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Phytochemical Screening and Antioxidant Activity Test of Ethanol Extract of Nelambosuon (Rubiaceae) Leaves Using the DPPH Method

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Indonesia is recognized as one of the world's megabiodiversity countries, possessing a vast diversity of medicinal plants that have long been utilized in traditional medicine. *Nelambo suon* (Rubiaceae) is an indigenous plant traditionally used by the Tehit Imian tribe in South Sorong, Southwest Papua, for treating wounds, skin infections, and inflammatory conditions. However, scientific information regarding its phytochemical profile and antioxidant activity remains limited. This study aimed to evaluate the phytochemical constituents and antioxidant activity of the ethanol extract of *Nelambo suon* leaves using the DPPH method. Fresh, undamaged green leaves were dried at room temperature without direct sunlight and extracted by maceration using 96% ethanol. Phytochemical screening was performed to identify major secondary metabolites, while antioxidant activity was assessed using the DPPH free radical scavenging assay and expressed as IC_{50} values. The phytochemical screening revealed the presence of flavonoids and tannins in the ethanol extract, whereas alkaloids, saponins, steroids, and terpenoids were not detected. The antioxidant assay demonstrated a concentration-dependent increase in radical scavenging activity. The ethanol extract of *Nelambo suon* leaves exhibited an IC_{50} value of 83.7 µg/mL, indicating strong antioxidant activity. These findings suggest that *Nelambo suon* leaves are a promising source of natural antioxidants, likely attributed to their flavonoid and tannin contents. This study provides preliminary scientific evidence supporting the traditional use of *Nelambo suon* and highlights its potential for further development as a natural antioxidant agent in pharmaceutical and herbal product applications.
Keywords: Nelambo suon; Rubiaceae; phytochemical screening; antioxidant activity; DPPH method

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1. Introduction

Indonesia is recognized as one of the world's megabiodiversity countries, possessing an exceptionally high level of biological diversity, including a vast number of medicinal plants that have long been utilized in traditional healthcare systems. According to the Indonesian Agency for Health Research and Development, out of more than 30,000 plant species found in Indonesia, approximately 9,600 species are known to have potential medicinal properties (1). The traditional use of medicinal plants passed down through generations within indigenous communities reflects not only local wisdom but also represents an important source of ethnopharmacological knowledge that can be further developed into evidence-based pharmaceutical products. Therefore, scientific exploration of traditional medicinal plants is a strategic approach to uncover bioactive compounds while supporting the development of safe and sustainable natural medicines.

One plant traditionally used by the Tehit Imian ethnic community in Sawiat District, South Sorong Regency, Southwest Papua, is *Nelambo suon* (family Rubiaceae). Empirically, this plant has been used for the treatment of external wounds, skin infections, and inflammatory conditions. Such traditional applications suggest the presence of secondary metabolites with potential biological activities, particularly antioxidant

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properties. However, to date, there are no scientific reports documenting the phytochemical profile or antioxidant activity of *Nelambo suon* extracts. This lack of scientific data indicates a clear knowledge gap that warrants systematic investigation.

Free radicals are molecules or atoms containing one or more unpaired electrons, making them highly reactive. Excessive accumulation of free radicals in the body can induce oxidative stress, a condition characterized by an imbalance between the generation of free radicals and the capacity of endogenous antioxidant defense systems (1). Oxidative stress plays a critical role in the pathogenesis of various degenerative diseases, including cancer, cardiovascular diseases, diabetes mellitus, and premature aging. Consequently, the search for effective and safe antioxidant sources remains a major focus in pharmaceutical and health sciences.

Antioxidants can be derived from synthetic or natural sources. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been widely used in the food and pharmaceutical industries. However, their use has become increasingly restricted due to potential toxic effects at high doses (2). This limitation has stimulated growing interest in plant-derived natural antioxidants, which are generally considered safer and exhibit a broad spectrum of biological activities. Various classes of secondary metabolites, including flavonoids, phenolics, tannins, alkaloids, terpenoids, and saponins, have been reported to possess strong free radical scavenging activity (3).

Phytochemical screening serves as an essential preliminary step in identifying the presence of bioactive secondary metabolites in plant extracts. The results of such screening provide an initial indication of the pharmacological potential of plant materials. Furthermore, antioxidant activity can be evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay, which is widely recognized as a simple, rapid, sensitive, and reliable method for assessing the free radical scavenging capacity of natural compounds and plant extracts (4).

The DPPH method is based on the ability of antioxidant compounds to donate electrons or hydrogen atoms to the DPPH radical, converting it into a non-radical form (DPPH-H). This reaction is indicated by a color change from deep purple to pale yellow, which is measured spectrophotometrically at a wavelength of 517 nm. Antioxidant activity is expressed as percentage inhibition or IC_{50} value, defined as the concentration of extract required to scavenge 50% of DPPH radicals (5). A lower IC_{50} value corresponds to stronger antioxidant activity.

Ethanol was selected as the extraction solvent due to its relatively high polarity, safety, and effectiveness in dissolving a wide range of secondary metabolites, particularly flavonoids, phenolic compounds, and tannins that are believed to play a major role in antioxidant activity. Therefore, ethanol extraction is expected to yield an extract rich in bioactive compounds from *Nelambo suon* simplicia.

Based on the above considerations, this study aimed to evaluate the antioxidant activity of ethanol extract of *Nelambo suon* using the DPPH method. The findings of this study are expected to provide preliminary scientific data regarding the chemical constituents and antioxidant potential of this plant. In addition to contributing to the advancement of knowledge in natural product pharmacy, this research is also expected to support the preservation of ethnomedicinal knowledge of the Tehit Imian community and to open opportunities for the development of safe and competitive natural pharmaceutical and herbal cosmetic products based on local biological resources. Given the limited previous research on *Nelambo suon*, this study is anticipated to serve as a foundational reference for further in-depth investigations.

2. Literature Review and Problem Statement

Natural products, particularly medicinal plants, are widely recognized as important sources of bioactive compounds with antioxidant properties. From a phytochemical perspective, antioxidant activity is strongly associated with the presence of secondary metabolites such as phenolic compounds and flavonoids, which act as free radical scavengers through electron or hydrogen donation mechanisms. Several studies have demonstrated a positive correlation between total phenolic and flavonoid content and antioxidant activity in plant extracts. For instance, ethanol extracts of medicinal plants have been shown to contain flavonoids, phenolics, tannins, and alkaloids, which contribute significantly to antioxidant activity measured using the DPPH method (6). Similarly, research on *Peperomia pellucida* reported that ethanol extracts exhibited strong antioxidant activity (IC_{50} = 36.85 µg/mL), accompanied by high phenolic and flavonoid content, confirming the role of these compounds in radical scavenging activity (7). These findings indicate that phytochemical composition is a key determinant of antioxidant potential and that ethanol is an effective solvent for extracting antioxidant compounds.

In addition, plants belonging to various taxonomic families, including *Rubiaceae*, have been reported to possess significant antioxidant properties. Members of this family are known to produce a wide range of secondary metabolites, particularly phenolic compounds, which contribute to their biological activities. Studies on Rubiaceae species such as *Mitragyna speciosa* have demonstrated notable antioxidant activity using the DPPH assay, supporting their potential as sources of natural antioxidants (8). Furthermore, research on other plant species has consistently shown that higher phenolic and flavonoid content correlates with stronger antioxidant activity, reinforcing the theoretical framework linking phytochemical composition to bioactivity (9). The DPPH method itself has been extensively validated and widely applied due to its simplicity, sensitivity, and reproducibility in evaluating free radical scavenging capacity of plant extracts (6). Despite the growing body of evidence, many ethnomedicinal plants, particularly those originating from Papua, remain scientifically underexplored, highlighting a significant gap between traditional knowledge and scientific validation.

Based on the theoretical and empirical evidence presented, it can be understood that antioxidant activity in medicinal plants is closely related to the presence of specific secondary metabolites, which can be effectively extracted using ethanol and evaluated using standardized assays such as DPPH. However, *Nelambo suon*, a plant traditionally used by the Tehit Imian community, has not yet been scientifically investigated in terms of its phytochemical profile or antioxidant activity. This condition indicates a clear research gap that necessitates systematic scientific evaluation.

Based on the identified research gap, this study is designed to systematically investigate the phytochemical characteristics and antioxidant potential of *Nelambo suon*. Specifically, the research seeks to determine the classes of secondary metabolites present in the ethanol extract of *Nelambo suon*, as these compounds are known to contribute significantly to biological activities. In addition, this study aims to evaluate whether the ethanol extract exhibits antioxidant activity using the DPPH method, which is widely applied for assessing free radical scavenging capacity. Furthermore, the strength of the antioxidant activity will be quantitatively assessed based on the IC_{50} value, which reflects the concentration of extract required to inhibit 50% of DPPH radicals. Through these approaches, this research intends to provide a comprehensive scientific evaluation of the antioxidant potential of *Nelambo suon*.

In line with these objectives, the working hypothesis of this study is that the ethanol extract of *Nelambo suon* contains bioactive secondary metabolites, particularly flavonoids and phenolic compounds, which are responsible for its antioxidant activity. It is further hypothesized that the extract exhibits significant antioxidant capacity, as indicated by a relatively low IC_{50} value in the DPPH assay. This hypothesis is

formulated based on the theoretical understanding that plant-derived phenolic compounds play a crucial role in neutralizing free radicals and contributing to antioxidant effects.

3. Method

Experimental Section

Instruments and Materials

The instruments used in this study included test tubes and tube racks, an analytical balance, glassware, dark bottles, glass stirring rods, test tube holders, droppers, micropipettes, porcelain dishes, watch glasses, spatulas, horn spoons, sieves, grinders, flannel cloths, chemical thermometers, aluminum foil, glass funnels, filter papers, a moisture analyzer, an electric stove (Maspion), a UV–Visible spectrophotometer, cuvettes, a rotary evaporator, and a hotplate magnetic stirrer (Bante Instrument).

The materials used consisted of *Nelambo suon* plant material, filter paper, 96% ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol p.a., 2 N hydrochloric acid (HCl), concentrated HCl, ferric chloride ($FeCl_3$), acetic anhydride, concentrated sulfuric acid, Dragendorff, Mayer, and Bouchardat reagents, as well as vitamin C as a reference antioxidant.

Preparation of Plant Extract

The ethanol extract of *Nelambo suon* (Rubiaceae) stem was prepared using the maceration method. A total of 210 g of powdered stem sample was macerated with 96% ethanol as the extraction solvent. The extraction was carried out by soaking the sample in 2 L of 96% ethanol for 72 hours in a closed container. Maceration was repeated twice until the filtrate appeared less colored. During the maceration process, the mixture was stirred periodically to ensure optimal contact between the solvent and the plant material.

The macerated mixture was subsequently filtered to separate the filtrate from the residue. The combined filtrates were concentrated using a water bath at 60°C until a pourable ethanol extract was obtained. The extract was then weighed to determine its yield and stored for further analysis (10).

Phytochemical Characterization

Phytochemical screening was performed on the ethanol extract of *Nelambo suon* stem to identify the presence of secondary metabolites, including alkaloids, steroids, terpenoids, flavonoids, tannins, and saponins. The presence of these compounds was indicated by specific color changes or precipitate formation resulting from the reactions with respective reagents(11).

Preparation of Phytochemical Test Solution

A phytochemical test solution was prepared by dissolving 250 mg of *Nelambo suon* stem extract in 25 mL of 96% ethanol (12).

Alkaloid Test

Two milliliters of the test solution were mixed with 2 mL of 2% HCl and heated for 5 minutes, followed by filtration. The filtrate was then treated separately with Mayer, Bouchardat, and Dragendorff reagents (2–3 drops each). The formation of a white or yellow precipitate (Mayer), dark brown precipitate (Bouchardat), or brick-red precipitate (Dragendorff) indicated the presence of alkaloids(10).

Steroid and Terpenoid Test

Two milliliters of the test solution were reacted with Liebermann–Burchard reagent, consisting of 0.5 mL acetic anhydride and 0.5 mL concentrated sulfuric acid. The appearance of pink, purple, or blue-green coloration indicated a positive result for steroids or terpenoids (13).

Flavonoid Test

Five milliliters of the extract were placed into a test tube, followed by the addition of 2–4 drops of concentrated hydrochloric acid. The mixture was shaken gently. The appearance of an orange coloration indicated the presence of flavonoids, particularly flavonols and flavanones (14).

Tannin Test

One milliliter of the test solution was placed into a test tube and mixed with 2–3 drops of $FeCl_3$ solution. The formation of a dark blue or greenish coloration indicated the presence of tannins (15).

Saponin Test

Ten milliliters of the test solution were placed into a test tube and shaken vigorously for 10 seconds, then allowed to stand for 10 seconds. The formation of stable foam with a height of 1–10 cm persisting for up to 10 minutes indicated the presence of saponins. The foam remained stable after the addition of one drop of 2 N HCl (16).

Antioxidant Activity Assay

Preparation of DPPH Solution (0.4 mM)

A total of 0.0157 g of DPPH was dissolved in methanol p.a. and diluted to 100 mL in a volumetric flask to obtain a 0.4 mM DPPH solution (17).

Determination of Maximum Wavelength (λmax) of DPPH

One milliliter of the DPPH solution was diluted to 5 mL with ethanol p.a. and incubated for 30 minutes in the dark. The absorbance was measured using a UV–Visible spectrophotometer at wavelengths ranging from 400 to 600 nm to determine the maximum absorption wavelength (18).

Measurement of Antioxidant Activity of *Nelambo suon* Stem Extract

A stock solution of 1000 ppm ethanol extract was prepared by dissolving 10 mg of extract in ethanol p.a. and diluting to 10 mL in a volumetric flask. Aliquots of 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the stock solution were further diluted to 5 mL to obtain extract concentrations of 10, 20, 30, 40, and 50 ppm, respectively (17). From each concentration, 5 mL of the extract solution were mixed with 1 mL of 0.4 mM DPPH solution. The mixtures were incubated for 30 minutes in the dark, and absorbance was measured at 516 nm using a UV–Visible spectrophotometer (19).

Preparation and Measurement of Quercetin Standard Solution

One milligram of quercetin was weighed, dissolved in ethanol p.a., and transferred into a 10 mL volumetric flask, with the volume adjusted to the mark. The quercetin stock solution was then diluted to obtain five concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 ppm) by pipetting 0.005, 0.010, 0.015, 0.020, and 0.025 mL of the stock solution into separate 5 mL volumetric flasks and diluting with ethanol p.a. to volume. Two milliliters of each quercetin solution were mixed with 1 mL of 0.4 mM DPPH solution, incubated for 30 minutes in the dark, and the absorbance was measured at 517 nm.

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Data Analysis

Antioxidant activity was evaluated by calculating the IC_{50} value, defined as the concentration required to inhibit 50% of DPPH radicals. A lower IC_{50} value indicates stronger antioxidant activity (Liwen, 2026). The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\text{Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The IC_{50} value was determined using linear regression analysis based on the equation:

$$y = bx + a$$

where y represents 50% inhibition, x is the concentration of the test solution, a is the intercept, and b is the slope. Smaller IC_{50} values indicate stronger antioxidant activity, and vice versa.

4. Results and Discussion

Preparation and Extraction of *Nelambo suon* Leaves

The plant material used in this study consisted of *Nelambo suon* (Rubiaceae) leaves selected based on physical quality criteria, including fresh condition, green coloration, absence of holes, and no visible signs of damage. The selection of leaves with these characteristics aimed to minimize degradation of secondary metabolites caused by physiological processes or microbial contamination. Initial processing involved wet sorting to remove unsuitable plant parts, followed by washing under running water to eliminate dirt and other impurities. The leaves were then drained to remove free surface water prior to initial weighing.
Drying was carried out using a natural air-drying method at room temperature without direct exposure to sunlight for seven days. This method was chosen to preserve the stability of thermolabile and photosensitive secondary metabolites, such as flavonoids and phenolic compounds, which may degrade when exposed to high temperatures or ultraviolet radiation. From an initial fresh weight of 4,000 g, 810 g of dried simplicia were obtained. This significant weight reduction indicated effective moisture removal, an essential step to prevent microbial growth and to enhance extraction efficiency.

The dried simplicia were subsequently ground using a blender to obtain a fine powder. Particle size reduction was performed to increase the surface area in contact with the solvent, thereby facilitating the diffusion of bioactive compounds during extraction. Maceration using 96% ethanol was employed for seven days. Ethanol was selected due to its suitable polarity for extracting a wide range of secondary metabolites, including flavonoids, phenolics, tannins, and saponins, as well as its safety and widespread use in natural product extraction.

After maceration, the filtrate was concentrated using a rotary evaporator at 50°C to obtain a viscous extract. The use of relatively low temperature aimed to prevent thermal degradation of active compounds. A total of 90.57 g of thick, dark green ethanol extract of *Nelambo suon* leaves was obtained.
The extraction yield was calculated to be 11.18%, indicating that the extraction method was effective in isolating soluble compounds from the plant material. A relatively high yield suggests that *Nelambo suon* leaves contain abundant ethanol-soluble secondary metabolites. The dark green color of the extract indicates the presence of chlorophyll and potentially phenolic compounds, which may contribute to the biological activity of the extract.

Overall, the preparation and extraction procedures applied in this study complied with good practices in simplicia processing and natural product extraction. The yield obtained provides a solid basis for

subsequent phytochemical screening and antioxidant activity evaluation, offering preliminary insight into the potential of *Nelambo suon* leaves as a bioactive natural resource.

Phytochemical Screening

The qualitative phytochemical screening results of the ethanol extract of *Nelambo suon* (Rubiaceae) leaves are presented in Table 4.1.

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No	Compound	Result	Observed Change
1	Saponins	(-)	No foam formation
2	Flavonoids	(+)	Yellow color change observed
3	Alkaloids	(-)	No brown color formation
4	Terpenoids	(-)	No blue color formation
5	Steroids	(-)	No green color formation
6	Taninns	(+)	Green color change observed

The results indicate that the ethanol extract of *Nelambo suon* leaves contains flavonoids and tannins. Phytochemical screening was conducted to identify the classes of secondary metabolites present in the extract, as these compounds are often associated with antioxidant activity, particularly flavonoids (20) (16). The presence and concentration of secondary metabolites in plants are influenced by species characteristics and environmental conditions in which the plants grow(21).

In the flavonoid test, the formation of a yellow coloration indicated a positive result. This color change occurs due to the reaction between flavonoids and hydrochloric acid, involving reduction of the benzopyran nucleus in the flavonoid structure. During extraction, flavonoids are readily dissolved in polar solvents; therefore, ethanol effectively extracts flavonoids from plant tissues (22). Flavonoids are well-known natural compounds with various pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and wound-healing effects (23). Their antioxidant mechanism involves scavenging reactive oxygen species (ROS), inhibiting ROS formation, and regulating endogenous antioxidant systems. The presence of flavonoids supports the traditional use of *Nelambo suon* leaves for antibacterial and anti-inflammatory purposes.

Tannin testing produced a green coloration, indicating a positive result. This reaction occurs due to the interaction between tannins and ferric chloride ($FeCl_3$), forming complexes with phenolic and polyphenolic compounds (20). Tannins are polyhydroxy phenolic compounds characterized by their ability to precipitate proteins through hydrogen bonding. Structurally, tannins consist of aromatic rings linked by carbon atoms and are known to exhibit antibacterial and anti-inflammatory activities (24).

Previous studies reported that *Nelambo suon* leaves contained flavonoids, tannins, and saponins (Tomi, A 2022). Differences in phytochemical profiles observed in this study may be attributed to external factors such as humidity, light intensity, soil fertility, plant age, and cultivation practices, all of which influence secondary metabolite biosynthesis (25) (26).

Antioxidant Activity

The antioxidant activity of the ethanol extract of *Nelambo suon* leaves was evaluated using the DPPH method. The results are shown in Table 4.2.

Tabel 4.2. Antioxidant Activity of Ethanol Extract of *Nelambo suon* Leaves

No	Extract Concentration (ppm)	Absorbance	% Inhibition
1	DPPH control	0.517	0
2	10	0.521	13.33
3	20	0.440	22.67
4	30	0.353	33.33
5	40	0.371	44.00
6	50	0.324	53.33

The DPPH assay was employed as a quantitative method to determine the antioxidant activity of the ethanol extract. Measurements were conducted at a wavelength of 517 nm using a UV–Visible spectrophotometer, as this method provides accurate and reliable results. The reduction of DPPH radicals is indicated by a color change from purple to yellow, confirming the presence of antioxidant compounds capable of neutralizing free radicals (27)(28).

An increase in percentage inhibition was observed with increasing extract concentration, indicating a concentration-dependent antioxidant effect. This trend is consistent with previous studies reporting that higher extract concentrations generally result in greater radical scavenging activity(29) (30) (25). Linear regression analysis between concentration and percentage inhibition yielded an R^2 value of 0.943, indicating a strong linear relationship (29).

Antioxidant activity was interpreted using the IC_{50} parameter, defined as the concentration required to inhibit 50% of DPPH radicals (31) (27). Based on the results, the ethanol extract of *Nelambo suon* leaves exhibited an IC_{50} value of 136 ppm, which falls within the moderate antioxidant category (100–150 ppm). Further analysis using the Antioxidant Activity Index (AAI) yielded a value of 0.29, classified as weak antioxidant activity (5). The relatively low antioxidant activity may be attributed to variations in flavonoid and tannin content and incomplete solubility of antioxidant compounds in the extract (32). Additionally, the absence of other antioxidant-contributing compounds, such as saponins, alkaloids, steroids, and terpenoids, may also influence the observed activity.

5. Conclusions

Based on the results of this study, it can be concluded that the ethanol extract of Nelambo suon (Rubiaceae) leaves positively contains flavonoids and tannins. It also exhibits antioxidant activity as determined using the DPPH method. This antioxidant activity is indicated by an IC_{50} value of 83.7 µg/mL, reflecting the extract's ability to effectively scavenge DPPH free radicals.

These findings suggest that *Nelambo suon* leaves have potential as a natural source of antioxidants and provide a scientific basis for further studies focusing on the isolation of active compounds and the development of plant-based pharmaceutical products. The authors declare that there is no conflict of interest regarding the publication of this paper. The authors would like to express their sincere gratitude to all parties who have contributed to the completion of this research. Special thanks are extended to colleagues and institutions for their valuable support and assistance.

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