

Comparative Macroscopic and Microscopic Characterization of Raw Herbal Drugs of *Abrus precatorius* L. and *Glycyrrhiza glabra* L.

Pankaj Kumar^{1,2}, Javaid Fayaz Lone^{1,2}, Sumeet Gairola^{1,2,*}

ABSTRACT

Background: The root of *Glycyrrhiza glabra* L., also known as 'Licorice,' is used as an expectorant in cough and cold preparations, used to treat hepatitis, and have therapeutic benefit against some other viruses. The roots of *Abrus precatorius* L. belonging to the family Leguminosae are commonly known as 'Indian wild Licorice.' The roots of both of these plants are used in Indian systems of medicine (I.S.M.) to treat various health ailments. The root of *A. precatorius* is used as a substitute or sometimes as an adulterant of *G. glabra*. **Objectives:** The present study aimed to develop comparative standards for easy and fast identification of root drug samples of two selected species. **Materials and Methods:** The present study involved a comparative macroscopic and microscopic characterization of the root drug samples of *G. glabra* and *A. precatorius* using compound and stereo microscopes. **Results:** Comparative study of qualitative and quantitative characters revealed significant differences in surface and microscopic characters. Anatomical characters such as cortex and phloem zone appearance, the appearance of vascular rays, and some powder characters, i.e., color and taste of powder and shape and size of starch grains, clearly differentiated the genuine 'Licorice' drug root samples from other drug species. **Conclusion:** Botanical characters compiled in the present study can be used as a reference standard for future identification of individual raw root drug samples and the comparative distinction of both species' root samples.

Key words: Raw root herbs, Licorice, Macroscopic and microscopic studies, Identification characters, Reference standards.

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INTRODUCTION

The Leguminosae family is the most prominent angiosperm family, with around 730 genera and more than 19,400 species.^[1] Several species of this family are used by various indigenous communities in India's different regions.^[2-6] *Glycyrrhiza glabra* L. and *Abrus precatorius* L. belonging to the family Leguminosae are commonly known as 'Licorice' and 'Indian wild Licorice,' respectively (Table 1). The roots of *A. precatorius* are used as an adulterant of genuine 'Licorice' or *G. glabra*.^[7-9] Roots of *A. precatorius* are considered as an emetic, diuretic, and alexiteric, used to treat sore throat, rheumatism, bronchitis, cold, cough, diarrhea, abdominal pain, gastritis, insomnia, gonorrhea, tumors, cancer, snake bite, heart diseases, kidney diseases, jaundice, hepatitis, and other viral infections.^[10-12] Root and rhizome of *G. glabra* affect memory, spatial learning, passive avoidance^[13,14] and minimize serum testosterone levels in men.^[15,16] *G. glabra* roots contain several significant bioactive chemicals, known with important pharmacological properties such as antimicrobial, antioxidant, anxiolytic, anti-carcinogenesis, and antidiabetic properties.^[17-19] Licorice is used as an expectorant in cough, cold preparations, and treating hepatitis, and

have therapeutic benefits against other viruses, including cytomegalovirus (CMV) and human immunodeficiency virus herpes simplex, etc.^[20] Roots of *A. precatorius* are reported to have abrol, abrasine, abraline, abricin, abrusogenic acid, abrusic acid, abruslactone A, methyl abrusgenate, precol, precasine, and glycyrrhizin.^[21,22] Roots of *Glycyrrhiza glabra* are reported to have triterpenoid compounds (Glycyrrhizin, Glycyrrhetic acid, and liquiritic acid), flavonoids, isoflavonoids (liquiritin, isoliquiritin, formononetin), and other chemical constituents.^[23-25]

The estimated annual trade of *A. precatorius* and *G. glabra* in the Indian herbal market is 200-500 MT and 2000-5000 MT, respectively.^[26] The root of *G. glabra* is considered as genuine source of 'Licorice,' which is often reported to be interchangeably used with the root of *A. precatorius*. Herbal samples of some other species are also reported to be used in place of both *A. precatorius* and *G. glabra* (Table 1). Herbal drug samples are generally procured in dried, fragmented, broken, or powdered form in the herbal industry. The raw material of dry root samples generally lacks specific vegetative

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Table 1: Details of the studied raw root drug samples (R.D.S.) of *A. precatorius* and *G. glabra*.

S.No.	Character	Plant species	
1	Botanical name ^[30]	<i>Abrus precatorius</i> L.	<i>Glycyrrhiza glabra</i> L.
2	Synonyms	<i>Abrus abrus</i> (L.) Wright, <i>Abrus minor</i> Desv., <i>Glycine abrus</i> L.	<i>Glycyrrhiza brachycarpa</i> Boiss., <i>Glycyrrhiza hirsuta</i> Pall., <i>Glycyrrhiza violacea</i> Boiss. and Noe
3	Distribution	Throughout the plains up to an altitude of 1200 m asl; ranging from Himalaya to Southern parts ^[31]	Sub-tropical and warm temperate regions, cultivated in many parts of India ^[7]
4	Collection place (asl)	Dadwara, Billawar, UT of J&K (wild)	IIIM campus, Jammu, UT of J&K (cultivated)
5	G.P.S. location	N 32°33.917' E 075°32.064'	N 32°43.855' E 074°51.059'
6	Altitude	621 m asl	304 m asl
7	Herbarium No.	RRLH-23814	RRLH-24233
8	Raw drug accession No.	CDR-4136	CDR-4157
9	Ayurvedic name ^[7,32]	Gunjaa, Gunjaka, Chirihintikaa, Raktikaa, Chirmiti, Kakanti, Kabjaka, Tiktikaa, Kaakananti, Kaakchinchii	Yashtimadhu, Madhuyashtyaahvaa, Madhuli, Madhuyashtikaa, Atirasaa, Madhurasaa, Madhuka, Yastikaahva, Yashtyaahva, Yashti, Yashtika, Yashtimadhuka
10	Local/Trade name	Jequirity, Indian wild Liquorice, Crab's Eye, Rosary pea, Ratti, Precatory Bean, Gundumani, Gunja, Gaunchi, Chirmati, Chinnoti, Mulati, Kakananti, Shangir ^[10,31]	Licorice, Liquorice root, Mulathi, Jeshtimadhu, Jethimadhu, Gulegafis, Yasti ^{7,26}
11	Estimated annual trade ^[26]	200–500 MT	2000–5000 MT
12	Habit and habitat	Much-branched, perennial, deciduous, woody twining herb, mature stem woody	A small perennial herb or undershrub, up to 2 m high, grows in sub-tropical and warm temperate regions. It can be seen growing in fertile, sandy, or clay soil near a river or stream
13	Distribution in India	It is distributed from the Himalayas to Southern parts throughout the plains, up to 1200 m. ^[31]	Cultivated in Punjab, Jammu and Kashmir, and South India.
14	Ayurvedic uses	Indralupta, Mukhasosa, Sula ^[32]	Kasa, Ksaya, Svarabheda, Vatarakta, Vrana ^[32]
15	Ayurvedic formulations	Nili Bhrngadi Taila ^[32]	Eladi Guika, Yastimadhuka Taila, Madhuyastyadi Taila ^[32]
16	Major chemical compounds	Abrasine, abrol, abrine, abruquinones, abrusgenic acid, choline, precatorine. ^[33]	Glycyrrhizin, glycyrrhizic acid, glabranins, Liquorice, liquiritic, glyzarin. ^[9]
17	Adulterants/ Potential confounding material	<i>Abrus fruticosus</i> Wight and Arn., <i>Abrus laevigatus</i> E. May. (Shveta Gunjaa). ^[7,33]	Some other species of <i>Glycyrrhiza</i> and root of <i>Abrus precatorius</i> Linn., ^[8] <i>Indigofera tinctoria</i> . ^[7,33]

taxonomic features, and it is comparatively challenging to identify than aerial herbal samples. Correct identification and authentication of raw herbal drugs are essential to ensure herbal medicines' correct identity, quality, and efficacy.^[27] Because of the above consideration, the present study aimed to develop botanical identification standards to distinguish both species' raw root drug samples. Detailed macroscopic and microscopic characterization (transverse sections and powder samples) were done on root drug samples of the selected two species using a stereo-microscope and light microscope. Comparative studies of crude drug samples revealed some characteristic features that can help identify and differentiate root drug samples of two species.

MATERIALS AND METHODS

Plant collection and identification

The authentic plant material (for herbarium sheets, crude raw specimens, and botanical studies) was collected from the two different locations of U.T. of Jammu and Kashmir (Table 1). For anatomical studies, samples were collected in Formalin-Acetic acid-Alcohol fixative (F.A.A.): Formalin (5ml) + Acetic acid (5ml) + 70% Ethyl alcohol (90ml); (stored for 24 hr then transferred to 70% alcohol). Herbarium sheets were prepared following standard herbarium procedures.^[28] Duly identified herbarium specimens were submitted to the internationally recognized

Janaki Ammal Herbarium (RRLH) at the Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu. Oven-dried raw herb samples were submitted to the Crude Drug Repository at CSIR-IIIM Jammu.

Botanical studies

Macroscopic studies

The macroscopic study involved color, texture, the root surface's appearance, and organoleptic characters of root powder samples were done using a stereo-microscope (LEICA S9i).

Anatomical studies

Fine transverse sections (T.S.) were obtained for the anatomical study by freehand sectioning using a razor blade. Fine sections were stained as per Kumar *et al.*,^[29] with minor modifications. The T.S. was dehydrated in a series of alcohol gradients (50% and 70% alcohol, each for 10-15 min), stained in safranin stain (5-10 min), and then was decolorized in 70% alcohol (5-10 min). The sections were again stained in fast green (3-5 min), decolorized in 70% alcohol (3-5 min), followed by dehydration in 90% alcohol and by absolute alcohol (5-7 min each). The sections were cleared in xylene and finally mounted in Canada balsam. The final sections were examined under a compound microscope (LEICA DM 750) and photographed with an attached camera (LEICA ICC50E).

Powder studies

The root powder samples were crushed into powdery mass, passed through a sieve to obtain a fine powder, and used for organoleptic and microscopic studies. The water-mounted slides were examined under a compound microscope to study the various cell types and cell contents.

Micrometric measurements

For micrometric measurements of transverse sections, 3-5 sections of nearly 2-5 mm diameter were selected. For each section, the size of various tissue zones was measured to the transverse section's radius. Besides, the dimensions of different cells, lumen diameter of xylem vessels, size of starch grains, etc., were also measured.

RESULTS

Taxonomical study

The taxonomic details and comparative macroscopic and microscopic observations have been given in Tables 1, 2, and Figure 1. *Abrus precatorius* is a perennial, much-branched climber with pinnately compound leaves having 10-15 pairs of oblong-shaped, compound paripinnate leaflets resembling tamarind leaves (Figure A1). *Glycyrrhiza glabra* is a small perennial herb or undershrub (2 m high) with 4-7 pairs of oblong, elliptical or lanceolate, compound imparipinnate leaflets (Figure B1).

Macroscopic characters of root

***Abrus precatorius*:** Root is woody, cylindrical, or irregularly curved to tortuous, 0.2-1.5 cm thick, with few branches (Figure A2). Surface light brown to buff-colored, rough, warty, corky, and flaky with transverse cracks (Figure A3). Cut root circular in outline, with thick, brown bark, internally lined with a thin silver-colored ring. The central zone comprised a light brown woody part with numerous randomly distributed pores of varying size, traversed by several creamish rays emerging in the form of

spokes of a wheel from the central region (Figure A4). The thickness of rays appeared nearly uniform from the center to the outer region.

***Glycyrrhiza glabra*:** Root is cylindrical, straight, or with bends, elongated, 0.2-1.0 cm thick, and with few secondary branches (Figure B2). Root yellowish brown, surface smooth with longitudinal cracks in young roots. Mature root surface rough and corky (Figure B3). Cut root circular in outline with light brown colored, thin, corky, bark region. Internal to the cork zone, cut root appeared of three main zones. The outermost zone formed of creamish white zone having alternating reddish-brown rays, followed by inner creamish white porous region alternated with light brown spoke like rays and central circular reddish-brown pith region (Figure B4).

Microscopic characters of root

A detailed comparative microscopic study of root sections of both species (*A. precatorius* and *G. glabra*) showed similar anatomical arrangements of some tissues (Figure A5, B5). Both species were observed with a circular outline of T.S. having outer irregular, broken, thin cork zone; inner cortex and phloem zone (comparatively broader in *G. glabra*), a thin cambium zone separating phloem and xylem tissue. Phloem and xylem tissue zones were present in a ray-like pattern, with each ray being separated from others by the parenchymatous medullary ray. Xylem vessels of variable lumen diameter were present in a spoke-like pattern in both species. A nearly circular to slightly angular pith was present in the center of the T.S. of the root of *G. glabra*. Root and stolon of *G. glabra* are reported similar in anatomical tissue arrangement, except for the absence of pith in T.S. of the root.^[9] Quantitative characters of transverse sections to the studied radius of the studied section are given in Table 2.

***Abrus precatorius*:** The T.S. of the root was circular in outline (Figure A5) with the outermost irregular, broken, thin cork zone (62.25±8.57 µm) having compact lignified, rectangular-shaped cells. Cork was followed by the parenchymatous cortex zone with compactly packed cells, interspersed with a continuous ring of the stone layer in the cortex zone.

Table 2: Quantitative microscopic characters of the T.S. of the root of *A. precatorius* and *G. glabra*.

Character	<i>A. precatorius</i> (µm)			<i>G. glabra</i> (µm)		
	Min	Max	Mean (±S.E.)	Min	Max	Mean (±S.E.)
The radius of studied root T.S.	953.63	1144.42	1073.35±23.93	1725.51	2758.99	1944.76±104.76
Cork zone	32.35	114.64	62.25±8.57	51.99	97.61	76.28±4.64
Cortex + Phloem zone	209.05	353.89	268.78±16.45	340.43	761.47	556.79±37.02
Xylem zone	642.23	845.59	747.13±17.33	477.46	1145.4	725.16±59.82
Vessel lumen diameter	16.4	92.26	52.32±8.19	34.50	74.6	54.48±4.79
Pith zone	-	-	-	501.00	802.14	588.11±28.23
Cork cell size						
Length	15.74	34.26	24.09±1.62	-	-	-
Breadth	11.95	17.31	13.91±0.56	-	-	-
Cortex cell size						
Length	18.44	38.40	25.78±2.30	51.71	78.00	61.47±2.75
Breadth	8.40	15.20	11.24±0.66	30.19	52.24	38.21±2.69
Pith cell size						
Length	-	-	-	31.70	121.30	79.69±8.49
Breadth	-	-	-	41.30	98.20	66.70±5.63
Starch grains size						
Length	4.50	12.70	7.65±0.79	8.70	15.50	10.88±0.70
Breadth	3.90	10.30	6.18±0.66	6.50	12.90	8.09±0.67



Figure 1: A- series). *A. precatorius*, B- series). *G. glabra*; A1 and B1). Plant habit, A2 and B2). Dry root drug sample, A3 and B3). Root surface appearance, A4 and B4). Cut root surface, A5 and B5). Transverse section of root, A6 and B6). Root powder sample, A7-A10 and B7-B10). Powder microscopic characteristics.

Cortex was followed by a distinct dark-colored phloem zone separated by thin parenchymatous ray-like structures. Cortex and phloem formed a thickness of $268.78 \pm 16.45 \mu\text{m}$. Xylem tissue present in the form of rays and formed a major part ($747.13 \pm 17.33 \mu\text{m}$) in the total radius of T.S. of the studied root (mean radius of $1073.35 \pm 23.93 \mu\text{m}$). Xylem consisted of distinct fibers, parenchyma cells, and xylem vessels. Xylem vessels observed of variable lumen diameter ($16.40 \mu\text{m}$ to $92.26 \mu\text{m}$) with a mean lumen diameter of $52.32 \pm 8.19 \mu\text{m}$, were observed present in a spoke-like pattern with uneven distribution. Each xylem ray was separated by thick medullary rays (2-8 cells wide). Pith absent.

***Glycyrrhiza glabra*:** The T.S. of the root was circular in outline (Figure B5) with outermost nearly circular, thin ($76.28 \pm 4.64 \mu\text{m}$), lignified cork zone formed of compactly packed cells. Cork was followed by a narrow cortex zone (3-5 cells wide) with parenchymatous, oval-shaped, compactly packed cells and inner radially elongated wide phloem zone. Phloem was present in a ray-like pattern and appeared patchy due to the presence of phloem parenchyma interspersed by phloem fibers. Each phloem's rays were separated from the other by parenchymatous cortical cells. Cortex and phloem formed a thickness of $556.79 \pm 37.02 \mu\text{m}$ in the total radius of the studied root (mean radius $1944.76 \pm 104.76 \mu\text{m}$). Next to the phloem, a thin cambium layer was present, which separated phloem and xylem tissue. Xylem present in ray-like structure, formed a thickness of $725.16 \pm 59.82 \mu\text{m}$ and consisted of distinct fibers, xylem parenchyma, and xylem vessels. Xylem vessels were present in spoke or ray-like pattern with variable lumen diameter ($34.50 \mu\text{m}$ to $74.60 \mu\text{m}$ with a mean lumen diameter of $54.48 \pm 4.79 \mu\text{m}$). Each xylem ray was separated by 4-7 cell thick medullary rays. A broad pith ($588.11 \pm 28.23 \mu\text{m}$) was present in the center of section.

Powder characteristics

***Abrus precatorius*:** Root powder was greyish brown with some creamish white fragments (Figure A6), odor was characteristic slightly pleasant, texture gritty to sandy, and no characteristic taste. The microscopic study revealed the presence of few fragments of cork cells (Figure A7), parenchyma cells, numerous vessels with pitted walls and reticulate thickening (Figure A8), few tracheids fragments, prismatic calcium oxalate crystals, and few parenchyma cells (Figure A9), round to oval-shaped starch grains (Figure A10), and several reddish-orange or varied colored fragments (Figures A7-A10).

***Glycyrrhiza glabra*:** Root powder was creamish yellow-colored (Figure B6) with a characteristic pleasant odor, flaky texture, and sweet taste. Microscopic examination of powder was observed with the presence of few prismatic crystals, few golden brown fragments, fragments of pitted xylem vessels with reticulate thickenings (Figure B7), cork cells (Figure B8), parenchyma cells with starch grains (Figure B9), prismatic crystals, and abundant spheroidal to elongated starch grains (Figures B7-B10).

DISCUSSION

Herbal drug samples in the crude form are difficult to identify and are often adulterated with other herbs. Plant samples with similar common names or samples belonging to the same family may show superficial resemblance, making identification of genuine herbal samples difficult.^[34,35] Root drugs of both species (*A. precatorius* and *G. glabra*) have different phytochemicals and pharmacological activities (Table 1). Botanical keys often lack taxonomic identification information on dry bark and underground drug samples making herbal drug identification difficult.^[36] In various modern pharmacopeia monographs, macro-morphological description (describing size, shape, relative form, and physical appearance of crude herbal drugs) and organoleptic description of plant drugs (flavor and nature of drugs) are helpful in the identification of medicinal plants.^[36] Among several identifications and

quality assurance methods, botanical identification of raw herbal drugs is considered simple, easy, reliable, time, and cost-effective.^[37,38] Detailed comparative botanical (macroscopic and microscopic) studies, including qualitative and quantitative features, can help identify herbal drugs.^[38,39]

The present study involved a detailed comparative macroscopic and microscopic characterization of root drug samples of *A. precatorius* and *G. glabra*, which revealed the significant characters of taxonomic value in distinguishing root drug samples of both species. A comparative study showed similarity in only a few characters in both species. The study of plant habit revealed compound pinnate leaves in both species; however, with a variable number of leaflets (Figure A1, B1). Similarly, root drug samples of both species were observed with nearly similar physical appearance of, nearly circular-cut root surface (with variation in internal appearance), spoke like appearance of vascular rays in both the species. Some qualitative and quantitative anatomical characters such as the width of medullary rays, the thickness of cork and xylem zone, lumen diameter of xylem vessels, etc. were slightly variable. However, both species significantly differed in some characters including surface features (color and texture of root surface), the appearance of cortex and phloem tissues (phloem being small patchy zone over xylem rays in *A. precatorius* while radially elongated and interspersed with parenchyma and phloem fiber in *G. glabra*), the arrangement of xylem vessels, dilation of medullary rays (more dilation in *G. glabra*), cortex cell size (larger in *G. glabra*, Table 2), presence of stone layer in the cortex (*A. precatorius*) (Figure A5), presence of pith (in *G. glabra*) (Figure B5). Powder study revealed nearly similar microscopic cell types and ergastic contents, but variation was observed in organoleptic and some selected microscopic features. Variation was observed in color of the powder sample (greyish brown in *A. precatorius* while creamish yellow in *G. glabra*) (Figure A6, B6), taste (sweet in *G. glabra*), and texture (gritty and sandy in *A. precatorius*, flaky in *G. glabra*); and in starch grains characters (abundant starch grains with spheroidal to elongated shape in *G. glabra* while few oval to rounded starch grains in *A. precatorius*) (Figure A10, B10).

Macroscopic and microscopic studies available on leaf, stem, and fruit of *Abrus precatorius*.^[40,41] Sawant et al.^[8] studied qualitative macroscopic and microscopic characters of the root of *G. glabra* and observed broad elongated phloem (with a group of fibers) and xylem rays (with xylem vessels, fibers), each ray separated by medullary rays, without pith in T.S. of the root. In several botanical studies, different microscopic characters were reported of taxonomic value in species characterization. Ozoemenam et al.^[42] observed variations in the comparative anatomical characters of herbal samples of various species of *Abrus*. Zhang et al.^[43] studied comparative microscopic studies on two *Abrus* species and observed xylem vessel arrangement, the number of xylem rays, and medullary ray thickness as characteristic features in the authentication of root samples of *A. cantoniensis* and *A. mollis*. Balazs et al.^[44] reported variations in stele structure and the thickness of various tissue types in the root cross-section of three species of *Helleborus* spp. Li et al.^[45] observed the presence of stone cells in the cortex and phloem of roots as helpful in species authentication. Various ergastic cell contents such as starch grains and prismatic crystals are also considered helpful in identifying and characterizing herbal drug material.^[46,47] Several quantitative anatomical characters such as the thickness of various tissue zones to studied transverse section,^[39] size of epidermal, hypodermal, cortical cells, the appearance of various tissues in cross-section, size of starch grains,^[48] are considered as characteristic features and helpful in the identification of raw herbal drugs of different species.

In the present study, various macroscopic and microscopic (anatomical and powder) features of root drug samples described for each species can be used as a reference standard for future identification of two species, in the authentication and distinction of raw root drug samples of genuine

'Liquorice' drug. A comparative study also revealed the significant botanical characters that can be used to distinguish raw root herbal samples of both species.

CONCLUSION

From botanical characters summarised in the present study, genuine 'Liquorice' (*G. glabra*) was distinguished from the adulterant drug (*A. precatوريوس*). Root drug samples of both species can be distinguished from botanical characters, including surface appearance, presence of stone layer in the cortex (in *A. precatوريوس*), the appearance of cortex and phloem tissue, cortex cell size, vascular rays, presence of pith, organoleptic characters of powder (color and taste), and shape and size of starch grains. Characters identified in the present study can be used as botanical reference standards in the future identification of raw root herbal samples of both species.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

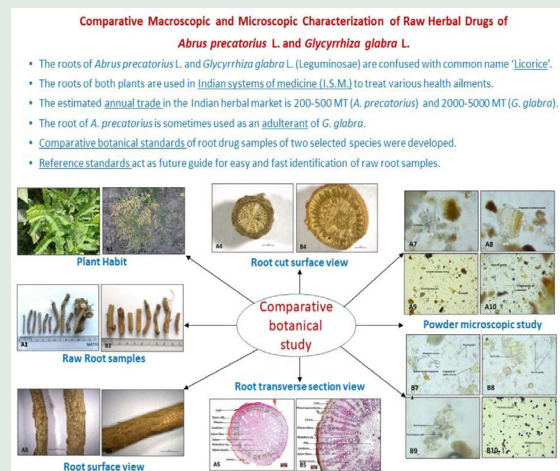
CMV: Cytomegalovirus; **FAA:** Formalin + Acetic acid + Alcohol (ethyl alcohol); **ISM:** Indian systems of medicine; **MT:** Metric Tonnes; **RDS:** Raw Drug Sample; **RRLH:** Regional Research Laboratory Herbarium; **TS:** Transverse Sections.

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GRAPHICAL ABSTRACT



SUMMARY

The roots of *Abrus precatorius* L. and *Glycyrrhiza glabra* L., belonging to family Leguminosae are used in Indian systems of medicine (I.S.M.) to treat various health ailments. The root samples of *G. glabra* are reported with comparatively higher trade value (2000-5000 MT) as compared to *A. precatorius* (200-500 MT). Raw herbal samples of *A. precatorius* is sometimes used as an adulterant of *G. glabra* due to confusion in common name 'Licorice'. Due to lack of proper reference identification standards, the correct identification of raw root samples of unknown background becomes difficult. Present study involved comparative botanical study of root samples of both species in terms of the macroscopic and microscopic characters. The comparative study revealed significant botanical characters that can potentially distinguish raw root samples of genuine 'Licorice' (*G. glabra*) from the adulterant drug (*A. precatorius*). The macroscopic and microscopic characters identified in this comparative study can be used as reference standard for future identification and for distinction of raw drug samples of these two species traded in herbal market.

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