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Establishment Of Pharmacognostic Standards And Physicochemical Parameters For Ruellia Prostrata Acanthaceae

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Abstract

Objective: *The present study aimed to establish comprehensive pharmacognostic standards and preliminary phytochemical profile of Ruellia prostrata Poiret to facilitate botanical authentication, quality control, and support its integration into evidence-based herbal medicine for treating inflammatory conditions and infections traditionally managed by this plant.*

Methods: *Fresh plant material collected from Hadapsar, Pune was authenticated (2024/SI/DD/0026). Macroscopic and microscopic evaluations were performed using standard pharmacognostic techniques. Quantitative microscopy parameters including stomatal number, stomatal index, vein-islet number, vein termination number, and palisade ratio were determined. Physicochemical parameters (ash values, extractive values, moisture content, foaming index, swelling index) were analyzed following Indian Pharmacopoeia guidelines. Successive solvent extraction using petroleum ether, chloroform, ethanol, and water was performed, followed by preliminary phytochemical screening using standard qualitative tests.* ***Results:*** *Macroscopic features revealed prostrate habit with pubescent ridged stems and purple bilabiate flowers. Microscopic examination showed dorsiventral leaf with single-layered palisade tissue and root with polyarch xylem containing oil glands and calcium oxalate crystals. Quantitative parameters showed stomatal number of 128.67±4.16 per mm² (abaxial) and 45.33±2.51 per mm² (adaxial), stomatal index of 18.72±1.23 (abaxial) and 12.45±0.89 (adaxial), and palisade ratio of 4.67±0.57. Physicochemical analysis revealed total ash 12.38±0.45%, water-soluble extractive 18.45±0.62%, alcohol-soluble extractive 14.72±0.58%, and moisture content 8.45±0.32%. Phytochemical screening confirmed presence of alkaloids, flavonoids, tannins, phenols, steroids, glycosides, and saponins.*

Conclusion: *The established pharmacognostic parameters provide reliable reference standards for quality control, authentication, and adulteration detection in crude drug formulations, supporting future clinical development of standardized R. prostrata preparations for therapeutic applications.*

Keywords: *Ruellia prostrata, Pharmacognosy, Quantitative microscopy, Physicochemical standardization, Phytochemical screening, Quality control, Acanthaceae*

INTRODUCTION

Medicinal plants continue to serve as primary healthcare resources for approximately 80% of the global population, particularly in developing nations where access to conventional pharmaceuticals remains limited [1]. The World Health Organization estimates that traditional medicine contributes significantly to healthcare systems, yet many medicinal species lack comprehensive pharmacognostic documentation and quality control standards. *Ruellia prostrata* Poiret, a prostrate herb belonging to family Acanthaceae, has been extensively utilized in traditional medicine systems across Asia and South America for treating various inflammatory conditions, skin disorders, and urogenital infections [2]. Despite its widespread ethnomedicinal applications, systematic pharmacognostic studies establishing authentication parameters and microscopic diagnostic features remain scarce in scientific literature. The absence of standardized pharmacognostic profiles creates significant challenges in quality assessment, species authentication, and prevention of adulteration in herbal drug markets. Furthermore, the increasing demand for evidence-based validation of traditional medicines necessitates comprehensive botanical and phytochemical characterization of therapeutically important species [3]. This knowledge gap hinders the development of quality control protocols and limits the integration of *R. prostrata* into modern phytopharmaceutical formulations, despite its documented therapeutic potential in traditional healing practices [4].

Phytochemical investigations of *R. prostrata* have revealed the presence of diverse bioactive constituents including flavonoids, alkaloids, phenolic compounds, steroids, and triterpenoids that contribute to its pharmacological properties [5]. The plant demonstrates significant anti-inflammatory, antimicrobial,

antioxidant, and hepatoprotective activities attributed to its rich phytochemical composition [6]. Flavonoid glycosides, particularly apigenin and luteolin derivatives, represent major active principles responsible for the plant's anti-inflammatory effects through inhibition of cyclooxygenase and lipoxygenase pathways [7]. Methanolic and aqueous extracts have exhibited pronounced antimicrobial activity against common pathogenic bacteria and fungi, validating traditional uses against infectious conditions [8]. The phenolic content correlates strongly with antioxidant capacity, suggesting potential applications in oxidative stress-related disorders. Previous pharmacological studies have documented dose-dependent therapeutic effects in various experimental models, demonstrating the plant's multi-targeted biological activities [9]. However, the therapeutic exploitation of these bioactive compounds requires precise botanical identification and establishment of diagnostic features to ensure correct plant material procurement. The chemical variability observed across different geographical populations further emphasizes the necessity for standardized microscopic and macroscopic evaluation protocols to maintain consistency in herbal preparations [10].

Pharmacognostic evaluation represents a fundamental approach for establishing authentic identity and quality parameters of medicinal plants through detailed examination of morphological, anatomical, and physicochemical characteristics [11]. This classical methodology provides reliable diagnostic features that remain stable across different growth conditions and serve as primary standards for botanical authentication [12]. Microscopic analysis reveals distinctive cellular structures, tissue arrangements, and diagnostic elements including trichomes, stomata, crystals, and specialized cells that facilitate species-level identification even in fragmented or powdered forms. Powder microscopy offers practical advantages for routine quality control in herbal industries where intact plant material may not be available [13]. Quantitative microscopic parameters such as stomatal index, vein islet number, and palisade ratio provide numerical standards for comparative authentication. Recent advances in pharmacognostic techniques incorporate digital imaging and morphometric analysis to enhance precision and reproducibility of diagnostic observations [14]. The integration of macroscopic, microscopic, and physicochemical parameters creates comprehensive monographs that serve as reference standards for regulatory compliance and quality assurance. This systematic approach enables detection of substitution, adulteration, and contamination while ensuring therapeutic efficacy and safety of herbal medicines [15].

The present investigation aims to establish comprehensive pharmacognostic standards for *Ruellia prostrata* through detailed microscopic examination of leaf, stem, and root anatomy. Specific objectives include documentation of diagnostic microscopical features, development of powder microscopy profiles, and determination of quantitative parameters for authentication purposes. This study seeks to generate a complete pharmacognostic monograph that facilitates quality control and species verification in herbal drug industries, ultimately supporting the safe and effective utilization of this traditionally important medicinal plant.

MATERIALS AND METHODS

MATERIALS

Chloral hydrate solution, phloroglucinol, concentrated hydrochloric acid, toluidine blue, phosphate buffer (pH 6.8), potassium hydroxide, safranin, and glycerin of analytical grade were procured from SciQuaint Chemicals, Pune, India. Petroleum ether (60-80°C), chloroform, ethanol (95% v/v) of analytical grade were obtained from Research Lab Fine Chem Industries, Mumbai, India. Dragendroff's reagent, Mayer's reagent, Wagner's reagent, Hager's reagent, and other phytochemical screening reagents were procured from Neeta Chemicals, Pune, India. Distilled water was prepared in laboratory. All other chemicals and reagents used were of analytical grade and used without further purification.

METHODS

Collection and authentication of plant material

The whole plant of *Ruellia prostrata* was collected during flowering season from Hadapsar, Pune, Maharashtra, India. After collection, plant material was thoroughly washed with running tap water to remove adherent soil, dust and pollutants, then blotted dry with absorbent paper. A complete herbarium specimen including roots, stems, leaves, flowers and fruits was prepared by pressing and shade drying at room temperature (25-28°C) for 7-10 days. The specimen was mounted on herbarium sheet with field labels and submitted to Sciquaint Innovations Pvt. Ltd., Pune for authentication. The plant was confirmed as *Ruellia prostrata* Poiret

(Family: Acanthaceae) and assigned reference number 2024/SI/DD/0026 for herbarium deposition. Remaining plant material was shade dried and stored in airtight containers for pharmacognostic investigations [16,17].

Macroscopic Evaluation

The macroscopic evaluation of *Ruellia prostrata* was performed to observe morphological characters of fresh and dried plant material using naked eye and magnifying lens (10x). Organoleptic features including color, odor, taste, texture and appearance were recorded for roots, stems, leaves and flowers. The shape, size, surface characteristics, fracture and distinguishing features were examined and documented. Fresh specimens were observed first to note natural color and texture, followed by shade-dried material to record drying changes. Measurements of leaf length, width, petiole length, stem diameter, root length and flower dimensions were taken using standard ruler and recorded in centimeters. The phyllotaxy, venation pattern, presence of trichomes and special characteristic features were noted systematically. Representative photographs were captured using digital camera for documentation. The macroscopic characters served as preliminary identification parameters for quality control and authentication (n=3 specimens examined) [18].

Microscopic Evaluation

The microscopic studies were conducted to examine anatomical features of *Ruellia prostrata*. For transverse section studies, fresh leaf, stem, root and petiole were collected and thin free-hand sections were cut using sharp razor blade. Sections were cleared with chloral hydrate solution and stained with phloroglucinol-HCl for lignin detection and toluidine blue (0.05% w/v in phosphate buffer, pH 6.8) for tissue differentiation. Stained sections were mounted in glycerin on glass slides and observed under compound microscope (Lawrence & Mayo, Model LM-2070, India) at magnifications of 10x, 40x and 100x. For surface studies, thin epidermal peels were obtained from adaxial and abaxial leaf surfaces by peeling method, treated with 5% potassium hydroxide for clearing and stained with safranin (1% w/v in 50% ethanol). Trichome studies were performed by examining leaf surface under microscope to observe type, distribution and density. Representative photomicrographs were captured using digital camera attached to microscope. Diagnostic features including epidermis type, stomata, trichomes, vascular bundles, cortex, pith and special cells or crystals were documented systematically (n=3 sections for each plant part) [19].

Quantitative Microscopy

Quantitative microscopic parameters were determined to establish numerical standards for identification of *Ruellia prostrata* leaf. For stomatal number and stomatal index determination, epidermal peels from both adaxial and abaxial surfaces of mature leaves were prepared and mounted in glycerin. The peels were observed under compound microscope (Lawrence & Mayo, Model LM-2070, India) at 40x magnification and number of stomata and epidermal cells were counted in 1 mm^2 area using calibrated eyepiece micrometer. Stomatal number was calculated as average number of stomata per mm^2 and stomatal index was calculated using formula:

$$\text{Stomatal index} = \left[\frac{S}{E+S}\right] \times 100$$

where S = number of stomata per unit area and E = number of epidermal cells in same area. For vein-islet number and vein termination number, leaf pieces were cleared by boiling in 5% sodium hydroxide solution, washed with distilled water and stained with safranin solution. The cleared leaves were mounted in glycerin and observed at 10x magnification. Vein-islet number was determined by counting number of vein-islets per mm^2 area and vein termination number was calculated by counting number of veinlet terminations per mm^2 area. Palisade ratio was determined by counting number of palisade cells beneath each epidermal cell in four continuous areas of leaf lamina in surface view at 40x magnification [20,21].

Physicochemical Parameters

Moisture content/Loss on drying

The moisture content was determined by loss on drying method as per Indian Pharmacopoeia. Accurately weighed 2 g of coarsely powdered plant material was taken in pre-dried and pre-weighed porcelain crucible and placed in hot air oven (Labline, Model LI-HOO-101, India) at 105°C for 3 hours. The crucible was cooled in desiccator containing anhydrous silica gel for 30 minutes and weighed. Heating, cooling and weighing

process was repeated until constant weight was achieved with difference not exceeding 0.5 mg between consecutive weighings. Moisture content (%) was calculated using formula:

$$\left[\frac{(\text{Initial Weight-Final Weight})}{\text{Initial Weight}}\right]\times 100$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [22].

Total Ash value

The total ash value was determined as per Indian Pharmacopoeia method to assess the amount of inorganic matter present in the plant material. Accurately weighed 2 g of air-dried coarse powder of *Ruellia prostrata* was taken in a pre-weighed silica crucible and spread evenly at the bottom. The crucible was placed in muffle furnace (Technico, Model TM-206, India) and heated gradually by increasing temperature to 450°C for 4-5 hours until the sample was completely incinerated and white or greyish-white ash was obtained. The crucible was cooled in desiccator for 30 minutes and weighed immediately. The heating, cooling and weighing was repeated until constant weight was achieved. Total ash value (%) was calculated using formula:

$$\left(\frac{\text{Weight of Ash}}{\text{Weight of Sample}}\right)\times 100$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [23].

Acid-insoluble ash

The acid-insoluble ash was determined as per Indian Pharmacopoeia to measure silica content. The ash obtained from total ash determination was boiled with 25 ml of dilute hydrochloric acid (2M HCl) for 5 minutes. The solution was filtered through ashless filter paper (Whatman No. 41) and residue was washed with hot distilled water until neutral to litmus. The filter paper with residue was transferred to pre-weighed silica crucible and incinerated in muffle furnace (Technico, Model TM-206, India) at 450°C for 3 hours. The crucible was cooled in desiccator for 30 minutes and weighed until constant weight was obtained. Acid-insoluble ash (%) was calculated using formula:

$$\left[\frac{\text{Weigth of acid-insoluble ash}}{\text{Weight of sample}}\right]\times 100$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [24].

Water-soluble ash

The water-soluble ash was determined as per Indian Pharmacopoeia. The ash from total ash determination was boiled with 25 ml of distilled water for 5 minutes and filtered through ashless filter paper (Whatman No. 41). The insoluble residue on filter paper was transferred to pre-weighed silica crucible and incinerated in muffle furnace (Technico, Model TM-206, India) at 450°C for 3 hours. The crucible was cooled in desiccator for 30 minutes and weighed until constant weight. Water-soluble ash (%) was calculated using formula:

$$\left[\frac{\text{Weight of total ash-weight of water insoluble ash}}{\text{Weight of sample}}\right]\times 100$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [25].

Alcohol-soluble extractive value

The alcohol-soluble extractive value was determined as per Indian Pharmacopoeia. Accurately weighed 5 g of coarsely powdered plant material was macerated with 100 ml of ethanol (95% v/v) in closed conical flask for 24 hours with occasional shaking for first 6 hours. The mixture was filtered through Whatman filter paper No. 1 and 25 ml of filtrate was transferred to pre-weighed flat-bottom petri dish and evaporated on water bath. The dish was dried in hot air oven (Labline, Model LI-HOO-101, India) at 105°C for 3 hours, cooled in desiccator for 30 minutes and weighed until constant weight. Alcohol-soluble extractive value (%) was calculated using formula:

$$\left(\frac{\text{Weight of Residue}}{\text{Weight of Sample}}\right)\times 100\times\left(\frac{100}{25}\right)$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [26].

Water-soluble extractive value

The water-soluble extractive value was determined as per Indian Pharmacopoeia. Accurately weighed 5 g of coarsely powdered plant material was macerated with 100 ml of chloroform water in closed conical flask for 24 hours with occasional shaking for first 6 hours. The mixture was filtered through Whatman filter paper No. 1 and 25 ml of filtrate was transferred to pre-weighed flat-bottom petri dish and evaporated on water bath. The dish was dried in hot air oven (Labline, Model LI-HOO-101, India) at 105°C for 3 hours, cooled in desiccator for 30 minutes and weighed until constant weight. Water-soluble extractive value (%) was calculated using formula:

$$\left(\frac{\text{Weight of residue}}{\text{Weight of sample}}\right)\times 100\times\left(\frac{100}{25}\right)$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [27].

Petroleum ether-soluble extractive value

The petroleum ether-soluble extractive value was determined as per Indian Pharmacopoeia. Accurately weighed 5 g of coarsely powdered plant material was macerated with 100 ml of petroleum ether (60-80°C) in closed conical flask for 24 hours with occasional shaking for first 6 hours. The mixture was filtered through Whatman filter paper No. 1 and 25 ml of filtrate was evaporated in pre-weighed petri dish on water bath. The dish was dried in hot air oven (Labline, Model LI-HOO-101, India) at 105°C for 3 hours, cooled in desiccator and weighed until constant weight. Petroleum ether-soluble extractive value (%) was calculated using formula:

$$\left(\frac{\text{Weight of Residue}}{\text{Weight of Sample}}\right)\times 100\times\left(\frac{100}{25}\right)$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [28].

Foaming index

The foaming index was determined as per WHO guidelines to detect saponins. Accurately weighed 1 g of coarsely powdered plant material was boiled with 100 ml of distilled water for 30 minutes, cooled, filtered and volume was adjusted to 100 ml. Ten test tubes were prepared with 1 ml to 10 ml of filtrate respectively and volume in each tube was adjusted to 10 ml with distilled water. The tubes were stoppered, shaken vigorously for 15 seconds and allowed to stand for 15 minutes. The height of foam in each tube was measured in centimeters. Foaming index was calculated using formula:

$$\text{Foaming index} = 100/a$$

where 'a' is the dilution in tube where 1 cm foam height was observed. The experiment was performed in triplicate (n=3) and mean value was calculated [29].

Swelling index

The swelling index was determined as per Indian Pharmacopoeia to measure the volume occupied by plant material after swelling in water. Accurately weighed 1 g of coarsely powdered plant material was transferred to a 25 ml stoppered measuring cylinder and volume occupied by powder was recorded. Then 25 ml of distilled water was added to the cylinder and mixed thoroughly by inverting. The cylinder was allowed to stand undisturbed for 24 hours at room temperature (25-28°C). After 24 hours, the volume occupied by the swollen plant material including any adhering mucilage was measured in milliliters. Swelling index was calculated using formula:

$$\text{Swelling index} = \frac{\left(\text{Final Volume - Initial Volume}\right)}{\text{Initial weight of sample}}$$

The experiment was performed in triplicate (n=3) and mean value with standard deviation was calculated [30].

Extraction of plant material

The dried leaves of *Ruellia prostrata* were coarsely powdered using mechanical grinder and passed through sieve no. 40. Accurately weighed 50 g of dried leaf powder was subjected to successive solvent extraction using Soxhlet apparatus (Borosil, Model BS-36, India) with petroleum ether (60-80°C), chloroform, ethanol and distilled water in sequential manner. Each extraction was carried out for 6-8 hours or until solvent in siphon tube became colorless with 4-6 cycles per hour. After each extraction, the marc was air-dried before proceeding with next solvent. The extracts were concentrated using rotary evaporator (Equitron, Model EQRE-5001, India) under reduced pressure at 40-45°C and further dried in desiccator [31].

Preliminary Phytochemical investigation

The preliminary phytochemical screening was performed on petroleum ether, chloroform, ethanol and aqueous extracts of *Ruellia prostrata* leaves to detect various phytoconstituents. Standard qualitative chemical tests were carried out for alkaloids (Dragendroff's, Mayer's, Wagner's test), flavonoids (Shinoda test, alkaline reagent test), tannins (ferric chloride, lead acetate test), saponins (foam test), steroids and triterpenoids (Salkowski, Libermann-Burchard test), glycosides (Keller-Kiliani, Legal test), phenolic compounds (ferric chloride test), proteins and amino acids (Biuret, Ninhydrin test), and carbohydrates (Molisch, Fehling's test). All tests were performed following standard phytochemical analysis procedures. The presence or absence of phytoconstituents was recorded as positive (+) or negative (-) based on observed color change or precipitation [32].

RESULTS AND DISCUSSION

RESULTS

The macroscopic evaluation of *Ruellia prostrata* revealed characteristic features consistent with Acanthaceae family. The prostrate habit, pubescent ridged stems with swollen nodes, and opposite decussate leaf arrangement represent diagnostic features. The ovate to elliptic leaves with reticulate unicostate venation and presence of trichomes on both surfaces serve as distinguishing characters. The purple to violet tubular bilabiate flowers with didynamous stamens confirm typical Acanthaceae family identity. The tap root system with cylindrical branched structure indicates typical dicotyledonous anatomy. The slightly bitter taste and aromatic odor suggest presence of bioactive secondary metabolites, correlating with traditional medicinal uses. These macroscopic features provide primary identification parameters for quality control and help prevent adulteration in herbal drug markets.

Table 1: Macroscopic characteristics of *Ruellia prostrata*

Plant Part	Parameters	Observations
Whole Plant	Habit	Prostrate herb
	Height	15-30 cm
	Odor	Characteristic, slightly aromatic
	Taste	Slightly bitter
Root	Type	Tap root system
	Shape	Cylindrical, branched
	Color (fresh)	Cream to light brown
	Color (dried)	Light brown to brown
	Surface	Rough with fine rootlets
	Fracture	Short, fibrous
	Length	8-15 cm
	Diameter	0.2-0.5 cm
Stem	Shape	Cylindrical, branched
	Color (fresh)	Green to purple-green
	Color (dried)	Greenish brown
	Surface	Pubescent, ridged

	Texture	Soft, herbaceous
	Fracture	Fibrous
	Nodes	Swollen, distinct
	Internodes	2-5 cm
	Diameter	0.1-0.3 cm
Leaf	Type	Simple
	Arrangement	Opposite decussate
	Shape	Ovate to elliptic
	Apex	Acute to obtuse
	Base	Rounded to cuneate
	Margin	Entire to slightly undulate
	Surface (upper)	Green, sparsely pubescent
	Surface (lower)	Pale green, pubescent
	Venation	Reticulate, unicostate
	Texture	Membranous
	Petiole	Short, 0.3-0.8 cm
	Length	2-4 cm
	Width	1-2.5 cm
Flower	Type	Solitary or in small clusters
	Position	Axillary
	Color	Purple to violet
	Shape	Tubular, bilabiate
	Size	1.5-2 cm long
	Calyx	5-lobed, green
	Corolla	5-lobed, zygomorphic
	Stamens	4 (didynamous)
	Ovary	Superior, bilocular

Microscopic study

The transverse section of *Ruellia prostrata* leaf (Figure 1) exhibited typical dorsiventral structure with distinct upper and lower epidermis covered with thick cuticle. The mesophyll was differentiated into single row of palisade cells and loosely arranged spongy parenchyma with intercellular spaces. Vascular bundles showed collateral arrangement with xylem towards upper side and phloem towards lower side. The presence of tannin-containing cells in mesophyll appeared as dark brown deposits. These anatomical features are consistent with mesophytic leaf structure and provide diagnostic characters for identification of the species. The transverse section of *Ruellia prostrata* root (Figure 2) displayed typical dicotyledonous anatomy with epidermis showing cork cells indicating secondary growth. The cortex contained parenchymatous cells with starch grains and crystal deposits. Vascular tissue showed radial arrangement with polyarch xylem at center and alternating phloem strands. The presence of oil glands and calcium oxalate crystals in cortical region represents important diagnostic features. Secondary growth was evident from cork cambium and secondary vascular tissues. These microscopic features provide authentic identification parameters for quality control of the crude drug.

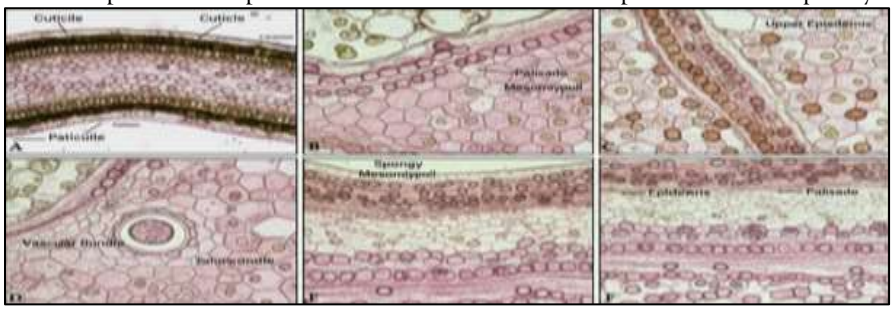

Figure 1: TS of leaf of *Ruellia prostrata*

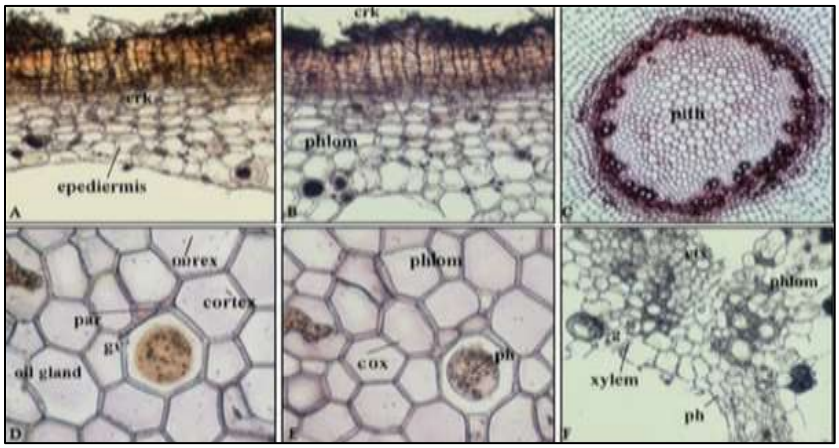

Figure 2: TS of *Ruellia prostrata* root

Quantitative microscopy

The quantitative microscopic parameters of *Ruellia prostrata* leaf (Table 2) revealed significant differences between adaxial and abaxial surfaces, providing numerical standards for authentication. The stomatal number was higher on abaxial surface (128.67 ± 4.16 per mm²) compared to adaxial surface (45.33 ± 2.51 per mm²), indicating hypostomatic leaf type which is common in mesophytic plants and helps reduce water loss. The stomatal index also showed higher value on abaxial surface (18.72 ± 1.23) than adaxial surface (12.45 ± 0.89), supporting the distribution pattern of stomata. The vein-islet number (14.33 ± 1.15 per mm²) and vein termination number (16.67 ± 1.52 per mm²) were consistent on both surfaces, indicating uniform venation density. The palisade ratio of 4.67 ± 0.57 represents the number of palisade cells beneath each epidermal cell, reflecting photosynthetic efficiency. These quantitative values serve as reliable diagnostic parameters for quality control and can differentiate *Ruellia prostrata* from adulterants or related species in powdered form.

Table 2: Quantitative microscopic parameters of *Ruellia prostrata* leaf

S. No.	Parameters	Upper surface (Adaxial)	Lower surface (Abaxial)
1	Stomatal number (per mm²)	45.33 ± 2.51	128.67 ± 4.16
2	Stomatal index	12.45 ± 0.89	18.72 ± 1.23
3	Vein-islet number (per mm²)	14.33 ± 1.15	14.33 ± 1.15
4	Vein termination number (per mm²)	16.67 ± 1.52	16.67 ± 1.52
5	Palisade ratio	4.67 ± 0.57	-

Pharmacognostic study

The physicochemical parameters of *Ruellia prostrata* (Table 3) established quality standards for the crude drug. The total ash value (12.38 ± 0.45%) indicates overall inorganic content, while acid-insoluble ash (2.67 ± 0.28%) represents silica content within acceptable limits. Water-soluble extractive (18.45 ± 0.62%) was higher than alcohol-soluble extractive (14.72 ± 0.58%), suggesting presence of polar compounds like glycosides and carbohydrates. Low petroleum ether extractive (3.28 ± 0.24%) indicates minimal lipophilic constituents. The moisture content (8.45 ± 0.32%) was within acceptable range, preventing microbial growth during storage. Foaming index below 100 suggests low saponin content. These standardized values serve as reference parameters for quality assessment and detection of adulteration.

Table 3: Physicochemical parameters of *Ruellia prostrata* powder

Parameters	Value (% w/w)
Ash value	
Total ash % w/w	12.38 ± 0.45
Acid insoluble ash % w/w	2.67 ± 0.28
Water soluble ash % w/w	6.82 ± 0.37
Extractive values	
Alcohol soluble extractive % w/w	14.72 ± 0.58

Water soluble extractive % w/w	18.45 ± 0.62
Petroleum ether soluble extractive % w/w	3.28 ± 0.24
Other parameters	
Loss on drying % w/w	8.45 ± 0.32
Foaming index	Less than 100
Swelling index (ml)	4.5 ± 0.35

All values are expressed as mean ± standard deviation (n=3)

Preliminary phytochemical investigation of plant

The preliminary phytochemical screening of *Ruellia prostrata* leaf extracts (Table 4) revealed presence of diverse bioactive compounds with varying polarity. Alkaloids were detected in chloroform, ethanol and aqueous extracts, indicating their moderate to high polarity. Carbohydrates and glycosides were present only in polar solvents (ethanol and aqueous), confirming their hydrophilic nature. Steroids were found exclusively in non-polar to moderately polar solvents (petroleum ether, chloroform, ethanol), demonstrating lipophilic characteristics. Flavonoids, tannins and phenols were abundant in chloroform, ethanol and aqueous extracts, suggesting significant antioxidant potential. Saponins were detected only in ethanol and aqueous extracts, correlating with low foaming index observed in physicochemical analysis. The presence of these phytochemicals validates traditional medicinal uses and provides scientific basis for pharmacological activities of the plant.

Table 4: Phytochemical screening of different extracts of *Ruellia prostrata* leaves

Phytochemicals	Test/Reagent	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
Alkaloids	Dragendorff's test	-	+	+	+
	Mayer's test	-	+	+	+
	Hager's test	-	+	+	-
	Wagner's test	-	+	+	+
Carbohydrates	Molisch's test	-	-	+	+
	Fehling's test	-	-	+	+
Glycosides	Keller-Killiani test	-	-	+	+
	Borntrager's test	-	+	+	-
Steroids	Libermann-Burchard test	+	+	+	-
	Salkowski test	+	+	+	-
Flavonoids	Shinoda's test	-	+	+	+
	Lead acetate test	-	+	+	+
Saponins	Foam test	-	-	+	+
Tannins	Lead acetate test	-	+	+	+
Phenols	Ferric chloride test	-	+	+	+

(+) indicates presence; (-) indicates absence

DISCUSSION

Discussion

The comprehensive pharmacognostic evaluation of *Ruellia prostrata* established systematic quality control parameters essential for botanical authentication and standardization of this medicinally important plant. The macroscopic examination revealed characteristic morphological features including prostrate habit, pubescent ridged stems with swollen nodes, opposite decussate leaf arrangement, and purple tubular bilabiate flowers typical of Acanthaceae family. These distinguishing features provide primary identification markers for crude drug authentication and help prevent substitution with morphologically similar species in herbal markets [33].

The microscopic analysis of leaf transverse section demonstrated typical dorsiventral structure with distinct cuticle, single-layered palisade parenchyma, well-developed spongy mesophyll, and collateral vascular bundles. The presence of tannin-containing cells in mesophyll tissue serves as diagnostic feature [34]. The root anatomy exhibited characteristic dicotyledonous structure with secondary growth, polyarch xylem arrangement, and presence of oil glands and calcium oxalate crystals in cortical region. These anatomical features are particularly valuable for identification of powdered drugs where macroscopic characters are lost during processing [35]. Quantitative microscopic parameters provided numerical standards with stomatal number significantly higher on abaxial surface (128.67 per mm^2) than adaxial surface (45.33 per mm^2), indicating hypostomatic leaf type adapted to mesophytic conditions. The consistent vein-islet number, vein termination number, and palisade ratio establish reliable reference values for quality assessment. These quantitative parameters are reproducible and can effectively differentiate authentic samples from adulterants [36].

The physicochemical evaluation revealed important quality indicators with total ash value of 12.38%, indicating moderate inorganic content. The higher water-soluble extractive (18.45%) compared to alcohol-soluble extractive (14.72%) suggests predominance of polar phytoconstituents [37]. Low petroleum ether extractive (3.28%) indicates minimal lipophilic compounds. The moisture content of 8.45% falls within acceptable limits for preventing microbial contamination during storage. These standardized values serve as reference benchmarks for routine quality control in pharmaceutical industries [38]. Preliminary phytochemical screening demonstrated presence of alkaloids, flavonoids, tannins, phenols, carbohydrates, glycosides, steroids, and saponins in different solvent extracts based on polarity. The detection of alkaloids in moderately polar solvents, flavonoids and phenolic compounds in ethanol and aqueous extracts, and steroids in non-polar solvents correlates with their chemical nature. The presence of these diverse bioactive compounds validates traditional therapeutic claims and provides scientific rationale for various pharmacological activities including anti-inflammatory, antimicrobial, and antioxidant properties reported in previous studies [39].

The established pharmacognostic profile creates comprehensive monograph encompassing macroscopic, microscopic, quantitative, and physicochemical parameters along with phytochemical composition [40]. These standardization parameters will facilitate correct botanical identification, quality assurance, and detection of adulteration in crude drug formulations. The numerical standards from quantitative microscopy and physicochemical analysis can be incorporated into pharmacopoeial monographs for regulatory compliance. This systematic documentation supports integration of *Ruellia prostrata* into modern phytopharmaceutical development while ensuring therapeutic efficacy, safety, and quality consistency in herbal medicine preparations. Future studies should focus on isolation and characterization of specific bioactive compounds responsible for observed pharmacological activities [41].

CONCLUSION

The present investigation successfully established comprehensive pharmacognostic standards for *Ruellia prostrata*, providing systematic quality control parameters for authentication and standardization of this traditionally important medicinal plant. The macroscopic evaluation documented distinctive morphological characteristics including prostrate growth habit, pubescent ridged stems with swollen nodes, opposite decussate leaves, and purple tubular bilabiate flowers that serve as primary identification markers. Microscopic examination revealed diagnostic anatomical features such as dorsiventral leaf structure with single-layered palisade tissue, collateral vascular bundles, tannin-containing cells, and root anatomy showing polyarch xylem with oil glands and calcium oxalate crystals in cortical region. Quantitative microscopic parameters established numerical standards with stomatal number of 128.67 per mm^2 on abaxial surface and 45.33 per mm^2 on adaxial surface, along with consistent vein-islet number (14.33 per mm^2) and palisade ratio (4.67). The physicochemical analysis determined standard values for total ash (12.38%), acid-insoluble ash (2.67%), water-soluble extractive (18.45%), alcohol-soluble extractive (14.72%), and moisture content (8.45%), which fall within acceptable pharmacopoeial limits. Preliminary phytochemical screening confirmed presence of alkaloids, flavonoids, tannins, phenols, carbohydrates, glycosides, steroids, and saponins, validating traditional medicinal applications. The standardization parameters documented in this study provide reliable reference standards for quality assessment, authentication, and detection of adulteration in

crude drug formulations. These findings contribute valuable data for development of pharmacopoeial monograph and support integration of *Ruellia prostrata* into evidence-based herbal medicine. The established quality control protocols ensure consistency in therapeutic efficacy and safety of herbal preparations, facilitating further phytopharmaceutical research and commercial exploitation of this medicinally important plant species.

Abbreviations

TS: Transverse Section; WHO: World Health Organization; HCl: Hydrochloric Acid; KOH: Potassium Hydroxide; SD: Standard Deviation; w/w: Weight by Weight; w/v: Weight by Volume; v/v: Volume by Volume; cm: Centimeter; mm: Millimeter; g: Gram; ml: Milliliter; °C: Degree Celsius; rpm: Revolutions Per Minute; pH: Potential of Hydrogen; UV: Ultra-Violet; No: Number.

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