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Studies on Anatomical and Phytochemical Analysis of *Oxystelma esculentum* (L.f.) R.br. Ex Schltes

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Abstract: The present investigation has been carried out to determine the requisite anatomical features of root, rhizome, stem, leaf, petiole and phytochemical analysis for evaluating the *Oxystelma esculentum,* important medicinal plant used in the traditional systems of medicine. This study provides referential pharmaco-botanical and phytochemical information for correct identification of this plant.

Key words: *Oxystelma esculentum* · Pharmocognosy · Phytochemical analysis · Anatomy

INTRODUCTION

Oxystelma esculentum (L.f.) R.Br. ex Schltes, an important medicinal plant belonging to the family Asclepiadaceae is used in the traditional systems of medicine for various ailments. The plant is hot, bitter, tonic, expectorant, pungent, dry and indigestible; causes flatulence, diuretic, laxative, aphrodisiac, anthelmintic, useful in leucoderma and bronchitis. The juice is used in gleet, gonorrhea, pain in the muscles, cough and given to children as an astringent. The milky sap forms a wash for ulcers. In combination with turpentine it is prescribed for itch [1]. The herb is reported to possess antiseptic, depurative and galactagogue properties. A decoction of the plant is useful as a gargle in infections of throat and mouth. The latex is bitter and used as a vulnerary. Fresh roots are prescribed for jaundice [2-5].

It is imperative that any crude drug for pharmacological or pharmaceutical use needs to be subjected to scrutiny for botanical identity. The role of anatomical and phytochemical analysis are sought at this juncture to provide a set of diagnostic features of the drug which will help to a considerable extent to ascertain the botanical identity of the drug in question. Anatomical perspective of medicinal plants in an integral component of pharmacognosy, especially while proposing diagnostic protocols for establishing the botanical identity and ascertaining the quality control of raw materials [6].

The present study has been carried out to standardize the anatomical features of leaf, stem, petiole, roots and rhizome and phytochemical analysis to serve as a possible tool for proper identification of *Oxystelma esculentum*.

MATERIALS AND METHODS

Anatomical Studies: For the present study, fresh plant was collected and authenticated using regional flora [7]. The fresh samples of leaf, petiole, stem, root and rhizome were cut in to small pieces and fixed immediately in FAA for 24 h. After fixation they were washed thoroughly in distilled water, dehydrated, embedded in paraffin wax after infiltration and sectioned using rotary microtome to the thickness of 8 to 14 µm [8]. Sections were stained with toluidine blue [9]. For the study of tracheary elements, the stem and roots were macerated employing Jeffrey's fluid [10] and stained with safranin. All the photomicrographs were taken using Nikon Eclipse 400 microscope.

Phytochemical Studies: For the chemical analysis the whole plant was shade dried and powdered. Powdered samples were subjected to physico-chemical analysis such as the percentage of water and alcohol soluble extractive, total ash, acid-insoluble ash [11] and preliminary phytochemical screening was carried out using standard procedures [12-14]. Total alkaloid content was also determined according to Abdelouaheb *et al*. [15].

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Cardiac glycosides (Cardenolide) were extracted as per the procedure followed by Wagner, H and S. Bladt [16] and used for the TLC analysis. For TLC precoated Silica Gel F_{254} (E.Merck) plate was used for stationary phase and Ethylacetate:Methanol:Water (100:13.5:10) used for mobile phase. After development the plate was sprayed with 10% ethanolic sulphuric acid and heated at 105°C in hot air oven for 5 to 10 min to develop the spots.

Observation

Macroscopic Structure of Root: Roots 15-40 cm long and 0.2-0.5 cm thickness with few lateral roots of smaller size, tap root branched at the tip, outer surface is brown to yellowish brown; odourless, slightly acrid taste.

Microscopic Structure of Root (Fig. 2, 3 and 8): The cross section of root measures about 4-5 mm in thickness and circular in outline with small fissures. Outermost zone consists of radial bands of rectangular, tangentially elongated, thin-walled cork cells in 4-5 rows. Secondary phloem composed of phloem fibres in small patches with thin walled parenchyma in between. Few druses type of calcium oxalate crystals were found scattered in phloem parenchyma (Fig. 3). Laticifers occur in the phloem cells. The vascular cylinder composed of dense solid secondary xylem with wide vessels. The secondary xylem consists vessel elements, xylem rays, xylem fibres and xylem parenchyma. Vessel elements are circular, wide, 125-150 x 400-450 μm in size, tailed, with simple pits, simple peroration plates, arranged in diffuse porous with pores solitary (Fig. 8). Xylem rays are not prominent and mostly uniseriate. Starch grains are absent (Fig. 3).

Macroscopic Structure of Rhizome: Rhizomes are short in length when compared to roots and the surface is yellowish to pale brown in colour, odourless, slightly acrid taste.

Microscopic Structure of Rhizome (Fig. 4 and 5): In cross section, 5-6 mm in diameter of rhizome shows circular or oval shape in outline with no fissures. Two layers of epidermis and outer layer consist prominent radially elongated cell with cuticle (Fig. 4). Cortex comprised heterogeneous cells of parenchyma and small nests of sclerenchyma. Parenchyma cells filled with abundant starch grains and crystals (Fig. 5). Xylem and phloem is continuously arranged. Xylem consists of mostly tracheids and xylem fibre. Pith is present (Fig. 4).

Fig. 1-9: Whole plant and anatomy of root, rhizome, stem and vessel elements
(C-Cork, Co-Cortex, Cr – Crystals, E-Epidermis, LV-Latex vessel, Phl–Phloem, PFi-Phloem fibre, Pi-Pith, PP-Perforation plate, Pt-Pit, SG-Starch grains, SX-Secondary xylem, T-Tail, VE-Vessel element, XR-Xylem rays, Xy-Xylem)

Macroscopic Structure of Stem: Greenish, thin, wirey, 2 to 3 meter long and 1.5 to 3 mm thickness, strong, fibrous, milky latex present.

Microscopic Structure of Stem (Fig. 6, 7 and 9): A thick mature stem cross section shows circular in outline with single layer of epidermis. The cortical zone is heterogeneous consisting of two layers of collenchymatous cells followed by two to three layers of chlorenchyma cells and five to seven layers of parenchyma. Patches of phloem fibres occurring in the cortex and form continuous ring. Druses type of calcium oxalate crystals are distributed in the cortex and in the phloem fibres and starch grains absent (Fig. 7). Prominent laticifers occur just above the phloem. Secondary xylem is pentagonal shaped (Fig. 6). Vessels are solitary, wide, short, circular or oval shaped in outline, barrel shaped,

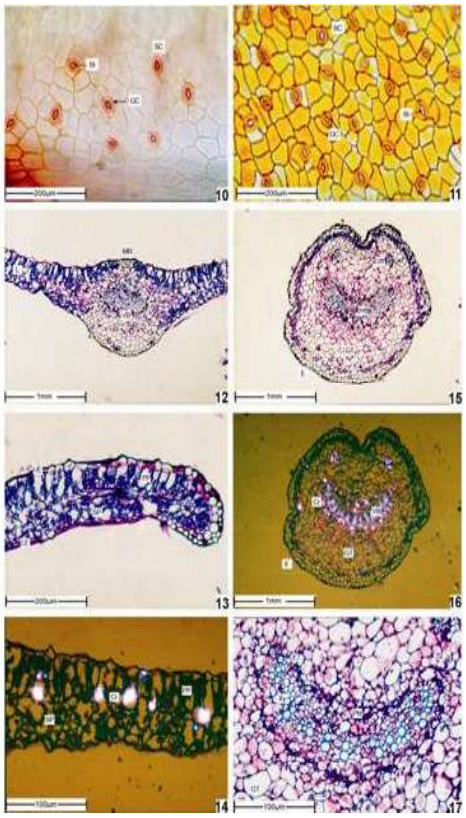

Fig. 10-17: Anatomy of leaf and petiole
(Cr-Crystal, E-Epidermis, GC-Guard cell, GT– Ground tissue, SC-Subsidiary cell, St-Stomata, MD-Midrib, VB-Vascular bundle, UE-Upper epidermis, LE-Lower epidermis, PP-Palisade, parenchyma, Phl-Phloem, SP-Spongy parenchyma)

with simple pits, simple perforation plate and no tailed (Fig.9). Parenchymatous pith present in the centre (Fig. 7).

Macroscopic Structure of Leaf: Lamina linear or linear-lanceolate, 6–11 × 0.7–2 cm, membranous, base rounded; lateral veins 9–12 pairs, marginal vein present, margin entire, acuminate, petiole 0.8 -1.2 cm long.

Microscopic Structure of Leaf (Fig. 10-14): The epidermis consists of cubical or somewhat conical shaped cells, covered by thick cuticle and is devoid of any trichomes (Fig. 12 and 13). Stomata are amphistomatic and anomocytic type. The stomatal index is higher in lower epidermis when compared to upper epidermis (Fig. 10 and 11). Mesophyll cells are differentiated in to palisade and spongy parenchyma. Palisade parenchyma present below the upper epidermis consists of continuous

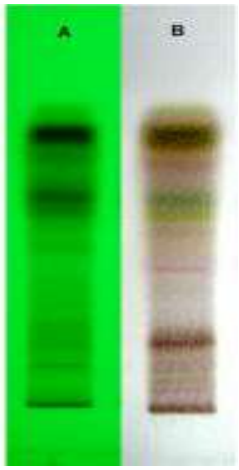

Fig. 18: TLC finger print of Cardenolide extract
A – Under UV-254 nm; B – After spray

single layered cells and vertically elongated. Spongy parenchyma lies below the palisade parenchyma and loosely arranged (Fig. 13). Spongy parenchyma consist druses type of calcium oxalate crystals (Fig. 14). Midrib and lamina regions are very distinct. The epidermis consists of a single layer of cells which are mostly cubical cells. Below the epidermis there is a wide zone of cortex composed of single layer of collenchyma showing angular thickening and wide zone of parenchymatous cells which are thin-walled and more or less isodiametric. In the vascular region phloem surrounds the central xylem bundle (Fig. 12).

Microscopic Structure of Petiole (Fig. 15-16): Petiole measuring about 1 mm thickness shows is circular in outline in cross sectional view with less distinct adaxial flat side and devoid of trichomes. Epidermis is single layered and consists of rectangular cells followed by two layers of collenchyma and two layers of chlorenchyma cells. Vascular bundles are open type. Three vascular bundles arranged as one dorsal, two laterals. Thus the arrangement of vascular bundles expressed as 1 + 2. Lateral bundles are very small and circular in shape when compared to dorsal bundle which is bowl shaped. Ground tissue made up of thin-walled parenchymatous cells (Fig. 15). Druses type of calcium oxalate crystals found abundantly scattered in the phloem tissue (Fig. 16).

Phytochemical Analysis: The results of physico-chemical analysis, presence and absence of different phyto-constituents, quantitative estimation of total alkaloids and TLC fingerprint profiles of cardenolide extract are presented in Tables 1-3 and Fig. 18.

Table 1: Physico-chemical analysis

Parameter	Results [Mean ± SD]
Water soluble extractive	26.45±1.56
Alcohol soluble extractive	7.5±0.65
Total ash	9.05±0.93
Acid insoluble ash	0.43±0.08

Table 2: Preliminary Phytochemical screening

Chemical constituents	Results
Tannins	+
Phlorotannins	-
Saponins	-
Flavonoids	+
Steroids	-
Terpenoids	+
Cardiac glycosides	+
Alkaloids	+
Quinones	-
Total Alkaloid content	0.075%

Table 3: TLC analysis cardenolide extracts

Rf Values	Colour of the spot
UV – 254 nm (Before spray)	
0.10	Black quenching
0.16	"
0.20	"
0.34	"
0.42	"
0.52	"
0.56	"
0.67	"
0.73	"
0.80	"
After spray (Day light)	
0.05	Light brown
0.07	Light brown
0.10	Light green
0.18	Dark brown
0.37	Pink
0.52	Light green
0.56	Green
0.65	Light brown
0.73	Black
0.80	Green

DISCUSSION AND CONCLUSION

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant and ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite [17]. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [18].

Oxystelma esculentum widely used in traditional medicines has tremendous medicinal potential owing to its multifaceted biological functions. However, there are no detailed pharmacognostic studies on this plant to help in the proper identification. Hence the present study was undertaken with the aim to provide key diagnostic tools of identification.

The following anatomical and phytochemical features of the above drug are the key features that can be used to diagnose this plant.

1.Root: Druses type of calcium oxalate crystals present and starch grains absent in the cortex region. Vessel elements circular in outline, wide, tailed, with simple pits, simple peroration plates, arranged in diffuse porous with pores solitary.

2. Rhizome: Epidermal cells large in size and radially elongated. Cortex with abundant starch grains and druses type of calcium oxalate crystals. Xylem elements mostly with fibres and tracheids.

3. Stem: Abundant druses type of calcium oxalate crystals and phloem fibres in the cortex. Pentagonal shape of secondary xylem. Vessels are solitary, wide, short, circular or oval outline, barrel shaped, with simple pits, simple perforation plate and without tail.

4. Leaf: Stomata are amphistomatic and anomocytic type. Druses type of calcium oxalate crystals in mesophyll.

5. Petiole: Circular in outline, vascular bundle open type; three vascular bundles, arrangement is 1 + 2; dorsal bundle large bowl shaped and laterals small circular. Vascular bundles surrounded by druses type of calcium oxalate crystals.

6. Secondary metabolites: Tannins, flavonoids, terpenoids, cardiac glycosides and alkaloids are present.

7. TLC profile (Before spray): Ten black quenching spots under UV 254 nm in cardenolide extract. After spray - ten spots along with three major spots at Rf. 0.18 (dark brown), 0.56 (green) and 0.73 – (black).

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