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A report on pharmacognostical evaluation of four *Adiantum* species, Pteridophyta, for their authentication and quality control

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Abstract: *Genus Adiantum* L., Pteridaceae, is an important fern used in traditional systems of medicine. Different species of *Adiantum* are known as avenca in Brazil; hansraj/hanmspadi in India; maiden hair fern in English. The present study aims to develop the quality control parameters of four similar looking *Adiantum* species viz. *A. capillus-veneris* L., *A. lunulatum* Burm. f., *A. peruvianum* Klotzsch, and *A. venustum* D. Don. Standard methods for macro-microscopic evaluation, physico-chemical parameters and HPTLC were used for authentication and identification. The salient distinctive characters under the microscope are the presence of slightly wavy elongated epidermal cells in *A. capillus-veneris*; epidermal cells strongly wavy in *A. lunulatum*; star shaped epidermal cells and mixed spores of regular and irregular shaped in *A. peruvianum*; stomata on both the surfaces of pinnule, absence of spinulus spores only in *A. venustum*. In addition, rachis anatomy showed different cellular and stellar characteristics as identifying characters of aforesaid four *Adiantum* species. Physico-chemical parameters and HPTLC finger print profiles along with stigmasterol and lupeol play significant role for the quality evaluation of raw drugs. The above finding will serve the purpose of quality control and assurance for the future studies.

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Introduction

Genus *Adiantum* L., Pteridaceae, has been used from ancient times medicinally, being mentioned by Dioscorides. It is antidysenteric, antiulcer (Puri & Arora, 1961), antihypoglycemic (Jain & Sharma, 1967), antimicrobial (Mahmoud et al., 1989; Reddy et al., 2001; Singh et al., 2008), antitumor (Hartwell, 1968; 1969), antiviral (Husson et al., 1986) and also used as astringent, demulcent, diuretic, emmenagogue, expectorant, tonic (Anonymous, 1985; Chopra et al., 1956; De Feo, 1992; Jain & Tarafdar, 1970; Kirtikar & Basu, 2005; Lokar & Poldini, 1988; Singh, 1973). The traditional uses of *Adiantum* species known to be for respiratory problems such as cough cold, fever, pneumonia, and mucous formation (Anonymous, 2004; Chopra et al., 1956; Kirtikar & Basu, 2005). However, during the survey it was observed that different species of *Adiantum* are being reported under the various vernacular names as avenca in Brazil; hansraj/ hanmspadi in India; maiden hair fern in English. Therefore, quality control markers are required for all similar looking *Adiantum* species for their validation and authenticity. In this context four *Adiantum* species viz. *A. capillus-veneris* L., *A. lunulatum* Burm., *A. peruvianum* Klotzsch., and *A. venustum* D. Don were studied. A number of phyto-constituents were reported in aforesaid *Adiantum* species like β-sitosterol, stigmasterol, compesterol (Marino et al., 1989), oleanane compounds (Nakane et al., 2002), astragalin, isoquercitine, rutin (Yoko & Masso, 1969), triterpenoids, keto-alcohol, α-carotene monoepoxide, leucopelargonidin, kaempferol, and quercitin glucosides (Rangaswami & Iyer, 1967).

Hence, realizing the importance or requirement of *Adiantum* in medical world, an attempt was made to evaluate the important four *Adiantum* species viz. *A. capillus-veneris, A. lunulatum, A. peruvianum* and *A. venustum* through macroscopic description, microscopic characteristics of pinnule and rachis, powder microscopy, physico-chemical parameters and HPTLC fingerprint profile. In addition, qualitative and quantitative estimation of stigmasterol and lupeol in these four *Adiantum* species was also done through high performance thin layer chromatography (HPTLC).

Materials and Methods

Plant material

Aerial parts of the four *Adiantum* species were collected from fern house of NBRI during the months of September and October 2009, their identity was confirmed by Dr. P. B. Khare after referring the identifying key characters of different species from floras and books (Borthakur et al. 2000; Khullar, 1994; Manickam & Irudayaraj, 1992). The samples were deposited in the Herbarium of the Institute wide voucher specimen number LWG-229703, LWG-229717, LWG-229612, and LWG-229707 for *A. capillus-veneris, A. lunulatum, A. peruvianum,* and *A. venustum*, respectively. The materials were dried at 40 °C in a hot-air oven, and then stored at 25 °C in an air-tight container.

Chemicals

Lupeol and stigmasterol standards were procured from Sigma, St. Louis, MO (USA). Methanol, toluene, ethyl acetate, anisaldehyde, glacial acetic acid, and sulphuric acid were AR grade, SD Fine, Mumbai (India).

Macroscopic and microscopic analysis

The macroscopy of *Adiantum* species was described with the help of floras and books. Hand/ Cryostat sections of 20-60 µm thickness in transverse (TS) view were taken and microscopy of the fronds and rachis were studied according to the methods of Brain & Turner (1975a) and the microscopic analysis of powder was done according to the method of Brain & Turner (1975b) and Kokate et al. (1986). The pinnules of all the samples were boiled separately with saturated chloral hydrate solution (2.5 g mL^{-1}) for surface studies. Leaf constant i.e. stomatal number and palisade ratio was studied according to the method of Evans (2003).

Physico-chemical analysis

Air dried plant material was used for the quantitative determination of ash and extractive values (WHO/QCMMPM guidelines, 1992).

Chromatographic analysis

For the preparation of the extract, the aerial parts of dried *Adiantum* species were powdered and sieved through 44 meshes. The powdered sample (10 g) was exhaustively extracted with methanol (4 x 25 mL, each time for 15 min) under reflux on a water bath at 100 °C. The extracts were filtered through Whatman no. 1 filter paper (separately for each sample), concentrated under reduced pressure, and lyophilized (Wagner, 1996; Khatoon et al., 2009). A solution (10 mg mL^{-1}) of these extracts was prepared in methanol for quantification of stigmasterol and lupeol.

Separate stock solutions (0.1 mg mL^{-1}) of lupeol and stigmasterol standards were prepared. Standard solutions for calibration containing 10-60 µg mL^{-1} were prepared. HPTLC was performed on 10 cm × 20 cm Higlachrosep plates coated with 0.2 mm layers of nano silica containing UV 254 fluorescent indicator (S.D. Fine Chemicals, India). Samples (20 µL) and standards (10 µL) were applied to the plates by means of a Camag (Switzerland) Linomat 5 sample applicator. The plates were developed to a distance of 8.0 cm with 20 mL toluene:ethylacetate (8:2) as mobile phase, in a Camag twin-trough chamber previously saturated with mobile phase vapor for 30 min at 24 °C. After removal from the chamber, plates were completely dried in air at room temperature (24 °C) and documented under UV 254 nm and UV 366 nm. The plates were dipped in anisaldehyde sulphuric acid, dried and heated at 110 °C for 5 min and documented in visible light after derivatization. The plates were scanned and quantified at 600 nm by using Camag TLC Scanner 3 with Wincats 3.2.1 software. The calibration graph was plotted and linear regression equation was calculated with the help of aforesaid TLC scanner software. The spectra were also recorded for stigmasterol and lupeol terpenoids.

Results and Discussion

Adiantum capillus-veneris

Macroscopic description of fronds

Fronds were of varying sizes, 10-28 cm long. Stipes were erect, usually 8-15 cm (up to 40 cm) long, glabrous, shining and paleate at base. Lamina was broad, ovate-deltoid in outline, 2-3 pinnate and 15-20 × 10-12 cm, rachis zigzag, and bearing 2-4 loosely branches on either side. Pinnules were 'fan shaped', or sub rectangular and limate dimidiate, base cuneate, 1.5-2.0 × 0.5-1.0 cm, outer margin cut up into 3-5 primary lobes, rounded, petioles usually short, thin black and wiry. In fertile pinnules, usually one sorus occured per secondary lobe of the upper margin. Sori elliptic or linear placed in roundish sinuses of the crenations; sporangia globose, small and short stalked, 20-21 celled annulus (Figure 1A).

Microscopic characteristics of fronds

Under the microscope, pinnule showed epidermal cells slightly wavy, elongated on both the

surfaces, stomata were present on lower surface only, anomocytic; stomatal number 0-2 per mm^2, mesophyll parenchymatous, 2-3 layered, cells mostly oval and elongated with large intercellular spaces (Figure 2).

Microscopic characteristics of rachis

TS rachis was almost circular with one end slightly flattened in shape. It showed thick cuticle followed by single layered epidermis. Hypodermis consists of 2-3 layers of sclerenchyma followed by compact collenchymatous ground tissue. At the centre, a bean shaped monostele was surrounded by single layered endodermis. Xylem present in the form of two arcs shows plectostelic and exarch condition which was surrounded by phloem (Figure 3A).

Powder

It showed groups of slightly wavy elongated epidermal cells, stomata, groups of tracheids, fragments of sporangia, and spores - brown coloured, regular, trilete, tetrahedral, non-perinate with smooth exine (Figure 4).

Adiantum lunulatum

Macroscopic description of fronds

Fronds were 15-30 × 7.5 cm. Stipes 10-15 cm long, tufted, wiry, naked, polished dark chestnut-brown, often elongated and scaly at base. Lamina simple pinnate, 12-30 cm long, rachis with a broad terminal pinna or sometimes extended with an apical vegetative bud. Pinnules semi-orbicular, subdimidiate, outer margin rounded almost entire with very shallow and remote, shallowly lobed into 4-8 lobes, each lobe may be further lobed, lower margin straight or slightly concave, the lower edge nearly in line or oblique with the petiole. Petioles of the lower ones were spreading, 6-13 mm long, texture herbaceous, the rachis and both surfaces naked. Sori in continuous line along the margin of the lobe which was slightly depressed from the general outline, crescent shaped, cup to 2 mm wide. Sporangia stalked with annulus 15-17 celled long (Figure 1B).

Microscopic characteristics of fronds

Under the microscope, pinnule showed epidermal cells somewhat elongated with strongly wavy margins. Stomata were present on the lower surface only, anomocytic; stomatal number varied 10-16 per mm^2, palisade ratio varied 12-13, followed by spongy parenchyma (Figure 2).

Microscopic characteristics of rachis

TS of rachis showed an adaxial groove and abaxial convex outline. The single layered epidermis was highly cutinized. Hypodermis consists of 2-4 layers of sclerenchyma followed by parenchymatous ground tissue. Monostele was located near the notch like a plate. Most of the part of protostele was occupied by xylem, protoxylem being at the both ends and metaxylem occupying the centre showing exarch condition which was patched up by less cells of phloem in the form of ceratenchyma. The cells at notch region were little stretched (Figure 3B).

Powder

It showed groups of strongly wavy somewhat elongated epidermal cells, stomata, fragments of sporangia, groups of annulus cells, fibers, and tracheids and spores - dark brown, trilete, tetrahedral, non-perinate with smooth margins (Figure 4).

Adiantum peruvianum

Macroscopic description of fronds

Fronds were large, 60-100 × 40-80 cm, obliquely horizontal with the upper part spreading, broadly ovate-deltoid in outline. Stipe was 30-50 cm long, black, polished and densely scaly at base. Lamina was 3-4 pinnate, rachis black, wiry and polished. Pinnules were petiolate and loosely placed, 5-8 × 3-6 cm, lower margin of pinnules straight or slightly concave and smooth; base smooth, convex; upper margin smoothly curved towards the base, becoming slightly concave and almost parallel to lower margin to a distance equal to the length of latter and then curved to an acute or blunt point from where it sharply turned backwards, running in a straight line to join the lower margin, sori occurred all over the upper margin except the extreme tip, sporangia long stalked, with an annulus approximately seventeen cells long (Figure 1C).

Microscopic characteristics of fronds

Under the microscope, pinnule showed star shaped epidermal cells with strongly wavy margins. Stomata were present on the lower surface only, anomocytic; stomatal number varied 15-20 per mm^2, palisade ratio varied 8-10, followed by spongy parenchyma (Figure 2).

Microscopic characteristics of rachis

TS rachis was gibbous shaped. It showed highly cutinized single layered epidermis. Hypodermis

consists of 3-5 layers of sclerenchyma followed by many layered collenchymatous ground tissue with circular and hexagonal compact cells. At the centre, 'V' shaped monostele was located. The xylem strands in each of the lateral arms of the V-shaped bundle was curved and hooked at the end, which appears as a sea horse and so was called hippocampus shape. A band of fibres was embedded in the xylem at both the broad ends of Phloem surrounds the xylem in protostele. Phloem surrounds the xylem (Figure 3C).

Powder

It showed groups of star shaped strongly wavy epidermal cells, stomata, trilete spores, sporangia embedded with spores, groups of fibers, and tracheids, spores - trilete, tetrahedral mixed with irregular shaped (Figure 4).

Adiantum venustum

Macroscopic description of fronds

Fronds were 20-25 cm long. Stipe was deep-brown, 10-20 cm long, erect and scaly at base but glabrous and glossy at above. Lamina was 3-4 pinnate, deltoids, rachis glossy and secondary rachis usually spreading. Pinnules were small, generally 0.5-0.75 cm across, ovate, cuneate, rarely subrhomboid accuminate, with two lateral margins, outer margin smoothly curved, and some shallowly lobed into 2 or 3 rounded, prominently dentate segments, each dentation with one veinlet entering it, venation prominent, and texture leathery. Sori occurred on the margin of pinnule notches. Sori 1-3 per pinnule, restricted to the outer margin. Sporangia stalked, 17-22 celled annulus, spores light brown (Figure 1D).

Microscopic characteristics of fronds

Under the microscope, pinnule showed elongated epidermal cells with somewhat dentated margins. Stomata were present on both the surfaces, anomocytic; stomatal number varied 8-10 per mm^2 on the lower surface and 2-5 per mm^2 on the upper surface. Mesophyll was not differentiated in palisade

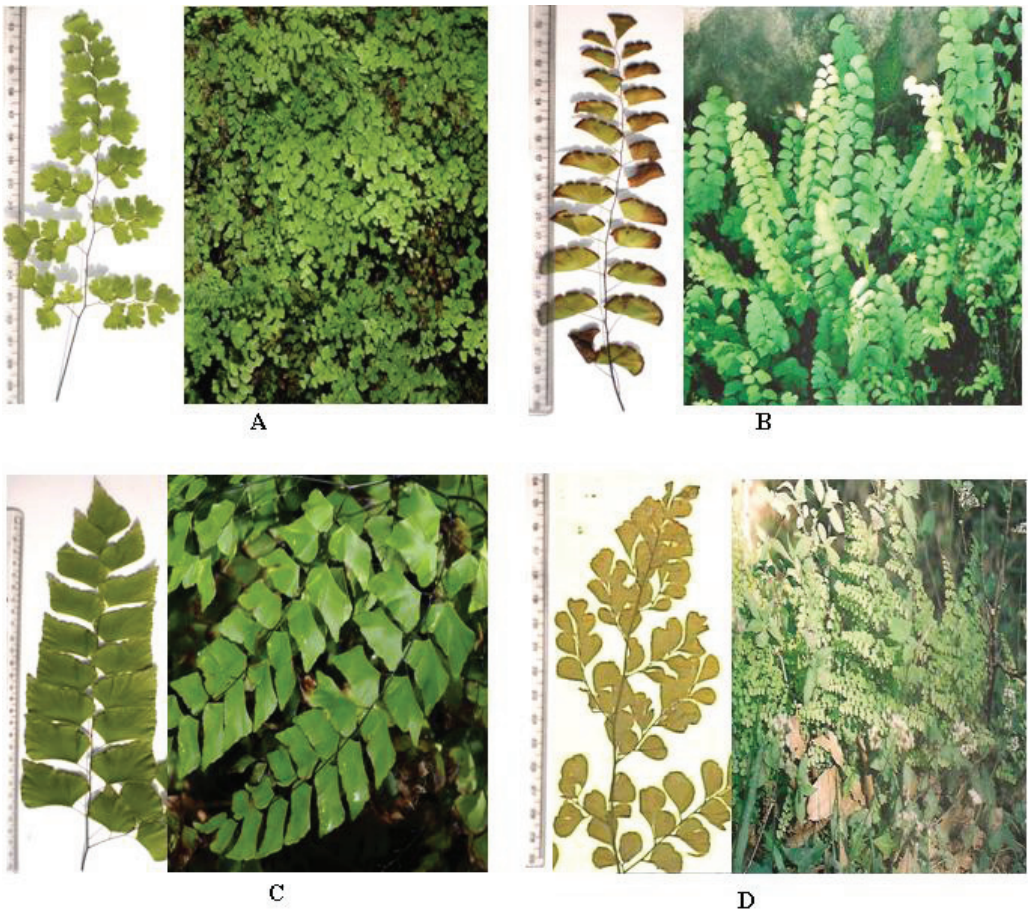

Figure 1. Frond and whole plant of *Adiantum* species. A. *A. capillus-veneris* L. B. *A. lunulatum* Burm. C. *A. peruvianum* Klotzsch. D. *A. venustum* D. Don.

and spongy parenchyma, parenchymatous cells oval to circulars with intercellular spaces (Figure 2).

Microscopic characteristics of Rachis

TS rachis was almost circular with two wild depressions. It showed thick cuticle followed by single layered epidermis. Hypodermis consists of 2-4 layers of sclerenchyma followed by collenchymatous ground tissue. Endodermis was thick walled. Below endodermis thin walled delicate tissues were present which breaks when cut the sections. The soft pericycle and cells of stellar region were damaged. A bean shaped monostele attached to the side leaving hollow remaining portion. Phloem surrounds the xylem in protostele (Figure 3D).

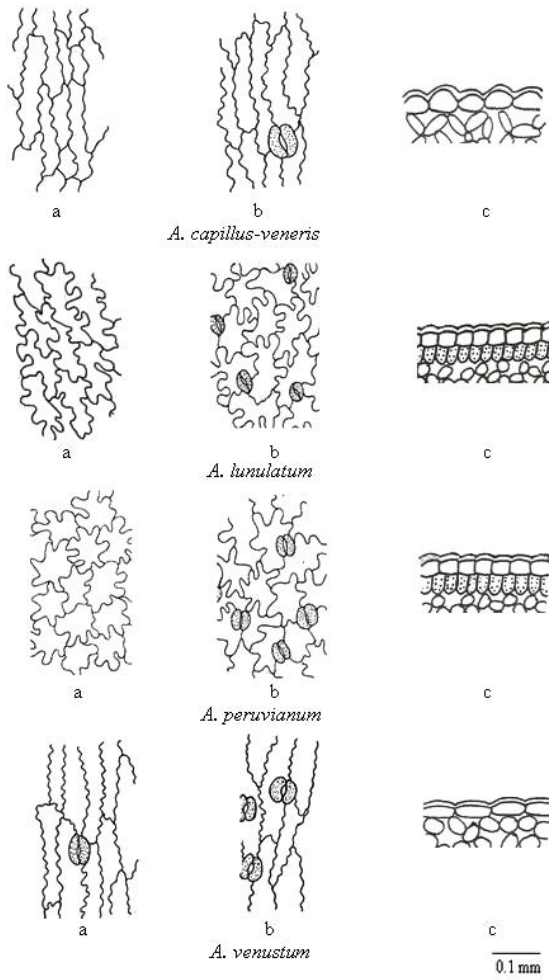

Figure 2. Microscopy of pinnule of *Adiantum* species; a. Upper surface; b. Lower surface; c. Epidermal cells and mesophyll in transverse view.

Powder

It showed groups of elongated epidermal cells with dentated margin, stomata, annulus cells, fibers, and tracheids, sporangia filled with spores, spores- light brown, spinulus, non-perinate (Figure 4).

In all the investigated *Adiantum* species morphological attributes were quite distinctive such as branching of fronds, type of lamina, rachis, shape and size of pinnules, distribution of sori and sporangia etc. The branching of a fern fronds and lamina play an important role for pteridophyte's taxonomy. In the case of genus *Adiantum* L. the identification key of species was also based on the frond branching and lamina (Borthakur et al. 2000; Manickam & Irudayaraj, 1992). From the studied four Adiantum species only *A. lunulatum* with simple pinnate fronds while remaining three *i.e. A. capillus-veneris, A. peruvianum*, and *A. venustum* varied to 2-4 pinnate. In *A. capillus-veneris* lamina was 2-3 pinnate and in *A. peruvianum* and *A. venustum* it was 3-4 pinnate. Similarly, the stipe of *A. capillus-veneris* was paleate at base while scaly at base in *A. lunulatum, A. peruvianum*, and *A. venustum*. The shape and margins of the pinnules were also characteristic to each species *e.g*. fan shaped, 3-5 lobed, rounded in *A. capillus-veneris*; semi-orbicular, 4-8 lobed, each lobe sometimes further lobed, limate subdimidiate in *A. lunulatum*; curved to an acute or blunt point from where it sharply turned backwards, margins straight or slightly concave, smooth with convex base in *A. peruvianum*; shallowly 2-3 rounded lobs with prominently dentate segments in *A. venustum.*

Likewise, the distribution of sori on the fertile pinnule is very important diagnostically. The distribution pattern of sori in afore said four species was very peculiar for their identification and quality evaluation. Sori elliptic or linear, placed in roundish sinuses of the cremations in *A. capillus-veneris*, continuous along the margin of the lobe in *A. lunulatum*, all over the upper margin except the extreme tip of fertile pinnule of *A. peruvianu*m, and 1-3 per pinnate, restricted to the outer margin in *A. venustum.* The sporangia are the bodies in which the spores are produced. Sporangia small and short stalked in *A. capillus-veneris* and *A. lunulatum* and long stalked in *A. peruvianum* and *A. venustum*. Sporangia dehiscence is caused by a region of cells with thick inner walls and thin outer walls, usually in a ring, called the annulus. The number of annulus cells also varied from species to species *i.e*. 20-21, 15-17, 16-17 and 17-22 in *A. capillus-veneris, A. lunulatum, A. peruvianum*, and *A. venustum* respectively.

As far as the microscopic investigations are concerned, the salient distinctive surface characters of pinnule were type of epidermal cells, stomata, and mesophyll tissues. Epidermal cells were elongated in *A. capillus-veneris, A. lunulatum*, and *A. venustum* while star

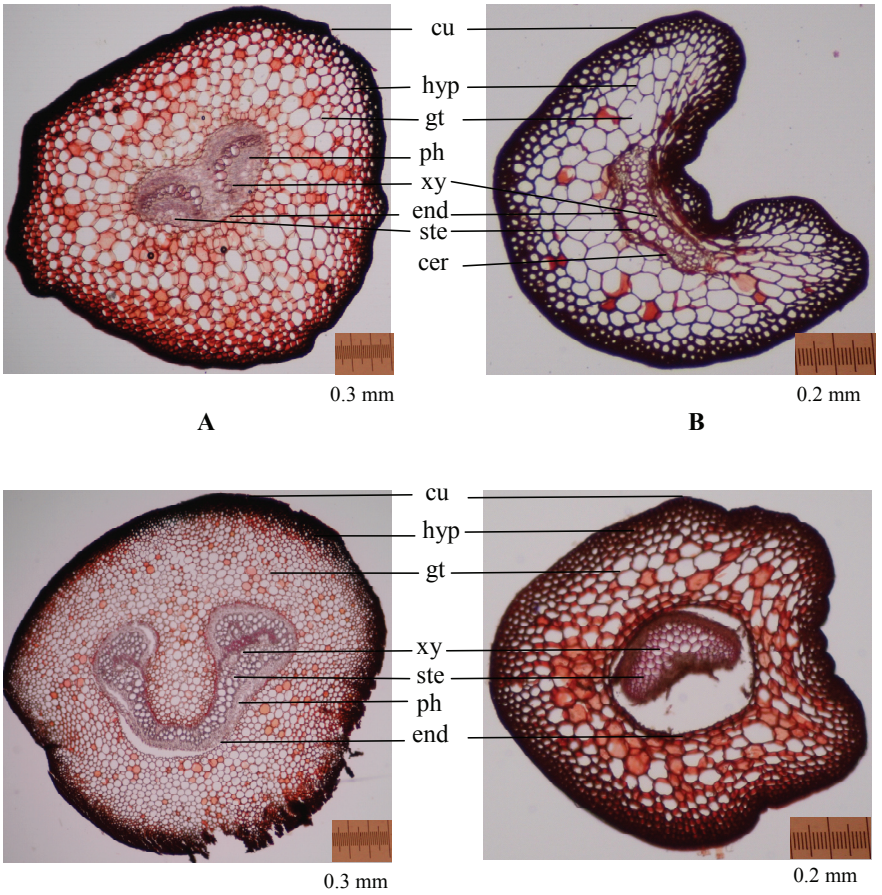

Figure 3. TS Rachis of *Adiantum* species. A. *A. capillus-veneris* L.; B. *A. lunula*tum Burm; C. *A. peruvianu*m Klotzsch; D. *A. venustum* D. Don.; cer: ceratenchyma; cu: cuticle; end: endodermis; gt: ground tissue; hyp: hypodermis; ph: phloem; ste: stele; xy: xylem.

shaped in *A. peruvianum*. The margins of epidermal cells were also varies from species to species viz. slightly wavy in *A. capillus-veneris*; strongly wavy in *A. lunulatum* and and dentate in *A. venustum*. Stomata were present in all the species but their distribution pattern varies *i.e.* only on lower surface 0-2, 10-16 and 15-20 in *A. capillus-veneris, A. lunulatum*, and *A. peruvianum* respectively. In contrast, stomata were present on both the surfaces of *A. venustum* with a frequency of 2-5 on upper surface and 8-10 on lower surface. The mesophyll was differentiated in palisade and spongy parenchyma in *A. lunulatum* and *A. peruvianum* but parenchymatous with intercellular spaces in *A. capillus-veneris* and *A. venustum.*

The transaction of rachis of all the investigated species showed thick cuticle, single layered epidermis, sclerenchymatous hypodermis and monostele although the shape varies from species to species *i.e.* almost circular with one end slightly flattened in *A. capillus-veneris,* adaxial grooved and abaxial canvex outline in *A. lunulatum*, gibbous shaped in *A. peruvianum*, almost circular with two wild depressions in *A. venustum*. Ground tissues were collenchymatous in *A. capillus-veneris, A. peruvianum*, and *A. venustum* but parenchymatous in *A. lunulatum*. The shape of monostele was also varied from species to species and was the characteristics of aforesaid four *Adiantum* species.

Powder microscopy also plays an important role in pharmacognostic evaluation and sometimes may be an identifying parameter of herbal drugs. The fragments of epidermal cells of pinnules, sporangia with annulus and spores were the identifying parameters in the powder microscopy of *A. capillus-veneris, A. lunulatum, A. peruvianum*, and *A. venustum e.g.* brown coloured, regular, trilete, tetrahedral, non-perinate with smooth exine in *A. capillus-veneris;* dark brown, trilete, tetrahedral, non-perinate with smooth margins in *A. lunulatum*; trilete, tetrahedral mixed with irregular shaped in *A. peruvianum*; and light brown, spinulus, non-perinate in *A. venustum*.

From the ongoing studies it is quite evident that aforesaid macroscopical and microscopical attributes of fronds, rachis, pinnules, and sporangia showed clear cut

identifier for different species of *Adiantum* L. and will provide the identification markers up to the specific level of crude drug Avenca or Hansraj/Hanmspadi.

Physico-chemical analysis

Air dried material was used for quantitative determination of physiochemical values. Extractive values (alcohol and water soluble) and ash values (total ash and acid insoluble) were determined for six times and it mean±SE was recorded (Table 1). Water soluble extractive was found to be very high when compared to other extractable matter in the drug.

HPTLC fingerprint profile

The common as well as differentiating bands were useful in identification and authentication of herbal drugs. Therefore, HPTLC profile of aerial parts of all the four species of *Adiantum* L. were studied (Figure 5) and it was found that some common bands at R_f 0.34, 0.52, 0.64, and 0.67 (all red) under UV 366 nm and at R_f 0.63 and 0.80 under UV 254 nm were present in all the four species. However, *A. capillus-veneris* could be clearly differentiated from other species by the presence of characteristic blue colored band at R_f 0.57 under UV 366 nm. On the contrary, a red coloured band at the same R_f 0.57 was observed in other three species viz. *A. lunulatum, A. peruvianum* and *A. venustum*. Likewise, *A. lunulatum* and *A. peruvianum* resembled to each other in having similar additional bands at R_f 0.22 and 0.27 under UV 366 nm and at R_f 0.74 under UV 254 nm. Similarly, a characteristic band at R_f 0.41 was observed only in *A. peruvianum* under UV 366 nm (Figure 5).

The bands of stigmasterol and lupeol were visible at R_f 0.40 and 0.58 respectively. The identity of

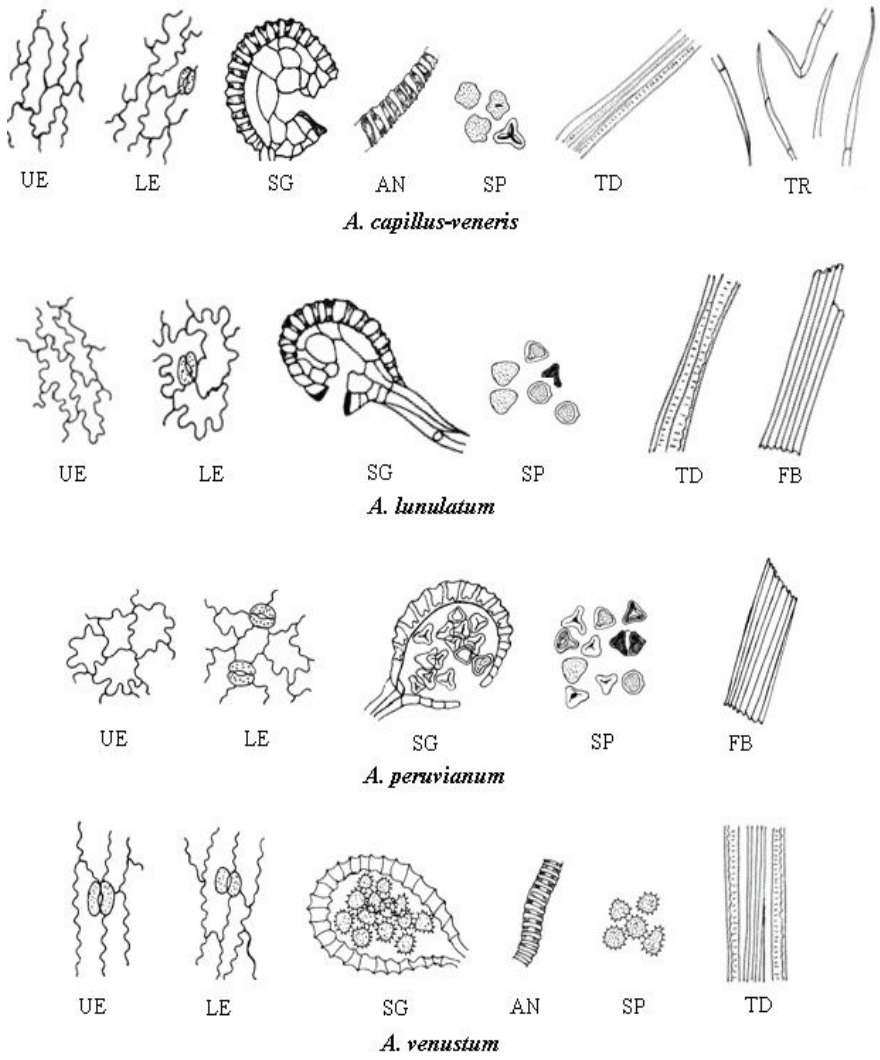

Figure 4. Fronds powder of *Adiantum* species under the microscope; UE: upper epidermal cell; LE: lower epidermal cells; SG: sporangia; AN: annulus cells; SP: spores, FB: fiber; TD: trachied.

Table 1. Physiochemical parameters of four *Adiantum* species, Pteridaceae.

Samples	Extractive values %		Ash values % (w/w)	
	Alcohol soluble	Water soluble	Total ash	Acid insoluble ash
A. capillus-veneris	3.20±0.50	8.86±0.22	16.28±0.35	1.21±0.43
A. lunulatum	4.35±0.56	7.21±0.45	14.93±0.20	1.99±0.32
A. peruvianum	5.61±0.61	18.07±0.56	12.61±0.02	1.56±0.01
A. venustum	9.42±0.66	11.04±0.29	8.09±0.14	1.86±0.36

Values are mean %±SD (n=6 per sample.

Table 2. Quantitative evaluation of stigmasterol and lupeol in four *Adiantum* species, Pteridaceae, by HPTLC.

Samples	Yield of extract	Stigmasterol	Lupeol
	g±SD	%±SD	%±SD
A. capillus-veneris	2.93±0.011	1.469±0.005	-
A. lunulatum	1.58±0.014	0.697±0.008	0.174±0.004
A. peruvianum	1.69±0.012	0.498±0.006	0.064±0.006
A. venustum	1.27±0.010	0.351±0.008	-

Values are mean %±SD (n=3 per sample.

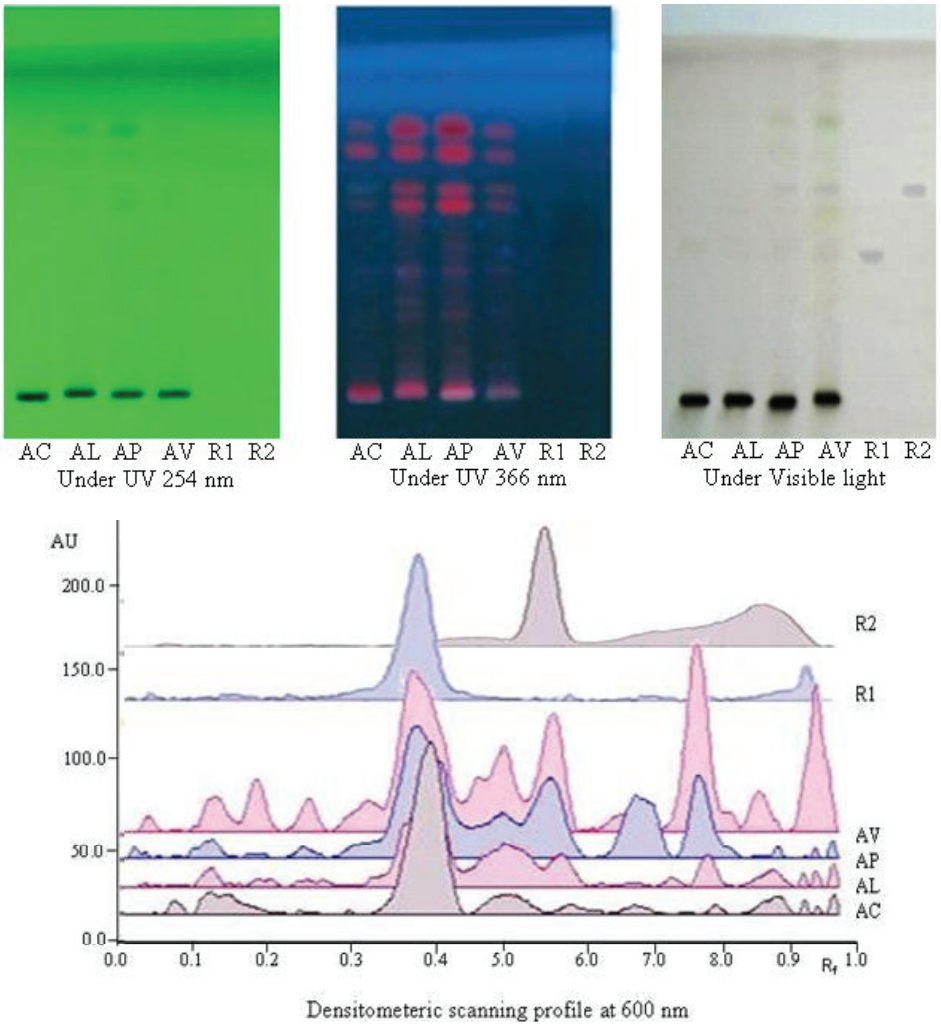

Figure 5. HPTLC fingerprint profile of methanolic extract of four *Adiantu*m species. AC: *A. capillus-veneris*; AL: *A. lunulatum*; AP: *A. peruvianum*; AV: *A. venustum*; R1: stigmasterol; R2: lupeol.

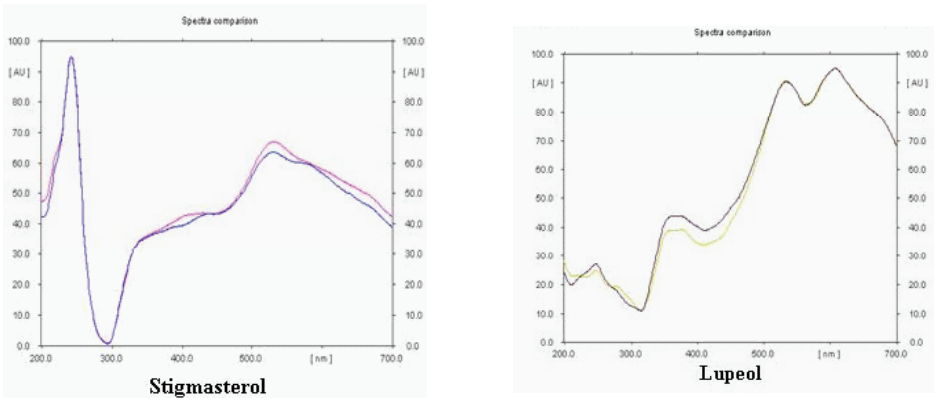

Figure 6. Overlaid absorption spectra of reference terpenoids and the corresponding peak from the extract of *Adiantum* species.

both the terpenoid bands from the sample extracts were confirmed by overlaying absorption spectra obtained from stigmasterol and lupeol standard solutions with the samples (Figure 6). It is interesting to note that stigmasterol was present in all the species while lupeol was observed only in *A. lunulatum* and *A. peruvianu*m. Besides, the variation in the amount of aforesaid markers was also noticed in different *Adiantum* species. Stigmasterol was estimated highest in *A. capillus-veneris* (1.469%) and lowest in *A. venustum* (0.351%). Likewise, the quantity of lupeol was 0.174% in *A. peruvianum* and 0.064% in *A. venustum* (Table 2).

Conclusions

Adiantum L. Pteridaceae, is a medicinally important fern and attributed to the traditional drug Avenca/Hanmspadi/Hansraj. Different species of *Adiantum* L. are being reported or sold under these vernacular names. It is a controversial drug and hence a well established quality control and identification parameters are highly essential for this traditional drug. In the present communication, the macroscopical and microscopical findings will lay down the standards which will be useful for the detection of the identity and authenticity of particular *Adiantum* species. The fronds type, shape, size, type of epidermal cells, distribution pattern of stomata, palisade tissue, rachis characteristics, sporangia, spores, physico-chemical parameters and HPTLC finger print profiles along with stigmasterol and lupeol play significant role for the quality evaluation of *A. capillus-veneris* L., *A. lunulatum* Burm., *A. peruvianu*m Klotzsch., and *A. venust*um D. Don. for the authentication of valid raw material and future studies.

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Authors' contributions

SS (Research Scholar) contributed in section cutting, running the laboratory work and data analysis. SK designed the study and contributed to microscopic description, HPTLC finger printing and drafted the paper. HS contributed to literature survey and physico-chemical analysis. SKB contributed to plant collection, herbarium confection and macroscopic description. PBK and AKS supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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