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Phytochemical Analysis of The Leaves, Roots, Stems, and Stem Bark of the Bengkal Plant (*Nauclea subdita*)

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Abstract

Bengkal (*Nauclea subdita* (Korth.) Steud) is a medicinal plant that has been traditionally used for various treatments and is known to contain a wide range of secondary metabolites. However, most previous studies have focused only on the leaves, so information regarding the phytochemical composition of other plant parts remains very limited. This research is important to provide a comprehensive overview of the bioactive contents in all morphological parts of *N. subdita*, which have the potential to support the development of biological and pharmacological applications, such as antibacterial, antioxidant, antidiabetic, and anticancer activities commonly associated with alkaloids, flavonoids, and phenolic compounds. Secondary metabolite analysis was conducted on the leaves, roots, stems, and bark using phytochemical screening. Samples were collected from Ketapang, West Kalimantan, then dried, ground, and extracted using sequential maceration with n-hexane and ethanol. The extracts were subsequently fractionated with dichloromethane (DCM) at pH 3 and pH 9. Moisture content was determined using the gravimetric method. Phytochemical screening was carried out using standard qualitative tests, including Mayer's and Wagner's tests for alkaloids, color tests for flavonoids and phenolics, the Liebermann–Burchard test for triterpenoids, the $FeCl_3$ test for tannins, and the foam test for saponins. The results showed that all parts of the plant contain alkaloids, flavonoids, phenolics, and triterpenoids, although there were variations among extracts and fractions. Tannins were detected only in the leaves, while saponins were found in the leaves, roots, and stems. These findings confirm that all parts of *N. subdita* possess important biological potential due to the presence of major bioactive compounds that play a role in the development of herbal medicines and nature-based therapeutic agents. This information serves as a significant initial foundation for further pharmacological research.

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INTRODUCTION

Bengkal (*Nauclea subdita* (Korth.) Steud) is one of the 35 species within the genus Nauclea, which belongs to the Rubiaceae family, and has long been recognized for its potential as a traditional medicinal plant (Rahmi et al., 2021). This species shows a clear ecological preference for wet habitats, including freshwater swamps and riverbanks. Morphologically, it possesses a fibrous taproot system, an upright cylindrical stem, and compound pinnate leaves (Ramadhani et al., 2023). he distribution of the genus Nauclea in Indonesia is relatively wide, covering major regions such as Kalimantan, Papua, Sumatra, and Sulawesi, reflecting its ecological adaptability to diverse tropical environmental conditions (Rahmi et al., 2021).

Bengkal has long been utilized in traditional medicine to address a variety of health conditions, including fever, pain, dental caries, malaria, oral infections, dysentery, diarrhea, and nervous system disorders such as epilepsy (Liew et al., 2023). This species is typically found in wetland ecosystems, such as swamps and riverbanks, which provide optimal conditions for its growth.

Environmental factors such as high rainfall, very high humidity, and alluvial/peat soils may stimulate the synthesis of bioactive compounds such as alkaloids, flavonoids, and tannins as part of the plant's adaptive response. Therefore, the natural habitat of Bengkal in these regions potentially enriches the composition of secondary metabolites that contribute to its pharmacological activities (F. Lestari & Andriani, 2021).

Empirically, local communities use the roots of Bengkal as a traditional remedy for acne caused by infections, commonly by boiling the roots (Vita Mayasari, 2023). The pharmacological effects of Bengkal are associated with the presence of secondary metabolite compounds contained within the plant. To identify secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, tannins, and saponins present in the plant, a phytochemical screening analysis needs to be conducted (Putri, 2013).

Bengkal consists of four main parts: leaves, roots, stems, and stem bark. Previous phytochemical screening studies by Ulfah et al. (2023), Fadlilaturrahmah et al. (2024), and Hidayatullah et al. (2023) have focused only on the leaves. These studies reported that Bengkal leaves contain alkaloids, steroids, tannins, and flavonoids, and exhibit antioxidant activity (Ulfah et al., 2024). In addition, the leaves have been reported to contain alkaloids, flavonoids, phenolics, saponins, steroids, and tannins, with potential as a sunscreen agent (Fadlilaturrahmah et al., 2024a). Another study also found flavonoids, triterpenoids, and tannins in Bengkal leaves, with a total flavonoid content of 21.508 mgQE/g (M. Hidayatullah et al., 2023).

However, there are currently no studies that have investigated the phytochemical content of the roots, stems, or bark of Bengkal. Therefore, this study aims to conduct a phytochemical screening analysis of secondary metabolites present in the leaves, roots, stems, and bark of Bengkal. The results of this research are expected to provide a valuable scientific reference for future studies, particularly in evaluating the biological activities and identifying the types of bioactive compounds contained in all parts of the Bengkal plant.

METHOD

Tools and Materials

The tools and materials used in this study include a drop pipette, measuring cylinder (Pyrex), glass funnel (Pyrex), analytical balance (Ohaus), desiccator (Normax), crucible, spatula, tongs, blender (Miyako), filter paper, vacuum rotary evaporator (IKA RV 8, Germany), oven (Memmert, Germany), Bengkal plant, 96% ethanol, n-hexane, dichloromethane (DCM), HNO_3 (Smartlab, USA), NH_3 (Smartlab, USA), Mayer's reagent, Wagner's reagent, HCl, magnesium powder, $FeCl_3$, and H_2SO_4.

Sample Collection and Determination of Moisture Content

Samples of the Bengkal plant, including the leaves, roots, stems, and stem bark, were collected from the Ketapang Regency area, West Kalimantan. After collection, the samples were washed with running water to remove impurities, then dried and ground into powder.

The analysis of moisture content was carried out using the following procedure. The test crucible was first heated in an oven at 105°C for 20 minutes, then weighed using an analytical balance. Next, 2 grams of the Bengkal sample were weighed and placed into the pre-weighed

crucible. The sample was then reheated in the oven at 105°C for 3 hours. After heating, the crucible containing the sample was cooled in a desiccator for 30 minutes and subsequently reweighed using an analytical balance. The moisture content was calculated using the following formula:

$$Moisture\ Content = \frac{b - (c - a)}{b}\ x\ 100\%$$

a = mass of empty crucible (grams)
b = initial mass of sample (grams)
c = mass of crucible and sample after drying (grams)
(Ndumuye et al., 2022)

Extraction and Fractionation

A total of 200 g of dried powder was extracted by maceration using 9 L of *n*-hexane for 3 × 72 hours. After the *n*-hexane extraction was completed, the process was continued using 9 L of 96% ethanol for 3 × 72 hours (Liew et al., 2014). The use of *n*-hexane aims to extract non-polar compounds such as lipids and non-polar terpenoids, allowing the subsequent extraction of polar and semi-polar fractions to occur more efficiently (Simões et al., 2022). Ethanol was selected because it is a polar solvent capable of extracting a wide range of polar to semi-polar secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolics. Moreover, ethanol is safe, volatile, and effective for extracting various bioactive compounds (Tourabi et al., 2025).

The resulting ethanol extract was then concentrated using a vacuum rotary evaporator at 40°C. The concentrated ethanol extract was subsequently acidified with 10% nitric acid (HNO_3) to reach pH 3. This acidification step converts alkaloids into their salt forms, allowing them to remain in the aqueous phase during fractionation (Leitão et al., 2021). The mixture was then fractionated using dichloromethane (DCM) to obtain the DCM fraction at pH 3 and the acidic ethanolic extract. DCM was chosen because it is a semi-polar solvent highly effective for separating non-ionic organic compounds from aqueous mixtures, particularly terpenoids, steroids, and non-protonated alkaloids (Leitão et al., 2021).

The acidic ethanolic extract was then basified with ammonia (NH_3) to reach pH 9, converting the protonated alkaloids back into their free-base forms (Leitão et al., 2021). Under alkaline conditions, the alkaloids become more non-polar, enabling them to migrate into the DCM layer. The mixture was subsequently fractionated again using DCM to obtain the DCM fraction at pH 9 and the basic ethanolic extract (Atta-ur-Rahman et al., 2009). This step aims to separate alkaloids based on their acid–base characteristics and to enhance the purity of the resulting fractions.

Phytochemical Screening

Phytochemical screening of Bengkal extracts was conducted to identify the presence of secondary metabolites, including alkaloids, flavonoids, triterpenoids, polyphenols, tannins, and saponins, using methods adapted from Masriani et al., 2023.

Alkaloid Screening

The alkaloid test was carried out as follows:

- The concentrated *n*-hexane extract of Bengkal stem bark was dissolved in 1 mL of *n*-hexane.
- The ethanolic extract of Bengkal was dissolved in 1 mL of 96% ethanol.
- Each dichloromethane (DCM) fraction of Bengkal was dissolved in 1 mL of DCM.

Each solution was then added with 2N HCl and divided into two portions, which were tested using Wagner's and Mayer's reagents, respectively (Masriani et al., 2023). A sample was considered positive for alkaloids if the Mayer's reagent produced a yellow color accompanied by a white precipitate, and the Wagner's reagent produced an orange-red color with an orange to yellowish-brown precipitate (S. M. Lestari et al., 2024).

Flavonoid Screening

The flavonoid test was carried out as follows:

- 10 mg of the concentrated *n*-hexane extract of Bengkal stem bark was dissolved in 1 mL of *n*-hexane.
- 10 mg of the ethanolic extract of Bengkal was dissolved in 1 mL of 96% ethanol.
- 10 mg of each DCM fraction of Bengkal was dissolved in 1 mL of DCM.

Each solution was then added with magnesium (Mg) powder and two drops of 2N HCl were added to the solution (Masriani et al., 2023). The extract is considered positive for flavonoids if a reddish, yellow, or orange color appears (Deti Andasari et al., 2020).

Triterpenoid Screening

Each 1 mL of concentrated n-hexane extract, ethanol extract, and DCM fraction of Bengkal was added with 2 mL of chloroform and three drops of concentrated H_2SO_4 (Masriani et al., 2023) . The presence of triterpenoids is indicated by the formation of a reddish-brown ring at the interface (Iradha et al., 2025).

Phenolic Screening

Each 1 mL of concentrated n-hexane extract, ethanol extract, and DCM fraction of Bengkal was reacted with $FeCl_3$ 1% solution (Masriani et al., 2023). The sample is considered positive for phenolic compounds if a dark green to black color appears (Septia et al., 2020).

Tannin Screening

The concentrated n-hexane extract, ethanol extract, and DCM fraction of Bengkal were each dissolved in distilled water and then heated, followed by the addition of 1% $FeCl_3$ solution (Masriani et al., 2023). A greenish-brown or bluish-green coloration indicates the presence of tannins (Putri et al., 2022).

Saponin Screening

Each concentrated n-hexane extract, ethanol extract, and DCM fraction of Bengkal was placed into a test tube, added with 1 mL of distilled water, and shaken vigorously for 10 minutes until foam was formed (Masriani et al., 2023). The extract is considered positive for saponins if stable foam persists for approximately 10 minutes (Rozaki et al., 2023).

RESULTS AND DISCUSSION

Sample Collection and Determination of Moisture Content

In this study, samples consisting of the leaves, roots, stems, and stem bark of Bengkal were used, collected from Ketapang, West Kalimantan, at the coordinates -1.4222640, 110.1352620. The moisture content of the samples was determined using the gravimetric method. This analysis was performed to minimize weighing errors, as high temperatures may cause the samples to undergo expansion, potentially affecting the measured mass (Wandira et al., 2023). The moisture content analysis of Bengkal (Table 1) showed that the root, stem, and stem bark parts met the simplicia quality standard of <10% (Andini & Putri, 2021). *N. subdita*, the leaf part did not meet this standard, which may be attributed to insufficient drying temperature and

humidity (Yasi et al., 2022). Low moisture content plays an important role in inhibiting microbial growth, thereby maintaining the physical and chemical quality of medicinal raw materials (Sutomo et al., 2021: Wandira et al., 2023). Additionally, low water content can extend the shelf life of raw materials (Farrel et al., 2020). Based on these data, the leaves have relatively lower stability compared to the other parts.

Figure 1. Bengkal plant

Table 1. Calculation of % Moisture Content of Bengkal Sample

Sample	leaves	root	stem	stem bark
Moisture Content (%)	10.35	8.30	7.39	9.20

Extraction and Fractionation

Table 2. Percent Yield of Bengkal Extract and Fractions

Sample	**n-Hexane Extract (%)**	**Ethanol Extract (%)**	**DCM Fraction pH 3 (%)**	**DCM Fraction pH 9 (%)**
leaves	6,9	7,2	0,8	0,04
root	7,1	9,1	0,7	0,02
stem	6,3	14,1	0,7	0,02
stem bark	4,6	14,8	0,7	0,67

Extraction was carried out using a successive extraction method to obtain a higher yield and to minimize the potential degradation of secondary metabolites that are unstable at high temperatures (Maria John et al., 2018). The successive extraction process employed several solvents with different polarity levels, namely n-hexane (nonpolar) and ethanol (polar). After obtaining the concentrated extracts, the next step was fractionation using dichloromethane (semi-polar) to optimize the separation of bioactive compounds based on gradual polarity differences, resulting in extracts and fractions with more specific and efficient chemical compositions (N. Hidayah et al., 2016). The extraction and fractionation results (Table 2) indicate that the differences in yield among the various parts of Bengkal are influenced by the polarity characteristics of the compounds present in each part. Based on the yield proportion, the stem bark and stem parts exhibited the highest yields.

Phytochemical Screening

Table 3. Phytochemical Screening Results of Bengkal *(Nauclea subdita)*

Parts of the plant	Sample	Alkaloid		Flavonoid	Phenolic	Triterpenoid	Tannin	Saponin
		Mayer	Wagner					
Leaves	n-Hex	-	+	-	+	+	-	-
	DCM 3	+	+	+	+	-	+	+
	DCM 9	+	+	+	-	-	-	-
	Et-OH	+	+	+	+	-	+++	+
Root	n-Hex	+	+	-	-	+	-	-
	DCM 3	+	++	+	-	-	-	+
	DCM 9	+	-	-	-	-	-	-
	Et-OH	+	++	+	+	-	-	-
Stem	n-Hex	+	-	+	-	+	-	-
	DCM 3	+	+	+	-	-	-	+
	DCM 9	+	+	-	-	-	-	-
	Et-OH	+	+	+	+	-	-	-
Stem bark	n-Hex	-	+	-	-	+	-	-
	DCM 3	+	+	+	-	-	-	-
	DCM 9	+	+	+	-	-	-	-
	Et-OH	+	+	+	+	-	-	-

The analysis of the extracts and fractions from various parts of the Bengkal plant showed that most of them contained alkaloids, flavonoids, phenolics, and triterpenoids. Meanwhile, tannins were detected only in the leaves, and saponins were found in the leaves, roots, and stems (Table 3). These findings are consistent with previous studies, which also reported that Bengkal contains alkaloids, flavonoids, phenolics, tannins, and triterpenoids (Liew et al., 2014; Ramadhani et al., 2023; Avanti et al., 2021)

Alkaloid Screening

Alkaloid testing was carried out based on the precipitation reaction between alkaloid compounds and heavy metal ions (Ngibad, 2019). Alkaloids are nitrogen-containing organic compounds commonly found in plants (Yang et al., 2024). The presence of a nitrogen atom gives alkaloids their basic properties, allowing them to interact with metal ions. The lone pair of electrons on the negatively charged nitrogen atom is highly reactive toward metal ions (Maheshwaran et al., 2024).

Alkaloid testing on various parts of Bengkal using Mayer's reagent was conducted to determine the presence of alkaloids, which is indicated by the formation of a white precipitate (Karneng et al., 2022). Based on the results, all parts of Bengkal produced a white precipitate, confirming that alkaloids are present in every part of the plant.

The formation of the white precipitate in the Bengkal extract tested with Mayer's reagent occurs due to the formation of a complex between potassium ions and alkaloids. This complex results from the reaction between the nitrogen atom of the alkaloid and potassium ions derived from potassium tetraiodomercurate(II) (Riwanti & Izazih, 2019) (Figure 2).

The results of the alkaloid test on Bengkal using Wagner's reagent showed that all parts of the plant contained alkaloids, as indicated by the formation of a brown precipitate (Karneng et al., 2022). Differences in the amount of precipitate formed suggest that each part of Bengkal contains varying levels of alkaloids (Rahmawati et al., 2023). The root part exhibited the

highest amount of brown precipitate, indicating that it contains a greater concentration of alkaloid compounds compared to the other plant parts.

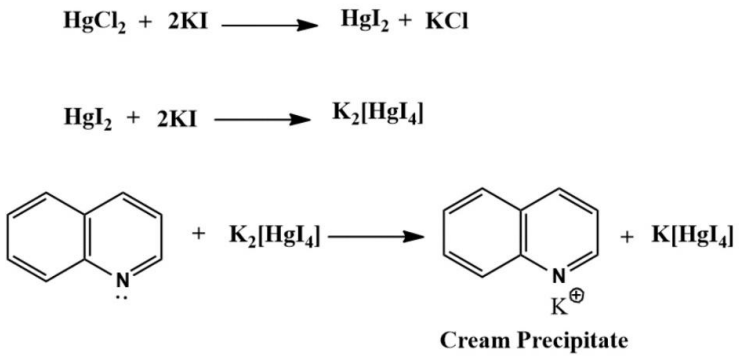

Figure 2. Mechanism of alkaloid identification using Mayer's reagent

The formation of the brown precipitate in the extract and fraction tests of Bengkal using Wagner's reagent is based on the formation of an ionic complex between alkaloid cations and triiodide ions, which produces a brown precipitate as a positive indication of alkaloids (Madhu Vishwakarma et al., 2025) (Figure 3).

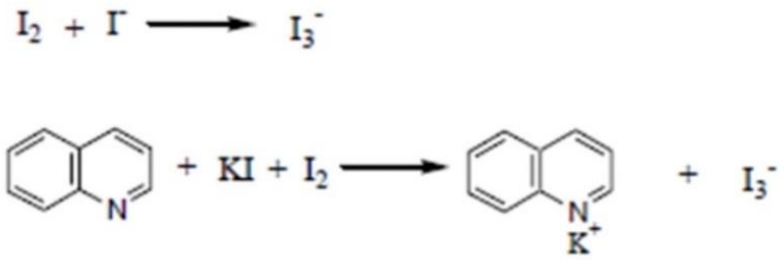

Figure 3. Reaction of alkaloids using Wagner's reagent

Based on the screening results, all parts of Bengkal were found to contain alkaloid compounds. These findings are consistent with previous studies, which reported that Bengkal particularly its leaves contains alkaloid compounds (Fadlilaturrahmah et al., 2024;Ulfah et al., 2024)

Flavonoid Screening

Flavonoids are compounds that are soluble in polar solvents such as ethanol. Flavonoid screening was carried out using the Shinoda test followed by the addition of concentrated sulfuric acid. Based on the results, all ethanol extracts from each part of Bengkal contained flavonoids, indicated by the formation of an orange-reddish color. These results are consistent with previous studies showing that Bengkal contains flavonoids (Ulfah et al., 2024; (Fadlilaturrahmah et al., 2024;M. Hidayatullah et al., 2023). The appearance of this orange coloration in the flavonoid test signifies a reduction reaction between the polyhydroxy groups of flavonoids and magnesium metal in hydrochloric acid, which subsequently produces benzopyrylium salts (Oktavia Dan, 2021) (Figure 4).

Based on the intensity of the color produced, the leaves exhibited the strongest orange-reddish coloration compared to the other plant parts. This indicates that the leaves contain higher levels of flavonoids. Differences in flavonoid content may be attributed to variations in physiological function, secondary metabolite distribution, and tissue structure in each part of the plant (Malik et al., 2021). Flavonoids belong to the phenolic compound group and are often associated with antioxidant activity (Laoué et al., 2022). Based on the screening results, the leaves are likely to possess antioxidant potent.

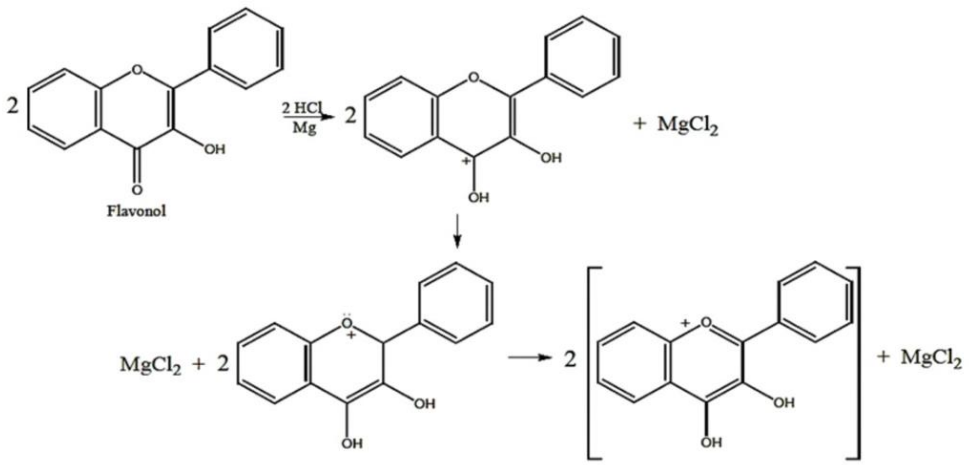

Figure 4. Shinoda Reaction in Flavonoid Test

Triterpenoid Screening

Triterpenoids are carbon-based compounds composed of six isoprene units synthesized from C36 squalene (H. Hidayah et al., 2023). n general, triterpenoids possess nonpolar characteristics, making them more readily soluble in nonpolar solvents (Fauziyah et al., 2022).

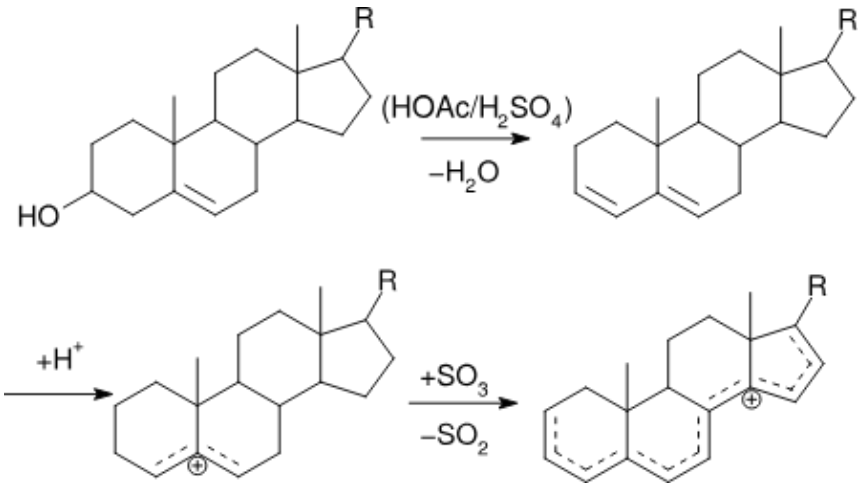

Figure 5. Mechanism Liebermann-Burchard reaction

Triterpenoid compounds were identified using the Liebermann–Burchard reagent, which consists of a mixture of chloroform and concentrated sulfuric acid (Figure 5) (Novia Fransiska et al., 2021). All n-hexane extracts from the Bengkal plant parts showed positive results for the triterpenoid test, indicated by the formation of a reddish-brown ring at the interface. These results are in accordance with previous studies (M. Hidayatullah et al., 2023).

Phenolic Screening

Based on the screening results, all ethanol extracts of Bengkal tested positive for phenolic compounds, indicated by a color change to dark green or blackish upon the addition of $FeCl_3$ solution these results are in accordance with previous studies (Fadlilaturrahmah et al., 2024) (Figure 6). Phenolic compounds are generally soluble in polar solvents and are widely associated with antioxidant activity in plants (Handayani et al., 2024: Zeb, 2020). The stem bark extract of Bengkal has been reported to exhibit antioxidant activity, which is most likely attributed to the presence of these phenolic compounds (Fatin et al., 2012).

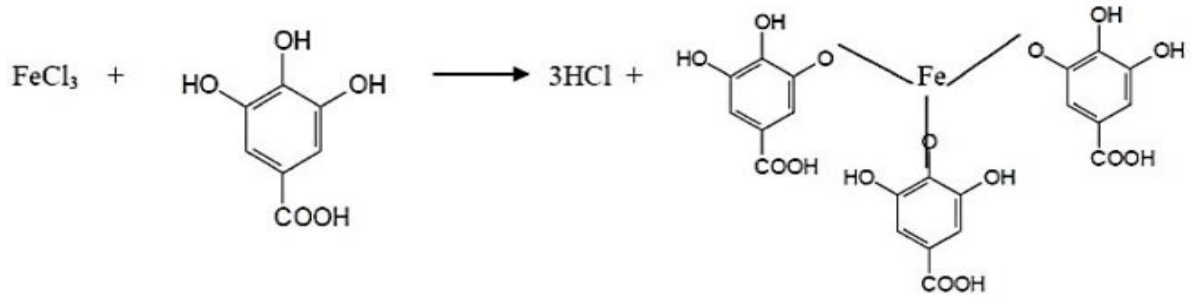

Figure 6. Reaction of Phenol with $FeCl_3$

Tannin Screening

The screening results showed that tannins were present in the ethanol extract and the DCM pH 3 fraction of the leaf part these results are in accordance with previous studies (Ulfah et al., 2024;Fadlilaturrahmah et al., 2024)(M. Hidayatullah et al., 2023). Tannins are polyphenolic compounds containing multiple hydroxyl (–OH) groups attached to aromatic rings (Pengelly, 2021). These compounds are known for their ability to form complexes with proteins, alkaloids, and heavy metals, and they exhibit various biological activities such as antioxidant, antibacterial, and anti-inflammatory effects (Wu et al., 2023).

Identification was carried out by reacting the sample with 1% $FeCl_3$, with a positive result indicated by a color change to greenish-brown. Chemically, this reaction occurs because Fe^{3+} ions from $FeCl_3$ form coordination complexes with the phenolic hydroxyl groups present in the tannin structure (Espina et al., 2022). The resulting complex produces a characteristic color due to intercondensation reactions and the formation of metal–phenolate complexes. This color change serves as the basis for identifying the presence of polyhydroxy phenolic groups within the tannin structure.

Saponin Screening

Saponins are polar compounds that are readily soluble in water and classified as glycosides of sapogenins. A positive indication of saponins is shown by the formation of foam after vigorous shaking. This foam appears because the hydrophilic portion of the saponin interacts with water, while the hydrophobic portion interacts with air (Godlewska et al., 2022). The stability of the foam after being left to stand for approximately 10 minutes indicates the presence of saponins that have been hydrolyzed in water into glycosides and other compounds (Góral & Wojciechowski, 2020). Saponins in the root and stem parts were found in the DCM pH 3 fraction, whereas in the leaves they were detected in both the ethanol extract and the DCM pH 3 fraction these results are in accordance with previous studies (Fadlilaturrahmah et al., 2024).This may be due to the amphiphilic nature of saponins, which results in different solubility profiles across plant parts (Rai et al., 2021). In the roots and stems, saponins with more nonpolar characteristics tend to be extracted into the DCM pH 3 fraction, while in the leaves which contain more polar constituents saponins dissolve in the ethanol extract and partially in the DCM pH 3 fraction.

CONCLUSION

Secondary metabolite compounds found in the leaves, roots, stems, and bark of Bengkal (*Nauclea subdita*) have been successfully identified, indicating that each part of the plant contains significant bioactive components. Alkaloids, flavonoids, phenolics, and triterpenoids were detected in all plant parts with varying intensities in each extract and fraction, while tannins were found only in the leaves and saponins were present in the leaves, roots, and stems.

These differences in extraction and fractionation results reflect the unique distribution of bioactive compounds based on the polarity of each plant organ.

This discovery affirms the potential of Bengkal as a valuable natural source for the development of natural product-based medicines. The presence of groups of compounds with important biological activities such as antioxidant, antibacterial, antidiabetic, or anticancer properties highlights Bengkal as a promising candidate in the search for new therapeutic agents. Therefore, these phytochemical screening results not only provide comprehensive initial information but also open up opportunities for the use of Bengkal in pharmaceutical research and the phytopharmaca industry.

To strengthen these findings, further research is urgently needed. More in-depth testing of specific compounds through isolation and structural characterization, as well as biological activity assays (for example, antimicrobial, antioxidant, cytotoxic, or antidiabetic tests), is required to ensure the therapeutic potential of each fraction or compound. Toxicity studies and evaluations of mechanisms of action are also necessary so that the use of these natural medicinal ingredients can be developed in a scientific and safe manner.

RECOMMENDATIONS

Based on the findings obtained in this study, it is recommended that future research include quantitative analyses of secondary metabolites in all parts of the Bengkal plant. Such analyses are necessary to generate comparative data on the concentrations of each metabolite contributing to the plant's bioactivity. In addition, further identification and more detailed characterization of the detected compounds are required to provide a more comprehensive understanding of their potential applications in pharmacology and the development of natural-product-based therapeutics. These findings are expected to serve as a scientific foundation for subsequent studies exploring the biological activities and broader utilization potential of Bengkal.

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