



# PHARMACOGNOSTICAL STANDARDIZATION AND PHYTOCHEMICAL SCREENING OF MORNING GLORY LEAVES

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## ABSTRACT

*Rivea hypocrateriformis* (Desr.) Choisy, a member of the Convolvulaceae family and commonly known as 'Morning Glory,' is a woody climbing shrub widely employed in Ayurvedic medicine. Despite its numerous medicinal properties, there is a lack of published research on the pharmacognostical characterization and phytochemical analysis of its leaves. This study aimed to explore the pharmacognostical, physicochemical, and preliminary profiles of *R. hypocrateriformis* leaves. The pharmacognostical characterization included an organoleptic examination, macroscopy, leaf constants, fluorescence analysis, and preliminary phytochemical screening. Organoleptic and macroscopic observations revealed that the leaves are initially dark green but become yellowish-green as they mature. The leaves are odorless but possess a slightly bitter and astringent taste. They are round-heart-shaped and have blunt apices, with dense velvet-like hairiness on the lower surface. The fruit is indehiscent or tardily dehiscent, dry-baccate, and approximately 2 cm long. Chemical analysis identified the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, and tannins. Analysis of leaf constants, powder microscopy, fluorescence characteristics, and physical parameters yielded valuable data for establishing standards for this plant. This pharmacognostical report on *R. hypocrateriformis* leaves serves as a crucial diagnostic tool for species identification, authentication, and the development of quality parameters. The data obtained in this study may be regarded as a reference for future research endeavors.

**Keywords:** Morning glory, *Rivea hypocrateriformis*, pharmacognostical standardization, phytochemical screening, etc.

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## INTRODUCTION

The utilization of plant-based or plant-derived products in human healthcare represents a significant milestone in the history of humanity [1-5]. However, accurately determining the total number of medicinal plants on Earth remains a complex task. Reports suggest that an impressive range of approximately 35,000 to 70,000 plant species has been utilized

globally to address various health conditions. In recent decades, pharmaceutical companies have focused their efforts on researching and developing new drugs derived from plants [6,7]. The estimated global market for medicinal plants is valued at around \$60 billion annually, with a consistent growth rate of 7% per year. This market exhibits varying proportions between developed and

developing countries. Researchers are actively disseminating knowledge about the importance and therapeutic properties of these valuable plants, thereby increasing awareness among the general population. One of the critical challenges is ensuring accountability in terms of standardization and the biological activities of these plants [8]. This step is vital to scientifically validate the therapeutic benefits offered by traditional medicine.

The *Rivea* genus comprises more than 47 plant species distributed worldwide, with only a limited number originating in India. Among these, *R. hypocrateriformis* (Desr.) Choisy stands out as a woody climbing shrub belonging to the Convolvulaceae family. This plant enjoys a wide distribution across regions including India, Nepal, Sri Lanka, Pakistan, Bangladesh, Myanmar, and Thailand. Traditional knowledge has long recognized its therapeutic potential, particularly in its bark, stems, and leaves, which are used to address a variety of health conditions, including malaria, cancer, mental disorders, and pain relief. For example, indigenous communities in Pakistan's Tharparkar region rely on this plant for treating malaria and easing pain [9,10].

*R. hypocrateriformis* has garnered attention due to its diverse biological properties, encompassing antioxidant, anti-implantation, antimicrobial, pregnancy interruption, anticancer, and antiarthritic activities [9, 11]. Furthermore, it holds a significant place in Ayurvedic medicine as a crucial component in the formulation known as "Rasa panchaka," used for managing asthma. Notably, similar to certain other species within its related genus, such as *Rivea corymbosa* Hall and

*Ipomoea violacea* L. found in Mexico, *R. hypocrateriformis* is also utilized as a hallucinogenic substance in India and serves as a psychoactive remedy in Pakistan [9].

The aim of this study was to create a comprehensive quality standard for *R. hypocrateriformis* leaves. The research primarily concentrated on documenting the organoleptic traits, macroscopic characteristics, quantitative parameters involving physical properties, phytochemical screening, and chromatographic profiles of these leaves. This undertaking marks a significant milestone in assessing the quality of the raw plant material, providing the groundwork for its future development and application.

## MATERIALS AND METHODS

### Collection and authentication of plant materials

Leaves of *R. hypocrateriformis* were collected from outskirts of Udaipur, Rajasthan in November 2022. The plant was identified, authenticated and certified by Botanist, Himachal Pradesh State Biodiversity Board, Shimla, Himachal Pradesh.

### Organoleptic evaluation

The leaves of *R. hypocrateriformis* were assessed for their influence on various sensory organs to determine organoleptic properties. This involved observing and recording parameters such as color, odor, size (both length and width), taste, and other diagnostic characteristics [12].

### Determination of leaf constants

Various leaf constant viz. stomatal number, stomatal index, vein-islet number, vein-termination number and palisade ratio

were determined as per the standard procedure.

**Stomatal Number:** Stomatal number refers to the quantity of stomata present per square millimeter of the leaf's epidermis.

**Stomatal Index:** Stomatal index represents the percentage of epidermal cells on a leaf that has undergone transformation into stomata. It indicates the proportion of epidermal cells that have developed into stomata.

$$\text{Stomatal index (I)} = \frac{S}{E + S}$$

Where, S = quantity of stomata per unit area and E = amount of ordinary epidermal cells in the same unit area.

**Vein-Islet Number:** Vein-islet number is calculated by counting the number of vein-islets within four adjacent squares located in the central area of the leaf lamina, positioned midway between the midrib and the leaf margin, and expressed per square millimeter.

**Veinlet Termination Number:** Veinlet termination number represents the count of veinlet terminations per square millimeter of the leaf's surface.

**Palisade Ratio:** The palisade ratio refers to the average number of palisade cells found beneath each upper epidermal cell on the leaf [12].

### Physicochemical parameters [12]

Various parameters viz. loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated ash, extractive values, foreign matter, and fluorescence analysis were evaluated. These analyses were conducted to ensure the quality and authenticity of the plant material under study.

**Loss on drying:** Approximately 3 grams of the sample were carefully transferred

into a separate bottle, ensuring an even distribution by gently shaking it from side to side, without exceeding a depth of 10 mm. The loaded bottle, with the stopper removed, was then placed in a drying chamber, maintaining a temperature not exceeding 100-105°C. The bottle, along with its contents, was weighed periodically and repeatedly until successive weights remained constant, indicating that the drying process had reached a state of constant weight. Afterward, the bottles were allowed to cool within a desiccator, and the percentage loss of weight was calculated in reference to the initial weight of the fresh drug. This process is crucial for determining the loss on drying, a parameter used to assess the moisture content of the sample.

**Total ash:** The process involved accurately weighing 3 grams of plant material, which was then placed in a silica crucible. This crucible had been previously ignited, cooled, and weighed to ensure accurate measurements. The plant material was incinerated by gradually increasing the heat, making sure not to exceed 450°C, until it was free from carbon. After the incineration process, the crucible with its contents was allowed to cool within a desiccator to ensure that it reached a constant weight. This constant weight indicated that all organic matter had been completely burnt away, leaving behind only the inorganic ash. The percentage of total ash was then calculated with reference to the weight of the air-dried drug, following the guidelines outlined in the Indian Pharmacopoeia. This process is crucial for determining the amount of inorganic residue left after incineration, providing valuable information about the purity and quality of the plant material.

**Acid insoluble ash:** The process involved boiling the total ash for 5 minutes using a 25.0 mL solution of 2 N HCl (approximately 70 g/L concentration). This step was aimed at dissolving any soluble components present in the total ash. Following this, the remaining insoluble matter was collected onto an ashless filter paper. Subsequently, this filter paper, along with the insoluble matter, was ignited at a temperature of approximately 500°C until a constant weight was achieved. This constant weight indicated the complete removal of all organic matter. The weight of the resulting insoluble matter was then subtracted from the initial weight of the total ash. The difference in weight represented the acid-insoluble ash content. Finally, the percentage of acid-insoluble ash was calculated with reference to the weight of the air-dried drug. This procedure is essential for assessing the quantity of inorganic residue that remains insoluble in acid, providing valuable insights into the purity and quality of the plant material.

**Water soluble ash:** The total ash was first subjected to a boiling process with 25 mL of water for 5 minutes. During this step, any soluble components were extracted from the total ash. Following boiling, the resulting insoluble matter was collected on an ashless filter paper. This collected material was then thoroughly washed with hot water to ensure the removal of any remaining soluble residues. Subsequently, the washed insoluble matter, still on the ashless filter paper, was ignited for 15 minutes. The ignition process was carefully conducted at a temperature not exceeding 450°C. The weight of the final insoluble matter after ignition was then subtracted from the initial weight of the

total ash. This difference in weight represented the water-soluble ash content. Finally, the percentage of water-soluble ash was calculated with reference to the weight of the air-dried drug. This procedure is essential for assessing the quantity of ash that can dissolve in water, providing crucial insights into the purity and quality of the plant material.

**Sulphated ash:** The process began by heating a silica crucible to redness for a duration of 10 minutes. Afterward, it was allowed to cool within a desiccator. Then, precisely 3 grams of the sample were accurately weighed and transferred into the crucible. Initially, the sample was gently ignited until it was completely burned. Following the combustion, the residue was cooled and saturated with 1 mL of concentrated sulfuric acid. It was then gently heated until no further white fumes were produced. Subsequently, the residue was ignited at a controlled temperature of 800°C±25°C until all individual black particles had disappeared. This ignition process was carried out in a muffle furnace. After cooling, a few drops of concentrated sulfuric acid were added, and the crucible was heated again. It was ignited as before and allowed to cool before being weighed. This entire operation was repeated until two successive readings, with no more than a 0.5 mg variation between them, were obtained. This meticulous procedure ensured the accurate determination of the ash content in the sample.

**Ethanol soluble extractive:** A 5-gram portion of the powdered drug was subjected to maceration with 100 ml of ethanol in a sealed flask for a duration of 24 hours. During the initial 6 hours, the flask was shaken successively, and

afterward, it was left undisturbed for 18 hours. Following this maceration period, the resulting extract was separated, and 25 ml of the filtrate was evaporated to dryness in a shallow dish. The dried residue was then heated at 105°C until constant weight was achieved, and its weight was recorded. Subsequently, the percentage of ethanol-soluble extractive, in relation to the weight of the air-dried drug, was calculated. This procedure is essential for assessing the quantity of constituents in the drug that can be extracted by ethanol, providing valuable information about the drug's solubility characteristics.

**Water soluble extractive:**

A 5-gram portion of the powdered drug underwent a maceration process with 100 ml of chloroform water in a sealed flask for a period of 24 hours. During the initial 6 hours, the flask was subjected to successive shaking, after which it was allowed to sit undisturbed for 18 hours. Following this maceration period, the resulting extract was separated, and 25 ml of the filtrate was evaporated to dryness in a shallow dish. The residue was then dried at 105°C until a constant weight was achieved and its weight was recorded. Subsequently, the percentage of water-soluble extractive, in relation to the weight of the air-dried drug, was calculated. This procedure is essential for evaluating the quantity of constituents in the drug that can be extracted by water, providing valuable insights into the drug's solubility characteristics.

**Determination of foreign matter:** Weigh 100-500 g of the sample, or the minimum quantity prescribed in the individual monograph, and spread it out in a thin layer. Examine for foreign matter by inspection with the unaided eye or by use

of a lens (6 ×). Separate foreign matter, weigh it and calculate the percentage present [12].

**Phytochemical Screening****Preliminary Phytoprofile [12]**

The preliminary phytoprofile of the powder of air-dried roots of *R. hypocrateriformis* indicates an initial analysis of the plant material's chemical composition and potential bioactive compounds. The steps involved in this process:

**Sample Preparation:** Air-dried roots of *R. hypocrateriformis* were ground into a fine powder. This powder is typically used for various analytical techniques to extract and analyze the plant's chemical constituents.

**Extraction:** The powder was subjected to extraction in a Soxhlet apparatus. The Soxhlet apparatus is a common laboratory equipment used for the extraction of compounds from solid materials using a solvent. It consists of a thimble, a condenser, a flask, and a heating element. The process involves continuous extraction and solvent recycling.

**Choice of Solvent:** The choice of solvent used in the Soxhlet extraction is critical, as it determines which compounds will be extracted. Different solvents can extract different classes of compounds, such as polar or nonpolar substances. Researchers often use a suitable solvent depending on the compounds they want to isolate.

**Extraction Process:** The Soxhlet extraction involves the following steps:

The sample (powdered leaves) is placed in a thimble. The thimble is loaded into the Soxhlet apparatus. The solvent is heated in the flask, and its vapor rises to the condenser. The vapor condenses and drips onto the sample in the thimble. The



solvent dissolves compounds from the sample and flows back into the flask. This cycle repeats continuously for several hours, ensuring thorough extraction.

**Collection of Extract:** The solvent that flows back into the flask contains the extracted compounds from the plant material. This extract is collected and further analyzed.

The powder of the air-dried leaves of *R. hypocrateriformis* was extracted in soxhlet apparatus with solvents such as ethanol, methanol and water.

Each time before extracting with the next solvent, the material was dried. Each extract was concentrated by distilling off the used solvent and concentrated extracts were evaporated using rotary evaporator to dryness and weighed. Colour and percentages of extracts were calculated with reference to air dried plant material.

#### **Qualitative Phytochemical Analysis**

Various extracts of *R. hypocrateriformis* leaves were subjected to phytochemical analysis. A series of identification tests were performed to detect presence of alkaloids, flavonoids, saponins, proteins and amino acids, fixed oils and fats, glycosides, tannins and steroids.

#### **Fluorescence analysis of leaf extracts**

The ethanol, methanol and water extracts of leaves of *R. hypocrateriformis* subjected

to fluorescence analysis in daylight and in UV- light (365 nm).

#### **Fluorescence analysis of leaf powder**

A small amount (1 gram) of dried and finely powdered *R. hypocrateriformis* leaves underwent a series of chemical treatments, including exposure to freshly prepared aqueous NaOH, alcoholic NaOH, 1N HCl (hydrochloric acid), concentrated H<sub>2</sub>SO<sub>4</sub> (sulfuric acid) in a 1:1 ratio, concentrated HNO<sub>3</sub> (nitric acid) in a 1:1 ratio, ammonia, iodine, 5% FeCl<sub>3</sub> (iron(III) chloride), and acetic acid.

Subsequently, these treated samples were subjected to fluorescence analysis under both daylight and UV-light with a wavelength of 365 nm. The colors and fluorescence patterns observed under these different conditions were meticulously documented and recorded. This comprehensive analysis aids in understanding the chemical composition and unique characteristics of the *R. hypocrateriformis* leaf powder, facilitating its identification and quality assessment.

### **Results and Discussion**

#### **Organoleptic characters**

In the initial stages of growth, the leaves exhibit a darker green color, gradually transitioning to a yellowish green hue as they mature (as depicted in Figure 1). These leaves lack a distinct odour but possess a mildly bitter and astringent taste.

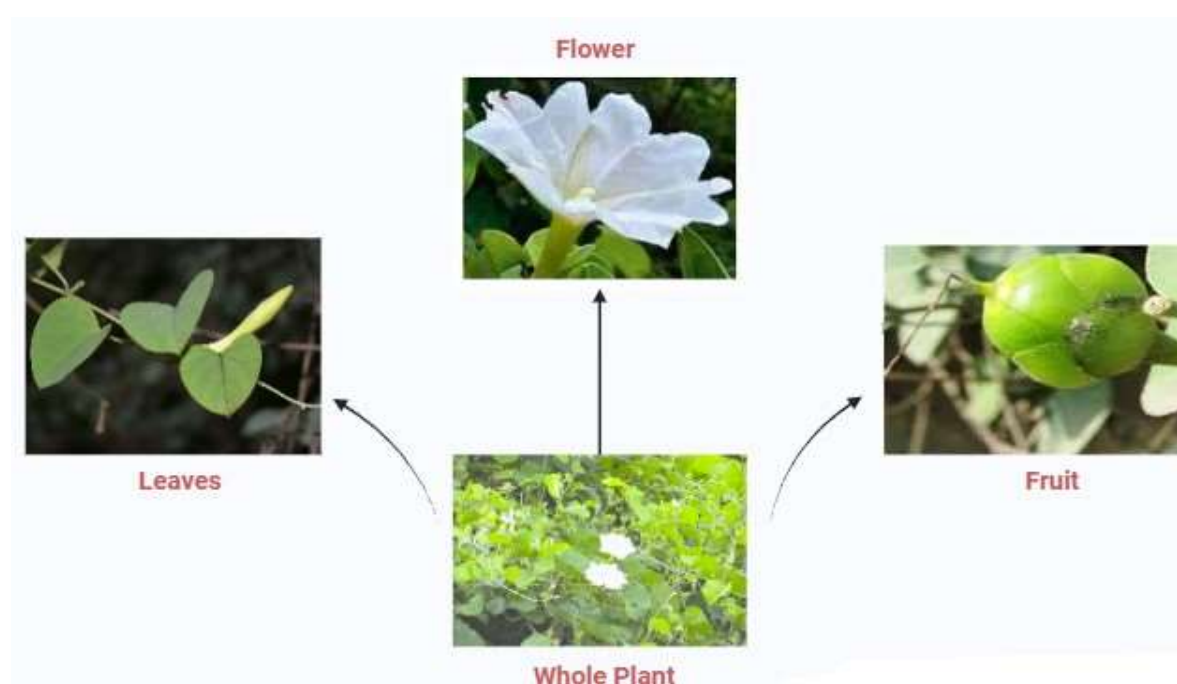


**Figure 1.** *R. hypocrateriformis* leaf

### Macroscopic characters

The morphological characteristics of the *R. hypocrateriformis* plant and its various parts are illustrated in Figure 2. The flowers, typically solitary, exhibit a creamy white color and possess the classic morning glory form, featuring a flat-faced structure. They measure approximately 6 to 9 centimeters in length. The sepals are uneven in size, ovate in shape, and blunt at the apex, measuring about 10 to 12 millimeters in length. These sepals are densely covered with short, fine hairs (villoses). The leaves of *R. hypocrateriformis* are round-heart-shaped

and have a blunt apex. They are densely covered with velvety, appressed hairs on the lower surface. The fruit of this plant is either indehiscent (does not split open upon maturity) or tardily dehiscent (splits open slowly), and it is dry-baccate, measuring about 2 centimeters in length. The seeds of *R. hypocrateriformis* are brown, smooth, and devoid of hair (glabrous). They have a slightly trigonous shape and are surrounded by a dry, white pulp. These morphological features provide valuable information for the identification and classification of this plant species.



**Figure 2.** Macroscopic characteristics of *R. hypocrateriformis* (leaves, fruit, and flower)

### Leaf Constants

On the adaxial (upper) surface, the stomatal density was found to be approximately 23.27 stomata per unit area, and the stomatal index was calculated as 16.6%. On the abaxial (lower) surface, the stomatal density was notably higher at approximately 72.56 stomata per unit area, and the stomatal index was measured as 27.33%. Additionally, the vein islet count was determined to be 5, while the vein termination count was 15. Furthermore, the palisade ratio, which is a measure of the arrangement of palisade cells in the leaf tissue, was calculated and found to be 32. These parameters provide valuable insights into the leaf structure and physiology of *R. hypocrateriformis*, aiding in the characterization and understanding of this plant species.

### Physicochemical parameters

The results of the physicochemical parameters are summarized in Table 1. It was observed that the sulphated ash value

was lower at 2.27% compared to the total ash value, which stood at 2.78%. The acid insoluble ash was determined to be 0.49%, and the water-soluble ash value was measured at 0.34%.

Further analysis revealed that the drug contained 8.72% moisture content. Notably, the foaming index and swelling index were both found to be nil, indicating a lack of foaming or significant swelling properties. Moreover, the foreign organic content was reported to be less than 1%.

In terms of extractive values, the drug exhibited varying solubility in different solvents. The extractive values for different solvents were as follows: Ethanol: 38.22%; Methanol: 13.9% Water: 8.34%. These findings provide important information regarding the chemical composition and characteristics of the *R. hypocrateriformis* drug, which is valuable for quality assessment and standardization purposes.

**Table 1.** Physicochemical parameters of *R. hypocrateriformis*



Physiochemical parameters		Percentage
Ash Value	Total Ash	2.78
	Acid insoluble ash	0.49
	Water soluble ash	0.34
	Sulphated ash	2.27
Extractive Value	Ethanol extract	38.22
	Methanol extract	13.9
	Aqueous extract	8.34
Moisture content		7.56
Foreign organic matter		Less than 1% (Presence of petiole stalks with leaves)
Foaming index		Nil
Swelling index		Nil

### Fluorescence analysis

The fluorescence characteristics of powdered drug samples play a crucial role in assessing the quality and purity of these materials. When subjected to ultraviolet (UV) light and visible light in the presence of specific chemical reagents, powdered drugs often exhibit distinctive fluorescence

patterns [13]. The fluorescence results of powdered *R. hypocrateriformis* leaves have been compiled and are presented in Table 2. These findings are essential for evaluating and verifying the authenticity and quality of the *R. hypocrateriformis* leaf material [14]

**Table 2. Fluorescence characteristics of leaves extracts**

S. No.	Treatment	Visible light	UV Light	
			254 nm (Short wavelength)	366 nm (Long wavelength)
1	Leaf powder	Dark Green	Green	Green
2	Leaf powder rubbed on filter paper	Light Green	Light Green	Light Green
3	Leaf powder + 1N NaOH	Reddish brown	Brown	Brown
4	Leaf powder + 1N HNO <sub>3</sub>	Light green	Light green	Light yellow
5	Leaf powder + 1N HCl	Light green	Light green	Light yellow
6	Leaf powder + 1N H <sub>2</sub> SO <sub>4</sub>	Light green	Green	Green
7	Ethanol extract of leaf	Green	Green	Black

	powder			
8	Methanolic extract of Leaf powder	Green	Green	Light Green
9	Aqueous extract of Leaf powder	Green	Green	Light Green

**Preliminary phytochemical screening**

Various phytochemical analysis tests supported that the extracts contain alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins and

glycosides, recorded in Table 3. The aqueous extract was found to be negative for the presence of alkaloids as compared to methanolic and ethyl acetate extract.

**Table 3. Results of phytochemical screening of different extracts of *R. hypocrateriformis* leaves extracts.**

Phytoconstituents	Leaf Extracts		
	Ethanol	Methanol	Aqueous
Carbohydrates	Present	Present	Present
Alkaloids	Present	Present	Present
Glycosides	Present	Absent	Present
Flavonoids	Present	Absent	Present
Phenolic compounds	Present	Present	Present
Tannins	Present	Absent	Present
Saponins	Present	Absent	Present
Steroids	Present	Absent	Present
Proteins	Present	Absent	Absent
Fixed oil and facts	Present	Absent	Absent

*R. hypocrateriformis* has a rich history of traditional medicinal use for treating a wide range of diseases. However, there are currently no established pharmacopeial standards for the accurate identification and authentication of the plant's leaves. In response, this study aimed to analyze the pharmacognostic features of *R. hypocrateriformis* leaves. The leaves were found to be odorless but possessed a slightly bitter and astringent taste. Morphologically, they exhibited round-heart-shaped characteristics with a blunt apex and were densely covered with appressed velvet-like hairs on the lower surface. The fruit was described as either indehiscent (not splitting open) or tardily dehiscent, with a dry-baccate structure

measuring 2 centimeters in length. Physicochemical evaluations provided various important parameters, including moisture content, ash values, and extractive values for different solvents. The ash values determined in this study can serve as essential benchmarks for establishing standards of purity and quality. The low total ash value indicates a minimal presence of inorganic salts, such as carbonates, phosphates, silicates of sodium, potassium, calcium, and magnesium. The very low acid-insoluble ash content suggests that only a small fraction of the inorganic components is insoluble in acid, which serves as a diagnostic tool. The moisture content of the fresh leaves was found to be 7.56%,

indicating that the leaves dried quickly after harvesting. Extractive values provide insights into the chemical constituents present in the plant, aiding in the estimation of specific constituents soluble in the selected solvent and determining the extent of exhausted materials. Notably, methanol and ethyl acetate were identified as superior solvents for extracting *R. hypocrateriformis* leaves. Furthermore, the fluorescence study revealed characteristic fluorescence patterns under both visible light and UV light (short and long wavelengths). This feature can be valuable in detecting potential adulterants in *R. hypocrateriformis* products. Overall, this comprehensive analysis contributes to the understanding, identification, and quality assessment of *R. hypocrateriformis* leaves, supporting their traditional medicinal use.

## CONCLUSION

Ensuring the quality of herbal products begins with rigorous control of the starting raw materials. Fundamental pharmacognostical parameters, such as leaf constants, microscopy, and physicochemical examinations, are crucial components of the standardization process for herbal products. These parameters provide essential information for the identification and authentication of herbs. The data obtained from basic phytochemical screening offers valuable insights into the chemical composition of the herbal medicine. Parameters such as the total ash value, brightness analysis, and extractive value play a significant role in the identification and authentication of the plant material. This research not only contributes to the accurate identification and authentication of the plant but also

provides valuable data that can serve as a reference for other researchers in their work. By maintaining these standards, researchers can ensure the consistency and quality of the plant material used in their studies, ultimately advancing the field of herbal medicine.

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