Morphoanatomic, physicochemical, and phytochemical standardization with HPTLC fingerprinting of aerial parts of Aerva lanata (Linn) Juss ex Schult

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Abstract
Objective: Aerva lanata (Linn) Juss ex Schult, family Amaranthaceae, is a common wayside weed. The herb is accepted by the Ayurvedic Formulary of India. This study was undertaken to establish morphoanatomic and physicochemical standards of A. lanata.
Methods: Leaf constants and high-performance thin layer chromatography fingerprint profiles of A. lanata were performed.
Results and conclusion: The physico–chemical, morphologic, and histologic parameters presented in this paper may be proposed as standards to establish the authenticity of A. lanata and help differentiate it from other species such as Aerva tomentosa.

Introduction
Aerva lanata (Linn) Juss ex Schult is known in Indian medical literature1 as a common wayside weed, locally known as "bui." It is identified by its small bunches of woolly flowers that bloom on axillary branches. The plant is
abundant on the plains in the warmer parts of India and is native in tropical Africa through Arabia and to the Philippines up to an altitude of 3000 m.

Aerva lanata contains β-sitosteryl palmitate, α-amyrin, and β-sitosterol. The plant is also reported to contain tannins, steroids, flavonoids, alkaloids, polysaccharides, and saponin. The phytochemical profile of A. lanata has been studied extensively. In ethanomedicine, A. lanata has broad uses. It is regarded as a valuable treatment for hemorrhage associated with pregnancy. The herb is also used for diarrhea, such as with cholera and dysentery. It is also known to treat hemorrhage associated with pregnancy. The flowers are used to treat gonorrhea and kidney stones.

Aerva lanata has been documented for its pharmacological properties including anti-asthmatic activity, nephroprotective activity, antidiabetic, antihyperglycemic, antimicrobial, cytoxic, anti-HIV, immunomodulatory, anti-inflammatory, analgesic, anti-ulcer, and antioxidant activities. Furthermore, the plant is often substituted by its allied species Aerva tomentosa Forsk. Since, A. lanata is widely used in ayurvedic medicine, confusion exists in the identity of the source material when the origin of a particular drug is assigned to more than one plant species, sometimes having the same synonym and only slight variation in their morphologic characters. Hence, we undertook this study to establish the morphoanatomic, physicochemical, and phytochemical characters of authenticated samples of A. lanata collected from wild sources. Histologic, histochemical, and physicochemical testing were performed using standard protocols. These standards for A. lanata have not been established previously. Leaf constants were also determined. Fingerprint profiles were performed using ethanol extraction.

Materials and methods

Collection and authentication of herb

Aerial parts of A. lanata were collected from wild sources during its flowering season of November–December and authenticated by Professor P. Jayaraman, Director, Plant Anatomy Research Centre, Chennai, India.

Morphology

The collected sample was subjected to morphologic study of its aerial parts.

Histology

Healthy samples of leaf, stem, and petiole were cut and removed from the plant and fixed in formalin-acetic acid solution (formalin 5 mL + acetic acid 5 mL + 70% ethyl alcohol 90 mL). After fixation for 24 hours the specimens were dehydrated with a graded series of tertiary-butyl alcohol solution (TBA solution). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58–60 °C) until the solution attained supersaturation. The paraffin-embedded specimens were sectioned using a rotary microtome into thicknesses of 10–12 μm. The sections were stained with toluidine blue O, a polychromatic stain that reveals histochemical properties.

For studying the stomatal index, vein-islet, vein termination number, venation pattern, and palisade ratio of paradermal sections as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s fluid (1:1 volumes of 10% nitric acid and 10% chromic acid) were prepared. Different cell components were studied and measured by Nikon Labo-phot 2 microscope system (Nikon Instruments, Melville, NY, USA). For study of leaf constants, standard methods were performed as described previously. Crystals, starch grains, and lignified cells, were assessed using polarized light. Since these structures have birefringent property, under polarized light they appear bright against a dark background.

Physicochemical analyses

Physicochemical analyses, such as moisture content, foreign organic matter, percentage of ash values (including acid insoluble and sulphated ash) as well as extractive values, were performed according to standard methodology and WHO guidelines.

Preliminary phytochemical screening

Powdered aerial parts of A. lanata were subjected to preliminary phytochemical screening for the detection of various plant constituents. Shade-dried coarsely powdered 800 grams of A. lanata were successively extracted with organic solvents in increasing order of polarity: petroleum ether (60–80 °C), chloroform, and ethanol using a Soxhlet apparatus and concentrated. The extraction was also carried out using distilled water. The extracts obtained were then subjected to qualitative tests for identification of plant constituents such as alkaloids, saponin, tannins, carbohydrate/glycosides, steroid, and falcon.

HPTLC analysis

For high performance thin layer chromatography (HPTLC), the concentrated ethanol extract was dissolved in 90% ethanol. Rf values were determined with a CAMAG TLC Scanner 3 (Muttenz, Switzerland) on precoated silica gel plates (EMD Millipore, Billerica, MA, USA). The plate was then eluted with chloroform:methanol:water: (70:30:4; v/v) to obtain HPTLC spectra.

Results

Morphology

Aerva lanata (Linn) is an erect or prostrate plant with a long, woody tap root that branches from near the base (Fig. 1). Branches: many, terete, pubescent or woolly tomentose, and striated 30–60 cm in height. Leaves: simple, alternate, 2.0–2.5 by 1.0–1.6 cm on the main stem, 6–10 mm by 5–6 mm on the branches, elliptical or obviate...
or sub-orbicular, small petiol, obtuse or acute, entire, with white cottony hairs beneath. Petioles: 3–6 mm long, often obscure, usually small in the flowering branches. Flowers: greenish white, very small, sessile, often bisexual in small dense sub-sessile auxiliary heads or spikes 6–12 mm long, filaments of the five stamens connect at the base with alternating linear staminodes. Bracteoles: 1.25 mm long, membranous, broadly ovate, concave, apiculate. Perianth: 1.25–1.5 mm long. Sepals: oblong, obtuse, silky hairy on the back. Utricle: broadly ovoid, acute, stigmas. Fruits: ovoid, acute, greenish, compressed utricle. Seeds: black, 0.85 mm diameter, smooth, polished.

Anatomic studies

Transverse section of leaf
The leaf is dorsiventral with a prominent midrib and thick lamina. The lamina is amphistomatic, that is, stomata are on both upper and lower surfaces. The adaxial epidermis has wide, rectangular, thin walled cells with a prominent cuticle; the cells are 20 μm thick. The mesophyll consists of an adaxial zone of palisade tissue of a single row of vertically oblong cylindrical cells up to 70 μm high. The spongy mesophyll is comprised of 6 or 7 lobed cells that form a filamentous structure. The midrib is 350 μm vertically and 250 μm horizontally. The vascular bundle of the midrib is single and collateral. It consists of a dense cluster of thick walled, narrow angular xylem elements and a narrow arc of phloem with their sclerenchymal layer beneath the vascular bundle. The leaf margin is thick and broadly conical. The marginal portion is 150 μm thick (Figs. 2 and 3).

Calcium oxalate crystals are widespread in the mesophyll tissue. On the leaf surface, the crystals appear scattered. The veins are also clothed with crystal granules. The crystals are 20 μm thick (Figs. 3 and 4).

Venation pattern. Veinlets are uniformly thin and straight forming wide, vein-islets of variable shape. Vein-islets have one or two vein terminations that are simple and unbranched or branched (Fig. 5).

Stomata. Stomata are anomocytic with no distinct subsidiary cells. Stomata are circular with slitlike stomata.
pores (Fig. 6). Epidermal cells are wide with thin, wavy anticlinal walls.

**Petiole.** The lower end of the petiole is triangular with a flat adaxial side and semicircular lower part. The outer part of the adaxial zone has chlorenchymatous tissue with small air spaces. The lower half of the petiole has circular compact parenchymatous cells. The vascular bundles are collateral and wedge shaped. The petiole is 430 μm along the longitudinal axis and 600 μm in horizontal plane. The petiole has two prominent lateral wings (Fig. 7).

**Transverse section of stem**
The stem is 750—800 μm thick and circular on transaxial view with opposing shallow, less prominent ridges. The epidermal layer of the stem is 50 μm thick including the cuticle. The cortex measures about 200 μm wide. The xylem is 350 μm thick. The fibers are 40 μm long and the vessels are 60—70 μm wide. The pith cells are large, circular, thick walled and compact. Large (nearly 100 μm) calcium oxalate crystals are present in the cortex (Fig. 8).

**Physicochemical content**
Determination of the physicochemical constants such as moisture, foreign organic matter, ash values, and extractive values of aerial parts of *A. lanata* were carried out to confirm the identity of the plant. These physical parameters are useful in establishing the profile quality of drugs that contain *A. lanata* and are important for their qualitative evaluation. These parameters were assessed by standard methods (Table 1).

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Results % (average value)</th>
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<tbody>
<tr>
<td>Moisture content</td>
<td>3.93</td>
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<tr>
<td>Foreign organic matter</td>
<td>1.30</td>
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<tr>
<td>Total ash value</td>
<td>8.43</td>
</tr>
<tr>
<td>Acid insoluble ash value</td>
<td>3.83</td>
</tr>
<tr>
<td>Sulphated ash value</td>
<td>3.31</td>
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</table>

**Figure 5** Paradermal section *Aerva lanata* leaf stained with toluidine blue O showing vein-islets (VI) and vein termination (VT).

**Figure 6** Transection of *Aerva lanata* leaf stained with toluidine blue O solution showing stomata (St), anticlinal walls (AW), and epidermal cells (EC).

**Figure 7** Transection of *Aerva lanata* leaf petiole stained with toluidine blue O solution showing adaxial side (AdS), epidermis (Ep), vascular bundle (VB), ground tissue (GT), wing (W), and calcium oxalate crystals (Cr).

**Figure 8** Transection of *Aerva lanata* stem stained with toluidine blue under brightfield microscopy showing pith (Pi), xylem (X), cortex (Co), calcium oxalate crystals (Cr), sclerenchyma (Scl), phloem (Ph), and epidermis (Ep) in the cortical tissue.
To evaluate whether or not a medicinal plant is exhausted or adulterated, assessment of extractable matter is performed. Extractable matter is comprised of constituents that can be extracted with solvents. Extractive values for *A. lanata* were assessed using standard methods (Table 2).

Preliminary phytochemical screening of extracts obtained by successive solvent extraction of *A. lanata* showed the presence of alkaloids, glycosides, saponin, tannin, and flavonoid (Table 3). *R*<sub>f</sub> values obtained from HPTLC profile of ethanol extract of *A. lanata* were obtained (Fig. 9).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Extractive values of air-dried aerial parts of <em>Aerva lanata</em>.</th>
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<tr>
<td>Solvent</td>
<td>Color</td>
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<tr>
<td>Petroleum ether (60–80°C)</td>
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<tr>
<td>Chloroform</td>
<td>Yellowish green</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Yellowish green</td>
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<td>Water</td>
<td>Yellowish green</td>
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<table>
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<tr>
<th>Table 3</th>
<th>Qualitative phytochemical screening of extracts of <em>Aerva lanata</em>.</th>
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<tbody>
<tr>
<td>Chemical test</td>
<td>Petroleum extract</td>
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<tr>
<td>Alkaloids</td>
<td>Mayer’s reagent</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s reagent</td>
</tr>
<tr>
<td></td>
<td>Hager’s reagent</td>
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<tr>
<td></td>
<td>Wagner’s reagent</td>
</tr>
<tr>
<td>Carbohydrates and glycosides</td>
<td>Benedict’s solution</td>
</tr>
<tr>
<td></td>
<td>Molisch’s reagent</td>
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<tr>
<td></td>
<td>Barfoed’s test</td>
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<tr>
<td>Fixed oils and fats</td>
<td>Spot test</td>
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<tr>
<td>Saponins</td>
<td>Foam test</td>
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<td></td>
<td>Hemolysis test</td>
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<td>Tannins</td>
<td>Ferric chloride solution</td>
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<td>Lead acetate solution</td>
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<td>Flavonoids</td>
<td>Magnesium turning test</td>
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<tr>
<td>Steroids</td>
<td>Liebermann Burchard’s test</td>
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</tbody>
</table>

Note: + Present, – absent.

Figure 9 HPTLC profile of ethanol extract of *Aerva lanata* at 256 nm (left) and 366 nm (right) in chloroform: methanol: water: (70:30:4) solvent system.
Discussion and conclusion

Before a botanical plant can be used for medicinal purposes, it should be studied in detail to ensure its therapeutic efficacy. Morphologic study, examination of cell structures and organization, and analysis of the tissue system are some of the pharmacognostic properties that are important for identifying the correct species of the plant and for distinguishing between closely related species of the same genus. These are the important initial steps toward standardizing a medicinal plant. The present pharmacognostic investigation of the aerial parts of *A. lanata* will help in its standardization and identification, including genuineness and differentiation from its allied species to ensure consistent results for certification and quality control.

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References