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Comparative Pharmacognostical Evaluation And HPTLC Analysis Of Three Different Species Of Bauhinia Leaves.

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ABSTRACT

Bauhinia purpurea L., Bauhinia variegata L., and Bauhinia acuminata L., commonly known as Kanchanar and mountain ebony belongs to family Caesalpiniaceae. The current study was carried out to provide comparative macro-microscopy, physicochemical parameters and HPTLC analysis of three different Bauhinia species. TS of leaf show different outline, variation in the size of ground tissue region as well as size of xylem vessels of three studied species of Bauhinia. The lower cortex region of B. acuminata is wide (6-7 layered) when compared to other species which are 3-4 layered. Bauhinia acuminata L. have large number of simple trichomes on lower surface while B. variegata L. have few trichome and trichomes are absent in B. purpurea L. The trichome size is long in B. acuminata L. when compared to B. variegata L. Quantitative microscopy revealed that B. purpurea L. and B. acuminata L. have paracytic stomata while B. variegata have anisocytic type of stomata. Stomatal index was found to be highest in lower side of B. purpurea L. leaf. Stomatal number is highest in lower side of B. purpurea L. leaf. Vein islet number and palisade ratio is highest in B. acuminata L. leaf. Comparative TLC profile showed presence of some common as well as differentiating bands in hydro-alcoholic extract of different Bauhinia species. However, the chemical markers, viz. lupeol and ursolic acid were present in all three species. The macro-microscopy and TLC profiles may play an important role for identification and quality evaluation of these three medicinally important Bauhinia species.

Keywords: Bauhinia purpurea L., Bauhinia variegata L., Bauhinia acuminata L., Pharmacognosy, HPTLC

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INTRODUCTION

Bauhinia purpurea L., Bauhinia variegata L. and Bauhinia acuminata L., commonly known as Kanchanar and mountain ebony belongs to family Caesalpiniaceae [1] with characteristic butterfly shaped leaves and are used in many traditional medicinal applications [2,3] commonly found in Southeast Asia [4,5,6]. The aerial parts of the plant are reported to contain amino acids, flavonoids, steroids, terpenoids, tannins, lactones, glycolipids, glycosyl steroids, quinines, phenyl fatty ester, β-sitosterol and flavanones from this plant [7,8,9]. All these three plant species have been used in traditional medicine for the cure of rheumatism, fever [10], dropsy, skin diseases, septicemia and diarrhoea, tonic, astringent, dysentery, haemorrhoids, piles, laxative, anthelmintic, antileprotic, antigoitrogenic, obesity, stomatitis, antidote for snake poisoning, dyspepsia, flatulence and as carminative [11, 12, 13]. Simultaneous determination of major flavonoids (apigenin, ursolic acid, rutin, luteolin and quercitrin) in Bauhinia variegata L. has been performed earlier [14]. Paper chromatography of flavonoids showed presence of kaempferol, ursolic acid, and apigenin in B. Acuminate [15]. Phytol, Sesquiterpenoids, β-caroyphyllene and caryophyllene oxide was identified as major constituent in B. acuminata L. leaf oil [16]. Several chemical compounds including palmitic acid, three phthalic acid esters, phthalic acid, gallic acid, ursolic acid were identified from the leaves of B. acuminata [17]. Some researchers have performed the qualitative HPTLC analysis of B. tomentosa L. leaves & flowers [18, 19]. We have earlier performed the simultaneous estimation of four phenolic compounds in B. purpurea L., B. variegata L. and B. acuminata L. flowers and floral buds [20]. However, there has been no study on the comparative pharmacognosy and HPTLC analysis of lupeol and ursolic acid in leaves of three different Bauhinia species. The quantification of lupeol and ursolic acid in hydro-alcoholic fraction has not yet been reported which may be utilized for the proper standardization of these species.

MATERIALS AND METHODS

Plant material

The plant material i.e. leaves of Bauhinia purpurea L., Bauhinia variegata L., and Bauhinia acuminata L. were collected from Lucknow, U.P, India. The plant was identified and authenticated by Dr. Tariq Hussain, CSIR-NBRI. A voucher specimen has been submitted in LWG herbarium.

Macro-microscopical studies

The macroscopy of leaf of Bauhinia species was described with the help of Floras [21]. Qualitative and quantitative microscopy, were done according to the standard methods [22-24].

Sample Preparation

The fresh leaves of different Bauhinia species were collected thoroughly washed with water to remove all debris. The plant material dried below 40°C for 48 hours and powdered by using electric grinder at 60 mesh size. Extraction was performed by soxhlation method. Firstly the powdered plant material was defatted under soxhlet assembly using 250 mL of 98% petroleum ether for 6 hours followed by 9 hours soxhlation of defatted powder by using 250 mL hydro-alcoholic solution (70:30). The final hydro-alcoholic extract obtained was passed through Whatman No. 1 filter paper. The filtrate obtained was concentrated under vacuum in a rotary evaporator at 40°C and stored at 4°C for further use. The dried extracts were dissolved in 98% methanol to obtain a stock solution of 10 mg/mL, which were used for application of spots on HPTLC plates.

Physicochemical and Phytochemical Studies

Physicochemical and Phytochemical studies like extractive values, total ash, acid insoluble ash, total sugar, starch, tannin, and phenols were calculated from the shade-dried and powdered plant material [25, 26].

Preparation of Standard Solutions

Stock solutions of lupeol and ursolic acid were prepared separately by dissolving those 0.1 mg/mL in methanol.

Development of HPTLC Fingerprinting of lupeol and ursolic acid

Instrumentation and Chromatographic Conditions

The following were the instruments and chromatographic conditions used. Spotting device: Linomat V automatic sample applicator; CAMAG (Muttenz, Switzerland), Syringe: 100 µL Hamilton (Bonaduz, Switzerland). TLC chamber: glass twin trough chamber (20 × 10 × 4 cm); CAMAG. Densitometer: TLC Scanner 3 linked to winCATS software V.4.06; CAMAG. HPTLC plates: 20 × 10 cm, 0.2 mm thickness precoated with silica gel 60 F254; E. Merck (Darmstadt, Germany). Experimental conditions: temperature, 25±2°C; relative humidity, 40%. Solvent system: toluene–ethyl acetate–formic acid (8:2:0.1). Detection wavelength: 650 nm for lupeol and ursolic acid. Visualization reagent: Anisaldehyde-Sulphuric acid; Slit dimension: 5.00 × 0.45 mm. Scanning speed: 10 mm s^{-1} and source of radiation: deuterium lamp.

RESULTS AND DISCUSSION

Standardization is an important tool for herbal drugs in order to establish their identity, purity, safety and quality. In order to standardize three different Bauhinia species various macroscopic, microscopic, physico-chemical, quantitative phytochemical estimations and HPTLC analyses were performed. Microscopy is one of the cheapest and simplest methods to start with establishing the correct identification of drug.

Morphological & Microscopical Studies

The morphological and microscopical study of the leaf enables to identify the aerial parts of different Bauhinia species. Comparative macroscopy of the leaf of three Bauhinia species **(Fig. 1)** is given in **Table 1** while comparative microscopical details **(Figs. 2, 3 & 4)** are given in **Table 2**. Results showed that macro-microscopical features give the identification markers [27] viz. size and shape of leaf macroscopically however, TS of leaf show different outline, variation in the size of ground tissue region as well as size of xylem vessels of three studied species of Bauhinia.

Table 1: Comparative macroscopy of three studied Bauhinia species

B. purpurea L.	B. variegata L.	B. acuminata L.
Leaves simple alternate, base rounded to shallow-cordate upto 12cmx12cm deeply 2 lobed at apex to 1/3-1/2 ca 7-12 cm long and surface smooth and glabrous and 9 or 11 nerved at the base the apex lobes rounded or obtuse or subacute, minute stipules 1-2 mm long, petioles, puberculous to glabrous 2.5-3.5 cm long	Leaves deciduous 4-6 inch across and rounded with lobed ends and heart shaped bases leaves are shaped a little like a cows hoofs Leaves have minute stipules 1-2mm, early caduceus, petiole puberulous to glabrous 3-4cm, lamina broadly ovate to circular, often broader than long 6-16cm diameter, 11-13 nerved, tips of lobes broadly rounded base cordate upper surface glabrous lower gloucous but glabrous when fully grown but glabrous	Bilobed, shaped like an ox hoof they are 6 to 15 cm long and broad with apical cleft upto 5cm deep the petiole is 1.5 to 4cm long

Table 2: Comparative microscopy of three studied Bauhinia species

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B. purpurea L.	B. vaerigata L.	B. acuminata L.
Midrib region is circular with comparatively small but irregular protuberance on the lower surface. Upper and lower epidermis single layer covered with thick striated cuticle Collenchymatous cell are absent Ground tissue is very compressed about 2-3 layer Bundle sheath cell are 3-4 layer but smaller in size Similar, except xylem vessels are comparatively larger and phloem region is comparatively narrow. Trichome absent	In the transverse section midrib region is circular in outline with large protuberance on the lower surface Similar Below the epidermis 1-2 layer of collenchymatous cells Ground tissue is comparatively compressed 3-4 cell layered cells are circular in shape. Bundle sheath sclerenchymatous 4-6 cell layer thick Stealer region is circular in shape consist of bicolletral open vascular bundle. Xylem consist of vessels and tracheids, cambium present in between xylem and phloem. Trichome unicellular very few on lower surface	Midrib region is pair shape in outline Similar Collenchymatous cell are absent Ground tissue is comparatively broader 4-5 layer with large size circular cells Similar to B. Variegata L. Stealer region is circular in shape consist of bicolletral open vascular bundle. Xylem consist of vessels and tracheids, cambium present in between xylem and phloem Trichomes uni or multi cellular, densely covered the lower surface

Figure 1 Leaves of three different *Bauhinia* species

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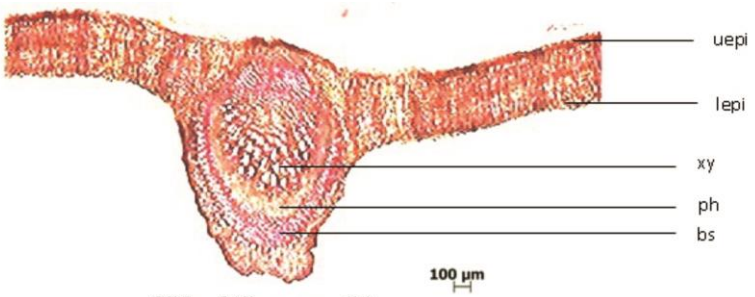
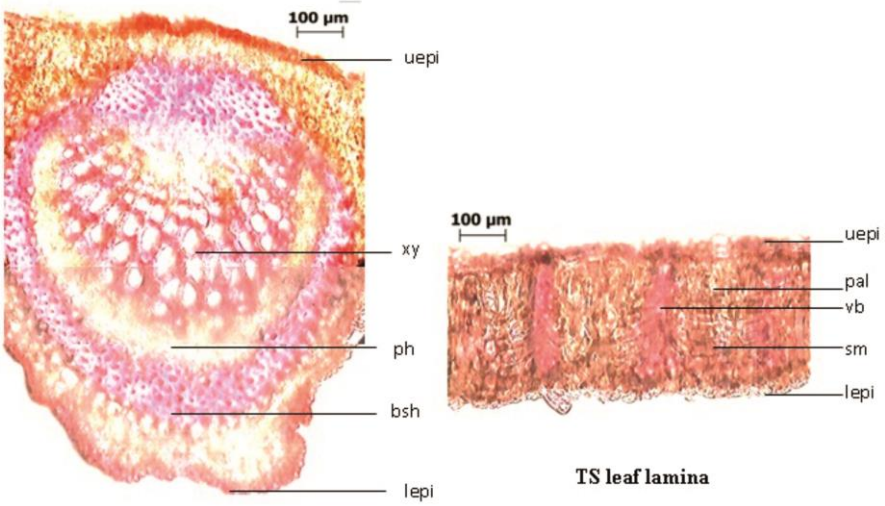


TS Leaf (diagrammatic)



TS leaf midrib

TS leaf lamina

Figure 2 Transverse Section of *Bauhinia Purpurea* L. Leaf

Abbreviations uepi, upper epidermis; bsh, bundle sheath; ph, phloem; xy, xylem; lepi, lower epidermis; pal, palisade layer; sm; spongy mesophyll.

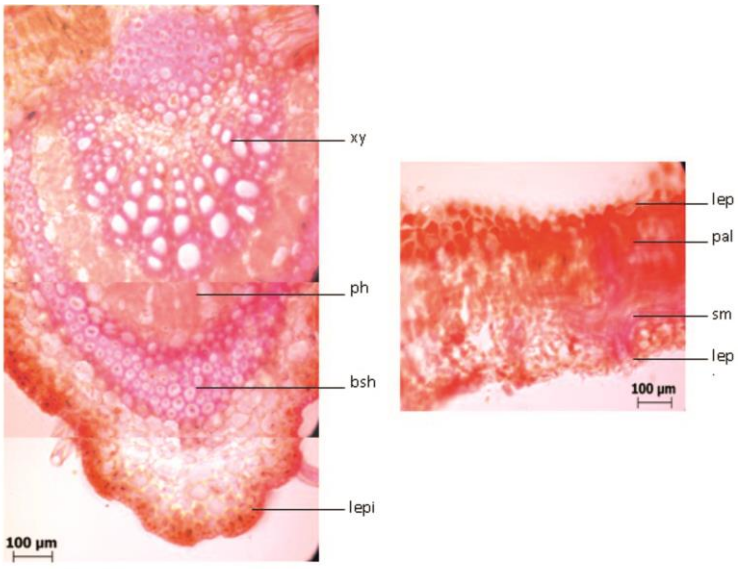


Figure 3 Transverse Section of *Bauhinia variegata* L. Leaf

Abbreviations uepi, upper epidermis: st, simple trichome; bsh, bundle sheath; ph, phloem; xy, xylem; lepi, lower epidermis; pal, palisade layer; sm; spongy mesophyll.

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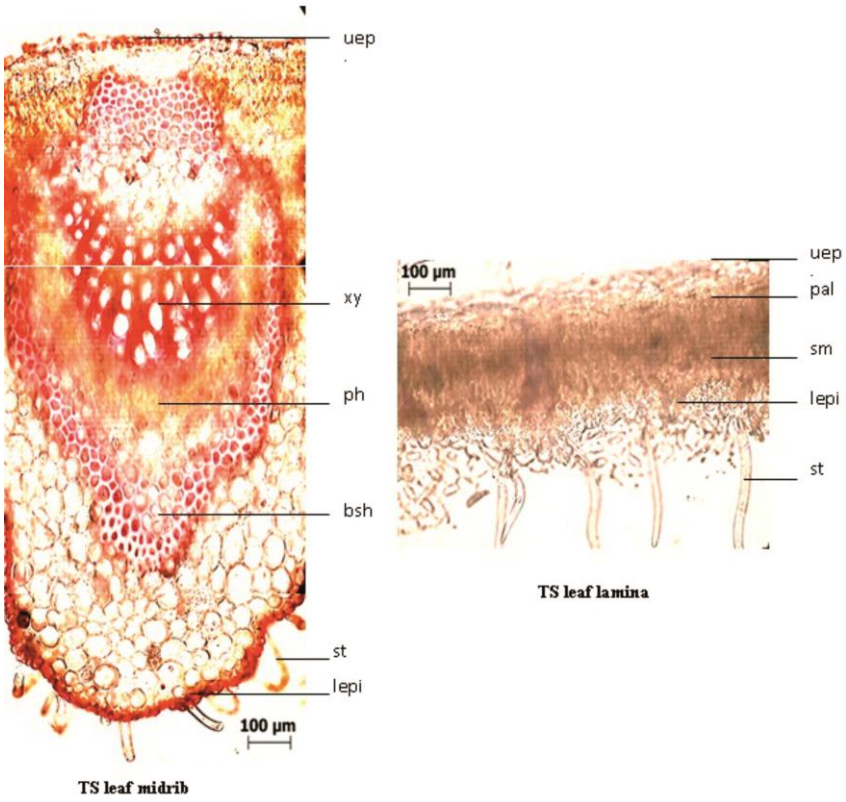

Figure 4 Transverse Section of *Bauhinia acuminata* L. Leaf

Abbreviations uepi, upper epidermis: st, simple trichome; bsh, bundle sheath; ph, phloem; xy, xylem; lepi, lower epidermis; pal, palisade layer; sm; spongy mesophyll.

The transverse section of leaves of selected Bauhinia species showed various distinct features. The lower cortex region of B. acuminata L. is wide (6-7 layered) when compared to other species which are 3-4 layered. B. acuminata have large number of simple trichomes on lower surface while B. variegata L. have few trichome and trichomes are absent in B. purpurea L. The trichome size is long in B. acuminata L. when compared to B. variegata L.In quantitative microscopy (Figure 5) B. purpurea L. and B. acuminata L. have paracytic stomata while B. variegata have anisocytic type of stomata. Vein islet number and palisade ratio was found to be highest in B. acuminata L. leaf, Table 3.

Table 3: Quantitative microscopy of different Bauhinia species

Plant name	Stomata type	Stomatal index	Stomatal no.	Vein islet no.	Palisade ratio
Bauhinia purpurea	Paracytic & Anisocytic	72.65-75.22	170-180	20-22	5-6
		87.23-90.43	190-200		
Bauhinia variegata	Anisocytic	70.47-72.55	160-170	20-22	5-6
		85.78-90.45	180-190		
Bauhinia acuminata	Paracytic	50.56-52.65	100-110	25-27	6-7
		55.75-60.23	120-130		

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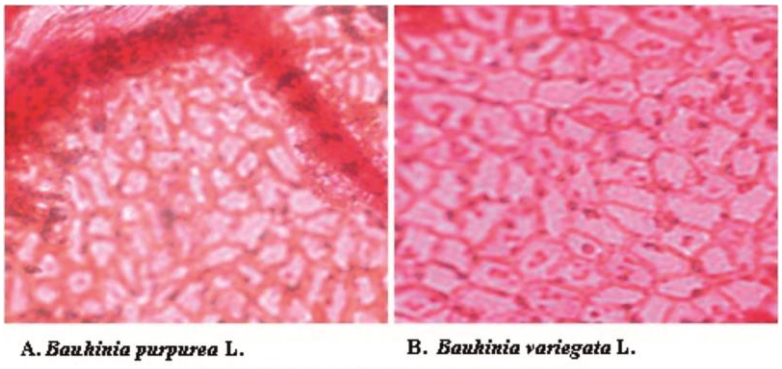

A. *Bauhinia purpurea* L. B. *Bauhinia variegata* L.

C. *Bauhinia acuminata* L.

Figure 5 Leaf surface showing vienation and stomata

Physico-chemical & Phytochemical Studies

The phytochemical research approach is considered effective in discovering bioactive profile of plants of therapeutic importance. During the present study samples were subjected to quantitative phytochemical screening of various phytochemicals by adopting standard methodology. Quantitative evaluation of total sugar & starch revealed the highest amount of total sugar, starch, phenol & flavonoid content in B. purpurea L. followed by B. variegata L. & B. acuminata L. Phytochemicals play an important role when used in food supplements as antimicrobial agents as well as antioxidants. Parameters such as moisture content, extractive values (Water and alcohol soluble), total ash and acid insoluble ash values, total sugar, total starch, total phenolics and total flavonoids were determined (**Fig. 6)**.

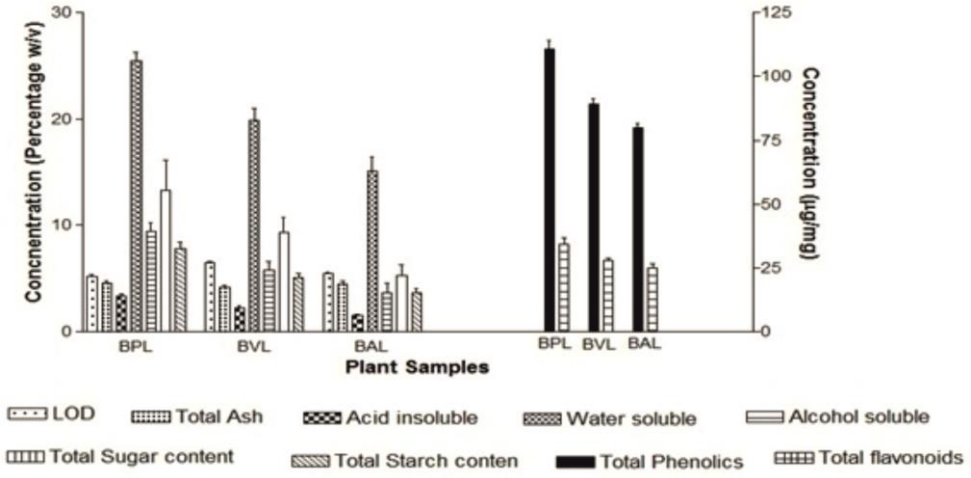

Figure 6 Physico-chemical & Phytochemical evaluation of three Bauhinia species (BPL: Bauhinia purpurea L., leaves; BVL: Bauhinia variegata L., leaves & BAL: Bauhinia acuminata L. leaves)

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HPTLC Studies

In this study mobile phase consisting of toluene: ethyl acetate: formic acid in the ratio of 8: 2: 0.1 v/v/v demonstrated compact spots with good resolution between other peaks of the extract in which lupeol and ursolic acid were quantified and are represented in **Table 4.** HPTLC chromatogram and densitograms were obtained from standard compounds and hydro-alcoholic fraction (**Fig. 7**) and both targeted compounds were identified by retention factor (R_f) and overlay UV-spectrum. The concentration of lupeol and ursolic acid in hydro-alcoholic fraction of B. purpurea L., B. variegata L. & B. acuminata L., are represented in **Table 4**.

Table 4: Quantification of lupeol and ursolic acid in different Bauhinia species

Plant Sample	Lupeol (%)	Ursolic acid (%)
BPLM	0.041	0.11
BVLM	0.049	0.08
BALM	0.19	0.17

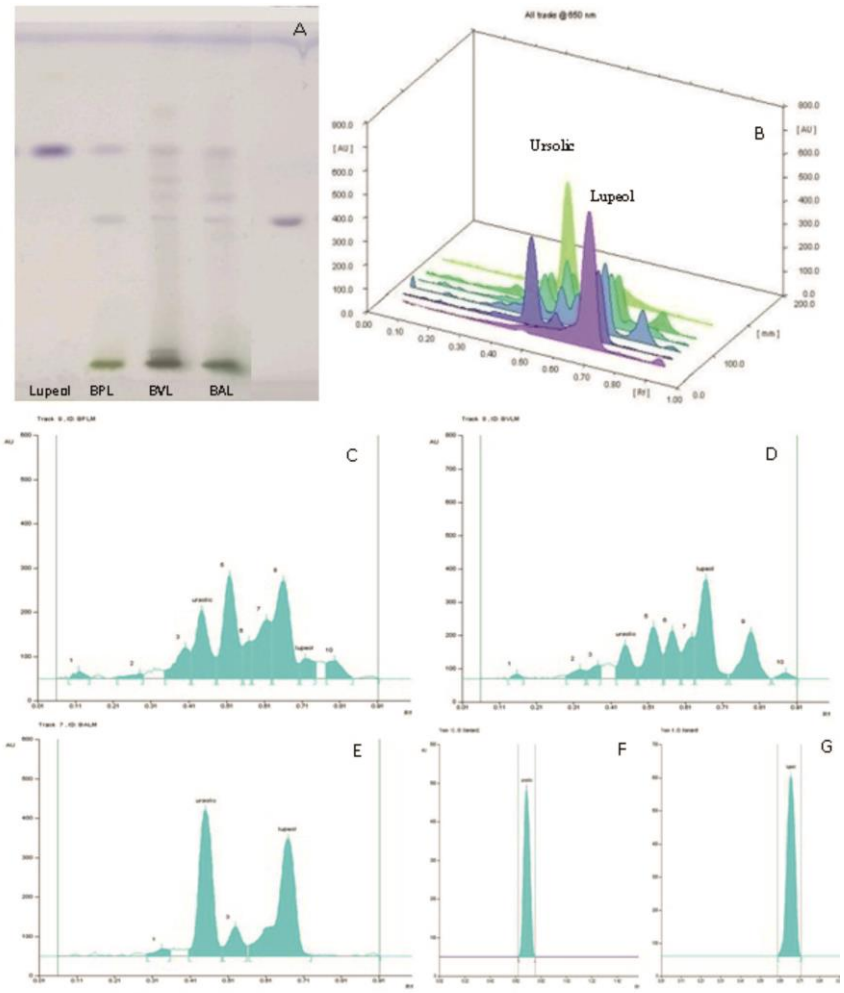

Figure 7 (A) HPTLC plate showing tracks of standard markers and samples

(B) Hptlc densitogram showing peaks of samples with Lupeol & Ursolic acid (C) Peaks of BPLM

(D) Peaks of BVLM (E) Peaks of BALM (F) Peak of Std. Lupeol (G) Peak of Std. Ursolic acid

CONCLUSIONS

The macroscopical & microscopical parameters reported here can be considered as distinctive enough to identify and decide the authenticity of reported Bauhinia species in herbal industry. Ash values, loss on drying, extractive values and quantitative phytochemical estimations were performed to standardize the crude plant materials. The results were established for the proper identification & to check the purity of the selected plant species. A HPTLC analytical method has been developed for the simultaneous determination of lupeol and ursolic acid in hydro-alcoholic fraction of different Bauhinia species. The method can be used to determine the purity of the Bauhinia available from various sources by detecting the related impurities as well as for quality control of herbal formulations containing Bauhinia leaves as ingredient. HPTLC analysis has indicated the presence of optimum amount of lupeol and ursolic acid in the samples. This can be used in the pharmaceutical industry as a pharmacognostical tool to identify these medicinally important plant species. In addition it can be adopted as a chemo-taxonomical tool in the plant systematic and can also help the manufacturers in identification and selection of the raw material for herbal drugs.

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