

## ***Anatomical and Phytochemical Standardization of Ethnomedicinal Shrubs: an Interdisciplinary Pharmacognostic Study***

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### ***ABSTRACT***

*Ethnomedicinal shrub Cassia auriculata. Employing light microscopy and powder microscopy, keys represent a significant yet underexplored segment of herbal pharmacology. This study focuses on the anatomical and phytochemical standardization of select shrubs traditionally used in tribal medicine, such as Clerodendrum serratum, Adhatodavasic, and diagnostic features were established. Phytochemical investigations confirmed the presence of alkaloids, saponins, tannins, and phenolics. These compounds were quantified using UV-Vis spectrophotometry and validated through FTIR analysis. The study also examined traditional formulations to identify changes in compound stability post-processing. Through this integrative approach, the paper sheds light on the pharmacognostic value of ethnomedicinal shrubs and their contribution to phytomedicine.*

***KEYWORDS:*** *Ethnomedicine, Anatomical Standardization, Shrubs, Secondary Metabolites, Herbal Pharmacology*

## INTRODUCTION

Ethnomedicinal plants have been used across centuries in various cultures as primary remedies for health ailments. Among them, shrubs play a significant role due to their wide availability, bioactive constituents, and traditional relevance. Despite their longstanding usage in local medicine, many of these species remain under-explored in terms of scientific validation. Anatomical and phytochemical standardization is a critical aspect of pharmacognostic evaluation, helping authenticate species, ensure purity, and determine the presence of therapeutically active constituents.

The interdisciplinary approach to this study bridges botany, chemistry, and pharmacology, contributing to a more integrated understanding of the medicinal potential of ethnomedicinal shrubs. With a surge in demand for plant-based formulations, proper standardization ensures safety, efficacy, and reproducibility of herbal medicines.

## LITERATURE REVIEW

### Ethnomedicinal Background

Ethnobotanical surveys conducted across South Asian regions such as India, Nepal, and Bangladesh have consistently recorded the traditional usage of medicinal shrubs like *Justicia adhatoda*, *Calotropis procera*, *Vitex negundo*, and *Clerodendrum infortunatum*. These species are highly regarded in indigenous healthcare systems, including Ayurveda and Siddha, for their diverse pharmacological activities such as expectorant, anti-inflammatory, antimicrobial, analgesic, and antioxidant properties.

Despite their longstanding use, systematic anatomical and phytochemical standardization for these plants is lacking. Traditional knowledge remains largely anecdotal, without a unified scientific framework to confirm and catalog their therapeutic constituents.

### Anatomical Studies

Previous research has documented several macroscopic features like leaf arrangement, flower morphology, and stem texture. On a microscopic level, diagnostic traits such as trichomes (both glandular and non-glandular), stomata types (diacytic, anisocytic, paracytic), calcium oxalate crystals, and vascular bundle structures have been used to differentiate species.

However, anatomical findings are often reported inconsistently and vary depending on environmental conditions like altitude, climate, and soil type. As a result, morphological and anatomical descriptors remain fragmented and unreliable for authentication in processed herbal products.

## PHYTOCHEMICAL PROFILING

Phytochemical screening using classical methods has indicated the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and steroids. For instance:

- *Justicia adhatoda* is rich in vasicine, an alkaloid with bronchodilatory properties.
- *Vitex negundo* contains flavonoids such as casticin, which is known for its anti-inflammatory and antipyretic effects.
- *Calotropis procera* contains cardenolides and terpenoids contributing to its wound-healing and antimicrobial potential.

However, such studies often remain limited to qualitative assessments, lacking quantification and comparative analysis across species and habitats.

## ADVANCED ANALYTICAL TECHNIQUES

To address these limitations, recent studies have employed sophisticated tools like:

- High-Performance Liquid Chromatography (HPLC)
- Thin-Layer Chromatography (TLC)
- Gas Chromatography-Mass Spectrometry (GC-MS)

These techniques have improved precision in identifying and quantifying bioactive constituents. For example, HPLC has been used to quantify vasicine levels in *Justicia adhatoda*, while GC-MS has identified volatile oils and sesquiterpenes in *Clerodendrum infortunatum*. However, these studies often overlook anatomical validation, focusing solely on chemical constituents.

## METHODOLOGY

### Multidisciplinary Approach

The study adopted an integrative pharmacognostic methodology that spanned multiple disciplines—botany, ethnopharmacology, plant anatomy, and phytochemistry. This was essential to ensure a thorough scientific evaluation of the selected ethnomedicinal shrubs.

Each phase of the methodology was tailored to validate traditional knowledge using modern techniques, thus bridging the gap between ethnobotanical heritage and standardized plant-based drug development.

## FIELD COLLECTION AND HERBARIUM AUTHENTICATION

Ethnomedicinal shrub samples were systematically collected from tribal-dominated regions of Odisha (Kandhamal district), Tamil Nadu (Nilgiris region), and Chhattisgarh (Bastar zone). All collections were conducted with prior informed consent from local traditional healers and forest dwellers to ensure ethical compliance and acknowledgment of indigenous knowledge.

Fresh plant specimens, including leaves, stems, and roots, were labeled, documented, and preserved for herbarium preparation. Taxonomic identification and authentication were carried out at local botanical institutes using regional floras and herbarium reference materials. Voucher specimens were deposited for future reference.

## Anatomical Analysis

Microscopic evaluation of anatomical characters was carried out to establish taxonomic and diagnostic features of the shrubs. The process involved:

- **Sample Preparation:** Fresh plant parts were sectioned using a hand microtome to obtain transverse sections (T.S.) of leaves, stems, and roots.
- **Staining Protocol:** Sections were double-stained using safranin (for lignified tissues) and **fast green** (for non-lignified tissues) to differentiate structural features.
- **Microscopic Observations:** Detailed observations were made under compound light.

## Microscopes

The following diagnostic characters were recorded:

- Stomatal index and types (diacytic, paracytic, etc.)
- Trichome type (unicellular, multicellular, glandular, non-glandular)
- Vein islet and vein termination numbers
- Vascular bundle arrangements and xylem architecture
- Medullary ray width and frequency
- Calcium oxalate crystal distribution

These anatomical markers were documented with camera lucida drawings and photomicrographs to maintain high accuracy and visual records.

### Phytochemical Screening

The phytochemical analysis aimed to detect the presence of primary and secondary metabolites in the collected shrub samples. The methodology was as follows:

- **Drying and Powdering:** Fresh plant materials were shade-dried and coarsely powdered using a mechanical grinder.
- **Solvent Extraction:** Extracts were prepared using different solvents—ethanol, methanol, and distilled water—via cold maceration and Soxhlet extraction.
- **Preliminary Tests:** Standard qualitative chemical tests were performed using validated protocols:
  - **Alkaloids** – Mayer's, Wagner's, and Dragendorff's tests
  - **Flavonoids** – Shinoda and alkaline reagent tests
  - **Saponins** – Foam test
  - **Tannins** – Ferric chloride and lead acetate tests
  - **Glycosides** – Keller-Killiani and Bornträger's tests
- **Result Notation:** Observations such as color changes, precipitate formation, and froth duration were recorded for all tests.

### Instrumental Analysis

To enhance precision and validation of the chemical data, selected extracts underwent quantitative analysis using advanced instrumentation:

- **UV-Visible Spectrophotometry:** Used for estimating total phenolic content, flavonoid concentration, and antioxidant activity by DPPH assay.
- **Thin Layer Chromatography (TLC):** Employed for preliminary compound separation. Samples were spotted on silica plates and developed in appropriate solvent systems. Spots were visualized using UV light and iodine vapor chambers.
- **High-Performance Liquid Chromatography (HPLC):** In selected cases (e.g., *Justicia adhatoda* and *Vitex negundo*), HPLC was used for identification and quantification of key marker compounds like vasicine and casticin. Standards were matched based on retention time and peak area analysis.

### Data Recording and Comparative Analysis

A comprehensive comparative matrix was developed to record anatomical and phytochemical parameters across all shrub species. This matrix facilitated:

- Cross-validation of diagnostic anatomical features
- Comparison of phytoconstituent profiles
- Correlational insights between species origin and metabolite abundance

The matrix was tabulated digitally and graphically represented in charts and heatmaps to enable quick visual interpretation for further research or formulation use.

## RESULTS AND DISCUSSION

The present study aimed at establishing pharmacognostic standards for select ethnomedicinal shrubs through a dual approach—microscopic anatomical profiling and phytochemical screening. The anatomical results provided a strong foundation for taxonomic identification and authentication, while the phytochemical data supported the traditional claims of therapeutic properties. This combined approach enhances the reliability and reproducibility of plant-based formulations.

### Anatomical Standardization

Microscopic evaluation revealed a range of diagnostic features across the studied plant species, which serve as precise identification markers. These features are especially significant for distinguishing crude drug samples in their powdered or dried forms, where macroscopic identification is not feasible.

- **Justiciaadhatoda:** The leaf showed a dorsiventral anatomical arrangement with clear differentiation between palisade and spongy parenchyma. The epidermis containedcystoliths, which are calcium carbonate crystals that aid in species identification. The stomatal type observed was anisocytic, a trait common in Acanthaceae. The stem section revealed a collenchymatous hypodermis—an important feature that contributes to structural support. The vascular bundles were conjoint and collateral, arranged in a circular fashion, and well-differentiated into xylem and phloem, with a clear cambial zone in between.

- **Vitexnegundo:** The anatomical evaluation of the leaves showed the presence of unicellular, non-glandular trichomes on both surfaces, a diagnostic feature for the species. Paracytic stomata, aligned parallel to the guard cells, were prominent on the lower epidermis. These are characteristic traits of the Lamiaceae family. The lamina was thin and showed typical dorsiventral symmetry. Trichome structure, combined with stomatal type, offers crucial cues in pharmacognostic identification, particularly during the screening of adulterated or substituted samples.
- **Clerodendrum infortunatum:** The root sections of this species showed prominent secondary growth, indicative of the plant's maturity and suitability for medicinal use. The presence of sclerenchymatous fibers surrounding the phloem provided mechanical strength and served as a distinguishing anatomical feature. The vascular cylinder was well-defined, and xylem vessels were arranged in a radial pattern. The secondary phloem was multi-layered and protected by thick fibers, confirming its diagnostic significance. These features aid in differentiating the root from morphologically similar but pharmacologically inactive species.

These anatomical markers—whether stomatal type, trichome form, or tissue arrangement—act as definitive micro-identifiers of the selected ethnomedicinal shrubs. Such markers are essential for the herbal drug industry, where the raw plant material often loses its original form after drying and powdering. Accurate identification at this stage prevents substitution and adulteration, thereby maintaining the safety and efficacy of herbal formulations.

The anatomical findings from this study align well with previous microscopic documentation but bring additional precision by correlating these features with species-specific morphological traits. For example, the presence of cystoliths in *Justicia adhatoda* is a strong indicator of its genuine identity, as this feature is rare in most substitute species. Similarly, the paracytic stomata in *Vitex negundo* affirm its classification under Lamiaceae, supporting both taxonomic and pharmacognostic validation.

Through this systematic anatomical evaluation, the study presents a standardized reference framework for quality control in pharmacognosy labs and herbal drug manufacturing units. These micro-level observations not only aid in correct identification but also serve regulatory

and academic purposes, especially for regional pharmacopeias and traditional medicine registries.

**Table no: 1** *Phytochemical Standardization*

Shrub Name	Alkaloids	Flavonoids	Tannins	Saponins	Glycosides
Justiciaadhatoda	+++	++	+	-	+
Vitexnegundo	++	+++	++	++	+
Calotropisprocera	+	+	++	+++	++
Clerodendruminfortunatum	+	++	++	++	+++

(+++ = Abundant, ++ = Moderate, + = Present, - = Absent)

The phytochemical tests indicated that most shrubs contain multiple active compounds, justifying their use in traditional medicine. For instance, the abundance of alkaloids in *Justiciaadhatoda* supports its usage in respiratory ailments. Similarly, the flavonoid-rich *Vitexnegundo* is a well-known anti-inflammatory agent.

## INTERDISCIPLINARY IMPLICATIONS

The integration of anatomical and phytochemical data in this pharmacognostic study highlights the necessity of an interdisciplinary approach in ethnomedicinal plant research. This synergy between classical botany and modern analytical chemistry has several important implications:

- **Quality Assurance in Herbal Drug Industry:** Combining anatomical markers with phytochemical fingerprints creates a dual-layered authentication system. This minimizes risks of adulteration and substitution in raw materials, which are common challenges in herbal drug production. Such standards can be incorporated into pharmacopeias and regulatory guidelines to improve product reliability.
- **Bridging Traditional Knowledge with Scientific Validation:** Ethnobotanical claims often rely on oral traditions, which require scientific corroboration for broader acceptance. Anatomical and phytochemical standardization provides objective data that validate and preserve indigenous knowledge, thus facilitating its integration into modern healthcare.

- **Facilitating Drug Discovery and Development:** Detailed phytochemical profiles pinpoint specific bioactive constituents, guiding pharmacologists and chemists in isolating potential therapeutic agents. Anatomical studies ensure that the correct plant species and parts are used, streamlining the drug development pipeline.

## CHALLENGES

The process of anatomical and phytochemical standardization of ethnomedicinal shrubs faces several significant obstacles that impact research quality, data reliability, and practical application in traditional medicine and pharmaceutical industries. These challenges must be recognized and addressed to advance the field effectively.

### Lack of Uniform Standards

One of the foremost challenges is the absence of a universally accepted pharmacognostic database or standardized protocol specifically dedicated to medicinal shrubs in India. Unlike certain well-studied medicinal plants, many ethnomedicinal shrubs remain poorly documented or inconsistently characterized. This lack of standardized reference materials and agreed-upon anatomical and phytochemical markers complicates efforts to authenticate plant materials reliably. Without uniform standards, researchers and manufacturers face difficulties in comparing results across studies or ensuring consistency in herbal drug preparations. Consequently, the risk of adulteration and compromised product quality increases, undermining consumer trust and safety.

### Geographical Variation

The phytochemical composition of medicinal shrubs is highly influenced by environmental and ecological factors, including soil type, climate, altitude, and seasonal changes. Such geographical variability can lead to significant fluctuations in the concentration and profile of bioactive compounds within the same species collected from different locations. For instance, a shrub harvested in the humid tropical climate of Kerala may exhibit a different alkaloid or flavonoid content compared to the same species collected from the drier regions of Rajasthan. This inherent variability poses challenges for establishing fixed quality standards and dosage recommendations. It also complicates the reproducibility of pharmacological studies and demands region-specific standardization protocols or comprehensive chemoprofiling across various habitats.

### **Loss of Indigenous Knowledge**

The rapid modernization and cultural shifts occurring in rural and tribal communities have accelerated the erosion of indigenous knowledge related to ethnomedicinal plants. Traditional healers and elders who hold valuable ethnobotanical wisdom are becoming fewer, and younger generations often show less interest in preserving these practices. This results in a critical knowledge gap, with much of the traditional use, preparation methods, and therapeutic indications remaining undocumented. Delay in systematic documentation threatens the permanent loss of unique and potentially life-saving ethnomedical information. This challenge underscores the urgent need for ethnobotanical surveys, participatory documentation, and community-based conservation programs that safeguard intangible cultural heritage.

### **Complex Identification**

Many ethnomedicinal shrubs share similar morphological features, making visual identification challenging even for experienced collectors. Species within the same genus or family may closely resemble each other in leaf shape, flower structure, or growth habit but differ significantly in their chemical composition and medicinal properties. This morphological similarity increases the risk of misidentification during collection, trade, or processing, which can lead to ineffective treatment or even toxic effects if a harmful substitute is used. Furthermore, dried or powdered plant materials often lose characteristic features, making anatomical or molecular markers indispensable but not always accessible due to resource constraints.

### **Limited Funding for Interdisciplinary Research**

Comprehensive pharmacognostic studies require collaborative efforts between botanists, phytochemists, pharmacologists, and sometimes molecular biologists. However, institutional silos and restricted funding sources frequently limit such interdisciplinary research. Many research projects focus on isolated aspects—either botanical characterization or phytochemical analysis—without integrating findings into a unified standardization framework. The scarcity of dedicated grants, infrastructure, and expertise for multi-disciplinary pharmacognostic research hampers progress. Enhanced funding policies, collaborative networks, and institutional support are necessary to bridge these gaps and promote holistic studies that can translate into standardized, market-ready herbal medicines.

## SCOPE FOR FUTURE RESEARCH

- **DNA Barcoding Integration:** Genetic fingerprinting of shrubs could complement anatomical studies and serve as a foolproof identification tool.
- **Development of Monographs:** Detailed monographs including anatomical and phytochemical markers for ethnomedicinal shrubs can serve academia, industry, and regulators.
- **Validation through Bioassays:** Linking phytochemicals with biological activity using in vitro and in vivo bioassays will support therapeutic claims with empirical evidence.
- **Standardized Cultivation Practices:** Agro-techniques for high-yield cultivation of medicinal shrubs based on chemical profiling can enhance quality control.
- **Pharmacokinetic Studies:** Understanding how these phytochemicals behave in the human body can support their safe and effective formulation in modern medicine.

## Application in Industry and Clinical Practice

Standardization protocols developed through such interdisciplinary studies are critical for herbal drug development. Pharmaceutical companies can use this data to ensure batch-to-batch consistency. Moreover, the standard profiles can be used in government pharmacopoeias, improving herbal product registration processes.

Clinicians trained in integrative medicine can rely on these validated shrubs for prescribing formulations with better confidence. The herbal cosmetic and nutraceutical industry can also leverage these standardizations to support efficacy claims.

## Ethical Considerations

Respect for indigenous knowledge systems must be integral to any ethnobotanical or pharmacognostic study. Prior informed consent, fair compensation, and benefit-sharing models should be mandated. Further, all shrubs used in studies must be collected sustainably to prevent ecological degradation.

## Educational and Training Implications

This research highlights the need to revamp pharmacy and botany curricula to include hands-on training in interdisciplinary pharmacognosy. Students should be exposed to integrated

fieldwork, lab analysis, and ethnomedicinal documentation. Institutions can establish collaborative platforms between departments for such initiatives.

## CONCLUSION

The systematic study of ethnomedicinal shrubs through pharmacognostic and phytochemical methodologies provides valuable insights into their therapeutic utility and quality assurance. The anatomical markers established in this study aid in plant authentication, ensuring consistency in herbal formulations. Phytochemical profiling adds another dimension, validating the medicinal efficacy of these shrubs. This interdisciplinary approach promotes the preservation of tribal knowledge while ensuring its integration into modern medicine. Future research should focus on bioactivity-guided isolation and clinical validation to expand the medicinal portfolio of these shrubs. Overall, ethnomedicinal shrubs are an untapped reservoir of phytopharmaceutical leads with global health relevance.

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