



RESEARCH ARTICLE

# Standardisation of *Adenostemma lavenia* (L.) Kuntze leaves: An unexplored folklore herb from Maharashtra

Khandekar Ritesh & Bindu Gopalkrishnan\*

Research Department of Botany, SVKM's Mithibai College of Arts, Chauhan Institute of Science and Amruthben Jivanlal College of Commerce and Economics, University of Mumbai, Vile Parle (West), Mumbai 400 056, Maharashtra, India

\*Correspondence email - [bindu\\_phd@rediffmail.com](mailto:bindu_phd@rediffmail.com)

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## Abstract

The herb *Adenostemma lavenia* (L.) Kuntze belongs to the family Asteraceae and commonly known as 'jangli jeera'. The leaves are traditionally used in the treatment of baldness and hair loss. Indigenous community of Dutch Indies has long employed the leaves in arresting baldness. They are also used in treatment of sunburn, ulcers and boils. Despite the traditional significance, this herb remains unexplored. The current study aims to introduce this less well-known medicinal herb to manifold. Pharmacognosy is the first step towards standardisation. The parameters investigated are macroscopy, microscopy, histochemical and powder study. The leaf powder was subjected to physicochemical, fluorescence and phytochemical analyses which yielded significant results. Microscopic investigation of leaf powder revealed key diagnostic features, including anisocytic stomata, palisade, spongy mesophyll cells, various types of trichomes, tannin, starch grains, prismatic calcium oxalate crystals, oil globules, etc. These diagnostic features go concurrent to the microscopy of whole leaf. Physicochemical parameters showed the following values, like total ash  $09.69 \pm 0.3$ , water soluble ash  $2.27 \pm 0.6$ , acid insoluble ash  $7.66 \pm 1.8$ , water soluble extractive value  $17.81 \pm 2.4$ , alcohol soluble extractive value  $24.10 \pm 1.8$ , etc. Preliminary phytochemical and histochemical analysis confirmed the presence of major phytoconstituents like alkaloids, flavonoids, saponins, etc. The present study sets the groundwork for standardising the leaf of *A. lavenia* as medicinal herb.

**Keywords:** jangli jeera; pharmacognosy; preliminary phytochemistry; sticky daisy

## Introduction

India is rich in the vast diversity of medicinal plants. It has been used for centuries in indigenous healthcare practices such as Ayurveda, Siddha and folk medicine. Despite their significant therapeutic potential, many of these lesser-known herbs remain underexplored and underutilised. One such plant is *Adenostemma lavenia* (L.) Kuntze.

*A. lavenia* belongs to family Asteraceae. It is commonly known as sticky daisy in English, *jangli jeera* in Hindi, *ghanerem* in Konkani, etc. It is an erect, branched annual herb, stem solitary, rigid, usually divaricately branched in the upper part, tinged with purple (1). The flowers are very small and white with the corolla hairy near the mouth, lobes 5, small and acute; androecium style arms white, twice as long as the corolla much exerted. Stigma bifid (2). The leaves of *A. lavenia* hold significant medicinal properties and are traditionally used by the Warli tribes of Maharashtra. The leaves are commonly employed in hair care, especially for preventing hair fall. Some of the tribals use salted leaves to treat sore throats. In Kerala, leaves serve as antiseptic. Additionally, the fresh juice is traditionally recognised for its stimulant, antispasmodic, antidiarrheal and sternutatory properties (3). Besides its wide range of ethnomedicinal applications, *A. lavenia* stands underutilised in modern herbal formulations. To promote the therapeutic potential of the said plant part pharmacognosy is

put forth for the first time. Pharmacognosy is the first step towards standardisation of any drug. Hence, the current study is of utmost important.

## Materials and Methods

### Collection, authentication and procurement of material

The mature leaves of *A. lavenia* were collected in the month of November from Sanjay Gandhi National Park (SGNP), Borivali, Mumbai. The plant specimen was identified by Mr. Pravin Kale curator at Blatter Herbarium, St. Xavier's College, Mumbai. The herbarium accession number is 22926. The voucher specimen is deposited at Research Laboratory, SVKM's Mithibai College, Vile Parle (W), Mumbai. The freshly collected leaves were preserved in formaldehyde: acetic acid: alcohol. For pharmacognostic analyses leaf materials were shade dried and then grounded to moderate coarse powder. It was stored in airtight container for further use (4).

### Leaf pharmacognosy

#### Macromorphology

The freshly collected leaves were studied for macroscopical and organoleptic features using stereo zoom microscope (5). Photographs showing abaxial and adaxial surfaces were taken for evidence.

### Microscopy

Free hand cut sections of leaves of the plant were stained and studied for microscopical features. The cellular contents were measured using stage and ocular micrometer. The Scanning Electron Microscopy (SEM) study was carried out at Sophisticated Analytical Instrument Facility (SAIF), IIT Bombay, Powai, Mumbai. The sections were observed under the magnification of 25–20000X (6).

### Leaf constants

The leaf constants including type of stomata, stomatal index, palisade ratio, trichome density, vein-islet and vein termination number were investigated (7).

### Histochemical study

The transverse hand cut section of leaves was treated with freshly prepared chemicals/reagents to observe the phytoconstituents localised in cellular region using standard methodology. Each test was carried out 5 times for reproducibility. Tests were carried out in triplicate (8).

### Powder study

The dried leaf powder was cleared using aqueous chloral hydrate solution. The powder was then stained using different freshly prepared stains and mounted in glycerin (50 %) and then observed under compound microscope at low and high magnification. As per the standard protocol the cell and cell content were measured with the help of stage and ocular meter (9, 10) and photographs were taken.

### Fluorescence study

The leaf powder of the plant was treated by various reagents and observed under wavelength short (254 nm), long (365 nm) and visible light separately. The test was carried out in triplicate (11).

### Physicochemical studies

Physicochemical parameters like moisture content and ash values were performed. The extractive values were performed using different polar and non-polar solvents as per standard protocol (12, 13).

### Preliminary phytochemical study

Dried leaf powder of 2 g was subjected to extraction using 50 mL solvents like water, alcohol and methanol. The obtained extracts were filtered using Whatman's filter paper and used for the phytochemical screening as per the standard procedure. The test was carried out in triplicate (14).

### Heavy metal analysis

The leaf powder of the plant part was subjected to heavy metal analysis as per standard methodology (12).

## Results

### Organoleptic study

The adaxial side of leaf is dark green and the abaxial side is light green in colour. It has characteristic aroma and slightly bitter taste.

### Macromorphology of leaves

The leaf is simple, opposite, lanceolate to ovate in shape. The size of leaf varies from 7.5–1.7 cm in length and 1.8–4.8 cm in breadth. The adaxial surface of the leaf is slightly smooth, while the abaxial surface is slightly rough in texture. The petiole of the leaf is

grooved, greenish smooth with 0.3–3.8 cm in length. The leaf base is symmetrical, acute, tapering to the petiole. The margin is slightly serrate; the apex is acute with unicostate reticulate venation. (Fig. 1, 2).



Fig. 1. Entire plant of *Adenostemma lavenia* (L.) Kuntze.

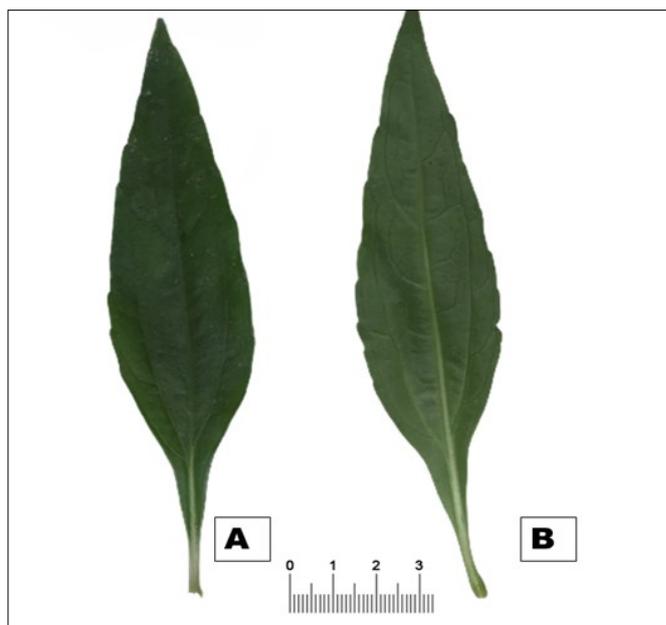


Fig. 2. Leaf of *Adenostemma lavenia*.

### Microscopic study of leaves

The leaf is isobilateral. Transverse section of leaf transversing the midrib shows:

#### Upper epidermis

It is composed of compactly arranged cells, 24–28.8  $\mu\text{m}$  in width, which are single-layered. The epidermis is externally covered with a thick cuticle and is interrupted by stomata. There are 4 types of trichomes. A few trichomes are multicellular, uniseriate, simple and smooth walled, measuring trichomes 29  $\mu\text{m}$  in length and 0.7  $\mu\text{m}$  in width. The second type of trichome is unicellular, uniseriate, smooth walled with 41  $\mu\text{m}$  in length and 0.8  $\mu\text{m}$  in width. The third type of trichome is multicellular, uniseriate, warty trichomes, measuring 26  $\mu\text{m}$  in length and 0.6  $\mu\text{m}$  in width. The fourth type of trichome is multicellular, uniseriate, smooth-walled with a blunt tip, measuring 20  $\mu\text{m}$  in length and 0.4  $\mu\text{m}$  in width.

### Midrib region

The upper epidermis is followed by 4–5 layers of thick walled compactly arranged collenchyma cells, 9.6–21.6  $\mu\text{m}$  in diameter. It is continued with compactly arranged polygonal parenchyma cells, 19.2–38.4  $\mu\text{m}$  in width. Parenchymatous cells are filled with oil globules and starch grains. The midrib region shows the presence of oil ducts lined by epithelial cells. Druse cells and prismatic calcium oxalate crystals are also observed in this region. Three vascular bundles were observed in matured leaves. Among the three, the central vascular bundle is bigger and sided by 2 small vascular bundles. In young leaves, only one vascular bundle is observed.

### Vascular bundles

The vascular bundles are arc-shaped, with the metaxylem facing the lower epidermis and the protoxylem towards the upper epidermis. The cambium is present between the xylem and phloem and a sclerenchyma patch surrounds each vascular bundle.

### Lower epidermis

It is a single layered compactly arranged cells, 7.1–9.5  $\mu\text{m}$  in width. A thick cuticle covers the epidermis. The epidermal cells are discontinued by uniseriate and multicellular simple, smooth walled trichomes/hair 31  $\mu\text{m}$  in length and 0.7  $\mu\text{m}$  in width. In comparison with the upper epidermis, a greater number of stomata are present on the lower epidermis. Just above the lower epidermis there are 3–4 layers of thick-walled collenchyma cells, 8.5–20.4  $\mu\text{m}$  in width.

Transverse section of fresh leaf transversing through lamina shows:

### Upper epidermis

It consists of a single layer, tangentially elongated, compactly arranged cells, measuring 5.8–8.6  $\mu\text{m}$  in length and 3.6–4.0  $\mu\text{m}$  in width. A moderately thick cuticle covers the epidermal layer. The epidermis is disconnected by 4 types of trichomes similar to that of upper epidermis of midrib region. Cells are filled with cellular content and the stomata are present at intervals.

### Mesophyll

Mesophylls are not differentiated into palisade and spongy tissues. Below the upper epidermis 3–4 layers of thick walled polygonal to rectangular chlorenchyma cells, with 19.6–23.4  $\mu\text{m}$  length and 10.4–13.6  $\mu\text{m}$  width. It is continued with 2–3 layers of polygonal cells, measuring 33.4–46.2  $\mu\text{m}$  in width. It also shows the presence of immature vascular bundles.

### Lower epidermis

It is similar to upper epidermis 16.2–20.4  $\mu\text{m}$  in length and 7.3–9.1  $\mu\text{m}$  in width. A thick cuticle covers the epidermis. The epidermal cells show uniseriate and multicellular, simple trichomes as well as warty trichomes and it also shows the stomata. The number of stomata is more than the upper epidermis (Fig. 3).

### Transverse section of *A. lavenia* leaf under SEM

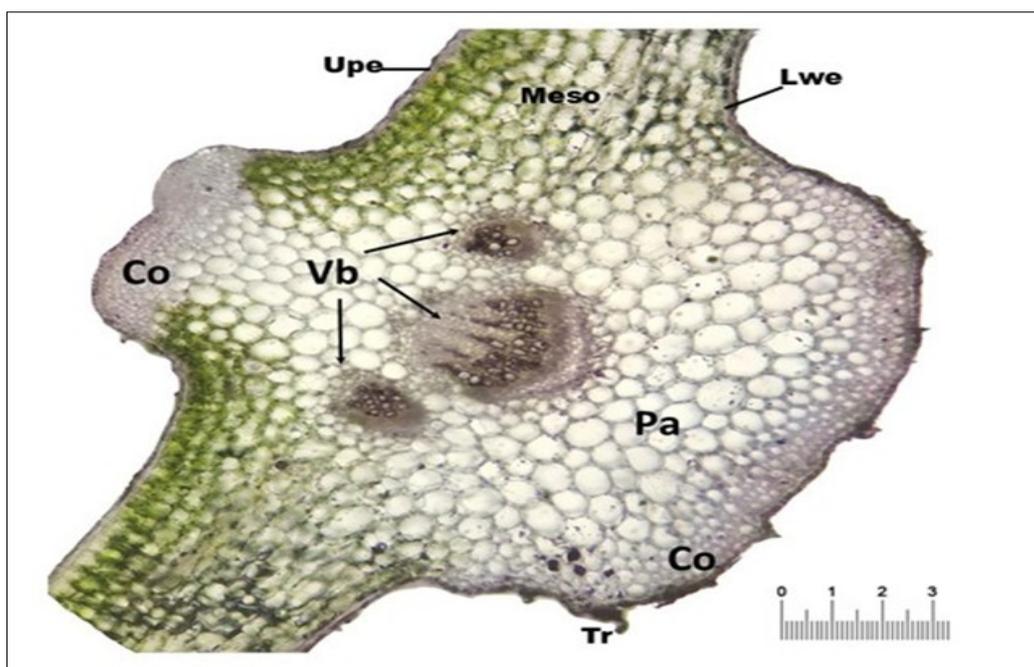
In cross section of leaf clearly shows the presence of trichomes and stomata. The midrib region shows a ring of vascular bundles surrounded by thick-walled sclerenchyma. The xylem vessels with annular thickening are clearly observed (Fig. 4, 5).

### Leaf constants

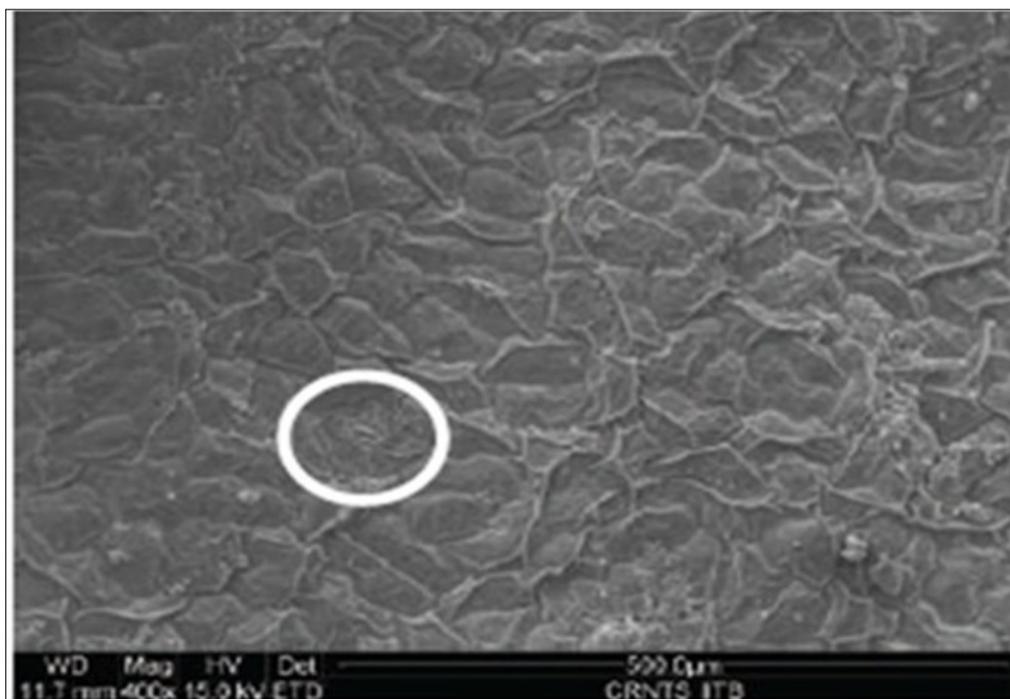
The leaves show anisocytic stomata. Stomatal index on upper epidermis is 3.6 and lower surface is 7.6. Palisade ratio of the leaf is 11.4. The trichome density varies, with 3.68 on the upper surface and 4.19 on the lower surface. The vein-islet termination number is 9.2 at the leaf tip, 6.9 in the middle region and 5 at the leaf base. The vein termination number is 11.8 at the leaf tip, 16.8 in the middle region and 11.4 at the leaf base (Fig. 6).

### Histochemical analysis

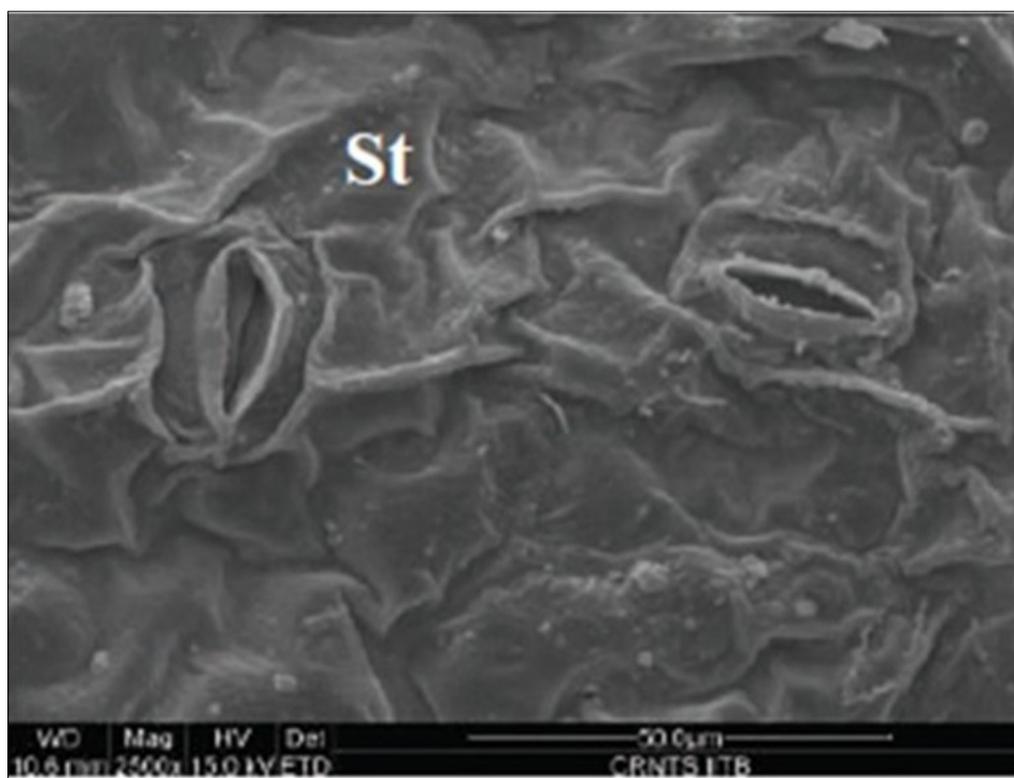
Histochemical analysis deals with identification and distribution of various chemical compounds within and in-between plant cell structure, using different stains and reagents. It helps to detect cell inclusions such as starch grains, calcium oxalate crystals, tannins, mucilage, oils etc. The results obtained are depicted in Table 1.



**Fig. 3.** T.S. of *Adenostemma lavenia* leaf. Upe- Upper epidermis; Tr- Trichome; Co- Collenchyma; Pa- Parenchyma; Vb- Vascular bundles; Lwe- Lower epidermis; Meso- Mesophyll



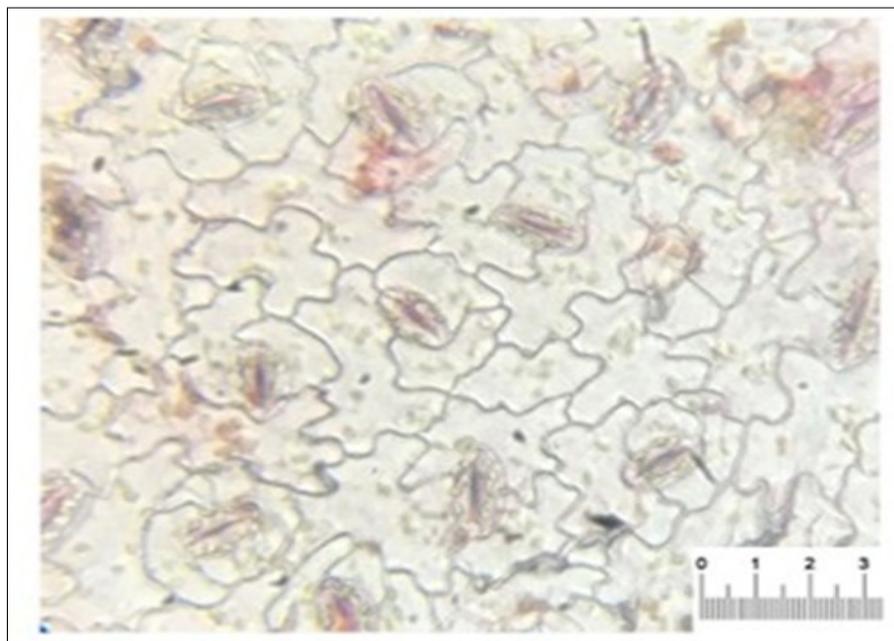
**Fig. 4.** Scanning electron micrograph of leaf surface showing waxy microstructure.



**Fig. 5.** Scanning electron micrograph of leaf surface showing stomata. St- Stomata

**Table 1.** Histochemical analysis of *Adenostemma lavenia* leaves

S. No.	Test	Observations
1.	Test for cellulose Zinc-chloride-iodide test	Present below the upper and lower epidermal cells represented by blue colour
2.	Test for starch Iodine test	Present in parenchyma cells of midrib region
3.	Test for tannins Ferric chloride	Present in parenchyma cells of midrib region
4.	Test for proteins Biuret test	Present in midrib, epidermal cell, vascular bundle, collenchyma and spongy cells
5.	Test for lipids Sudan III	Present in vascular bundles represented by pink colour
6.	Test for calcium oxalate crystals Sulphuric acid test Hydrochloric acid test	Present in parenchyma cells of midrib region Present in parenchyma cells of midrib region
7.	Test for alkaloids Mayer's reagent test Wagner's reagent test	Present in vascular bundles, collenchyma and spongy cells Present in vascular bundles, collenchyma and spongy cells
8.	Test for enzymes Methylene blue	Present in collenchyma, upper and lower epidermis and vascular bundles



**Fig. 6.** Leaf constant-anisocytic stomata.

### Powder study of leaf

The dried leaf powder is dark green, with characteristic aroma and slightly bitter taste. Microscopically the leaf powder shows epidermal cells are thin-walled elongated cells, 15.5  $\mu\text{m}$  in length and 8.5  $\mu\text{m}$  in width. Four types of trichomes were observed, they are: a) Smooth-walled, unicellular, uniseriate, 43  $\mu\text{m}$  in length and 0.6  $\mu\text{m}$  in width at the base; b) Multicellular, uniseriate with blunt tip, 29  $\mu\text{m}$  in length and 0.45  $\mu\text{m}$  in wide at the base and with a curved tip; c) Multicellular, uniseriate, smooth walled trichomes, 31  $\mu\text{m}$  in length and 0.7  $\mu\text{m}$  in wide at the base; d) Some trichomes are multicellular, uniseriate with warty surface 26  $\mu\text{m}$  in length and 0.7  $\mu\text{m}$  in wide at base. Vessels of different types are frequently observed, i.e., pitted vessels, 26  $\mu\text{m}$  in length and 4.5  $\mu\text{m}$  in width and annular lignified vessels, 28  $\mu\text{m}$  in length and 5.6  $\mu\text{m}$  in width. The calcium oxalate crystals (prismatic) measure 35  $\mu\text{m}$  in length and 13  $\mu\text{m}$  in wide. Oil globules are small, spherical, shiny inclusion measuring up to 6  $\mu\text{m}$  in diameter. Tannin is irregular in shape, amber brown coloured up to 42  $\mu\text{m}$  in diameter. Starch grains are small, simple, globular measuring up to 19  $\mu\text{m}$  in diameter. Anisocytic stomata are frequently observed. Collenchyma cells are unevenly thick walled, polygonal shaped, compactly arranged, less frequent, lignified measuring up to 29  $\mu\text{m}$  in diameter. Fiber is thin walled, long, elongated, frequent measuring up to 69  $\mu\text{m}$  in length and 24  $\mu\text{m}$  in wide (Fig. 7a–i).

### Fluorescence study

The leaf powder showed prominent fluorescence with different chemicals/solvents. The results are summarised in Table 2.

### Physicochemical studies

The physicochemical constants like moisture content, ash values (total ash, water soluble, acid insoluble ash and sulphated ash and extractive values were performed with different solvents. Results are represented in Table 3.

### Preliminary phytochemical analysis

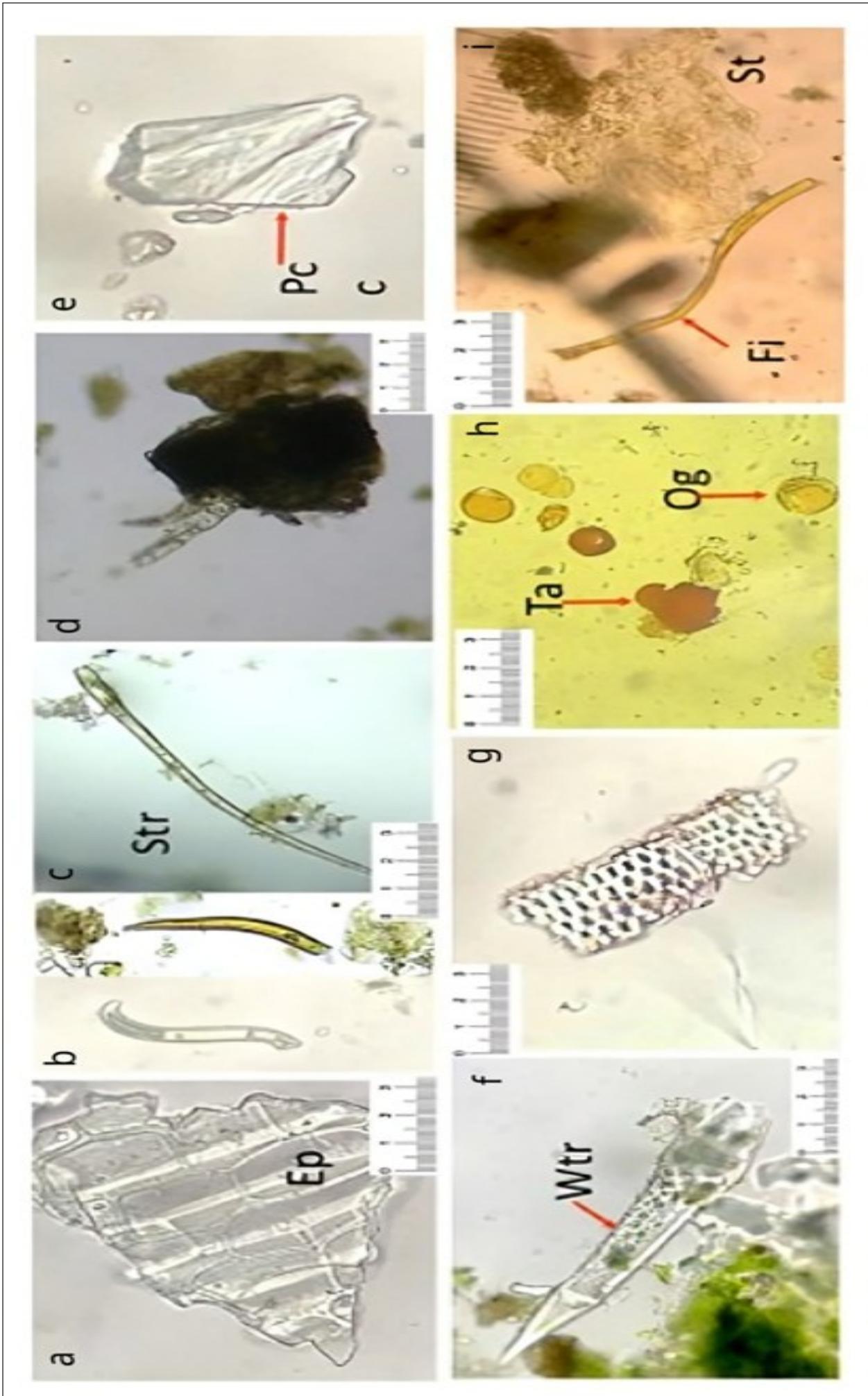
The qualitative analysis of powder drug reveals presence of phytoconstituents as summarised in Table 4.

### Heavy metal analysis

Heavy metal analysis revealed the plant material to be non-toxic, as shown in Table 5.

### Discussion

The leaves of *A. lavenia*, is used in treating hair problems by the indigenous people. Besides cosmetic use, it is also recommended to treat lung congestion, pneumonia, edema, inflammation, etc. (3). Before this medicinal plant is introduced to market the pharmacopeial standards need to be laid down hence the said plant part was studied. This study is carried out for the first time. The macroscopic characters as shape of leaves, petiole and organoleptic parameters like taste and colour will help in authenticating the plant on the field. The microscopical investigation along with powder microscopy revealed the 4 different types of trichomes, anisocytic stomata, etc. as diagnostic feature in identification of crude drugs. The presence of waxy microstructure on the leaf surfaces, trichome types and stomata was prominently observed in SEM which was not clearly sighted through compound microscope. The leaf constants confirmed the type of stomata as anisocytic, while vein islet number, vein termination number were significant. The preliminary phytochemical and histochemical analysis of leaves showed promising results which go concurrent with each other. The Physicochemical parameter, extractive values provided insights into the selection of appropriate solvent systems for phytochemical analysis. Moisture content and ash values showed significant results. The powdered leaves of said plant showed characteristic fluorescence, which can be used to distinguish authentic drugs. The heavy metal toxicity study proved the safety of the said plant part, as the concentration of heavy metal falls within the permissible limits. Quantitative phytochemical analysis and evaluation of hair growth activity using albino rat model are in progress.



**Fig. 7.** Powder study of *Adenostemma lavenia* leaf. (a) Epidermal cells x 450; (b,c) Simple trichome and Multicellular trichome x 100; (d) Tannin filled cells x 100; (e) Prismatic calcium oxalate crystals x 450; (f) Warty trichome x 100; (g) Pitted vessel cell and oil globule x 450; (h) Tannin filled cell and oil globule x 450; (i) Fibre and stomata x 100. Upe- Upper epidermis; Tr- Trichome; St- Stomata; Ep- Epidermal cell; Pa- Parenchyma; Vb- Vascular bundles; X- Xylem; Lwe- Lower epidermis; Wtr- Warty trichome; Ta- Tannin filled cell; Og- Oil globules; Fi- Fibre; meso- Mesophyll

**Table 2.** Fluorescence analysis of *Adenostemma lavenia* leaves

S. No.	Test	Visible	254nm	365nm
1.	Powder: Powder on dried slide	Dark Green	Dark Green	Dark Green
2.	Aq. NaOH solution: Powder with 1N aqueous sodium hydroxide solution.	Yellowish green	Yellowish	Dark green
3.	Alc. NaOH solution: Powder with 1N alcoholic sodium hydroxide solution.	Green	Green	Fluorescentorange
4.	Aq. HCl solution: Powder with 1N dilute hydrochloric acid solution.	Green	Green	Green
5.	Conc. H <sub>2</sub> SO <sub>4</sub> solution: Powder with concentrated sulphuric acid solution.	Green	Green	Green
6.	Dil. H <sub>2</sub> SO <sub>4</sub> solution: Powder with (1:1) conc sulphuric acid solution.	Green	Green	Green
7.	Conc. HNO <sub>3</sub> solution: Powder with conc nitric acid solution.	Orange	Light orange	Green
8.	FeCl <sub>3</sub> solution: Powder with 5% dilute Ferric chloride solution	Yellowish green	Yellow	Dark Green
9.	NH <sub>3</sub> solution: Powder with Conc. ammonia solution	Yellowish green	Green	Green
10.	Benzene solution: Powder with Conc. Benzene solution	Green	Green	Fluorescent orange
11.	Petroleum ether solution: Powder with Conc. Petroleum ether solution	Light green	Green	Fluorescent orange
12.	Chloroform solution: Powder with Conc. chloroform solution	Dark green	Green	Fluorescent orange
13.	Acetone solution: Powder with Conc. Acetone solution	Light green	Green	Fluorescent orange
14.	Ethyl acetate solution: Powder with Conc. Ethyl acetate solution	Green	Green	Fluorescent orange
15.	Acetonitrite solution: Powder with Conc. Acetonitrite solution	Green	Green	Fluorescent orange
16.	Diethyl ether solution: Powder with Conc. Diethyl ether solution	Green	Green	Fluorescent orange
17.	Picric solution: Powder with 5% dilute Picric solution	Yellowish green	Yellowish green	Green
18.	Propanol solution: Powder with Conc. 2-Propenol solution	Green	Green	Fluorescent orange
19.	Methanol solution: Powder with Conc. Methanol solution	Green	Green	Fluorescent orange
20.	Ethanol solution: Powder with Conc. Ethanol solution	Light yellow	Greenish yellow	Fluorescent orange
21.	Distilled water: Powder with Distilled water solution	Light green	Green	Green
22.	Iodine solution: Powder with 5% Iodine solution	Light yellow	Green	Green
23.	Hexane solution: Powder with Hexane solution	Light yellow	Greenish yellow	Fluorescent orange
24.	Xylene solution: Powder with Xylene solution	Green	Green	Pink
25.	Acetic acid solution: Powder with Acetic Acid solution	Yellowish green	Yellow	Fluorescent orange
26.	Nitrocellulose solution: Powder with Nitrocellulose and few drops of Amyl acetate solution.	Light green	Yellowishgreen	Pink
27.	Nitrocellulose and Alc NaOH solution: Powder with 1N Alcoholic NaOH solution, let it dry. Put Nitrocellulose and few drops of Amyl acetate solution	Light green	Green	Light pink
28.	Nitrocellulose and HCl solution: Powder with 1N HCl solution, let it dry. Put Nitrocellulose and few drops of Amyl acetate solution	Green	Green	Green

**Table 3.** Physicochemical parameters of *Adenostemma lavenia* leaves

Physicochemical parameters		Observations
<b>Moisture content (%)</b>		4.11 ± 0.98
<b>Ash values (not more than)</b>		
i.	Total ash (% w/w)	09.69 ± 0.3
ii.	Water soluble ash (% w/w)	2.27 ± 0.6
iii.	Acid insoluble ash (% w/w)	7.66 ± 1.8
iv	Sulphated ash (% w/w)	1.10 ± 0.35
<b>Extractive values (not less than)</b>		
i	Water soluble extractive	17.81 ± 2.4
ii	Alcohol soluble extractive	24.10 ± 1.8
iii	Butanol soluble extractive	2.20 ± 2.56
iv	Chloroform soluble extractive	3.45 ± 1.18
v	Methanol soluble extractive	9.89 ± 1.11
vi	Benzene soluble extractive	1.46 ± 1.19
vii	Ethyl acetate soluble extractive	2.52 ± 0.90

**Table 4.** Preliminary phytochemical screenings of *Adenostemma lavenia* leaves

Sr. No.	Test	Leaf			
		Aq	Alc	Met	
1.	Starch	Lugol's test:	+	+	+
		Molisch's test:	+	+	+
2.	Carbohydrates	Fehling's test:	+	+	+
		Benedict's test:	+	+	+
		Bial's Orcinol test:	+	+	+
3.	Mucilage	Ruthenium Red test:	-	-	-
4.	Proteins	Millon's Test:	+	+	+
		Test for tyrosine:	+	+	+
5.	Amino acids	Folin's test:	+	+	+
		Sudan red III test:	+	+	+
6.	Lipids	Ferric chloride test:	+	+	+
		Lead acetate test:	+	+	+
8.	Steroids	Lieberman Burchard test:	-	-	-
		Trichloroacetic acid test:	-	-	-
		Shinoda test:	+	+	+
9.	Flavonoids	Sulphuric acid test:	+	+	+
		Lead acetate solution	+	+	+
10.	Cardiac glycoside	Keller-Killani test:	+	+	+
11.	Anthraquinone glycosides	Bontrager's test for anthraquinone glycosides	+	+	+
12.	Cyanogenic glycoside	Sodium picrate test	-	-	-
13.	Coumarin glycosides	NaOH test	+	+	+
14.	Saponin glycosides	Foam test:	+	+	-
		Dragendorff's test:	+	+	+
15.	Alkaloids	Mayer's test:	+	+	+
		Wagner's test:	+	+	+
		Chloroform test:	+	+	+

Key: Aq- Aqueous, Alc- Alcohol, Met- Methanolic extracts. "+" Present and "-" Absent

**Table 5.** Heavy metal analysis of *Adenostemma lavenia* leaves

Sr. No.	Metals	Permissible limit (mg/kg)	Observed value
1	Lead (Pb)	2	0.3265
2	Manganese (Mn)	500	0.8883
3	Iron (Fe)	1.00	0.0000
4	Nickel (Ni)	67.9	0.0000
5	Cobalt (Co)	0.01	0.01

## Conclusion

The comprehensive pharmacognostic evaluation of *A. lavenia* leaves is carried out for the first time. It will provide valuable information in establishing essential diagnostic features for field identification and quality control. Importantly, heavy metal analysis confirmed the safety profile of the leaf powder within permissible limits. These findings form a foundational basis for the safe introduction of *A. lavenia* into herbal markets. The detailed studies on quantitative phytochemistry and pharmacology are in progress.

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## Authors' contributions

KR carried out all the experiments pertaining to the designing of pharmacognostical analysis mentioned in the manuscript under the guidance of BG. Writing of manuscript was carried out by KR and BG. Final correction of manuscript was done by BG. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None

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