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Qualitative identification of alkaloid compounds in *Moringa oleifera* leaf extract

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Abstract

Background: *Moringa oleifera* (moringa leaves) is a biological resource with significant pharmacological potential. Empirically and scientifically, this plant extract has been proven effective as a hematinic (antianemic) agent due to its essential mineral and vitamin content. In addition, moringa is rich in secondary metabolites, including alkaloids, which have strong antioxidant activity. This antioxidant activity is crucial because it has the potential to protect erythrocyte membranes from damage due to oxidative stress, thus supporting synergistic antianemic efficacy. **Objective**: This study sought to qualitatively validate the existence of alkaloid compounds as bioactive constituents in a 96% ethanol extract of moringa leaves. **Methods:** Dried moringa leaf powder was extracted using maceration with 96% ethanol solvent for 72 hours. The concentrated extract was tested qualitatively using a precipitation test with specific reagents: Mayer, Dragendorff, Bouchardat, and Wagner. **Results:** The phytochemical screening results showed a strong positive response to the third test reagent, which proved that alkaloid compounds were present. The reaction is indicated by the formation of a white precipitate (Mayer), an intense orange-red precipitate (Dragendorff), and a Reddish-Brown Precipitate (Bouchardat). **Conclusion:** The 96% ethanol extract of Moringa oleifera leaves was proven to contain alkaloid compounds. These qualitative findings confirm the role of alkaloids as supporting phytochemicals, supporting the potential of Moringa leaves as a natural source of ingredients for antianemia therapy.

Keywords: *Moringa oleifera*; alkaloids; antianemia; antioxidants; phytochemical screening.

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INTRODUCTION

Moringa oleifera, also known as the Moringa leaf, is a biological resource widely recognized worldwide, particularly in tropical and subtropical regions, due to its superior nutritional profile and diverse pharmacological potential.[1] This plant has long been used in traditional medicine to treat a variety of ailments. Modern research has confirmed its biological activities, including anti-inflammatory, anti-diabetic, and antioxidant properties [2,3]

Moringa leaves play a prominent and widely studied role as a hematinic or anti-anemic agent.[4] This effectiveness is primarily associated with the content of essential nutrients such as iron, vitamin C, and folate, which are vital in hemoglobin synthesis and red blood cell maturation.[5] Consumption of Moringa extract has been shown to significantly increase hemoglobin levels and red blood cell counts in white rats (Rattus norvegicus) as a model of iron deficiency anemia.[6,7]

The overall effectiveness of Moringa leaf extract is not only determined by its macro- and micronutrient content but is also strongly supported by the presence of secondary metabolites that act as the main bioactive components.[8] One of the most widely known groups of secondary metabolites due to their strong biological activity is alkaloids, which have great potential as antioxidant agents.[9,10] Chemically, alkaloids in plants can be in the form of quaternary ammonium compounds or other compounds that have a nitrogen base.[11,12]

Erythrocyte membrane protection is an important mechanism in managing anemia, particularly those resulting from deficiencies or oxidative stress conditions, such as hemolysis.[13] Oxidative stress in erythrocytes can cause membrane lipid peroxidation, leading to cell damage and worsening anemia.[14] Alkaloids, which function as antioxidants, can stabilize and protect red blood cells from free radical damage.[14,15] Therefore, the presence of alkaloids in Moringa leaf extract is hypothesized to work synergistically, supporting the antianemic effect through two pathways: nutritional enhancement and cellular protection.[15]

Despite the proven antianemic activity of Moringa oleifera, we still need to thoroughly confirm the specific components that underpin its cellular protection mechanism. [16] In this study, the focus of the research was directed at 96% ethanol extract, which was chosen because its polar solvent properties are effective in extracting polar and semi-polar compounds, including alkaloids, flavonoids, and phenolics.[17,18] Therefore, this study aims to qualitatively confirm the presence of alkaloid compounds as supporting phytochemical components in the 96% ethanol extract of Moringa oleifera leaves using a series of specific reagent precipitation tests (Mayer, Dragendorff, Bouchardat). The results of this identification are expected to strengthen the scientific basis for the potential of Moringa leaves as a source of natural ingredients for antianemia therapy.

METHODS

Study design and setting

This research is an observational descriptive study conducted at the Laboratory of the Faculty of Science and Technology, Jambi University, from August - September 2025. This study aims to identify the content of phytochemical compounds qualitatively.

Population, samples and sampling

The samples used were Moringa oleifera leaves collected from the Jambi region and dried into a simple drug. The samples were identified in the Laboratory of the Faculty of Science and Technology, Jambi University.

Sample preparation and extraction

The Moringa oleifera L. leaf samples taken were fresh leaves picked one by one from the leaf stem. The collected Moringa oleifera L. leaf samples were cleaned by washing them in running water, then dried by airing them, after which they were partially dried, powdered, and then subjected to an extraction process and then subjected to chemical identification tests.

Dried powder from Moringa oleifera leaves was prepared for the extraction process. Extraction was carried out using the maceration method. A total of 200 grams of Moringa oleifera simplicia was macerated using 96% ethanol solvent (with a ratio of 1:10) and left for 72 hours (3 days) at room temperature [19,20]. The filtrate obtained from the maceration was then concentrated. The concentration process was carried out using a rotary evaporator at a controlled temperature (50°C) until a thick extract of 20 grams was produced. The yield obtained was 10%.

Qualitative identification of alkaloid compounds

Qualitative identification of alkaloid compounds in concentrated moringa leaf extract was performed using a precipitation test. This procedure involved sample preparation and testing with three specific reagents [21]. Reagent preparation

1) Mayer's reagent

5 grams of potassium iodide are dissolved in 10 ml of distilled water. Then, a solution of 1.36 grams of HgCl in 60 ml of distilled water is added. The solution is shaken, and distilled water is added to make up to 100 ml.

2) Dragendorff's reagent

8 grams of bismuth nitrate are dissolved in 20 ml of nitric acid, then mixed with a solution of 27.2 grams of potassium iodide in 50 ml of distilled water. The mixture is allowed to stand until completely separated. The clear solution is taken and diluted with sufficient water to make 100 ml.

3) Bouchardat's reagent

4 grams of potassium iodide are weighed, dissolved in sufficient distilled water, then 2 grams of iodine are added, and distilled water is added to make a 100 ml solution.

Statistical analysis

The data is presented descriptively and qualitatively based on color changes and the formation of deposits in phytochemical tests.

Ethical concideration

This research involved only laboratory tests on plant material and did not involve human or animal subjects, so specific ethical considerations were not required.

RESULTS

The phytochemical screening results of the 96% ethanol extract of Moringa leaves showed a strong positive response to the alkaloid test, confirming the presence of alkaloid compounds. Figures 1 to 3 present the visual results of the alkaloid test, while Table 1 summarizes the results. The positive reaction indicated by the formation of a precipitate with the three alkaloid precipitating reagents (Mayer, Dragendorff, and Bouchardat) definitively proves that the ethanol extract of Moringa leaves contains alkaloid compounds [21,22].

Table 1. Alkaloid test results of 96% ethanol extract of *Moringa oleifera* leaves.

Test Reagents	Test Results (precipitation formation)	Interpretation
Mayer	White or yellowish-white sediment	Positive Alkaloid
Dragendorff	Intense orange-red sediment	Positive Alkaloid
Test Reagents	Test Results (precipitation formation)	Interpretation

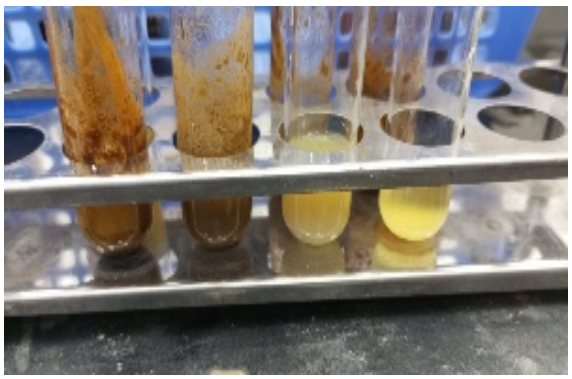

Figure 1. Results of the Alkaloid Test with Mayer's Reagent (White Precipitate)

Figure 2. Results of the Alkaloid Test with Dragendorff's Reagent (Intense Orange-Red Precipitation)

Figure 3. Results of the Alkaloid Test with Bouchardat Reagent (Reddish Brown Precipitate)

DISCUSSION

This research has qualitatively confirmed the presence of alkaloid compounds in a 96% concentrated ethanol extract of Moringa oleifera leaves. Conclusive evidence for the presence of alkaloids was obtained through a reaction mechanism typical of natural product chemistry. The key to this confirmation lies in the ability of the acidified

extract to form insoluble salt complexes when mixed with specific precipitating reagents. Chemically, this occurs when the heavy metal complex ions or bismuth/iodine salts contained in these reagents react with the alkaloid ions present in the test solution [23,24]. The results of the reagent tests provide a clear indication: Mayer's reagent (containing potassium tetraiodomercurate (II)) produces a specific white precipitate. This precipitate is formed due to the presence of alkaloid ions that react to form an insoluble coordination complex. Dragendorff's reagent (containing potassium tetraiodobismuthate) produces an intense orange to red precipitate. This positive reaction is considered a strong and specific indicator for identifying alkaloids. In addition, the use of Bouchardat's reagent (a solution of iodine in potassium iodide) also strengthens and supports the conclusion that alkaloid compounds are one of the main phytochemical components successfully extracted by 96% ethanol from Moringa leaves [21,22].

The results of this study show strong consistency with various previous phytochemical studies, which have widely reported that Moringa oleifera leaves are a natural resource rich in various secondary metabolites. The active compounds that have been successfully identified in Moringa leaves include, but are not limited to, alkaloids, along with flavonoids, saponins, tannins, and glucosinolates [15,17,18]. Among the identified secondary metabolites, several major alkaloids have been successfully isolated and specifically identified from M. oleifera. These important compounds include niazimine, niaziminin, their isomers (such as niazimine A and B), and moringin [25,26] Structurally, these alkaloids are characterized by the presence of a nitrogen atom within their molecular framework. This nitrogen atom provides the base site, a fundamental property that allows the alkaloid compound to interact through salt formation with precipitating reagents used in phytochemical screening [11,21,22].

The successful confirmation of the presence of alkaloids in Moringa leaf extract is highly relevant in efforts to strengthen the pharmacological potential of this plant, particularly as an antianemic agent. Fundamentally, the primary antianemic benefits of Moringa oleifera extract are primarily driven by its essential macro- and micronutrient content. These nutrients, particularly iron, folate, and vitamin C, are key components that directly support erythropoiesis (red blood cell formation) and hemoglobin synthesis in the body [13,16]. However, research shows that alkaloids are not merely passive but rather provide synergistic support to this overall effect. This support stems from their powerful antioxidant activity. This antioxidant function is crucial because it acts as a line of defense: it protects the erythrocyte (red blood cell) membrane from structural damage triggered by oxidative stress in the body's internal environment. Thus, alkaloids ensure that newly formed red blood cells can function optimally and survive longer [13,14].

Oxidative stress, which occurs due to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of cells, is a major triggering factor in hemolysis and the pathophysiology of anemia.[27] Alkaloids and other phenolic compounds in Moringa oleifera act as free radical scavengers, neutralizing ROS before they can attack the lipid membranes of erythrocytes.[14,27] Recent studies have also shown that Moringa extract can increase the activity of endogenous antioxidant enzymes (such as superoxide dismutase and catalase) in erythrocytes, providing double protection against oxidative hemolysis.[25,27] By protecting red blood cells from damage, alkaloids function as supporting bioactive components, which protect red blood cells from oxidative hemolysis, thereby increasing the overall efficacy of moringa leaf extract in antianemia therapy [25,27].

This study is limited to qualitative identification only. For further studies, quantitative identification of alkaloids, as well as isolation and specific identification of alkaloid species (e.g., using High-Performance Liquid Chromatography/HPLC or Gas Chromatography-Mass Spectrometry/GC-MS), is recommended to definitively link specific compounds to the observed erythrocyte-protective effects.

CONCLUSIONS

A 96% ethanol extract of Moringa oleifera leaves was shown to contain alkaloid components after positive qualitative identification using Mayer, Dragendorff, Bouchardat, and Wagner reagents. The presence of these alkaloids validates their significance as important supportive phytochemical components, adding to Moringa leaves' potential as a natural antianemia therapeutic element due to their antioxidant function in giving cellular protection to erythrocytes.

CONFLICT OF INTEREST

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

We hereby confirm that artificial intelligence (AI) was not used during the data collection, analysis, and visualization stages. An AI-based language model was used for language refinement (improving grammar, sentence structure, and readability) and technical writing assistance (providing suggestions for more effective technical descriptions) during the preparation of this manuscript. The authors have critically reviewed all AI-assisted processes to ensure the integrity and reliability of the results. The authors alone make the final decisions and interpretations presented in this article.

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