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Green Extraction of Cherry Flowers (*Muntingia calabura* L.) and Antioxidant Activity by Ferric Reducing Antioxidant Power Method

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ABSTRACT: The cherry plant (*Muntingia calabura* L.) is known as a versatile medicinal plant, with its leaves, fruits, and flowerstraditionally used for various therapeutic purposes. This plant contains several bioactive compounds, including flavonoids, tannins, triterpenes, saponins, and polyphenols, which play a crucial role in its antioxidant activity. The present study aimed to determine the antioxidant activity level of *Muntingia calabura* L. using the Ferric Reducing Antioxidant Power (FRAP) method. The ethanol extract of cherry plant material was obtained using the Microwave-Assisted Extraction (MAE) technique, which had been previously optimized to achieve the highest flavonoid yield through variations in temperature and extraction time, employing a green solvent system. The antioxidant activity was measured by the FRAP method, and the absorbance was recorded using a UV–Visible spectrophotometer at a wavelength of 715 nm. The results were expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g) based on the absorbance data. The antioxidant activity values of the ethanol extract from three replications were 908.56 mg QE/g, 910.6 mg QE/g, and 955.5 mg QE/g, respectively, with an average value of 924.88 mg QE/g extract. These findings indicate that *Muntingia calabura* L. exhibits strong antioxidant potential, likely attributed to its high flavonoid and polyphenol content, and therefore has promising applications as a natural antioxidant source in traditional and modern medicine.

KEYWORDS: MAE; extraction; phytochemical; antioxidant; bioactive.

1. INTRODUCTION

Since ancient times, humans have relied on natural resources, particularly plants, to fulfil various needs, including for traditional medicine and healing practices. This long-standing dependence illustrates the vital role of plant-derived medicines in improving and maintaining public health. One such plant is the cherry plant (*Muntingia calabura* L.), a wild species commonly found growing along roadsides and frequently used as a shade tree in tropical regions (Nurholis & Saleh, 2019).

Phytochemical investigations have revealed that *Muntingia calabura* L. contains various bioactive compounds, notably flavonoids (including flavones, flavanones, flavans, and biflavans), as well as tannins, triterpenes, saponins, and polyphenols, which contribute significantly to its antioxidant activity (Tahir et al., 2022). Antioxidants are molecules capable of donating electrons to free radicals, thereby stabilizing them and preventing oxidative chain reactions that can damage biological cells (Maryam & Suhaenah, 2023). Many natural antioxidants are derived from plants, making them valuable candidates for preventing oxidative stress–related diseases.

Several studies have reported additional pharmacological properties of *Muntingia calabura*, including its ability to inhibit α-glucosidase enzyme activity (Maryam et al., 2023), exhibit strong antioxidant potential with an IC_{50} value of 9.271 µg/mL using the DPPH method (Maryam et al., 2019), and show antibacterial activity against acne-causing bacteria (Maryam et al., 2023).

Among the various methods used to evaluate antioxidant capacity, the Ferric Reducing Antioxidant Power (FRAP)method is widely applied. This method is based on an electron transfer reaction in which antioxidants reduce the Fe^{3+}–TPTZ complex to Fe^{2+}–TPTZ, producing a blue-colored complex measurable by UV–Visible spectrophotometry (Aryanti et al., 2021; Arifin et al., 2025). The intensity of the color change, typically measured at 593 nm, reflects the antioxidant strength of the sample. Compared to other assays such as DPPH (which measures radical scavenging ability via IC_{50} values) (Ezra et al., 2023) and CUPRAC (which measures Cu^{2+} reduction at 450 nm) (Maryam et al., 2016), the FRAP method offers several advantages: it is simple, fast, inexpensive, requires easily prepared reagents, and does not demand specialized instruments (Aryanti et al., 2021; Ainunnisa et al., 2025).

Based on these considerations, the present study aims to evaluate and optimize the antioxidant activity of *Muntingia calabura* L. extracts obtained using green solvents and the FRAP method. The results are expected to provide scientific evidence supporting the potential of the cherry plant as a natural source of antioxidant compounds for use in pharmaceutical and nutraceutical applications.

2. EXPERIMENTAL SECTION

2.1. Sample Collection and Extraction

The Cherry plant samples *(Muntingia calabura* L.*)* obtained were peeled and washed thoroughly using running water, then dried by airing without exposure to sunlight for approximately a week after which the sample was powdered. Cherry plant extract was made using a method referred to from previous research with several modifications (Mutammimah et al., 2022). The extraction used was the Microwave-Assisted Extraction method with a ratio of 1:20 between the material and distilled water. Each sample was weighed as much as 1 gram and added with 20 mL of distilled water. Then the sample was put into the microwave and set based on the time treatment (1, 5, and 10 minutes) and temperature variations (low, med low, and medium). Furthermore, the sample was filtered and concentrated until a dry extract was obtained. The sample used was the sample extracted at 1 minute and medium temperature because it had the highest flavonoid content.

2.2. Materials

The tools and instruments used in this study included a Microwave-Assisted Extraction (MAE) unit, beakers (50 mL and 250 mL), stirring rods, blender, porcelain crucibles, pH meter, horn spoon, micropipettes, centrifuge, vortex mixer, UV–Visible spectrophotometer, measuring flasks, centrifuge tubes, vials, analytical balance, oven, and water bath. The materials and reagents used in this study were cherry plant extract (*Muntingia calabura* L.), distilled water, 10% trichloroacetic acid (TCA), 0.1% $FeCl_3$ solution, phosphate buffer (0.2 M, pH 6.6), 1% potassium ferricyanide solution, 96% ethanol, aluminum foil, label paper, tissue, and quercetin standard as a comparator.

2.3. Reagents Preparation

The quercetin standard solution was prepared by accurately weighing 10 mg of quercetin and dissolving it in 96% ethanol in a 10 mL volumetric flask, then homogenized to the mark. A series of quercetin standard solutions was subsequently prepared by pipetting 1 mL of the stock solution into a 10 mL volumetric flask to obtain a 100 µg/mL concentration. From this, 0.75, 1.25, 1.75, 2.25, and 2.75 mL aliquots of the 100 µg/mL solution were each pipetted into 5 mL volumetric flasks to produce standard concentrations of 15, 25, 35, 45, and 55 µg/mL, respectively, then diluted to volume with 96% ethanol and homogenized. The 0.2 M phosphate buffer solution (pH 6.6) was prepared by dissolving 2 g of NaOH in 250 mL of CO_2-free distilled water and 6.8 g of KH_2PO_4 in another 250 mL of CO_2-free distilled water. A total of 16.4 mL of the NaOH solution was then mixed with 50 mL of the KH_2PO_4 solution, adjusted to pH 6.6 using a pH meter, and diluted to 200 mL with CO_2-free distilled water. The 0.1% $FeCl_3$ solution was prepared by dissolving 0.1 g of $FeCl_3$ in distilled water and diluting it to 100 mL in a volumetric flask. The 10% trichloroacetic acid (TCA) solution was prepared by dissolving 10 g of TCA in distilled water and diluting it to 100 mL, while the 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution was prepared by dissolving 1 g of $K_3Fe(CN)_6$ in distilled water and making up the volume to 100 mL in a volumetric flask (Fawwaz et al., 2022).

2.4. Quantitative Analysis of Antioxidant Activity Using the FRAP Method

2.4.1 Determination of Maximum Wavelength (λmax)

The maximum wavelength (λmax) was determined by measuring the absorbance of a 35 µg/mL quercetin standard solution (Damongilala, 2013). A total of 1 mL of the quercetin solution was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% potassium ferricyanide ($K_3Fe(CN)_6$), then vortexed until homogeneous. The mixture was incubated at 50°C for 20 minutes, followed by the addition of 1 mL of 10% trichloroacetic acid (TCA). The solution was then centrifuged at 3000 rpm for 10 minutes, and 1 mL of the supernatant was transferred into a vial. To this, 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. Absorbance was measured within the wavelength range of 400–800 nm using a UV–Vis spectrophotometer, and the maximum absorbance was observed at 715 nm (λmax).

2.4.2 Determination of the Quercetin Standard Curve

A 10 mg quercetin standard was dissolved in 10 mL of 96% ethanol to obtain a 1000 µg/mL stock solution. From this, 1 mL was pipetted and diluted to 10 mL to yield a 100 µg/mL standard solution. Subsequently, 0.75, 1.25, 1.75, 2.25, and 2.75 mL of the 100 µg/mL solution were each transferred into 5 mL volumetric flasks, diluted to volume with ethanol, and homogenized to produce 15, 25, 35, 45, and 55 µg/mL standard concentrations. For the FRAP assay, 1 mL of each standard solution was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$, then vortexed and incubated at 50°C for 20 minutes. After incubation, 1 mL of 10% TCA was added, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant (1 mL) was then combined with 1 mL of distilled waterand 0.5 mL of 0.1% $FeCl_3$, incubated for 5 minutes, and the absorbance was recorded at 715 nm using a UV–Vis spectrophotometer (Fawwaz et al, 2022).

2.4.3 Determination of Antioxidant Activity in Cherry Plant Extract

A total of 10 mg of cherry plant extract (*Muntingia calabura* L.) was dissolved in 5 mL of 96% ethanol to obtain a 2000 µg/mL stock solution. From this, 0.125 mL was pipetted and diluted to 5 mL with ethanol to produce a 50 µg/mL working solution. Then, 1 mL of this extract solution was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1

mL of 1% $K_3Fe(CN)_6$, vortexed to homogenize, and incubated at 50°C for 20 minutes. After incubation, 1 mL of 10% TCA was added, followed by centrifugation at 3000 rpm for 10 minutes. The supernatant (1 mL) was transferred into a vial, combined with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and incubated for 5 minutes. The absorbance was measured at a wavelength of 715 nm using a UV–Vis spectrophotometer. All measurements were performed in triplicate to ensure accuracy and reproducibility (Fawwaz et al, 2022).

2.5. Data Analysis

The data analysis for determining the total antioxidant activity of the cherry plant (*Muntingia calabura* L.) extract was conducted by first measuring the maximum wavelength (λmax) using a UV–Visible spectrophotometer. This was followed by the determination of antioxidant activity using the Ferric Reducing Antioxidant Power (FRAP) method. A calibration curve was constructed by plotting the absorbance values against the concentrations of the quercetin standard solutions, and a linear regression equation of the form y = a + bx was obtained. The FRAP value, representing the antioxidant capacity of the sample, was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

3. RESULTS AND DISCUSSION

Antioxidants are defined as compounds capable of neutralizing the harmful effects of oxidizing agents by donating electrons to stabilize free radicals and prevent oxidative damage (Balqis Hira, 2024). In general, antioxidants function by inhibiting the oxidation of molecules, thereby protecting biological systems from oxidative stress and cellular damage (Fawwaz et al., 2023).

One of the plants known to exhibit strong antioxidant activity is the cherry plant (*Muntingia calabura* L.). In this study, cherry flowers were used as the test sample. The samples were collected from Makassar City, South Sulawesi Province. The flowers were harvested directly from the plant, then air-dried for approximately one week to remove moisture and subsequently ground into a fine powder for extraction.

The mechanism of antioxidant action involves delaying, preventing, or neutralizing oxidative damage to biomolecules by quenching free radicals, chelating metal ions, reducing pro-oxidant enzyme activity, and stimulating endogenous antioxidant enzymes (Arnanda & Nuwarda, 2019; Fawwaz et al., 2024). Flavonoids, one of the main classes of polyphenolic compounds, act as exogenous antioxidants through metal chelation and free radical scavenging. Their ability to chelate transition metals, such as Fe^{2+} and Cu^{2+}, prevents these ions from catalyzing oxidative reactions that generate free radicals (Mutammimah et al., 2022).

The Microwave-Assisted Extraction (MAE) technique was employed in this study due to its advantages of higher extraction efficiency, reduced solvent use, and shorter extraction time compared to conventional methods such as maceration (Fadiyah et al., 2020). Previous research supports that MAE provides a faster and more efficient extraction process, allowing for improved recovery of heat-stable bioactive compounds. In this study, the extract yield found in the sample is 34% as shown in **Table 1**.

The selection of cherry flower extract in this study was based on the results of a flavonoid content optimizationexperiment using the MAE method. Among the tested conditions, extracted at medium temperature for 1 minute, exhibited the highest total flavonoid content, recorded as 16.708 ± 0.59 mg QE/g extract. Flavonoids, as secondary metabolites of the polyphenol group, are recognized for their potent antioxidant capacity through free radical scavenging and metal chelation mechanisms. Numerous studies have highlighted the antioxidant potential of flavonoids, underscoring their role in protecting the body from oxidative stress and related degenerative diseases (Arnanda & Nuwarda, 2019).

Table 1. Extraction results of sample from the MAE method and percentage yield.

Sample weight (g)	Extraction weight (g)	Yield (%)
0.34	1	34

Next, antioxidant activity testing was conducted using the Ferric Reducing Antioxidant Power (FRAP) method with quercetin as the standard reference compound. Prior to the analysis, the maximum wavelength (λmax) was determined using a UV–Visible spectrophotometer to ensure optimal detection sensitivity. Compounds measurable by UV–Vis spectrophotometry must be in solution form, contain chromophore groups (color-bearing functional groups), and possess conjugated double bonds that enable light absorption within the UV–Visible range. The determination of λmax was performed by scanning the absorbance of the quercetin standard solution over the wavelength range of 400–800 nm. Quercetin, a flavonoid derivative known for its strong antioxidant properties, was used as the comparator standard. The results showed that the maximum absorbance wavelength (λmax) for quercetin occurred at 715 nm. The purpose of determining λmax was to identify the wavelength that provides the highest absorbance per unit concentration, thereby achieving maximum analytical sensitivity during measurement. Once λmax was established, absorbance measurements were performed by preparing a series of quercetin standard solutions at concentrations of 15, 25, 35, 45,

and 55 µg/mL, which were then used to construct the standard calibration curve for subsequent antioxidant activity determination.

Table 2. Results of measuring the absorbance of the comparative solution of quercetin

Concentration (µg/mL)	Absorbance
15	0.255
25	0.363
35	0.472
45	0.545
55	0.656

Based on the measurement data of the standard quercetin solution, a standard curve of the regression results from the concentration (x) with the absorbance value (y) was made, the linearity equation y = 0.0098x + 0.1138 was obtained with an R2 value = 0.9961 and an r value = 0.998. The linearity requirement is the correlation coefficient value which is said to be good if the r value ≥ 0.998 is obtained and this is in accordance with the requirements (Widiastuti, 2019). So it can be said that absorbance and concentration have a very strong correlation.

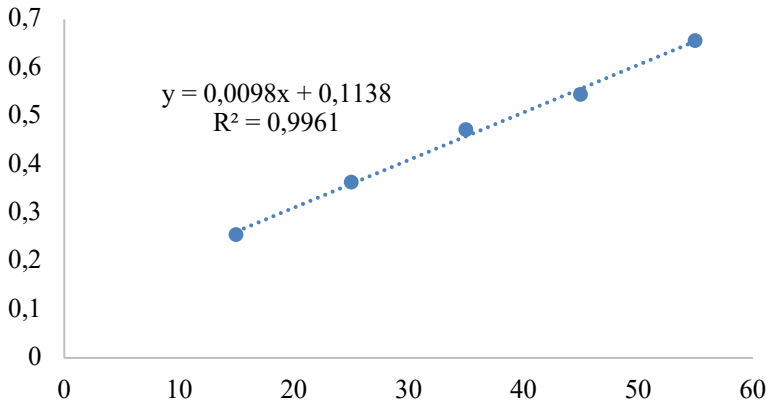

Figure 1. Linier regression of quercetin

Table 3. Results of absorbance measurements and activity values of Cherry plant extract *(Muntingia calabura* L.*)*

Ethanol extract of Cherry plant	Absorbance (715 nm)	Antioxidant Activity (mgQE/g)	Average (mgQE/g)
Replication 1	0.559	908.56	924.88
Replication 2	0.560	910.6	
Replication 3	0.582	955.5	

The high antioxidant activity observed in the cherry plant extract (*Muntingia calabura* L.), with an average value of 924.88 mg QE/g, indicates that this plant possesses strong reducing power and radical-scavenging ability. This value reflects the extract's capability to donate electrons and convert free radicals or oxidizing agents into more stable molecules, thereby preventing oxidative stress and cellular damage. The remarkable antioxidant potential of *M. calabura*is likely attributed to its abundant flavonoid and polyphenolic compounds, which are known to act as primary antioxidants through mechanisms such as hydrogen atom transfer and metal ion chelation (Arnanda & Nuwarda, 2019; Mutammimah et al., 2022).

Comparable findings have been reported in previous studies, where *M. calabura* leaf and flower extracts demonstrated strong antioxidant capacity, with IC_{50} values ranging from 9.27 µg/mL to 15.42 µg/mL using the DPPH radical scavenging method (Maryam et al., 2023). The antioxidant strength obtained in the present study using the FRAP method supports these earlier observations, confirming that *M. calabura* is a potent source of natural antioxidants. Such activity suggests that cherry plant extracts have potential applications as natural antioxidant agentsin preventing diseases related to oxidative stress, including cardiovascular disorders, neurodegenerative diseases, and premature aging. The high

FRAP value also highlights the effectiveness of the Microwave-Assisted Extraction (MAE) technique, which enhances the recovery of bioactive compounds while maintaining their structural integrity.

4. CONCLUSION

Based on the results of this study, it can be concluded that the cherry plant exhibits strong antioxidant activity, as demonstrated by the FRAP assay. The extract obtained through the MAE method produced an average antioxidant activity of 924.88 mg QE/g extract, indicating a high capacity to reduce ferric ions and scavenge free radicals. This potent antioxidant potential is closely related to the presence of flavonoid and polyphenolic compounds in the extract, which act as natural reducing agents and metal chelators. These findings suggest that *Muntingia calabura* L. can serve as a promising natural source of antioxidants, supporting its traditional use in herbal medicine and offering potential applications in pharmaceutical and nutraceutical formulations aimed at mitigating oxidative stress–related diseases.

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