*Original Research Article*

Determination of Total Tannin Content and Antioxidant Activity of the Ethanol Extract from Senduduk Leaves (*Melastoma malabathricum* L.)

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ABSTRACT

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| **Background:** Antioxidants are molecules capable of donating one or more electrons to free radicals, thereby stabilizing and neutralizing them. By doing so, antioxidants help protect the body from oxidative stress and its associated health risks.**Aims:** This study aims to determine the total tannin content and antioxidant activity of Senduduk leaves using a UV‑Vis spectrophotometer method.**Study design:** Experimental laboratory study involving maceration extraction, qualitative and quantitative phytochemical analysis, and DPPH antioxidant assay.**Place and Duration of Study:** Department of Pharmacy, Faculty of Pharmacy, Universitas Perintis Indonesia, Padang, West Sumatera, Indonesia January-June 2023.**Methodology:** Extraction was performed by maceration with 70% ethanol solvent. Tannins in the ethanol extract of Senduduk leaves were identified qualitatively (bluish‑black color and precipitate formation with 5% FeCl₃ and gelatin) and quantified by measuring absorbance at 764 nm using a UV‑Vis spectrophotometer. Antioxidant activity was determined via the DPPH method, measuring absorbance at 519 nm.**Results:** Qualitative analysis confirmed presence of tannins. The maximum absorption wavelength of tannic acid was found at 764 nm. Total tannin content was 32.71% w/w ± 0.06. Validation parameters for tannin determination were BD = 1.63 ppm, BK = 5.44 ppm, and CV = 0.18%. For antioxidant activity, the IC₅₀ value of the ethanol extract was 24.00 ppm, with a GAE value of 10.61 mg. The antioxidant activity of the ethanolic extract of Senduduk leaves was assessed using the DPPH radical scavenging method, a widely accepted and simple assay to evaluate free radical inhibition potential. The extract exhibited an IC₅₀ value of 24.00 µg/mL, which classifies it as having moderate antioxidant activity.**Conclusion:** Senduduk leaves possess very strong antioxidant activity. The findings suggest that the tannin-rich extract contributes significantly to the antioxidant potential of Senduduk leaves, indicating its potential use as a natural antioxidant in nutraceutical or pharmaceutical formulations. Future studies should focus on the isolation, fractionation, and mechanistic evaluation of specific active compounds to further explore their therapeutic potential. |

*Keywords: Melastoma malabathricum, tannin content, antioxidant activity, DPPH assay, UV-Vis spectrophotometry*

1. INTRODUCTION

Excessive oxidation reactions in the human body can lead to the formation of highly reactive free radicals (Chaudhary et al. 2023). These free radicals can cause damage to cellular structures, disrupt cellular functions, and contribute to the development of degenerative diseases such as aging, cancer, and other chronic conditions (Yoshikawa and You 2024). To prevent these harmful effects, the formation and activity of free radicals must be inhibited, and this can be achieved through the use of antioxidant compounds (Mukherjee et al. 2024).

Antioxidants are molecules capable of donating one or more electrons to free radicals, thereby stabilizing and neutralizing them. By doing so, antioxidants help protect the body from oxidative stress and its associated health risks. Many natural antioxidants are found in plants, particularly in secondary metabolites such as flavonoids, which are well known for their potent antioxidant properties (Chaudhary et al. 2023). One plant that has shown promise as a natural antioxidant source is *Melastoma malabathricum* L., commonly known as Senduduk (Mayasari et al. 2025).

Among the various parts of the Senduduk plant, the leaves are the most widely used in traditional medicine. Empirically, Senduduk leaves have been used to treat ailments such as dysentery, diarrhea, skin infections, wounds, and mouth ulcers (Sapitri, Lara, and Sitorus 2020). Phytochemical investigations have revealed that Senduduk leaves contain a variety of secondary metabolites, including flavonoids, tannins, saponins, glycosides, steroids, and terpenoids (Hainil, Rachdiati, and Prawita 2022).

Tannins, in particular, are active compounds known for their astringent, antidiarrheal, antibacterial, and antioxidant properties. They are complex phenolic substances that interact with proteins and metals, contributing to a range of biological activities, including antioxidant functions (Cosme et al. 2025). Tannins are generally classified into two main types: hydrolyzable tannins and condensed tannins (Motta et al. 2025).Several analytical techniques can be employed to determine tannin content, each with its own advantages. These methods include acid-base titration, UV-Visible spectrophotometry, fluorescence, infrared spectrophotometry, volumetry, chromatography (such as HPLC and GC), and atomic absorption spectrophotometry (Watrelot 2021).

Although previous studies have identified the presence of flavonoids, tannins, and saponins in Senduduk leaf extracts, the total tannin content has not yet been quantified. In addition, while antioxidant activity of the ethanol extract has been demonstrated using the FRAP (Ferric Reducing Antioxidant Power) method, there is limited information on its activity as measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Therefore, this study aims to determine the total tannin content and evaluate the antioxidant activity of Senduduk leaf extract using the DPPH method.

2. material and methods

**Plant Material Collection, Identification, and Extraction Procedure**

The sample used was Senduduk leaves (*Melastoma malabathricum* L.), approximately 2 kg, collected from the Ampang Kualo area, Solok City, West Sumatra. Plant identification was performed to ensure the species used in the research. The sample was identified at the ANDA Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University, Padang, West Sumatra. Fresh Senduduk leaves, approximately 2 kg, were sorted, with stems removed, and washed thoroughly with water. The leaves were then air-dried until they became crisp when crushed. The dried leaves were then ground into powder using a blender, weighed to 250 g, and placed in a dark-colored maceration bottle or container. A 70% ethanol solvent was added, and the mixture was kept in a dark place for 3 x 24 hours (3 days), with occasional stirring during the first 6 hours and then left undisturbed. The maceration was filtered using filter paper, and the residue was returned to the container for re-maceration, continuing until the filtrate became clear or colorless. The combined filtrate was then concentrated using a rotary evaporator to obtain a thick extract (Sapitri, Lara, and Sitorus 2020).

**Organoleptic Examination, Yield Calculation, Drying Loss, Ash Content**

The ethanolic extract of *Melastoma malabathricum* leaves was evaluated for its organoleptic properties, including form, color, and odor, through visual and olfactory assessments under standard laboratory conditions. The extract yield was calculated by comparing the weight of the dried extract to the initial weight of the dried plant material, expressed as a percentage. Loss on drying was determined by drying a known amount of the extract at 105°C until a constant weight was achieved, with the percentage weight loss indicating moisture and volatile content. Total ash content was measured by incinerating a weighed sample at 550°C until only inorganic residue remained, and the ash percentage was calculated to assess the mineral content and possible impurities in the extract.

**Qualitative Analysis of Tannins**

One gram of ethanol extract from Senduduk leaves (*Melastoma malabathricum* L.) was dissolved in 100 mL of distilled water, and then 1 mL of 2% NaCl was added. The solution was divided into two parts. Part A was treated with three drops of 5% FeCl3 reagent. A positive result for tannins is indicated by the formation of a bluish-black color. Extract B was mixed with gelatin solution containing 2% NaCl; if a precipitate forms, it indicates the presence of tannins.

**Quantitative Analysis of Tannins**

Fifty mg of tannic acid was dissolved in distilled water in a 100 mL volumetric flask up to the mark, resulting in a stock solution concentration of 500 ppm. One of the concentrations from the series was taken to determine the maximum absorption wavelength; in this study, a standard solution concentration of 30 ppm was used. The maximum absorption wavelength of the tannic acid solution was measured in the wavelength range of 400-800 nm using UV-Vis spectrophotometry. From the stock solution of 500 ppm tannic acid, 2, 4, 6, 8, and 10 mL were pipetted and diluted with distilled water in a 100 mL volumetric flask up to the mark, yielding concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm of tannic acid. Each concentration of the solution was pipetted into a dark vial (0.5 mL), mixed with 1 mL of Folin-Ciocalteu reagent, and then 2 mL of 15% sodium carbonate solution was added, allowing it to stand for 15 minutes. The absorbance was measured using UV-Vis spectrophotometry at the maximum absorption wavelength of tannic acid at 764 nm, and a standard calibration curve was created. Ten mg of the concentrated ethanol extract of Senduduk leaves was weighed, dissolved in distilled water in a 100 mL volumetric flask up to the mark, resulting in a concentration of 100 µg/mL. A 0.5 mL aliquot of the concentrated ethanol extract of Senduduk leaves was pipetted into a vial, followed by the addition of 1 mL of Folin-Ciocalteu reagent and then 2 mL of 15% sodium carbonate in the dark vial, shaken to homogenize. It was allowed to stand for 15 minutes, and then the absorbance was measured with the UV-Vis spectrophotometer at 764 nm, with three repetitions performed. The sample concentration was calculated using the linear regression equation, and the total tannin content was then determined.

**Antioxidant Activity Assay**

The DPPH radical scavenging assay was performed to evaluate the antioxidant activity of the ethanolic extract of Senduduk leaves, following modifications of established protocols (Molyneux, 2004; Mosquera et al., 2007). A stock solution of DPPH (100 µg/mL) was prepared by accurately weighing 10 mg of 2,2-diphenyl-1-picrylhydrazyl (DPPH, ≥ 95% purity, Sigma-Aldrich) and dissolving it in 100 mL of analytical grade methanol (Merck). This solution was diluted to 35 µg/mL with methanol for use in the assay. The maximum absorption wavelength of the DPPH solution was determined to be 519 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800) within the 400–800 nm range. Gallic acid (≥ 98% purity, Sigma-Aldrich) was used as a positive control antioxidant. A stock solution of gallic acid (500 µg/mL) was prepared by dissolving 12.5 mg in 25 mL methanol, then diluted with a methanol: distilled water mixture (1:1) to 50 µg/mL. Serial dilutions were made to obtain final concentrations of 1, 2, 3, 4, and 5 µg/mL. The ethanolic extract stock solution was prepared by dissolving 25 mg of the extract in 25 mL methanol (1000 µg/mL) and further diluted to obtain working concentrations of 5, 10, 15, 20, and 25 µg/mL using the methanol: distilled water (1:1) mixture. For the assay, 2 mL of each sample or standard solution was mixed with 4 mL of the 35 µg/mL DPPH solution in a 10 mL amber vial to protect from light. The mixture was homogenized by vortexing for 10 seconds and then incubated in the dark at room temperature (25 ± 2 °C) for 30 minutes. After incubation, the absorbance was measured at 519 nm against a blank (methanol) using the UV-Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\% Inhibition = \frac{A0-As}{A0}$$

Where A0 is the absorbance of the control (DPPH solution without sample) and as is the absorbance of the sample or standard. The IC₅₀ value, defined as the concentration of sample required to scavenge 50% of DPPH radicals, was determined by plotting the % inhibition against concentration and calculated using linear regression analysis. All tests were conducted in triplicate to ensure accuracy and reproducibility.

3. results and discussion

**Macroscopic and Physicochemical Characteristics of the Extract**

The ethanolic extract of Senduduk leaves exhibited a thick consistency, dark green coloration, and a characteristic herbal odor. These organoleptic properties serve as preliminary indicators of extract identity and quality, assessed using the human senses. Such evaluations are commonly applied in herbal drug standardization for rapid and non-instrumental verification of authenticity and consistency (Patel 2025).

**Table 1. Macroscopic and Physicochemical Properties of the Extract**

|  |  |
| --- | --- |
| **Parameter** | **Observation/Result** |
| Form | Thick extract |
| Color | Dark green |
| Odor | Characteristic |
| Extract yield | 7,431 % |
| Loss on drying | 8,2 % |
| Total ash content | 6,1 % |

The physicochemical parameters—including extract yield, drying loss, and total ash content—were also determined to assess the extract’s quality and stability. As shown in Table 1, the extract yield was 7.431%, which meets the acceptable range specified by the Indonesian Ministry of Health (≤ 15%). This yield reflects the extraction efficiency from the dry simplicia. The drying loss was 8.2%, indicating the proportion of volatile matter or moisture content removed during the dryingprocess (Kim et al. 2013). Additionally, the total ash content was 6.1%, representing the residual inorganic material after incineration, which provides insight into the mineral composition and possible contamination with extraneous matter such as soil or sand. These parameters are critical for evaluating extract purity and are essential components of pharmacognostic analysis (Prakash et al. 2019).

**Qualitative Analysis of Tannins**

The presence of tannins in the ethanolic extract of Sendudukleaves was confirmed through qualitative phytochemical screening using ferric chloride (FeCl₃) and gelatin reagents. A bluish-black coloration observed upon the addition of 5% FeCl₃ to Filtrate I + 2% NaCl indicates a positive reaction for hydrolyzable tannins, which are known to form colored complexes with iron ions.

**Table 2. Qualitative Test for Tannins in the Ethanolic Extract of Senduduk Leaves**

|  |  |  |  |
| --- | --- | --- | --- |
| **Filtrate** | **Reagent** | **Observation** | **Conclusion** |
| Filtrate I + 2% NaCl | 5% FeCl₃ | Formation of bluish-black color | **+** |
| Filtrate II + 2% NaCl | Gelatin | Formation of precipitate | **+** |

Note: “+” indicates a positive result for the presence of tannins based on qualitative identification in the extract.

Additionally, the formation of a precipitate in the reaction between Filtrate II + 2% NaCl and gelatin reagent further supports the presence of tannins, particularly condensed tannins, which interact with proteins to form insoluble complexes. These results are consistent with the known phytochemical profile of *M. malabathricum*, a plant widely reported to contain various types of polyphenolic compounds, including tannins. The identification of these compounds is important due to their potential antioxidant, antimicrobial, and astringent properties.

**Total Tannin Content and Antioxidant Activity**

The total tannin content of the ethanolic extract of Senduduk leaves was determined to be 32.711 ± 0.0615 µg/mL. Tannins, as phenolic compounds, contribute significantly to the antioxidant potential of plant extracts due to their ability to donate hydrogen atoms or electrons and stabilize free radicals.

**Table 3. Total Tannin Content and DPPH Radical Scavenging Activity**

|  |  |  |
| --- | --- | --- |
| **Parameter** |  | **Value** |
| Total Tannin Content |  | 32.711 ± 0.0615 µg/mL |
| IC₅₀ of Ethanolic Extract of Senduduk Leaves |  | 24.00 µg/mL |
| IC₅₀ of Gallic Acid |  | 2.26 µg/mL |
| Equivalence of Ethanolic Extract to Gallic Acid |  | 10.61  |

The antioxidant activity of the ethanolic extract of Senduduk leaves was assessed using the DPPH radical scavenging method, a widely accepted and simple assay to evaluate free radical inhibition potential. The extract exhibited an IC₅₀ value of 24.00 µg/mL, which classifies it as having moderate antioxidant activity. According to Blois (BLOIS 1958), substances with IC₅₀ values below 10 µg/mL are considered to have strong antioxidant activity, those between 10–50 µg/mL as moderate, and values above 100 µg/mL as weak. Therefore, the observed value supports the potential of this plant extract as a viable natural antioxidant.

When compared to gallic acid, which exhibited a significantly lower IC₅₀ value of 2.26 µg/mL, the extract was less potent. However, this is expected, considering that gallic acid is a pure compound known for its strong antioxidant capacity, while the extract is a crude mixture containing a variety of phytochemicals with differing antioxidant potentials. The calculated equivalence to gallic acid (10.61) indicates that approximately 10.61 mg of the extract is required to exert an antioxidant effect equivalent to 1 mg of gallic acid. This ratio serves as a useful reference for future formulation and dosage development in herbal antioxidant products.

The moderate antioxidant activity observed is likely attributable to the presence of tannins, which are known to act as hydrogen donors and radical scavengers. This is consistent with previous reports that identified high levels of polyphenolic compounds, especially tannins and flavonoids, in M. malabathricum leaves.

Overall, these findings reinforce the potential application of *M. malabathricum* leaf extract as a natural antioxidant agent, particularly in nutraceutical and pharmaceutical formulations targeting oxidative stress-related disorders. Further fractionation and characterization of the active constituents may help to identify the most potent antioxidant components within the extract.

4. Conclusion

The ethanolic extract of Senduduk leaves yielded 7.43% w/w, with acceptable moisture (8.2%) and ash (6.1%) contents. Qualitative tests confirmed the presence of both hydrolyzable and condensed tannins. Quantitatively, the extract contained 32.711 ± 0.062 µg/mL of total tannins. In the DPPH assay, it exhibited a moderate antioxidant activity with an IC₅₀ of 24.00 µg/mL—equivalent to 10.61 mg of extract per 1 mg of gallic acid. These findings suggest that the tannin-rich extract contributes significantly to the antioxidant potential of Senduduk leaves, indicating its potential use as a natural antioxidant in nutraceutical or pharmaceutical formulations. Future studies should focus on the isolation, fractionation, and mechanistic evaluation of specific active compounds to further explore their therapeutic potential.

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