Comparative Morphological, Anatomical and Phytochemical Evaluation of Three Variants of Justicia Gendarussa Burm. F

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ABSTRACT

Justicia gendarussa Burm. f., an important medicinal shrub of the Acanthaceae family, shows notable variation among its violet-stemmed, green-stemmed and variegated-leaf forms, creating challenges for standardization in herbal formulations. This study compares the morphology, anatomy and phytochemical profiles of the three variants to determine the most suitable type for pharmacological use. Clear morphological differences were observed: the violet-stemmed variant showed purplish pigmentation and elliptic leaves; the green-stemmed type had uniformly green stems and lanceolate leaves; and the variegated form exhibited violet stems with white-striped leaves. Anatomically, the violet variant possessed a thicker cortex, compact collenchyma and more lignified xylem. Phytochemical tests revealed flavonoids, alkaloids, terpenoids, glycosides and phenolics in all variants, with the violet-stemmed type showing higher intensity of metabolites. FTIR analysis further indicated stronger functional group peaks, confirming richer phytochemical diversity. Overall, the violet-stemmed variant demonstrates superior phytochemical richness and is the most promising for therapeutic applications. The study highlights the need for variant-specific standardization and recommends molecular and in vivo studies to support future pharmacological development.

Keywords: *Justicia gendarussa*, variants, phytochemical screening, FTIR, traditional medicine.

INTRODUCTION

Traditional systems of medicine have led to the development of many important drugs that are still in use today. Natural products derived from plants have historically played a crucial role in the discovery of new and effective pharmaceuticals. Even now, the plant kingdom contains numerous medicinal species with valuable therapeutic properties that are yet to be fully explored or discovered [1]. Recent years, there's been a growing effort to document how tribal and indigenous communities across India use medicinal plants. These traditional practices often serve as a starting point for drug discovery and many modern medicines like aspirin, digoxin, quinine, and morphine were originally derived from plants. Plant compounds show incredible chemical variety, with the most common categories being alkaloids, glycosides, polyphenols and terpenes.

However, only a small fraction of these compounds have been rigorously studied or integrated into modern medicine. Acanthaceae is believed to have evolved from Scrophulariaceae or a common ancestral stock. This family is known for a wide variety of ornamental and medicinal plants, attributed to the presence of alkaloids, particularly in the leaves [2]. The genus *Justicia*, with around 600 species, is distributed across tropical and temperate regions [3]. Species within this genus are traditionally employed in the treatment of numerous health conditions, including anemia, epilepsy, kidney disorders, respiratory and gastrointestinal diseases [3].

Justicia gendarussa Burm. f., a herb or shrub belonging to the family Acanthaceae is widely distributed across several Asian countries, including India, China, the Philippines, Indonesia, Malaysia, Sri Lanka, Pakistan, Thailand and the Andaman Islands. The genus *Justicia* is predominantly found in tropical regions worldwide. *J. gendarussa* is considered native to China. There are approximately 300 species of *Justicia* globally, with around 50 species reported in India [1]. Notable species within the genus include *Justicia bentonia* L., *Justicia glabra* J. Koenig ex Roxb., *Justicia diffusa* Willd., *Justicia glauca* Rottler, *Justicia prostrata* (C.B. Clarke) Gamble, *Justicia procumbens* L., *Justicia simplex* D. Don, *Justicia bentonica*, *Justicia traquebariensis*L.f., *Justicia spicigera* and *Justicia beddomei* (Clarke) Bennett [4].

In traditional medicine systems, various parts of *Justicia gendarussa* are used to treat a wide range of ailments. The plant has been widely employed by native healers and tribal communities for managing conditions such as inflammation, liver disorders, tumours and skin diseases. In Ayurveda, it is considered beneficial for the treatment of inflammation, myringitis, bronchitis, vaginal discharges, eye diseases, dyspepsia and fever [5]. The plant is characterized by a strong pungent odour and is described as bitter, hot and dry in nature. It is regarded as emetic, febrifuge, emmenagogue and diaphoretic. Recent observations during field and cultivation studies have revealed the existence of three distinct variants within the species *Justicia gendarussa*, A dark violet-stemmed type commonly used in traditional medicinal preparations, Green-stemmed type that, although similar, is less frequently mentioned in classical medicinal texts And a dark violet-stemmed type with variegated leaves, which appears to be primarily ornamental and often found in home gardens. The presence of such morphological variation within the same species raises serious concerns regarding accurate identification and the reliability of its use in medicinal formulations. This situation presents multiple challenges: misidentification during collection is common, especially among non-specialists and traditional practitioners who rely solely on visible morphological features. As a result, different variants— possibly representing different chemotypes may be unintentionally mixed in medicinal infusions, potentially leading to inconsistencies in phytochemical composition and, therefore, variability in therapeutic efficacy. These inconsistencies may contribute to reduced medicinal effects or, in some cases, adverse health outcomes when non-medicinal or ornamental types are used inadvertently.

In the context of medicinal use, this variation poses significant implications. Traditional formulations and dosages rely heavily on the accurate identification of the plant. The chemical constituents—such as flavonoids, alkaloids and lignans—may vary between variants, leading to compromised efficacy, safety and standardization of herbal preparations. Furthermore, such misidentification can hinder pharmacological research, affect quality control processes and disrupt the development of reliable phyto-therapeutic agents derived from *J. gendarussa*. The essential oils extracted from *J. gendarussa* are rich in volatile compounds with strong aroma and notable therapeutic actions. Furthermore, ethyl acetate fractions of the plant's aerial parts have yielded several novel and known compounds with documented antioxidant and anti-inflammatory effects. These diverse chemical constituents underpin the traditional and pharmacological uses of the plant, making it a valuable source for herbal medicine development [6,7,8,9]. antioxidant efficacy and potential for managing oxidative stress-related disorders [10]. With growing resistance to synthetic antifungal drugs, *J. gendarussa* offers a promising natural alternative for developing antifungal therapies [11,12]. *Justicia gendarussa* has shown promising antibacterial properties through various extracts [13]. Traditionally, the plant is used in regions of China such as Guangdong and Taiwan to manage rheumatic conditions and injuries, supporting its ethnomedical relevance [14-16]. However, further investigation is necessary to isolate and identify the specific bioactive compounds responsible for this hepatoprotective activity [17-21]. Moreover, methanolic leaf extracts stabilize cellular membranes and inhibit the release of inflammatory mediators, further supporting its anti-inflammatory efficacy [5,22,23]. Although preliminary, such results indicate that *Justicia gendarussa* may serve as a valuable source for anticancer agents, warranting further detailed mechanistic and clinical studies [24,25]. The aim of present study is to assess whether the observed variants differ significantly in terms of their medicinal properties, and to identify the specific type(s) most suitable for therapeutic use. Such a comparative approach is essential for ensuring the safety, efficacy and standardization of *J. gendarussa* based formulations in both traditional and modern systems of medicine.

Plant material

Willow-Leaf Justicia (*Justicia gendarussa*), a member of the Acanthaceae family, is an erect, smooth and well-branched undershrub that typically reaches a height of 0.8 to 1.5 meters. The plant is characterized by its slender, lance shaped leaves which measure between 7 to 14 cm in length and 1 to 2.5 cm in width, tapering to pointed tips. The inflorescences are borne as spikes ranging from 4 to 12 cm in length, emerging either terminally at the end of branches or from the axils of leaves. The sepals are linear and smooth, forming tiny cuplike structures approximately 3 mm long. The flowers, though relatively small at about 1.5 cm in length, are quite attractive, displaying white or pale pink corollas adorned with distinct purple spots, which enhance their ornamental appeal. The plant produces smooth, club-shaped capsules around 1.2 cm long that contain the seeds. Willow-Leaf Justicia is widely distributed throughout India and Southeast Asia, where it often grows in moist, shaded locations.

Within the species *Justicia gendarussa*, three distinct morphological types are commonly recognized. One is a dark violet-stemmed variety, which is most frequently used in traditional medicinal preparations due to its prominence in classical Ayurvedic, Siddha and Unani texts. Another is the green-stemmed type, which closely resembles the medicinal form but is less frequently cited in ancient medicinal literature and appears to be used less often in traditional therapies. The third type is a dark violet- stemmed form with variegated leaves, primarily cultivated as an ornamental plant. This variety, characterized by its striking foliage, is commonly found in home gardens and is not typically associated with medicinal applications (Figure 1a-c).

Methodologies

Variants analysis

Variants analysis involves studying and distinguishing different forms (variants) of a species based on their external (and sometimes internal) morphological features. For plants like *Justicia gendarussa*, identifying and analyzing variants is important to avoid misidentification—especially in medicinal applications where chemical composition may vary between forms.

Morphological characterization

It is the most fundamental and widely used method for variants analysis. It involves the systematic observation and documentation of various visible features of the plant. Key parameters assessed include stem color, Leaf characteristics are also closely examined, including their shape, size, coloration, margin type and venation pattern. Floral features such as flower structure, size and color, along with the type of inflorescence, provide additional taxonomic clues. The morphology of the capsule or fruit, including its shape and surface texture, is noted, as are root traits. For precise documentation, basic tools such as a hand lens, ruler and photographic equipment are used to measure and record these morphological traits systematically.

Anatomical studies

It involves the microscopic examination of cross-sections of plant parts such as the stem, root and leaf to identify internal structural differences among variants. These studies help in understanding the arrangement and organization of tissues that are not visible externally. Key anatomical features examined include the vascular bundle arrangement, distribution of xylem and phloem tissues. The leaf mesophyll structure, such as the proportion of palisade to spongy tissue, provides insights into photosynthetic adaptations. Stem cortex thickness and the nature of cortical cells can vary between variants and are often linked to structural or functional specializations. Additionally, differences in internal tissue organization, such as the presence or absence of sclerenchyma, secretory cells, or trichomes, can further help distinguish between plant types.

Analytical Studies

Hot continuous soxhlet extraction

Fresh leaves of *Justicia gendarussa* (100g) were chopped, shade dried and sequentially extracted with 250 ml of water (non-polar to polar) for 6h using soxhlet hot continuous extraction method. The resulted extract was concentrated using rotavapour at 45°C and were stored at -20°C for further studies.

Preliminary phytochemical analysis (Qualitative)

The aqueous extract from hot continuous soxhlet method was evaluated for the presence of secondary metabolites according to the standard protocols Khandelwal [26].

Fourier transform infrared spectroscopy (FTIR) analysis

The leaves of *Justicia gendarussa* were collected and dried in oven for 2 days at 60 C. Tablets for FTIR spectroscopy were prepared in agate mortars, by mixing powder (2 mg) with KBr (1:100 p/p). The absorbance spectra was measured between 300 and 4500/cm. At least three spectra were obtained foreach sample [27]. A FTIR spectrometer was (FTIR Shimadzu Prestige 21) used to collect spectra. Spectra were obtained in 32 scans co-added, 4000 resolution and 2.0 gains. The parameters for the Fourier self-deconvolution were a smoothing factor of 15.0 and width factor of 30.0 cm-1. De- convolved and seconddderivative spectra were calculated for Fourier self-deconvolution and the bands selected and normalized to unity with Omnic 7 software. Curve-fitting of the original spectra was performed with Origin 7 software. The band position of functional groups was monitored with Knowitall 7.8 software. The spectral region between 3000and 2800/cm was selected to analyze lipids. The spectral region between 1800and 1500/cm for proteins and between 1200 and 1000/cm for carbohydrates. Triplicate experiments (N = 3) were conducted and spectra from the first two experiments were used for establishment of chemometric models and the thirdspectra of the experiment were used for model validation.

RESULT AND DISCUSSION

Plant material

***Justicia gendarussa* Burm.f.**

Herbaceous plants or upright, ascending shrubs with opposite leaves that are either crenate or have entire margins. The flowers are arranged in terminal or axillary inflorescences, which may be sessile or pedunculated and occur either singly or in cymes within the axils of the bracts, typically forming spikes or panicles. The calyx and corolla, divided into 4–5 parts, exhibit a range of colours including purple, red, lilac, white, yellow, and orange. The corolla features a bilabiate limb, with the inner upper lip often narrow, erect or curved and sometimes concave, ending in an entire, bifid, or slightly bilobed apex. The lower lip is broader, sometimes curved or spreading, and typically trilobed. There are two stamens with filaments attached near or above the middle of the slender tube, sometimes slightly widened at the base. The anthers are bilocular with oblong, occasionally curved or kidney-shaped thecae. Each ovary chamber contains two ovules. The fruit is a capsule, generally oblong, elliptical, or obovate in shape, with a solid base that is laterally compressed and an upper part that may be cylindrical, ovoid, or subspherical (Figure1a-c).

Figure 1 : Plant material
a: Accession 1 (Violet stemmed)
b: Accession 2 (Green stemmed)
c: Accession 3 (Violet stemmed with variegated leaf)

Comparative analysis of Morphological Characters of the three observed variants of *Justicia gendarussa*

Variants are distinct forms or variants within a species that differ in observable morphological traits such as stem or leaf colour, leaf shape or size, flower structure, or overall growth habit. These differences can result from underlying genetic variation or environmental influences like soil type, light conditions, or cultivation practices. In the case of *Justicia gendarussa*, for example, three variants may be observed: a dark violet-stemmed type, a green stemmed type and a variegated violet-stemmed type. Although these forms belong to the same species, they exhibit noticeable physical differences that may correspond with variations in their medicinal properties or phytochemical composition. Variants are not considered separate species or subspecies but represent intraspecific diversity—variation within a single species. Studying variants is crucial in ethnobotany, pharmacognosy and conservation, as it helps identify the most therapeutically effective and chemically potent forms of a medicinal plant.

The comparative analysis of the three variants of *Justicia gendarussa*— namely the dark violet-stemmed type, the green-stemmed type and the dark violet-stemmed variant with variegated leaves—reveals significant differences in their morphological traits and potential medicinal value. The table 1 shows that while all three types share core botanical characteristics, variations exist in stem pigmentation, leaf margin and venation, and inflorescence length. Notably, the dark violet-stemmed variants, traditionally used in medicinal preparations, exhibited more pronounced structural features such as thicker stems and denser inflorescence spikes (Figure 1a-c). The green-stemmed variant, although morphologically similar, showed slightly reduced floral development and is less frequently cited in ethnomedicinal texts. The variegated type, often cultivated ornamentally, displayed more decorative traits like irregular leaf coloration and lower phytochemical intensity, as confirmed by preliminary screening. These observations suggest that the traditional use of the dark violet stemmed variants may be justified by its distinct morphological robustness and potentially richer phytochemical content. This variants-specific differentiation is critical for ensuring the authenticity, safety and efficacy of *Justicia gendarussa* based formulations in traditional and modern phytotherapeutics. Similarly, studies on *Datura* species have highlighted significant morphological and phytochemical variation among different populations and variants, underscoring the necessity for comparative evaluation. Abdelkader et al. [28] investigated twelve Algerian samples of *Datura stramonium* and reported noticeable diversity in plant morphology and the levels of alkaloids like hyoscyamine and scopolamine. Such variability suggested that variants-based differences could greatly influence the plant's pharmacological potential.

In another comprehensive study, Partap et al. [29] compared different populations and variants of *Datura innoxia* and *Datura metel* from the Punjab plains and revealed significant variation in key phytoconstituents. These findings emphasize the need for standardized phytochemical profiling across various morpho and ecotypes to ensure therapeutic consistency. Our study on *Justicia gendarussa* aligns with this perspective, where distinct variants— characterized by differences in stem colour and leaf morphology— exhibited variations in phytochemical composition and traditional medicinal usage. Like *Datura*, the identification and selection of the most pharmacologically active variants of *J. gendarussa* is essential for developing standardized herbal formulations, validating traditional knowledge and ensuring product safety and efficacy in broader medicinal markets.

Anatomy of the *Justicia gendarussa* Stem

The transverse section of the stem of *Justicia gendarussa* shows a typical dicotyledonous organization. The outermost epidermis is a single layer of tightly packed rectangular cells covered by a thin cuticle, sometimes bearing unicellular trichomes. Below it lies the cortex, which is differentiated into 2–3 layers of supportive collenchyma followed by several layers of loosely arranged parenchyma containing chloroplasts and occasional starch grains. The cortex ends with a well-defined endodermis made up of barrel-shaped cells that may store starch, forming a starch sheath. Just inside the endodermis is the pericycle, often sclerenchymatous, adding extra mechanical strength. The vascular bundles are arranged in a ring, with phloem on the outer side and xylem toward the centre, separated by a thin cambial layer that indicates secondary growth. At the centre lies the pith, composed of large, thin-walled parenchyma cells with noticeable intercellular spaces, sometimes also containing starch grains (Figure 2a-c).

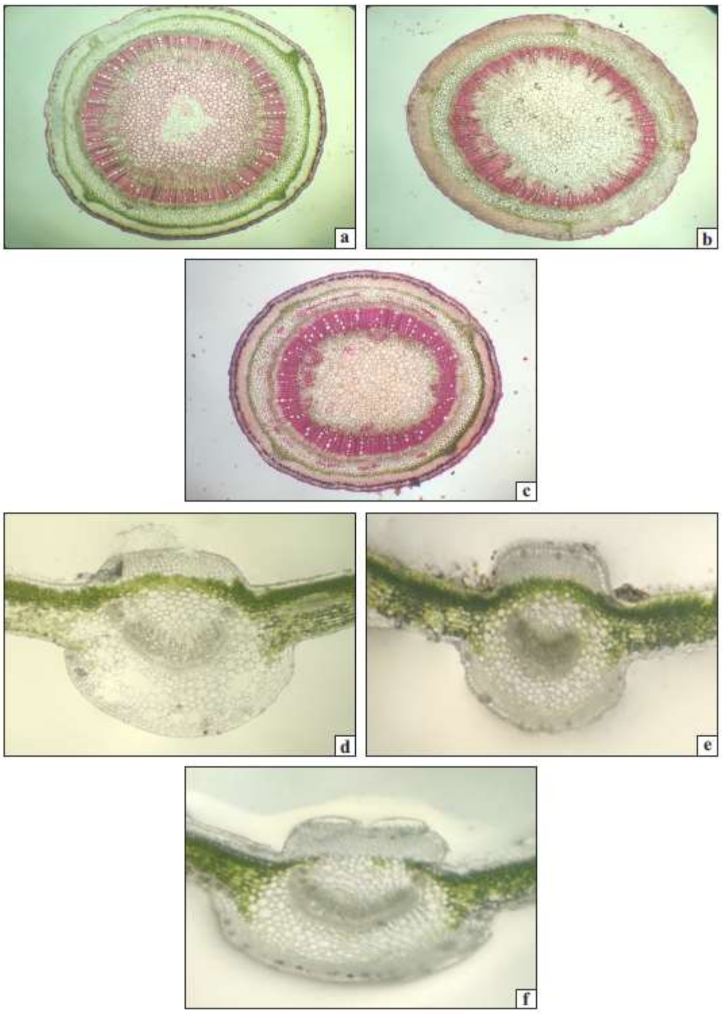


Figure 2: Stem and leaf anatomy of *Justicia gendarussa*
a: Accession 1 (violet stemmed stem anatomy)
b: Accession 2 (green stemmed stem anatomy)
c: Accession 3 (violet stemmed with variegated leaf stem anatomy)
d: Accession 1 (violet stemmed leaf anatomy)
e: Accession 2 (green stemmed leaf anatomy)
f: Accesssion 3 (violet stemmed variegated leaf anatomy)

Anatomy of *Justicia gendarussa* Leaf

The leaf of *Justicia gendarussa* is dorsiventral, with distinct anatomical regions: The transverse section of the leaf of *Justicia gendarussa* exhibits a typical dorsiventral anatomy. The upper epidermis is single-layered, consisting of tightly packed rectangular cells covered by a thick cuticle, with stomata either absent or very sparse. In contrast, the lower epidermis is also single-layered but contains a higher number of stomata, making the leaf hypostomatic; the guard cells are evident and the cuticle is comparatively thinner. The mesophyll is distinctly differentiated into two regions: the palisade parenchyma, consisting of one to two layers of vertically elongated, chloroplast-rich cells situated just beneath the upper epidermis and specialized for photosynthesis; and the spongy parenchyma, located below the palisade cells, composed of

Table 1: Comparison of morphological characters of the three observed variants of *Justicia gendarussa*

Morphological Feature	Dark Violet-Stemmed Type	Green-Stemmed Type	Variegated Violet-Stemmed Type
Stem Color	Dark violet	Light to medium green	Dark violet with visible striping or marbling
Leaf Color	Uniform dark green	Pale to medium green	Green with pale yellow or cream variegation
Leaf Shape	Lanceolate, slightly curved	Lanceolate or elliptic	Narrow-lanceolate to elliptic, slightly asymmetrical
Leaf Size	Medium to large (7–14 cm × 1.5–3 cm)	Smaller (6–10 cm × 1–2 cm)	Variable; often smaller than the other two
Leaf Margin	Entire	Entire	Entire
Venation	Pinnate, prominent midrib	Pinnate, less prominent	Pinnate, less visible due to variegation
Inflorescence Type	Terminal and axillary spikes (4–12 cm long)	Axillary spikes, sparse	Shorter, less conspicuous terminal/axillary spikes
Flower Color	White to pale pink with purple spots	White with less prominent markings	White-pink with faint markings, occasionally sterile
Flower Size	~1.5 cm long	Slightly smaller (~1.2 cm)	Small and often less developed
Sepal Features	4–5 narrow linear lobes (~3 mm), smooth	Similar, less pigmented	Similar, possibly distorted in ornamental variants
Fruit (Capsule) Shape	Club-shaped, smooth, ~1.2 cm long	Club-shaped, slightly smaller	Rarely fruiting; capsules underdeveloped
Growth Habit	Erect, bushy, up to 1.5 m	Erect to spreading, ~1.2 m	Dwarf or ornamental habit, ~0.5–1 m tall
Habitat/Use	Common in medicinal gardens; widely used in Ayurveda	Found in wild or less documented use in classics	Primarily cultivated as ornamental in home gardens

irregularly shaped, loosely arranged cells with prominent intercellular spaces that facilitate gaseous exchange. The vascular bundles, present in the midrib and smaller veins, are surrounded by a bundle sheath, with xylem oriented toward the upper side and phloem on the lower side. The midrib region prominently protrudes on the lower side of the leaf and is reinforced with collenchymatous tissue, providing mechanical support, while the centrally placed vascular bundles ensure efficient transport (Figure 2d-f).

Qualitative Analysis (Preliminary Screening)

The qualitative phytochemical screening of *Justicia gendarussa* was carried out using methanol and water extracts obtained through hot continuous Soxhlet extraction. Both extracts revealed the presence of major classes of secondary metabolites, including alkaloids, terpenoids, phenols, steroids, flavonoids, phlobatannins and phytosterols, while anthraquinones were absent in all cases (Table 2). When comparing the three morphological variants—violet-stemmed, green-stemmed and variegated—the results showed that all variants contained flavonoids, alkaloids, terpenoids, glycosides and phenolics. However, the violet-stemmed variant consistently exhibited stronger reactions and higher intensity of these metabolites, indicating a richer phytochemical profile overall.

Table 2: Qualitative analysis of different phytochemicals in hot solvent extracts

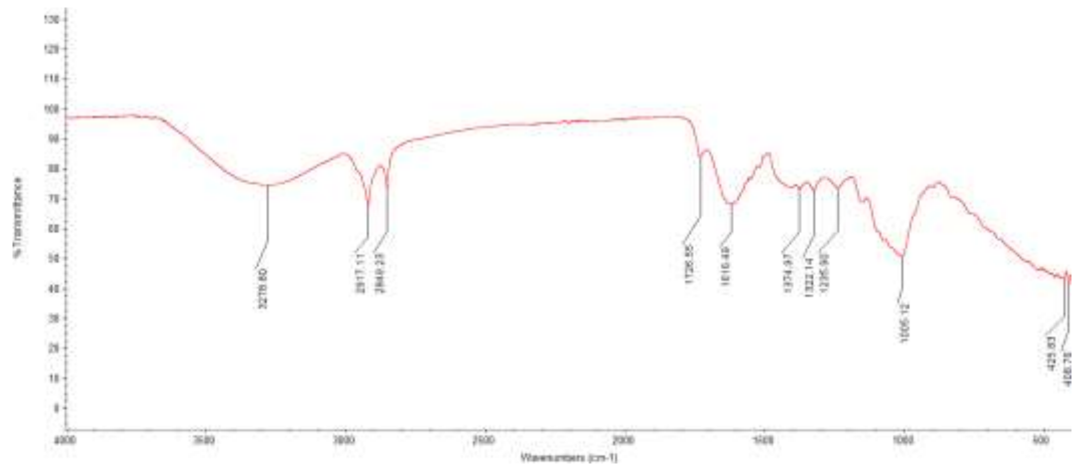
Phytochemical	Methanol	Water
Alkaloid	+	+
Terpenoid	+	+
Phenol	+	+
Flavonoid	+	+
Phytosterol	+	+
Phlobatannins	+	+
Tannins	+	+
Saponins	+	+
Quinone	+	+
Anthaquinone	_	_
Glycosides	+	+
Emodin	+	+
Steroid	+	+

Methanol extract exhibited a more diverse and abundant presence of phytochemicals compared to the water extract. It showed significant amounts of phenols, flavonoids, tannins, quinones and emodins. This highlights methanol's superior efficiency in extracting a broad spectrum of phytoconstituents. The water extract, while also demonstrating a rich phytochemical profile, had comparatively fewer detectable amounts, although flavonoids, tannins, quinones and emodins were still present in notable levels. These findings suggest that methanol is a more effective solvent for extracting secondary metabolites from *J. gendarussa*. This is particularly important for phytopharmaceutical applications, where the efficacy of medicinal formulations depends on the concentration and diversity of active compounds.

Fourier Transform Infrared (FTIR) Spectroscopy analysis

FTIR is a powerful analytical technique used to identify the functional groups present in plant powders by analysing the absorption of infrared radiation by chemical bonds within molecules. FTIR helps determine the presence of specific bioactive compounds by identifying characteristic functional groups (–OH, –NH, –C=O, –CH, etc.) in the plant powder. This is especially valuable for confirming phytoconstituents like phenols, flavonoids, terpenoids, alkaloids and others.

FTIR spectroscopy reveals the presence of various functional groups that correlate with secondary metabolites such as phenolics, flavonoids, alkaloids, esters and more. A comparative study of accession 1, 2 and 3 shows both common and distinct peaks, indicating shared and unique chemical constituents (Figure 3a-c)(Table 3,4,5).



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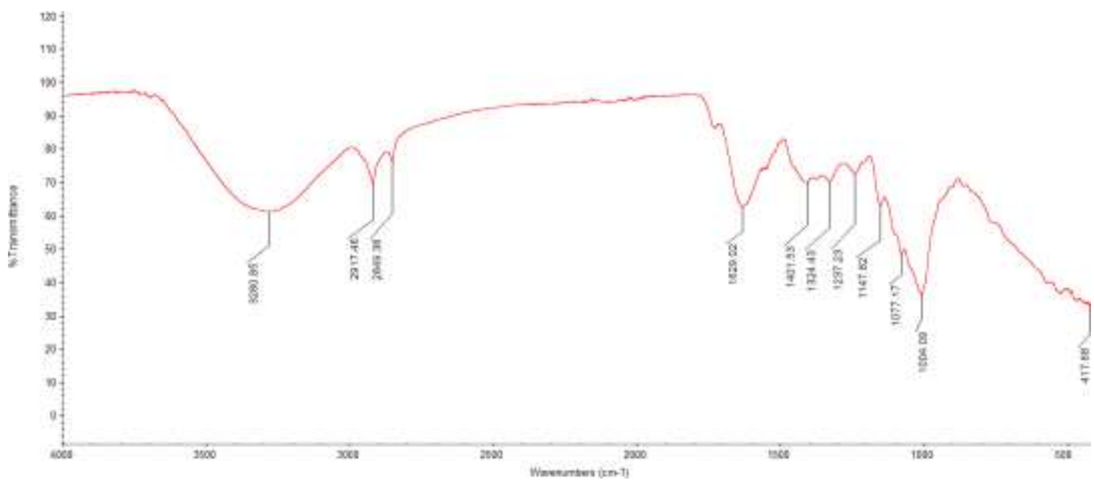

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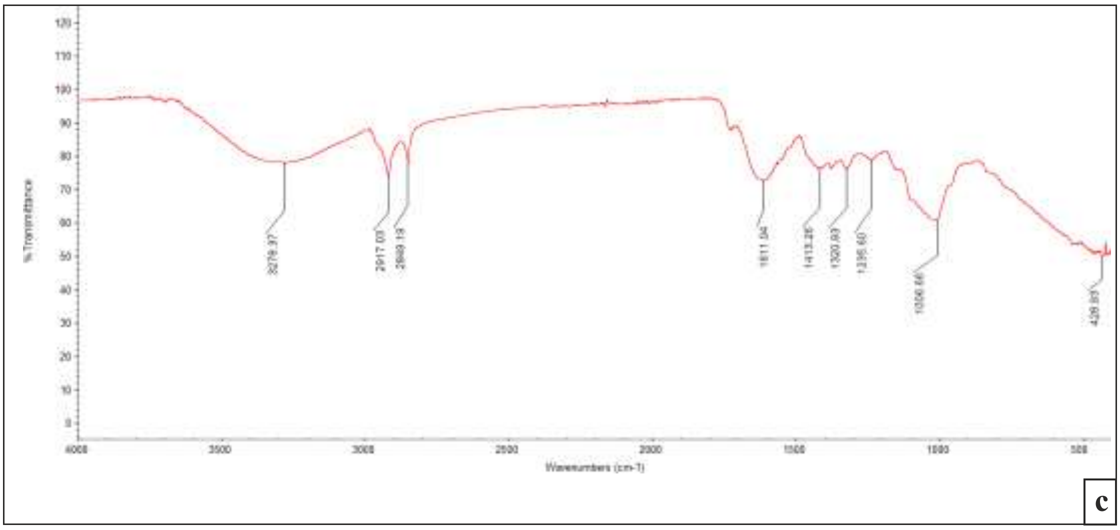

Figure 3: FT-IR chromatogram of *Justicia gendarussa*

a: Accession 1 (Violet stemmed)

b: Accession 2 (Green stemmed)

c: Accession 3 (Violet stemmed variegated leaf)

Accession 1

Unique Peaks: 1726.55 cm⁻¹ (Anhydride): Suggests the presence of complex esters or organic acids with anhydride linkages**.** 1374.97 cm⁻¹ (Alcohol): Indicates presence of free alcohol groups. 1235.90 cm⁻¹ (Aromatic Ester): Suggests presence of aromatic-based secondary metabolites like flavonoid esters. Accession 1 may contain more esters and hydroxylated aromatic compounds, suggesting richer presence of flavonoid glycosides or related secondary metabolites.

Table 3: FT-IR Spectroscopy of Accession 1

FTIR Peak	Functional group
3278.80	Carboxyl acid
2917.11	Alkane
2849.23	Alkane
1726.55	Anhydride
1616.49	Alkane
1374.97	Alcohol
1322.14	Aromatic amine
1235.90	Aromatic ester
1005.12	Fluro compound

Accession 2

Unique Peaks: 1147.82 cm⁻¹ (Aliphatic Ether): Indicates possible sugar residues or ether- linked compounds (e.g., saponins). 1077.17 cm⁻¹ (Primary Alcohol): Suggests simple alcohols or hydroxyl-containing molecules (like phenolics or sugars). 1237.23 cm⁻¹ (Amine): Primary or secondary amines often relate to alkaloids or amino acids. Accession 2 may be rich in sugar- or ether- linked phytoconstituents and alkaloid-based compounds, possibly contributing to its bioactivity.

Table 4: FT-IR Spectroscopy of Accession 2

FTIR Peak	**Functional group**
3280.85	Carboxyl acid
2917.46	Alkane
2849.38	Alkane
1629.02	Alkane
1401.53	Fluoro compound
1324.43	Aromatic amine
1237.23	Amine
1147.82	Aliphatic ether
1077.17	Primary alcohol
1004.09	Fluoro compound

Accession 3

Unique Peaks: 1611.04 cm⁻¹ (Unsaturated Ketone): May indicate presence of conjugated ketones, often found in quinones or flavonoids. 1413.26 cm^{-1} (Sulfate): Uncommon in plant FTIR but may suggest sulfur-containing compounds or external contamination. 1235.60 cm^{-1} (Amine): Similar to accession 2, accession 3 likely contains conjugated ketones and nitrogenous compounds. The sulfate signal could be related to sulfur-based metabolites or processing agents.

Table 5: FT-IR Spectroscopy of accession 3

FTIR Peak	Functional group
3278.37	Carboxyl acid
2917.30	Alkane
2849.19	Alkane
1611.04	Unsaturated ketone
1413.26	Sulfate
1320.93	Aromatic amine
1235.60	Amine
1006.66	Fluoro compound

The FTIR analysis of the three plant powder accessions reveals both shared and distinct functional groups, suggesting a common phytochemical framework with individual variations. All three accessions exhibit prominent peaks around 3278– 3281 cm^{-1}, 2917–2849 cm^{-1} and 1320–1324 cm^{-1} indicating the presence of carboxylic acids, alkanes and aromatic amines respectively. These are characteristic of phenolic compounds, terpenoids and nitrogenous metabolites commonly found in medicinal plants. Fluoro compounds are also consistently detected in all three, though their presence may indicate halogenated plant metabolites or artifacts introduced during accession preparation. Accession 1 is distinguished by the presence of anhydride (1726.55 cm^{-1}), aromatic ester (1235.90 cm^{-1}) and a specific alcohol group (1374.97 cm^{-1}) suggesting a rich profile of esters and hydroxylated compounds such as flavonoid glycosides. Accession 2 uniquely shows aliphatic ether (1147.82 cm^{-1}), primary alcohol (1077.17 cm^{-1}) and additional amine signals (1237.23 cm^{-1}), pointing to sugar- based compounds and alkaloids, possibly linked to the presence of saponins or glycosidic linkages. In contrast, accession 3 reveals an unsaturated ketone (1611.04 cm^{-1}) and sulfate group (1413.26 cm^{-1}), which may correspond to quinonoid structures and sulfur-containing phytochemicals. These findings suggest that while the three accessions share a foundational phytochemical composition, they differ in specific functional groups that reflect variations in their secondary metabolite profiles.

Conclusion

The present study highlights significant morphological, anatomical and phytochemical variations among the three *Justicia gendarussa* variants—violet-stemmed, green-stemmed and variegated. All variants contained key secondary metabolites, but the violet-stemmed type consistently exhibited higher metabolite intensity and stronger FTIR functional group signals, indicating superior phytochemical richness. Anatomical features such as thicker cortex and more lignified vascular tissues further distinguish the violet-stemmed variant, supporting its enhanced bioactive potential.

Overall, the findings identify the violet-stemmed variant as the most promising for therapeutic use, underscoring the need for variant-specific standardization in herbal drug development. Future studies involving advanced phytochemical profiling, molecular characterization and in vivo validation will be essential to fully establish its pharmacological efficacy and ensure safe, consistent medicinal applications.

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