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Preliminary Phytochemical Screening of Moringa Leaves (*Moringa oleifera*): *An Ethnomedicinal Investigation in Kaway XVI, West Aceh*

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Abstract

Background: *Moringa oleifera Lam. represents a crucial botanical asset globally, yet its localized phytochemical expressions under specific coastal microclimates remain under-explored, threatening the standardization of community-based traditional medicine. M. oleifera Lam. is a versatile, multipurpose tree species that holds significant importance in the ethnomedicine of the West Aceh community. While previous studies heavily rely on dried extracts, this study establishes the baseline phytochemical integrity of fresh, unextracted coastal specimens, bridging the gap between empirical local preparation methods and laboratory validation. This study aimed to characterize the qualitative phytochemical profile of M. oleifera leaves sourced from Kaway XVI Subdistrict and to document their traditional applications as a basis for scientific validation.* **Methodology:** *Employing a qualitative experimental, phytochemical screening was performed directly on fresh leaf samples using standardized assays-including Mayer's, Wagner's, and Dragendorff's tests for alkaloids, the Shinoda test for flavonoids, and the ferric chloride test for phenolics—thereby minimizing the degradation of secondary metabolites often associated with intensive solvent extraction processes.* **Findings:** *The qualitative analysis revealed the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, and steroids, while terpenoids were notably absent. The identification of these bioactive constituents provides scientific substantiation for the local ethnomedicinal use of M. oleifera as both a nutritional supplement and a galactagogue agent. This study provides a baseline phytochemical profile derived from fresh, non-extracted plant material, offering a more ecologically relevant representation of bioactive compounds as utilized in traditional practices.* **Contribution:** *The findings contribute to bridging the gap between empirical ethnomedicine and laboratory validation, and support the standardization of Moringa as material for nutraceutical, and functional food development.*

Keywords: *Moringa oleifera; Phytochemical Screening; Ethnomedicine; Kaway XVI; Secondary Metabolites*

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INTRODUCTION

Moringa oleifera Lam., commonly known as Moringa, is a fast-growing tree from the Moringaceae family characterized by its exceptional adaptability to drought conditions (Amin et al., 2024; Kaur & Praba, 2025). This plant is globally recognized for its resilience in marginal environments, earning the moniker "the miracle tree" (Rajput et al., 2025; Ali et al., 2022). Native to the sub-Himalayan regions, *M. oleifera* has become naturalized and is now extensively cultivated throughout the Indonesian archipelago (Panova et al., 2025). The intersection of ethnomedicinal practices and phytochemical screening provides a scientific paradigm to validate empirical traditional knowledge. While local communities utilize plants based on cross-generational observations, screening identifies the precise secondary metabolites responsible for these physiological activities, establishing a necessary foundation for safety and standardized efficacy.

Botanically, *M. oleifera* can reach heights of 10–12 meters, featuring an upright cylindrical trunk and light-colored fibrous bark (Jalani et al., 2024; Ligo et al., 2025). The leaves are bipinnate or tripinnate compounds, ranging from 30–60 cm in length, which provide the canopy with a distinct feathery appearance (Ramappa et al., 2025). The species exhibits significant morphological diversity among accessions, including variations in leaf shape and branching patterns, representing vital genetic resources for crop improvement (Bharathi et al., 2024). Furthermore, the flowering process produces small white flowers, followed by the development of slender seed pods measuring 15–45 cm in length (Pareek et al., 2023). Its capacity to tolerate extreme water stress positions it as a cornerstone for sustainable food security in developing nations (Zeru et al., 2025).

Beyond its morphological robustness, Moringa represents a significant botanical resource in the Indonesian public health landscape, utilized to address malnutrition, immune deficiencies, diabetes mellitus, hypertension, and inflammation (Japaries et al., 2023; Musyaropah & Cahyanto, 2025). The plant is rich in secondary metabolites that function as potent natural antioxidants, neutralizing free radicals that trigger degenerative diseases, regulating metabolic pathways, and mediating targeted anti-inflammatory responses within human biological systems (Ligo et al., 2025).

In rural areas of Indonesia, Moringa leaves have long been integral to traditional medicinal systems (Rahayu & Hasibuan, 2023). The most prominent applications include supporting lactation in breastfeeding mothers and preventing stunting in toddlers (Pratiwi et al., 2023; Yasin et al., 2024). The most widely adopted preparation method is "*sayur bening*" (a traditional clear soup), which is culturally preferred by postpartum mothers and families (Irwandi et al., 2024). Contemporary innovations in West Aceh are now leveraging the combination of Moringa with local marine resources to create practical, nutrient-dense products (Rumapea et al., 2025). Additionally, the community utilizes Moringa to treat gastrointestinal infections and manage obesity, reflecting an empirical efficacy observed across generations (Yohandini et al., 2024; Yunita et al., 2024).

Despite its widespread use, the phytochemical composition of *M. oleifera* can vary significantly based on local agro-climatic factors. Therefore, phytochemical screening is fundamental to understanding the chemical identity and ensuring the

safety of these herbal formulations (Kumar et al., 2025; Dwaipayan et al., 2025). Given this variability, an investigation of specimens from Kaway XVI is of urgent importance to support standardization efforts and validate the nutritional security of local communities (Sukmawaty et al., 2024; Rana et al., 2025).

This lack of localized, fresh-tissue chemical evaluation constitutes a critical gap, as agro-climatic variations heavily dictate the synthesis and yield of secondary metabolites. Therefore, this study aims to systematically characterize the qualitative phytochemical profile of fresh *M. oleifera* leaves from Kaway XVI, West Aceh, using a direct-tissue screening approach, thereby providing the first explicit scientific validation of the raw botanical materials driving local ethnomedicinal applications.

METHOD

This study utilized a qualitative descriptive and experimental laboratory-based research design integrated with an ethnobotanical contextual approach.

Sample Collection and Preparation

Moringa oleifera leaf specimens were collected on October 17, 2023, from a naturally occurring population in Kaway XVI Subdistrict, West Aceh (Geographic Coordinates: 4°11'49.5"N 96°09'22.1"E). The collection environment is characterized by a humid coastal lowland climate, with a dominant mixture of red-yellow podzolic and alluvial soil types (BPS Kabupaten Aceh Barat, 2015; BPS Kabupaten Aceh Barat, 2025; Pemerintah Kabupaten Aceh Barat, 2023). Mature green leaves in their active vegetative growth phase were selectively harvested from disease-free, unpest-infested branches to ensure uniform physiological maturity. To ensure the integrity of the bioactive constituents and minimize the degradation of secondary metabolites, a rigorous pre-analytical protocol was executed: immediately post-harvest, samples were washed thoroughly under running tap water to remove physical debris, rinsed with distilled water, subjected to manual sorting, and gently surface-dried using lint-free tissue paper. The fresh leaf samples were processed within 24 hours of collection without prior drying or solvent extraction.

Qualitative Phytochemical Screening

Qualitative phytochemical screening was performed directly on the fresh plant tissues using established protocols to identify the primary groups of secondary metabolites (Maheshwaran et al., 2024). This direct-tissue approach was selected to facilitate the rapid and accurate detection of bioactive compounds (Sanwal et al., 2025). All assays were conducted in triplicate to ensure reproducibility, following these standardized procedures,

Alkaloids

For alkaloids, 2.0 grams of finely chopped fresh leaves were subjected to extraction using 5.0 mL of 2% aqueous HCl, with the mixture being warmed in a water bath for 5 minutes before filtration. The resulting filtrate was evenly distributed into three test tubes, each containing 1.0 mL. Each test tube was then individually treated with three drops of Mayer's reagent, which serves as an indicator for white or cream

precipitate, three drops of Wagner's reagent, indicating a reddish-brown precipitate, and three drops of Dragendorff's reagent, which indicates an orange-red precipitate. Following these treatments, the samples were incubated for five minutes at room temperature prior to the final visual assessment (Sanwal et al., 2025).

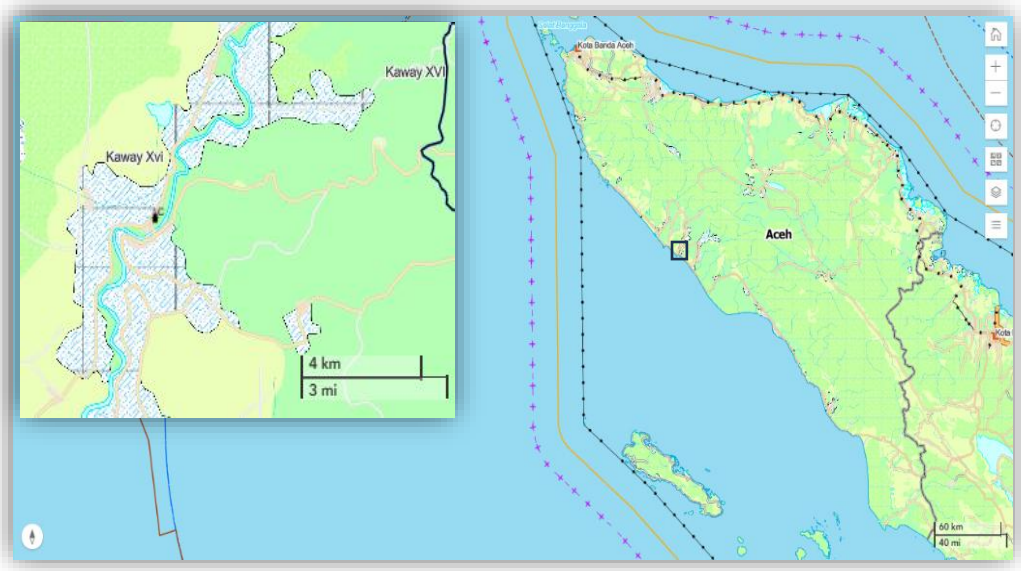

Figure 1. Map view of Kaway XVI (Source: https://tanahair.indonesia.go.id/map)

Flavonoids

The evaluation of flavonoids was conducted using the Shinoda test. Sample of 2.0 grams of fresh of *M. oleifera* leaf was placed in a test tube containing 3.0 mL of 96% ethanol. Subsequently, 10 mg of magnesium powder or ribbon was added, followed by the slow, dropwise addition of 1.0 mL of concentrated hydrochloric acid (HCl). The rapid appearance of a yellow or red coloration within 2 minutes was indicative of a positive result (Apriani, 2026).

Phenolics and Tannins

The analyze of phenolics, 2.0 grams of fresh *M. oleifera* leaves were boiled in 5.0 mL of distilled water for 5 minutes, followed by filtration. Subsequently, 2.0 mL of the filtrate was treated with 3 drops of a freshly prepared 1% Ferric Chloride (FeCl3) solution. The development of a green-black or blue-black coloration served as an indicator of phenolic presence. For tannin confirmation, a separate 2.0 mL aliquot of the filtrate was combined with 1.0 mL of a 1% gelatin solution containing 10% NaCl. The formation of a white precipitate within 3 minutes was indicative of tannins (Sanwal et al., 2025; Durri & Walid, 2024).

Saponins

The froth test was employed to assess the sample. A quantity of 2.0 grams of fresh *M. oleifera* leaves was placed in a graduated test tube containing 10.0 mL of distilled water. The mixture underwent vigorous manual mechanical agitation for precisely 2 minutes. Observations were made to determine the formation of a stable

and dense foam column, which was required to reach a minimum height of 1 cm and persist for more than 15 minutes (Apriani, 2026).

Steroids and Terpenoids

The Liebermann-Burchard reagent, comprising glacial acetic acid and concentrated H_2SO_4, was utilized for analysis. A 2.0-gram sample of fresh *M. oleifera* leaves was extracted using 3.0 mL of chloroform and subsequently filtered. The resulting filtrate was combined with 2.0 mL of glacial acetic acid and thoroughly mixed. Subsequently, 1.0 mL of concentrated H2SO4 was carefully added along the side of the inclined test tube to form distinct layers. The appearance of a blue-green color indicated the presence of steroids, whereas a red or orange color signified the presence of terpenoids (Mikail et al., 2025).

Data Analysis

The results from the triplicated qualitative phytochemical screenings were systematically evaluated based on visual color transformations, foam stability heights, and precipitate formations. Data were recorded using a qualitative matrix scoring system, where (+) denoted clear, reproducible presence across all three replicates, and (-) denoted absolute absence or lack of visible reaction.

RESULT AND DISCUSSION

Qualitative Phytochemical Profile

The qualitative screening results indicate that the fresh leaves of *Moringa oleifera* from Kaway XVI are rich in secondary metabolite compounds (Table 1). Table 1 presents the findings from the qualitative phytochemical analysis of fresh *Moringa oleifera* leaves sourced from the Kaway XVI region. The analysis identified several key groups of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, saponins, tannins, and steroids, while terpenoids were absent. This pattern strongly suggests that fresh leaves of *M. oleifera* from this area have significant bioactive potential, especially in natural pharmacology. The detection of alkaloids was confirmed using three different reagents—Mayer, Wagner, and Dragendorff—enhancing the reliability of this finding.

Flavonoids were identified through the Shinoda test, which resulted in a yellow-red color change, indicating the presence of compounds with flavone or flavonol structures. The presence of phenolic compounds was indicated by a greenish-black color change in the $FeCl_3$ test, further supporting the extract's antioxidant properties. The combination of flavonoids and phenolics in this sample suggests a synergistic potential for antioxidant activity.

Saponins were identified by the formation of a stable foam lasting over 15 minutes, indicating the presence of compounds with natural surfactant properties. Additionally, tannins were detected through the formation of a white precipitate in the gelatin test. Tannins are known for their protein-precipitating ability and antimicrobial and astringent activities, further enhancing the therapeutic potential of *M. oleifera*. Steroids were detected via the Liebermann-Burchard reaction, which resulted in a blue-green color, indicating the presence of phytosterols.

Table 1. Qualitative Phytochemical Screening Results of Fresh *Moringa oleifera* Leaves from Kaway XVI

Compound Group	Results	Positive Indicator Observation
Alkaloids (Mayer's Reagent)	+	Cream/White precipitate formation
Alkaloids (Wagner's Reagent)	+	Reddish-brown precipitate formation
Alkaloids (Dragendorff's Reagent)	+	Orange-red precipitate formation
Flavonoids (Shinoda Test)	+	Yellow-red color transformation
Phenolic Compounds ($FeCl_3$ Test)	+	Greenish-black color development
Saponins (Froth Test)	+	Stable dense foam column > 1 cm persisting > 15 minutes
Tannins (Gelatin Test)	+	White precipitate formation
Steroids (Liebermann-Burchard)	+	Blue-green (Liebermann-Burchard)
Terpenoids (Liebermann-Burchard)	-	No significant or characteristic color change

Note: (+) *Detected uniformly across all replicates;* (-) *Not detected.*

On the other hand, terpenoids were not detected, as shown by the lack of significant color change in the Liebermann-Burchard test. This absence could be due to factors such as environmental conditions, leaf age, extraction methods, or compound concentrations below the qualitative method's detection limit. Geographic variability is known to affect the secondary metabolite profile of plants (Zhang et al., 2023).

Comparative Analysis and Bioactivity

The detection of alkaloids, flavonoids, and phenolics is consistent with studies conducted on *Moringa* foliar tissues in other tropical regions (Ben-Uwabor et al., 2025). Flavonoid and phenolic compounds have been proven to have a strong positive correlation with antioxidant activity (Ligo et al., 2025). Previous evaluations using the DPPH method on *Moringa* leaf extracts showed IC_{50} values in the moderate to strong category, indicating high efficiency in scavenging free radicals (Ligo et al., 2025).

The absence of terpenoids in this study is likely influenced by geographical variations specific to the West Aceh coastal environment (Sukmawaty et al., 2024). However, the presence of steroids and saponins supports the plant's potential for hepatoprotective activity and metabolic regulation (Al-Muzafar & Amin, 2025; Chigurupati et al., 2022). Beyond its medicinal aspects, the richness of protein and minerals in *Moringa*, when combined with Lumi-lumi fish (*Harpodon nehereus*)—which boasts a protein content of up to 70% and calcium levels of 2500 mg/100g—in a snack bar formulation, has been shown to provide a balanced macronutrient profile (Rumapea et al., 2025).

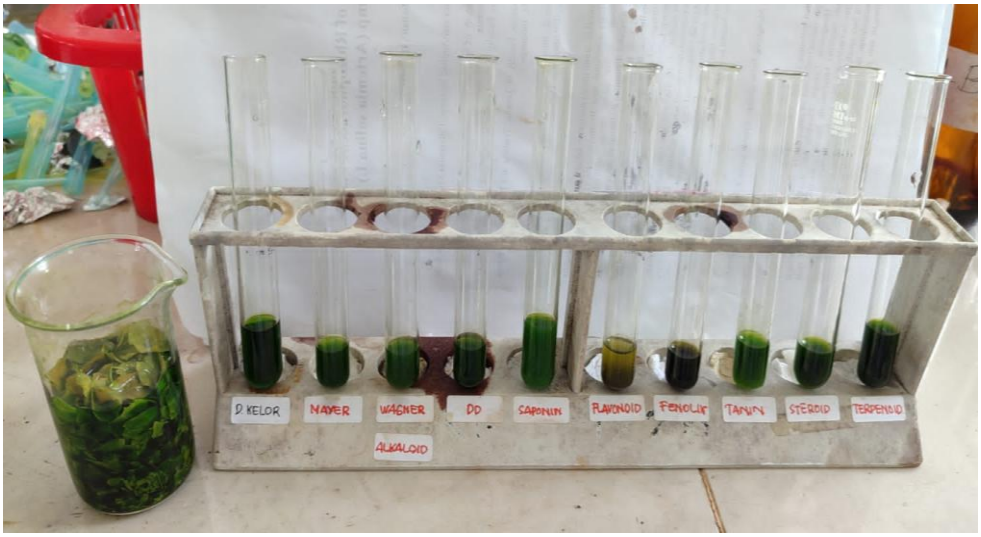

Figure 2. Phytochemical Screening Results

Pharmaceutical and Food Innovation Implications

This characterization establishes a foundation for the quality control of herbal products and functional foods in Indonesia. The utilization of *Moringa* in snack bar products demonstrates that specific formulations (P1) are organoleptically preferred by consumers (Rumapea et al., 2025). Furthermore, the robust antioxidant bioactivity data (Ligo et al., 2025) reinforces the position of *Moringa* as a primary raw material for nutraceutical development.

CONCLUSION

This study successfully establishes the qualitative secondary metabolite blueprint of fresh, unextracted *Moringa oleifera* leaves from the coastal zone of Kaway XVI, West Aceh, utilizing a direct-tissue screening approach that bypasses drying-induced chemical alteration. The qualitative identification of rich phenolic, alkaloid, steroid, tannin, and flavonoid profiles provides a reliable chemical basis consistent with local traditional medicinal practices and galactagogue applications. These baseline qualitative findings offer chemical validation for traditional applications and suggest excellent raw material suitability for future downstream nutritional innovations, such as functional food enrichment. A primary limitation of this study is its purely qualitative nature; it does not provide absolute quantitative yields or high-resolution chromatographic profiling of the active components. Consequently, future research must prioritize quantitative biomarker determination and subsequent clinical efficacy evaluations to fully standardize these local resources.

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