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Binitha Raj RV
Drug Standardisation Unit, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India

MA Shajahan
Dept. of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India

Sudhakaran MV
University of Calicut, Kerala, India

Sreedeepthi GN
National Ayurveda Research Institute for Panchakarma, Thrissur, Kerala, India

Rajesh KS
Drug Standardisation Unit, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India

Resny AR
Research Fellow, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India

Correspondence
Binitha Raj RV
Research Officer (Ayurveda)
Drug Standardization Unit, Govt. Ayurveda College, Thiruvananthapuram, Kerala, India

Pharmacognostic studies of *Lobelia alsinoides* Lam.

Binitha Raj RV, MA Shajahan, Sudhakaran MV, Sreedeepthi GN, Rajesh KS and Resny AR

Abstract
Introduction: *Lobelia alsinoides* Lam. is a herbaceous medicinal plant belonging to the family Lobeliaceae. It is an ethnomedicine used by tribal practitioners in South India for the treatment of jaundice.
Objective: The present work has been carried out in a view to delineate the pharmacognostic profile of root, stem, and leaf of *Lobelia alsinoides* Lam.
Methods: Macroscopic study, microscopic evaluation, powder analysis, fluorescence standards and quantitative evaluations of root, stem, and leaf of *Lobelia alsinoides* Lam. were carried out.
Results: Macroscopy and histological features revealed *Lobelia alsinoides* Lam. was a dicotyledonous plant, having circular vascular bundles, idioblasts with prismatic crystals of calcium oxalate, air filled chambers in stem and root and anomocytic stomata in leaves. These were the diagnostic features of the taxon. The presence of reticulate venation, small palisade ratio, starch grains and idioblasts containing Calcium oxalate crystals were also the diagnostic characters of leaf. The distinguishing characters of triangular stem were the presence of idioblasts with abundant deposition of calcium oxalate crystals, simple starch grains, chlorenchyma and aerenchymatous cortex. Circular roots showed trabaculate cortex with prominent air cavities, annular xylem vessels with crystal, fibers and simple starch grains.
Conclusions: The present study provides the scientific data on macro, microscopic, powder, quantitative and fluorescence standards for the proper identification and authentication of *Lobelia alsinoides* Lam.

Keywords: *Lobelia alsinoides* Lam, pharmacognosy, palisade ratio, powder analysis, fluorescence standards, standardization

1. Introduction
Lobelia alsinoides Lam. is a herbaceous medicinal plant belongs to the family Lobeliaceae. Traditional Ayurvedic practitioners of Kerala are using this plant (known as *Cheriyamanganari* in Malayalam) for the treatment of Jaundice. It is a small perennial aquatic herb widely distributed in marshy areas [1, 2] and wetlands [3] (Fig.1A). It has almost cosmopolitan distribution and 12% is found in Asia [4]. Lobelia is the largest group of Lobelioidea (subfamily of companulaceae) comprising over 400 species [5]. The genus is characterized by simple, alternate leaves, two-lipped tubular flowers and is a rich source of specific alkaloids of lobeline group [6].
Lobelia alsinoides Lam. grows up to a height of 15-30 cm at altitude 300-1600m in clayey soil. It has three angled week succulent stem creeping at the base with roots at nodes and oozes milky white latex on cutting. Flowering and fruiting time is throughout year especially September to December [2].
It is included in the IUCN red list of threatened species [7]. International Union for Protection of new Variety of plants (UPOV) assigned a UPOV code for *Lobelia alsinoides* Lam. as LOBAL [8]. European and Mediterranean Plant Protection Organization (EPPO) gave EPPO Code as LOBEL-ALS [9]. Basionym of the plant is *Lobelia hancei* H.Hara. In Indochina and in the Malesian area the name *Lobelia alsinoides* Lam. is replaced by *L. dopatrioides* Kurz var. cantonensis (Danguy) W.J.de Wilde & Duyfjes. In the remaining area of *L. alsinoides*, mainly India, that species is divided into two varieties, viz. var. *alsinoides* and var. *trigona* (Roxb.) W.J.de Wilde & Duyfjes [10].
Leaf epidermal morphology of Lobelia species was studied by Li et al, 2017 using light microscopy and scanning electron microscopy and found that it retains primitive leaf epidermal features. Diagnostic characters of leaf epidermis could be used to differentiate species of lobelia4. *Lobelia chinensis* is reported to possess analgesic, anti-inflammatory, antimicrobial and bile secretion enhancing effects [11, 12]. Research works exploring physical, chemical and biological properties of the plant are scarcely available in the current databases of pubmed, google scholar etc.

The description about this plant is seen in Hortus malabaricus which is a compilation of folklore practices by Henry Vanrheeed. It is prescribed for *pitta* disorders which includes liver disorders in this book [1]. It is also included in the wild food plants of Thailand [13].

According to WHO guidelines of research and evaluation of traditional medicine, scientific research is needed to evaluate the safety and efficacy of traditional medicine [14]. Identification of different species of *lobelia* is difficult [15]. Authentication of botanicals is a critical step to ensure sustained quality of herbal preparations. The present study is aimed to evaluate the pharmacognosy of root, stem and leaves of *Lobelia alsinoides* Lam. (family- Lobeliaceae) as it may assist in standardization of samples of whole, cut or powdered plant material which could ensure precise means of identifying crude drug.

2. Materials and Methods

2.1 Plant Material

Lobelia alsinoides Lam. is a decumbent herb, forming many spreading branches from its base, rooting from lower nodes, growing to 15-30cm tall. Succulent stem is 3-angled, creeping at the base, and 10-25cm long (Fig. 1D, 1E). Whitish roots are adventitious with hairs, soft in nature, simple or branched and are superficial. Leaves are simple, alternate, 1.5-2 x 1-1.5 cm, base rounded to attenuate, margin serrate, apex acute, subsessile, upper leaves ovate-elliptic or subcircular, sessile and smaller; lower leaves(sub)circular, glabrous on both surfaces, petiolate; petiole 3 mm broad and 3-5mm long (Fig.1B). Flowers are white or blue colored, occurs singly in leaf axils in terminal raceme. Two-lipped flowers with 3 lobes in upper lip and 2-lobes in lower lip (Fig.1C). Pedicels are slender 2-3 times longer than leaves (3-4cm) and bibracteolate at the base.

Sepals are 5 in number, 5 mm long and lanceolate. Petals are blue or white, 5 in number, 10 mm long, having an oblong and broad neck, upper 3 lobes obovate, and lower 2 lobes narrower. 5 white stamens capped with apices. Ovary is glabrous, inferior with numerous ovules and axile placentation (Fig.1F). Fruits are capsules with many 0.5mm long, brownish black, glabrous and trigonous seeds.

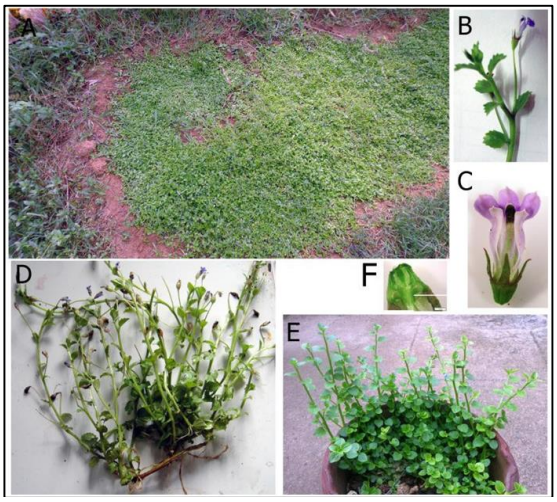

Fig 1: (A) *Lobelia alsinoides* in its natural habitat, (B) Stem, leaves and flowers of *L.alsonoides*, (C) 2-lipped Flower with 3 lobes in upper lip and two lobes in lower lip, (D) Whole plant of *L.alsonoides*, (E)Leaves and stem of *L.alsonoides*, (F) Axile placentation of Ovary.

2.2 Methods

Lobelia alsinoides Lam. was collected from its natural habitat at Thiruvananthapuram District of Kerala state (8°31'26.9"N 76°56'11.8"E), India during rainy season. The plant material was taxonomically identified by Botanist of University of Kerala, India. Fresh full-grown and healthy plant was selected. Roots, stem, leaves, flower, pedicel and petiole were separated for macro and microscopic evaluation. The investigation on trasverse, paradermal and powder of *L.alsinoides* was carried out in the Drug Standardization laboratory of Government Ayurveda College, Thiruvananthapuram and the voucher specimen was retained in department herbarium (1022/DG/AVC) for future reference. Microphotographs of sections and powder analysis were made by using Olympus Microscope (Model CX 41; Tokyo, Japan) with CCD camera 2 mega pixel and quantitative measurements were taken using Olympus Image-Pro Plus, version 5.1 software. Fine hand sections of lamina, midrib, petiole, stem, pedicel, root, and epidermal peels were taken using standard procedures and were stained with Aqueous Safranin 1% and mounted in glycerin. Fluorescence analysis of the powder was carried out in UV light (256 nm & 366 nm) using Camag UV apparatus. Microscopic descriptions of tissues of various plant parts are supplemented with micrographs wherever necessary and magnifications are indicated by the scale-bars in the figures. The number of epidermal cells, stomatal number, stomatal index were calculated per square millimeter of leaf area from intercostal areas of fresh leaves and vein islet number, vein termination number, palisade ratio and size of Guard Cell Area (GCA) were calculated from cleared leaves. Stomatal index was calculated by using formula S×100/ (E+S). Where S is the stomata per unit area, E is the number of epidermal cells in the same unit area [16]. For analysis of the Fluorescence properties of crude drug, the solvents of HPLC/ Chromatographic Grade purchased from Merck and Qualigens Fine Chemicals, India were used.

3. Results and Discussion

3.1 Microscopic evaluation of leaf

The leaf of *Lobelia alsinoides* was dorsiventrally differentiated and its average length was 1.5 cm and width 1.0 cm. In the midrib region, adaxial side (upper) was somewhat flat and abaxial (lower) side was semicircular in shape. Lamina was flat and much reduced in dimension. Midrib consisted of epidermis, collenchyma, mesophyll and vascular tissues. Below the epidermis, 2-3 layers of Chlorenchymatous tissue were seen followed by parenchymatous ground tissue.

Vascular bundles were arranged in crescent shape at the middle of ground tissue. Xylem vessels were arranged radially in 4 or 5 groups with phloem on the abaxial side. Idioblasts with prismatic crystals of calcium oxalate were present in the parenchymatous ground tissue as shown in Fig. 2A. Two lateral vascular bundles were seen on either side of the midrib.

Both the epidermis of the lamina was uniseriate, composed of compactly arranged rectangular cells with cuticle in the outer walls. The mesophyll was differentiated into upper single layered palisade and lower 2-3 layered spongy tissue as shown in Fig.2B. Single layered columnar palisade cells were filled with plenty of chloroplasts and the palisade ratio was found to be about 2.5. Spongy parenchymatous cells were 3 layered and loosely arranged. A few simple starch grains were present in the palisade tissue and prismatic crystals of calcium oxalate were present in mesophyll tissue.

Epidermal characters: In epidermal peel, guard cells of stomata were present in both epidermal layers. Stomata were fairly numerous on lower epidermis and were bigger in size. The guard cells were surrounded by 3 or 4 irregularly shaped wavy epidermal cells and stomata was of anomocytic type as shown in Fig.2C. In abaxial side, length and breadth of stoma was observed as27.35 µm × 10.59 µm and Guard cell area (GCA) was found to be 295.54 µm2. In adaxial side, measurements of stoma was 26.03×20.31 µm and GCA was 424.37 µm^2. Average number of stomata per square millimeter area of leaf was found to be 3.5 in abaxial side and 2.5 in adaxial side (Table 1). The number of epidermal cells per square millimeter area of the leaf was observed as 8 in abaxial side and 5 in adaxial side and stomatal index for the lower surface was found to be 30.43 and 33.33 for upper surface.

Venation pattern

Leaf architectural characters could provide useful anatomical information for characterization of the taxon [17].Venation patterns of cleared leaves were studied. Petiolate simple leaves with serrate margins had observed reticulate venation with prominent midrib and two diverging lateral nerves. Areolation was poorly developed. Polygonal shaped areoles were small and their area ranges from 40.03 to 20.67 mm (Figure 2D, 2E). Within the areoles a few terminal vein-endings were present.

The mean number of vein islets/mm^2 of leaf was found to be 15 and vein termination number/mm^2 was found to be 4.

Table 1: Quantitative microscopy of *Lobelia alsinoides* Lam.

Parameters		Mean value	Range
Stomatal length(µm)	Upper	26.03	23.41-27.42
	Lower	27.35	23.79-30.69
Stomatal width(µm)	Upper	20.31	18.01-22.09
	Lower	10.59	9.25-12.77
Guard cell Area GCA(µm^2)	Upper	424.37	369.82-490.5
	Lower	295.54	224.58-329.17
Average no. of stomata/mm^2	Upper	2.5	2-3
	Lower	3.5	3-4
Average no. of Epidermal cell /mm^2	Upper	5	4-6
	Lower	8	7-9
Stomatal index	Upper	33.33	32.7-33.91
	Lower	30.43	30-31.4
Vein-islet Number/ mm^2		15	12-19
Vein-termination Number/ mm^2		4	4-5
Palisade ratio		2.5	2-3

Petiole

In cross sectional view, the petiole was boat shaped with flat adaxial side and semicircular abaxial side (Figure 2G). A chlorenchyma zone consisting of 2–3 layers were located beneath the epidermis, followed by parenchymatous ground tissues. The 8–9 vascular bundles were arranged in crescent shape above in the parenchymatous ground tissue and phloem on abaxal side. Idioblasts with crystals were present in the parenchymatous ground tissue

Pedicel

Transverse section of pedicel of *L. alsinoides* appeared quadrangular in shape. Single layer of epidermis was seen followed by 2-3 layers of chlorenchyma. 9-11 vascular bundles were arranged in circular fashion in the parenchymatous ground tissue with phloem on the abaxial side. A small pith was present and Idioblasts containing calcium oxalate crystal were seen in the chlorenchymatous region as shown in Fig.2F.

3.2 Microscopic evaluation of Stem

Stem of *L. alsinoides* Lam**.,** appeared more or less trigonous in outline, quadrangular or circular in lower parts (Fig.2H). Epidermis was single layered rectangular or oval shaped cells, covered with thin cuticle (Fig.2L). Cortex was broad, 2 or 3 layers of chlorenchyma were present just below the epidermis. Inner cortex was aerenchymatous. A few simple starch grains were present in parenchyma. Some idioblasts contained Calcium oxalate crystals as shown in Fig.2M. A prominent endodermis was present demarcating the cortical and stelar regions. Stele showed outer pericycle and vascular bundles having 20-25 groups of xylem strands arranged in a circular fashion. In a xylem strand, 3- 4 prominent, circular or polygonal shaped xylem vessels were arranged radially with protoxylem pointing towards the center. Phloem was arranged outer to xylem vessels and central large parenchymatous pith was present as shown in Fig.2I.

3.3 Microscopic evaluation of Root

Transverse section of root of *L. alsinoides* Lam. appeared more or less circular in outline with hairs (Fig.2J). It consists of 3 distinct regions- piliferous layer, cortex and stele. The outer piliferous layer showed single row of cells, followed by single layer of parenchyma. Cortical cells appeared trabaculate with prominent air cavities somewhat regularly alternate with parenchymatous cells and the endodermis was

seen as the innermost layer. Stele showed pericycle and vascular bundles. Xylem vessels were prominent with metaxylem pointing towards the center. Phloem was arranged as a strip above xylem (Fig.2K).

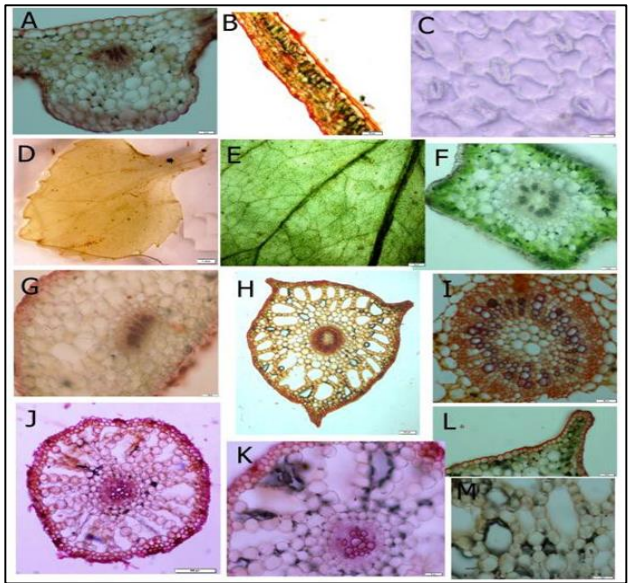

Fig 2: Histological features of *Lobelia alsinoides* Lam. (A) T.S of leaf midrib 10x. (B)T.S of lamina 10x. (C) Epidermal peal of lower leaf surface 40 x. (D) Venation pattern of leaf (E) Vein islets and vein terminations in leaf 10x (F) T.S of pedicel 10x. (G)T.S of petiole 10x. (H)T.S of stem 2x. (I)T.S of stellar part of stem 10x. (J) T.S of root 2x. (K) T.S of root 10x. (L) Edge of stem 10x. (M) Cortex of stem 40x

3.4 Powder microscopy

The dried leaves, root and stem of *Lobelia alsinoides* were analyzed for powder characteristics. Microscopic examination showed fragments of leaf epidermis with stomata and ependymal cells and annular xylem vessels, phlioem fibres and simple starch grains (Figure 3A, 3Band 3C). Root powder showed prismatic calcium oxalate crystals, annular xylem vessel, crystal fibers and starch grains were observed (Figure 3D, 3E, 3F, 3G, 3H and 3I). In stem powder, annular xylem vessel showing lumen filled with brown resin, crystal fibres, group of fibres and trachieds (Figure 3J) were also observed.

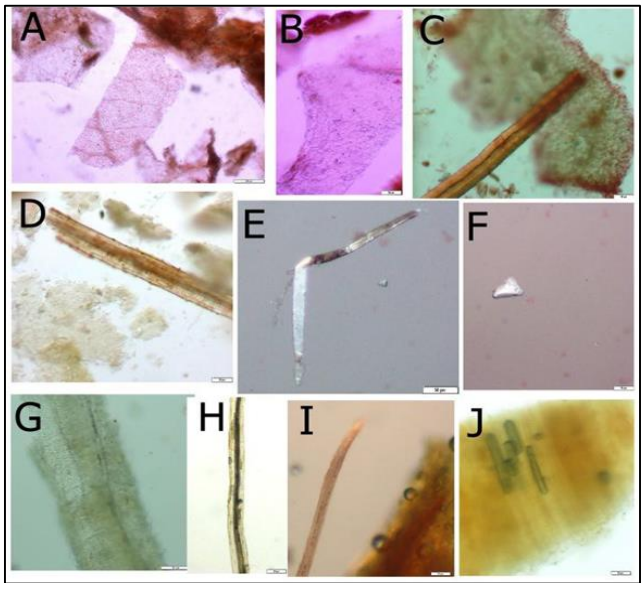

Fig 3: Powder microscopy of *L. alsinoides*: (A) Leaf fragments 4x. (B) Ependymal cells in leaf epidermis 10x. (C) Fibre in leaf powder.10x (D) Annular xylem vessels in leaf powder.10x. (E) Crystal fibers in root powder 10x. (F)Prismatic calcium oxalate crystal in root powder, 10x (G) Xylem vessels in root powder, 40x. (H) annular xylem vessels with crystals in root powder 40x. (I) Tracheids in root powder 10x. (J) Annular xylem vessels with crystals in stem powder 40x

3.5 Fluorescence analysis

The use of fluorescence can be very useful adjunct to botanical pharmacognosy, since it can be applied as rapid and easy test to verify certain identifications of the botanicals. When exposed to the day light, root powder of *Lobelia alsinoides* was found to be greyish white color, stem powder was green and leaf powder was found to be of dark green in color. Powder of whole plant mixture was found to be light green in color. The Fluorescence property of the powdered drug extracts taken in different solvent systems was analyzed under UV light (long and short). Specimens were recorded as either fluorescent (with color and intensity) or not fluorescent and their responses under UV light are presented in Table 2.

Table 2: Fluorescence properties of the extract of root, stem, leaves of *Lobelia alsinoides* Lam. in various solvents.

Solvent	Under UV (256nm)	Under UV (366nm) (Color & Intensity)
Powder form	$NF^{R, S, L}$	$Yellow^{R, S}$, mild red^{L}
Aqueous	$NF^{R, S, L}$	$Yellow^{R, S}$, red^{L}
Methanol	$NF^{R, S, L}$	Bluish green $^{R, S}$, red^{L}
Ethyl alcohol	$NF^{R, S, L}$	Bluish green $^{R, S}$, red ^{L}
Acetone	$NF^{R, S, L}$	Yellow $^{R, S}$, red ^{L}
Chloroform	$NF^{R, S, L}$	Yellow $^{R, S}$, red^{L}
Ethyl acetate	$NF^{R, S, L}$	$NF^{R, S}$ red^{L}
Toluene	$NF^{R, S, L}$	Yellow $^{R, S}$, red^{L}
Cyclohexane	$NF^{R, S, L}$	Yellow $^{R, S}$, Yellowish $green^{L}$
Diethyl ether	$NF^{R, S, L}$	$NF^{R, S}$ red ^{L}
Acetone+methanol	$NF^{R, S, L}$	Yellow $^{R, S}$, red ^{L}
NaOH(3%)	$NF^{R, S, L}$	Yellow $^{R, S, L}$
Picric acid	$NF^{R, S, L}$	$NF^{R, S, L}$
Con.HNO_3	$NF^{R, S, L}$	$NF^{R, S, L}$
$FeCl_3$	$NF^{R, S, L}$	$NF^{R, S, L}$
Dil.NH_3	$NF^{R, S, L}$	Yellow $^{R, S, L}$
IN HCl	$NF^{R, S, L}$	$NF^{R, S, L}$
$H2SO_4$	$NF^{R, S, L}$	$NF^{R, S, L}$
10% Iodine	$NF^{R, S, L}$	$NF^{R, S, L}$

NF = not fluorescent, R = extract of root, S = extract of stem, L = extract of leaf

4. Conclusion

The present study evaluated the pharmacognostic properties of *Lobelia alsinoides Lam.*, for standardizing the plant based on the procedures in API (Ayurvedic pharmacopoeia of India), a legally valid Ayurvedic drug document [18]. Histological features showed that it was a dicotyledonous plant, having circular vascular bundles, idioblasts and air filled chambers in stem, anomocytic stomata in leaves. Idioblasts with calcium oxalate crystals were seen in all parts of the plant. The histological features of *Lobelia alsinoides* Lam. was found similar to aquatic plants like *Brahmi* (*Bacopa monnieri*)[19] and *Jalakumbhi* (*Eichhornia crassipes* Mart.)[20]. The above studies provide objective scientific data with respect to the identification of *Lobelia alsinoides* Lam. Morphological and Microscopical studies will enable to identify the crude drug. In conclusion, the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug and this can be included as standards in Herbal Pharmacopeia.

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