ORIGINAL ARTICLE

Comparative pharmacognosy of medicinal plant species used as *Prsniparni*

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Background: Substitution or adulteration of a particular genuine drug with other species due to demand exceeding the supply of the original species, is rampant in the present trade scenario. As a result, proper authentication of the drug for safe administration as an herbal medicine assumes paramount significance. **Aim:** *Prsniparni, Uraria picta* (Jacq.) DC., is one such drug for which three different botanical entities are commonly used as substitutes, namely *U. lagopodoides* (L.)DC; *Desmodium gangeticum* (L.) DC., and *Pseudarthria viscida* (L.) Wight and Arn.; all belonging to the family *Fabaceae.* The anatomical, histochemical and powder microscopic characters of the four species were compared in the field-collected samples to validate the quality herbal drug and to find the similarity and dissimilarity of the substitute species. **Materials and Methods:** Histological and histochemical characters were studied using sectioned materials following standard protocols. Histochemical methods were adopted to localize the presence of the primary metabolites such as starch, lipids, total proteins and amino acids and the secondary metabolites such as volatile oils, resins, tannins, lignin and pectin. **Results:** The present study shows that the authentic species *U. picta* and substitute species *U. lagopodoides* showing higher similarities of 90% based on histology, histochemistry and powder microscopy analysis. Other two candidates, *D. gangeticum* and *P. viscida* showing 60 % and 55% similarities, respectively, when compared to *U. picta.* Thus, the similarity matrix were developed using characters based on anatomical, histochemical and powder microscopy. **Conclusion:** Ayurvedic texts suggest use of substitute herbs for the rare species. The substitution is proved to be logical by our studies that *U. lagopodoides* can be used as a substitute species in the place of *U. picta* under *Prsniparni* and also the present study validates the genuinity of the drug by anatomical, histochemical as well as powder microscopy characters to quality checking of the raw drug.

Key words: Ayurveda, *Desmodium gangeticum*, histochemistry, powder microscopy, *Pseudarthria viscida*, similarity matrix, *Uraria lagopodoides, Uraria picta*

INTRODUCTION

The annual demand of botanical raw drugs in India was estimated to be 3,19,500 Metric Tons (MT) during the year 2005-06 amounting to a trade value of Rs. 1,069 crores.[1] This demand however, is often not met with the supply of the original raw drugs alone and is topped up with other species of plants that are substitutes or adulterants. As a result, the quality of herbal products in many cases may become compromised. In Ayurvedic medicine, "*Dasamula*" (10 roots) plants are a top traded group and their annual demand is >1000 MT.[1] *Dasamula* group of plants are integrated in a number of Ayurvedic formulations like *Dasamula Rasnadikwath, Dasamuladi ghrita, Dasamularishta, Dasamuladikwatha,* and *Dasamula Haritaki leha.*[2] Roots of *Prsniparni* are one of the 10 ingredients of the *Dasamula* group of plants.[3] *Uraria picta* (Fam. *Fabaceae*) is an original *Prsniparni* species[4] while in trade or use *Uraria lagopodoides* (L.) DC.,[3] *Desmodium gangeticum* (L.)DC.[4] and *Pseudarthria viscida* Wight and Arn.,[5] are observed. Roots of *Prsniparni* are used in formulations other than of *Dasamula* of Ayurveda such as *Amrtarishta, Sirah, suladi, vajra, rasa,* etc., and in many instances also used as single drug (*Dasamula taila, Dasamularishta*).[3] The therapeutic properties of *Prsniparni* are analgesic, anti-inflammatory and wound healing.[6] In order to characterize and compare the drugs used as *Prsniparni* in the raw drug markets, the candidate species traded under the name *Prsniparni* were subjected to morphological, anatomical, histological, histochemical and powder microscopic studies.

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MATERIALS AND METHODS

Collection and Authentication of Samples

Dried and fresh roots of *U. picta, U. lagopodoides, D. gangeticum* and *P. viscida,* were collected from different locations of India. Collected plant samples were authenticated and each sample was assigned with a specific laboratory identification number (FRLH IDs) as indicated in Table 1. Voucher specimens

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were deposited in the Herbarium and Raw Drug Repository (FRLH)-Institute of Ayurveda and Integrative Medicine (I-AIM), Bangalore, India.

Morphology

All root samples were subjected to macroscopical analysis based on shape, size (thickness) color, odor and taste.

Microscopy

Transverse sections

Portions of fresh roots as well as those preserved in Formalin-Acetic Acid-Alcohol (FAA; 40% Formalin – 5 ml, 50% Ethanol – 90 ml, Glacial Acetic Acid – 5 ml)[7] were used for the study. The samples were soaked in water before sectioning. Transverse sections were taken and stained according to the standard protocols.[8] Stains was prepared using either safranin (0.5% in distilled water) or TBO [Toluidine Blue O 0.05% in benzoate buffer (benzoic acid 0.25 g, sodium benzoate 0.29 g in 200 ml water), pH 4.4].[7] The stained sections were washed with water, mounted on clean slide, observed under the microscope (Olympus BX 41, Tokyo, Japan) and the required photographic images were captured using a digital Olympus camera fixed with the microscope; the processing of the images was done using the Image Pro Express 6.0.

Powder microscopy

Powder microscopy was done on coarse powder of 1 mm size (Bureau of Indian Standards. Mesh no 16).[9] The prepared powder was examined for specific microscopic characters. The powders were macerated further with Jeffery's maceration fluid (1:1 of 10% nitric acid and 10% chromic acid mixed in a beaker and heated in water bath until a bleaching effect was observed).[9] Remaining acid was decanted and the bleached Powder fragments were repeatedly washed with water and neutralized by adding a few drops of ammonium hydroxide. The macerated powder was then stained in TBO or safranin and observed under Olympus BX 41 microscope for powder characters.

Histochemical studies

Portions of fresh roots, and roots preserved in FAA were used. The samples were soaked in water before taking the sections. Using a sharp blade, transverse sections were taken. The sections were stained using specific reagents (Lugol's iodine; TBO; Fast Green FCF; Sudan Red; Ferric chloride, Phloroglucinol, Lugol's iodine and Sulphuric acid; Ruthenium red) to observe and locate starch, polyphenols, total proteins and amino acids, fats, oils, volatile oils and resins, tannins, lignin, pectin as per the protocols.[7] The stained sections were then washed in water to remove the excess stain and observed for microscopical features. Similar staining methods were followed for powders (which were not macerated). Photographic images were captured as above.

RESULTS AND DISCUSSION

Morphology

The dried and fresh roots of *U. picta*, *U. lagopodoides*, *D. gangeticum*, and *P. viscida* [Figure 1] were subjected to morphological study.

Figure 1: Morphology of the roots respectively of: (a) *Uraria picta*; (b) *U. lagopodoides*; (c) *Desmodium gangeticum* and (d) *Pseudarthria viscida*

Table 1: Details of samples taken up for study

Species	FRLH ID	Source
Uraria picta	L/09/12/035	Jabarra MPCA, Dugli range, Dhamtari, Chhatisgarh, Orissa
Uraria picta	L/09/11/030	MPCA, Tharbaaz, Nawali, Orissa
Uraria lagopodoides	L/09/11/017	Vellapparkovil hill range, Aundipatti Taluka, Theni district, Tamil Nadu
Uraria lagopodoides	L/09/07/042	Balaghat, Madhya Pradesh
Uraria lagopodoides	L/09/11/023	Rampur, Kalabandi, Orissa
Desmodium gangeticum	L/09/07/025	Savandurga forest, Magadi, Karnataka
Desmodium gangeticum	L/09/11/012	Vellapparkovil hill range, Aundipatti Taluka, Theni district, Tamil Nadu
Desmodium gangeticum	L/09/11/018	Vellapparkovil hill range, Aundipatti Taluka, Theni district, Tamil Nadu
Pseudarthria viscida	L/09/07/026	Savandurga forest, Magadi, Karnataka
Pseudarthria viscida	L/09/11/014	Kurangani, Muthuvarkudi (Tribal hamlet), Tamil Nadu
Pseudarthria viscida	L/09/11/024	Nayagarh Forest Development Area, Orissa

The roots of all the four species were tap roots. *U. picta* roots were thicker (26 mm) as compared to those of *U. lagopodoides* (18 mm), *D. gangeticum* (12 mm) and *P. viscida* (10 mm). The branching was more in roots of *P. viscida* as compared to the other three roots. *U. picta* and *U. lagopodoides* were light brown in color while those of *D. gangeticum* and *P. viscida* were darker brown. Dried roots of *U. picta* had a characteristic muddy odor while the other three had an agreeable herbaceous odor. The roots of *U. lagopodoides* and *P. viscida* were slightly sweet while *D. gangeticum* had a bitter taste and that of *U. picta* was bitter with slight sweetness.

Anatomy

Microscopic features of transverse sections of all the species used as *Prsniparni* are summarized in Table 2 and Figures 2 and 3. The table compares the anatomical characters of the four *Prsniparni* candidates. Presence of pith is observed only in the root of *P. viscida*. The two species of *Uraria* have a ring porous wood while the other two have a diffuse porous. There is difference in the type of parenchyma exhibited by the two *Uraria* species as compared to those of *P. viscida* and *D. gangeticum*. Both the *Uraria* species show only apotracheal banded axial parenchyma while *D. gangeticum* and *P. viscida* show apotracheal banded as well as paratracheal aliform type.

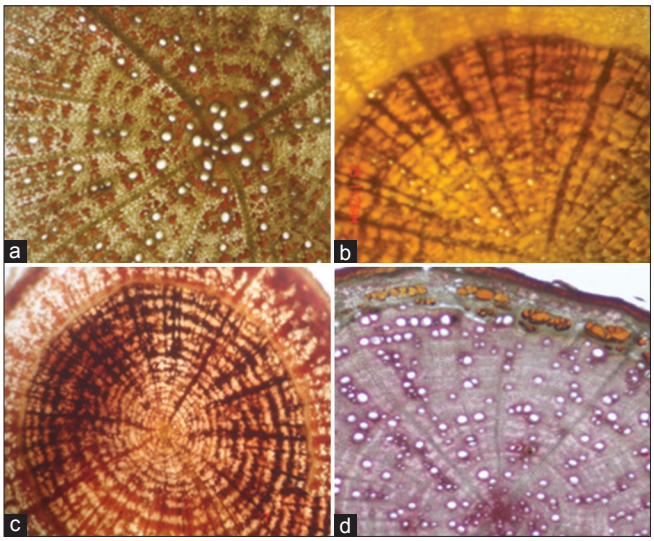

Figure 2: Anatomy of the roots respectively of: (a) *Uraria picta*; (b) *Ulagopodoides*; (c) *Desmodium gangeticum* and (d) *Pseudarthria viscida*

Powder Microscopy

The details obtained on *Prsniparni* candidates using powder microscopy studies are tabulated in Table 3. The powder study of all the four candidates reveals the similar characters like the presence of libriform fibers, cork cells and columnar sclereids [Figures 4-6].

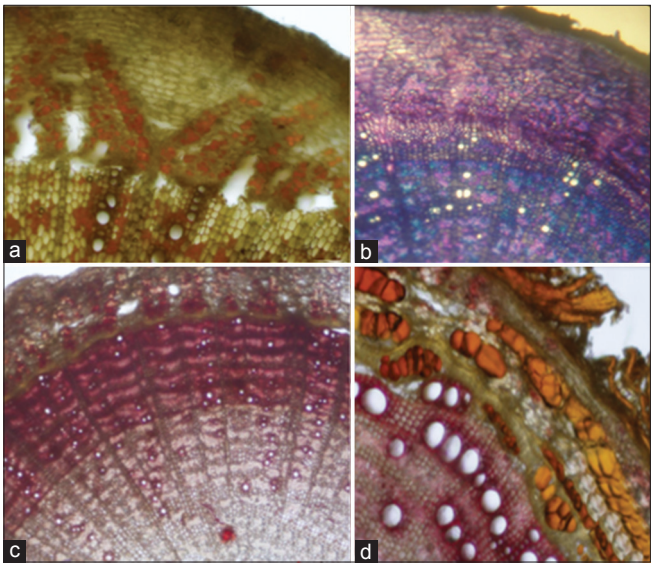

Figure 3: Anatomical structure of the peripheral parts of the roots respectively of: (a) *Uraria picta*; (b) *U. lagopodoides*; (c) *Desmodium gangeticum* and (d) *Pseudarthria viscida*

Table 2: Comparison of the features seen in the transverse sections of the four species used as *Prsniparni*

Character	*Uraria picta*	*Uraria lagopodoides*	*Desmodium gangeticum*	*Pseudarthria viscida*
Pith	Absent	Absent	Absent	A narrow pith present
Primary xylem	4-5 protoxylem groups	4-5 protoxylem groups	4-5 protoxylem groups	4-5 protoxylem groups
Secondary xylem	Ring porous	Ring porous	Diffuse porous	Diffuse porous
Axial parenchyma	Apotracheal banded type	Apotracheal banded type	Apotracheal and occasional paratracheal aliform banded type	Apotracheal and occasional paratracheal aliform banded type
Ray parenchyma	1-3 cell thick with contents, probably phenols	1-4 cell thickness. Starch grains seen	1-2 cell thick ray cells contain starch and tannins	1-2 cell thick cells contain starch grains
Fibers	Groups forming concentric discontinuous rings	Groups forming concentric discontinuous rings	Seen in groups	Seen in groups
Phloem	Secondary phloem outside secondary xylem	Secondary phloem outside secondary xylem	Secondary phloem on the outside of cambium	Secondary phloem on the outside of cambium
Secondary cortex	Uniformly parenchymatous	Uniformly parenchymatous	Parenchymatous with groups of fibers scattered	Parenchymatous with groups of fibers scattered
Cork	Stratified	Stratified	Stratified	Stratified
Parenchyma cell contents	Starch grains and phenols	Starch grains	Starch grains, tannins, phenols	Starch grains

Table 3: Powder characters of the *Prsniparni* candidates

Character	*Uraria picta*	*Uraria lagopodoides*	*Desmodium gangeticum*	*Pseudarthria viscida*
Starch grains	Simple	Simple	Simple	Simple and compound (2-3 grains together)
Fibers	Libriform, tips blunt	Libriform, tips narrow	Libriform, tapering towards tips	Libriform, tips tapering
Cork cells	Cells lignified, radial arranged parenchyma cells	Cells lignified, radial arranged parenchyma cells	Cells lignified, radial arranged parenchyma cells	Cells lignified, radial arranged parenchyma cells
Vessel elements	Short, broad, pitted, simple and terminal perforation; perforation plate more or less horizontal	Simple perforation; perforation plate horizontal or slightly oblique	Short, simple terminal perforation; perforation plate slightly oblique	Short, simple terminal perforation; perforation plate more or less horizontal
Tracheids	Vasicentric; short and broad, reticulate or pitted	Vasicentric; short and broad, reticulate thickenings	Vasicentric, short and broad; reticulate thickening	Vasicentric; short and broad; spiral or reticulate thickening
Sclereids	Columnar	Columnar	Columnar or macro sclereids; thick walled, narrow lumen	Columnar, small, rectangular to square; thin walled

Figure 4: Powder microscopy of *Uraria picta* root: (a) libriform fiber; (b) cork cells in radial files; (c) vessel element with simple, slightly oblique perforation; (d) vasicentric tracheid with reticulate thickened; (e) columnar sclereid and (f) phenol-filled parenchyma cells

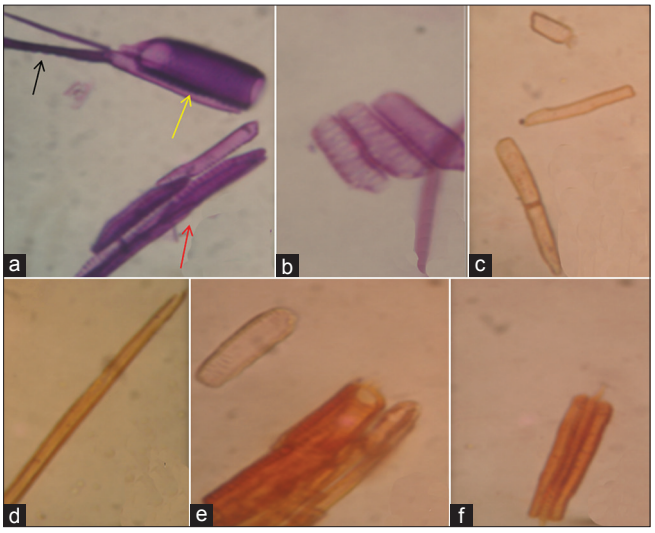

Figure 5: Powder microscopy of *Desmodium gangeticum* root: (a) Libriform fiber (black arrow) vessel element (yellow arrow), vasicentric tracheid (red arrow); (b) Tracheid and *Pseudarthria viscida* root; (c) fiber with axial parenchyma cells; (d) libriform fiber; (e) vessel element and (f) tracheid

Histochemistry

The detail histochemical studies carried out on the *Prsniparni* candidates are shown in Table 4. Histochemical studies on all the four species revealed that all the four contain starch [Figure 7], polyphenols, total proteins, amino acids, fats, oils [Figure 8], volatile oils and lignin. Tannins were present in *U. picta, D. gangeticum* and *P. viscida* and not observed in *U. lagopodoides*. Based on the morphological, anatomical, histochemical, and powder study a scoring for all the four candidates were made so as to find the percentage similarity between the four *Prsniparni* candidates, considering *U. picta* as the authentic source of *Prsniparni* the remaining candidates were marked against the characters which were similar to *U. picta* was given a score of 1 and the dissimilar ones, was given a score of 0. The similarity percentage was worked out based on these scores, between *U. picta* and the other species used as substitutes. Similarly the percentage of similarity between the other substitute species was also worked out and these results were expressed in the form of similarity matrix [Figure 9].

Morphological comparison shows that roots of *U. picta* differ from those of others in color, branching intensity and thickness. While the roots of *U. picta* as observed by us, and as per API[4,10] and Database on Medicinal plants used in Ayurveda[11] were light brown in color, those of *D. gangeticum* and *P. viscida* were dark brown. The roots of *U. picta* are the thickest as compared to other three. The intensity of branching of roots is greater in *P. viscida* when compared to others. Among the four species studied, the transverse sections of the roots showed the presence of small pith only in *P. viscida*. The two species of *Uraria* have ring porous wood while the other two have diffuse porous and this difference

Table 4: Histochemical features of the four species used as *Prsniparni*

Chemical constituents	Tests	*Uraria picta*	*Uraria lagopodoides*	*Desmodium gangeticum*	*Pseudarthria viscida*
Starch	Lugol's iodine	+	+	+	+
Polyphenols	TBO	+	+	+	+
Total proteins and amino acids	Fast green FCF	+	+	+	+
Fats, oils, volatile oils	Sudan red	+	+	+	+
Tannins	Ferric chloride	+	–	+	+
	Lugol's	+	–	+	+
Lignin	Phloroglucinol/HCl	+	+	+	+
	Lugol's iodine and 60-70% Sulphuric acid	+	+	+	+
Pectin	Ruthenium Red	+	+	+	+
	TBO	+	+	+	+

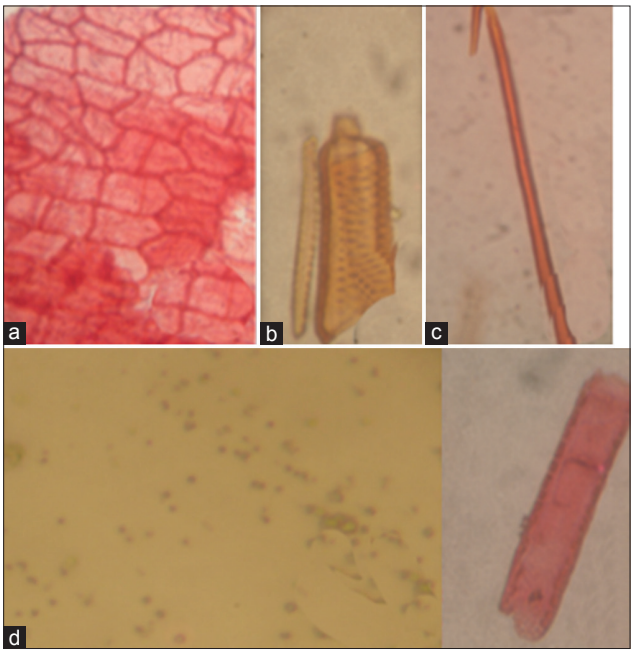

Figure 6: Powder microscopy of *Uraria lagopodoides* root: (a) cork cells in radial files; (b) vessel element and vasicentric tracheid; (c) libriform fiber; (d) presence of starch grains and (e) phloem parenchyma cell

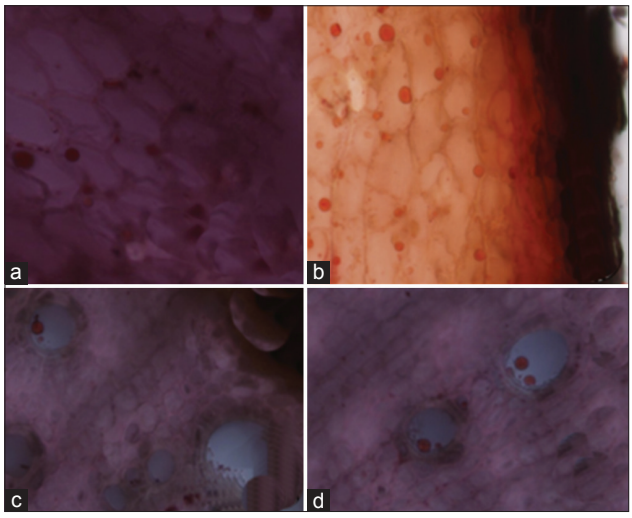

Figure 8: Histochemical analysis for Lipid droplets: (a) *Uraria picta*, (b) *U. lagopodoides*, (c) *Desmodium gangeticum* and (d) *Pseudarthria viscida*

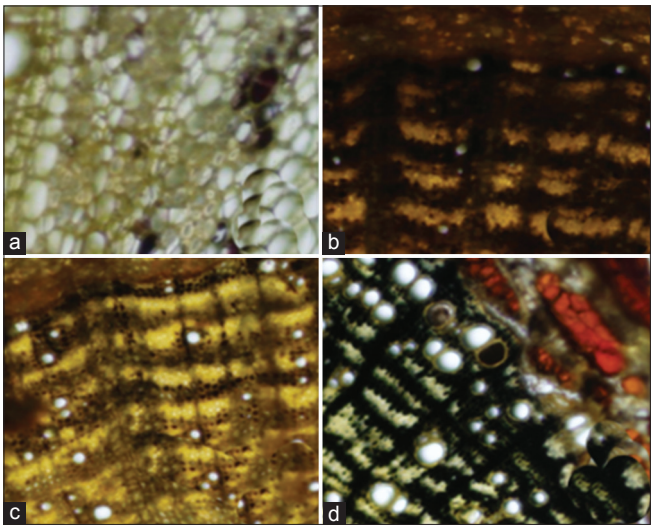

Figure 7: Histochemical analysis, Starch: (a) *Uraria picta*, (b) *U. lagopodoides*, (c) *Desmodium gangeticum* and (d) *Pseudarthria viscida*

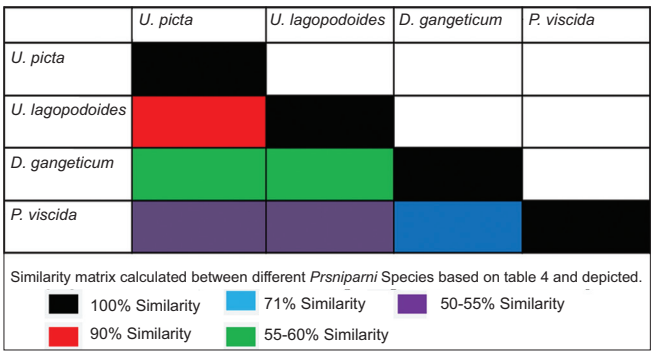

Figure 9: Similarity matrix showing the differences between the *Prsniparni* candidate species

is due to their accession from North India where there is a seasonal periodicity in the type of wood elements produced, especially relating to diameter of vessel pores.[12] The two species of *Uraria* also showed only simple apotracheal banded axial parenchyma, while other two species showed apotracheal band as well as paratracheal aliform type known in legumes which grow in places where there are no conspicuous seasonal changes.[12] Calcium oxalate crystals and starch grains were observed in all four species and this

Table 5: Similarity scores of the different species as compared to *Uraria picta*

Characters compared with those of *U. picta*	*U. lagopodoides*	*D. gangeticum*	*P. viscida*
Color	1	0	1
Microscopic features			
Pith	1	1	0
Primary xylem	1	1	1
Wood	1	0	0
Axial parenchyma	1	0	0
Ray	1	0	0
Fibers	1	0	1
Phloem	1	0	0
Secondary cortex	1	0	0
Powder microscopy			
Starch	1	1	0
Cork	1	1	0
Vessel	0	0	0
Tracheids	1	1	1
Sclereids	1	0	0
Histochemistry			
Starch	1	1	1
Phenols	1	1	1
Proteins	1	1	1
Tannins	0	1	1
Lignin	1	1	1
Pectin	1	1	1
Total	18	12	11
Percentage similarity	90	60	55

observation is in agreement with prior observations made on *U. picta.*[10,11] *D. gangeticum* [10,13] and *P. viscida* [4,10,14]

Microscopic study of the powders of the roots of the four species revealed similar characteristics like the presence of libriform fiber, cork cell in radial stratified files, vasicentric tracheids and columnar sclereids. Stratified cork has also been reported earlier for *U. picta,*[11] *D. gangeticum* and *P. viscida.*[14]

The histochemical studies carried out revealed that starch, polyphenols, total proteins and amino acids, fats, oils, volatile oils, resins, and lignin were detected in all four species but tannins were not detected in *U. lagopodoides* and pectin was not detected in *P. viscida*. The presence of tannins in *P. viscida* has been reported[14] and our results confirm the same. The analysis of the overall macro- and microscopic characters as well of histochemical characters of the four *Prsniparni* candidates reveal that *U. lagopodoides* possesses 90% similarity in characters with *U. picta* the original *Prsniparni*, as compared to *D. gangeticum* which has only 60% similarity and *P. viscida* which has only 55 % similarity in characters to *U. picta* as in Table 5. From the above study we can infer that *U. picta* and *U. lagopodoides* are more similar to one another than the other two candidates. By anatomical features, original *Prsniparni* can still being eminent from the substitute species *U. lagopodoides, D. gangeticum* and *P. viscida*. These differences are likely to be reflected in their bioactivities, which of path needs to be confirmed, particularly the type of ailment for which one may have better effect than the other.

In the Ayurvedic industry, many a times different botanical entities are used under the same name as a living practice, as four species are known to be used as *Prsniparni*. At least four species are known to be used as *Daruharidra*, namely *Berberis aristata, B. lycium, B. asiatica, Coscinium fenestratum*. It is important to research into the legitimacy of substitution using objective pharmacognostic and pharmacological methods. In this article we have suggested a methodology to score the similarities of pharmacognostic features and to arrive at a similarity matrix. The same may be extended to pharmacology as well. Similar methods are commonly used in Numerical Taxonomy, where all characters, irrespective of whether they are morphological, anatomical, biochemical etc., are used after giving equal weightage to them and to work out similarity matrices. Such matrices can then be used to indicate the degree to which materials investigated are similar to one another from a functional perspective.

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