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Phytochemical Study of Active Compounds in the Stem Extract of *Nelambo suon* (*Rubiaceae*)

Studi Fitokimia Senyawa Aktif Ekstrak Batang *Nelambo suon* (*Rubiaceae*)

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Abstract

Background: Papua has high biodiversity with significant potential as a source of traditional medicinal plants, including *Nelambo suon* (Rubiaceae), which has been empirically used by local communities. However, scientific information regarding the active compounds in its stem bark remains limited. **Objective:** This study aimed to identify the secondary metabolite groups present in the ethanol extract of *Nelambo suon* stem bark through phytochemical screening. **Methods:** The research was conducted experimentally using the maceration method with 80% ethanol as the solvent. The stem bark simplicia was extracted and qualitatively screened for alkaloids, flavonoids, tannins, and saponins using specific reagents. **Results:** The results showed that the ethanol extract of *Nelambo suon* stem bark contained alkaloids, flavonoids, tannins, and saponins, indicated by characteristic color changes, precipitate formation, and foam production. **Conclusion:** These findings suggest that *Nelambo suon* stem bark is a potential source of bioactive compounds and may be further developed as a herbal medicinal material based on local wisdom.

Keywords: *Stem, Nelambo suon, Rubiaceae, Phytochemical screening.*

Abstrak

Latar Belakang: Papua memiliki keanekaragaman hayati tinggi dengan potensi besar sebagai sumber tanaman obat tradisional, salah satunya *Nelambo suon* (Rubiaceae) yang dimanfaatkan secara empiris oleh masyarakat lokal. Namun, data ilmiah mengenai kandungan senyawa aktif pada batang tanaman ini masih terbatas. **Tujuan:** Penelitian ini bertujuan untuk mengidentifikasi golongan metabolit sekunder pada ekstrak etanol batang *Nelambo suon* melalui skrining fitokimia. **Metode:** Penelitian dilakukan secara eksperimental menggunakan metode maserasi dengan pelarut etanol 80%. Simplisia batang *Nelambo suon* diekstraksi, kemudian diuji secara kualitatif untuk mendeteksi alkaloid, flavonoid, tanin, dan saponin menggunakan pereaksi spesifik. **Hasil:** Hasil penelitian menunjukkan bahwa ekstrak etanol batang *Nelambo suon* positif mengandung alkaloid, flavonoid, tanin, dan saponin yang ditandai dengan perubahan warna dan pembentukan endapan atau buih sesuai karakteristik masing-masing senyawa. **Kesimpulan:** Keberadaan metabolit sekunder tersebut menunjukkan bahwa batang *Nelambo suon* berpotensi sebagai sumber senyawa bioaktif dan dapat dikembangkan lebih lanjut sebagai bahan obat herbal berbasis kearifan lokal.

Kata Kunci: *Batang, Nelambo suon, Rubiaceae, Skrining fitokimia.*

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Introduction

Indonesia is recognized as one of the countries with the highest levels of biodiversity in the world, encompassing a wide range of tropical ecosystems, including lowland areas, mountainous regions, as well as coastal and marine ecosystems. This high biodiversity is reflected in the large number of flora and fauna species and the high degree of endemism. The Papua region is considered an area of strategic importance and high priority for conservation efforts and scientific research development. One of the most significant forest resources for local communities is medicinal plants, which are commonly used as traditional remedies to treat various diseases. Indigenous communities in Papua have utilized medicinal plants for generations. Although modern medicines are now widely available, some Papuan communities continue to rely on traditional medicine due to limited access to public healthcare services. The use of medicinal plants in Papua generally varies among ethnic groups, geographical locations, and cultural backgrounds [1].

One local plant with notable pharmacological value is *Nelambo suon* from the family Rubiaceae. Communities in several areas of Southwest Papua traditionally use parts of this plant for specific medicinal purposes, although scientific information regarding its specific chemical constituents remains very limited. According to local knowledge from the Walma and Heriapini districts in Jayapura City, Papua, *Nelambo suon* is believed to possess antimalarial properties [2]. Previous research has also reported that *Nelambo suon* is traditionally used for wound treatment. Medicinal plants generally contain potential secondary metabolites that contribute to their biological activities [3].

Based on evidence-based scientific approaches, phytochemical analysis or preliminary phytochemical screening is a strategic step in identifying major groups of bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, phenolics, and terpenoids, which play important roles in determining the biological activity of plants [4] [5]. Information on the phytochemical profile of a plant serves as a crucial foundation for the development of herbal medicines and the exploration of novel compounds with potential pharmaceutical applications. Previous screening results reported by [6], the bark extract of *Nelambo suon*, obtained from the Yali tribe in Heriapini District Yahukimo Regency, contains active secondary metabolites including alkaloids, flavonoids, terpenoids, steroids, saponins, tannins, and quinones. The presence of diverse classes of bioactive compounds indicates that *Nelambo suon* has strong potential as a promising natural resource for further investigation. Scientific exploration of local plants such as *Nelambo suon* contributes to the preservation of ethnopharmacological knowledge and enhances the added value of biodiversity through science-based approaches.

Based on the foregoing description, this study aims to generate scientific data regarding the secondary metabolite content present in the stem of *Nelambo suon* (Rubiaceae), collected from Sawiat Village, South Sorong Regency, using the maceration method with 80% ethanol as the extraction solvent. This approach was employed to obtain a preliminary and systematic characterization of the plant's phytochemical profile in a measurable and reproducible manner. The findings of this study are expected to provide a scientific foundation for further research, particularly in relation to the evaluation of biological activities, elucidation of the mechanisms of action of bioactive compounds, and the development of herbal medicinal products derived from natural resources.

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Experimental Section

Plant Sample Collection

The stem of *Nelambo suon* (*Rubiaceae*) was collected from Sawiat Village, South Sorong Regency, Southwest Papua, Indonesia (1.5050°S, 132.2864°E). The species naturally grows and is distributed along cliff margins and hilly terrains at elevations ranging from 2.000 to 3.000 meters above sea level. The plant typically reaches a height of approximately 12–18 m, with a trunk diameter of 20–30 cm, and remains green throughout the year. For traditional utilization, the stem bark is harvested from relatively young stems with a diameter of approximately 5–10 cm.

Materials and Apparatus

The instruments used in this study included an analytical balance (Fujitsu FSR-A220, Japan), rotary evaporator (Buchi R-100, Switzerland), water bath (Memmert WNB10, Germany), test tubes, test tube racks, droppers, porcelain dishes, glassware (Pyrex®), a blender, a 65-mesh sieve, and a maceration container.

The materials employed in this study consisted of 80% ethanol, distilled water (aquadest), Dragendorff's reagent, Bouchardat's reagent, ferric chloride ($FeCl_3$), lead(II) acetate (Pb^{2+} acetate), sodium hydroxide (NaOH), hydrochloric acid (2 N HCl), aluminum foil, and filter paper.

Preparation of *Nelambo suon* Stem Powder

The stem bark of *Nelambo suon* was cleaned by removing the outer bark, followed by wet sorting and washing under running water. The material was cut into small pieces and air-dried at room temperature. The dried samples were ground into powder using a blender and sieved through a 65-mesh sieve to obtain a fine and homogeneous powder [7].

Preparation of *Nelambo suon* Stem Extract

Extraction of *Nelambo suon* (Rubiaceae) stem bark was performed using the maceration method with 80% ethanol as the solvent. A total of 250 g of stem bark powder was immersed in 1000 mL of 80% ethanol (1:4, w/v) in a closed container protected from light and allowed to stand for 72 h with occasional stirring. The mixture was then filtered to separate the filtrate and residue. The residue was subjected to remaceration with 1000 mL of 80% ethanol for an additional 48 h to enhance compound extraction. The combined filtrates were concentrated using a rotary evaporator at 40°C, followed by evaporation in a water bath to obtain a viscous crude extract [8]. Subsequently, the concentrated extract obtained was weighed, and the percentage yield was calculated using the following formula [9].

$$\text{Percentage Yield (\%)} = \frac{Weight\ of\ concentrated\ extract}{Initial\ weight\ of\ crude\ drug\ material}\ x\ 100\%$$

Phytochemical Screening

Alkaloid Test

A total of 2 mL of the sample was transferred into a test tube and mixed with 2 mL of 2% HCl. The mixture was heated for 5 min and subsequently filtered. The obtained filtrate was treated with two drops of Dragendorff's reagent and Bouchardat's reagent. The presence of alkaloids was indicated by the formation of an orange or reddish-orange precipitate with Dragendorff's reagent and a reddish-brown precipitate with Bouchardat's reagent [10] [11].

Flavonoid Test

A total of 2 mL of the sample was placed into a test tube, followed by the addition of 2–3 drops of lead(II) acetate (Pb^{2+} acetate) solution. The presence of flavonoids was confirmed by the formation of a yellow precipitate [12] [13].

Tannin Test

A total of 2 mL of the sample was introduced into a test tube and treated with ferric chloride ($FeCl_3$) reagent. The presence of tannins was indicated by the formation of a dark blue or greenish-black coloration [14].

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Saponin Test

A total of 2 mL of the sample was mixed with 10 mL of hot distilled water in a test tube, allowed to cool, and then vigorously shaken to produce foam. The solution was left to stand for 10 min, followed by the addition of one drop of 2 N HCl. The formation of stable foam indicated the presence of saponins [15].

Results and Discussion

Extraction is a crucial initial step for obtaining secondary metabolites from natural materials. Extraction methods are generally classified into conventional methods (maceration, percolation, infusion, reflux, and Soxhlet extraction) and modern methods, such as Ultrasonic-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE). In this study, the maceration method was employed because it allows the extraction of various compounds without the application of heat, thereby reducing the risk of compound degradation. Maceration operates based on the principle of diffusion of active compounds from plant tissues into the solvent at room temperature, making it particularly suitable for extracting thermolabile constituents such as flavonoids, phenolic compounds, and saponins that are susceptible to degradation at elevated temperatures [16]. Maceration is frequently employed as a comparative method in solvent optimization studies due to its simplicity, which enables researchers to evaluate the influence of extraction parameters such as time, temperature, and solvent concentratio extraction yield and phytochemical content [17]. In the context of the herbal industry, maceration is also applied at small to medium production scales because it does not require complex equipment, is easily adaptable, and can be utilized for a wide variety of plant materials.

Extraction yield is defined as the ratio of the amount of extract obtained to the initial quantity of plant material used in the extraction process. A higher yield indicates a greater amount of extract produced. The requirement for the yield of a concentrated extract is that its value should not be less than 10% [18]. Determination of extraction yield is essential to quantify the amount of extract produced during the extraction process. Moreover, the yield is associated with the presence of active constituents in a given sample; thus, a higher extraction yield generally indicates a greater amount of bioactive compounds contained in the extract. As reported by [19], a high concentration of active compounds in a sample is reflected by a correspondingly high extraction yield. The percentage yield obtained in this study was 4%. The low extraction yield observed in this study may be attributed to the predominance of nonpolar compounds, such as essential oils, whereas ethanol is relatively polar in nature. Nevertheless, ethanol is considered safer and more environmentally friendly compared to nonpolar solvents such as *n*-hexane. However, this study did not include an analysis of residual solvent content, which is also an important parameter in assessing the safety of the extract. Extraction yield is influenced by multiple factors, including the type of solvent, solvent concentration, duration of maceration, particle size of the simplicia, and extraction temperature [20]. The yield obtained from the stem extract of *Nelambo suon*is presented in Table 1.

Table 1. Percentage Yield (%) of *Nelambo suon* (Rubiaceace) Stem Extract

Sample Weight (g)	Extract Weight (g)	Extraction Yield (%)
250	10	4

Subsequently, phytochemical screening was performed. Phytochemical screening is a qualitative analytical method used to identify the presence of secondary metabolites in a sample. Solvent selection is a critical factor in the extraction process, as it significantly influences the quality, quantity, and selectivity of the extracted compounds. An appropriate solvent should possess the ability to dissolve bioactive components according to the polarity of the target compounds, while also being safe and stable during the extraction process. Each solvent exhibits specific properties that enable the extraction of compounds with particular polarities. In recent years, ethanol has become one of the most widely used solvents in phytochemical research due to its ability to extract a broad range of secondary metabolites, such as flavonoids, alkaloids, tannins, and phenolic compounds [21] [22]. The use of ethanol is also recommended because it is relatively safe, non-toxic, and suitable for natural product research. Ethanol at a concentration of 80% has been reported to be the most effective solvent for the extraction of polyphenols [23]. Based on studies conducted by [24] [25], phytochemical screening of plants belonging to the Rubiaceae family has revealed the presence of various compounds, including alkaloids, terpenoids, tannins, flavonoids, iridoids, sterols, triterpenes, lactones, anthraquinones, methylxanthines, diterpenes, and monoterpenes. The results of the phytochemical screening test on the stem

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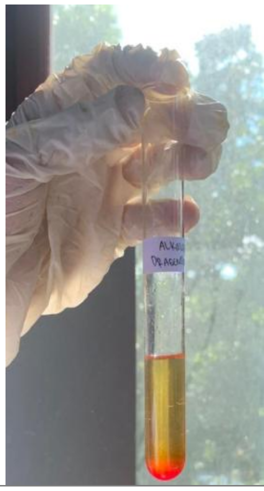
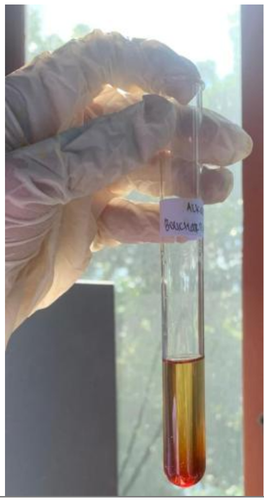
extract of *Nelambo suon* (Rubiaceae) were conducted to determine the chemical constituents present in the extract. Based on the phytochemical screening results presented in **Table 2.** the stem extract of *Nelambo suon* was found to contain alkaloids, flavonoids, tannins, and saponins.

Table 2. Results of Phytochemical Analysis of *Nelambo suon* (Rubiaceae) Stem Extract

Compound Class	Reagent	Result	Remark
Alkaloids	Dragendorff reagent		Positive (+): orange or reddish-orange precipitate
	Bouchardat reagent		Positive (+): reddish-brown precipitate
Flavonoids	lead(II) acetate (Pb^{2+} acetate)		Positive (+): yellow precipitate

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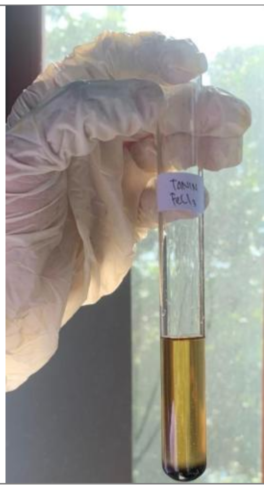
Tannins	$FeCl_3$		Positive (+): dark blue or greenish-black coloration
Saponins	Water+ HCl		Positive (+): foam indicated

The presence of alkaloid compounds was indicated by the formation of an orange precipitate with Dragendorff's reagent and a reddish precipitate with Bouchardat's reagent. The precipitate formed in the Dragendorff test is a potassium–alkaloid complex resulting from the interaction between alkaloid compounds and the active components of the reagent. Dragendorff's reagent contains bismuth nitrate, which provides Bi^{3+} ions; however, these ions are prone to hydrolysis, forming bismuthyl ions (BiO^{+}). Therefore, bismuth nitrate is dissolved in an acidic medium using HCl to inhibit hydrolysis by shifting the equilibrium toward the formation of stable Bi^{3+} ions. The Bi^{3+} ions subsequently react with iodide ions from potassium iodide to form bismuth(III) iodide, which then dissolves in excess iodide to produce a potassium tetraiodobismuthate complex. In the alkaloid test, the nitrogen atom in the alkaloid structure plays a crucial role by forming a coordinate covalent bond with potassium metal ions (K^{+}), resulting in the formation of a characteristic precipitate that serves as a positive indicator of alkaloid presence [26] [27]. The test using Bouchardat's reagent also showed a positive reaction for alkaloids, as evidenced by the formation of a brown precipitate. This precipitate is formed due to the coordinate covalent interaction between potassium ions (K^{+}) and alkaloid compounds, producing an insoluble potassium–alkaloid complex. Bouchardat's reagent consists of potassium iodide and iodine, which function to form complexes with alkaloids and serve as the basis for the qualitative identification of these compounds [28].

Flavonoid analysis was conducted using reagent lead(II) acetate (Pb^{2+} acetate). In the flavonoid test of the *Nelambo suon* stem extract obtained through the maceration method, the use of lead(II) acetate (Pb^{2+} acetate) reagent yielded a positive result, as indicated by a change in the solution color to yellow. This color change occurs due to the formation of acetophenone compounds resulting from the reaction between flavonoid components in the sample and the lead(II) acetate (Pb^{2+} acetate), thereby indicating the presence of flavonoids in the extract [12] [13].

The identification of tannins was carried out using iron(III) chloride ($FeCl_3$) reagent, as indicated by a color change of the solution to dark green or bluish-black. This color change occurs due to the interaction

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between tannin compounds and Fe^{3+} ions, resulting in the formation of an iron(III)–tannin complex, which produces a characteristic color serving as an indicator of the presence of tannins in the extract [29].

The presence of saponin compounds was identified by the formation of foam. Saponins are steroidal or triterpenoid glycosides commonly found in various plants. Structurally, saponins are glycosides composed of an aglycone moiety known as sapogenin [30]. Saponins possess the ability to reduce the surface tension of water, leading to foam formation on the surface of the solution after shaking.Saponins consist of two groups with different properties, namely hydrophilic and hydrophobic groups. The addition of h ydrochloric acid (HCl) during the saponin test increases the polarity of the compounds, causing a change in the orientation of their constituent groups. Under these conditions, the polar (hydrophilic) groups tend to orient outward, while the nonpolar (hydrophobic) groups orient inward, forming micellar structures. The formation of these micelles results in stable foam, which serves as a positive indicator of the presence of saponins in the extract [31].

Based on a study conducted by [32], *Carica papaya* contains several bioactive compounds that have been investigated for their antimalarial properties, including flavonoids, alkaloids, saponins, and tannins. The presence of these compounds is reflected in the literature, which reports that *C. papaya* exhibits antimalarial activity and, in certain cases, demonstrates synergistic effects when combined with extracts from other plants, such as *Moringa oleifera*, thereby enhancing its efficacy. The presence of flavonoids, alkaloids, and saponins in *Carica papaya* is presumed to contribute to its antiplasmodial activity, with additional antipyretic effects that help alleviate the clinical symptoms of malaria [33]. A study conducted by [34] reported that a commercial drug containing active flavonoids demonstrated significant antimalarial potential, both through *in vivo* testing in malaria-infected mice and *in vitro* evaluation against chloroquine-resistant *Plasmodium falciparum*. These findings indicate that flavonoids function as bioactive compounds capable of inhibiting the growth and development of the parasite, including strains that have developed resistance to conventional therapies. Therefore, flavonoids hold considerable promise for further development as alternative therapeutic agents or as components of combination therapy in the management of malaria, particularly in cases involving drug resistance.

Conclusions

Based on the results of this study, it can be concluded that the ethanol extract of *Nelambo suon* (Rubiaceae) stem obtained through the maceration method contains alkaloids, flavonoids, tannins, and saponins. These findings indicate that the stem of *Nelambo suon* has potential as a source of bioactive compounds and support its traditional use as a medicinal plant by local communities in Papua. For future research, quantitative analysis, isolation, and characterization of the active compounds using instrumental methods are recommended. In addition, *in vitro* and *in vivo* biological activity assays, particularly those related to antimalarial and wound-healing activities, are necessary to support its development as a raw material for herbal medicine.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this review article.

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