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Ethanolic Soxhlet Extract of Harendong Leaf (*Melastoma malabathricum*): A Study of Total Phenolic Content and Antioxidant Activity

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Abstract. As a traditional medicinal plant that has been used for generations by the Baduy community to treat various ailments, *Melastoma malabathricum* L. (*harendong*) leaves represent a promising source of bioactive compounds, particularly phenolic constituents that contribute to antioxidant activity. This study aimed to determine the total phenolic content and antioxidant activity of harendong leaf extract obtained through Soxhlet extraction using 96% ethanol. The total phenolic content was quantified using the Folin–Ciocalteu assay and expressed as mg gallic acid equivalents (GAE)/g extract. At the same time, antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay at a wavelength of 593 nm. The extract exhibited a total phenolic content of 66.48 ± 3.97 mg GAE/g extract and an antioxidant activity of 1.46 ± 0.14 mmol Fe^{2+}/g, which was lower than that of ascorbic acid (3.99 ± 0.12 mmol Fe^{2+}/g (n=3 for both). These findings are consistent with the known relationship between phenolic content and antioxidant activity, highlighting the potential of M. malabathricum leaves as a scientifically supported natural source of antioxidants.

Keywords: *phenolic compounds, antioxidants, harendong leaves, M. malabathricum L.*

Abstrak. Sebagai tanaman obat tradisional yang secara turun-temurun digunakan masyarakat Baduy untuk mengatasi berbagai penyakit, daun harendong (*Melastoma malabathricum* L.) berpotensi menjadi sumber senyawa bioaktif, khususnya senyawa fenolik yang berperan sebagai antioksidan. Kajian ini dilakukan untuk mengukur kandungan fenol total serta aktivitas antioksidan pada ekstrak daun hasil sokletasi dengan etanol 96%, di mana kadar fenol total ditentukan menggunakan metode Folin–Ciocalteu (mg GAE/g ekstrak) dan aktivitas antioksidan diukur menggunakan metode FRAP pada panjang gelombang 593 nm. Diperoleh kadar fenol total sebesar 66,48 ± 3,97 mg GAE/g dan aktivitas antioksidan sebesar 1,46 ± 0,14 mmol Fe^{2+}/g — lebih rendah daripada asam askorbat (3,99 ± 0,12 mmol Fe^{2+}/g (n=3 untuk masing-masing). Hasil ini sejalan dengan hubungan yang telah diketahui antara kandungan fenolik dan aktivitas antioksidan, sehingga membuka peluang pengembangan daun harendong sebagai antioksidan alami berbasis bukti ilmiah.

Kata kunci: senyawa fenolik, antioksidan, daun harendong (*M. malabathricum* L.)

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INTRODUCTION

Indonesia's remarkable biodiversity as a tropical country positions it as a region with substantial potential as a source of medicinal plants. The practice of utilizing plants for traditional medicine has been passed down for generations across various indigenous communities, grounded in local knowledge inherited from one generation to the next. One community that continues to preserve this traditional wisdom is the Baduy people of Lebak Regency, Banten Province, who to this day rely on a variety of plants as herbal remedies, one

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of which is harendong leaf (*M. malabathricum* L.) (Kameswari et al., 2023; Nursaadah et al., 2017).

Traditionally, harendong leaf has been used to treat various health complaints, including diarrhea, dysentery, leukorrhea, hemorrhoids, wounds, infections, toothache, abdominal pain, and mouth ulcers. Several studies have reported that this plant contains secondary metabolites, including flavonoids, tannins, saponins, alkaloids, and steroids or terpenoids, which contribute to a range of biological activities, including anticancer, antibacterial, antidiabetic, anti-inflammatory, and antioxidant effects (Novasella et al., 2022; Harahap et al., 2022). Furthermore, LC-MS/MS analysis has confirmed the presence of several phenolic compounds such as quercetin, kaempferol, myricetin, catechin, rutin, and gallic acid; these compounds are known to contribute to antioxidant activity (Lestari et al., 2024).

These compounds belong to a class of secondary metabolites; phenolic compounds scavenge free radicals by donating electrons or hydrogen atoms. This capacity is what makes phenolic compounds important in inhibiting oxidative damage associated with various degenerative diseases. Consequently, total phenolic content serves as an important indicator for assessing a plant's antioxidant potential. The Folin–Ciocalteu method is commonly employed for this measurement, utilizing gallic acid as the standard. Results are expressed in Gallic Acid Equivalents (GAE).

Several previous studies have reported strong antioxidant activity of harendong leaf extract based on both DPPH and ABTS methods. Apmarja et al. (2025), for instance, reported that ethanolic extract of harendong leaf exhibited IC_{50} values of 12.51 µg/mL (DPPH method) and 8.21 µg/mL (ABTS method). Similar studies on phenolic content, flavonoid content, and antioxidant activity have been reported in many plant species, including a comparison of phenolic content, flavonoid content, and antioxidant activity between robusta and arabica coffee (Ngibad et al., 2023), as well as a study on antioxidant activity and total flavonoid content in papasan leaf extracts obtained using different polar solvents (Sari et al., 2021). Both studies confirm that the choice of extraction method and solvent type influences the yield of phenolic-flavonoid compounds as well as the resulting magnitude of antioxidant activity. Nevertheless, studies on the antioxidant activity of harendong leaf using the FRAP method remain limited. The FRAP method relies on the Single Electron Transfer (SET) mechanism to evaluate the capacity of antioxidant compounds to reduce Fe^{3+} ions to Fe^{2+}. The measured value indicates the reducing capacity of the analyzed sample. Unlike DPPH and ABTS, which are predominantly Hydrogen Atom Transfer (HAT)-based assays and primarily reflect radical-scavenging capacity, the SET-based FRAP assay evaluates the electron-donating (reducing) power of antioxidant compounds toward a metal ion. Applying FRAP to harendong leaf extract therefore provides a mechanistically distinct perspective on its antioxidant potential, complementing the radical-scavenging data already reported for this species.

This study aims to determine the total phenolic content of ethanolic harendong leaf (*Melastoma malabathricum* L.) extract using the Folin–Ciocalteu method and to measure its antioxidant activity with the FRAP method. This study aims to expand scientific knowledge of

harendong leaf as a natural antioxidant and to strengthen the evidence supporting traditional medicinal plants.

MATERIAL AND METHODS

Materials

The materials utilized in this study comprised harendong leaves, 96% ethanol, $FeCl_3$, NaOH, chloroform, concentrated H_2SO_4, Mayer's reagent ($HgCl_2$ + KI), Wagner's reagent (I_2 + KI), concentrated HCl, glacial acetic acid (CH_3COOH), 96% p.a. ethanol solvent, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, sodium acetate trihydrate ($CH_3COONa \cdot 3H_2O$), distilled water, TPTZ (2,4,6-tripyridyl-s-triazine), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ascorbic acid, and iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$).

Instrumentation

The Instrumentation used in this study included a blender, a Soxhlet extraction apparatus, a condenser, a round-bottom flask, a heating mantle, filter paper (thimble), measuring cylinders, test tubes, dropper pipettes, an analytical balance, a watch glass, a spatula, a volumetric pipette, a volumetric flask, a beaker, a desiccator, a cuvette, and a UV-Vis spectrophotometer.

Procedure

Sample collection, preparation, and extraction

Harendong leaf samples (*M. malabathricum* L.) were obtained from Kp. Talun, Panancangan Village, Cibadak District, Lebak Regency, Banten Province. Debris was removed from the samples with running water. After draining, the samples were left to air-dry at room temperature, kept out of direct sunlight. Once dried, they were ground with a blender and sieved to achieve a uniform particle size of the simplicia.

Extraction was conducted using the Soxhlet method with 96% ethanol as the solvent. Sixty grams of simplicia powder were extracted with 750 mL of 96% ethanol at a 1:12.5 (w/v) ratio for approximately 6 hours, until the solvent returning to the flask was clear. The resulting filtrate was concentrated with a rotary evaporator until the volume decreased, yielding a viscous extract that still contained residual solvent. The extract was stored in a sealed container for later phytochemical screening, total phenolic content determination, and antioxidant activity testing.

Phytochemical screening

Qualitative phytochemical screening was performed to identify the presence of secondary metabolite classes, including phenols, tannins, flavonoids, alkaloids, saponins, steroids, terpenoids, and glycosides.

For **the phenol and tannin test**, add 2 mL of 2% $FeCl_3$ solution to the ethanolic extract. A color change to blue-green or black indicates the presence of phenolic and tannin compounds. For **the flavonoid test**, add 2 mL of 2% sodium hydroxide solution to the ethanolic extract, then add 10 drops of dilute acid. A change to a colorless solution indicates the presence of flavonoid compounds. For **the steroid test**, 2 mL of chloroform was added to the ethanolic extract, forming two layers: an upper and a lower phase. Concentrated H_2SO_4 was then slowly added along the tube wall. The presence of steroid compounds was indicated if the upper chloroform layer turned red. For **the terpenoid test**, the concentrated ethanolic extract should be dissolved in 2 mL of chloroform and evaporated to dryness. Subsequently, 2 mL of concentrated H_2SO_4 is

added, and the mixture is heated in a water bath for approximately 2 minutes. The appearance of a grayish color indicates the presence of terpenoid compounds. For **the alkaloid test**, the ethanolic extract was added to two test tubes, each with 2 mL of 1% HCl, and gently heated in a water bath. Mayer's reagent was added to the first tube and Wagner's reagent to the second. The presence of a precipitate in each tube indicated the presence of alkaloids in the extract. **To test for saponins**, the ethanolic extract was diluted with 5 mL of distilled water and shaken vigorously. The presence of stable foam indicated saponin compounds. For **the glycoside test**, the ethanolic extract was mixed with 2 mL of glacial acetic acid and 1–2 drops of 2% $FeCl_3$ solution. This mixture was carefully layered over 2 mL of concentrated sulfuric acid in a separate test tube. A brown ring at the interface indicated the presence of glycosides (Purnamasari *et al*., 2022).

Quantification of total phenolic content

Total phenolic content was measured using the Folin–Ciocalteu method and a modified gallic acid standard curve. A 100 ppm stock solution was prepared by dissolving 2.0 mg of gallic acid in analytical-grade ethanol and adjusting the final volume to 20.0 mL. The solution was serially diluted to prepare standards at 10, 20, 30, 40, and 50 ppm. To each 5.0 mL standard, 0.4 mL of 10% (v/v) Folin–Ciocalteu reagent was added and allowed to stand for 4 to 8 minutes. Then, 3.0 mL of 7% Na_2CO_3 was added, and the mixture was diluted with distilled water to a final volume of 10.0 mL. Absorbance at 744.8 nm was recorded with a UV-Vis spectrophotometer after incubating the sample for 2 hours at room temperature (25 °C) (Mukhriani *et al*., 2019).

The same procedure was used for the sample solution, which was prepared by dissolving 10.0 mg of ethanolic harendong leaf extract in p.a. ethanol to a final volume of 10.0 mL. A 5.0 mL aliquot of this solution was then analyzed using the same method as the standard solutions. All measurements were performed in triplicate. Total phenolic content was determined using the regression equation from the gallic acid calibration curve and is expressed as mg GAE/g extract (Mukhriani *et al*., 2019).

Quantification of antioxidant capacity by the FRAP method

Antioxidant activity was evaluated using a modified ferric reducing antioxidant power (FRAP) assay. The FRAP reagent consisted of acetate buffer (pH 3.6), 10 mmol/L TPTZ solution, and 20 mmol/L $FeCl_3 \cdot 6H_2O$. The acetate buffer was prepared by dissolving 0.775 g of sodium acetate trihydrate and 4 mL of acetic acid, diluting the mixture to 250 mL, and adjusting the pH to 3.6. A 40 mmol/L HCl solution was prepared by diluting 0.828 mL of concentrated HCl to a volume of 250 mL; this solution was used to dissolve 0.031 g of TPTZ in a final volume of 10 mL, resulting in a 10 mmol/L TPTZ solution. A 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution was prepared by dissolving 0.054 g of $FeCl_3 \cdot 6H_2O$ in 10 mL of distilled water. The three components—25 mL of acetate buffer, 2.5 mL of TPTZ solution, and 2.5 mL of $FeCl_3 \cdot 6H_2O$ solution—were combined and diluted with distilled water to a total volume of 100 mL to prepare the working FRAP reagent (Dewi, 2019; Mukhriani *et al*., 2019).

A standard curve was constructed using $FeSO_4 \cdot 7H_2O$; 0.013 g of $FeSO_4 \cdot 7H_2O$ was dissolved in 50 mL of distilled water to prepare

a 1000 µmol/L stock solution, which was then diluted to obtain a concentration series of 10, 20, 30, 40, and 50 µmol/L. The optimum operating time was determined by reacting the 30 µmol/L standard solution with the FRAP reagent and monitoring it over 30 minutes (Dewi, 2019; Mukhriani *et al*., 2019)

Antioxidant activity was measured for both the ethanolic harendong leaf extract and ascorbic acid as a comparator. Dissolve 0.7 mg of extract and 0.6 mg of ascorbic acid separately in distilled water to a final volume of 100 mL. Mix 1 mL of each solution with 3 mL of FRAP reagent and incubate at 37°C for 30 minutes. Measure absorbance at 593 nm. All measurements were performed in triplicate (Dewi, 2019; Mukhriani *et al*., 2019)

This study was intended as a characterization of harendong leaf extract rather than an optimization study; consequently, FRAP activity was assessed at a single extract concentration rather than across a concentration series, and only one solvent/extraction method (96% ethanol, Soxhlet) was evaluated. These scope limitations should be considered when comparing the present FRAP values with IC_{50}-based results from other harendong studies employing multiple solvents or concentration series.

RESULT AND DISCUSSION

Phytochemical Screening

Phytochemical screening revealed the secondary metabolite classes present in the ethanolic extract of harendong leaf (*M. malabathricum* L.), including phenols and tannins, flavonoids, steroids, terpenoids, alkaloids, saponins, and glycosides, using specific reagents. These results provide a preliminary indication of the bioactive compounds that may contribute to the extract's antioxidant activity, as shown in Table 1.

Table 1. Phytochemical screening of harendong leaf

No.	Compound Class	Result
1	Phenols and tannins	+
2	Flavonoids	+
3	Steroids	+
4	Terpenoids	+
5	Alkaloids	+
6	Saponins	+
7	Glycosides	+

According to the phytochemical screening results (Table 1), the ethanolic extract of harendong leaf contains several types of secondary metabolites. These include phenols and tannins, flavonoids, alkaloids, saponins, glycosides, steroids, and terpenoids. The diversity of these secondary metabolites indicates the potential of harendong leaf extract as a source of biological activity, particularly as a natural antioxidant.

Total Phenolic Content

The next stage following phytochemical screening was the determination of total phenolic content, which began with the construction of a gallic acid calibration curve at concentrations of 10–50 ppm, as shown in Figure 1.

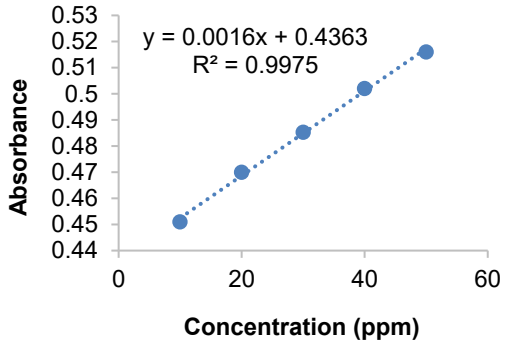

Figure 1. Gallic acid calibration curve

The gallic acid calibration curve produced a linear regression equation of y = 0.0016x + 0.4363, with R^2 = 0.9975, indicating a strong linear correlation between concentration and absorbance. This curve was thus considered suitable for use in determining the total phenolic content of the ethanolic harendong leaf extract.

Table 2. Sample results for the determination of total phenolic content (TPC)

Replicate	Absorbance	Phenolic Content (mg GAE/g)
1	0.689	64.19
2	0.688	64.19
3	0.699	71.06
Mean ± SD		66.48 ± 3.97

Table 2 reports the total phenolic content of ethanolic harendong leaf extract as 66.48 ± 3.97 mg GAE/g extract (n=3). This total phenolic content of 66.48 ± 3.97 mg GAE/g indicates a significant phenolic compound content in this plant. Hydroxyl (-OH) groups in phenolic compounds allow them to donate electrons or hydrogen atoms. This helps them act as antioxidants because they can stop oxidation reactions. A direct TPC benchmark within Melastoma malabathricum itself is not yet available in the literature; the closest comparable value is that of Mukhriani et al. (2019), who reported a phenolic content of 95.28 mg GAE/g extract in grape leaves (Vitis vinifera), used here only as a secondary reference given the lack of a Melastoma-specific TPC dataset. The lower value obtained in the present study relative to that reference may be attributed to several factors, including extraction method, solvent type and concentration, solvent-to-sample ratio, plant habitat conditions, leaf age, and sample drying and storage methods. Notably, the strong antioxidant activity reported for M. malabathricum leaf in DPPH- and ABTS-based studies (IC_{50} of 12.51 µg/mL and 8.21 µg/mL, respectively; Apmarja et al., 2025) is consistent with a substantial phenolic content in this species, supporting the TPC value obtained here.

Antioxidant Capacity by the FRAP Method

The $FeSO_4 \cdot 7H_2O$ calibration curve, constructed using concentrations ranging from 10 to 50 µmol/L, produced a regression equation of y = 0.004x + 0.0218 with R^2 = 0.9549 (Figure 2). This linear relationship between standard concentration and absorbance served as the basis for calculating the antioxidant activity of the ethanolic harendong leaf extract using the FRAP method. This R^2 value is noticeably lower than the R^2 = 0.9975 obtained for the gallic acid calibration curve used for TPC, and below the ~0.99 threshold generally desired for reliable quantitative spectrophotometric determination. This lower linearity may modestly reduce the precision of the derived FRAP concentrations and is a limitation to consider when interpreting the absolute antioxidant activity values; repeating the $FeSO_4 \cdot 7H_2O$ calibration with additional replicates or a narrower concentration range is recommended in future work to improve curve fit.

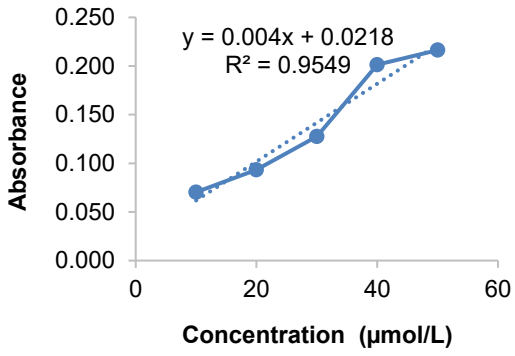

Figure 2. $FeSO_4 \cdot 7H_2O$ calibration curve

The $FeSO_4 \cdot 7H_2O$ calibration curve in Figure 2 shows a linear relationship between standard concentration and absorbance. The regression equation was used to determine the antioxidant activity of ethanolic harendong leaf extract (*M. malabathricum* L.) using the FRAP method.

Table 3. Antioxidant activity measurement results for harendong leaf (*M. malabathricum* L.)

Replicate	Sample Absorbance	Antioxidant Activity (mmol Fe^{2+}/g)
1	0.200	1.40
2	0.199	1.36
3	0.206	1.61
Mean ± SD		1.46 ± 0.14

Table 4. Antioxidant activity measurement results for ascorbic acid

Replicate	Ascorbic Acid Absorbance	Antioxidant Activity (mmol Fe^{2+}/g)
1	0.260	4.13
2	0.255	3.93
3	0.255	3.93
Mean ± SD		3.99 ± 0.12

According to Table 3 and Table 4, the antioxidant activity of the ethanolic harendong leaf extract was measured at 1.46 ± 0.14 mmol Fe^{2+}/g. This value is lower than that of ascorbic acid, which served as the positive control (3.99 ± 0.12 mmol Fe^{2+}/g), indicating that the reducing capacity of the extract remained below that of the reference standard. An independent-samples (Welch's) t-test confirmed that this difference was statistically significant ($t = -24.84$; $p < 0.001$, $n = 3$ per group), supporting the conclusion that the antioxidant activity of the extract was significantly lower than that of ascorbic acid.

The antioxidant activity measured at 1.46 ± 0.14 mmol Fe^{2+}/g shows that the extract can reduce Fe^{3+} ions to Fe^{2+} using the FRAP method. However, this value remained lower than that of ascorbic acid (3.99 ± 0.12 mmol Fe^{2+}/g). This difference is reasonable, given that ascorbic acid is a pure compound with high reducing power. In contrast, harendong leaf extract consists of a mixture of various secondary metabolites, such that its antioxidant activity is highly dependent on the type and proportion of bioactive compounds it contains.

Mechanistically, the phenolic content of harendong leaf extract is expected to relate closely to its antioxidant activity, although this was not tested here as a formal statistical correlation, since only a single extract condition was analyzed. Phenolic compounds function as electron donors, so higher concentrations of these compounds are expected to enhance the potential for Fe^{3+}-to-Fe^{2+} reduction. In addition to phenolics, flavonoids and tannins identified in the phytochemical screening likely contribute to the extract's antioxidant capacity. This FRAP-based reducing activity is consistent in direction with the strong radical-scavenging activity previously reported for harendong leaf using DPPH and ABTS assays (IC_{50} of 12.51 µg/mL and 8.21 µg/mL, respectively; Apmarja et al., 2025; Harahap et al., 2022), although the two families of assays measure different mechanisms (SET versus HAT) and are expressed in different units, so a direct numerical comparison is not appropriate. Overall, these results indicate that ethanolic harendong leaf extract holds promise for development as a source of natural antioxidants. However, its activity remains lower than that of ascorbic acid as the reference standard.

CONCLUSION

The ethanolic extract of harendong leaf (*M. malabathricum* L.) contains secondary metabolites that exhibit potential antioxidant activity. The total phenolic content, determined by the Folin–Ciocalteu method, was 66.48 ± 3.97 mg GAE/g, while testing with the FRAP method yielded an antioxidant activity value of 1.46 ± 0.14 mmol Fe^{2+}/g. Although this value remained lower than that of ascorbic acid used as the reference standard (3.99 ± 0.12 mmol Fe^{2+}/g), these findings support the potential of ethanolic harendong leaf extract for development as a source of natural antioxidants, consistent with its phenolic compound content. Future work should evaluate multiple solvents and extraction methods, include complementary DPPH/ABTS assays for direct comparison with prior harendong studies, and assess FRAP activity across a concentration series (dose-response) to further strengthen these findings.

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