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Special paper

Morpho-anatomical standardization of six important RET medicinal plants of Astavarga group from Western Himalaya, India

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Abstract

Astavarga is a group of eight important medicinal plants used in Ayurveda found in Indian Himalayan region between an altitude of 800 and 4000 m asl. These plants are used in variety of Ayurvedic formulations such as Chyavanprasha. Due to scarcity of these plants in wild, non-existing cultivation practices and confusing vernacular names there is widespread problem of adulteration or substitution of these plants with cheaper unrelated plants. Dried underground parts of these plants are used for medicinal purpose, due to which identification of raw drug material is very difficult. Out of the eight plant species of Astavarga group, six plant species were selected for the present study *i.e.*, *Habeneria intermedia*, *Habeneria edgeworthii*, *Malaxis muscifera*, *Crepedium acuminatum*, *Polygonatum verticillatum* and *Polygonatum cirrhifolium*. Extensive literature review was conducted to compile information about habit, habitat, distribution, plant part used, RET status and substitutes of the selected plant species. Authentic specimens were collected from different locations of Western Himalaya India. Duly identified voucher specimens were submitted to the Janaki Ammal Herbarium (RRLH) at CSIR-IIIM, Jammu. Macroscopic and microscopic characters of the plant parts use as a drug of the selected plant species were studied in detail. They were characterised morphologically in terms of size, shape, colour, texture, external appearance and other distinguishing morphological features. Transverse sections were observed under compound light microscope and powder study was done with main focus on starch grains and calcium oxalate crystals in the cells. The size and shape of starch grains and calcium oxalate crystals can be helpful in identifying some of the studied species. For example, in case of pseudobulbs of *C. acuminatum* raphids are present and starch grains are absent, whereas in *M. muscifera* raphids are absent and starch grains are present. Difference in starch grain size and raphid length may be taken as a key characters for identification of *H. intermedia* and *H. edgeworthii*. The morpho-anatomical standards developed in the present study will help in easier, faster and efficient

identification of the traded plant parts of the Astavarga plants in fresh, dried and powder forms.

Keywords: Astavarga, Orchidaceae, Asparagaceae, raw drugs, substitutes, distribution, standardisation.

Introduction

Astavarga is a group of eight important Rare, Endangered and Threatened (RET) medicinal plants used in Ayurveda found between an altitude of 800 and 4000 m asl in Indian Himalayan region *viz*., Uttarakhand, Himachal Pradesh, Jammu & Kashmir, Manipur and Sikkim [2, 16 & 19]. Eight medicinal plant species of Astavarga group are *Crepedium acuminatum* (D.Don) Szlach., *Malaxis muscifera* (Lindl.) Kuntze, *Habenaria intermedia* D.Don and *Habenaria edgeworthii* Hook.f. ex Collett of family Orchidaceae; *Lilium polyphyllum* D.Don of family Liliaceae; *Polygonatum cirrhifolium* (Wall.) Royle and *Polygonatum verticillatum* (L.) All. of family Asparagaceae and *Roscoea purpurea* Smith. of family Zingiberaceae. In Ayurveda Astavarga group of plants are claimed to possess rejuvenating, health promoting, immune system strengthening, anti-oxidant and cell regenerating properties [13, 16 & 17]. They are also claimed to promote body fat, healing fractures, control fever, abdominal thirst, diabetic condition, seminal weakness, and as a cure for vata, pitta and rakta doshas [13, 16 & 17]. Astavarga plants are used in various formulations in Ayurveda of which most prominent is Chyavanprasha. They are processed and used as medicine in different forms such as oil (*Taila*), medicated clarified butter (*Ghritam*) and powder (*Churana*) [8].

Natural habitat of Astavarga plants in Indian Himalayan Region is threatened due to various natural and anthropogenic causes such as rapid habitat destruction, fragmentation and degradation, climate change, exotic species invasion, land use change, forest fire, etc [9, 12], whereas over exploitation of these plants from wild for medicinal purpose, poor seed germination or poor seedling establishment, overgrazing, population fragmentation and other anthropogeneic threats are adversely effecting their survival in natural habitats [7, 12]. Due to specific habitat, population bottleneck and narrow range of distribution Astavarga plants are already rare, this coupled with anthropogenic threats have now brought these plants under endangered and threatened in wild category [4, 11 & 21]. Heavy demand of Astavarga plants by pharmaceutical industry, non existing cultivation practices and overexploitation has mounted pressure on natural population in wild. There are number of substitutes and adulterants of Astavarga plants available in the market, which because of their easy availability and low cost are used commercially [2, 5, 19, 24 & 26]. Mostly dried underground parts of Astavarga plants are used in Ayurvedic formulations, due to which it becomes very difficult to morphologically authenticate their identity. Moreover multiple vernacular names may lead to misidentification of the plant material and can cause intentional or unintentional use of adulterants or substitutes [18, 23]. Adulteration or substitution with plants having different pharmacological activities may lead to severe degradation of quality and credibility of these plants, which may further result in severe health complications for the end users. Confusion in vernacular names and similarity in appearance of dried plant drug of different species makes it very tough to correctly identify raw drugs. Keeping the aforesaid facts in view the present study was conducted to develop morpho-anatomical

standards for faster and efficient identification of economically important plant parts of the selected six Astavarga plant species from Western Himalaya, India.

Material and methods

Out of the eight plants species of Astavarga group, six plant species were selected for the present study *i.e.*, *H. intermedia*, *H. edgeworthii*, *M. muscifera*, *C. acuminatum*, *P. verticillatum* and *P. cirrhifolium.* Extensive literature review was conducted to compile information about habit, habitat, distribution, plant part used in trade, RET status and substitutes of the selected plant species (Table 1 and Table 2). Field tours were conducted in different seasons in temperate, sub-alpine and alpine regions of Western Himalaya covering Uttarakhand, Himachal Pradesh and Jammu & Kashmir states of

Table 1: General details of the studied species of Astavarga plants.

S. No.	Botanical name	Family	Ayurvedic name [2]	Vernacular names [8, 20]	Synonym [22]	Plant part used
1	*Crepidium acuminatum* (D.Don) Szlach.	Orchidaceae	*Jeevak*	Chirnjivi, Dirghayu, Harsanga, Ksveda, Kurchashira, Kurchkakaar, Madhur, Madhurak, Pranda, Shringaka, Svadu.	*Crepidium bilobum* (Lindl.) Szlach. ex Lucksom, *Malaxis acuminata* D.Don, *Microstylis wallichii* Lindl.	Pseudobulb
2	*Malaxis muscifera* (Lindl.) Kuntze	Orchidaceae	*Rishbhak*	Bandhura, Dheera, Durdhara, Gopati, Inderaksa, Kakuda, Lashunkand, Matrika, Nissar, Suksampatrak, Varishnabha, Vishani, Vrisha, Vrishshringvat.	*Malaxis muscifera* (Lindl.) Grubov, *Microstylis muscifera* (Lindl.) Ridl.	Pseudobulb
3	*Habenaria intermedia* D.Don	Orchidaceae	*Riddhi*	Lakshmi, Mangala, Rathanga, Rishisrista, Saravajanpriya, Siddhi, Sukha, Talgranthisamakand, Vamavartal, Vasu, Vrisya, Yuga.	*Ochyrorchis intermedia* (D.Don) Szlach.	Tuber
4	*Habenaria edgeworthii* Hook.f. ex Collett	Orchidaceae	*Vriddhi*	Dakshinavarta, Himadrija, Lakshmi, Mangala, Rathanga, Rishisrista, Saravajanpriya, Siddhi, Sukha, Vasu, Yuga.	*Habenaria edgeworthii* Hook.f. ex Collett, *Platantheroides edgeworthii* (Hook.f. ex Collett) Szlach.	Tuber
5	*Polygonatum verticillatum* (L.) All.	Asparagaceae	*Meda*	Basuchidra, Dhara, Evamani, Manichhidra, Mitha Dudhia, Pandra, Saat Ashee, Salammishri, Sel, Shakakul, Shalyaparni, Vasuchhidra, Whorled Solomon's.	*Convallaria verticillata* L. *Polygonatum macrophyllum* Sweet, *Sigillum verticillatum* (L.) Montandon	Rhizome
6	*Polygonatum cirrhifolium* (Wall.) Royle	Asparagaceae	*Maha meda*	Basuchidr, Devamani, Dharaa, Manichhidra, Nakhchechi, Shalyaparni, Subrakand, Vasuchhidra.	*Convallaria cirrhifolia* Wall., *Polygonatum cirrhifoliodes* D. M.Liu & W.Z.Zeng	Rhizome

India for collection of authentic specimens of the selected plants (Figure 1). The collected herbarium specimen were dried, pressed, poisoned with 1% mercuric chloride and mounted on herbarium sheet following usual herbarium procedures. The collected specimens were identified with the help of available floras, monographs, literature and by comparing with authentic specimens available at internationally recognized Janaki Ammal Herbarium (RRLH) at CSIR-IIIM Jammu. Duly identified herbarium voucher specimens were submitted to the Janaki Ammal Herbarium (RRLH). Collected fresh as well as dry samples of the selected Astavarga plants were studied in detail.

Plant parts of the selected plants used as drug were characterised morphologically in terms of size, shape, colour, texture, external appearance or other distinguishing morphological features. For anatomical study, fresh plant samples or samples stored in FAA fixative were used. Transverse sections (T.S.) of the plant parts of each of the selected plant species were obtained by hand sectioning using razor blade. T.S. were serially dehydrated in different concentrations of alcohol (30%, 50%, 70%, 90% and finally in absolute alcohol) along with staining by using safranin and fast green stains [3]. Some plant material being delicate and mucilaginous was studied without alcoholic dehydration in water mount. Powder study was done with main focus to study starch grains and calcium oxalate crystals in the cells. Iodine test was conducted to test the presence of starch grains in the powder of the studied plant part. Sections were observed under compound light microscope and images of T.S. were captured using LEICA DM 750 light microscope with LEICA ICC50E camera. Dimensions of starch grains and raphids were taken using LEICA LAS V 4.9.0 software.

Table 2: Details about distribution, RET status and substitutes of the studied Astavarga plants.

S.No.	Botanical name	Geographical distribution in India	Altitudinal distribution (m asl)	RET status	Substitutes used in trade
1.	*Crepidium acuminatum* (D.Don) Szlach.	Himachal Pradesh, Uttarakhand, Assam, Nagaland, Manipur, Mizoram, Tripura, Andaman islands [2]	1800-2300 [8], 1200-2100 [1]	Rare [10, 15 & 25]	*Centaurea behen* L., *Malaxis cylindrostachya* (Lindl.) Kuntze, *Malaxis mackinnonii* (Duthie) Ames, *Pueraria tuberosa* (Willd.) DC., *Tinospora sinensis* (Lour.) Merr. [19]
2.	*Malaxis muscifera* (Lindl.) Kuntze	Sikkim, Himachal Pradesh, Uttarakhand and Jammu & Kashmir [2]	1800-3500 [8], 2400-3600 [1]	Rare, Threatened [25], Endangered [7]	*Centaurium roxburghii* (D. Don) Druce., *Pueraria tuberosa* (Willd.) DC. [5]
3.	*Habenaria intermedia* D.Don	Temperate Himalaya, Kashmir to Sikkim, Uttarakhand and Himachal Pradesh [2]	2000-3000 [6], 800-2800 [8], 1500-2500 [1]	Common, Endangered [6, 25]	*Asparagus filicinus* Buch.-Ham. ex D.Don, *Sida cordifolia* L., *Tacca integrifolia* Ker Gawl. [5]
4.	*Habenaria edgeworthii* Hook.f. ex Collett	Himachal Pradesh, Jammu & Kashmir, Himachal Pradesh and Uttarakhand [2]	800-2500 [8], 2500-3000 [1]	Rare [25]	*Dactylorhiza hatagirea* (D.Don) Soo, *Habenaria griffithii* Hook.f., *Sida acuta* Burm.f., *Tacca integrifolia* Ker Gawl. [5]
5.	*Polygonatum verticillatum* (L.) All.	Sikkim, Kashmir, Himachal Pradesh and Uttarakhand [14, 16 & 20]	2000-3600 [8], 1600-4000 [1]	Threatened [25]	*Asparagus racemosus* Willd., *Eulophia campestris* Wall. [14]
6.	*Polygonatum cirrhifolium* (Wall.) Royle	Himachal Pradesh, Uttarakhand, Sikkim, Manipur [2]	2000-3600 [8], 1200-3300 [1]	Rare [25]	*Asparagus racemosus* Willd., *Paederia foetida* L., *Polygonatum multiflorum* (L.) All., *Sida veronicifolia* Lam. [2]

Results

General details of the studied plant species such as botanical name, family, Ayurvedic name, vernacular names, synonyms and plant parts used in trade are given in Table 1. Details about geographical distribution, altitudinal distribution, RET status and substitutes used in trade of the studied plant species are given in Table 2. Details of the collected specimens such as place of collection, altitude, herbarium number and pictures of fresh and dry specimens have been given in Figure 1. Comparative microscopic observations between pseudobulbs of *C. acuminatum* and *M. muscifera* are given in Figure 2, between tubers of *H. intermedia* and *H. edgeworthii* are given in Figure 3 and between rhizomes of *P. verticillatum* and *P. cirrhifolium* are given in Figure 4. Detailed morphological and microscopic characters of the traded plant parts of the studied six plant species of Astavarga group observed in the present study are given in the following sections.

Crepidium acuminatum (D.Don) Szlach.

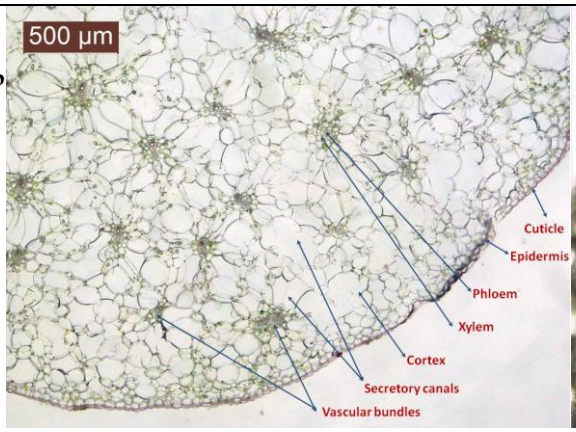
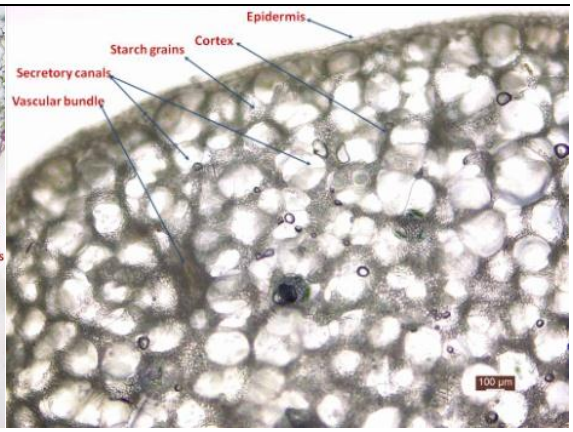
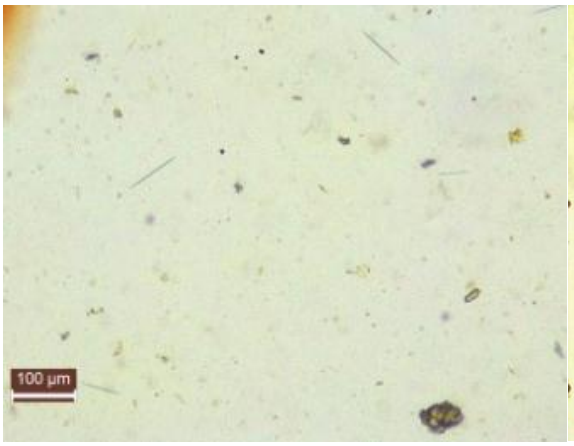
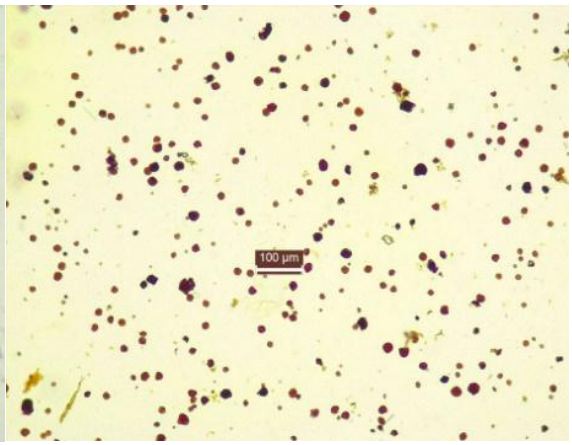
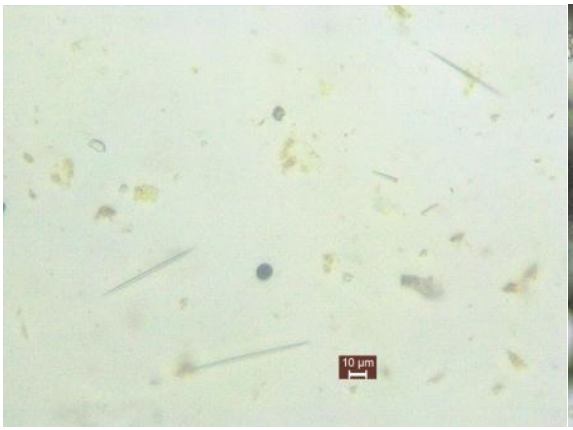
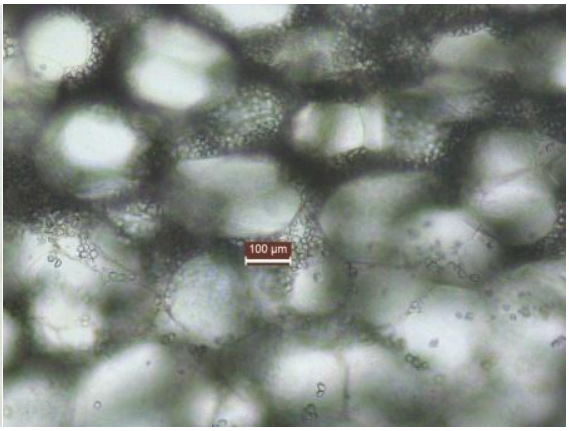
Morphological characters: Lenth of pseudobulbs of *C. acuminatum* varied from 0.7 to 9.0 cm. Pseudobulbs were of 2 types *i.e.*, small oval shaped and large elongated (Figure 1). Small pseudobulbs were round, oval or conical in shape. Their length varied between 1.0 and 2.2 cm, whereas breadth varied between 0.5 and 1.5 cm near mid region. Scales were present on small pseudobulbs. Large pseudobulbs were elongated, cylindrical, fleshy, light green coloured with 3 to 4 alternate sheathing scale leaves having parallel venation. Leaves arise in ring like pattern at some interval from each other. Length of large pseudobulbs can be up to 9.0 cm, whereas breadth varied from 0.4 to 1.7 cm. Base of the pseudobulb was swollen while at upper part of pseudobulb was tapering. Fibrous roots were present at basal swollen part of pseudobulb.

Microscopic characters: Transverse section of pseudobulb was circular in outline with outermost single layered cuticularised epidermis followed by parenchymatous cortical tissue (Figure 2). Cells in cortex were arranged in small chain like pattern with much intercellular spaces among parenchymatous cells. Vascular bundles were present randomly in cortex with xylem encircling phloem. Several secretory canals were also present in cortex. Cortical cells near vascular bundles and near epidermis were small in size as compared to the other cortical cells (Figure 2). Starch grains were not present in the powder of the pseudobulbs. Some needle like calcium oxalate

Figure 1: Details of the collected specimens of plant parts used as drug of the studied six Astavarga plant species.

Botanical name	***Crepidium acuminatum* (D.Don) Szlach.**	***Malaxis muscifera* (Lindl.) Kuntze**	***Habenaria intermedia* D.Don**	***Habenaria edgeworthii* Hook.f. ex Collett**	***Polygonatum verticillatum* (L.) All.**	***Polygonatum cirrhifolium* (Wall.) Royle**
Place of collection	Chakrata, Dehradun district (Uttarakhand)	Saroli, Udhampur district (Jammu & Kashmir)	Latti, Udhampur district (Jammu & Kashmir)	Latti, Udhampur district (Jammu & Kashmir)	Chattergalla, Bhaderwah, Doda district (Jammu & Kashmir)	Mandi, Mandi district (Himachal Pradesh)
Altitude (m asl)	1800	2699	1962	1962	3182	1974
RRLH Herbarium No.	23712	23717	23715	23925	23718	23714
Picture of the plant						
Fresh samples of the plant part used as a drug						
Dry samples of the plant part used as a drug						

Figure 2: Comparative microscopic observations between pseudobulbs of *C. acuminatum* and *M. muscifera*.

	***Crepidium acuminatum* (D.Don) Szlach.**	***Malaxis muscifera* (Lindl.) Kuntze**
Transverse section of pseudobulb		
Starch grains		
	Absent	**Present** (6.77×7.10 µm to 25.10×25.98 µm with an average size of 17.68±4.99 µm×18.86±5.82 µm)
Raphids		
	Present (48.56 to 79.29 µm with an average length of 60.66±8.74 µm)	**Absent**

crystals were observed in pseudobulb cells (Figure 2). Length of raphids varied from 48.56 to 79.29 µm with an average length of 60.66±8.74 µm.

***Malaxis muscifera* (Lindl.) Kuntze**

Morphological characters: Pseudobulbs of *M. muscifera* were round or slightly conical in shape with broad base of 1.0 to 1.8 cm, slightly tapering at apical region, resembling garlic bulb, white creamish in colour, covered with 3 to 4 shiny scaly leaves, with parallel venation, which arise alternatively from the basal region and covered the whole bulb (Figure 1). Outermost 1 or 2 scaly leaves surrounded pseudobulb incompletely, while inner scaly leaves formed a complete covering over pseudobulb. Adventitious roots were present at the base of the pseudobulbs. Internally pseudobulbs were mucilaginous in touch.

Microscopic characters: Transverse section of pseudobulb was circular in outline with single layered epidermis followed by parenchymatous cortex (Figure 2). Cortical cells were much larger than epidermal cells. Numerous starch grains and secretory canals were present in the cortex. Very few vascular bundles were present in the cortex with little differentiation in different vascular cells. Starch grains were present in the powder of pseudobulbs (Figure 2). Size of starch grains varied from 6.77×7.10 to 25.10×25.98 µm with an average grain size of 17.68±4.99×18.86±5.82 µm. Raphids were absent from the pseudobulbs of *M. muscifera* (Figure 2).

***Habenaria intermedia* D. Don**

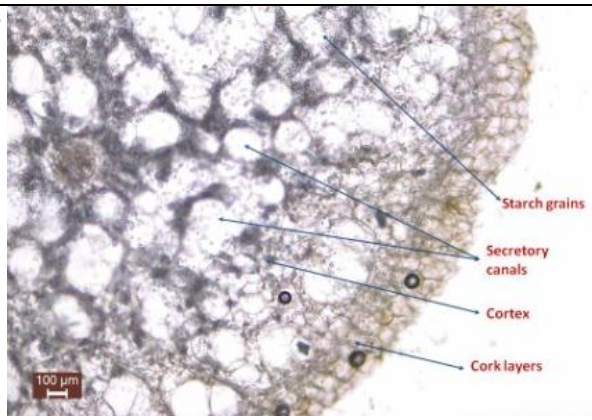
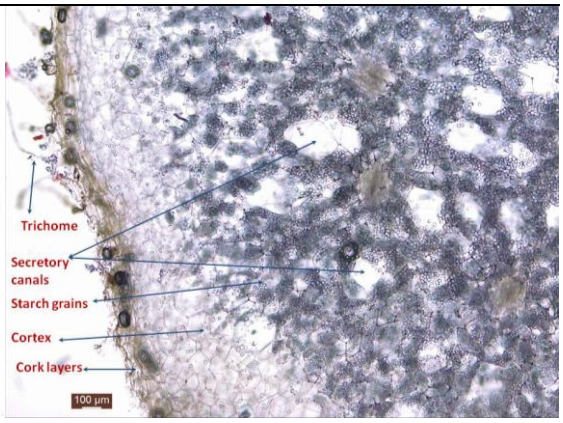
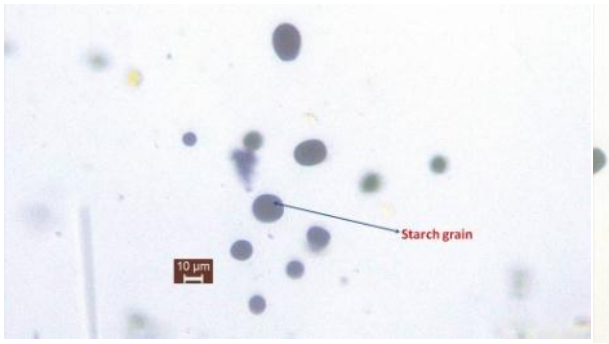
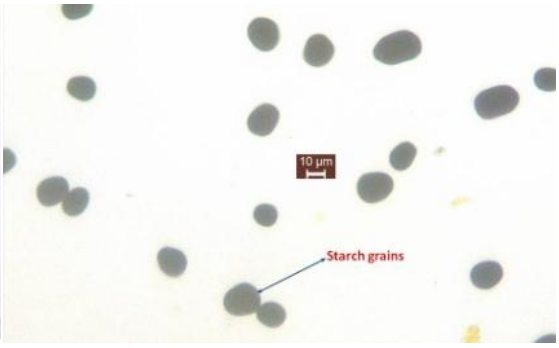
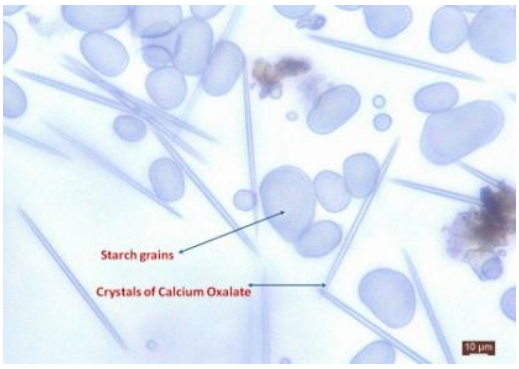
Morphological characters: Fresh tubers of *H. intermedia* were fleshy, oval or oblong, broader at basal region and tapering towards apical region. Tip of tuber possessed a scar of aerial stem. Tuber surface was smooth with groove like pits present at some point. Surface was covered with white hairs. Tubers were buff coloured and mucilaginous internally**.** Length ranged from 1.6 to 4.0 cm, whereas breadth varied from 1.0 to 2.4 cm at basal region, 1.4 to 2.6 cm at mid region and 0.8 to 1.3 cm at tip.

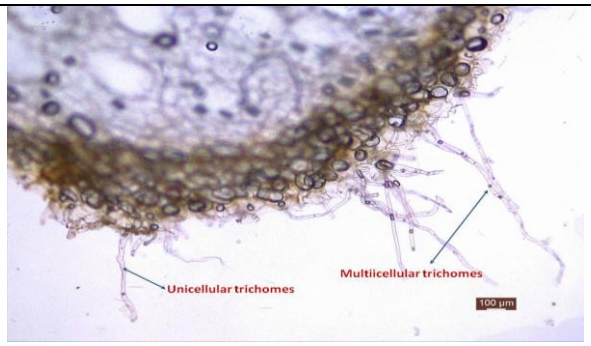
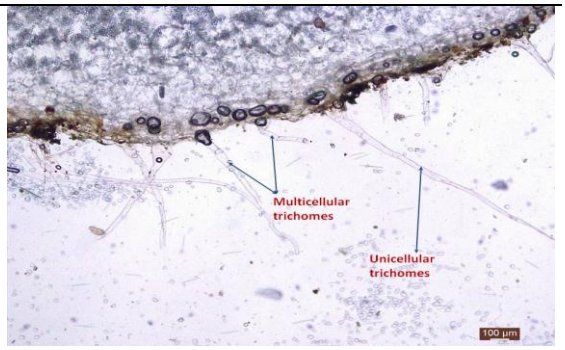
Microscopic characters: Transverse section of tuber appeared nearly circular in outline with less distinguishable epidermis being replaced by few cork layers. It also possessed several unicellular to uniseriate multicellular epidermal hairs or trichomes (Figure 3). Multilayered parenchymatous cortex was present, cells of which were rich in starch grains. Several mucilage secretory canals and few vascular bundles were also present in cortical region. Vascular bundles were not distinguishable into individual xylem and phloem cells. Round or oval starch grains were observed in tuber with iodine test (Figure 3). Size of starch grains varied from 8.78×8.79 to 14.03×20.13 µm with an average grain size of 12.03±3.28×12.93±3.81 µm. Calcium oxalate crystals were present in the powder of tuber (Figure 3). Length of raphids varied from 33.12 to 98.09 µm with an average length of 68.72±21.85 µm.

***Habenaria edgeworthii* Hook.f. ex Collett**

Morphological characters: Fresh tubers of *H. edgeworthii* were fleshy, oval, oblong or fusiform in shape, buff in colour and internally mucilaginous (Figure 1). Tip of tuber possessed a scar of aerial stem. Tuber surface was smooth with some groove like pits on surface at some points. Surface was covered with numerous small white

Figure 3: Comparative microscopic observations between the tubers of *H. intermedia* and *H. edgeworthii*.

	***Habenaria intermedia* D.Don**	***Habenaria edgeworthii* Hook.f. ex Collett**
Transverse section of tuber		
Starch grains		
	Present (8.78×8.79 µm to 14.03×20.13µm with an average size of 12.03±3.28×12.93±3.81 µm)	**Present** (6.18×6.39 µm to 27.27×49.51 µm with an average size of 20.75± 3.39×29.29±3.83 µm)
Raphids		
	Present (33.12 to 98.09 µm with an average length of 68.72±21.85 µm)	**Present** (92.51 to 116.02 µm with an average length of 99.08±19.01µm)

Trichomes		
	Unicellular or uniserriate multicellular	**Unicellular or uniserriate multicellular**

hairs. Tuber length ranged from 1.5 to 3.0 cm, whereas breadth ranged from 0.8 to 1.1 cm at basal region, 1.2 to 1.6 cm at mid region and 0.4 to 0.9 cm at tip region.

Microscopic characters: Transverse section of tuber was nearly circular in outline with epidermis not much distinguishable and appeared replaced by few cork layers with several epidermal hairs (Figure 3). Epidermal hairs or trichomes were unicellular to uniseriate multicellular. Cork was followed by multilayered parenchymatous cortex, cells of which were rich in starch grains. In cortex, several mucilage secretory canals were present. Few vascular bundles were also present in cortical region with less distinguishable xylem and phloem cells. Starch grains were observed in tuber with iodine test (Figure 3). Starch grains were oval or round in shape. Size of the starch grains ranged from 6.18×6.39 to 27.27×49.51 µm with an average grain size of 20.75±3.39×29.29±3.83 µm. Numerous calcium oxalate raphids were also observed in tuber cells (Figure 3). Length of raphids varied from 92.51 to 116.02 µm with an average length of 99.08±19.01 µm.

Polygonatum verticillatum (L.) All.

Morphological characters: Fresh rhizomes of *P. verticillatum* were fleshy, creamish white in colour with glabrous surface bearing nodes and internodes. Their size generally ranged from 0.5 to 1.5 cm or more (Figure 1). Elongated adventitious fibrous roots were present with thickness of 0.035 to 0.05 cm. Small bud like branches arises at some points of rhizome from where new plants emerge.

Microscopic characters: Transverse section of rhizome was circular with wavy outline (Figure 4). Outermost layer with cuticularised epidermis was followed by parencymatous cortex. Numerous secretory canals and vascular bundles were present in the cortex. In vascular bundles, xylem was present outside phloem in circular or semi circular pattern. Transverse section of root was circular in outline with single layered epidermis having root hairs (Figure 4). Next to epidermis, single layered hypodermis was present, which was followed by multilayered parenchymatous cortex. Centre was occupied by vascular bundles having outer single layered endodermis and single layered pericycle, surrounding both phloem and xylem in the centre. Starch grains were not observed in the rhizome (Figure 4). Numerous needle like, elongated calcium oxalate raphids were observed in rhizome powder (Figure 4). Length of the raphids ranged from 43.61 to 232.68 µm with an average length of 158.76±59.60 µm.

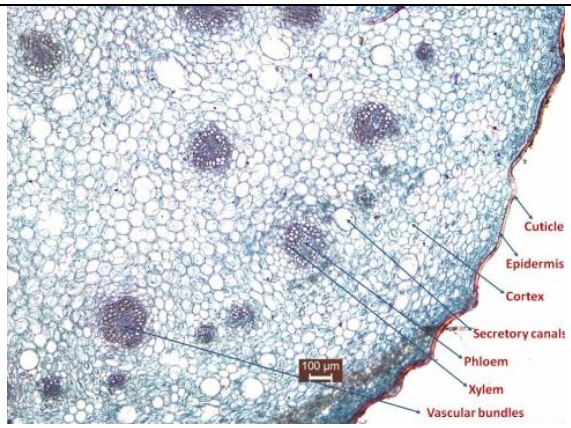
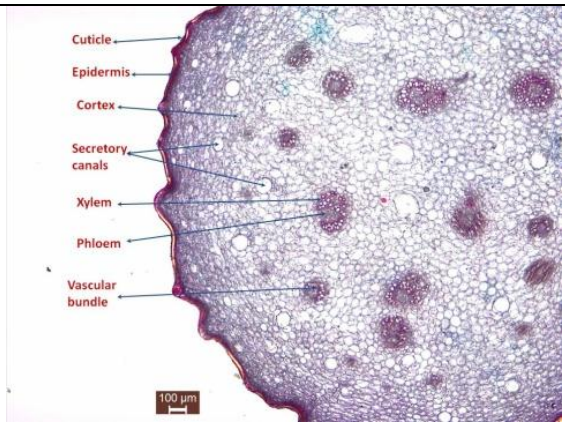
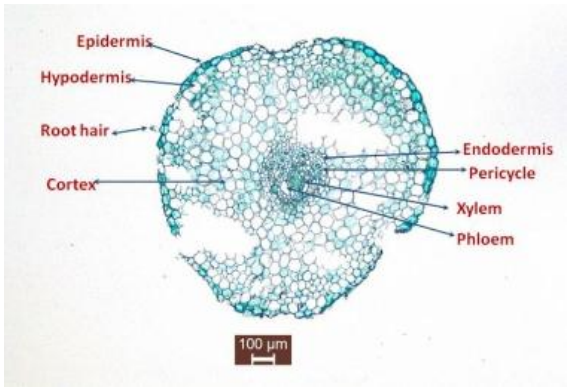
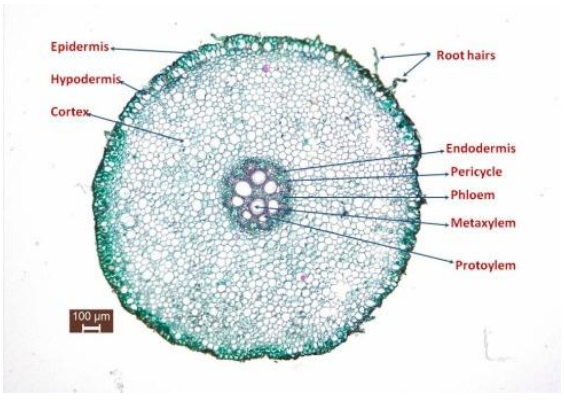
Polygonatum cirrhifolium (Wall.) Royle

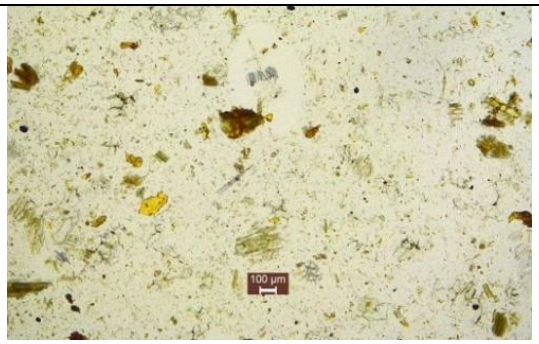
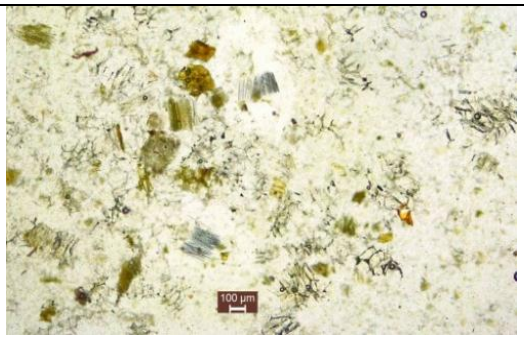
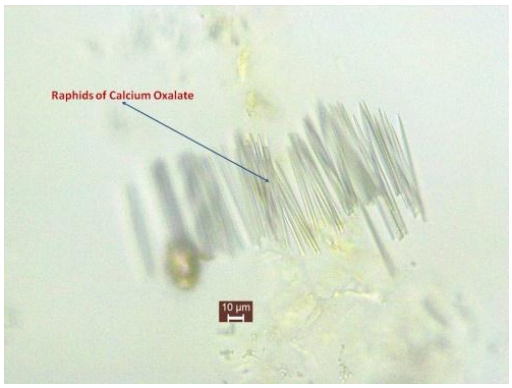
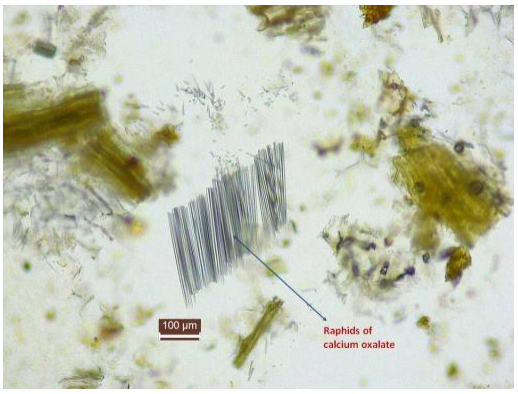
Morphological characters: Fresh rhizomes of *P. cirrhifolium* were swollen, fleshy, bearing

nodes and internodes, forming small bud like branches at tips from where new plants emerged (Figure 1). Fresh rhizomes were creamish white in colour, surface glabrous with breadth generally ranging from 1.0 to 3.5 cm or more. A number of elongated, adventitious roots were present on rhizomes with 0.1 to 0.2 cm in breadth.

Microscopic characters: Transverse section of rhizome was circular with wavy outline (Figure 4). Outermost single layered cuticularised epidermis was followed by parencymatous cortex. Numerous secretary canals and vascular bundles were observed in cortex in scattered pattern. Xylem was present outside phloem in circular or semi circular pattern. Transverse section of root was circular in outline, with single layered outer epidermis

Figure 4: Comparative microscopic observations between rhizomes and roots of *P. verticillatum* and *P. cirrhifolium*.

	***Polygonatum verticillatum* (L.) All.**	***Polygonatum cirrhifolium* (Wall.) Royle**
Transverse section of rhizome		
Transverse section of root		

Test for starch		
	Absent	**Absent**
Raphids		
	Present (43.61 to 232.68 µm with an average length of 158.76±59.60 µm)	**Present** (127.06 to 221.94 µm with an average length of 152.53±26.91 µm)

having root hairs at some points (Figure 4). Epidermis was followed by single layered hypodermis and then by multilayered parenchymatous cortex. Centre was occupied by vascular bundles. Single layered endodermis and then pericycle were present in the stellar region, which surrounded both phloem and xylem present in centre of the vascular bundles. Starch grains were absent in the rhizome (Figure 4). Numerous needle like, elongated calcium oxalate raphids were observed in the rhizome powder (Figure 4). Length of raphids ranged from 127.06 to 221.94 µm with an average length of 152.53±26.91 µm.

Discussion

Astavarga group of plants are in high demand in trade and are being collected from wild at very rapid rate, which has brought these plants under threatened category. Less availability and high market demand of these plants is the primary reason for prevalence of adulterants in the market. For use of authentic plant material correct identification of the plant material is primary step. Macroscopic and microscopic characters of the medicinally important plant parts of the selected plant species were studied in detail. It was observed that plant parts of the species belonging to the same genera showed many similarities in morphological and microscopic characters. Due to which they can be easily misidentified based on either morphological or anatomical characters alone. Moreover confusion in vernacular names may add to the problem of misidentification. For example, according to [2] vernacular name of *P. cirrhifolium* is 'Mahameda' and of *P. verticillatum* is 'Meda', whereas according to [8] vernacular name of *P. cirrhifolium* is 'Meda' and *P. verticillatum* is "Mahameda'.

Comparative studies between plant parts of the same genera in the present study showed that for correct identification both microscopic as well as macroscopic characters should be used together. Various morphological features of the traded plant parts such as shape, size, texture and external morphology, anatomical features characterising the pattern of tissue arrangement and powder studies describing features such as starch grains or crystals types can be very helpful in identification (Figure 1 to 4). In the present study, it was observed that anatomical evaluation by transverse sectioning can be difficult in fresh samples due to mucilaginous nature of plant parts, which makes hand sectioning difficult. Serial dehydration and staining was also observed to be difficult in plant parts with much parenchymatous cells. Further, anatomical arrangement of tissues may not be much varying in species belonging to the same genera however morphological characters were proved to be helpful in some species. The size and shape of starch grains and calcium oxalate crystals can be helpful in distinguishing some species. For example, in pseudobulbs of *C. acuminatum* raphids were present and starch grains were absent, whereas in pseudobulbs of *M. muscifera* raphids were absent and starch grains were present (Figure 2). Also difference in starch grain size and raphid length may also be taken as a character for faster and efficient identification between *H. intermedia* and *H. edgeworthii* (Figure 3). The morpho-anatomical standards developed in the present study will help in easier, faster and efficient identification of the studied plant species in fresh, dry and powder forms.

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