Methanol Extract Using the *2,2-Diphenyl 1-Picrylhydrazyl* (DPPH) Method. *Indonesian Journal of Pharmaceutical Research,* 2020; *9*(1), 31–36. https://doi.org/10.51887/jpfi.v9i1.800

Fawwaz, M., Purwono, B., Sidiq, Y., Arwansyah, Arsul, M. I., Fitriana & Baits, M. Anti-inflammatory, Antioxidant, and Antibacterial Activities with Molecular Docking Studies of Vitex Trifolia L. Targeting Human COX-2 and Peroxiredoxin-5. *ChemistrySelect*, 2024; *9*(39), e202403834.

Fina Fitria Sari, St Maryam, M. T. (2019). Antioxidant Activity Test of Methanol Extract of Kersen Flowers (*Muntingia calabura* L.) Using the DPPH (*1,1-Diphenyl-2 Picrylhydrazil*) Free Radical Scavenging Method.

Hasanah, N., Dahlia, A. A., & Handayani, V. Antioxidant Activity Test of Sea Kedondong Leaf Extract (*Nothopanax Fructicosum* (L.) Miq) Using the DPPH Free Radical Scavenging Method. 2023; 1(2), 10–17.

Ilkafah, I. Kersen Leaves (*Muntingia calabura* L.) as an Alternative Therapy for Gout Arthritis Patients. *Jurnal Farmasi Medica/Pharmacy Medical Journal (PMJ)*, 2018; 1(1). https://doi.org/10.35799/PMJ.1.1.2018.19649

Ilmu, J., Upadhye, M., Kuchekar, M., Pujari, R., Kadam, S., Gunjal, P., & Muntingia, K. *Muntingia Calabura*: A Comprehensive Review. 2021; 9(2), 81–87.

Irawan, A. (2019). Spectrophotometer Calibration as Quality Assurance for Measurement Results in Research and Testing Activities. *Indonesian Laboratory Journal*, 1(2), 1. Https://Doi.Org/10.22146/Ijl.V1i2.44750

Lestari, P. A., Supriyono, T., & Rahayu, C. Analysis of Sugar Content, pH, Organoleptic Quality, and Acceptability of Goutseel Beverage with Proportions of Cherry Leaf and Apple Fruit Extracts. *Sentri: Journal of Scientific Research*, 2023; 2(12), 5501–5516. Https://Doi.Org/10.55681/Sentri.V2i12.1966

Maryam, S. Comparison of the Antioxidant Activity of Ethanol Extracts of Soursop Leaves (*Annona Muricata* L.) Based on Growing Location Using the DPPH Scavenging Method. 2019; 3(1), 146–150.

Molyneux, P. The Use of the Stable Free Radical *Diphenylpicryl-Hydrazyl* (DPPH) to Estimate Antioxidant Activity. *Songklanakarin Journal of Science and Technology*, 2004; 211–219. https://doi.org/10.1287/isre.6.2.144

Mutammimah, S., Supriyanto, S., & Mu'tamar, M. F. F. Antioxidant and Antibacterial Activity of Kersen Leaf Extract (*Muntingia calabura* L) Using Microwave-Assisted Extraction Method. *Engineering*, 2022; 15(1), 21–28. Https://Doi.Org/10.21107/Rekayasa.V15i1.13229

Riadini, R. K., Sidharta, B. B. R., & Pranata, F. S. Antioxidant Activity Test of Sambung Nyawa Leaf Extract (*Gynura Procumbens* (Lour.)Merr.) Based on Differences in Extraction Methods and Harvest Age. *Ind J Pharm*, 2015; 1, 1–16.

Rivai, M., & Mardiyanto, M. R. Identification of Smoke in the Air Using a Spectrophotometer and Artificial Neural Network. 2015.

Salimi, Y. K. Miana Leaves as Antioxidants and Anticancer Agents. In *Angewandte Chemie International Edition*, 2021; 6(11), 951–952.

Sari, B. L., Triastinurmiatiningsih, T., & Haryani, T. S. Optimization of *Microwave-Assisted Extraction* (MAE) Method to Determine Total Flavonoid Content of Brown Algae Padina Australis. *Alchemy Journal of Chemical Research*, 2020; 16(1), 38. https://doi.org/10.20961/alchemy.16.1.34186.38-49

Susiloningrum, D., Erliani, D., & Sari, M. Antioxidant Activity Test and Determination of Total Flavonoid Content of Temu Mangga (*Curcuma Mangga Valeton & Zijp*) Extract with Variations in Solvent Concentration. 2021; 5(2), 117–127.

Subandrate and Fatmawati. Comparison of Antioxidant Activity Between Extract and Nanofiber Forms of Ethanol Extract from Yellow Wood (*Arcangelisia flava*) Stems. *Bachelor's Thesis*, University of Sriwijaya. 2024.

Syarif, R. A., Muhajir, Ahmad, A. R., & Malik, A. Identification of Antioxidant Compound Groups Using the DPPH Radical Scavenging Method of Ethanol Extracts from *Cordia Myxa* L. Leaves. *Indonesian Journal of Phytopharmaceuticals*, 2015; 2(1), 83–89.

Integrated Taxonomy Information System. *Taxonomic Hierarchy: Manihot Esculenta* (P. https://www.itis.gov.). 2015.

Tahir, M., Maryam, S., Nurfauziah, P., & Nazhifah, N. Activity of Flavonoid Compounds from Ethanol Extract of Cherry Flower (*Muntingia calabura* L) as Sunscreen. Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy. 2022; 14(2), 97–104.

Tristantini, D., Ismawati, A., Pradana, B. T., & Gabriel, J. Testing Antioxidant Activity Using the DPPH Method on Tanjung Leaves (*Mimusops elengi* L). University of Indonesia, 2. 2016.

Tri Sapatri H, Triastinurmiatiningsih, Bina Lohita, I.N.S. Phenolic Content and Antioxidant Activity of Ethanol Extracts of Brown Seaweed (*Padina australis*)', Αγαη, 2019; 8(5), p. 55.

Vadivel, K., Kumar, G. S., & Babu, S. M. Ex Vivo Antispasmodic Activity of Aqueous Extract of Flowers *of Muntingia calabura* Linn. on Excised Rabbit's Jejunum. 2017; 301–303. https://doi.org/10.4103/pr.pr

Wulansari, A. N., Farmasi, F., Padjadjaran, U., & Ungu, C. Alternatif Cantigi Ungu (*Vaccinium Varingiaefolium*) Sebagai Antioksidan Alami. 16, 419–429.

Zela, Z., & Diah, A. W. M. Testing the Antioxidant Activity of Kersen Fruit Extract (*Muntingia calabura* L.) Using *1,1-Diphenyl-2-Picrylhydrazine*. *Media Eksakta*, 2021; 17(2), 85–90. Https://Doi.Org/10.22487/Me.V17i2.1108.