Pharmacognostic specifications of Abroma augusta stems and Cissus quadrangularis aerial part

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ABSTRACT
In present communication pharmacognostic specification of medicinal plants used in veterinary products have been described for the purpose of their standardization. The plants undertaken for the study were Abroma augusta stem (Sterculiaceae) and Cissus quadrangularis (Vitaceae). These plants are official in Ayurvedic Pharmacopoeia of India. The observed microscopic characters of Abroma augusta stems are suberized cork cells, pericyclic fibers, polygonal parenchymatous cells. Microscopic characteristics of Cissus quadrangularis aerial part shows chlorenchymas hypoderms, sclerenchyma sheath; large cells of mucilage along with clusters and bundles of acicular crystals of calcium oxalate were seen in abundant and were scattered throughout the section. Powder microscopy of both the plants was also studied and identifying features were noted down. The improved qualitative microscopic features would prove useful for their quick identification and laying down their pharmacopoeia standards. Additionally, present study also provides the information with respect to crude drug and may serve as a reference point for the proper identification of Abroma augusta stems and aerial parts of Cissus quadrangularis. The present study would also fill the gap in providing evidential support concerning quality determination of plant material used in veterinary medicinal product. Emphasis is upon aiming to increase documentation on primary studies on medicinal plants used in veterinary medicines.

Keywords: Abroma augusta, Cissus quadrangularis, Microscopy.

INTRODUCTION
Animals inherently rely on nature for their wellbeing and use of appropriate herbs in veterinary medicinal product bring them very close nature. Plant derived medicines are used primarily in prophylaxis, diagnosis and therapeutic action in animal health. Herbal medicines used in veterinary medicines are prepared/administered by mixing the herbs primarily with food; in the form of infusions, decoctions, tinctures, powders and tablets for commercial purpose [1]. Herbal drugs are easily available and are economical in use and also have minimum side effects. Most of them are inspired by traditional system of medicine used in locality. Herbal global market is increasing day by day and it generates huge revenue for the country/state. Hence, there is a need for standardization of crude plants to maintain the quality, reproducibility and consistency of herbs used in veterinary medicinal products. Use of herbal medicines for animal health care by rural people covers knowledge, skills, and methods and practices for caring their livestock’s. It is mainly ayurvedic plants which have been in use for thousands of years and have proven to be safe and effective. Modern studies have proven that it is secondary metabolites in herbs/herbal medicines are responsible for considerable range of pharmacological effects are thus benefitting animal health [2]. Each herb has characteristic pharmacological effect and has its own health benefit; also, when combined with other under one medicine can serve the purpose of prescribing herbal medicines in animal health.

There is an increase in demand for herbal drugs these days and to meet the demand it is easily mixed with degraded plant material or adulterated with foreign matters to build the weight and to cut the cost. The misuse of herbal products starts with wrong identification of raw materials. Problems of identification can easily be resolved by pharmacognostic studies of medicinal plants. Standardization parameters for evaluation include macroscopic and microscopic study, powder study, phytochemical analysis and physicochemical analysis [3]. The selected plants for the present study were Abroma augusta stems and Cissus quadrangularis aerial part which accounts for their role in various autoimmune diseases, antimicrobial, adaptogenic, analgesic, anti-inflammatory properties, uterine tonic, galactagogue and growth [4,5], in livestock and other animals. The popular formulations of these plants are Heatont Vet® by Dr. Johns, Ayucal D®, Ayufytase 500K®, Golder, KalDhan B® by Ayurved, Baddi, Rum axon® by Indian herbs, Saharanpur, INDIA. Taking into account above mentioned facts i.e., 1) adulteration menace, 2) scarce of Pharmacognostic study, 3) important pharmacological attributes of selected plants; the objective of the present study is to improve the anatomical description along with estimation of the value of microscopy for the proof of identity and purity of selected plants.
MATERIALS AND METHODS

Collection of plant material

Different samples of plant material were obtained from the Department of stores, Ayurvet Limited Baddi, Himachal Pradesh, India on which further macroscopic and microscopic studies were carried out.

Chemicals

Chloral hydrate - HIMEDIA, Safranin - (Sd. Fine Chem Limited, Mumbai) Phloroglucinol - Sigma Aldrich, Japan) Conc. Hydrochloric acid and glycerin of analytical grade were purchased and used in the study.

Macroscopic analysis

The macroscopic features of Abroma augusta stems and Cissus quadrangularis aerial parts were studied for shape, size, color, surface character, texture and odor. Microscopic studies were carried out by boiling plant parts in 20% solution of chloral hydrate to remove chlorophyll and fatty substances. Further studies were carried out by making very thin sections as per the standard procedures. Hand sections were made by simply holding the moistened plant part to be sectioned between the thumb and forefinger of one hand and other hand for cutting the sections using blade. Wherever necessary, sections were stained with safranin, phloroglucinol, concentrated hydrochloric acid and mounted in glycerin medium. Images were recorded using Zeiss Digital Microscope (Primo Star) in resolution 4X, 10 X, 40 X with PROCAM camera [6].

RESULTS AND DISCUSSION

Abroma augusta is a large, spreading shrub or small tree (normal height 5 meters) sometimes growing up to 10 meters tall. The plant provides a high-quality fiber that is mainly used locally. The branches are horizontal and velvety. Leaf varies in size, and are about 10 – 30 cm long and 6 -18 cm broad. They are ovate or lanceolate, more or less cordate, three to five lobed with 1 - 12 cm long petiole, finely acuminate, membranous, entire. The dorsal surface is glabrous while ventral surface is pubescent. The fragmented stem bark is dark brown in color and thickness ranges from 0.2 to 0.3 cm. The external surface is blackish brown with longitudinally wrinkled small warty markings, while the inner surface is whitish-yellow and finely longitudinally striated, slimy and tough, without any characteristic odour and taste

The transverse sections (T. S.) of mature stem bark shows periderm, consisting of 6-8 layers of cork, 1-2 layers of phellogen and a 3 to 4 of phelloderm. A tangentially elongated thin walled 6 - 9 cell layered suberized cork cells are also seen (Figure 2). The phelloderm consisted of parenchymatous, thin-walled, circular or elliptical to polygonal cells. These parenchymatous cells are characterized by irregular divisions, large mucilaginous cavities, starch grains and rosette crystals of calcium oxalate. Pericyclic fibers are also present. The secondary phloem is wedge-shaped with sieve tubes, phloem parenchyma and companion cells, interspersing with the strands of phloem fibers crossed by medullary rays. Additionally in transverse sections, the phloem parenchymatous cells are found to be polygonal. The thick-walled lignified fibers with sharply pointed chisel like or truncated extremities were also noted. These fibers have entire walls with tapering ends on one side (Figure. 1, 2).

Powder microscopy of Abroma augusta show the presence of group of fibers, stone cell, sclerenchyma, spiral xylem vessels and sclerenchyma cells and may serve as diagnostic features for the powdered stem bark of Abroma augusta (Figure 3).

Figure 1: Microscopy of Abroma augusta stem: (A, B 10x): fibres associated with parenchymatous cells, (C 40x): parenchymatous cells, (D 40x): fibres; stained with safranin (E-H): 40x): (E,F) Cork cells, (G) Parenchyma cells, (H) Pitted aseptate fibres.

Figure 2: Microscopy of Abroma augusta: (F 4X): Parenchyma cells with fibres, (D, E 10X): Cork cells, (C 40X): Pitted fibres with cork cells, (A, B 40X): enlarged view of cork cells.

Figure 3: Powder microscopy of Abroma augusta: (A, F, H): group of fibres, (B, E): stone cell, (C): sclerenchyma, (D, G): spiral xylem vessels, (I): sclerenchyma
Cissus quadrangularis

*Cissus quadrangularis* is a perennial shrub which attains a height of 1.5 m (4.9 ft) and has characteristic quadrangular-sectioned branches with internodes (10 – 12 cm x 1.2 – 1.5 cm) with a leathery edge. Leaves (2-5 cm) appear at the nodes are toothed trilobe. Each leaf is characterized by a tendril emerging from the opposite side of the node. The plant has small white racemes, yellowish-greenish flowers and red globular berries when ripe.

The diagnostic features *Cissus quadrangularis* L. stem are the characteristic quadrangular stem with winged corners; invaded internodes on four sides are depressed deeply in the middle and the corners. Stem have reddish brown to black colored margins (3-4 cm long). *Cissus quadrangularis* is an evergreen climber grows to 5 meters at a fast rate. It grows well in light (sandy), medium (loamy) and heavy (clay) soils.

**Microscopy**

Diagrammatic transverse section of the *C. quadrangularis* stem is four angled and shows primarily single layer epidermis followed by hypodermis. It has narrow cortex and centrally located large pith which occupies almost 2/3rd region of the section and is surrounded by numerous, small, discontinuous band of vascular bundles. Further on magnification the detailed section shows rectangular - pentagonal, 1-2 layered epidermis which is covered by thin cuticle. It is followed by 3-4 layered, circular-polygonal, chlorenchymas hypodermises which are deposited more near the angle. Cortex is very narrow and has 5-7 layers of cortical parenchyma. Pith is very large, parenchymatous in nature and surrounded by discontinuous band of numerous, small, conjoint, collateral vascular bundles. Each vascular bundle is shielded with sclerenchyma sheath, formed into strip and capped with collenchyma band. Few starch grains and rosette crystals and abundant large cells of mucilage, clusters and bundles of acicular crystals of calcium oxalate can be seen scattered throughout the section (Figure 4, 5, 6).

**Powder microscopy**

The fine powdered plant material is observed as green colored and has faint odor. The identifying features of powder are presence of plenty of cluster, rosette and acicular crystals of calcium oxalate which are scattered as such throughout or are embedded with parenchymatous cells. Starch grains are seen as simple and compound, sometimes with 2-celled starch grains; scattered as such or embedded in parenchyma. Various fragments of epidermis in surface view embedded with amniocytic stomata are also observed. The fibers either isolated or in groups, are thin walled, occasionally exhibiting dentate margin. The vessels are annular, reticulate and boarded pitted thickening. Cells of the medullar rays have pitted thickening (Figure 7).
CONCLUSION

Microscopic examination of the raw material received in industry offers a scientific basis for use of right species in making veterinary medicinal products. It is also very economical. Sections are made and with use of proper reagents anatomical structures are visualized differentiated and compared and finally standardization parameters are proposed. The identified characters can be used as identifying features and to differentiate closely related species. Presence of amniocytic stomata, epidermis, chlorenchymas hypodermis; cortex, cortical parenchyma, characteristic pith, collateral vascular bundles, xylem vessels etc. can be seen in Cissus quadrangularis samples. The observed identifying characters of mature stem bark of Abroma augusta shows periderm, phelloderm, cork cells parenchyma, phloem parenchyma (polygonal), pericyclic fibers (lignified); while that of Cissus quadrangularis are mucilaginous cavities, starch grains and rosette crystals of calcium oxalate. In transverse sections, the phloem parenchymatous cells are polygonal. Powder microscopy of Abroma augusta and Cissus quadrangularis show characteristic features as shown. The observed characteristics can be used as diagnostic features for the raw material samples of the mentioned plant and finally check piracy and accordingly make available the true botanicals to the manufacturers of drugs and consumers.

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Conflict of Interest

None declared.

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