PHARMACOGNOSTIC STUDY AND ESTABLISHMENT OF QUALITY PARAMETERS OF LEAVES OF *OXYSTELMA ESCULENTUM*

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ABSTRACT
Oxystelma esculentum is a perennial twiner growing near water-logged areas in the Indian subcontinent. The leaves are traditionally used as diuretic, galactagogue, anthelmintic, antiulcer, laxative and antiperiodic. The plant is also used ethnomedicinally in throat infections, skin diseases and jaundice. The present investigation deals with the pharmacognostical study of the leaves of the plant and establishment of its quality parameters. The study revealed important diagnostic features. Transverse section of leaf showed the presence of a sheath of calcium oxalate rosette crystals in the mid-rib and 2-3 layered palisade in the lamina. Microscopy of the powdered leaves revealed the presence of anomocytic stomata, various types of covering trichomes and parenchymatous tissue filled with numerous rosette crystals. Various physico-chemical parameters and quantitative microscopic parameters were established. From the phytochemical screening, the leaves were found to contain cardenolides, flavonoids, phenolics and sugars, which were estimated by their respective procedures. The present work can serve as a useful tool in the identification, authentication and standardization of the plant material.
Keywords: Asclepiadaceae; Dudhlata; Jaldudhi; *Oxystelma secamone*

1. Introduction

Oxystelma esculentum R. Br. syn. *Oxystelma secamone*, *Periploca esculenta*, *Periploca secamone*, *Sarcostemma secamone*, *Sarcostemma esculentum* and *Asclepias rosea* (Family – Asclepiadaceae) is commonly known as 'Jaldudhi' or 'Dudhlata' and is found near water logged areas of the plains and lower hills of the Indian subcontinent and Java. The whole plant, especially leaves, have been reported to possess antiperiodic, anthelmintic, diuretic, laxative, antiulcer and galactagogue activity. It is used ethnomedicinally in throat infections, skin diseases and also in the treatment of jaundice[1-3]. The present study deals with the pharmacognostical study of the leaves of the plant and establishment of its various quality parameters.

2. Materials and Methods

O. esculentum was collected in the flowering stage from Nadiad, Central Gujarat in November 2006. Voucher specimen (No. LM/COG/PG/17) was deposited in the Department of Pharmacognosy, L. M. College of Pharmacy, Ahmedabad. Authentication of herbarium was done by Dr. H. A. Solanki, Department of Botany, Gujarat University.

Fresh leaves were used for pharmacognostical studies and quantitative microscopy. The leaves were dried under shade and powdered to 60# separately and stored in airtight containers and used for physico-chemical evaluation and phytochemical studies.

Macroscopical and microscopical studies of the leaves were performed. For microscopical studies, chloral hydrate was used as clearing agent and phloroglucinol with conc. HCl was used for staining. Photomicrography of the transverse sections and the powdered drug was performed using light microscope (Lab-o-Med) and WinDVR camera and software. Quantitative microscopic study was performed using camera lucida and stage micrometer scale[4] (Table 1).

Physico-chemical evaluation included determination of moisture content, ash values (total ash, acid insoluble ash and water soluble ash) and extractive values (water and alcohol soluble extractives)[5] (Table 2). Phytochemical screening was performed (Table 3)[6-10]. Estimation of phytoconstituents included that of cardenolides, phenolics, flavonoids and sugars [11-14] (Table 4).

3. Results

3.1 Macroscopy: Leaves are simple, 4-12cm X 2-8mm, opposite, dorsiventral, linear-

lanceolate, glabrous, having acute apex, entire margin, membranous texture, reticulate venation and symmetrical base. Stem is cylindrical, slender, 4-6 mm in diameter, glabrous and light green in color, with a distance of 5-11 cm between the nodes. The leaves and stems do not have any particular odor or taste. Flower is solitary, 2-3 cm in diameter, drooping, having saucer-shaped corolla with purple veins. Fruit is follicle, 4-6 cm long, solitary, ovate-lanceolate and tapering towards the end (Fig. 1).

Figure 1. Aerial parts of *Oxystelma esculentum*

3.2 Microscopy: Both the upper epidermis and lower epidermis show straight walled, polygonal epidermal cells, underlying palisade cells (Pal), collapsed and semi-collapsed anomocytic stomata (St), and various types of covering trichomes including 2-5 celled multicellular trichomes, thick-walled warty trichomes, collapsed cell trichomes and knee-shaped trichomes (Fig. 2). Diagrammatic and detailed T. S. of leaf (Fig. 3, 4) shows a dorsiventral lamina consisting of a single layered epidermis (Ep) having various types of trichomes (Tr). Underneath the epidermis are 2-3 layered palisade cells (Pal) having scattered rosette crystals of calcium oxalate (Cr). The spongy mesophyll region (Sm) in the lower lamina consists of polygonal parenchymatous cells and shows obliquely cut vessels. The midrib has a single layered upper and lower epidermis (Ep) followed by a well developed collenchymatous hypodermis (Co). Bicollateral vascular bundles (Vb) are found in the centre of parenchymatous ground tissue. Perimedullary phloem (PPh) is found above the vascular bundles. A sheath of rosette crystals of calcium oxalate (Cr) is found in the lower phloem region of the vascular bundles. Various types of covering trichomes are found predominantly on the lower surface.

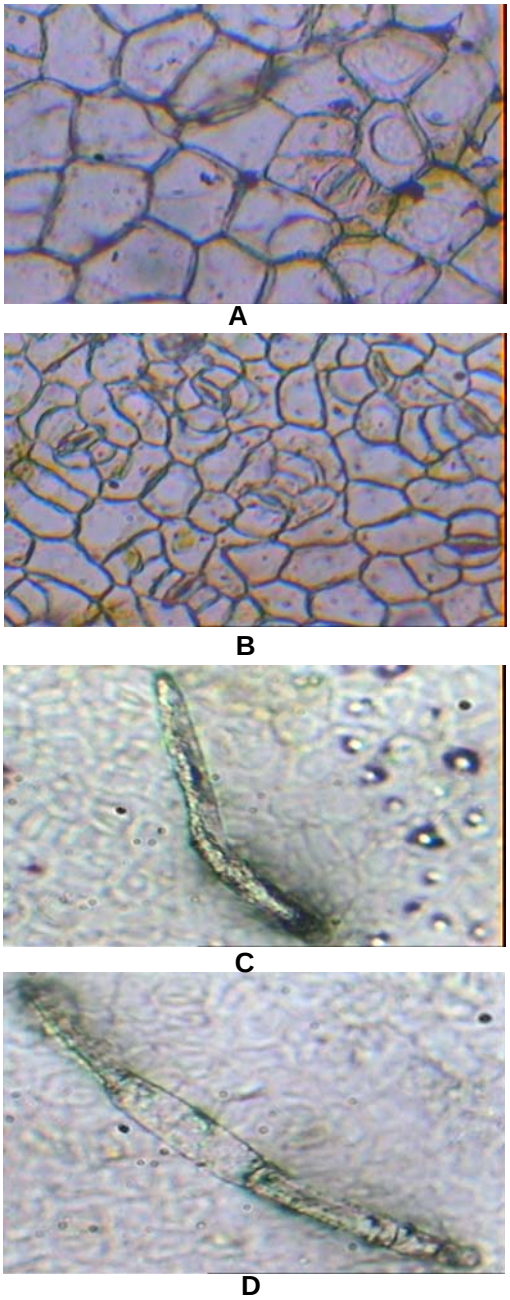
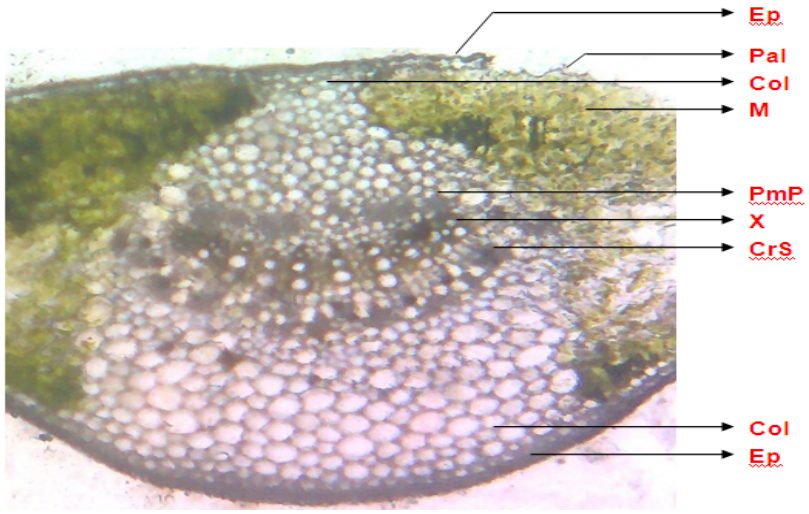


Figure 2. Surface preparation of leaf

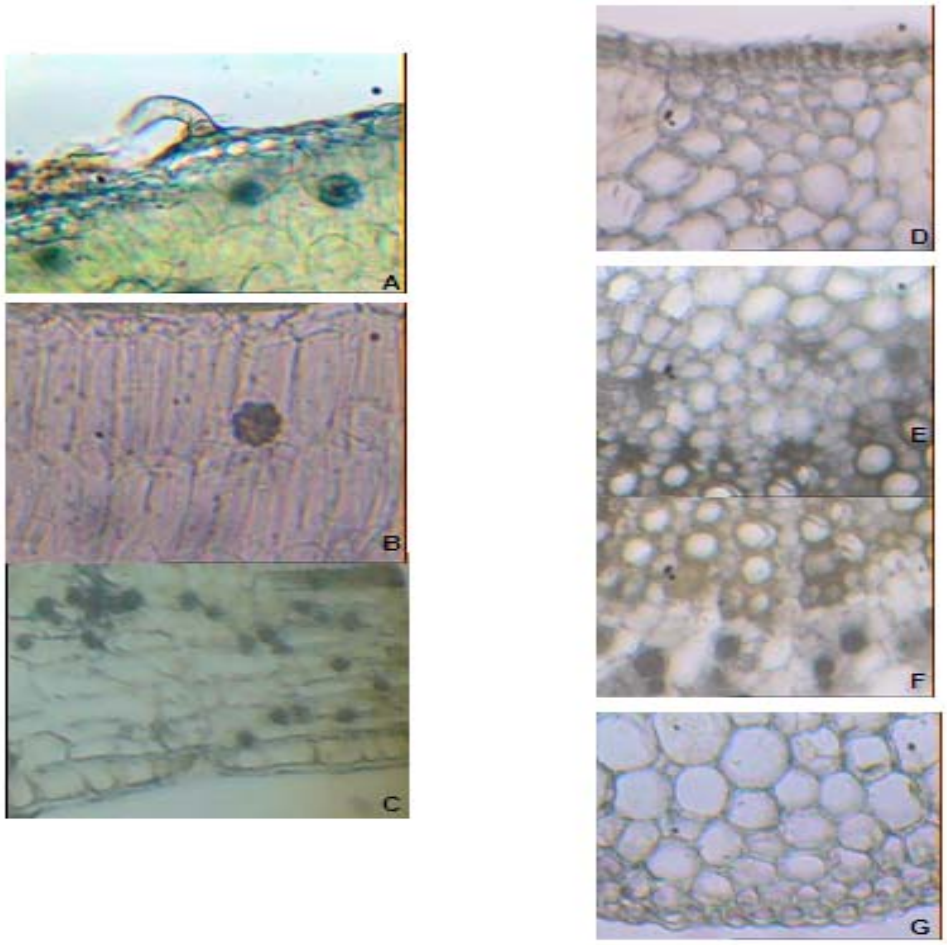
A, Upper epidermis showing anomocytic stomata and underlying palisade cells (X100); **B,** Lower epidermis showing anomocytic stomata (X100); **C**, Knee-shaped covering trichome (X400); **D**, Collapsed-cell covering trichome (X400).

Figure 3. T. S. of leaf (X100)



Ep, Epidermis**; Col,** Collenchyma**; Pal,** Palisade**; X,** Xylem**; PmP,** Perimedullary phloem**; CrS,** Calcium oxalate rosette crystals sheath; **Tr,** Trichomes; **PPh,** Perimedullary phloem; **M,** Spongy mesophyll.

Figure 4. Enlarged portions of detailed T. S. (X400)



A, Upper lamina having covering trichome, palisade cells and rosette crystals**; B,** Palisade cells having rosette crystal; **C,** Lower part of lamina; **D,** Upper collenchyma of mid-rib; **E,** Perimedullary phloem; **F**, Rosette sheath underlying the vascular bundles; **G**, Lower collenchyma of mid-rib.

***3.3 Powder characteristics*:** It is a green coloured powder with no distinct odour or taste. The most important diagnostic feature of the powder is a parenchymatous tissue filled with large number of rosette crystals of calcium oxalate (Cr). Xylem vessels with pits or annular thickening (Xy), parts of epidermis in surface view showing anomocytic stomata (St) are the other powder characteristics. Various types of trichomes are found which include 2-5 celled multicellular covering trichomes, warty trichomes, curved trichomes, collapsed cell trichomes, unicellular dagger-shaped trichomes and knee-shaped trichomes (Tr) (Fig. 5).

Figure 5. Powder study (X400)

A, Xylem vessels with annular thickening; **B**, Parenchymatous tissue filled with rosette crystals of calcium oxalate; **C,** Anomocytic stomata; **D**, Unicellular dagger-shaped covering trichome; **E**, Multicellular covering trichome; **F**, Hooked-top covering trichome.

Table 1. Quantitative microscopy

PARAMETERS	MEASURED VALUE
Stomatal Index Upper surface Lower surface	 5.35±0.08 14.96±0.08
Palisade ratio	8±1
Vein islet no.	13 ±0.09
Vein termination no.	28 ±1
Rosette diameter (µ)	12.88 - 23.39 – 37.2

Number of observations =10

Table 2. Physico-chemical evaluation

PARAMETER	% w/w± SD
Loss on drying	77.83 ± 0.07
Ash values Total ash Acid insoluble ash Water soluble ash	 11.34±0.13 0.66±0.17 5.89±0.16
Extractive values Water soluble extractive Alcohol soluble extractive	 4.72 ± 0.06 1.87 ± 0.05

Number of observations =10

Table 3. Phytochemical screening

Phytoconstituent	Test	Result
Alkaloids	Dragendorff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
Flavonoids	Shinoda test	+ve
	Lead acetate test	+ve
Sterols	Salkowski test	+ve
	Libermann Buchardt t	+ve
Cardiac glycosides	Legal's test	+ve
	Baljet test	+ve
	Keller Killiani test	+ve
	Kedde's test	+ve
Saponin glycosides	Foam test	-ve
	Lead acetate test	-ve
Phenolics	Ferric chloride test	+ve
	Folin ciocalteu test	+ve
Sugars	Fehling's test	+ve
	Molisch test	+ve
Gums	Ruthenium red test	-ve

Table 4. Estimation of phytoconstituents

PHYTOCONSTITUENTS	% w/w± SD
Cardenolides	0.82 ±0.02
Phenolics	1.10±0.04
Flavonoids	0.56±0.01
Sugars	2.38±0.03

Number of observations =10

4. Results and Discussion

A detailed pharmacognostic study of leaf of *Oxystelma esculentum* has been performed. Powder study showed the presence of parenchymatous tissue filled with abundant rosette crystals, various types of covering trichomes, xylem vessels with pits or annular thickening and anomocytic stomata. Various leaf constants and physico-chemical parameters were established. Values of water soluble extractives were greater than those of alcohol soluble extractives, indicating the presence of water soluble phytoconstituents in higher amounts. Cardenolides, phenolics, flavonoids and sugars were detected and estimated by their respective methods. The present study can be very useful in standardizing the formulations of *O. esculentum* as well as serve as a guide for isolating novel phytoconstituents.

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