Pharmacognostic studies ofLeucasbiflora(Vahl) R. Br. (Lamiaceae):A less known ethnomedicinal plant

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ABSTRACT
The present study evaluates the pharmacognsotic standardization of a medicinal plant, Leucasbiflora based on its botanical and physico-chemical parameters and HPTLC fingerprinting. Powder microscopical studies and anatomical studies were carried out. Physico-chemical parameters such as loss on drying at 105 ^{0} C, total ash value, acid insoluble ash, water soluble extractive and alcohol soluble extractive were also determined. High performance thin layer chromatographic study (HPTLC) was performed and the chromatograms were documented. Thus, physico-chemical standardisation and HPTLC fingerprinting helped to ensure the quality of the herbal drug and to identify the presence of phyto-components based on its R_f values.
Keywords: Leucasbiflora, powder microscopy,pharmacognostic, HPTLC

I. INTRODUCTION

Leucasbifloraand its variety procumbens has extent distribution from Uttar Pradesh, Bihar, West Bengal, Orissa, Madhya Pradesh, Karnataka, Tamil Nadu, Kerala, Andaman and Nicobar Islands and also found in Bangladesh and Sri Lanka. Ethno-medicinal usage: The mature leaf decoction is used as eye drop twice a day in case of conjunctivitis. The mature leaves ground with the leaves of Centellaasiatica (L.) Urban. (Thankuni) in a ratio of 2:1 and the juice extracted from this mixture is applied directly to stop instance of bleeding from nose (Nose bleed). Four to five leaves are also prescribed to chew with a leaf of Piper betel L. (Pan Pata) for the women who suffering from white discharge(Majumdar&Datta, 2011). The other species of Leucas, L. aspera is well known for its medicinal properties. In Siddha medicine, the samoolam of this plant is crushed and boiled with water, the steam is used for inhalation in conditions like nasal congestion, cough, cold, fever, headache etc.The oil prepared by using the flowers is effective in headache, sinusitis etc. (Mudaliyar, 2002).

So far, the anatomical and pharmacognosticstudies of L. biflora has not attempted. Hence, the present study focuses on the anatomical, powder microscopical, physic-chemical and HPTLC fingerprinting of L. biflora.

II. MATERIALS AND METHODS
Materials and methods
Collection of plant materials:
The plant material is collected from the SRRI campus,Poojappura, Thiruvananthapuram, Keralaand authenticated as LeucasbiflorabyDr.Ghanthi Kumar, Research Officer (Botany), SRRI, Thiruvananthapuram.

Anatomical studies
Hand sections of various parts of the plant material are taken and stained with saffranin and mounted in Glycerin under 10X as well as 40X objective of microscope.

Powder microscopy studies
The powdered form of L. biflorawas mounted in glycerin at room temperature for 24 h and observed under 10X and 40X objective of bright field microscope for different fragments of tissues and diagnostic powder features.

Extraction for HPTLC studies
Alcohol extract of L. biflorawas taken by refluxing 1g each of the material with 10 mL alcohol at a temperature of 60°C for 10 minutes. The extractswere filtered and concentrated to desired volume.

Physico-chemical evaluation
Physico-chemical constants like total ash value, acid insoluble ash value, water soluble extractive value, alcohol soluble extractive value, volatile oil content and loss on drying at 105 °C

were determined as per standard protocol(Trease& Evans, 2002).

HPTLC fingerprinting

The alcohol extract of R. tuberosa and its powder ingredients were subjected to HPTLC analysis. The instrument employed was CAMAG HPTLC system (Muttenz, Switzerland) equipped with a sample applicator TLC autosampler 4 with win CATS software version 1.4.4. Each extract was applied as two tracks of volume 1-5 μL and 2-10 μL. The plate was developed using the solvent system, Toluene: Ethyl acetate (6: 2)in a twin trough chamber. The plate was developed up to 7 cm, removed from the chamber and allowed to dry. The developed plate was scanned using TLC Scanner 3 and analyzed with win CATS software version 1.4.4. at λ_{max} 254 nm using deuterium light source, at λ_{max} 366 nm with mercury light source and the slit dimensions were 8.00 mm × 0.40 mm. Densitometric documentation was done. After scanning, the plate was observed under 254 nm and 366 nm and TLC chromatograms were recorded.

Then the plate was dipped in vanillin-sulfuric acid reagent and dried at 105°C on a hot plate till thecolour of the bands appears. The plate was visualized under white light and scanned at 575 nm. TLC chromatograms, R_f values and fingerprint data were recorded by win CATS software.

III. RESULTS

The cross section of the stem is quadrangular in outline. Single layered epidermis made of rectangular cells covered by a thick cuticle. Uniseriate multicellular trichomes are seen as epidermal outgrowths**(Fig. 1)**. Hypodermis is made of 2-3 layered collenchyma tissues followed by a 3-4 layer of chlorenchyma cells. Xylem forms a continuous ring that surrounds the pith. Phloem elements are seen as a continuous ring around the xylem vessels. Isolated patches of sclerenchyma are seen above phloem elements. Pith is large composed of thin walled polygonal parenchyma cells.
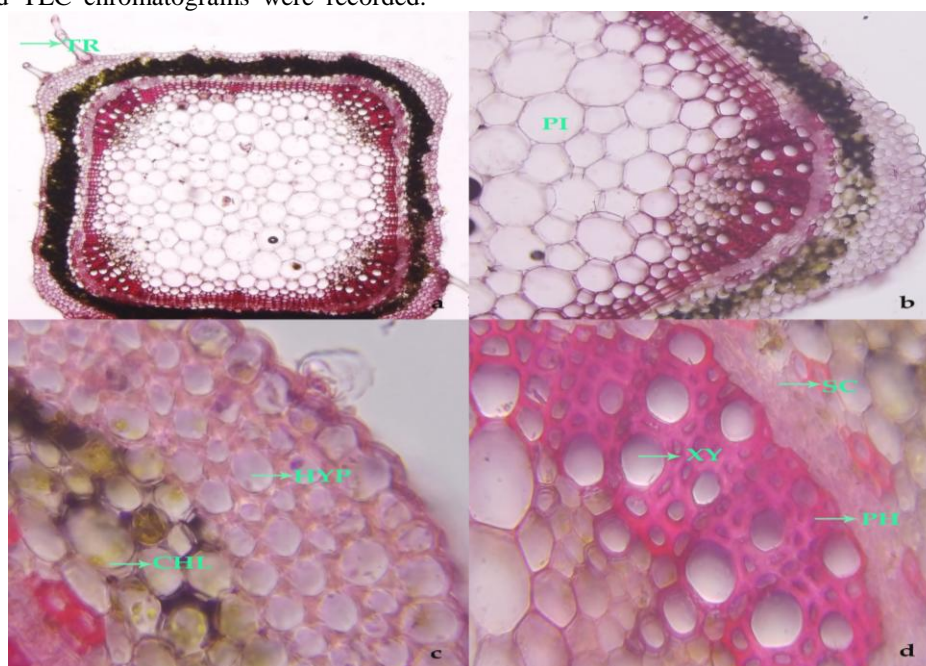

Fig. 1: Cross section of stem of L. biflora. TR- Trichome, HYP-Hypodermis, CHL-Chlorenchyma, XY-Xylem, PH-Phloem, SC-Sclerenchyma, PI-Pith.

Leaf

Midrib of the leaf shows uniseriatetrichomes on the epidermal layer. Collenchyma tissues are absent. Vascular bundle was seen to be embedded in the parenchymatous cortex **(Fig. 2).** Lamina is composed of mesophyll tissues which are clearly differentiated into palisade and spongy parenchyma tissues.

The cross section of the petiole is kidney shaped with uniseriatetrichomes seen to be protruded from the epidermal cells. Epidermis is single layered which is enveloped by a thick cuticle. Followed by, 1-2 layered hypodermal tissue made of collenchyma cells are present.Two-three layers of chlorenchyma tissues are also present. Two

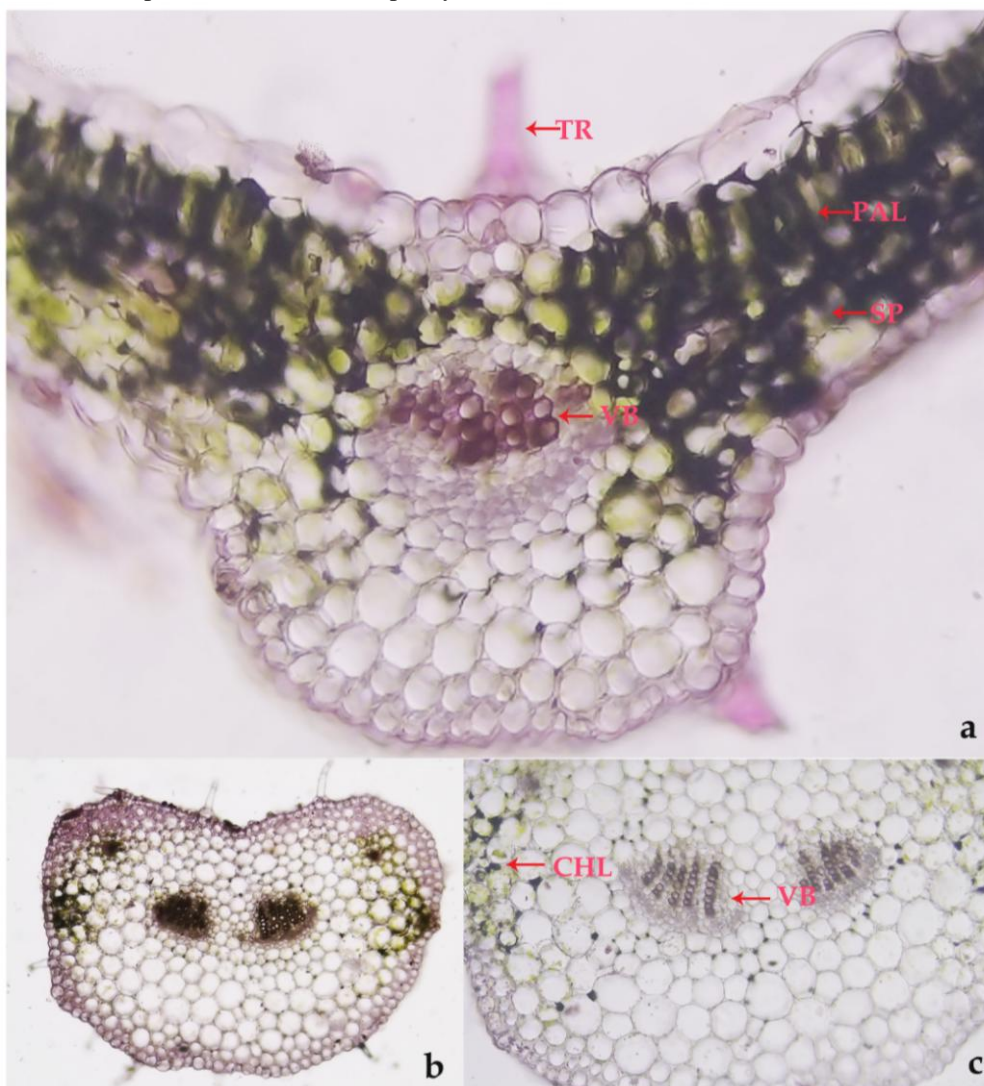vascular bundles are embedded in ground tissue which is composed of compactly arrangedparenchyma cells.

Fig.2:a. Cross section of leaf, b. cross section of petiole, c. a portion enlarged. PAL-Palisade cells, SP- Spongy cells, VB- Vascular bundle, CHL- Chlorenchyma, VB- Vascular bundle
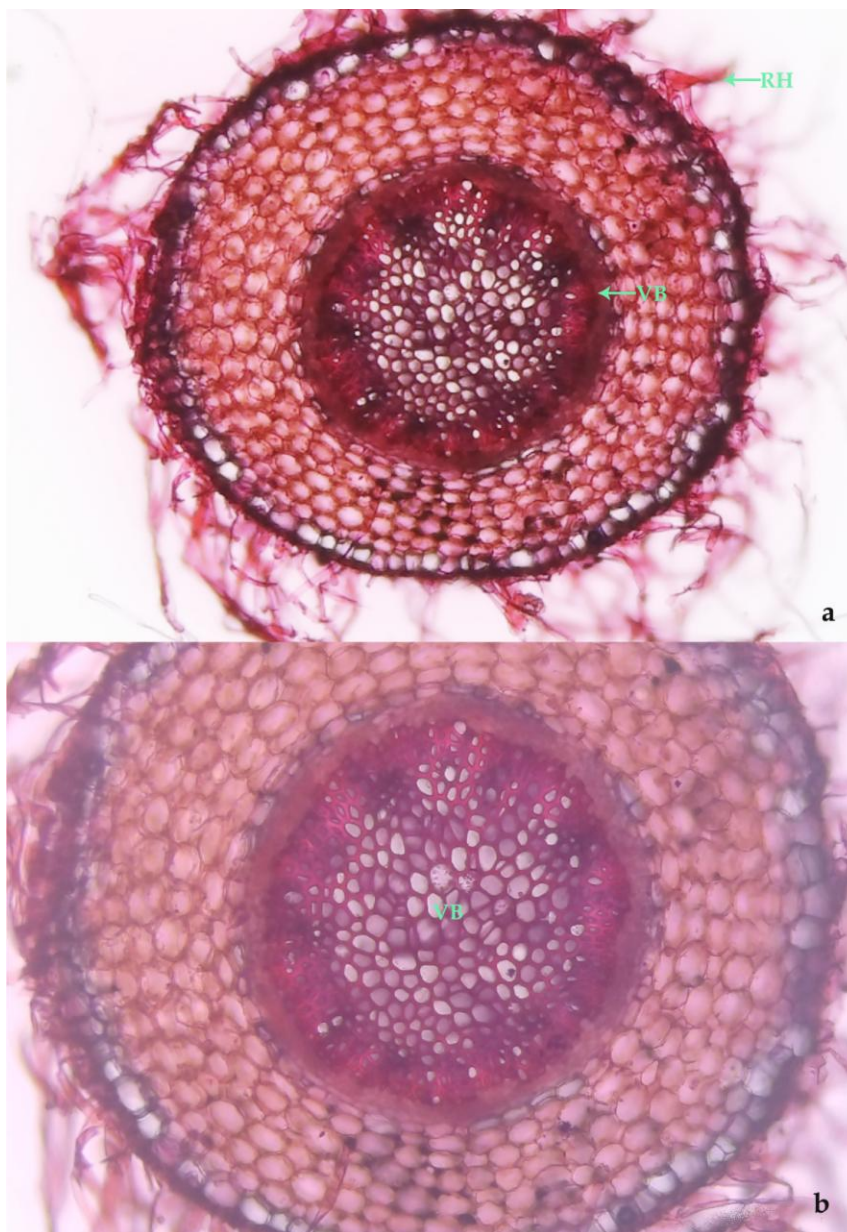
Root

Fig. 3: Cross section of root. RH- Root hair, VB- Vascular bundle

Cross section of the root is circular in outline with numerous root hairs are seen as epidermal outgrowths. Epidermis is composed of rectangular thick walled cells which are highly cuticularised**(Fig. 3).** Ground tissue is composed of thin walled, oval parenchyma cells which are compactly arranged and vascular bundle is embedded in the ground tissue.

Powder

The powder is dark green in colour and revealed various cellular characteristics like prismatic Calcium oxalate crystal, starch grains, smooth trichomes, spiral vessels etc.**(Fig. 4).**These characters can be utilized for standardization of drugs as well as used for preparation of plant monograph and also reduces the possibilities of adulteration when the drug is available in the powdered form(Venkateswarlu&Ganapathy, 2018).
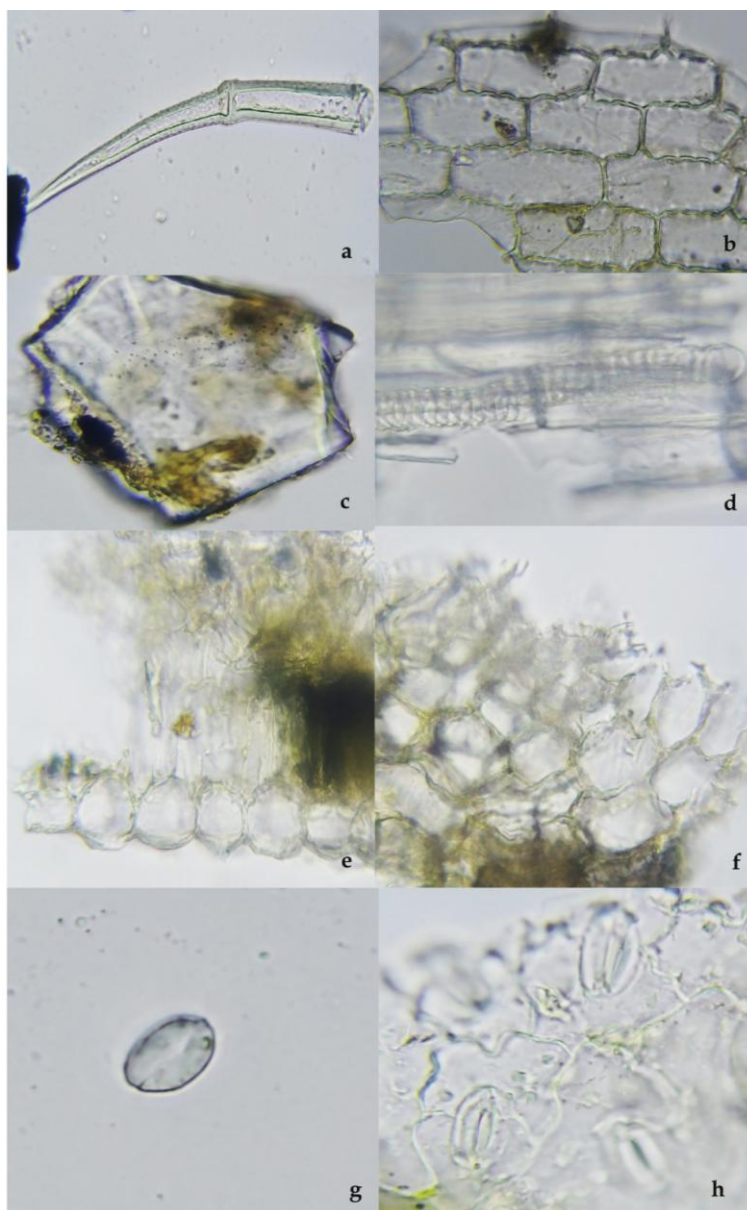
Fig. 4: a: Trichome, b: Epidermal cells, c: Prismatic calcium oxalate crystal, d: Spiral vessel, e: Epidermal and palisade cells, f: Surface view of epidermal cells, g: Starch grain, h: Diacytic stomata

Physicochemical evaluation

The physico-chemical parameters such as loss on drying at 105°C, total ash content, acid insoluble ash and extractive values (water soluble extractive and alcohol soluble extractive) were evaluated and results were tabulated **(Table 1)**. Studies of physicochemical parameters can serve as an important source to judge the purity and quality of crude drugs. Ash values are utilized to establish the quality and purity of the crude drug. It implies the existence of various impurities like carbonate, oxalate, and silicate. The acid insoluble ash comprises mostly silica and indicates contamination with earthy matter(Prasanth et al., 2016). The moisture content of drugs might be at a minimum level in order to suppress the growth of microorganisms like bacteria, yeast or fungi during storage. The extractive values are helpful to judge the chemical constituents present in the crude drug and also assist in the evaluation of particular constituents soluble in a specific solvent. Acid insoluble ash measures the amount of silica present, especially sand(Dave et al., 2010).

Sl. No.	Physicochemical constants	Values (%)
1.	Alcohol soluble extractive	8.54
2.	Water soluble extractive	16.2
3.	Total ash value	10.43
4.	Acid insoluble ash	0.64
5.	Loss on drying at 105 °C	13.05

Table 1: Physico-chemical parameters of L. biflora

HPTLC Fingerprinting

In the present study, the presence of various phytochemical constituents were identified based on the patterns of colour zones in the chromatogram obtained during the HPTLC analysis under three wavelengths of light (254 nm, 366 nm and 575 nm). HPTLC chromatogram of L. biflora is shown in **Fig. 5**. HPTLC fingerprinting profile, R_f values and their corresponding densitograms are given in Fig. 6. The HPTLC fingerprinting analysis revealed several peaks having different R_f values Toluene: Ethyl acetate (6: 2) was the suitable solvent system which resolved various bands on the chromatogram and it indicates various phytochemicals present in the plant.

At 254 nm, Rf value of 0.76 showed maximum area percentage in the form of dark green band. At 366 nm, prominent bands were observed at R_f positions like 0.6, 0.72 and 0.8. inderivatized chromatogram, maximum area percentage were observed at positions like 0.39, 0.52, 0.61, 0.8 and 0.92. It is obvious from **Fig. 5** that 0.61 is the common R_f point at which a prominent band was present at all three wavelength of light and it represent the presence of a specific phytocompound(Kamboj&Saluja, 2017).
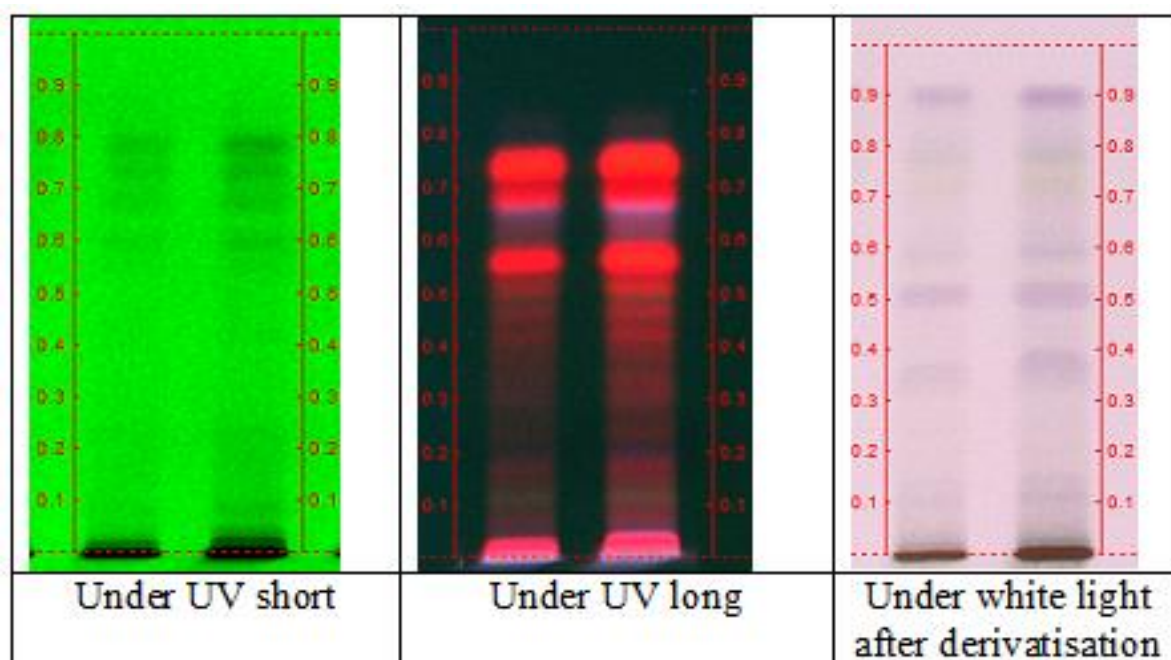

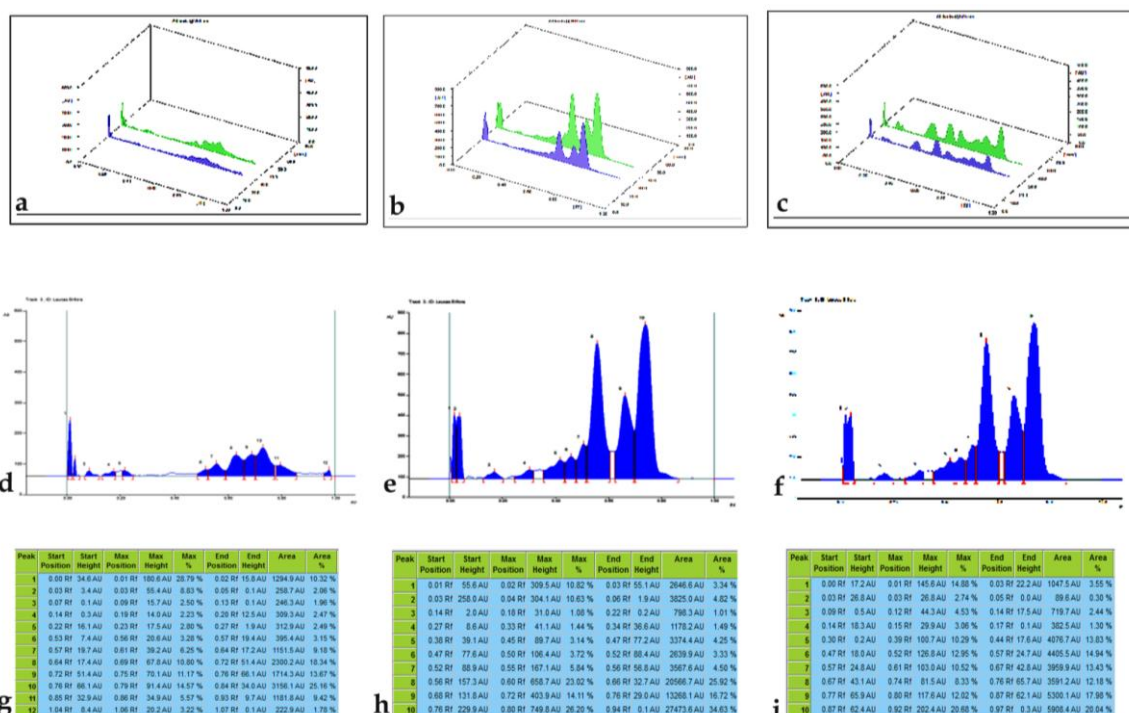**Fig. 5:** HPTLC profile of L. biflora viewed in UV short; UV long and under white light after derivatisation.

Fig. 6: HPTLC finger print profile of L. biflora. a,d,g: under 254 nm, b,e,h: under 366 nm, c,f,i: under 575 nm.

IV. CONCLUSIONS

Since there is no pharmacognostic work documented on this traditionally significant valued drug, the current work had been taken up with a view to lay down standards that could be helpful to establish the authentication of this medicinally important plant

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