

Unravelling the identity of *Justicia beddomei* (C.B.Clarke) Bennet (Acanthaceae) through pharmacognostic characterization

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Abstract

Justicia beddomei (C.B.Clarke) Bennet is commonly known as *chittadalodakam* or *cheriya aadalodakam* and belongs to the family Acanthaceae. It is an evergreen medicinal plant used in the traditional systems of medicine for the treatment of various respiratory disorders. *J. adhatoda* L. also a member of family Acanthaceae is morphologically very similar to *J. beddomei*. Nowadays, *J. adhatoda* is more commonly used than *J. beddomei* due to the latter's limited geographical distribution and the two species are often confused because of their morphological similarities. *J. beddomei*, is considered medicinally superior to the allied species. The present study was conducted to determine the pharmacognostic features of *J. beddomei* for the detailed characterization and authentication of the plant. Dried leaf powder as well as fresh leaves were subjected to pharmacognostic analysis. Macroscopic characters show simple, elliptic-lanceolate dark green colour leaves with a prominent midrib on the lower surface. Microscopic study revealed the presence of diacytic stomata and bowl-shaped vascular strands with one circular lateral vascular bundle on either side of the strand. Powder microscopy showed the presence of characteristic spirally thickened xylem tracheids, thin-walled phloem with sieve plate elements and multicellular non-glandular trichomes. The fluorescence analysis of leaf powder with chemical reagents showed different colours under UV and daylight. Physico-chemical parameters were evaluated by pH, loss on drying at 105°C, total ash, acid insoluble ash, water soluble ash, swelling index, foaming index and extractive values. The results obtained from this study are useful for establishing the pharmacognostic profile of *J. beddomei* and thus help in the standardization and proper identification of drugs derived from this plant.

Keywords: *Justicia adhatoda*, Organoleptic, Physico-chemical, Powder microscopy

1. Introduction

The Acanthaceae family is a rich source of plants with medicinal properties and is used in traditional systems of medicine (Sharma and Kumar, 2016). The largest genus of Acanthaceae is *Justicia*, which comprises more than 600 species distributed in tropical and pan-tropical regions, nearly 50 species occur in India and are distributed in the temperate regions (Mabberley, 2008). Among them, two are medicinally important viz. *Justicia adhatoda* L. (synonym: *Adhatoda vasica* (valiya aadalodakam) and *J. beddomei* (C.B.Clarke) Bennet (synonym: *A. beddomei* (*chittadalodakam*/*cheriya aadalodakam*)). Early reports suggest that *J. beddomei* is endemic to India occurring in the Travancore hills of the Southwestern Ghats, Valparai (South Arcot), Akkamalai (Coimbatore district) and Mahendragiri, Kanyakumari district (Joy *et al.*, 2001). This

species was reported from Kerala in recent literatures (Sasidharan, 2013; Prabhukumar *et al.*, 2018). As the plant flowers rarely or not at all in temperate habitats, the propagation of *J. beddomei*, through seed is limited. Seed germination and propagation through stem cutting are also very poor (Mathew *et al.*, 1998). Morphologically, *J. beddomei* is similar to *J. adhatoda* in its habit and other floral characteristics (Prabhukumar *et al.*, 2018). The leaves of *J. beddomei* are dark green and smaller, while the leaves of *J. adhatoda* are larger and pale green.

The preliminary phytochemical screening of *J. beddomei* revealed the presence of diverse phytochemical constituents. The leaves and roots of this plant are the main ingredients of many Ayurvedic preparations. Phytochemical investigations of *J. beddomei* have revealed

the presence of various bioactive constituents including alkaloids, tannins, flavonoids, phenolics, glycosides and disaccharides (Srinivasan *et al.*, 2014). Among these, quinazoline alkaloids such as vasicine have been extensively studied for their pharmacological activities (Jiang *et al.*, 2019). Previous pharmacognostic reports indicate that the leaves contain vasicine, which is effective in the management of cough, chronic bronchitis and asthma (Haleshi *et al.*, 2020). Comparative pharmacognostic and phytochemical analysis of *J. beddomei* and *J. adhatoda* have further confirmed the presence of vasicine in both species (Nandhini and Ilango, 2020). In addition to vasicine, other compounds such as anisotine, deoxyvasicinone, vasicinol, vasicinolone and vasicolinone have been reported; however, their pharmacological potential remains insufficiently explored (Marathakam *et al.* 2012; Haleshi *et al.*, 2020). Although the plant is traditionally used for various therapeutic purposes (Patel and Venkatakrishna, 1984), most studies have primarily focused on its phytochemical aspects and detailed pharmacognostic standardization remains limited and incomplete, particularly in comparison with *J. adhatoda*. This lack of comprehensive authentication data may contribute to substitution and misidentification in herbal practice.

The novelty of the present study lies in establishing a detailed pharmacognostic and physico-chemical standardization profile of *J. beddomei*, which has not been comprehensively addressed in previous reports. While earlier studies described phytochemical constituents and general morphological features (Haleshi *et al.*, 2020), the present work provides detailed microscopic diagnostics, fluorescence characteristics and quantitative physicochemical parameters essential for authentication and quality control. Additionally, the study identifies distinguishing features that help to differentiate *J. beddomei* from the closely related *J. adhatoda*, thereby minimizing misidentification. Therefore, the present investigation was undertaken to establish a comprehensive set of pharmacognostic parameters, including macroscopic, microscopic, powder, fluorescence and physicochemical evaluations, to support accurate identification and standardization of the species.

2. Materials and methods

The plants were collected from Irinjalakuda (10.3804° N, 76.2074° E), Thrissur district, Kerala. They were maintained at the Medicinal Plant Garden, Department of Botany, University of Kerala, Thiruvananthapuram, Kerala, India. A voucher specimen was maintained at the Herbarium, Department of Botany, University of Kerala (voucher no.: KUBH 11520). For comparative morphological analysis, authenticated samples of *J. adhatoda* were collected from Porathissery (10.36281° N, 76.2048° E), Thrissur district, Kerala. A total of five mature plants of *J. beddomei* and *J. adhatoda* were randomly selected for the study. The collected plants were shade-dried and powdered for further analysis. All experiments were carried out in triplicate ($n = 3$) and results were expressed as mean $\pm$ standard error (SE).

2.1. Morphological characterization

The macromorphological features of plants were evaluated using both qualitative and quantitative characteristics. The characters were studied using five individual mature plants for both species. Quantitative parameters were measured from three fully expanded leaves per plant. A comparative morphological analysis of both *J. adhatoda* and *J. beddomei* were performed by assessing qualitative and quantitative characters, including plant height, leaf size (length and breadth), leaf colour, odour, taste, leaf texture, powder colour, leaf nature, habit, midrib prominence, leaf shape and number of lateral veins. Each measurement was recorded in triplicate. All data were expressed as mean $\pm$ standard error (SE) based on five biological replicates.

2.2. Anatomy

Fresh leaf samples were collected. Freehand sections were made and stained in aqueous 1% safranin for two minutes. Then they were mounted in 50% glycerine, and photographs were taken using an image analyser (Leica DM2000). The leaf peel was taken and stained with safranin. Then, stomatal characters were analysed.

2.3. Powder microscopy

The powdered leaves were treated with 1-2 drops of phloroglucinol and observed under the microscope. The characteristics of xylem and phloem elements were studied (Phloroglucinol test) (Evans, 2008).

2.4. Organoleptic characterization

The characteristic colour, texture and odour of both leaves and leaf powder were evaluated (World Health Organization, 1998).

2.5. Fluorescence analysis

The behaviour of leaf powder was studied with various chemical reagents. It was then observed under short UV (254nm), long UV (365nm) and under visible light. The characteristic colour for each treatment was noted (Ansari, 2006).

2.6. Physico-chemical parameters

All physico-chemical tests were carried out in triplicate according to standard protocols and the results were expressed as mean $\pm$ standard error (SE) ($n = 3$).

2.6.1. Determination of pH of leaf powder: About one gram of leaf powder was taken in a beaker and 10 mL of distilled water was added. The mixture was stirred thoroughly and allowed to stand for ten minutes at room temperature. The pH was determined using a pH meter (World Health Organization, 1998).

2.6.2. Foreign matter analysis: About 10 grams of leaf powder was taken and the sample was spread on white paper or a clean surface. The sample was visually inspected and any foreign matter such as other plant parts, soil particles, sand, insect parts, or other contaminants, was carefully separated with the help of forceps. The separated material was then weighed, and the percentage of foreign

organic matter was calculated using the World Health Organization (1998) formula;

Foreign organic matter (%w/w) = (Weight of the foreign matter/Weight of the sample taken) × 100

2.6.3. Alcohol soluble extractive value: Five grams of leaf powder were weighed and transferred to a conical flask and 100 mL of alcohol (95%) was added. The mixture was mixed thoroughly and allowed to stand for 24 hrs. The solution was then filtered and evaporated to dryness. The percentage of alcohol soluble extractive was calculated using the standard equation (Evans, 2008; Ghosh and Rahaman, 2016);

Extractive value (%) = (Weight of the extract after evaporation/Weight of the powder taken) × 100

2.6.4. Water soluble extractive value: Five grams of leaf powder was weighed and transferred to a conical flask and 100 mL of water was added. The mixture was mixed thoroughly and allowed to stand for 24 hrs. The solution was then filtered and evaporated to dryness. The percentage of water-soluble extractive was calculated using the standard equation (Evans, 2008; Ghosh and Rahaman, 2016).

2.6.5. Determination of ash value: The total ash, acid-insoluble ash and water-soluble ash values of leaf powder were evaluated according to standard procedures (Indian Pharmacopoeia, 1996).

2.6.6. Determination of loss on drying at 105°C: A measured quantity of leaf powder was transferred to a pre-weighed 50 mL beaker. Then it was subjected to drying in a hot air oven at 105°C for five hours. The sample was then cooled and weighed. The process was repeated until it reached a constant weight (Indian Pharmacopoeia, 1996). The percentage of loss on drying at 105°C was calculated.

Percentage of loss on drying at 105°C = (Loss in weight of the sample/Weight of the powder taken) × 100

2.6.7. Determination of swelling index and foaming index: The swelling index and foaming index were calculated using standard procedures (Indian Pharmacopoeia Commission, 2007).

2.7. Histochemical localization

The collected leaf samples of *J. beddomei* were sectioned and then stained with safranin. The sections were then observed under the microscope to identify various tissues and used as a control. The histochemical localization of the leaf petiole was done to detect the presence of various plant metabolites such as alkaloids (Furr and Mahlberg, 1981) proteins (Ramasheshan *et al.*, 2017), carbohydrates (Sass, 1951), resins (David and Carde, 1964), tannins (Mace and Howell, 1974) and terpenoids (de Alcantara *et al.*, 2016) using the standard protocols.

3. Results and discussion

3.1. Morphological characterization

The plant is a glabrous, perennial shrub attaining 1-3 m in

height with an erect and non-dense branching habit. The stem is cylindrical, smooth and ivory to pale green in colour, supported by a taproot system. Leaves are simple, opposite, decussate and petiolate with an elliptic-lanceolate lamina measuring 12-14 cm in length and 3-5 cm in breadth. The apex is acute, the base attenuate and the margin entire. The adaxial surface is dark green and smooth, whereas the abaxial surface is comparatively lighter. A prominent midrib is observed on the abaxial surface and venation is pinnate with 8-9 pairs of lateral veins that are not prominently raised. The combination of smaller stature, dark green smooth leaves, fewer lateral veins, erect leaf orientation and non-dense shrub habit serves as key diagnostic macroscopic characters distinguishing it from *J. adhatoda*, which exhibits greater height, rough pale-green leaves and 11-13 prominently raised lateral veins. The habit of *J. beddomei* (Fig. 1) and herbarium specimens deposited at the Department of Botany, University of Kerala (Fig. 2).



Fig. 1. Habit of *Justicia beddomei* (C.B. Clarke) Bennet



Fig. 2. Herbarium specimen at KUBH (No: 11520)

The comparative morphological study revealed clear diagnostic features distinguishing *J. beddomei* from *J. adhatoda*. *J. beddomei* is smaller in height, with dark green smooth-surfaced leaves and fewer, less prominent lateral veins, whereas *J. adhatoda* is taller, with pale green rough-surfaced leaves and prominently veined foliage. These contrasting traits, particularly in leaf texture, venation and midrib prominence, provide reliable markers for species identification. The identifying characters of both *J. beddomei* and *J. adhatoda* are depicted in Table 1.

3.2. Anatomy

The leaf anatomy of *J. beddomei* shows a typical dorsiventral organization with a well-defined midrib and bilaterally differentiated lamina. The epidermis is single-layered and covered by a distinct cuticle, which is comparatively thicker on the adaxial surface. Epidermal cells in the midrib region are small and compact, while the underlying ground tissue consists of large, thin-walled parenchymatous cells. The mesophyll is differentiated into palisade and spongy parenchyma. The midrib contains a prominent collateral vascular bundle composed of radially arranged, compact xylem elements and a continuous phloem strand. The vascular bundle is enclosed by a thin sclerenchymatous bundle sheath. Additionally, two small circular accessory vascular bundles are present in the lower region of the adaxial ridge. Glandular trichomes are present on the abaxial epidermal layer, with characteristic multicellular, funnel-shaped bases and darkly stained cells (Nandhini and Ilango, 2020), as shown in Plate 1. The transverse section (T.S) of the leaf petiole is also depicted in Plate 1.

The absence of stomata on the adaxial epidermis and the presence of numerous diacytic stomata on the abaxial surface observed in the present study are consistent with previous reports on *J. adhatoda* (Nandhini and Ilango, 2020). The diacytic stomatal type is a characteristic anatomical feature of members of the family Acanthaceae and serves as an important diagnostic character for species

identification. The quantitative anatomical parameters were expressed as mean $\pm$ standard deviation (SD) ($n = 3$). The guard cells measured $21.90 \pm 1.41 \mu\text{m}$ in length and $3.97 \pm 0.23 \mu\text{m}$ in breadth. The subsidiary cells measured $30.48 \pm 0.20 \mu\text{m}$ in length and $26.63 \pm 0.75 \mu\text{m}$ in breadth. The epidermal cells were $56.43 \pm 0.80 \mu\text{m}$ in length and $19.11 \pm 0.82 \mu\text{m}$ in breadth. The stomatal pore measured $11.76 \pm 0.47 \mu\text{m}$ in length and $2.18 \pm 0.40 \mu\text{m}$ in breadth. The entire stomatal complex measured $40.90 \pm 0.90 \mu\text{m}$ in length and $37.24 \pm 1.20 \mu\text{m}$ in breadth. The average stomatal number was 39.10 ± 0.10 per mm^2 . The diacytic stomata of *J. beddomei* is shown in Plate 2.

3.3. Powder microscopy

Powder microscopy revealed the presence of spiral-thickened xylem tracheids, round to oval xylem vessels with distinct spiral thickening, elongated thin-walled phloem elements with visible sieve plate remnants, simple round starch grains predominantly located in parenchymatous cells and multicellular non-glandular trichomes. The xylem tracheids exhibited prominent spiral wall thickening, which serves as a characteristic diagnostic feature. The vessels occurred singly or in small groups along with tracheids. Starch grains were simple and mostly spherical. Xylem vessels were $25.00 \pm 0.01 \mu\text{m}$ in diameter, while starch grains ranged from $6.10 \pm 0.10 \mu\text{m}$ in diameter. The trichomes were short, prominent, multicellular and measured $130.00 \pm 0.01 \mu\text{m}$ in length and $10.00 \pm 0.02 \mu\text{m}$ in width ($n = 3$). The presence of spiral thickened tracheids along with characteristic multicellular trichomes constitutes important pharmacognostic diagnostic markers for species identification (Plate 3).

3.4. Organoleptic observations

Organoleptic observations play a significant role in pharmacognostic studies, as they provide valuable information about the physical and sensory properties of a plant drug. Organoleptic analysis of the powdered sample is one of the suitable methods for identification, quality control, standardization and authentication (Sreelekshmi et al.,

Table 1. Comparative features of *J. adhatoda* and *J. beddomei*

Characters*	<i>J. beddomei</i>	<i>J. adhatoda</i>
Height of the plant	1.5-3 m	3-6 m
Leaf colour	Dark green	Pale green
Odour	Characteristic odour	Characteristic odour
Taste	Bitter	Slightly bitter
Leaf texture	Smooth surface	Rough surface
Powder colour	Willow green	Dark green
Leaf nature	Straight or erect	Wavy
Habit	Shrub, not dense	Dense shrub
Leaf size	Small, 10-12 cm length, 3-4 cm width	Big, 12-16 cm length, 3-6 cm width
Midrib	Prominent midrib at the lower surface	Prominent midrib at the lower surface
Leaf shape	Elliptic lanceolate	Ovate lanceolate
Lateral veins	8-9 pairs in number, not prominent	11-13 in number, prominent
*Data taken as triplicates and represented as mean $\pm$ standard error (SE) ($n = 3$)		

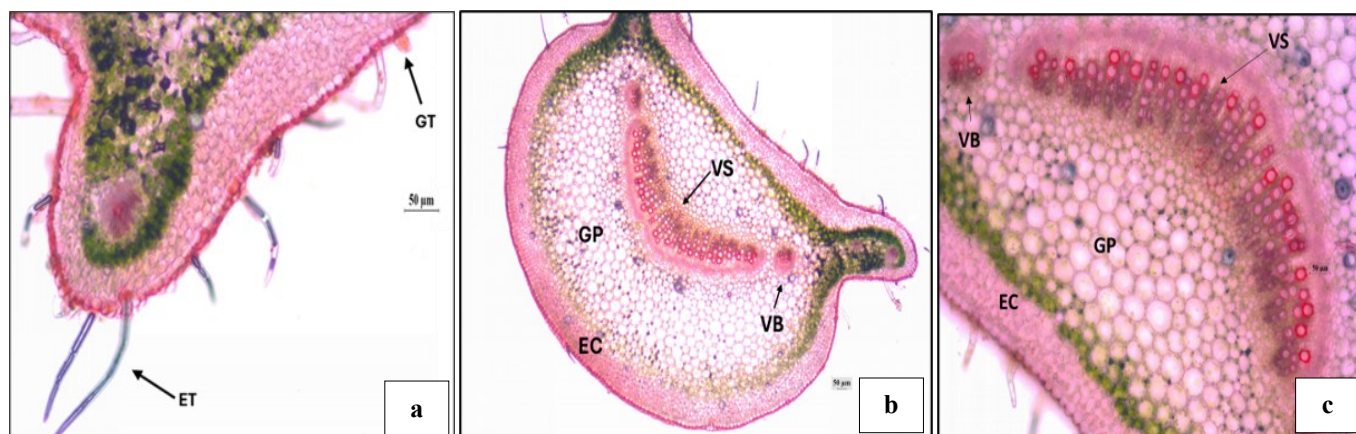


Plate 1. (a). Transverse section of leaf with Glandular trichomes (GT) and non-glandular Epidermal trichomes (ET); (b). Transverse section (T.S) of petiole of *J. beddomei* showing VS- vascular strand, GP-ground parenchyma, EC-epidermal cells and VB- vascular bundles; (c). Enlarged view of the T.S.

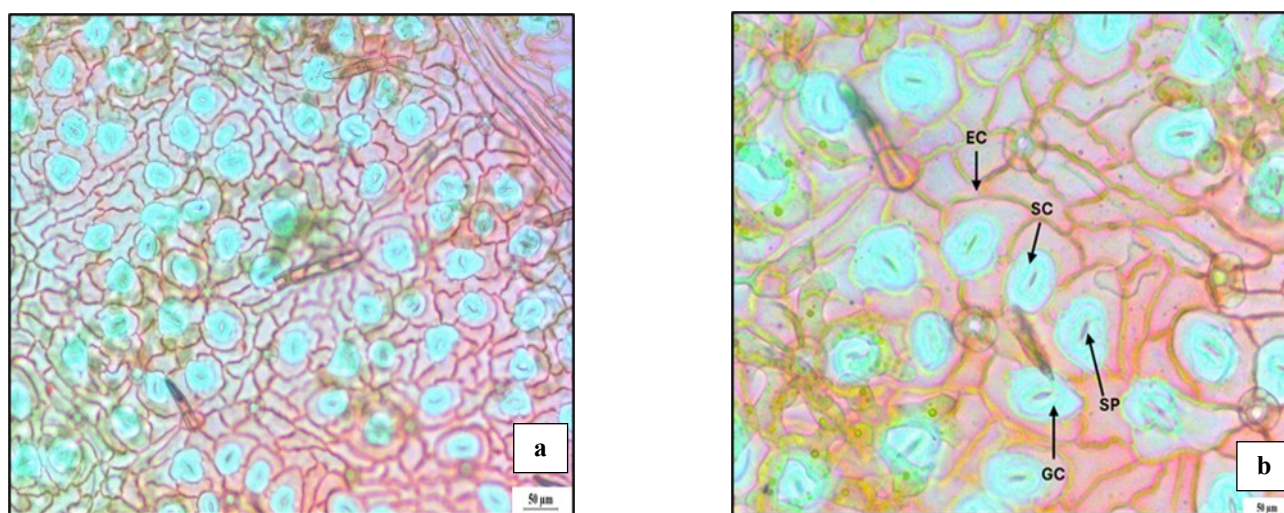


Plate 2. (a). Diacytic stomata of *J. beddomei*; (b). Enlarged view showing EC- epidermal cells, SC-subsidiary cells, SP-stomatal pore; and GC-guard cells

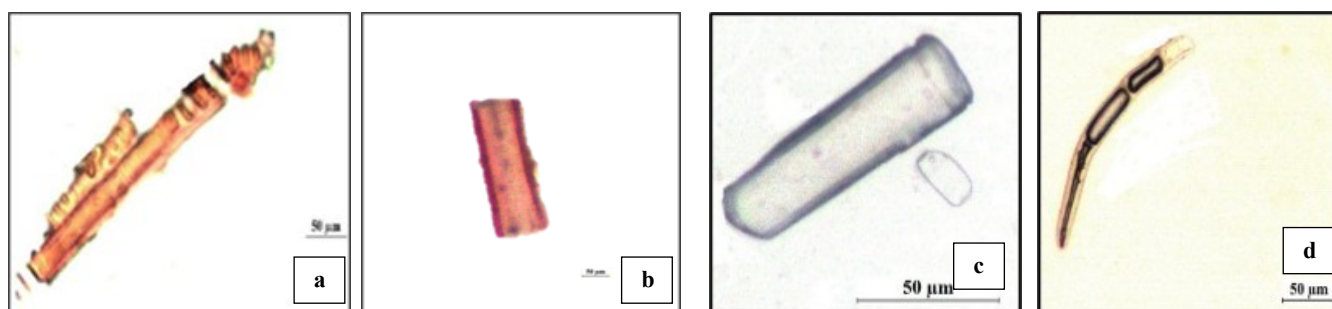


Plate 3. Powder microscopy of leaf showing diagnostic elements: (a). Spiral thickened xylem tracheids; (b). Round to oval xylem vessel; (c). Phloem cells; (d). Multicellular non glandular trichomes

2017). The leaves of *J. beddomei* were deep green with a bitter taste and smooth texture. The adaxial surface of the leaf is darker green when compared with the abaxial surface. Similarly, the leaf powder also has a characteristic smell with a bitter taste. It was coarse in texture and willow green in colour. Any kind of variation can be considered a sign of adulteration.

3.5. Fluorescence analysis

Fluorescence analysis is considered an important method, that provides valuable information about the phytochemicals and authenticity of the plant material. The fluorescence characteristics of any powder drug are unique, and these distinguishing characteristics will help in the determination of adulteration (Jahan, *et al.*, 2008). In the present study, the

colour variations under fluorescence analysis of leaf powder were recorded (Wilson, 1938). The results are given in Table 2. These data will be helpful for the determination of adulterants.

Fluorescence analysis of the powdered drug of *J. beddomei* revealed characteristic colour changes under short UV (254 nm), long UV (365 nm) and visible light. The untreated powder exhibited green shades under all conditions, indicating mild natural fluorescence. Treatment with nitric acid produced a distinct Naples yellow colour under both UV wavelengths, suggesting the presence of chromophoric compounds. Sulphuric acid, hydrochloric acid and distilled water treated samples retained greenish shades, indicating limited fluorescence enhancement. Organic solvent-treated samples such as methanol, ethanol, chloroform and acetone showed slight variations in green tones under UV light, reflecting the presence of fluorescent phytoconstituents. The samples exhibited mild fluorescence under UV light, which may be attributed to the presence of flavonoids, alkaloids and other phenolic compounds.

3.6. Physico-chemical parameters

Physico-chemical constants were evaluated to check the purity and quality of the drug. Foreign organic matter refers to the presence of extraneous organic material in a plant powder that is not part of the intended plant material. The leaf powder of *J. beddomei* did not contain any foreign organic matter in the present study. Hence, it is essential to monitor and control foreign organic matter in plant powder to ensure quality, safety and efficacy.

The pH of the plant powder was found to be 8.2, which is slightly alkaline in nature. The total ash of the plant powder refers to the residue left after incineration of the powder at high temperature. It may consist of inorganic minerals and salts present in the plant material. The acid-insoluble ash of the plant powder is the residue left after acid treatment. This process dissolves the acid-soluble minerals, leaving behind the acid-insoluble minerals and other inert materials. Similarly, water-soluble ash is the residue left after treating the plant powder with water. The total ash value was

13.05%, acid-insoluble ash was 0.57% and water-soluble ash was 4.40% (Table 3).

The extractive values give an idea about the chemical constituents that can be extracted from the powder. When compared with water-soluble extractive value, alcohol soluble extractive value was higher in *J. beddomei* leaf powder. This indicates that chemical compounds present in this plant may be more soluble in alcohol than in water. The moisture content of the drug can be evaluated by the loss on drying at 105°C. A high moisture content may promote bacterial growth and lead to deterioration of the drug. The leaf powder of *J. beddomei* had low moisture content (6.90%).

The swelling index serves as a quality control parameter to ensure consistency in the physical properties of the plant powder. The low swelling index value of *J. beddomei* leaf powder (4.6%) indicates that it may not disintegrate easily when it comes in contact with a solvent. The foaming index is a measure of the ability of the plant powder to produce foam when shaken with water. The higher foaming index value in *J. beddomei* leaf powder indicates that it contains saponins that can cause foam when an aqueous decoction is shaken.

Table 3. Physico-chemical parameters of leaf powder of *J. beddomei*

Sl. No.	Parameters	Result*
1	Foreign organic matter (%w/w)	Nil
2	Total ash value (%w/w)	13.05±0.21
3	Acid insoluble ash (%w/w)	0.57±0.01
4	Water soluble ash (%w/w)	4.40±0.26
5	Swelling index (%w/w)	4.6±0.09
6	Foaming index (%w/w)	111.20
7	Loss on drying at 105°C (%w/w)	6.90±0.32
8	Water soluble extractive value (%w/w)	20.17±0.26
9	Alcohol soluble extractive value (%w/w)	23.31±0.62
10	pH	8.2
*Data taken as triplicates and represented as mean ±standard error (SE) (n = 3)		

Table 2. Fluorescence analysis of leaf powder of *J. beddomei* according to Wilson's colour chart

Sl. No.	Reagents	Short UV (254nm)*	Long UV (365nm)*	Visible light*
1	Powder alone	Parsley Green, 00962	Parsley Green, 00962	Willow green, 000862
2	Powder + HNO ₃	Naples Yellow, 403	Naples Yellow, 403	Naples Yellow, 403
3	Powder + H ₂ SO ₄	Ivy green, 0001060	Ivy green, 0001060	Ivy green, 0001060
4	Powder + Acetic acid	Parsley Green, 00962	Parsley Green, 00962	Parsley Green, 00962
5	Powder + Ethanol	Parsley Green, 00962	Parsley Green, 00962	Scheeles green, 860
6	Powder + Methanol	Lettuce green, 861	Lettuce green, 861	Scheeles green, 860
7	Powder + Acetone	Sage green, 000861	Sage green, 000861	Pod green, 061
8	Powder + NaOH	Parsley Green, 00962	Parsley Green, 00962	Parsley Green, 00962
9	Powder + Chloroform	Spinach green, 0960	Spinach green, 0960	Pod green, 061
10	Powder + Benzene	Parsley Green, 00962	Parsley Green, 00962	Pod green, 061
11	Powder + Distilled water	Ivy green, 0001060	Ivy green, 0001060	Ivy green, 0001060
12	Powder + HCl	Ivy green, 0001060	Ivy green, 0001060	Parsley Green, 00962
*Colour variations were noted according to Wilson's colour chart				

The physicochemical parameters of *J. beddomei* were evaluated and compared with the standards prescribed for the closely related species *J. adhatoda* in the Ayurvedic Pharmacopoeia of India. The total ash value (13.05%) was well within the pharmacopoeia limit of not more than 21%, indicating acceptable levels of total inorganic content and minimal contamination with extraneous matter. Similarly, the acid-insoluble ash value (0.57%) was below the prescribed limit of 1%, suggesting low silica and earthy matter contamination, thereby confirming the purity of the crude drug. Overall, the values suggest that the studied sample possesses acceptable purity.

3.7. Histochemical localization

Medicinal plants contain many secondary metabolites (Dhar *et al.*, 1968). These include different groups of phytochemicals, such as phenols, alkaloids, flavonoids, glycosides and terpenoids, which are therapeutically important for treating various diseases. The histochemical localization of *J. beddomei* leaf petiole was carried out to detect various phytochemicals present in it. The results indicate that *J. beddomei* leaves contain metabolites such as proteins, carbohydrates, resins, tannins, terpenoids and alkaloids (Plate 4).

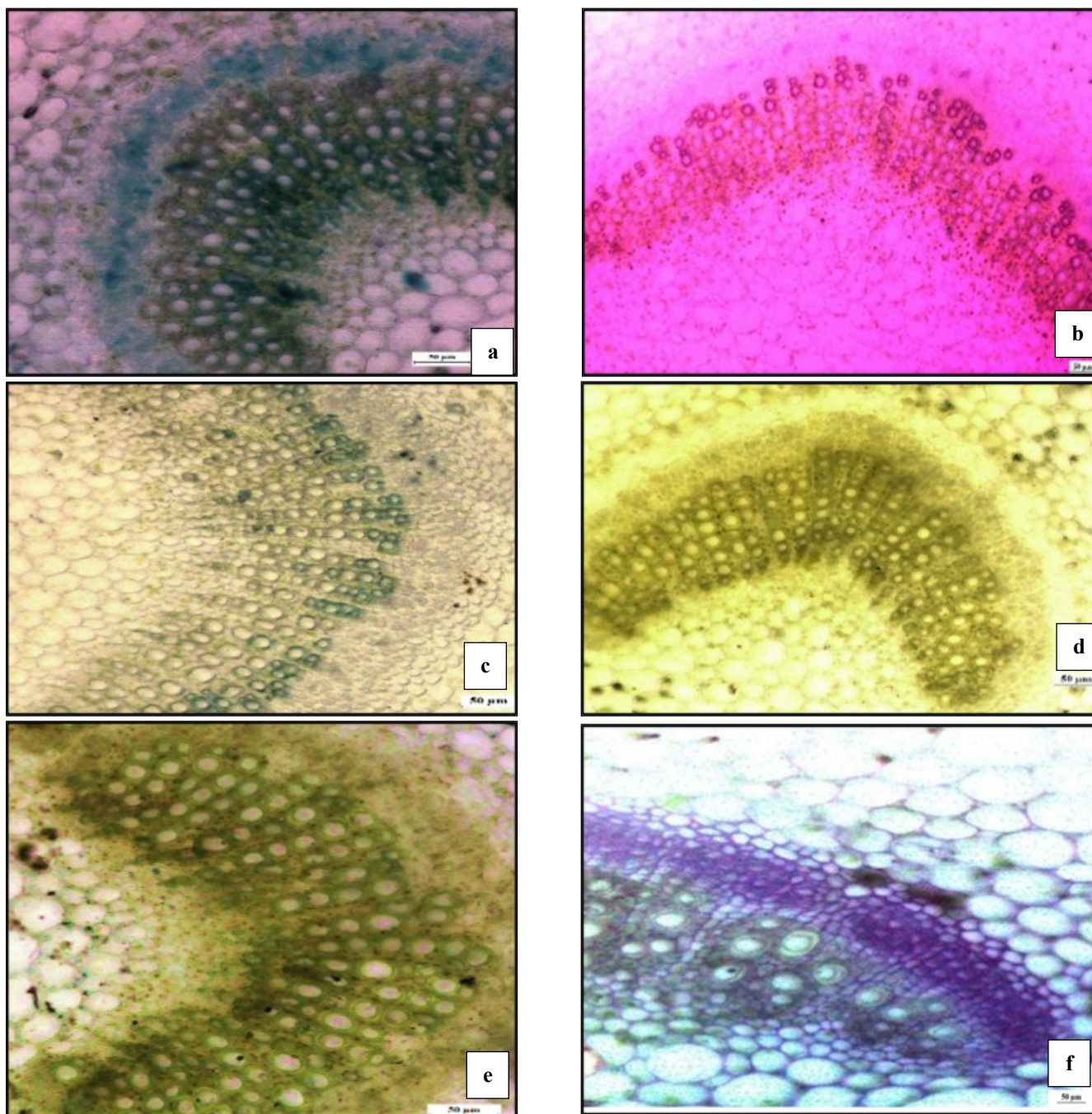


Plate 4. (a). Histochemical localization of protein; (b). Carbohydrates; (c). Resins; (d). Tannins; (e). Terpenoids; (f). Alkaloids

The presence of protein was detected by the light blue coloration in the vascular bundle region of the petiole. In the case of carbohydrates, the vascular tissues appeared dark pink in colour. The presence of resins was detected by the blue colour of the vascular tissues. The golden yellow colouration in vascular tissues indicates the presence of tannins and terpenoids. The purple colour in vascular tissues indicates the presence of alkaloids. The histochemical study of *J. beddomei* leaf petiole revealed the presence of key phytochemicals such as proteins, carbohydrates, resins, tannins, terpenoids and alkaloids, localized mainly in the vascular tissues. These findings highlight the tissue-specific distribution of bioactive compounds, supporting the therapeutic potential of *J. beddomei* and its relevance in developing plant-based medicinal formulations.

The present study establishes reliable diagnostic parameters for the identification and standardization of *J. beddomei* and provides a comparative perspective with *J. adhatoda*. Morphologically, *J. beddomei* differs from *J. adhatoda* in its comparatively smaller stature, smoother dark green leaves and less conspicuous venation, which may serve as distinguishing taxonomic markers. While both species share general anatomical features typical of Acanthaceae, subtle morphological distinctions observed in the present study aid in resolving possible misidentification.

The organoleptic characteristics recorded for *J. beddomei*, particularly the bitter taste and green coloration, are consistent with reports on related species, suggesting similarity in phytochemical composition. However, slight variations in texture and colour intensity may reflect species-specific chemical profiles or ecological influences. Fluorescence behaviour under UV light exhibited moderate characteristic responses, indicating the presence of secondary metabolites such as alkaloids and flavonoids. These fluorescence traits provide an additional authentication parameter and may help detect adulteration or substitution. The physico-chemical parameters obtained in this study fall within acceptable purity limits when compared with pharmacopoeial standards prescribed for related species, suggesting minimal inorganic contamination. Histochemical localization of bioactive constituents within vascular tissues further supports the medicinal relevance of *J. beddomei* and aligns with the distribution pattern reported for pharmacologically active compounds in related species. Collectively, these findings contribute novel standardization data for *J. beddomei*, for which detailed pharmacognostic evidence remains limited.

4. Conclusion

The present study establishes reliable pharmacognostic standards for the identification and authentication of *J. beddomei*. Diagnostic macroscopic features include its smaller stature, dark green smooth leaves, erect orientation and 8-9 pairs of less prominent lateral veins. Microscopically, the presence of diacytic stomata restricted to the abaxial epidermis, a prominent collateral vascular bundle with a sclerenchymatous sheath and accessory

vascular bundles serves as key anatomical markers. Powder microscopy revealed characteristic spiral-thickened xylem tracheids and multicellular non-glandular trichomes. The fluorescence profile under UV light and the physicochemical parameters provides reproducible quality control standards. Histochemical localization of major phytoconstituents in vascular tissues further supports its diagnostic distinctiveness. These combined parameters provide definitive authentication markers for *J. beddomei*.

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Declarations

Conflict of Interest Statement: The authors declare no competing financial, institutional, or personal conflicts of interest.

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Data Availability Statement (DAS): The empirical datasets supporting the conclusions of this article are available from the corresponding author upon reasonable academic request.

Author Contributions (CRediT Taxonomy): Shilpa K. S. (Author 1): Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Validation, Visualization, Writing – original draft. Rajalakshmi R. (Author 2): Supervision, Methodology, Resources, Project administration, Writing – review & editing. Manisha S. J. Kurup (Author 2): Writing – review & editing.

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References

- Ansari, S. H. 2006. *Essentials of Pharmacognosy*. New Delhi: Birla Publications Pvt. Ltd., pp. 367–369.
- David, R. and J. P. Carde. 1964. Coloration différentielle des inclusions lipidiques et terpéniques des pseudophylles du pin maritime au moyen du réactif NADI. *Comptes Rendus de l'Académie des Sciences, Paris*. 258: 1338–1340.
- de Alcantara Guimarães, A. L., R. P. C. Costa, L. M. Cabral and A. C. de Macêdo Vieira. 2016. Comparative anatomy and chemical analysis of the vegetative organs of three species of *Stigmaphyllon* (Malpighiaceae). *Flora*. 224: 30–41. <https://doi.org/10.1016/j.flora.2016.06.007>.
- Dhar, M. L., M. Dhar, B. N. Dhawan, B. N. Mehrotra and C. Ray.

1968. Screening of Indian plants for biological activity. *Indian Journal of Experimental Biology*. 6(4): 232–247.
- Evans, W. C. 2008. *Pharmacognosy*. 15th ed. Singapore: Saunders/Elsevier.
- Furr, M. and P. G. Mahlberg. 1981. Histochemical analyses of laticifers and glandular trichomes in *Cannabis sativa*. *Journal of Natural Products*. 44(2): 153–159. <https://doi.org/10.1021/np50014a002>.
- Ghosh, P. and C. H. Rahaman. 2016. Pharmacognostic studies and phytochemical screening of aerial and root parts of *Cyanotis tuberosa* (Roxb.) Schult. & Schult.f., an ethnomedicinal herb. *World Journal of Pharmaceutical Research*. 5(2): 1580–1601.
- Haleshi, C., A. N. Sringswara and V. Dinajpur. 2020. Pharmacognostic study of *Justicia beddomei* (C.B. Clarke) Bennet. *European Journal of Medicinal Plants*. 31(9): 83–88. <https://doi.org/10.9734/EJMP/2020/v31i930269>.
- Indian Pharmacopoeia Commission. 2007. *Indian Pharmacopoeia*. Vol. 2. New Delhi: Government of India, The Controller of Publications.
- Indian Pharmacopoeia. 1996. 4th ed. New Delhi: Controller of Publications, Government of India, Ministry of Health and Family Welfare, pp. A53–A54.
- Jahan, N., S. H. Afaque, N. A. Khan, G. Ahmad and A. Ansari. 2008. Physico-chemical studies of gum *Acacia*. *Natural Product Radiance*. 7 (4): 335–337.
- Jiang, T., L. Zhang, M. Ding and M. Li. 2019. Protective effect of vasicine against myocardial infarction in rats via modulation of oxidative stress, inflammation and the PI3K/Akt pathway. *Drug Design, Development and Therapy*. 13: 3773–3784. <https://doi.org/10.2147/DDDT.S223147>.
- Joy, P. P., J. Thomas, S. Mathew, G. Jose and J. Joseph. 2001. Aromatic plants. *Tropical Horticulture*. 2: 633–733.
- Karhikeyan, S., M. Sanjappa and S. Moorthy. 2009. *Flowering Plants of India: Dicotyledons*. Vol. 1. Kolkata: Botanical Survey of India.
- Mace, M. E. and C. R. Howell. 1974. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. *Canadian Journal of Botany*. 52(11): 2423–2426. <https://doi.org/10.1139/b74-314>.
- Marathakam, A., N. Kannappan, S. Jasemine, A. Santhiagu, M. Sreejith and M. P. Ajith. 2012. Studies on phytochemical and in vitro antioxidant potential of *Justicia beddomei* (Clarke) Bennett. *Free Radicals and Antioxidants*. 2(4): 26–31. <https://doi.org/10.5530/ax.2012.4.6>.
- Mathew, A. S., K. N. Patel and B. K. Shah. 1998. Investigation on antifeedant and anthelmintic potential of *Adhatoda vasica* Nees. *Indian Journal of Natural Products*. 14(1): 11–16.
- Nandhini, S. and K. Ilango. 2020. Comparative study on pharmacognostical, phytochemical investigations and quantification of vasicine content in the extracts of *Adhatoda vasica* Nees and *Adhatoda beddomei* C.B. Clarke. *Pharmacognosy Journal*. 12(4): 884–896. <https://doi.org/10.5530/pj.2020.12.126>.
- Patel, V. K. and H. Venkatakrishna-Bhatt. 1984. In vitro study of antimicrobial activity of *Adhatoda vasica* Linn. (leaf extract) on gingival inflammation: A preliminary report. *Indian Journal of Medical Sciences*. 38(4): 70–72.
- Prabhukumar, K. M., B. Farsana, G. Satheesh and B. Indira. 2018. Revisiting the taxonomy of *Justicia beddomei* (Acanthaceae). *Phytotaxa*. 350(1): 71–78. <https://doi.org/10.11646/phytotaxa.350.1.7>.
- Ramashesan, Shantha T., R. Prathapa, P. Maramreddy, P. Pitchaiah, K. Kishore, R. Ramakrishna, V. Bharti, G. Venkateshwarlu, D. Tewari, A. K. Mangal, N. Srikanth and K. S. Dhiman. 2017. Pharmacognostical and histochemical studies on Apakva Kadali (unripe banana fruit): *Musa × paradisiaca* L. *Journal of Drug Research in Ayurvedic Sciences*. 2(1): 10–17. <https://doi.org/10.5005/jdras-10059-0013>.
- Sasidharan, N. 2013. *Flowering Plants of Kerala*. Version 2.0 (CD-ROM). Peechi, Kerala: Kerala Forest Research Institute.
- Sass, J. E. 1951. *Botanical Microtechnique*. Ames, Iowa: Iowa State College Press.
- Sharma, A. and A. Kumar. 2016. Acanthaceae: Taxonomy and uses in traditional medicinal system. *World Journal of Pharmaceutical Research*. 5(7): 403–412.
- Sreelekshmi, M., K. S. Vimala, P. Raiby, N. Chandran, P. A. Shine and N. A. Salam. 2017. Drug adulteration: A threat to efficacy of Ayurveda medicine. *Journal of Medicinal Plants Studies*. 5(4): 1–6.
- Srinivasan, M., B. Padmaja and S. Nair. 2014. GC-MS profiling and in vitro radical scavenging effect of *Adhatoda beddomei*. *Journal of Pharmacognosy and Phytochemistry*. 2(5): 55–59.
- Wilson, R. F. 1938. *Royal Horticultural Society Colour Chart*. 1st ed. London: British Colour Council.
- World Health Organization. 1998. *Quality Control Methods for Medicinal Plant Materials*. Geneva: World Health Organization. Available at: <https://apps.WorldHealthOrganization.int/iris/handle/10665/41986>.