

Unveiling the nutritional treasures of *Moringa drouhardii*, *Moringa hildebrandtii* (endemic to Madagascar), and *Moringa oleifera* leaves

Abstract

This study comprehensively investigated three *Moringa* species in Madagascar *Moringa drouhardii*, *M. hildebrandtii* and *M. oleifera*. Recognizing the significant nutritional and medicinal potential of *M. oleifera*, this research aimed to expand our understanding of the endemic Malagasy species. A multi-faceted approach was employed, encompassing phytochemical screening, macronutrient and micronutrient analyses, and acute toxicity assessments. Results demonstrated that the leaves of all three species possess significant nutritional value, with high protein content, particularly in *M. oleifera*. They are also rich in essential minerals such as potassium, magnesium, and phosphorus, while supplementation with calcium-rich foods is recommended. Phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, tannins, flavonoids, and saponins, suggesting potential health-promoting properties. Acute toxicity studies in animal models did not reveal any significant adverse effects, indicating the potential safety of *Moringa* leaf consumption. To fully characterize the nutritional composition of these species, further in-depth studies, including detailed amino acid and vitamin analyses, are warranted. To promote the sustainable utilization of these valuable resources, a multi-pronged approach is necessary. This includes public awareness campaigns, the development of value-added products, the establishment of a nursery for the conservation and propagation of endemic species, and the promotion of sustainable cultivation and harvesting practices. This research provides a foundation for the sustainable utilization of these underutilized Malagasy resources, promoting both human health and environmental sustainability.

Keywords: *Moringa*, Madagascar, nutritional value, phytochemicals, sustainable utilization

Volume 13 Issue 1 - 2025

Letsara Rokiman,^{1,2} Mihaja Sam Bryanne Murielle,³ Ratalata Baovola,⁴ Manjovelo Sambany Christian,^{5,6} Ralaivaon-Tsitonta Jumaël Edith Fabrice,⁶ Koto-Te-Nyiwa Jean-Paul Ngbolua,^{7,8} Robijaona Rahelivololoniaina Baholy^{1,3,9}

¹Engineering, Industrial, Agricultural and Food Process and Systems, University of Antananarivo, Antananarivo, Madagascar

²Tsimbazaza Botanical and Zoological Park, Antananarivo, Madagascar

³Polytechnic High School of Antananarivo, University of Antananarivo, Antananarivo, Madagascar

⁴Geochemistry and Medicinal Chemistry Graduate School, University of Fianarantsoa, Fianarantsoa, Madagascar

⁵Institute of Higher Education of Toliara, University of Toliara, Toliara, Madagascar

⁶Geosciences, Physics, Environmental Chemistry and Pathogenic Host Systems Graduate School, University of Toliara, Toliara, Madagascar

⁷Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

⁸National Scientific Council, Ministry of Scientific Research and Technological Innovation, Democratic Republic of the Congo

⁹Laboratory for the Valorization of Natural Resources, Polytechnic High School of Antananarivo, Madagascar

Correspondence: Jean-Paul Ngbolua, Department of Biology, Faculty of Science, University of Kinshasa, Kinshasa, Democratic Republic of the Congo

Received: January 3, 2025 | **Published:** February 5, 2025

Introduction

Moringa oleifera has garnered significant scientific attention due to its multifaceted potential across nutritional, medicinal, and environmental domains. The leaves of this remarkable plant exhibit a rich profile of vitamins and minerals. Furthermore, *Moringa* demonstrates considerable promise in addressing global malnutrition challenges through sustainable approaches. Notably, its seeds possess potent water purification properties, while its historical significance in traditional medicine underscores its potential for novel therapeutic applications. This confluence of attributes renders *Moringa* a captivating and highly promising subject for ongoing scientific investigation.

Madagascar serves as a habitat for three distinct *Moringa* species: *Moringa oleifera*, a widely cultivated species, and the two endemic species *Moringa drouhardii* and *Moringa hildebrandtii*. While *Moringa oleifera* has garnered significant scientific attention for its recognized nutritional and medicinal properties, research efforts have primarily centered on this species, overshadowing the potential of the native Malagasy species. Despite their presence on the island, *Moringa drouhardii* and *Moringa hildebrandtii* remain largely unexplored.

This paper aims to address this knowledge gap by conducting an in-depth comparative analysis of the three *Moringa* species found in Madagascar. The study will encompass phytochemical analyses of leaf extracts from each species, alongside oil extraction from the seeds of the two endemic species and *Moringa oleifera*. This multifaceted investigation seeks to elucidate the unique characteristics of each species and unlock their hitherto untapped potential.

Materials

Moringa drouhardii

Moringa drouhardii is an endemic semi-succulent tree native to southwestern Madagascar. It primarily inhabits arid biomes and dry scrublands,¹ occurring both in wild populations and under cultivation within this region. The generic name "*Moringa*" originates from the Tamil vernacular term "*Murungai*." The specific epithet "*drouhardii*" honors the French botanist Eugène-Jean Drouhard.

Moringa drouhardii exhibits a variable growth habit, attaining heights ranging from 4 to 10 meters, with reports indicating specimens reaching 15 meters² or even 18 meters.³ (Figure 1–Figure 4)



Figure 1 *Moringa drouhardii* trunk.

Source:Wikipédia



Figure 2 *Moringa drouhardii* leaves.

Source:Wikipédia



Figure 3 *Moringa drouhardii* Jum. Flowers.

Source: Boutique végétale



Figure 4 Pod containing *Moringa drouhardii* seeds.

Source: Mihaja Sam, B. M., 2024

- I. The tree possesses a robust trunk, reaching diameters of up to 2 meters,² and a crown characterized by short, contorted branches. The bark displays a smooth texture and a characteristic whitish coloration.
- II. Leaves are tri-impairipinnate, measuring approximately 25 cm in length. The petiole, secondary petioles, and petiolules are glabrous, with stipulated glands evident at the base. The petiole measures 10 to 15 cm in length, the secondary petioles range from 2 to 3 cm, and the petiolules measure 3 to 4 mm. Leaflets, arranged in opposite pairs (5 to 9 pairs per leaf), are oval-elongate, measuring 1.5 to 3 cm in length and 0.5 to 1.2 cm in width. They exhibit a light green coloration, an acuminate apex, and a prominent midrib (Au cactus Francophone, 2008).

Inflorescences are arranged in axillary panicles, attaining lengths of up to 30 cm. Each panicle bears numerous bisexual, actinomorphic, and fragrant flowers. These flowers exhibit a whitish-yellow coloration and a cup-shaped morphology, with pedicels measuring 1 to 2 mm in length. The sepals, glabrous in nature, measure 5 to 6 mm in length and 1.8 mm in width. The oval-shaped petals, measuring 7 to 10 mm in length and 1.8 mm in width, are glabrous on the exterior surface and exhibit slight pubescence on the interior. The filaments, characterized by strong pubescence, measure 6 to 8 mm in length. Au cactus Francophone, 2008.

- I. The fruits of this species are dehiscent capsules, exhibiting a characteristic brown coloration and a persimmon-like morphology. They typically measure between 30 and 50 centimeters in length and approximately 4 centimeters in diameter. The fruit surface is smooth and exhibits a slightly triangular, circular cross-section with distinct constrictions between each seed.
- II. The seeds are predominantly whitish in color and exhibit an ovoid shape. They typically measure 2 to 2.5 cm in length and 1.8 to 2 cm in width. Three distinct ridges are discernible along the seed surface, although they lack wings. The seeds are glabrous, Au cactus Francophone, 2008.

Leaf samples of *Moringa drouhardii* were collected in August from individuals residing in Saint-Augustin, a coastal fishing village situated approximately 30 kilometers south of the city of Toliara in the Atsimo Andrefana region of Madagascar. In a subsequent sampling effort conducted in December, pods of *M. drouhardii* were also harvested from the same location. Saint-Augustin experiences a dry, hot semi-arid climate (Köppen BSh), characterized by consistently warm temperatures throughout the year, with an average annual temperature of 26.5°C. Despite the high temperatures, rainfall in this region is relatively low, averaging 350 mm per year. These specific climatic conditions are considered favorable for the growth and development of *M. drouhardii* in this particular environment.

While sandy-loam soils are prevalent throughout the Toliara region, the soil observed within the Saint-Augustin village exhibits a ferrallitic composition.

***Moringa hildebrandtii* Engl.**

Moringa hildebrandtii Engler, an endemic tree of Madagascar, is the sole representative of the Moringaceae family on the island.⁴ The specific epithet “*hildebrandtii*” honors Johann Maria Hildebrandt (1847-1881), the German botanist who collected the type specimen. Ethnobotanical evidence suggests that the ancestral range of *M. hildebrandtii* was situated in the extreme southwest of Madagascar.⁵ This region, historically inhabited by the Maroseranana,

the descendants of King Olombetsitoto, encompassed a significant portion of the island from 1550 to 1921.⁶

The Sakalavan'i Menabe, an ethnic group currently inhabiting much of Madagascar's west coast, recognizes *M. hildebrandtii* by the common name "hazomaroseranana" or "hazomaroserana," a term derived from "hazo" (tree) and "Maroseranana," signifying its association with the Maroseranana people or their ancestral territory.

Despite its cultural significance and widespread cultivation, field surveys conducted within the historical domain of the Maroseranana, particularly in the region of Tuléar, have failed to locate any wild populations of *M. hildebrandtii*.

This tree can reach up to 25 meters in height, with a pachycaule trunk and more or less twisted branches. Its bark is smooth and varies from white to brown, Au cactus Francophone, 2008 (Figure 5– Figure 8).



Figure 5 Arbre *Moringa hildebrandtii* Engler.

Source: (Au cactus Francophone, 2008)



Figure 6 *Moringa hildebrandtii* leaves.

Source: (Mihaja Sam, B. M., 2024)



Figure 7 *Moringa drouhardii* Jum. Flowers.

Source: Boutique végétale



Figure 8 Gousses et graines de *Moringa hildebrandtii*.

Source: (Mihaja Sam, B. M., 2024)

- I. *Moringa hildebrandtii* exhibits bi- to tri-impairipinnate leaves composed of 2 to 3 pairs of opposite leaflets. These leaflets are characterized by a gray-green coloration, an obovate shape, and a pointed apex. They typically measure 4.5 to 7 cm in length and 2 to 3.5 cm in width. The main petiole ranges from 5 to 10 cm in length, while the secondary petioles measure 4 to 5 cm. Petiolules are relatively short, measuring 5 to 7 mm. All leaf parts are glabrous, and stipulated glands are present at the base of each leaf. A prominent midrib traverses the length of each leaflet (Au cactus Francophone, 2008).
- II. Inflorescences of *M. hildebrandtii* are arranged in axillary panicles, attaining lengths of up to 25 cm. Each panicle bears numerous bisexual, actinomorphic flowers, exhibiting a color range from cream to whitish-yellow. These flowers possess a cup-shaped morphology, with pedicels measuring 3 to 3.5 mm in length. Oblong sepals, measuring 5 to 6 mm in length and 3 mm in width, subtend the corolla. Elongated oblong petals, measuring 8 to 9 mm in length and 1 to 2 mm in width, are characteristic of the flowers. The petal margins exhibit a ciliated appearance, while the interior surface of the flowers displays prominent pubescence. The filaments, measuring 7 to 8 mm in length, are also densely pubescent (Au cactus Francophone, 2008).
- III. Fruits of *M. hildebrandtii* are dehiscent capsules, exhibiting a characteristic khaki-brown coloration. They typically measure 45 to 65 cm in length and 2 to 3 cm in diameter, with a slightly triangular, circular cross-section. The fruit surface is smooth and exhibits constrictions between each seed.
- IV. Seeds are pale brown, more or less ovoid, 3.5 to 4 cm long and 2.2 to 2.5 cm wide. They have three pronounced ribs and are winged.

Moringa hildebrandtii exhibits a natural distribution within the Taolagnaro region and along the western coastline of Madagascar. The specific geographic distribution of this species within these regions is likely influenced by a complex interplay of environmental factors, including climatic conditions, soil characteristics, altitude, and potential biotic interactions.

Specimens of *Moringa hildebrandtii* were collected in the vicinity of the Onilahy River within the Toliara region of Madagascar. In the southern regions of the island, this species is commonly referred to by the local name "Maroseranana." *Moringa hildebrandtii* demonstrates a remarkable capacity for adaptation and exhibits robust growth within the characteristically dry and arid climatic conditions prevalent in the southern regions of Madagascar. These specific environmental conditions appear to favor the vigorous development of this *Moringa* species.

Moringa oleifera

In developing nations, *Moringa oleifera* has garnered significant recognition, often referred to as the “miracle tree” due to its exceptional nutritional and medicinal properties. This designation is particularly evident in Senegal, where it is locally known as “nébéday,” translating to “that which never dies.” This evocative term aptly describes the plant’s remarkable resilience. Following pruning, young *Moringa* plants exhibit a rapid regenerative capacity, promptly re-flowering with the onset of the rainy season.

Moringa oleifera Lam. (Family Moringaceae), a member of the Brassicales order, is a tropical tree widely cultivated for its diverse applications.

Moringa oleifera is a deciduous tree characterized by alternate, imparipinnate leaves that may be compound up to four times. The leaves are often tuberous, bearing more or less opposite leaflets. Notably, the leaflets are exstipulate but may exhibit glands at the base of the pinnae and petioles.⁴

Inflorescences are arranged in axillary panicles, bearing numerous fragrant flowers. These flowers typically exhibit actinomorphic symmetry, although zygomorphic forms may occasionally occur. They are bisexual, displaying a range of colors from white to yellow or red, and possess a cup-shaped or tubular morphology.⁷

Flowers possess five (5) sepals, typically unequal in size, and five (5) free petals. The androecium comprises five (5) outer stamens alternating with three to five inner staminodes. The stamens and staminodes exhibit basal fusion. The ovary, positioned superiorly, is unilocular and composed of three (3) fused carpels. The anthers are unilocular and dehisce longitudinally.⁷ (Figure 9– Figure 12)



Figure 9 *Moringa oleifera*.

Source: (Mihaja Sam, B. M., 2024)

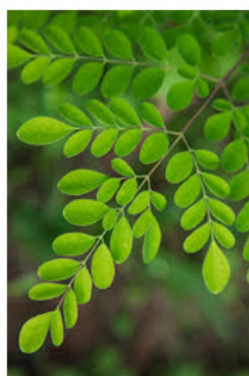


Figure 10 *Moringa oleifera* leaves.

Source: (Mihaja Sam, B. M., 2024)



Figure 11 *Moringa oleifera* flowers.

Source: INPN



Figure 12 Different parts of *Moringa oleifera*: pods, flowers, leaves.

Source: Blogs mighty

Fruit development culminates in a dehiscent capsule, splitting open along three (3) valves. Each capsule contains numerous black, rounded, winged seeds. These seeds are exalbuminous, characterized by a straight embryo.⁷

Moringa oleifera exhibits a remarkably broad geographical distribution, a testament to its exceptional capacity for acclimatization. This widespread dispersal is concomitant with a diverse array of vernacular names, reflecting local adaptations and acknowledging its recognized functional utility within various geographical contexts.

Leaf samples of *Moringa oleifera* were collected in Antananarivo, specifically within the commune of Ampitatafika in the Antananarivo Atsimondrano District. The collection site is geographically located at 18° 54' South latitude and 047° 31' East longitude. The soil at the collection site was characterized as silty-clay, a soil type generally considered to be of low fertility. Despite these suboptimal soil conditions, *Moringa oleifera* demonstrated a remarkable capacity for growth and development.

Antananarivo experiences a high-altitude tropical climate, characterized by subtle variations compared to more conventional tropical climates. These climatic nuances can exert significant influence on plant growth and development, including that of *Moringa oleifera*. The region typically receives annual rainfall ranging from 1,000 to 2,000 millimeters.

The following cartographic representations delineate the geographic distribution of the Malagasy endemic species *Moringa drouhardii* and *Moringa hildebrandtii*, contrasted with the cosmopolitan *Moringa oleifera*.

The cartographic representations delineate the geographic distribution of the Malagasy endemic species *Moringa drouhardii* and *Moringa hildebrandtii*, contrasted with the cosmopolitan *Moringa oleifera*.

Methods

Preparation of the *Moringa* leaves studied

Leaf samples of *Moringa oleifera* (collected from Antananarivo) and the endemic Malagasy species, *M. drouhardii* and *M. hildebrandtii* (collected from Toliara), were subjected to a standardized sample preparation protocol. After washing with distilled water, the leaves were shade-dried for 12–15 days. Subsequently, the dried leaves were meticulously ground into a fine powder using a kitchen blender, preparing them for further analysis (Figure 13).

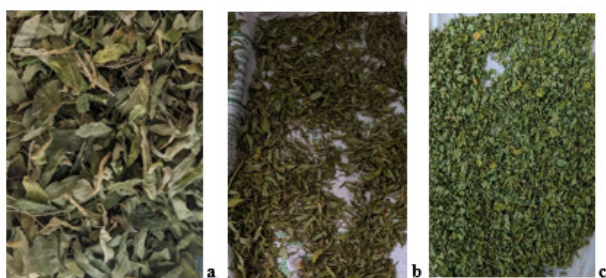


Figure 13 *Moringa* leaves in the drying process: (a) *M. drouhardii*, (b) *M. hildebrandtii*, (c) *M. oleifera*.

Source: (Mihaja Sam, B. M., 2024)

Analysis of elemental micronutrients in *Moringa* leaves

Micronutrient analysis of the extracts was performed at the Office des Mines Nationales et des Industries Stratégiques (OMNIS) laboratory. Dried leaf samples were meticulously ground into a fine powder prior to analysis. X-ray fluorescence (XRF) spectroscopy was employed to determine the elemental composition. XRF involves irradiating the sample with primary X-rays, which excite atoms within the sample. Upon de-excitation, these atoms emit characteristic secondary X-rays, allowing for the identification and quantification of elements based on the unique wavelengths and intensities of the emitted radiation.⁸

Macronutrient analysis of *Moringa* leaf samples

Macronutrient analyses of the leaf samples were conducted at the Laboratoire de Chimie et Microbiologie (LCM) Nanisana, while protein content determination was performed at the Centre National de Recherches sur l'Environnement (CNRE).

Determining moisture content

Water constitutes the primary component of living matter. Accurate determination of moisture content in foodstuffs is crucial for standardizing analytical results on a dry matter basis. This is essential as dry matter, primarily composed of organic matter, serves as the primary source of energy for the human organism.

The Guilbot method, specifically designed to quantify free water content, was employed in this study. A pre-weighed capsule was loaded with a 5-gram sample of the foodstuff. Subsequently, the sample was subjected to a drying process at a temperature of 103°C for a duration of four hours within a drying oven. Following the drying period, the samples were transferred to a desiccator for a cooling period of one hour before final mass determination.

The moisture content is obtained using the following equation:

$$H\% = \frac{M_1 - M_2}{M_1 - M_0} \times 100 \quad (1)$$

H%: Water content expressed in grams per 100g of sample

M_0 : mass of empty capsule in grams

M_1 : mass in grams of capsule with sample before drying

M_2 : mass in grams of capsule with sample after drying

From the water content, it is possible to determine the dry matter content (MS%) of each sample.

$$MS\% = 100 - H\% \quad (2)$$

Fat determination

Lipids, a class of hydrophobic biomolecules, were extracted from the samples using the Soxhlet method. Hexane, a nonpolar solvent, was employed for efficient lipid extraction. The Soxhlet apparatus facilitates continuous solvent extraction through a cycle of vaporization, condensation, and dripping of the solvent onto the sample. Following extraction, the solvent was removed using rotary evaporation.

The lipid content was determined gravimetrically. The sample was initially weighed, and subsequently, the weight of the lipid-free flask was recorded. The difference in weight between the initial and final measurements represents the amount of extracted lipids. This method provides an accurate and efficient means of quantifying lipid content in various samples.

The lipid content is given by the relationship:

$$MF\% = \frac{M_2 - M_0}{M_1} \times 100 \quad (3)$$

MF%: Fat content in grams per 100g of raw material

M_0 : empty balloon mass in grams

M_1 : test sample weight in grams

M_2 : mass of balloon with test sample after steaming

Determination of ash content

Crude ash, representing the inorganic residue remaining after complete combustion, provides valuable insights into the mineral content of food samples. Ash determination employs the gravimetric principle, measuring the mass loss upon combustion.

In this study, porcelain crucibles were used to incinerate 5-gram samples in a muffle furnace at 550°C for 12–24 hours. The completion of calcination was indicated by the formation of a white, gray, or yellowish ash. After cooling in a desiccator, the mass of the crucibles and their ash content were recorded. These measurements were used to calculate the percentage of crude ash in the original samples.

The crude ash content, expressed in grams per 100g of sample, is obtained using the following formula:

$$CB\% = \frac{M_2 - M_0}{M_1} \times 100 \quad (4)$$

C%: Content in grams of ash per 100g of raw material

M_0 : Empty incineration capsule mass in grams

M_1 : Mass in grams of test sample

M_2 : Mass in grams of sample capsule after incineration.

Determining protein content

The Kjeldahl method, a two-stage analytical procedure, was employed to determine the protein content of the *Moringa* leaf samples.

Mineralization: In the first stage, organic nitrogen within the sample is converted to ammonium nitrogen through oxidative digestion with concentrated sulfuric acid in the presence of a catalyst (e.g., copper sulfate and potassium sulfate). The catalyst accelerates the digestion process by elevating the boiling point of the acid mixture.

Distillation: Following mineralization, the ammonium ions are liberated from the ammonium sulfate salt by the addition of excess sodium hydroxide, shifting the medium to an alkaline pH. The liberated ammonia gas is then distilled with steam and trapped in a boric acid solution.

Quantification: The amount of ammonia trapped in the boric acid solution is determined by titration with standardized sulfuric acid. The volume of sulfuric acid consumed is directly proportional to the amount of ammonia, which in turn is directly related to the protein content of the original sample.

The total nitrogen content is given by the following formula:

$$N\% = \frac{V_{CB} \times N \times 0,014}{PE} \times 100 \quad (5)$$

The total protein content of the sample was determined by multiplying the total nitrogen content by a conversion factor of 6.25. This conversion factor is based on the empirical observation that proteins typically contain approximately 16% nitrogen by weight.

$$P\% = N\% \times 6,25 \quad (6)$$

P%: Total protein content in grams per 100g of sample

N%: Nitrogen content in grams per 100g of sample

V_{CB} : Volume in ml of 0.1N sulfuric acid required to obtain the turn (Burette drop)

N: Sulfuric acid normality

PE: Mass in grams of test sample

M (N) = 14g/mol; N=0.1N

Determining carbohydrate content

The total carbohydrate content of the sample is calculated by subtracting the sum of the protein, lipid and crude ash contents from the dry extract content.

Carbohydrate content, expressed as a percentage of the dry weight, was calculated using the following equation:

$$G\% = 100 - (H\% + MG\% + P\% + CB\%) \quad (7)$$

G%: Total carbohydrate content in grams per 100g of sample

H%: Water content in grams per 100g of sample

MG%: Fat content in grams per 100g of sample

P%: Protein content in grams per 100g of sample

CB%: Crude ash content in grams per 100g of sample

Determining overall energy value

The total energy value of a food represents the amount of energy released upon the complete oxidation of its constituent macronutrients: proteins, lipids, and carbohydrates. This energy value considers the physiological availability of each nutrient, specifically the proportion that is effectively absorbed and utilized by the human body.

The Atwater system provides a widely accepted framework for estimating the energy content of foods. Atwater factors are empirical coefficients that convert the mass of each macronutrient into its corresponding metabolizable energy:

- 1 g glucide = 4 kcal
- 1 g protein = 4 kcal
- 1 g lipid = 9 kcal

The total energy value of a food is determined by adding up the metabolizable energies of its carbohydrate, fat and protein components. The total energy value, expressed in kcal, is calculated from the relationship:

$$E(Kcal) = (9 \times L) + (4 \times G) + (4 \times P) \quad (8)$$

E: Energy value of sample, expressed in kcal

L: Total lipid content in g per 100g of sample

G: Total carbohydrate content in g per 100g sample

P: Protein content in g per 100g sample

Phytochemical screening of three plant species

Phytochemical screening is a method for identifying the chemical families and organic substances present in a plant sample. Phytochemical screening of samples was carried out at the Laboratoire de Chimie et de Microbiologie (LCM) Nanisana.

Principle of phytochemical screening

Phytochemical screening involves a series of qualitative tests aimed at detecting the presence of various classes of secondary metabolites within plant extracts. This study employed a comprehensive screening protocol to investigate the presence of a diverse range of compounds, including phenolic compounds such as total phenols, tannins (both gallic and catechic), anthocyanins, leucoanthocyanins, flavonoids, and coumarins. Furthermore, the screening encompassed the detection of terpenoids, specifically saponins and carotenoids; nitrogenous compounds such as alkaloids; and glycosides, particularly iridoids. Additionally, the presence of other classes of compounds, including quinones, sterols, and polyterpenes, was also investigated. This comprehensive screening approach provided valuable insights into the phytochemical diversity of the plant extracts under examination (Table 1).⁹

Table 1 Tests identifying chemical families by phytochemical screening

Chemical families	Tests
Alkaloids	Mayer, Dragendorff and Wagner.
Triterpenes and steroids	Salkowski, Liebermann-Burchard and Badjet-Kedde.
Flavonoids and Leucoanthocyanins	Modified Wilstater and Bate-Smith tests.
Anthraquinones	Börnstrager
Irridoids	Kedde, Trim-Hill
Saponosides	Foam Test
Deoxyoses	Keller-Kiliani
Tannins and polyphenols	1% gelatin, Salted gelatin, FeCl ₃ (MeOH)

Acute toxicological test on leaves of three *Moringa* species: *oleifera-drouhardii* and *hildebrandtii*.

Extraction procedure

A hot extraction method was employed. Ten grams of leaf powder were accurately weighed and dissolved in 100 mL of 80% ethanol, resulting in a 1:10 (w/v) solution. The mixture was subjected to continuous magnetic stirring to ensure thorough mixing. Subsequently, the mixture was allowed to macerate for a duration of one hour, facilitating the transfer of bioactive compounds from the plant matrix into the solvent. Following maceration, the mixture was filtered to remove insoluble plant debris. The filtrate, containing the extracted compounds, was then subjected to evaporation to obtain a crude extract.

Acute toxicity assessment

Acute toxicity assessments were conducted on Swiss-albino mice to evaluate the potential toxicity of the *Moringa* leaf extracts. Three mice were utilized for each extract tested. Prior to administration, the mice were subjected to a 24-hour fasting period. The crude leaf extracts were appropriately diluted with distilled water prior to oral administration. The volume of the administered extract was carefully calculated for each mouse. Following administration, the mice were closely monitored for a period of 24 hours for any signs of toxicity, including behavioral changes, mortality, and other relevant clinical observations.

The observed toxicity symptoms, including the time of onset and severity, were recorded. These observations provided valuable insights into the potential toxicity of the extracts and facilitated the identification of any adverse effects.

The determination of acute toxicity consists in knowing the single lethal dose of the extract at one time in a determined time (24 h). Trevan,¹⁰ method was used to determine the Lethal Dose LD₅₀, given by the curve of percentage mortality of mice as a

function of the decimal logarithm of the doses administered. The values found are then confirmed by the calculation method of Berhens and Karber.¹¹

$$LD_{50} = [LD_{100} - \Sigma (a \times b)]/n'$$

n' the average number of animals per batch,

a, the average of deaths between doses and

b, the difference between two successive doses.

The toxicity values scale used to locate the toxicity effect of extracts is given in Table 2. Animals were selected by stratified randomization and then divided into three groups of three mice each. Groups II and III were given 1,200 mg/kg body weight of each plant extract orally (force-feeding at a rate of 0.19-0.24 mL) in a single dose. NaCl 0.9% served as the vehicle and was used to prepare the dose. Group I served as the control group and received the NaCl 0.9% vehicle only. The mice were fasted for 24 hours before administration of the extracts. They were observed for 24 hours for signs of toxicity, mortality and general behaviors. Animals were fed ad libitum with standard feed, and had free access to water. They were also maintained under standard conditions of humidity, temperature, and 12 hours light/dark cycle. The animals were acclimatized for a week before the commencement of the study. A standard protocol was drawn up in accordance with current guidelines for the care for laboratory animals and ethical guidelines for investigations of experiments in conscious animals. The first day of dosing was taken as day 0. During the two-week period, all the animals were observed daily for clinical signs and mortality patterns once before dosing, immediately after dosing and up to two to four hours after dosing.¹²

Table 2 Toxicity class, According to the **Hodge and Sterner 1943**. Toxicity scale

Toxicity index class	Commonly used term	Toxicological parameter (LD ₅₀)
1	Extremely toxic	LD ₅₀ ≤ 1 mg/Kg
2	Highly toxic	1 mg/Kg ≤ LD ₅₀ ≤ 50 mg/Kg
3	Moderately toxic	50 mg/Kg ≤ LD ₅₀ ≤ 500 mg/Kg
4	Slightly toxic	500 mg/Kg ≤ LD ₅₀ ≤ 5 g/Kg
5	Almost toxic	5 g/Kg ≤ LD ₅₀ ≤ 15 g/Kg
6	Relatively harmless	LD ₅₀ ≥ 15 g/Kg

Results

Elemental micronutrient analysis results for the three species of *Moringa* leaf samples

Elemental micronutrient analysis of the leaves of the three *Moringa* species was carried out at the OMNIS laboratory (Table 3).

Table 3 Results of the micronutrient content of the three *Moringa* species (in g/100g dry matter)

Code	Ca	P	Mg	K	Fe	Zn	Mn	Cu	As	Si
<i>M.drouhardii</i>	0.08	0.39	1.96	3.66	0.63	0.02	0.04	0.03	0.01	1.16
<i>M.hildebrandtii</i>	0.09	0.68	1.96	2.7	0.54	0.01	0.09	0.02	0.01	1.24
<i>M.oleifera</i>	0.07	0.56	1.91	2.16	0.57	0.01	0.07	0.02	0.01	1.22

The leaves of *M. oleifera*, *M. drouhardii*, and *M. hildebrandtii* were found to be rich in both macro- and micronutrients. Potassium and magnesium emerged as the predominant macronutrients across all three species. Among the micronutrients, silicon and iron were notably abundant.

Potassium plays a crucial role in regulating blood pressure and maintaining proper muscle and nerve function. Magnesium is an essential cofactor in numerous cellular processes, particularly in energy production. Iron, a key component of hemoglobin, is indispensable for oxygen transport within the bloodstream. These essential minerals are vital for human health and well-being.

Results of the determination of macronutrients present in leaf samples of the three *Moringa* species

Humidity test results are recorded in the following table. The proportion of dry matter was determined from the water content (Table 4).

Table 4 Results of leaf moisture content for three *Moringa* species

Macronutrients	<i>Moringa drouhardii</i>	<i>Moringa hildebrandtii</i>	<i>Moringa oleifera</i>
Moisture content (%)	9.89	9.26	9.2
Lipids (%)	7.59	17.57	4.78
Proteins (%)	19.265	22.26	24.67
Total ash (%)	3.6	6.73	3.31
Carbohydrates/ Glucides (%)	59.66	44.23	58.02
Energy value (kcal/100g)	373.96	383	424.09
Dry matter: DM (%)	90.11	90.74	90.8

Moisture and dry matter content

The analysis revealed slight variations in moisture content among the three *Moringa* species. All species exhibited low moisture levels, minimizing the risk of microbial growth. Notably, the dry matter content was consistently high across all species, indicating a significant proportion of nutrients within the leaf samples. These low moisture levels are likely attributable to the drying and pulverization process employed prior to analysis.

Lipid content

Significant interspecific variations in lipid content were observed. While *Moringa oleifera* exhibited a lipid content of 4.78%, *M. drouhardii* and *M. hildebrandtii* demonstrated higher lipid levels, with values of 7.59% and 17.57%, respectively. Notably, *M. hildebrandtii* exhibited the highest lipid content among the three species. These variations in lipid content can be attributed to a range of environmental factors, including climatic conditions and soil composition, which directly influence the plant's metabolism and lipid biosynthesis.

Protein content

Protein content analysis, conducted at the CNRE Tsimbazaza laboratory, revealed that all three *Moringa* species are rich in protein. *Moringa oleifera* exhibited the highest protein content with an average of 24.67%, highlighting its nutritional significance. *Moringa hildebrandtii* also demonstrated a substantial protein content, averaging 22.260%. While exhibiting slightly lower protein content,

Moringa drouhardii still exhibited a respectable level, averaging 19.265%. This observed diversity in protein content among the three species underscores their potential as valuable sources of dietary protein.

Ash content

Ash content, an indicator of the mineral content within the samples, varied among the species. *Moringa hildebrandtii* exhibited the highest ash content, followed by *M. drouhardii* and *M. oleifera*. However, the ash content observed in all three species was relatively low when compared to previous findings reported by Youssouf (2001) for *M. oleifera* from Tuléar (14 g/100g dry matter).

Carbohydrate (Glucid) content

Carbohydrates, a primary source of energy, were found to vary among the species. *Moringa drouhardii* exhibited the highest carbohydrate content, followed by *M. oleifera* and *M. hildebrandtii* (Figure 14).

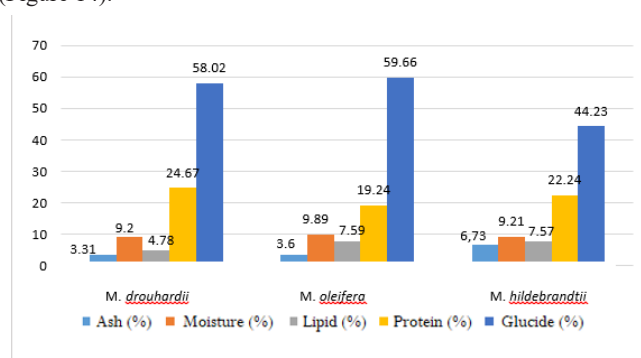


Figure 14 Summary of macronutrient content results for the three *Moringa* species.

This figure demonstrates that *Moringa* leaves from all three species contain a spectrum of essential macronutrients, suggesting their nutritional suitability for human consumption.

Phytochemical screening of *Moringa oleifera* - *Moringa drouhardii* and *Moringa hildebrandtii* leaves

The results of the phytochemical screening carried out on *Moringa oleifera*-*Moringa drouhardii* and *Moringa hildebrandtii* leaf powders are shown in the following table: Table 5

Phytochemical screening of *Moringa oleifera*, *M. drouhardii*, and *M. hildebrandtii* leaf powders revealed the presence of several key secondary metabolites. All three species exhibited the presence of alkaloids, flavonoids, and tannins. Notably, *M. oleifera* also demonstrated the presence of saponins, anthraquinones, and steroids.

The presence of alkaloids in these *Moringa* species may be linked to their traditional medicinal uses. However, it is important to note that the observed alkaloid levels were likely low or present in trace amounts, suggesting minimal toxicity.

Flavonoids, a class of polyphenols, are widely recognized for their antioxidant properties. These compounds are believed to contribute significantly to the medicinal properties of *Moringa* leaves Sallau et al.,¹³ by effectively scavenging free radicals, which are implicated in various human diseases.¹⁴

While extensive phytochemical research has been conducted on *M. oleifera*, comparatively less research has been dedicated to *M. drouhardii* and *M. hildebrandtii*.

Table 5 Phytochemical screening results for the three *Moringa* species

Chemical families	Tests	Moringa species studied		
		<i>M.oleifera</i>	<i>M.drouhardii</i>	<i>M.hildebrandtii</i>
Alkaloids	Mayer	+	+	+
	Wagner	-	-	-
	Dragendorff	-	-	-
	Wilstater	+	++	++
Flavonoids and Leucoanthocyanins	Modified Wilstater	++	+++	-
	Bate Smith	-	-	-
	1% gelatin	+	+	-
Tannins and polyphenols	Salted gelatin	+	-	-
	FeCl ₃ (MeOH)	-	-	-
Saponosides	Foam test	+	+	-
Anthraquinones	Börnstrager	+	-	-
	Salkowski	-	-	-
Steroids and Triterpenes	Libermann-Burchard	-	-	-
	Badjet-kedde	+	-	-
Deoxyoses	Kelleer Killiani	-	-	-
Irridoids		-	-	-

(-) : absence ; (+) : trace ; (++) : average presence ; (+++) : abundance

Toxicological testing of the leaves of each *Moringa* species

The acute toxicity test was carried out at the Parc Botanique et Zoologique de Tsimbazaza, where Swiss mice weighing at least 23g were chosen specifically for this study. The criteria for toxicity assessment was based on lethality, specific behavioral changes (Table 6).

Table 6 Results of acute toxicity test on mice

Sample extract	Volume of diluted extract for force-feeding (ml/ mouse)	Observed symptoms	Number of deaths
<i>M. drouhardii</i>	0.20	None	None
<i>M. hildebrandtii</i>	0.19	None	None
<i>M. oleifera</i>	0.24	None	None

Acute toxicity assessment

Acute toxicity studies were conducted on three groups of mice (n=3/group) to evaluate the potential adverse effects of the *Moringa* leaf extracts. Prior to the study, all mice were subjected to a 24-hour fasting period. The crude extracts were appropriately diluted with distilled water before oral administration to the mice.¹⁵

Clinical signs noted after force-feeding the crude extracts

A few moments after gavage of plant extracts at a dose of 1200 mg/Kg, a lack of appetite were noted. This behavior can be explained by the altered physiological processes for transient period due to administered dose. About twenty minutes later, all the animals resumed their normal behavior. This shows that the plant extract seems to exert a stressful effect on the mice.

Effect of gavage of the extract on the mortality of mice

During the present investigation, the extracts administered at a dose of 1200 mg/Kg, did not cause the death of the mice. No signs of toxicity were observed in any of the mice during the 24-hour

observation period following extract administration. This suggests that, within the tested dose range, the *Moringa* leaf extracts exhibited low acute toxicity. The absence of any observable adverse effects indicates that these extracts may be consumed safely, at least within the tested dose limits.

Determination of the LD₅₀

The Lethal Dose LD₅₀ which is the dose causing the death of 50% of the mice could not be determined. In fact, no mouse died at the dose of 1200 mg/kg; the LD₅₀ would therefore be higher than this dose. The present study revealed that the tested plant materials are not (slightly) toxic to mice. It is obvious that the results obtained on mice cannot be applied directly to humans; however, these results reassure the safety of the two plant extracts.

The Maximum Tolerated Dose (MTD) is higher than 1200 mg/kg and could therefore potentially be used experimentally in a subacute or chronic toxicity study. Thus, the value of the lethal dose 50 greater than 1200 mg/Kg of body weight in mice makes it possible to classify the extracts as slightly toxic substance, on the classified toxicity scale of Hodge and Sterner as reported by Cotonat.¹⁶

In addition, for this LD₅₀ value, a 50 kg person should receive at least 1200 mg/Kg x 50, i.e. 60,000 mg of product in a single dose to run the same symptoms. These 60 g dose of extract on the Gosselin Smith and Hodge classification scale could be classified as non-toxic to humans.

Thus, Malagasy endemic *Moringa* could also be used as nutraceuticals by associating them with *Aloe* as fortifiant for solving Helminthiasis problem in pregnant women.¹⁷

Nutritional and phytochemical composition

Comprehensive analyses of *Moringa oleifera*, *M. drouhardii*, and *M. hildebrandtii* leaves revealed a rich nutritional profile. All three species exhibited high protein content, with *M. oleifera* demonstrating the highest levels. Furthermore, the leaves were rich in essential minerals, including potassium and iron. Phytochemical

screening revealed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, and, in *M. oleifera*, saponins, anthraquinones, and steroids. Notably, the presence of flavonoids, known for their antioxidant properties, suggests potential health-promoting effects. Acute toxicity studies in animals did not reveal any significant adverse effects, indicating potential safety for human consumption. However, further research is warranted to fully elucidate the safety and efficacy of these *Moringa* species for human consumption.

Discussion

Macronutrients found in the leaves of three *Moringa* species

The role of proteins

Proteins, macromolecules composed of amino acids linked by peptide bonds, play crucial roles in various cellular processes. In *Moringa* leaves, they contribute significantly to nutritional value. Proteins serve as essential structural components, forming cell membranes and organelles. They also act as enzymes, catalyzing biochemical reactions; as transporters, facilitating molecular movement; as receptors, mediating cellular signaling; and as antibodies, crucial for immune function. Furthermore, proteins are vital for regulating metabolic processes, including gene expression and cellular signaling pathways. This diverse array of functions underscores the critical importance of proteins for cellular and organismal homeostasis.¹⁸

The role of carbohydrates

Carbohydrates constitute a major component of the *Moringa* leaf biomass, with observed values of 58.02% in *M. oleifera*, 59.66% in *M. drouhardii*, and 44.23% in *M. hildebrandtii*. Carbohydrates are major energy sources, fueling cellular respiration and ATP synthesis. They also function as energy storage molecules.¹⁸

The role of lipids

Lipid content varied significantly among the three *Moringa* species, with *M. hildebrandtii* exhibiting the highest lipid content (17.57%), followed by *M. drouhardii* (7.59%) and *M. oleifera* (4.78%). Lipids play crucial roles in various physiological processes. They serve as an important energy reserve, providing more than twice the energy per gram compared to carbohydrates or proteins. Lipids are vital energy sources, providing more energy than carbohydrates or proteins. They serve as precursors for hormones and signaling molecules. Lipids are also crucial for vitamin absorption and membrane structure (Park et al., 2021).

The role of ash

Ash content, representing the inorganic mineral residue remaining after combustion, provides valuable insights into the mineral composition of the *Moringa* leaves. The analysis revealed variations in ash content among the species, with *M. hildebrandtii* exhibiting the highest content. These minerals, including calcium, phosphorus, potassium, sodium, magnesium, and trace elements, play crucial roles in various physiological processes. They are essential for bone and tooth formation, nerve signal transmission, blood pH regulation, muscle contraction, and enzyme function. Additionally, minerals play a critical role in maintaining fluid and electrolyte balance within the body.¹⁹

The role of water

Moisture content is a critical parameter in food quality and stability. Water content significantly influences the physical and chemical properties of food materials, including texture, sensory characteristics, and susceptibility to microbial spoilage. High moisture content can increase the risk of microbial growth and spoilage, while low moisture levels can lead to desiccation and loss of quality. Therefore, the observed low moisture content in the *Moringa* leaf samples is advantageous, minimizing the risk of microbial contamination and enhancing their shelf-life.²⁰

Elemental micronutrients

The role of magnesium

Magnesium serves as an essential cofactor for numerous enzymes involved in critical metabolic processes, including nucleic acid and protein synthesis, ATP production, and cellular signaling. It plays a vital role in maintaining cellular membrane stability and regulating ion transport across cell membranes. Furthermore, magnesium is crucial for neuromuscular function, blood pressure regulation, and cardiac activity. Importantly, magnesium plays a significant role in calcium homeostasis by regulating calcium transport and storage within bone and muscle tissues.²¹

The role of calcium

Calcium, a divalent cation, is indispensable for a wide range of physiological processes. As a major component of the skeletal system, it is essential for bone and teeth formation and maintenance. Calcium also plays a crucial role in muscle contraction, including cardiac function, and in the transmission of nerve impulses. Additionally, it functions as a cofactor in various enzymatic reactions and plays a critical role in blood coagulation. Calcium also regulates cellular processes such as membrane permeability, hormone secretion, and neurotransmitter release. These diverse roles highlight the critical importance of calcium for maintaining cellular homeostasis and overall human health.²²

The role of phosphorus

Phosphorus is an essential mineral ubiquitous in living organisms and plays a crucial role in numerous biological processes. As a major component of DNA, RNA, and phospholipids, phosphorus is indispensable for cellular growth, gene expression, and membrane structure.

Furthermore, phosphorus is essential for energy metabolism, playing a key role in the synthesis and utilization of ATP. It also contributes to bone and tooth mineralization as a major component of hydroxyapatite. Additionally, phosphorus plays a crucial role in maintaining acid-base balance, regulating intracellular and extracellular pH. Finally, phosphorus is involved in nerve impulse transmission, muscle contractility, and other vital physiological processes.²³

The role of potassium

Potassium, a major intracellular cation, plays a critical role in maintaining cellular homeostasis. It is essential for maintaining osmotic balance, regulating cell volume, and facilitating the transmission of nerve impulses. Potassium also plays a crucial role in regulating muscle contractility, including cardiac function, and in the release and regulation of hormones and neurotransmitters.²⁴

The role of iron

Iron is an essential trace element crucial for various physiological functions. As a key component of hemoglobin, iron is indispensable for oxygen transport and cellular respiration. Additionally, iron plays a critical role in DNA synthesis, cell division, and gene expression. It is also essential for the production of essential proteins, such as myoglobin and enzymes involved in energy metabolism and neurotransmitter synthesis. Furthermore, iron plays a crucial role in immune function, antioxidant defense, and the prevention of oxidative stress. Iron deficiency can have significant health consequences, including anemia, impaired growth and development, and cognitive dysfunction.²⁵

The role of zinc

Zinc is an essential trace element that plays a crucial role in a wide range of biological processes. As an essential cofactor for over 300 enzymes, zinc is involved in various metabolic pathways, including carbohydrate, lipid, and protein metabolism. It is also essential for growth and development, regulating gene expression and cell differentiation. Zinc plays a critical role in immune function, wound healing, and antioxidant defense. Furthermore, zinc is essential for sensory perception, including taste and smell, and is crucial for maintaining healthy skin, hair, and nails. Zinc also plays a vital role in reproductive health, including spermatogenesis and fetal development.²⁶

The role of manganese

Manganese is an essential trace element involved in various physiological processes. As an essential cofactor for numerous enzymes, manganese plays a crucial role in various metabolic pathways, including amino acid biosynthesis, photosynthesis, and antioxidant defense.

Manganese is also essential for bone and cartilage formation, contributing to the synthesis of connective tissue components. Additionally, manganese plays a crucial role in central nervous system function, blood sugar regulation, and immune function. Manganese deficiency can lead to a variety of health issues, including metabolic disorders and neurological abnormalities.²⁷

The role of arsenic

Arsenic is a metalloid with both essential and toxic properties. While some forms of arsenic are essential for certain microorganisms, it is primarily known for its toxicity in higher organisms. Arsenic can interfere with cellular respiration, disrupt cell signaling pathways, and induce oxidative stress. Furthermore, arsenic is a known carcinogen, increasing the risk of various cancers, including skin, lung, and bladder cancer.²⁸

The role of copper

Copper is an essential trace element that plays a crucial role in various physiological processes. As an essential cofactor for numerous enzymes, copper is involved in various metabolic pathways, including neurotransmitter biosynthesis, cellular energy production, and connective tissue formation. Copper is also essential for iron metabolism, immune function, and antioxidant defense. Furthermore, copper is crucial for the proper function of mitochondria, lysosomes, and iron transport proteins.²⁹

The role of silicon

Silicon is an essential trace element that plays a crucial role in various physiological processes. As a major component of the

extracellular matrix, silicon is essential for the formation and maintenance of connective tissues, including skin, hair, nails, and cartilage. Silicon also plays a role in bone mineralization and contributes to the synthesis of collagen and other structural proteins. Additionally, silicon plays a role in immune function, wound healing, and protection against oxidative stress. It is also involved in glucose metabolism, detoxification of heavy metals, and the regulation of blood pressure.³⁰

Secondary metabolites in the leaves of three *Moringa* species

Phytochemical screening of *Moringa oleifera*, *M. drouhardii*, and *M. hildebrandtii* leaf powders revealed the presence of a diverse array of secondary metabolites, including alkaloids, flavonoids, leucoanthocyanins, tannins, polyphenols, saponins, steroids, and triterpenes. *M. oleifera* exhibited a broader range of secondary metabolites compared to *M. drouhardii*, which lacked polyphenols and anthraquinones. The presence of these compounds, particularly alkaloids, flavonoids, and tannins, suggests that these *Moringa* species may possess a range of pharmacological activities.

Role of alkaloids

Alkaloids constitute a diverse class of naturally occurring organic compounds, predominantly of plant origin. These nitrogen-containing compounds often exhibit complex chemical structures, including heterocyclic rings. Alkaloids exhibit a wide range of biological activities, including analgesic, antipyretic, and anti-inflammatory properties. However, they are also known for their potential toxicity and psychotropic effects. Due to their diverse pharmacological activities, alkaloids continue to be extensively studied in the fields of pharmacology and medicinal chemistry.³¹

Role of leucoanthocyanins

Leucoanthocyanins belong to the flavonoid class of plant pigments. These colorless compounds lack the chromophore groups responsible for the characteristic color of anthocyanins. However, under specific enzymatic conditions, leucoanthocyanins can be converted into anthocyanins, playing a crucial role in plant pigmentation. Furthermore, leucoanthocyanins possess significant antioxidant properties, effectively neutralizing reactive oxygen species and contributing to the protection against oxidative stress and associated diseases.³²

Role of flavonoids

Flavonoids are a large class of polyphenolic compounds widely distributed in the plant kingdom. These compounds are characterized by a C6-C3-C6 carbon skeleton consisting of two aromatic rings connected by a three-carbon bridge. Flavonoids exhibit a diverse range of biological activities, including antioxidant, anti-inflammatory, anticancer, and neuroprotective properties. Their potential health benefits have generated significant interest in their utilization as therapeutic agents and dietary supplements.³³

Role of saponins

Saponins are a class of glycosides characterized by their amphiphilic nature, possessing both hydrophilic and hydrophobic moieties. This amphiphilic nature allows saponins to form stable complexes with lipids, exhibiting surface-active properties. In addition to their industrial applications (e.g., detergents, emulsifiers), saponins have demonstrated a range of biological activities, including antifungal, antiviral, and immunomodulatory properties, making them promising candidates for the development of novel therapeutic agents.³⁴

Role of tannins

Tannins constitute a class of polyphenolic compounds widely distributed in the plant kingdom. They are characterized by their ability to form complexes with proteins and polysaccharides, imparting astringent properties. Tannins play a crucial role in plant defense mechanisms, deterring herbivory and protecting against microbial pathogens. Furthermore, tannins have been extensively studied for their potential antioxidant, anti-inflammatory, and anticancer properties. These diverse biological activities have generated significant interest in their potential applications in various fields, including medicine, food science, and industry.³⁵

Role of steroids and Triterpenes

Steroids and triterpenes are a class of structurally related isoprenoid compounds. Steroids, characterized by a cyclopentanophenanthrene ring system, constitute a diverse group of compounds, including hormones (e.g., sex hormones, corticosteroids) and cholesterol. Triterpenes are characterized by a 30-carbon skeleton derived from six isoprene units.

Both steroids and triterpenes exhibit diverse biological activities. They serve as precursors for various hormones and bioactive molecules, including saponins and cardiogenic glycosides. Furthermore, they have been implicated in various physiological processes, including anti-inflammatory, immunomodulatory, and anticancer activities. These compounds continue to be extensively studied for their potential therapeutic applications.³⁶

Role of anthraquinones

Anthraquinones are a class of aromatic compounds derived from anthracene, characterized by the presence of one or more keto and hydroxyl groups. They exhibit a wide range of biological activities, including laxative, antioxidant, and antimicrobial properties. Due to their laxative properties, anthraquinones have been traditionally used in herbal medicine for the treatment of constipation. Additionally, they have been investigated for their potential therapeutic applications in the treatment of skin disorders.^{37,38}

Conclusion

This study comprehensively investigated three *Moringa* species endemic to Madagascar: *Moringa oleifera*, *M. drouhardii*, and *M. hildebrandtii*. Analyses encompassed phytochemical screening, determination of macronutrients and micronutrients, and acute toxicity assessments. The results demonstrated that the leaves of all three species possess significant nutritional value, being rich in essential minerals such as potassium, magnesium, and phosphorus. While supplementation with calcium-rich foods is recommended, the leaves exhibited high protein content, particularly in *M. oleifera*, making them a valuable dietary source. Furthermore, the carbohydrate content of the leaves provides a significant source of energy.

Phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, tannins, flavonoids, leucoanthocyanins, and saponins, suggesting potential health-promoting properties. Acute toxicity studies in animal models did not reveal any significant adverse effects, indicating the potential safety of *Moringa* leaf consumption.

To fully characterize the nutritional composition of these species, further in-depth studies, including detailed amino acid and vitamin analyses, are warranted. To promote the sustainable utilization of these valuable resources, a multi-pronged approach is necessary. This

includes public awareness campaigns to educate the local population about the nutritional and health benefits of *Moringa* leaf consumption, the development of value-added products such as infant formula enriched with *Moringa* leaf powder, the establishment of a nursery for the conservation and propagation of the endemic *M. drouhardii* and *M. hildebrandtii* species, and the promotion of sustainable cultivation and harvesting practices. This research provides a foundation for the sustainable utilization of these underutilized Malagasy resources, promoting both human health and environmental sustainability.

Acknowledgments

None.

Conflicts of interest

The authors declare that there is no conflict of interest.

Funding

None.

References

1. Plants of the world online. *Moringa drouhardii* Jum. 2023.
2. Rauh W. *Succulent and xerophytic plants of Madagascar*. Vol 2. Strawberry Press. 1998.
3. Eggl U. (Edn). *Illustrated handbook of succulent plants: Dicotyledons*. Springer. 2002.
4. Verdcourt B. A synopsis of the Moringaceae. *Kew Bulletin*. 1985;40(1):1–23.
5. Olson ME, Razafimandimbison SG. *Moringa hildebrandtii* (Moringaceae): A tree extinct in the wild but preserved by indigenous horticultural practices in Madagascar. *Adansonia, série 3*. 2000;22(2):217–221.
6. Poirier MCh. *Genealogy of the Maroseranana kings south of Onilahy*. Bulletin Trimestriel de l'Académie Malgache. 1953;18:29–35.
7. Adanson M. Familles des plantes. 1763.
8. Valérie V, Thirion-Merle. Spectrométrie de fluorescence X. *HAL open science*. 2016.
9. Arab K, Bouchenak O, Yahiaoui K. Phytochemical study and evaluation of the antimicrobial and antioxidant activity of essential oils and phenolic compounds of Pistacia Lentiscus L. *Journal of Fundamental and Applied Sciences*. 2014;6(1):7.
10. Trevan J. The error of determination of toxicity. *Proc R Soc*. 1927;101(712):483–514.
11. Gosselin RE, Smith RP, Hodge HC. *Clinical toxicology of commercial products*. 5e éd. Baltimore (MD): Williams and Wilkins. p. II330, 1984.
12. Ngbolua KN, Mpiana PT, Tshibangu DST, et al. In vitro anti-sickling and radical scavenging activities of a poly-herbal formula (Drepanoalpha®) in Sickle cell erythrocyte and acute toxicity study in Wistar albino rats. *European Journal of Medicinal Plants*. 2014;4(10):1251–1267.
13. Sallau AB, Mada SB, Ibrahim S. Effect of boiling, simmering and blanching on the antinutritional content of *Moringa oleifera* leaves. *International Journal of Food Nutrition and Safety*. 2012;(2):1–6.
14. Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. *Journal of Pharmaceutical Research*. 2008;7(3):1089–1099.
15. Enegeide C, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. *Toxicol Int*. 2013;20(3):224–226.
16. Cotonat J. La toxicologie, Paris, Presses Universitaires de France (PUF), 1996.

17. Randrantoarimbola L, Rafalimanantsoa J, Ratiarimananjatovo N, et al. Formulation of *Moringa oleifera* Lam. based Bio-fortified food supplement for pregnant women in Madagascar, Indian Ocean. *Britain International of Exact Sciences (BioEx) Journal*. 2020;2(2):533–540.
18. Albert CM, Ma J, Rifai N, et al. Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. *Circulation*. 2002;105(22):2595–2599.
19. Ali MY, Khalil MI, Jahan FN, et al. *Moringa oleifera*: a review on nutritional attributes, therapeutic applications and value-added product generation. *SAARC Journal Agric*. 2022;20(2):1–15.
20. Fontana Jr AJ, Carter BP. Measurement of water activity, moisture sorption isotherm, and moisture content of foods. In G. V. Barbosa-Cánovas, A. J. Fontana Jr., S. J. Schmidt, & T. P. Labuza (Edn.), *Food engineering fundamentals* (2nd edn. pp. 221–250). Wiley-Blackwell. 2020.
21. Castiglioni SC, Farruggia GF, Cappadone CC. Edn. *Magnesium in human health and disease*. MDPI. 2021.
22. Vannucci L, Fossi C, Quattrini S, et al. Calcium intake in bone health: A focus on calcium-rich mineral waters. *Nutrients*. 2018;10(12):1930.
23. Khan F, Siddique AB, Shabala S, et al. Phosphorus plays key roles in regulating plants' physiological responses to abiotic stresses. *Plants*. 2023;12(15):2861.
24. Udensi UK, Tchounwou PB. Potassium homeostasis, oxidative stress, and human disease. *Int J Clin Exp Physiol*. 2017;4(3):111–122.
25. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. *J Res Med Sci*. 2014;19(2):164–174.
26. Saleem MH, Usman K, Rizwan M, et al. Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. *Front Plant Sci*. 2022;13:1033092.
27. Li L, Yang X. The essential element manganese, oxidative stress, and metabolic diseases: links and interactions. *Oxid Med Cell Longev*. 2018;2018:7580707.
28. Ullah N, Khan MF, Jan SU, et al. Study of the effect of inorganic and organic complexes of arsenic metal on the status of GSH in T. cells and B. cells of blood. *Pak J Pharm Sci*. 2015;28(2):457–464.
29. Tapiero H, Townsend DM, Tew KD. Trace elements in human physiology and pathology. Copper. *Biomed Pharmacother*. 2003;57(9):386–398.
30. Jugdaohsingh R. Silicon and bone health. *J Nutr Health Aging*. 2007;11(2):99–110.
31. Ferreira MJU. Alkaloids in future drug discovery. *Molecules*. 2022;27(4):1347.
32. Bueno JM, Ramos-Escudero F, Sáez-Plaza P, et al. Analysis and antioxidant capacity of anthocyanin pigments. Part I: general considerations concerning polyphenols and flavonoids. *Critical Reviews in Analytical Chemistry*. 2012;42(2):102–125.
33. Dias MC, Pinto DCGA, Silva AMS. Plant flavonoids: chemical characteristics and biological activity. *Molecules*. 2021;26(17):5377.
34. Timilsena YP, Phosanam A, Stockmann R. Perspectives on saponins: food functionality and applications. *Int J Mol Sci*. 2023;24(17):13538.
35. Swanson BG. *Tannins and polyphenols*. 2003.
36. Nes WD. Biosynthesis of cholesterol and other sterols. *Chemical Reviews*. 2011;111(10):6423–6451.
37. Malik EM, Müller CE. Anthraquinones as pharmacological tools and drugs. *Med Res Rev*. 2016;36(4):705–748.
38. Hodge HC, Sterner JH. *Determination of substances acute toxicity by LDB50B*. Amer. Industrial Hyg. Assoc. 1943;10:93.