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Histo-Anatomical characterization of aerial and root crude samples of *Withania somnifera* (L.) Dunal

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In the herbal drug industry, raw herbal drug samples of several important plant species are often reported interchangeably used with other drug samples. Correct identification for the use of genuine species in herbal preparation is essential to ensure the safety and quality of herbal medicines. Developments of standard botanical monographs are known to help in the easy, cost-effective and authentic identification of raw herbal samples of genuine species. The present study involved the botanical characterization of plant material (leaf, fruit, stem, and root) of *Withania somnifera* (L.) Dunal. The study of macroscopic and microscopic characters using a stereomicroscope and compound microscope was observed with unique qualitative and quantitative features including characteristic C- shaped vascular bundle arrangement (in leaf); solitary, linear, and grouped xylem vessels in root anatomy; and multi-cellular branched trichomes (in stem, leaf and fruit); the presence of starch in root powder, and prismatic crystals in root and fruit powder as characteristic features. Also, the size of various cells and tissue zones were also provided for studied plant samples. Detailed botanical characters compiled in the present study can be used as a reference standard by herbal drug industries to check the adulteration of *W. somnifera* by correct identification of raw herbal samples in intact or fragmented form.

Keywords: Macroscopic and Microscopic characterization, Raw herbal drugs, Reference identification standard, *Withania somnifera*

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Introduction

Withania somnifera (L.) Dunal belonging to the Solanaceae family, is an erect, evergreen, perennial shrub, up to 0.5-1.5 m tall, generally grows in wastelands, open grounds, and cultivated field. It is reported to be medicinally used over 3000 years in Ayurvedic and indigenous system of medicines with use in more than 100 traditional formulations[1-2]. The plant grows throughout India especially in the drier and subtropical parts upto an altitude of 1500 m above sea level and also widely cultivated in certain areas of Jammu and Kashmir, Punjab, Uttar Pradesh, Madhya Pradesh, Gujarat, Maharashtra, Rajasthan and Andhra Pradesh[2-4]. Common names of the species are Winter Cherry, *Aswagandha*, *Asgandh*, *Askagandha*, Indian ginseng; Ayurvedic names being *Ashwagandhaa*, *Turangi-gandha*, *Ashwakanda*, *Hayagandhaa*, *Gandharvagandhaa*, etc.[3,5]. Three synonyms are available in The Plant List TPL including *Physalis somnifera* L., *Withania kansuensis* Kuang & A. M. Lu, and *Withania microphysalis* Suess. The plant is reported to have withanolides and steroidal lectones and of significant therapeutic value, especially roots are used for its rejuvenative and immunomodulatory properties[4-5]. The plant reported used for stomachache, memory enhancer in obesity, for boils, as sedative, alterative, aphrodisiac, in fevers, cold, cough, dropsy, rheumatism, and for general weakness, wound healing, diabetes, snakebite, to prevent miscarriage, increases fertility in women for conception, and also known with a detrimental role to male sexual competence[6-12]. Root drug samples specifically known as 'Asgand' are reported used in several important ethnomedicinal uses. In Ayurveda, mature roots of *W. sominifera* are reported beneficial for different health issues including *Sotha*, *Ksaya*, *Daurbalya*, *Vataroga*, *Klaibya* and are used in some Ayurvedic formulations including *Asvagandhadyarista*, *Asvagandhadi Leha*, *Balasvagandha Laksadi Taila*[3].

Different plant parts of *W. somnifera* are also reported with some important pharmacological properties including antimicrobial, anticancer, antidiabetic, anti-proliferative and anti-oxidative, anti-

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cancer, anti-bacterial, anti-inflammatory, antioxidant, antiparkinson, antitumor, immunomodulatory, thyroidal activities, and have hypoglycemic, diuretic and hypocholesterolemic effects[13-18]. *W. somnifera* is reported to possess important phytoconstituents including withaferin, withanone, sitoindosides, stigmasterol, ashwandhanolide etc[19].

Due to its immense medicinal properties, *W. somnifera* is in high demand in the herbal drug industry. The estimated annual trade value of the root drug of *Ashwagandha* in the Indian herbal market is 2000-5000 MT[5]. *W. somnifera* is used as a substitute for *Kaakoli* (*Lilium polyphyllum* D. Don.) and *Kshira-Kakoli* (*Fritillaria cirrhosa* D. Don). Some other species are also confused with the common names, for example, *Physalis alkekengi* L. is also known as Winter Cherry[20]. In the herbal drug industry, herbal samples of different species are reported with adulteration problems due to several reasons[21]. For developing any botanical product, herbal drug authentication is considered an important step for experimental studies on traditional medicine drugs and reliable clinical applications. Correct identification and authentication of raw herbal drug samples are required to ensure the efficacy, purity and quality of herbal medicines. Dried samples in fragmented and powdered form can be identified from characteristic anatomical characters and some other microscopic features[22]. Anatomical characteristics have been used for the identification of herbal drugs in several studies[23-27].

The present study aimed at the botanical characterization of raw plant samples of *W. somnifera* to develop a reference standard for the identification of species. Botanical standardisation in the present study involved detailed macroscopic, anatomical and powder studies on stem, leaf, fruit and raw root herbal samples. Qualitative and quantitative characterization of each sample with corresponding photographs provided in the present study acts as a complete reference standard for botanical-based identification of intact or fragmented samples in fresh or dried form.

Materials and Methods

Plant material was collected (June, 2018) from species cultivated and growing as wild in CSIR-Indian Institute of Integrative Medicine (IIIM campus), Jammu, J&K. Herbarium sheets were prepared following the usual herbarium procedure[28], and duly identified herbarium specimens were submitted to Janaki Ammal Herbarium at CSIR-IIIM, Jammu (Herbarium accession no.- 23434). Oven-dried raw herbal samples were submitted to the Crude Drug Repository (CDR) at CSIR-IIIM, Jammu with accession nos. 4114 (whole plant samples) and 4124 (root samples). Different collected samples were studied for morphological, anatomical, and powder microscopy study.

The macroscopic study involved the study of drug sample's shape, size, colour, texture, appearance, surface characters, etc. by hand lens and stereomicroscope (LEICA S9i).

For the anatomical study, transverse sections of the freshly collected stem, leaf, and root samples were obtained by free hand sectioning using a razor blade. Fine sections were subjected to dehydration with counter staining in safranin and fast green according to Kumar *et al.*[29], with little modifications. Samples were dehydrated in 30, 50, and 70% alcohol gradients each for 10 min, followed by staining in safranin (5-7 min leaf samples, 10-15 min root and stem samples). Leaf, root, and stem sections were decolourised in 70% alcohol for 5-10 min, followed by staining in fast green (leaf for 2-3 min, stem and root for 4-5 min). All sections were dehydrated in 90% and absolute alcohol (each for 5-7 min each). Sections were cleared in xylene (1-2 min), mounted in Canada balsam, and observed under a compound microscope (LEICA DM 750) with an associated camera (LEICA ICC50E).

For powder study, samples were crushed to powdery mass and passed through a sieve to obtain a fine powder. Each sample was characterized for organoleptic features. For the microscopic study, water-mounted powder slides were viewed under a compound microscope to study various cell types and cell contents. An iodine test was also performed to study starch grain's characters in powder samples. Various micrometric measurements were made with LEICA LAS V 4.9.0 software.

Results

Morphological characters

The plant is an erect small shrub, leaves are simple, ovate, and with an entire margin (Fig. 1a, b); fruits enclosed in the persistent calyx (Fig. 1k, l) present in small berries (orange-red when mature) (Fig. 1m). Seeds were small, reniform, and yellow to orange in colour with characteristic surface ornamentation (Fig. 1n). Root light brown to buff coloured, woody and hard, cylindrical, of variable thickness (0.2-2 cm or more) stout and less branched, rough, wrinkled,

with longitudinal furrows and ridges (Fig. 2a, b). Cut root nearly circular to irregular in outline, outer zone thin, corky, greenish-brown coloured, followed by inner comparatively broad, creamish green cortex region. Major of the central region is occupied by a woody xylem region (with circular or dilated xylem origin), xylem pores of variable size observed with uniformly distributed pores present in a spoke-like

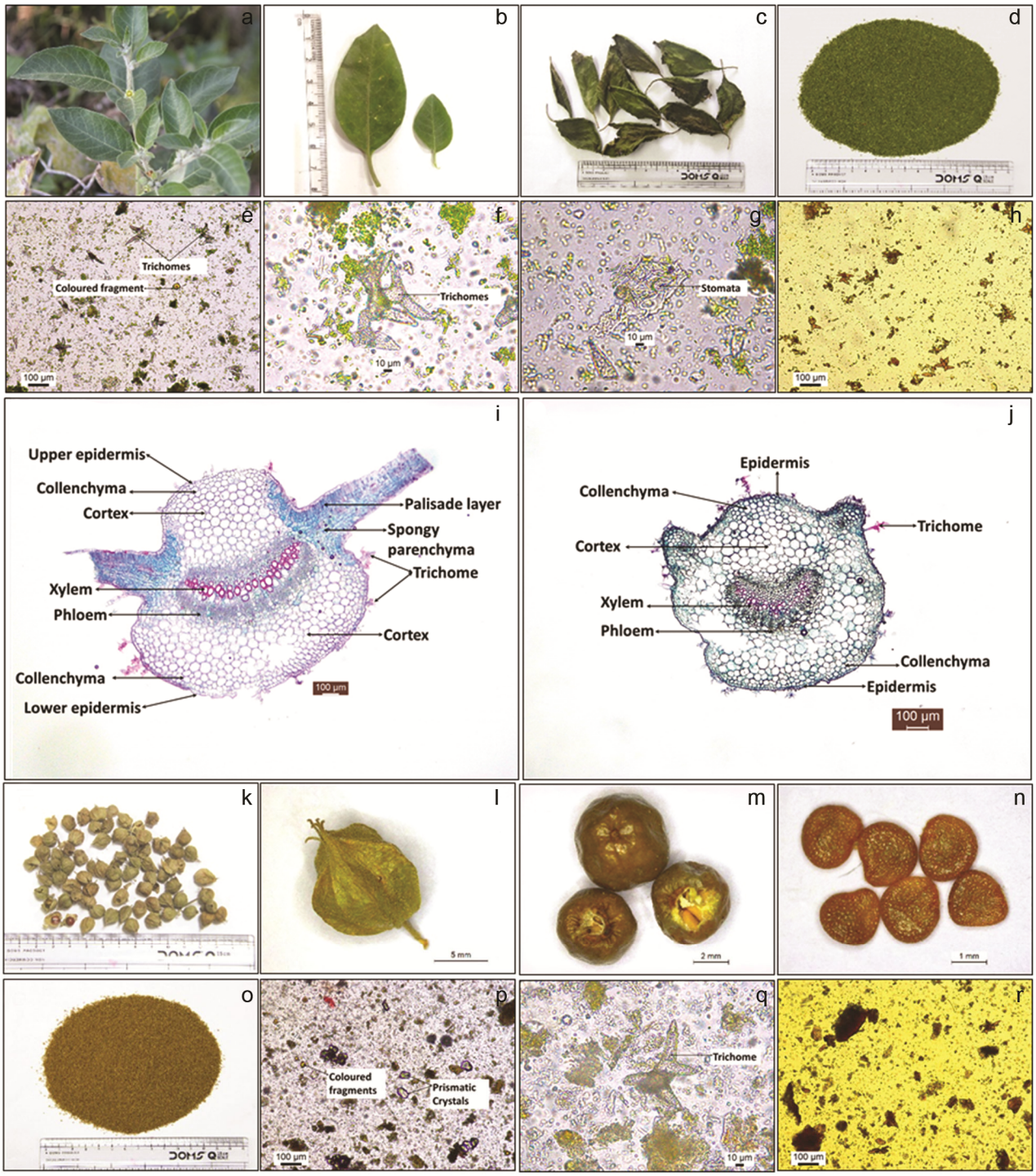

Fig. 1 — Macroscopic and microscopic details of *W. somnifera.* a) Plant habit; b) Fresh leaf samples; c) Raw dried leaf sample; d) Leaf powder sample; e-h) Common microscopic structures in leaf powder study; i) T.S. of Leaf; j) T.S. of petiole; k) Raw fruit samples; l) Single fruit sample with calyx; m) Fruit without calyx; n) Seed samples; o) Fruit powder sample; and p-r) Common microscopic structures in fruit powder study.

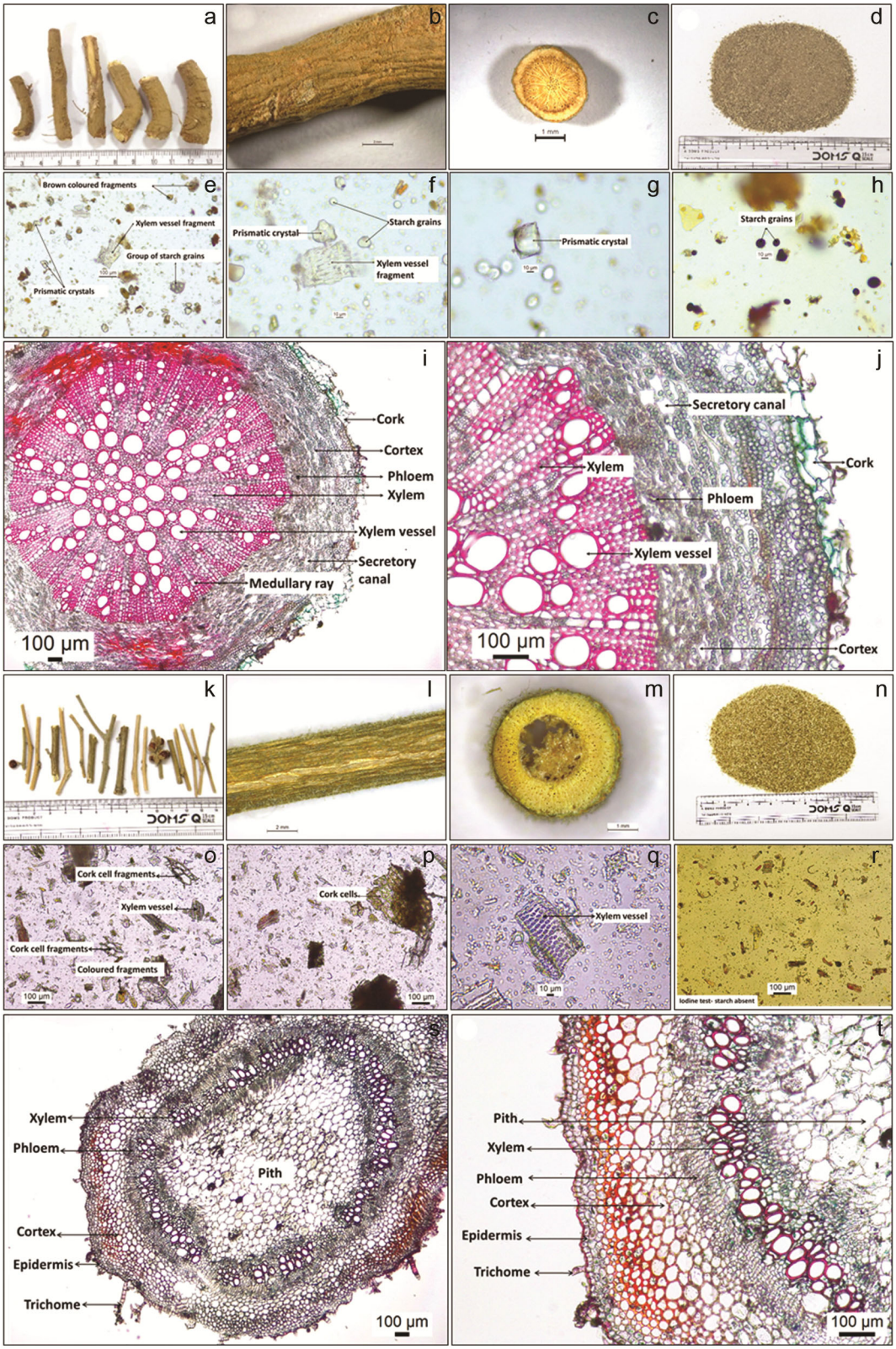

Fig. 2 — Macroscopic and microscopic details of *W. somnifera*. a) Root herbal samples; b) Root surface view; c) Root cut surface view; d) Root powder sample; e-h) Common microscopic structures in root powder study, i) T.S. of root; j) T.S. of root (magnified); k) Stem raw herbal samples; l) Stem surface view; m) Stem cut surface view; n) Stem powder sample; o-r) Common microscopic structures in stem powder study; s) T.S. of stem; and t) T.S. of stem (magnified view).

pattern (Fig. 2c). Stem samples occur as cylindrical, woody, branched pieces of variable thickness (0.2-1.5 cm or more), surface light brown, young samples smooth and hairy, while mature samples smooth, longitudinally wrinkled and without hairs (Fig. 2k, l). The cut surface of the stem is circular in outline with an outer hairy, light green zone, followed by a circular, woody, porous zone (may appear with dilated thickness) and a central hollow pith region with disintegrated cells (Fig. 2m).

Anatomical characters

T.S. of leaf

The transverse section of the leaf showed a typical dicot anatomical arrangement and consisted of lamina and midrib (Fig. 1i). Lamina has a single-layered upper epidermis, palisade layer, spongy parenchyma layers, and lower epidermis. The palisade is single-layered, thick (88.35±2.80 µm), with compactly packed elongated cells present below the upper epidermis. Spongy parenchyma (present below the palisade layer) consisted of loosely arranged, elongated to oval-shaped cells. The lower epidermis is single-layered with multi-cellular (only a few multi-cellular un-branched) trichomes. Midrib consisted of the upper and lower single-layered epidermis, each with multicellular branched trichomes (more trichomes on the lower epidermis). The upper and lower epidermis was followed by 2-3 layers of collenchymas cells and a broad multilayered cortex (5-7 cell layered). Cortex followed by vascular bundles with xylem and phloem. The vascular bundle was collateral with phloem on both sides of the xylem.

T.S. of petiole

The transverse section of the petiole was observed nearly circular in outline with two small projections (Fig. 1j). Single layered epidermis formed the outermost layer with several multicellular branched trichomes (only a few multi-cellular un-branched). Epidermis followed by 2-3 cell layered collenchymatous zone and inner comparatively broad (5-7 layered), parenchymatous cortical zone. The centre of the cross-section was occupied by a flattened or slight C-shaped, collateral vascular bundle. Xylem vessels were distinguishable, arranged in a ray-like pattern with variable lumen diameter (12.32-41.26 µm). Two small circular vascular bundles were observed in the protruded wing-like structure of the petiole.

Anatomy of root

T.S. of the root was nearly circular in outline (Fig. 2i) with the outermost broken, thin cork zone with rectangular-shaped parenchyma cells, followed by a broad (10-12 cell layered) cortex zone with oval-shaped, compactly packed, starch grains filled parenchyma cells. Cortex cells are followed by a continuous thin phloem zone and central circular xylem region. Xylem region comprised of well distinct xylem vessel traversed by less distinguishable, 1-2 cell wide medullary rays filled with starch grains. Xylem vessels showed a slight spoke-like arrangement and were of variable size with solitary, linear (in 3-5 units), or grouped arrangement (up to 2 units). Central vessels were of larger lumen diameter compared to xylem vessels at the peripheral region (Fig. 2i, j).

Anatomy of stem

T.S. of the stem was slightly elongated or can be nearly circular in outline (Fig. 2s) with the outermost single-layered epidermis with rectangular to square-shaped cells and several multicellular branched trichomes (only a few multi-cellular un-branched). Epidermis was followed by 5-7 cell layered collenchymatous zone and then by multilayered parenchymatous cortex zone with oval to polygonal-shaped cells with or without intercellular spaces. The cortex was followed by a collateral vascular bundle with phloem and xylem. The vascular bundles were present in a circular ring-like pattern (Fig. 2t). A prominent circular or oval-shaped parenchymatous pith was present in the centre of the section.

Quantitative anatomical data

Quantitative microscopic characters such as the size of various tissue zones, size of epidermal, cork, cortical, and pith cells, trichome thickness, xylem vessel lumen diameter, size of starch grains, etc. are given in Tables 1 and 2.

Powder study

The studied samples showed characteristic sensory characteristics in terms of colour, odour, taste, and texture. Organoleptic characters of studied powder samples are shown in Table 3. Powder microscopic studies of each sample showed characteristic microscopic structures. Microscopic examination of leaf powder was observed with multicellular branched trichomes (few multi-cellular un-branched), epidermal fragments with stomata and few coloured fragments

Table 1 — Quantitative microscopic characters (tissue zones) of the T.S. of stem, leaf and root of *Withania somnifera*

Character	Min	Max	Mean (±S.D.)	Character	Min	Max	Mean (±S.D.)
	Stem (µm)				Root (µm)		
Total radius	1090.62	1666.65	1339.97±62.85	Total radius	827.82	2016.16	1534.67±113.43
Cortex thickness	280.75	420.32	343.35±17.32	Cork thickness	55.51	271.58	162.50±19.94
Phloem thickness	78.61	133.08	103.72±5.41	Cortex+Phloem	207.43	658.76	451.87±37.47
Xylem thickness	104.83	216.77	164.83±10.32	Xylem thickness	396.43	1248.94	926.51±86.25
Pith radius	475.97	898.68	712.02±45.01	Xylem vessel diameter	28.12	136.31	75.42±14.57
Xylem vessel diameter	12.68	55.97	37.69±37.69	Vessel per square area	65.00	85.00	75.70±2.16
Trichome thickness	17.51	36.78	22.54±1.99				
	Petiole (µm)				Leaf (µm)		
Cortex thickness	360.83	654.17	560.06±27.54	Palisade thickness	74.02	101.94	88.35±2.80
Phloem thickness	66.73	102.64	81.22±5.18	Xylem length	104.52	168.53	135.64±7.64
Xylem thickness	67.28	195.78	141.23±11.95	Xylem vessel diameter	14.00	43.99	28.07±3.61
Xylem vessel diameter	12.32	41.26	23.60±3.06	Trichome thickness	11.95	37.40	25.92±3.14
Trichome thickness	9.54	18.8	14.62±0.93				

Table 2 — Quantitative microscopic characters (cell size) of the leaf, stem, root and fruit of *Withania somnifera*

Character	Length			Breadth		
	Min	Max	Mean (±S.D.)	Min	Max	Mean (±S.D.)
			Leaf (µm)			
Upper epidermis (midrib region)	10.38	26.22	16.69±1.75	12.55	24.22	18.62±1.11
Upper epidermis (lamina region)	17.37	37.98	27.20±1.92	11.3	21.87	17.33±1.12
Lower epidermis (midrib region)	8.53	14.96	11.76±0.73	12.77	20.29	16.09±0.64
Adaxial cortical cell size	37.47	88.09	66.97±4.72	26.78	70.13	46.96±4.77
Abaxial cortical cell size	91.58	36.38	61.18±5.39	24.32	60.94	39.47±3.56
			Petiole (µm)			
Epidermis	14.73	23.15	18.86±0.90	15.32	19.34	17.41±0.42
Cortex cells	45.77	97.35	67.29±5.35	28.39	76.75	51.78±5.71
			Stem (µm)			
Epidermis	11.07	19.28	15.11±0.82	14.09	27.11	19.44±1.13
Cortex cells	27.56	71.65	47.53±4.24	16.26	47.24	32.54±3.41
Pith cell size	30.98	103.57	67.95±7.66	20.09	67.33	43.95±4.62
			Root (µm)			
Cortex cells	39.24	90.8	66.22±4.05	25.96	63.28	40.94±4.04
Cork cells	49.94	93.02	65.71±5.05	23.21	59.02	42.35±4.10
Starch grains	6.7	19.1	12.56±1.01	5.50	15.90	10.68±0.99
			Fruit (mm)			
Fruit (Berry) size	5.05	6.24	5.59±0.11	5.16	5.66	5.45±0.05

Table 3 — Comparative organoleptic powder characteristics of the various plant parts of *Withania somnifera*

Plant part	Colour	Odour	Taste	Texture
Leaf	Dull green to soil coloured (Fig. 1d)	Slightly characteristic odour	Slightly characteristic taste	Slightly sandy or gritty
Fruit	Reddish green (Fig. 1o)	Characteristic odour	Smooth	Slightly bitter
Root	light brown to soil coloured (Fig. 2d)	Unpleasant and slightly pungent	No characteristic taste	Flaky and gritty
Stem	Light brown to soil coloured (Fig. 2n)	No characteristic odour	Flaky	No characteristic taste

(Fig. 1e-h). Powder microscopy of fruit samples was observed with few coloured fragments, prismatic crystals, few multicellular branched trichomes and few cork cell fragments (Fig. 1p-r). Microscopic examination of root powder sample was observed with cork cells, pitted xylem vessel fragments, starch grains (in groups or solitary), brownish fragments and prismatic crystals (Fig. 2e-h). A stem powder sample was observed with cork cell fragments, xylem vessels and a few coloured fragments (Fig. 2o-r).

Discussion

Macroscopic and microscopic characteristics have been observed as taxonomically significant in several studies for the distinction of raw herbal drugs from adulterants. Some previous studies has reported that the botanical characteristics such as the shape of cross-section (stem or other samples); shape and size of epidermal, ground tissue cells, endodermis and sclerenchyma; shape, number, and arrangement of vascular bundles; characters of stomata, trichomes, crystals type, starch grains, etc. as taxonomically important characters in the identification of herbal samples[25,29-31]. Hassan *et al.*[32], described the thickness of the epidermis, cortex, phloem, xylem, palisade, and spongy parenchyma to the total thickness of cross-section and vessel diameter as important quantitative characters for the description of species.

In a botanical study of fruit and seed samples of *W. coagulens*, fruits were observed with calyx covering, ear-shaped seeds, characteristic fruity odour, branched and un-branched trichomes[33]. In the present study, the anatomical study revealed characteristic dicot anatomical patterns in different herbal samples. Some characteristic anatomical features of studied samples were-multicellular branched trichomes, collateral vascular bundles in stem, characteristic crescent-shaped vascular bundles in leaf and petiole, two small circular vascular bundles in petiolar projections; stem anatomy characterized by massive, broad collechymatous zone (5-7 layered) with a ring-like arrangement of vascular bundles and a broad circular to the oval-shaped central pith. Root anatomy was observed with square-shaped cork cells with slight lignification, cortex zone with secretory canals, and characteristic spoke like arrangement of xylem vessels (solitary, linear to grouped) in root transverse section. In the microscopic study of powder samples, some characteristic cell and tissue types are also helpful in the identification of herbal constituents[34]. In present study, the powder samples were observed with characteristic organoleptic and microscopic features. The microscopic study was observed with characteristic features in powder samples of the leaf (trichomes, anomocytic stomata), fruit (prismatic crystals), stem (cork cells, xylem vessels), and root (cork cells, xylem vessels, crystals, starch grains, and coloured fragments).

Anatomical description for various herbal drug samples of *W. somnifera* has been provided in some previous studies[3,9,35]. Quantitative description for root macroscopic and microscopic characters was provided in API[1], however, with no corresponding images. Uddin *et al.*[9], reviewed some qualitative and quantitative root botanical features of *W. somnifera*, however, no corresponding structural details or images were provided. Calalb *et al.*[35], studied detailed qualitative characters of different herbal samples of *W. somnifera*, but no quantitative description was provided for cells of tissues. Compared to earlier studies, the present study involved botanical studies with a detailed qualitative and quantitative description of macroscopic and microscopic features with corresponding digital images for each studied herbal sample.

Conclusion

Herbal drug misidentification and adulteration is a burning problem in the herbal drug industry and requires correct identification and quality assurance of raw herbal samples. Present study revealed some of characteristic microscopic features of leaf (multicellular branched trichomes), petiole (C-shaped vascular bundle in leaf and petiole anatomy), stem (multicellular branched trichomes and collateral vascular bundles) and root (xylem vessels with solitary, linear and grouped arrangnement). Powder microscopy revealed characteristic presence of prismatic crystals (in fruits and root), starch grains (in root) and trichomes (in leaf, stem, and fruits). Detailed botanical information compiled in the present study including a quantitative and qualitative description with corresponding images can be used as reference standard for taxonomic studies on species. In addition, the reference monographs can be used by herbal drug industry for easy, fast, and authentic distinction of crude herbal samples from adulterants

Conflict of interest

The authors declare that there are no conflict of interest.

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