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Physicochemical, pharmacognostic standardisation and phytochemical analyses of the homoeopathic drug Justicia adhatoda

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Abstract

Background: *Justicia adhatoda* (*J. adhatoda*), also well known as Vasaka in India is a medicinal plant chiefly used to treat respiratory ailments in traditional and alternative medical systems. Objective: This study aimed to establish the physicochemical, pharmacognostic, and phytochemical standardisation parameters of the drug. The study involved the measurement of physicochemical parameters and chemical profiling of the raw drug. The chemical characteristics of the in-house mother tincture (prepared from in-house raw drug) and commercial mother tinctures available in India were also evaluated to assess the quality of commercially available mother tinctures. Materials and Methods: The present study included pharmacognostic parameters like macroscopic, microscopic, powder studies and physicochemical parameters involving foreign matter, loss on drying, extractive values in different solvents, ash value measurements, total alkaloid content, total fixed oil and total volatile oil of the raw drug. This study also included measurement of various mother tincture parameters, such as organoleptic characteristics, specific gravity, pH, total solids, UV–Vis spectrum, high performance thin-layer chromatography (HPTLC), phytochemical screening, total alkaloid content, and chemical profiling of commercial and in-house mother tinctures to assess their comparative quality. Results: The macroscopic and microscopic studies revealed the pharmacognostic characteristics of the leaves. Low ash values indicated low metal contents. The chemical profiling and analysis indicated the presence of the same alkaloid in the raw drug and in the mother tinctures. The drug also contained several phytochemicals. Conclusion: The present study provides pharmacopoeial standards for *J. adhatoda*.

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ORIGINAL ARTICLE

Physicochemical, pharmacognostic standardisation and phytochemical analyses of the homoeopathic drug *Justicia adhatoda*

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ABSTRACT

Background: *Justicia adhatoda* (*J. adhatoda*), also well known as Vasaka in India is a medicinal plant chiefly used to treat respiratory ailments in traditional and alternative medical systems. **Objective:** This study aimed to establish the physicochemical, pharmacognostic, and phytochemical standardisation parameters of the drug. The study involved the measurement of physicochemical parameters and chemical profiling of the raw drug. The chemical characteristics of the in-house mother tincture (prepared from in-house raw drug) and commercial mother tinctures available in India were also evaluated to assess the quality of commercially available mother tinctures. **Materials and Methods:** The present study included pharmacognostic parameters like macroscopic, microscopic, powder studies and physicochemical parameters involving foreign matter, loss on drying, extractive values in different solvents, ash value measurements, total alkaloid content, total fixed oil and total volatile oil of the raw drug. This study also included measurement of various mother tincture parameters, such as organoleptic characteristics, specific gravity, pH, total solids, UV–Vis spectrum, high performance thin-layer chromatography (HPTLC), phytochemical screening, total alkaloid content, and chemical profiling of commercial and in-house mother tinctures to assess their comparative quality. **Results:** The macroscopic and microscopic studies revealed the pharmacognostic characteristics of the leaves. Low ash values indicated low metal contents. The chemical profiling and analysis indicated the presence of the same alkaloid in the raw drug and in the mother tinctures. The drug also contained several phytochemicals. **Conclusion:** The present study provides pharmacopoeial standards for *J. adhatoda*.

Keywords: Justicia adhatoda, Homoeopathy, Mother tincture, Drug standardization

Introduction

Justicia adhatoda (L.) Nees (synonym *Adahatoda vasica* Nees), belonging to the family Acanthaceae, is a diffuse perennial shrub distributed widely in tropical areas of Southeast Asia.[1] It is commonly known as vasaka and malabar nut in India and grows all over India up to an altitude of 1,300 m.[2] The plant is evergreen and highly branched. It can go up to one to two and a half meters in height. The stem is quadrangular to terete in shape, erect, opposite ascending branching, cylindrical, solid, having inflated nodes, and green to pale-green. The leaves are 10–15 cm in size, precisely hairy, elliptic-lanceolate in shape, and acute apex with entire margins. Ascending branches have white, pink or purple flowers. Flowers

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are marked with pink, long peduncle having axillary spikes and with large, leafy, ovate, glabrous bracts. Calyx are 1.5 cm long and glabrous. Corolla is cylindrical in shape and white with red to purple buds. The fruit is capsule, clavate, longitudinally channeled, 4-sided, small-haired and with orbicular, rugose seeds.

This medicinal plant is widely used in traditional and alternative medical systems, including Homoeopathy.[3,4] This medicinal plant is used to treat different respiratory-related ailments in Homoeopathy, chiefly related to cough and coryza.[5] The therapeutic importance of this medicinal plant has been further recognised by the modern medical system. In modern biomedical research, the chemical constituents of *J. adhatoda* have been studied for their biological activities. *Vasicine*, a quinazoline alkaloid, is considered one of the major alkaloids present in the plant and shows similar bioactivities to *J. adhatoda* (Fig. 1).[6] *Vasicine* is reported to be a bronchodilator.[7] Interestingly, it shows contraction of the tracheal muscle at high concentrations and relaxation at low concentrations. Further, it protects the tracheal muscle from histamine-induced bronchospasm. In vitro and in vivo studies showed bronchodilatory activity of *Vasicine* comparable to theophylline. Similar to *vasicine*, *vasicinone* related but minor alkaloid in *J adhatoda* also shows similar bronchodilatory activity. However, a 1:1 mixture of these two compounds shows much greater activity than that of the individual compound indicating the importance of synergistic effect.[8] Besides their effects on respiratory tract, *vasicine* was also studied for its anti-oxidant and anti-inflammatory activities.[9] *Vasicine* was found to decrease lipid peroxidation and to increase the level of several anti-oxidant enzyme, superoxide dismutase and catalase.[10,11] Its activity towards glutathione peroxidase was manifested by a decrease in glutathione. *Vasicine* was also found to have uterotonic activity.[10] Considering these immense bioactivities of the chemical constituents, several natural product analogues have also been synthesised. Those synthetic alkaloids also show similar but sometimes enhanced activities. Besides synthetic analogues, semi-synthetic derivative of *vasicinone*, *Bromhexine* is an approved medicine for treatment of respiratory ailments.[12]

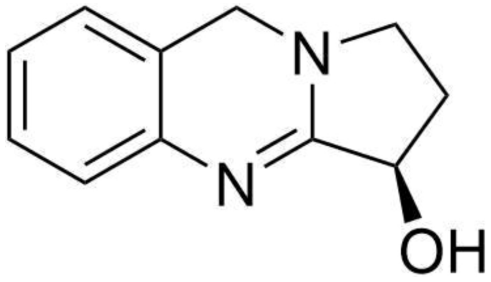

Fig. 1. 2D structure of *Vasicine*.

Despite extensive research in other medical systems, the Homoeopathic Pharmacopoeia of India (HPI) does not yet provide physicochemical profiles of the raw drug or its mother tincture.[13] Therefore, a standardisation study consisted of both physicochemical and pharmacognostic study would be helpful to improve or update existing pharmacopoeial standards as per latest Pharmacopoeia Commission for Indian Medicine and Homoeopathy (PCIM&H) guidelines. Here, pharmacognostic and physicochemical standardisation of the homoeopathic drug *Justicia adhatoda* were reported. Besides regular standardisation study phytochemical screening of the drug in different solvents and the mother tincture to gauge the plant's phytochemical constituents were also carried out. Furthermore, a comparative study evaluated quality and compositional variations between commercial and in-house mother tinctures.

Material and methods

Material

***Plant collection*:** The plant specimen of *Justicia adhatoda* L. (family: Acanthaceae), was collected from the Centre of Medicinal Plants Research in Homoeopathy (CMPRH) Herbal Garden, Emerald, in the Nilgiri district of Tamil Nadu, India during the south-west monsoon season on 16.09.2020. The collection was recorded under Field Book No. 059 and Field/Voucher No. 9646. The site falls under a heavy rainfall agroclimatic condition with black soil type. The plant, a shrub by habit, was found to be cultivated and commonly available in the region. Leaves were collected for the study. The GPS coordinates of the collection site are Latitude 11.31344° and Longitude 76.629424°. The collection was carried out and authenticated by JK, Scientist, Survey Officer/Scientist-2. The leaves were shade-dried for 24 hours. The mother tincture was prepared as per HPI.[13] Dried leaves were ground, and the coarse powder (10/44 mesh) was used for phytochemical and physicochemical studies.[14]

***Chemicals*:** All reagents used in the study were of analytical grade. Chemicals were used only after a thorough assessment of their Safety Data Sheets (SDS).

Methods

The reported study comprehensively evaluated both the raw drug and its corresponding mother tincture through pharmacognostic, physicochemical,

and chemical profiling. The pharmacognostic part consisted of macroscopic, microscopic, and powder studies, while the physicochemical analysis included determination of the raw drug's foreign matter, loss on drying, extractive values in water, alcohol, and hexane, ash values, total alkaloid content, total fixed oil, and total volatile oil content. Additionally, the in-house and three commercial mother tinctures were examined for organoleptic characteristics, specific gravity, pH, total solids, UV–Vis spectral profile, high-performance thin-layer chromatography (HPTLC) fingerprinting, phytochemical screening, and total alkaloid content. A comparative assessment of commercial and in-house mother tinctures was performed to determine variations in quality and composition across different sources.

Macroscopy studies: The leaves and leaf powders were independently examined for organoleptic and morphological characters like venation, color, shape, taste, size, odor, texture, touch, and so on as per the prescribed methods.[15,16]

***Microscopy studies*:** Qualitative and quantitative microscopic examination was performed using binocular microscopes (Model: CH-20i and CX-21i, Manufacturer: Olympus,). In qualitative evaluation, leaves were boiled separately, cut into tiny pieces and proceeded for the paraffin method of microtomy as described by *Johansen*.[17] The section of leaf was incised through midrib with a small portion of lamina and thin section was double stained with hematoxylin and safranin. In leaf quantitative evaluation, different leaf constants like stomatal number, stomatal index, vein islet number and palisade ration of leaf were studied as per prescribed procedure and formula.[17]

***Powder studies*:** The powdered samples were examined by boiling them in distilled water and mounting in various reagents; cellular structures and diagnostic cell inclusions were then observed.

***Organoleptic characters*:** Organoleptic characters of the leaf powder (raw drug) were examined by spreading a small quantity of the powder on a white background and visually inspecting it for general appearance, including nature, colour, odour, taste, and texture.

Experimental procedures for physicochemical study and chemical profiling

1. ***Foreign matter*:** The foreign matter was measured following Ayurvedic Pharmacopoeia of India guidelines.[18] No mold or significant amount of foreign matter, including parts other than leaves, was detected.
2. **Loss on Drying (LOD):** The standard procedure for vegetable products, as described in the HPI, was adopted for LOD measurement.[19] The weighing was done one an analytical weighing balance (Model: IG-204ES, Manufacturer: iGeneLabserve).

The experiment was done thrice, and the mean value and standard deviation were determined.

[LOD is reported as $\mu \pm \sigma$, (μ = mean, σ = standard deviation)]

3. ***Preparation of mother tincture(φ)*:** The mother tincture was prepared as per the procedure described in HPI.[13]
4. ***Extractive values*:**
   a. **Alcohol soluble extractive:** This method adopted the procedure as described in HPI.[20] A hot air oven (Model: BPI-22; Manufacturer: Ambassador) and an analytical balance (Model: IG-204ES, Manufacturer: iGeneLabserve) was used for this experiment.
   b. **Water Soluble extractive:** In closely related experiments, a water-soluble extract was evaluated using 50 mL of chloroform–water (1:40 v/v) instead of ethanol. The methodology followed the guidelines outlined in HPI.[21]
   c. **Hexane Soluble extractive:** The hexane-soluble extractive value was determined in the same manner as the alcohol-soluble extractive value, using 50 mL of hexane instead of alcohol.

The extractive value experiments were repeated thrice and reported as $\mu \pm \sigma$.

5. ***Ash values*:**
   a. **Determination of total ash value:** This has been done as per the reported procedure at Ayurvedic Pharmacopoeia of India.[22] The above experiment was performed six times, and the mean value and standard deviation were calculated. The total ash value is reported as $\mu \pm \sigma$. Out of six samples of total ash three samples were used to measure acid insoluble ash and rest three for water soluble ash.
   b. **Determination of acid-insoluble ash value:** Methodology described in the Ayurvedic Pharmacopoeia of India for acid insoluble ash determination was adopted.[23]
   c. **Determination of water-soluble ash value:** Method described in the Ayurvedic Pharmacopoeia of India for water soluble ash determination was followed.[24]

Overall, for ash values determination, a Muffle furnace (Model: BPI-18 Manufacturer: Ambassador) and an analytical weighing balance (Model: IG-204ES, Manufacturer: iGeneLabserve). were used for these experiments. The acid insoluble ash and water soluble

ash were reported as $\mu \pm \sigma$ based on three experiments for each parameter.

6. ***Determination of total solids*:** The total solid was measured according to the protocol delineated in the HPI.[25] The hot air oven, water bath (Model: BPI-22; Manufacturer: Ambassador), and weighing balance were used for this experiment. The above experiment was performed thrice, and the average value and standard deviation were reported. The total solid is reported as $\mu \pm \sigma$.
7. ***Weight per mL:*** This parameter was measured following a previously reported method.[26]
8. ***pH*:** The pH was determined as previously reported method using a digital pH meter, Model: 707 pH Meter, Manufacturer: Design Electronics Services).[27]
9. λ_{max}**:** The mother tincture solvent system was used as the blank. The λ_{max} was measured by diluting the mother tincture ~100 times using the same solvent system by a UV-Vis Spectrophotometer (Model: Lambda 25 Manufacturer: Perkin Elmer).[27]
10. ***Estimation of total alkaloid*:** This procedure was adopted from the procedure described in Ayurvedic Pharmacopoeia of India.[28] The alkaloid content was determined in triplicate, and $\mu \pm \sigma$ was reported.

Another experiment was done where the procedure was scaled down for 1g of the drug. In that case the dried final basic extract obtained after removal of the solvent on a water bath. Then the material was diluted with 5 mL of chloroform. The chloroform solution was then analysed by TLC using 5% methanol in chloroform. The plates were stained with universal staining agents iodine vapour and phosphomolybdic acid stains, wherein only one spot was detected having the same R_f value as that with the Dragendorff stain.

11. ***Fixed oil determination*:** About 5 g of the ground raw drug was extracted with 140 mL of petroleum ether (bp 40–60 °C) for 8 h. Thirty millilitres of the extract was evaporated on a water bath and dried at 105 °C to constant weight. The experiment was performed in triplicate, and the mean and standard deviation were calculated. The value was reported as $\mu \pm \sigma$.

The total fixed oil content was calculated as follows:

Drug − weight = W_{drug}
Empty Glassware weight = W_{empty}
Beaker + dried Extract weight = W_{+drug}
Wt. of the extract = $(W_{+drug} - W_{empty}) = W_{extract}$
% of fixed oil = $(W_{extract}/W_{drug}) \times 100$ %w/w

12. ***Volatile oil determination*:** The volatile oil content was first tested (% v/w) using a Clevenger apparatus, but no oil layer formed due to its low concentration. Therefore, gravimetric estimation (% w/w) was used. The powdered sample was mixed with water (1:8), extracted in a Clevenger for 6 h, and the organic layer was extracted with dichloromethane, dried over anhydrous calcium chloride, and evaporated at ~40 °C.

Drug − weight = W_{drug}
Empty Glassware weight = W_{empty}
Beaker + dried Extract weight = W_{+drug}
Wt. of the volatile oil = $(W_{+drug} - W_{empty}) = W_{vo}$
% of volatile oil = $(W_{vo})/W_{drug}\} \times 100$ %w/w

13. ***Thin Layer Chromatography (TLC)/Qualitative High Performance Thin Layer Chromatography (HPTLC)*:** About 20 mL of mother tincture was heated on a water bath (Model: BPI-22; Manufacturer: Ambassador) to remove alcohol, and the resultant semi-solid residue was extracted thrice with 20 mL portions of chloroform. The combined chloroform layer was dried over anhydrous sodium sulfate, filtered, and concentrated to around 5 mL. This chloroform extract was used for TLC/HPTLC on silica gel 60 F_{254} plates (0.25 mm, Merck) using chloroform: methanol (95:5 v/v) as the mobile phase. Spots were visualised under 254 nm and 365 nm UV light, with Dragendorff reagent for alkaloids and anisaldehyde–sulfuric acid for general detection under white light.
14. ***Phytochemical screening*:** Phytochemical screening was carried out as per the procedure reported in the referenced paper.[29]

Results

Pharmacognostic study

Macroscopy

Leaves were elliptic-lanceolate, acute apex with entire margin and having size 8–15×3–5 cm (Figs. 2 and 3A). Petiole was 2.5–3.5cm long and 1–1.5 mm wide and covered with white hairs. Upper surface of leaves was green, glabrous and slightly grooved on the upper surface whereas lower surface was light green in colour and have distinct veins. Leaves have unpleasant odor and slightly bitter taste.

Qualitative microscopic studies

Leaf

The transverse section of the leaf lamina showed a single-layered epidermis on both sides, with rectangular, compactly arranged epidermal cells. The epidermis was followed by the mesophyll, which was

Fig. 2. Habit of *Justicia adhatoda* L.

differentiated into two layers: compactly arranged palisade cells containing chloroplasts, and loosely arranged spongy parenchymatous cells with intercellular air spaces. Cystolith cells were also observed in spongy tissue (Fig. 3B).

Midrib: The transverse section of the midrib was convex on the abaxial side and slightly depressed on the adaxial surface. Single layered epidermis with slightly elongated thick-walled epidermal cells and were covered with cuticle. Epidermis followed by 6 to 8 layered irregular, thick walled collenchymatous cells. Uniseriate trichomes (2 to 3 celled rarely 5 celled) were present on both sides. It was observed that more trichomes were present on abaxial side as compare to adaxial surface. Ground tissues were composed of 10 to 12 layers, thin-walled parenchymatous cells. starch grains and intercellular space were also seen in ground tissue. Collateral vascular bundles were present in center. One large vascular bundle was arc shaped present on abaxial surface and two small vascular bundles were in adaxial surface (Fig. 3C,3D).

Petiole: The transverse section of the petiole showed a reniform shape. Single layered epidermis with slightly elongated, cuticular epidermal cells. Uniseriate trichome was present. Epidermis was followed by cortex. Outer cortex composed of 4 to 10 layered collenchyma cells with angular thickenings and inner cortex composed of thin-walled parenchymatous cells with intercellular spaces. Vascular bundles arrangement was similar to midrib (Fig. 3E,3F).

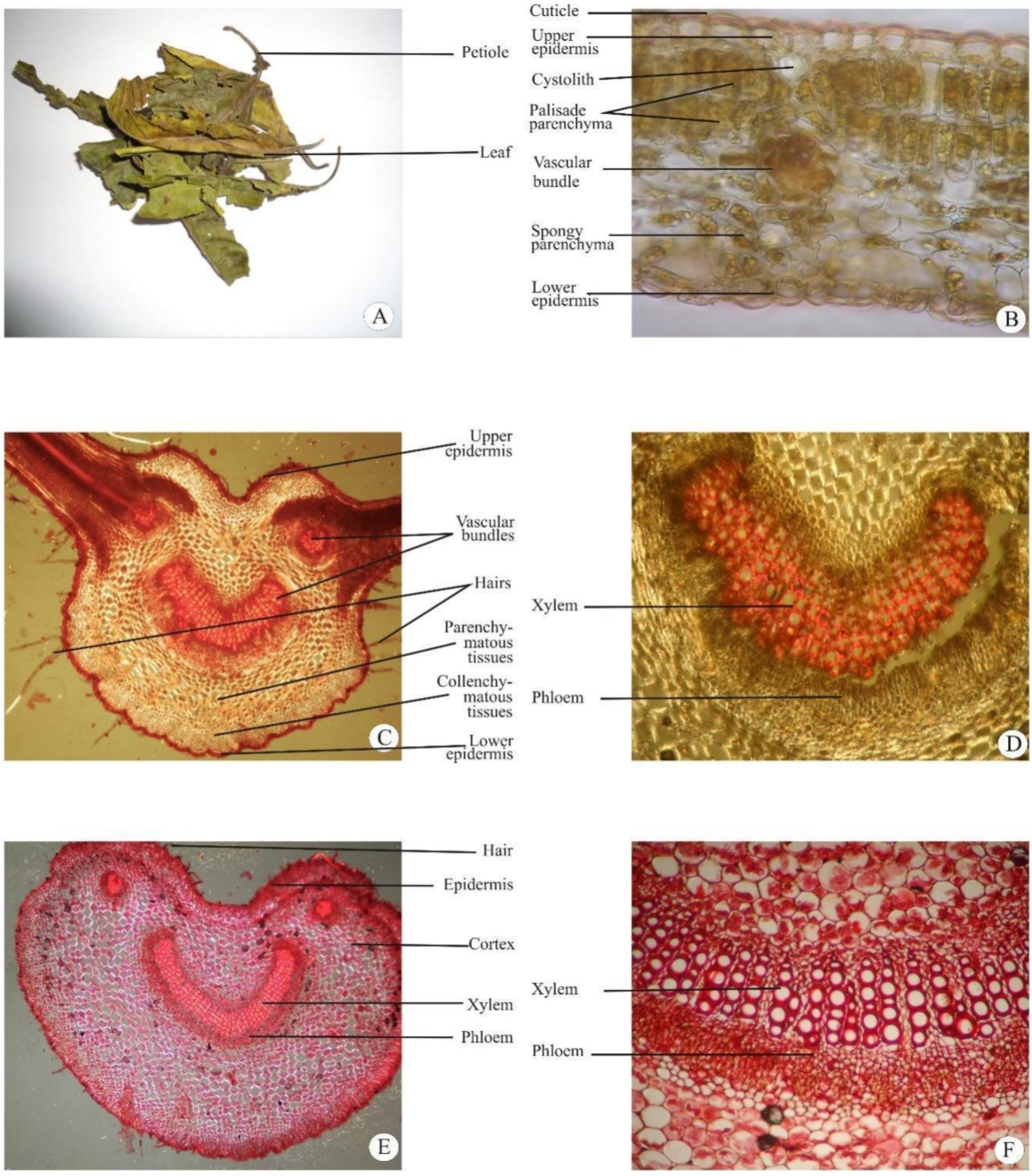

Fig. 3. *Justicia adhatoda* L.**:** A) Raw drug; B) Vertical section of leaf lamina showing epidermis with thick cuticle, two layered palisade layers, cystolith; C) Vertical section of leaf midrib showing epidermis with hairs, one central arc shaped vascular bundle and two small vascular bundle on adaxial side; D) Enlarged view of midrib vascular bundle; E) Transverse section of petiole showing reniform outline, epidermis with hairs, cortex, one large arc shaped vascular bundle, two small lateral vascular bundle; F) Enlarged view of petiole vascular bundle showing xylem and phloem.

Quantitative microscopic studies

Epidermal cells are 5–8 sided and polygonal and size ranging from 30–70 × 20–40 μm. Anticlinal sides of epidermal cells are slightly wavy and thin. Contents are dense in some of epidermal cells. Adaxial surface epidermal cells are very slightly sinuous. Trichomes were present on both surface and are multicellular, uniseriate, non-glandular with pointed apex, straight, peltate base. Diacytic stomata present more on abaxial surface and very few on adaxial surface, elliptic in shape. Subsidiary cells were slightly distinct. Stomatal number were ranged from 0–1 and 130–172 on adaxial and abaxial side respectively and

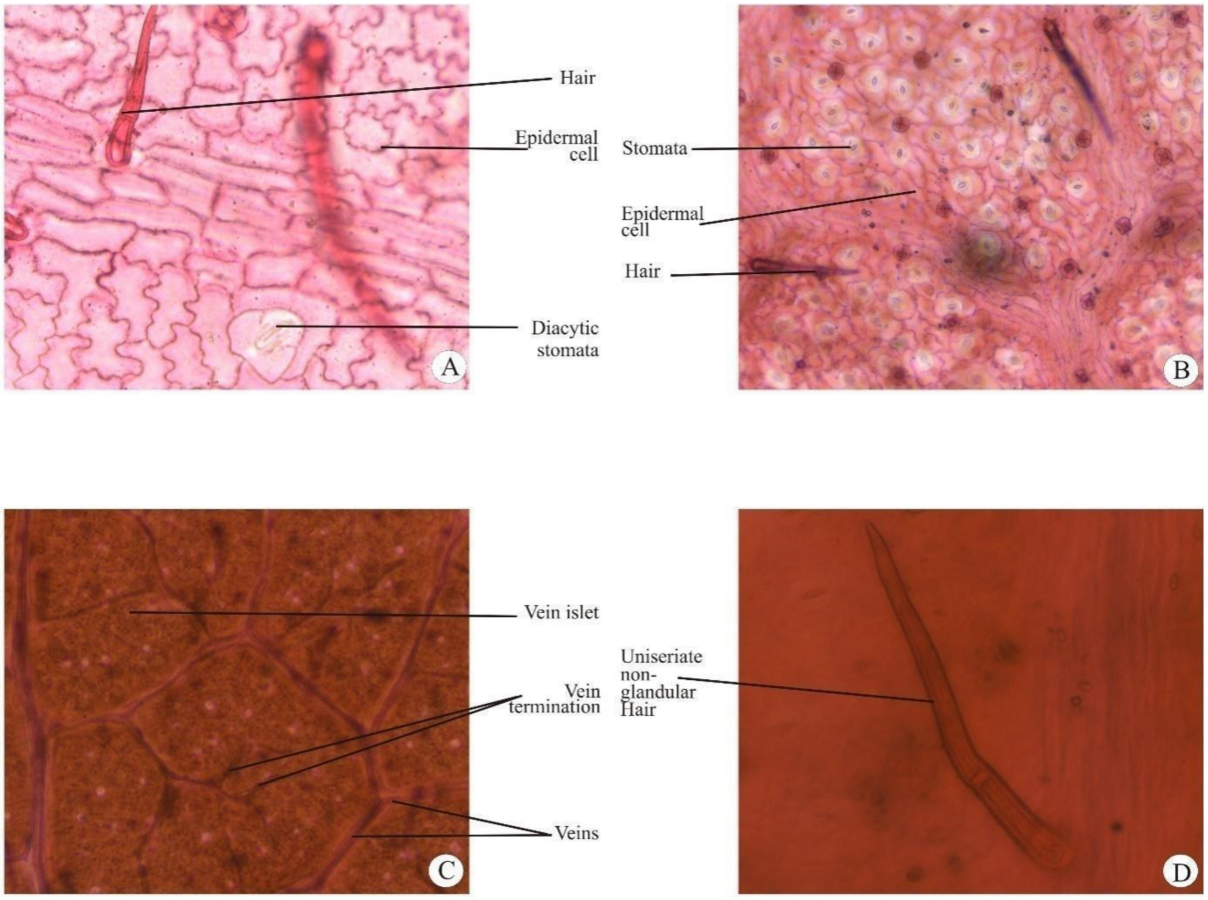

Fig. 4. *Justicia adhatoda* L.: A) Leaf peel of upper epidermis showing diacytic stomata, epidermal cells, hairs after cleaning with chloral hydrate solution; B) Leaf peel of lower epidermis showing diacytic stomata, epidermal cells, hairs after cleaning with chloral hydrate solution; C) Leaf lamina showing veins, vein-islet and vein termination after cleaning with chloral hydrate solution; D) Leaf peel showing uniseriate, non -glandular hair after cleaning with chloral hydrate solution.

stomatal index was found to be 0–1.05 and 11.34–17.02 per mm square on adaxial and abaxial surface. Leaf constant palisade ratio, vein islet number and vein termination number were found to be 4.5–9 per epidermal cell, 3–6.5 per mm square 10–18 per mm square respectively (Fig. 4A-D).

Powder studies

The powder microscopy shown that fragments of uniseriate non-glandular trichome, multicellular uniseriate non-glandular hair, fragments of palisade cells, spongy cells, diacytic stomata with epidermal cells, fragment of epidermal cells with papilla- like hairs, fragments of parenchymatous cells, some lignified xylem vessel, and tracheary elements with spiral thickening are present (Fig. 5A-F).

Organoleptic characters

Colour: Green
Touch: Slightly smooth
Odor: Tea like
Taste: Bitter

Physicochemical studies and chemical profiling

The foreign matter was only ~0.20 % w/w (Entry 1, Table 1). The moisture content of the air-dried leaves was 7.69 ± 0.13% (Entry 2, Table 1). The total ash, acid-insoluble ash, and water-soluble ash values were found to be 7.75 ± 0.45%, 0.81 ± 0.06%, and 1.91 ± 0.14%, respectively (Entries 3–5, Table 1). The raw drug showed very high- water soluble extractive (31.10 ± 0.52%) (Entry 7, Table 1), significant alcohol soluble extractive (13.70 ± 0.30%) (Entry 6, Table 1) and very low hexane soluble extractive (0.41 ± 0.28%) (Entry 8, Table 1).

The total fixed oil was found to be considerably high (3.36 ± 0.02%) (Entry 10, Table 1) compared to the hexane soluble extractive values. The total alkaloid content, representing the most important secondary metabolites of this drug, was found to be

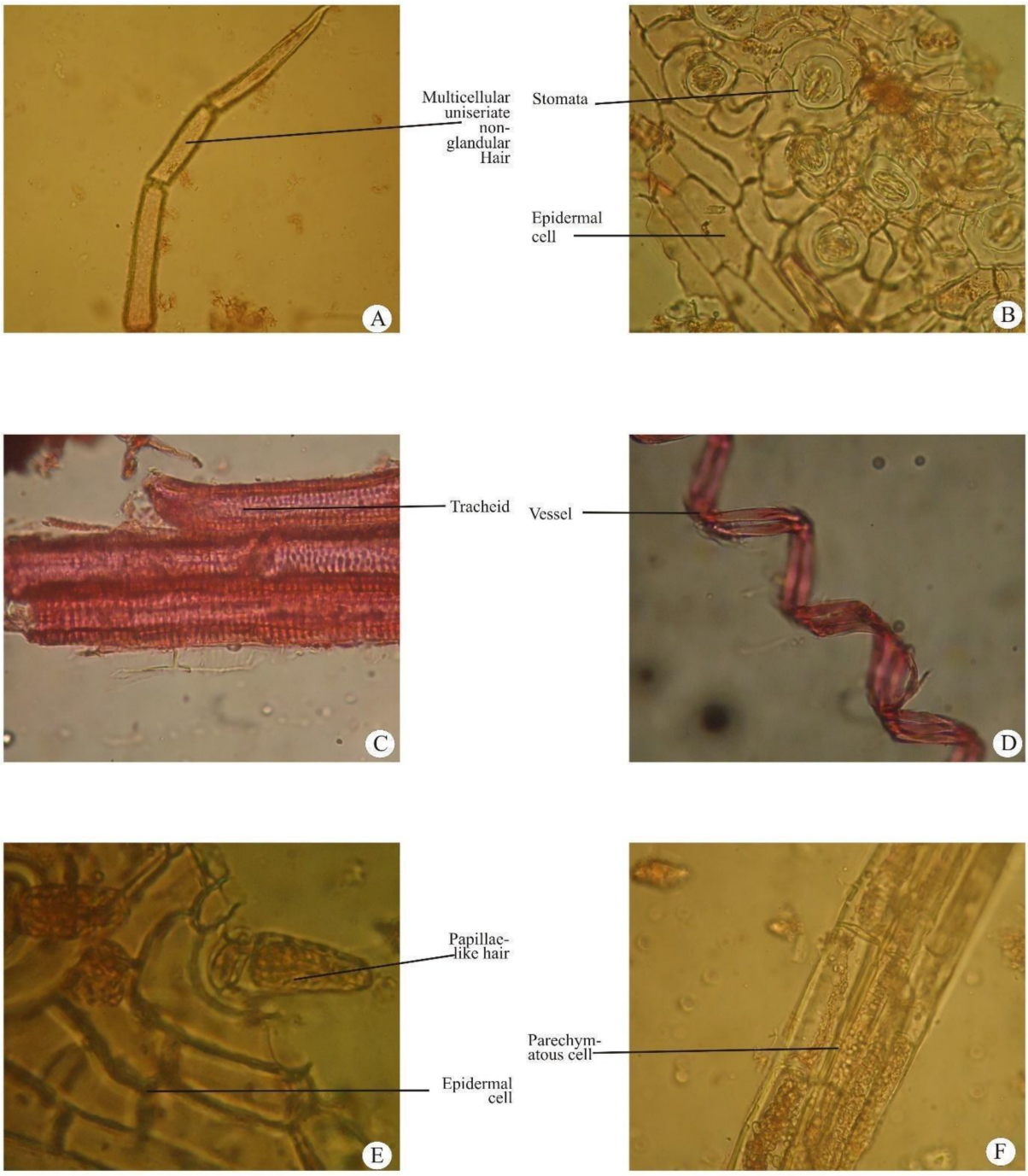

Fig. 5. *Justicia adhatoda* L.: Powder microscopy A) Multicellular uniseriate hair; B) Fragment of epidermal cells with diacytic stomata; C) Fragment of tracheids; D) Fragment of vessels; E) Fragment of epidermal cells with papilla like hairs; F) Fragment of parenchymatous cells.

1.31 ± 0.20% w/w (Entry 9, Table 1). The volatile oil content was trace (Entry 11, Table 1). After obtaining the raw drug's physicochemical parameters, the mother tincture was prepared following the protocol mentioned at Homoeopathic Pharmacopoeia of India (HPI). [13] Then the different parameters of the mother tinctures (inhouse and commercial samples) were measured and given in Table 2. A, B, and C are three commercial samples and D is the in-house mother tincture prepared from the raw drug internally.

The colour of A, C were green while B and D have some brown tinge (Fig. 6; Entry 1, Table 2a).

All mother tinctures are sediment-free (Entry 2, Table 2). The weight per mL of the mother tinctures

Table 1. Physicochemical parameters of the raw drug.

Entry	Parameter	Value
1.	Foreign matter	~0.20 % w/w
2.	Moisture content (LOD at 105˚c)	7.69 ± 0.13 % w/w
3.	Total ash	7.75 ± 0.45% w/w
4.	Acid insoluble ash	0.81 ± 0.06% w/w
5.	Water soluble ash	1.91 ± 0.14% w/w
6.	Alcohol soluble extractive	13.70 ± 0.30% w/w
7.	Water soluble extractive	31.10 ± 0.52% w/w
8.	Hexane soluble extractive	0.41 ± 0.28% w/w
9.	Total alkaloid content	1.31 ± 0.20% w/w
10.	Total fixed oil content	3.36 ± 0.02% w/w
11.	Volatile oil content	0.13% w/w

Table 2. Physicochemical parameters of the mother tincture.

Entry	Parameters	A	B	C	D
1.	Organoleptic profile				
a.	Appearance	Clear	Clear	Clear	Clear
b.	Colour	Green	Brownish green	Green	Brownish green
c.	Odour	Characteristics	Characteristics	Characteristics	Characteristics
2.	Sediments	Absent	Absent	Absent	Absent
3.	Weight per ml (in g)	0.85	0.87	0.85	0.87
4.	Total solids	1.74 ± 0.02% w/v	1.07 ± 0.02% w/v	0.53 ± 0.01 w/v	1.14 ± 0.08% w/v
5.	Alcohol content	75%*	75%*	86%*	75%
6.	pH	7.51	7.37	7.21	7.47
7.	λ_{max}	225 nm, 284 nm, 304 nm, and 403 nm	284 nm, 304 nm, and 403 nm	281 nm, 304 nm, and 400 nm	220 nm, 281 nm and 390 nm
8.	Alkaloid content (w.r.t. mother tincture volume)	0.20 ± 0.01% w/v	0.06 ± 0.00% w/v	0.01 ± 0.00% w/v	0.15 ± 0.01% w/v

*From the label of the mother tincture.

varied from 0.85–0.87 g (Entry 3, Table 2). The total solid of B and D are all 1–2% w/v (Entry 4, Table 2). Interestingly, C has an exceptionally low total solid (Entry 4, Table 2) < 0.6% w/v. The alcohol content of A, B, and D is 75% v/v and conforming the HPI value while C has exceptionally high, 86% v/v alcohol content (Entry 5, Table 2). All the mother tinctures' pH are greater than 7.00 indicating the basic nature of the mother tincture (Entry 6, Table 2). The UV-Vis λ_{max} values (Entry 7, Table 2) indicate that all of the mother tinctures' spectra are similar. The total alkaloid content in terms of %w/v, the order of alkaloid content in mother tinctures is A > D > B> C≈0 and varied between 0.20–0.01 % w/v (Entry 8, Table 2). The alkaloid content of commercial sample-C is almost zero. After these, a phytochemical screening was carried out on the mother tinctures (A, B, C, and D), and chloroform, methanol and water extracts of the raw drug. The result of the phytochemical screening is summarized in Table 3.

A positive test for alkaloids is expected considering the reported phytochemical constituents (Entry 3, Table 3). However, for C we could not detect any alkaloid by the qualitative screening test. This result is in agreement with negligible alkaloid content of the mother tincture. Overall, the phytochemical screening of A, B and D matched while that of C differed considerably.

A qualitative HPTLC study on the chloroform extract of the mother tinctures (A, B, C, and D) was carried out. The developed Chromatogram are given here (Fig. 7(I–IV)). The R_f values are reported in

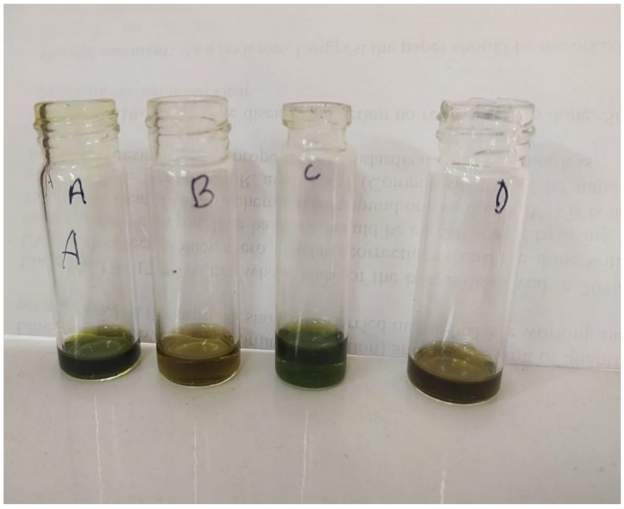

Fig. 6. Colour of the mother tinctures a,b,c, and d.

Table 3. Phytochemical screening of the mother tinctures, chloroform and methanol extracts of *Justicia adhatoda.*

S No.	Name of test	Phytochemical detected	A	B	C	D	Chloroform extract	Methanol extract	Water extract
1.	Salkowski test-	Terpenoids and Sterols	(+)	(+)	(+)	(+)	(+)	(+)	(–)
2.	Keller Kiliani test	Cardiac Glycosides	(–)	(–)	(–)	(–)	(–)	(–)	(–)
3.	Dragandroff test	Alkaloids	(+)	(+)	(–)	(+)	(+)	(+)	(+)
4.	Sodium Hydroxide test	Coumarins	(+)	(+)	(–)	(+)	(+)	(+)	(+)
5.	Shinoda Test	Flavonoids	(–)	(–)	(–)	(–)	(–)	(–)	(–)
6.	Ferric chloride test	Phenols and tannins	(+)	(+)	(–)	(+)	(–)	(–)	(–)
7.	Lead acetate Test	flavonoids	(+)	(+)	(–)	(+)	(+)	(+)	(+)
8.	Froth test	Saponins	(–)	(–)	(–)	(–)	(+)	(–)	(–)

*(+) : Present; (–): absent.

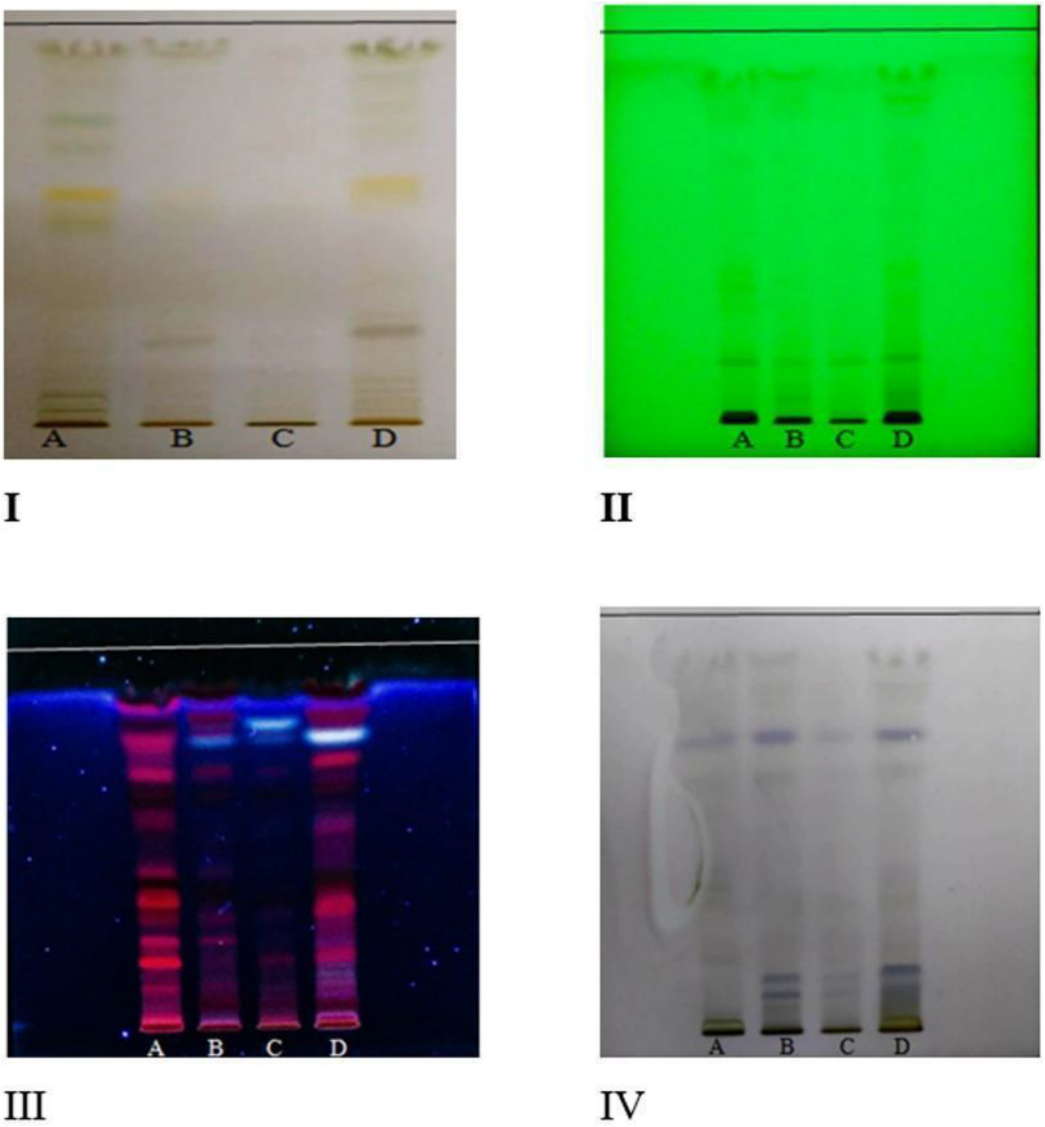

Fig. 7. HPTLC chromatogram of mother tinctures a,b, c and d eluted with 5% methanol in chloroform. I: Under visible light, II: under 254 uv light, iii: under 365 uv light, and IV: Under visible light with anisaldehyde sulphuric acid stain.

Table 4. The concentration of the chloroform extracts of A, B, C, D were 7.6 mg/mL, 5.4 mg/mL, 2.6 mg/mL and 9.0 mg/mL respectively. The concentration of the standard was 5.5 mg/mL.

Even though HPTLC pattern of A, B and D are not identical, they are similar. However, the HPTLC pattern for sample C, though similar to the others, appeared much fainter and did not produce the

Table 4. R_f values of the spots for qualitative HPTLC analysis.

Sample	R_f values under visible light	R_f values under 254 nm UV light	R_f values under 365 nm	R_f values under visible light with Anisaldehyde-sulfuric acid stain	R_f values under visible light with Dragendorff stain
A	0.05(green), 0.08(green), 0.60(yellow), 0.77(green), 0.92(green blob)	0.16(black), 0.92(black)	0.03(pink), 0.13(pink), 0.16(pink),0.26(pink), 0.32(red), 0.42(pink), 0.52(pink), 0.62(brown), 0.70(pink) ,0.80(pink), 0.83(brown), 0.86(pink).	0.03(green), 0.61(violet), 0.72(violet), 0.92(black blob)	0.03
B	0.23(brown), 0.60(yellow), 0.92(green blob)	0.16(black), 0.92(black)	0.08(brown), 0.26(pink), 0.32(pink), 0.52(pink), 0.62(brown), 0.70(pink), 0.80(blue), 0.83(pink),	0.10(violet), 0.13(violet), 0.61(violet), 0.72(violet), 0.92(black blob)	0.03
C	0.23(brown)	0.16(black)	0.08(brown), 0.16(pink), 0.62(brown), 0.70(pink), 0.80(blue), 0.83(blue)	0.72(violet)	-
D	0.23(brown), 0.60(yellow), 0.92(green blob)	0.16(black), 0.92(black)	0.03(pink), 0.08(brown), 0.13(pink), 0.16(pink), 0.26(pink), 0.32(reddish pink), 0.52(pink), 0.62(brown), 0.70(pink), 0.80(blue), 0.83(reddish pink), 0.86(violet)	0.03(green), 0.10(violet), 0.13(violet), 0.61(violet), 0.72(violet), 0.92(black blob)	0.03

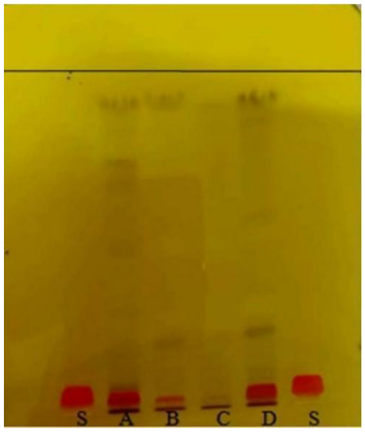

Fig. 8. HPTLC chromatogram of mother tinctures A,B, C, D, and S: Alkaloid isolated from *J. adhatoda* leaves. eluted with 5% methanol in chloroform. under visible light with Dragendorff stain.

characteristic orange coloration with Dragendorff's reagent (Figs. 7 and 8; Table 4).

Discussion

The pharmacognostic study provided the essential botanical features for identification. The foreign matter was extremely low indicating high purity of the raw drug. The moisture content was around 8% w/w expected for an air-dried drug. The total ash was also around 8% w/w indicating low metallic or silicates contamination. Less than 1% w/w acid insoluble ash demonstrates low level of heavy metals and silicates contents in the leaves. These results comply with the limits prescribed in the Ayurvedic Pharmacopoeia of India for *Justicia adhatoda* leaves, wherein the upper limit for total ash is 21% w/w and that for acid-insoluble ash is 1% w/w.[30] The water soluble ash value, around 2% w/w was significantly higher than acid insoluble ash suggesting appreciable contribution from the alkali metals for the total ash. In the cited Ayurveda Pharmacopoeia Monograph, the water soluble ash was not given. Overall, all the ash values reported here are appreciably less than the already reported ash values of the leaves of J. adhatoda, wherein it was reported that the total ash value was 12% w/w, the acid-insoluble ash 1.5% w/w, and the water-soluble ash 4% w/w.[31] This indicated that our sample is less contaminated with inorganic materials, especially metals and silicates, compared to their sample. The raw drug showed more than 30% w/w- water soluble extractive, considerable level of alcohol soluble extractive (~14% w/w) and less than 1% w/w hexane soluble extractive. This clearly indicates highly polar nature of the chemicals present in the leaves of the plant. The Ayurvedic Pharmacopoeia of India specifies lower limits for water- and alcohol-soluble extractive values as 22% w/w and 3% w/w, respectively. Therefore, our sample certainly complies pharmacopoeially with the water- and alcohol-soluble extractive values. In a previous study, the hexane-soluble extractive value and total ash were reported to be 0.13% w/w and 7.82% w/w, respectively.[32] These reported results are in agreement with our findings. In another study, the total ash, acid-insoluble ash, water-soluble ash, water-soluble extractive, and alcohol-soluble extractive were reported as 20% w/w, 0.82% w/w, 4.5% w/w, 18.45% w/w, and 6.8% w/w, respectively.[33]

Overall, these reported values are in good agreement with the results of the present study. The low hexane soluble extractive value and comparatively high fixed oil percentage suggest incomplete extraction by soaking method, necessitating exhaustive Soxhlet extraction for accurate quantification of fatty matter. Then the total alkaloid content of the raw drug was more than ~1% w/w supporting a previous reports.[34] Among the mother tinctures, sample C uniquely displayed high alcohol content and the lowest total solids, resulting in poor extraction efficiency of polar constituents. The pH values >7.0 are consistent with the basic nature imparted by the alkaloids. The UV-visible λmax bands are in agreement with the chromophoric systems of alkaloids and polyphenolic compounds. Phytochemical screening confirmed the presence of alkaloids, terpenoids, sterols, and coumarins in most samples, whereas flavonoids and cardiac glycosides were absent. Sample C consistently showed negative results for alkaloids. The HPTLC profiles of A, B, and D were similar, confirming comparable quality and chemical composition, while C deviated markedly. Dragendorff staining further confirmed alkaloid presence, with visible intensity in the order A≈D>B>>C≈0 (Fig. 8). As for the sample C the UV–Vis spectral data, and overall HPTLC fingerprinting are similar to those of the other mother tinctures, the quality issue is unlikely to have arisen from a spurious raw drug. The actual cause of such discrepancies requires further in-depth investigation and may ultimately necessitate regulatory intervention to resolve. Mass spectrometry, LC-MS (Liquid chromatography-Mass Spectrometry) may be needed for the detection of minor alkaloids.

Further, the commercial mother tinctures A and B and the in-house mother tincture D clearly showed similar chemical characteristics, while C differed significantly with higher alcohol content, lower total solids, and negligible alkaloids, suggesting lower quality. These dissimilarities highlight the importance of regulatory attention and enforcement to ensure manufacturing uniformity.

Conclusion

In this study, the pharmacognostic and physicochemical characteristics of the leaves of the raw drug of *Justicia adhatoda* were evaluated. Extensive physicochemical and chemical profiling studies on the mother tinctures prepared from the leaves of *Justicia adhatoda* as reported in HPI and three commercially available mother tinctures were evaluated as well. The raw drug exhibited lower ash values and similar alkaloid content compared to the already reported literature, demonstrating superior quality and reduced inorganic contamination.

Notably, this work emphasises that finished pharmaceutical product standardisation is at least as critical as raw drug quality standardisation, if not more so, as finished pharmaceutical products are ultimately administered to patients and directly affect public health. Considering the present standardisation data available in HPI for *Justicia adhatoda*, such studies provide crucial data for establishing robust quality benchmarks for batch-to-batch uniformity in homoeopathic drug preparations.

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Conflict of interest (On behalf of all authors)

None

Contribution details

Bibaswan Biswas: Concepts, Design, Definition of intellectual content, Literature search, Experimental studies (physicochemical work, Chemistry related Data acquisition), Data analysis, Manuscript preparation, editing, and revision.

Satish Patel: Experimental studies, Data acquisition, and Supervision of the Pharmacognosy related work.

Digvijay Verma: Manuscript review.

Satyajit Maity: Experimental studies (Chemistry), Literature search, Chemistry Data analysis, and Manuscript editing, review, and revision.

Paulami Majumdar: Manuscript editing, review, and revision.

Shilpi Singh: Experimental studies, Data acquisition of of the Pharmacognosy related work.

G.V. Narasimha Kumar: Manuscript review.

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Analyses physicochimiques, standardisation pharmacognostique de la plante homéopathique *Justicia adhatoda* et phytochimiques

Contexte: *Justicia adhatoda* (*J. adhatoda*), également connue sous le nom de Vasaka en Inde, est une plante médicinale principalement utilisée pour traiter les affections respiratoires dans les systèmes de médecine traditionnelle et alternative. **Objectif**: Cette étude visait à établir les paramètres de standardisation physico-chimiques, pharmacognostiques et phytochimiques de la drogue. Elle a impliqué la mesure des paramètres physico-chimiques et le profilage chimique de la drogue brute. Les caractéristiques chimiques de la teinture mère préparée en interne (à partir de la drogue brute produite en interne) et des teintures mères commerciales disponibles en Inde ont également été évaluées afin d'apprécier la qualité de ces dernières. **Matériel et méthodes**: L'étude a porté sur les paramètres pharmacognostiques (examens macroscopique, microscopique et de poudre) et les paramètres physico-chimiques (matières étrangères, perte à la dessiccation, extraits dans différents solvants, teneur en cendres, teneur totale en alcaloïdes, en huile fixe totale et en huile essentielle totale) de la drogue brute. Elle a également inclus la mesure de divers paramètres de la teinture mère, tels que les caractéristiques organoleptiques, la densité, le pH, l'extrait sec total, le spectre UV-visible, la chromatographie sur couche mince haute performance (HPTLC) et l'analyse phytochimique. Analyse comparative de la qualité des teintures mères commerciales et internes : dosage des alcaloïdes totaux, profilage chimique et analyse de la teneur en alcaloïdes totaux. **Résultats**: Les études macroscopiques et microscopiques ont révélé les caractéristiques pharmacognostiques des feuilles. Les faibles teneurs en cendres indiquent de faibles teneurs en métaux. L'analyse et le profilage chimique ont indiqué la présence du même alcaloïde dans la plante et dans les teintures mères. La plante contenait également plusieurs composés phytochimiques. **Conclusion:** La présente étude fournit des normes pharmacopéiques pour *J. adhatoda.*

Physikochemische, pharmakognostische Standardisierung und phytochemische Analysen des homöopathischen Arzneimittels *Justicia adhatoda*

Hintergrund: *Justicia adhatoda (J. adhatoda*), in Indien auch als Vasaka bekannt, ist eine Heilpflanze, die hauptsächlich zur Behandlung von Atemwegserkrankungen in traditionellen und alternativen Medizinsystemen eingesetzt wird. **Ziel:** Ziel dieser Studie war die Bestimmung der physikochemischen, pharmakognostischen und phytochemischen Standardisierungsparameter des Arzneimittels. Die Studie umfasste die Messung physikochemischer Parameter und die chemische Profilierung des Rohmaterials. Die chemischen Eigenschaften der im Haus hergestellten Urtinktur (aus dem im Haus hergestellten Rohmaterial) sowie handelsüblicher Urtinkturen aus Indien wurden ebenfalls untersucht, um die Qualität der im Handel erhältlichen Urtinkturen zu beurteilen. **Material und Methoden:** Die vorliegende Studie umfasste pharmakognostische Parameter wie makroskopische, mikroskopische und pulveranalytische Untersuchungen sowie physikochemische Parameter wie Fremdkörper, Trocknungsverlust, Extraktionswerte in verschiedenen Lösungsmitteln, Aschegehalt, Gesamtalkaloidgehalt, Gesamtgehalt an fettem Öl und Gesamtgehalt an ätherischem Öl des Rohdrogenpräparats. Darüber hinaus wurden verschiedene Parameter der Urtinktur gemessen, wie beispielsweise Organoleptische Eigenschaften, spezifisches Gewicht, pH-Wert, Gesamtfeststoffgehalt, UV-Vis-Spektrum, Hochleistungsdünnschichtchromatographie (HPTLC), phytochemisches Screening, Gesamtalkaloidgehalt und chemisches Profil von kommerziellen und selbst hergestellten Urtinkturen wurden zur Beurteilung ihrer vergleichenden Qualität bestimmt. **Ergebnisse:** Die makroskopischen und mikroskopischen

Untersuchungen zeigten die pharmakognostischen Eigenschaften der Blätter. Niedrige Aschewerte wiesen auf einen geringen Metallgehalt hin. Das chemische Profil und die Analyse ergaben das Vorhandensein desselben Alkaloids in der Rohdroge und in den Urtinkturen. Die Droge enthielt außerdem mehrere Phytochemikalien. **Schlussfolgerung:** Die vorliegende Studie liefert Arzneibuchstandards für *J. adhatoda.*

होम्योपैथिक दवा *जस्टिसिया अधातोडा* का फिजियोकेमिकल व फार्माकोग्नोस्टिक स्टैंडर्डाइजेशन और फाइटोकेमिकल एनालिसिस

पृष्ठभूमि: *जस्टिसिया अधातोडा* (*J. adhatoda*), जिसे भारत में वसाका के नाम से भी जाना जाता है, एक औषधीय पौधा है जिसका इस्तेमाल मुख्य रूप से पारंपरिक और वैकल्पिक मेडिकल सिस्टम में सांस की बीमारियों के इलाज के लिए किया जाता है। **उद्देश्य:** इस अध्यन्न का मकसद दवा के फिजियोकेमिकल, फार्माकोग्नोस्टिक और फाइटोकेमिकल स्टैंडर्डाइजेशन पैरामीटर तय करना था। अध्यन्न में मूल दवा के फिजियोकेमिकल पैरामीटर और केमिकल प्रोफाइलिंग को मापना शामिल था। भारत में उपलब्ध इन-हाउस मदर टिंक्चर (इन-हाउस मूल दवा से तैयार) और बाज़ारी मदर टिंक्चर की केमिकल विशेषताओं का भी मूल्यांकन किया गया, ताकि बाज़ारी रूप से उपलब्ध मदर टिंक्चर की क्वालिटी का भी पता लगाया जा सके। **विधि:** इस अध्यन्न में मैक्रोस्कोपिक, माइक्रोस्कोपिक, फार्माकोग्नोस्टिक पैरामीटर और पदार्थ सुखाने पर असर, अलग-अलग सॉल्वैंट्स में एक्सट्रैक्टिव वैल्यू, ऐश वैल्यू माप, मूल दवा की कुल एल्कलॉइड मात्रा, पूर्ण फिक्स्ड ऑयल और पूर्ण वोलाटाइल ऑयल से जुड़े फिजियोकेमिकल पैरामीटर शामिल थे। इस अध्यन्न में अलग-अलग मदर टिंक्चर पैरामीटर्स, जैसे ऑर्गेनोलेप्टिक कैरेक्टरिस्टिक्स, स्पेसिफिक ग्रेविटी, pH, कुल सॉलिड्स, UV–Vis स्पेक्ट्रम, हाई परफॉर्मेंस थिन-लेयर क्रोमैटोग्राफी (HPTLC), फाइटोकेमिकल स्क्रीनिंग, टोटल एल्कलॉइड मात्रा, और कमर्शियल और इन-हाउस मदर टिंचर्स की केमिकल प्रोफाइलिंग को भी शामिल किया गया, ताकि उनकी तुलनात्मक गुणवत्ता का पता लगाया जा सके। **परिणाम:** मैक्रोस्कोपिक और माइक्रोस्कोपिक अध्यन्नों से पत्तियों की फार्माकोग्नोस्टिक विशेषताओं का पता चला। कम ऐश वैल्यूज़ ने कम मेटल मात्रा का इशारा दिया। केमिकल प्रोफाइलिंग और एनालिसिस ने कच्ची दवा और मदर टिंक्चर्स में एक ही एल्कलॉइड की मौजूदगी का इशारा दिया। दवा में कई फाइटोकेमिकल्स भी थे। **निष्कर्ष:** यह अध्यन्न *जे. अधातोडा* के लिए मानकों को दर्शाता है।

Análisis fisicoquímicos y farmacognósticos de estandarización del medicamento homeopático *Justicia adhatoda* y fitoquímicos

Antecedentes: *Justicia adhatoda* (*J. adhatoda*), también conocida como Vasaka en India, es una planta medicinal utilizada principalmente para tratar afecciones respiratorias en la medicina tradicional y alternativa. **Objetivo**: Este estudio tuvo como objetivo establecer los parámetros fisicoquímicos, farmacognósticos y fitoquímicos de estandarización del medicamento. El estudio incluyó la medición de

parámetros fisicoquímicos y el perfil químico del medicamento crudo. También se evaluaron las características químicas de la tintura madre casera (preparada a partir de la misma) y de las tinturas madre comerciales disponibles en India para evaluar la calidad de las tinturas madre disponibles comercialmente. **Materiales y métodos**: El presente estudio incluyó parámetros farmacognósticos como estudios macroscópicos, microscópicos y de polvos, así como parámetros fisicoquímicos que incluyen materia extraña, pérdida por secado, valores extractivos en diferentes solventes, medición del valor de cenizas, contenido total de alcaloides, aceite fijo total y aceite volátil total de la droga cruda. Este estudio también incluyó la medición de diversos parámetros de la tintura madre, como características organolépticas, gravedad específica, pH, sólidos totales, espectro UV-Vis, cromatografía en capa fina de alta resolución (HPTLC) y fitoquímica. Análisis, contenido total de alcaloides y perfil químico de tinturas madre comerciales y de elaboración propia para evaluar su calidad comparativa. **Resultados**: Los estudios macroscópicos y microscópicos revelaron las características farmacognósticas de las hojas. Los bajos valores de cenizas indicaron un bajo contenido de metales. El perfil químico y el análisis indicaron la presencia del mismo alcaloide en la droga cruda y en las tinturas madre. La droga también contenía varios fitoquímicos. **Conclusión**: El presente estudio proporciona estándares farmacopeicos para *J. adhatoda*.

顺势疗法药物鸭嘴花（*Justicia adhatoda*）的理化性质、药材学和植物化学标准化分析

背景：鸭嘴花（*Justicia adhatoda*，*J. adhatoda*），在印度也被称为Vasaka，是一种主要用于治疗呼吸系统疾病的药用植物，广泛应用于传统医学和替代医学体系中。**目的**：本研究旨在建立该药物的理化性质、药材学和植物化学标准化参数。研究内容包括测定该药物的理化性质参数和化学成分。此外，还评估了本实验室自制母酊剂（由本实验室自制的生药制备）和市售印度市售母酊剂的化学特性，以评价市售母酊剂的质量。**材料与方法**：本研究包括药材的生药学参数，如宏观、微观、粉末研究，以及理化参数，包括杂质、干燥失重、不同溶剂中的提取物含量、灰分测定、总生物碱含量、总固定油和总挥发油含量。本研究还包括对母酊剂的各种参数进行测定，例如感官特性、比重、pH值、总固形物、紫外 可见光谱、高效薄层色谱（HPTLC）和植物化学成分分析。对市售和自制母酊剂进行筛选、总生物碱含量测定和化学成分分析，以评估其相对质量。**结果**：宏观和微观研究揭示了叶片的药材学特征。低灰分值表明金属含量低。化学成分分析表明，原药材和母酊剂中含有相同的生物碱。该药材还含有多种植物化学成分。结论：本研究为鸭嘴花（*J. adhatoda*）提供了药典标准。