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Histological and Physico-Chemical Evaluation of *Hybanthus enneaspermus* (L.) F. Muell

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Abstract – This paper presents a detailed pharmacognostical study of the plant drug *Hybanthus enneaspermus* (L.) F. Muell (Violaceae), an important drug in the Indian system of medicine. The leaf and stem samples were studied using procedures of light, confocal microscopy, WHO recommended physico-chemical determinations and authentic phytochemical procedures. The physico-chemical, morphological, and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *H. enneaspermus* and may possibly help to differentiate the drug from its adulterants.
Keywords – *Hybanthus enneaspermus*, Violaceae, pharmacognosy, stem and leaf

Introduction

Hybanthus enneaspermus (L.) F. Muell (Violaceae) (Singh, 1988) known as Lakshmisheshta, Padmavati, Padmacharini or Purusharathna in Sanskrit, is an important plant in the Indian system of medicine. It is a small suffrutescent perennial herb found in the regions of former Madras Presidency in India, Ceylon, tropical Asia, Africa, and Australia. It grows 15 - 30 cm in height with many diffuse or ascending branches and is pubescent in nature (Kirtikar and Basu, 1991). Traditionally the plant is used as an aphrodisiac, demulcent, tonic, diuretic, in urinary infections, diarrhea, leucorrhoea, dysuria, and sterility (Yoganarasimhan, 2000). Moreover, the plant is reported, in ancient ayurvedic literature, to cure conditions of "kapha" and "pitta", urinary calculi, strangury, painful dysentery, vomiting, burning sensation, wandering of the mind, urethral discharges, blood troubles, asthma, epilepsy, cough, and to give tone to the breasts (Kirtikar and Basu, 1991). The plant is also attributed to its antimicrobial and antiplasmodial action (Rajakaruna *et al.*, 2002; Weniger *et al.*, 2004). Various phytoconstituents viz. dipeptide alkaloids, aurantiamide acetate, isoarborinol, and β-sitosterol have been isolated from different parts of this plant (Majumder *et al.*, 1979; Prakash *et al.*, 1999; Yoganarasimhan, 2000; Retnam and De Britto, 2003).

In spite of the numerous medicinal uses attributed to this plant, there is no pharmacognostical report on the anatomical and other physico-chemical standards required for the quality control of the crude drug. Hence, the present investigation includes morphological and anatomical evaluation, determination of physico-chemical constants and preliminary phytochemical screening of the different extracts of *H. enneaspermus*.

Experimental

Plant material – The whole plant of *H. enneaspermus* was collected from the Chickballapur fields in Kolar district of Karnataka state, India in the month of July, 2001. The plant was authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (PP 509) has been deposited in the herbarium of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

Chemicals and instruments – Compound microscope, stage micrometer, camera lucida, drawing sheets, glass slides, cover slips, watch glass and other common glass wares were the basic apparatus and instruments used for the study. Microphotographs were taken using a Leica DMLS Microscope attached with Leitz MPS 32 camera. Solvents *viz;* ethanol (95%), hexane, petroleum ether, diethyl ether, chloroform, acetone, n-butanol and reagents *viz.* phloroglucinol, glycerin, hydrochloric acid, chloral hydrate and sodium hydroxide were procured from Ranbaxy Fine Chemicals Ltd, Mumbai, India.

Macroscopic and microscopic analysis – The macros-

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copy and microscopy of the plant were studied according to the method of Brain and Turner (1975a). For the microscopical study, cross sections were prepared and stained as per the procedure of Johansen (1940). The micro powder analysis was done according to the method of Brain and Turner (1975b) and Kokate (1986a). Leaf constants *viz.* vein islet number, veinlet termination, and stomatal index were studied according to the method of Wallis (1953) and Evans (2003).

Physico-chemical analysis – Physico-chemical values such as the percentage of ash values and extractive values were performed according to official methods prescribed (Indian Pharmacopoeia, 1996) and the WHO guidelines on quality control methods for medicinal plant materials (WHO/QCMMPM guidelines, 1992).

Preliminary phytochemical screening – Preliminary phytochemical screening was carried out by using standard procedures described by Kokate (1986b) and Harborne (1998)

Results and Discussion

Macroscopic characters (Fig. 1) – *H. enneaspermus* is a small suffrutescent perennial herb, 15 - 30 cm in height, with many diffuse or ascending branches, glabrous or more or less pubescent. The leaves are linear or lanceolate, 4 - 5 cm in length and 3 - 8 mm in breadth. They are sub-sessile with serrated margin, stipules, gland-tipped and subulate. The flowers are red, 6 - 12 mm in length, auxiliary, solitary, pedicels shorter than the leaves, erect and slender. Bracts are small, which are grown above the pedicel. Sepals are 2.5 mm in length, lanceolate and very acute. Petals are unequal, the upper ones oblong, slightly longer than the sepals, the lower ones much larger than the others, having an orbicular or obviate limb with a long claw which is curved behind into a short spur. Capsules are about 6mm in diameter and sub-globose. Seeds are ovoid, acute, often longitudinally striate, yellowish white and about 1.5 mm in length.

II. Microscopic characters Leaf (Fig. 2) – The transverse section of the leaf *H. enneaspermus* shows a dorsiventral nature. The section is broadly divided into lamina and midrib region. The lamina of the leaf shows three distinct regions *viz.* upper and lower epidermis, spongy parenchyma and mesophyll. The upper epidermis is single layered with more or less rectangular cells with cuticularised walls. Covering trichomes which emerge from the epidermal layers are unicellular, short and warty. Paracytic stomata are seen on the lower and upper epidermis. Being a dorsi-ventral leaf, mesophyll is differentiated into palisade and spongy parenchyma. The palisade is single layered, cells are elongated, compact and continuous over the midrib region. The spongy parenchyma is many-layered, loosely arranged with intercellular spaces. Few spheraphides are seen in the parenchyma. Lower epidermis is identical to the upper epidermis but has numerous stomata.

Fig. 1. Macroscopy of the aerial aspect of *Hybanthus enneaspermus*.

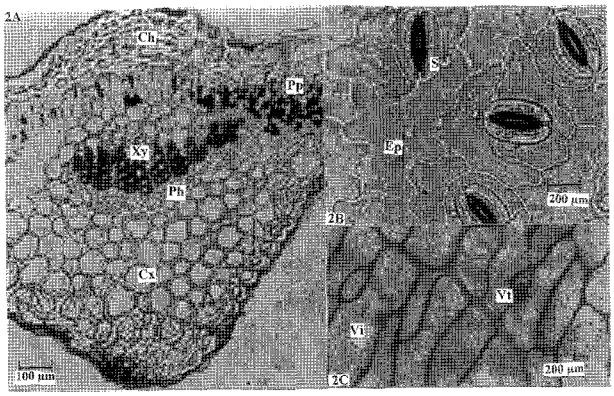

Fig. 2. Microscopy of the leaf of *Hybanthus enneaspermus*.
A. Photomicrograph showing the transverse section of the leaf of *Hybanthus enneaspermus*.
B. A portion of leaf showing stomata.
C. A portion of leaf showing the vein islets and terminations.
Ch-collenchyma, **Cx**-cortex, **Ep**-epidermal cell, **Ph**-phloem, **Pp**-palisade parenchyma cell, **S**-stomata, **Vi**-vein islet, **Vt**-vein termination, **Xy**-xylem.

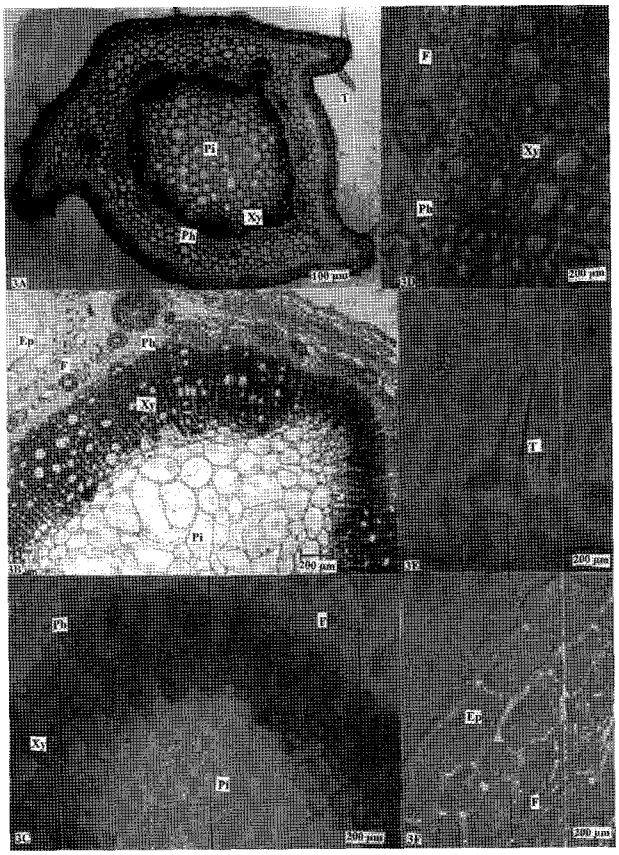

Fig. 3. Microscopy of the stem of *Hybanthus enneaspermus.*
A. Photomicrograph showing the transverse section of the stem of *Hybanthus enneaspermus.*
B. A portion of the transverse section (enlarged).
C. A section of stem showing the vascular structures.
D. A section showing fibres and vascular elements in the stem.
E. A portion of stem showing trichome.
F. A section showing the outer regions of the stem.
Ep-epidermal cell, **F**-fibres, **Ph**-phloem, **Pi**-pith, **T**-trichome, **Xy**-xylem.

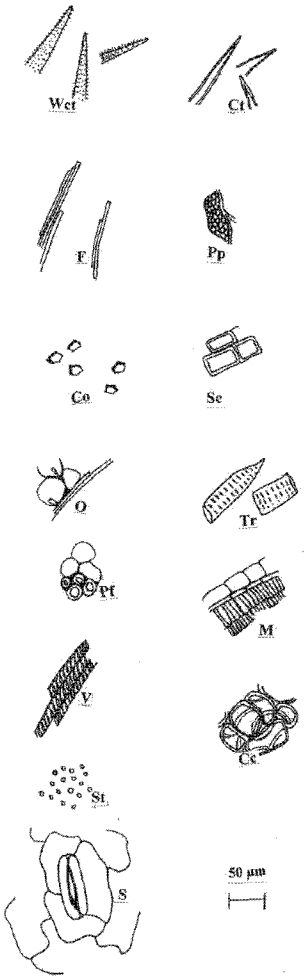

Fig. 4. Powder microscopy of the leaf and stem of *Hybanthus enneaspermus.*
Cc-cortical cells, **Co**-calcium oxalate prisms, **Ct**-covering trichomes, **F**-fibres, **M**-mesophyll tissue, **O**-oil droplets in cell, **Pf**-pericyclic fibres, **Pp**-palisade parenchyma cells, **S**-stomata, **Sc**-sclereids, **St**-starch grains, **Tr**-trachieds, **V**-vessels, **Wct**-warty covering trichomes.

The epidermal layers of the lamina are continuous in the midrib region also. Strips of collenchyma appear below the upper and above the lower epidermis. This is followed by several layers of cortical parenchyma containing microsphenoidal crystals embedded in the central region of a cortical parenchyma. The vascular bundle is collateral.

Stem (Fig. 3) – The transverse section of the stem is more or less triangular with three ridges. The margin is wavy and the following tissues are seen from the periphery to the centre.

Epidermis – This consists of a single layer of more or less circular cells, with cuticularised outer walls. Abundant covering trichomes emerge from the upper epidermal layer. The covering trichomes are uniseriate, unicellular and warty with blunt tips.

Cortex – The outermost 2 - 3 layers of cortical parenchyma cells appear like collenchyma cells and contain chloroplasts. Scattered lignified fibres in groups of 3 - 4 occur in the innermost layers of circular cortical parenchyma.

Vascular bundle – This is arranged in a ring and is conjoint and collateral with distinct phloem towards the outside. Groups of discontinuous lignified pericyclic fibres crown the phloem on its outside. Well developed xylem, consisting of vessels, tracheids, fibro-tracheids and parenchyma are seen. The xylem in the stem occurs as a well developed continuous band.

Pith – This occupies a major part of the transverse section and is made up of large thick-walled polygonal

Table 1. Preliminary phytochemical screening of the leaf powder of *H. enneaspermus*

test	hexane	benzene	chloroform	acetone	ethanol	water
alkaloids	–	–	+	–	+	+
carbohydrates	–	–	–	–	+	+
glycosides	–	–	–	+	+	+
phenolic compounds and tannins	+	–	–	+	+	–
flavonoids	–	–	–	+	+	+
volatile oil	–	–	–	–	–	+
gums and mucilages	–	–	–	–	–	+
saponins	–	–	–	–	–	–
phytosterols	+	–	–	–	–	–

+ denotes the presence of the respective class of compounds.

Table 2. Preliminary phytochemical screening of the stem powder of *H. enneaspermus*

test	hexane	benzene	chloroform	acetone	ethanol	water
alkaloids	–	–	+	+	+	+
carbohydrates	–	–	–	–	+	+
glycosides	–	–	–	+	+	+
phenolic compounds and tannins	+	–	–	+	+	–
flavonoids	–	–	–	+	+	+
volatile oil	–	–	–	–	–	–
saponins	–	–	–	–	–	–
gums and mucilage	–	–	–	–	–	+
phytosterols	+	–	–	–	–	–

+ denotes the presence of the respective class of compounds

parenchyma with intercellular spaces.

III. Powder characters (Fig. 4) – The powder is pale green in colour, with a characteristic odour and mucilaginous taste. Numerous covering warty trichomes, which are unicellular and lignified with blunt tips are seen in the powder. Paracytic type of stomata are seen occasionally. Bundles of palisade cells are observed along with veins. Calcium oxalate crystals occur as prisms which are observed as a sheath of cells around the fibers. Few elongated fibres, tracheids and sclereids are also observed. Oil droplets are seen rarely but starch grains occur throughout the powder.

IV. Preliminary phytochemical screening – Preliminary phytochemical screening mainly revealed the presence of carbohydrates, alkaloids, glycosides, sterols, flavonoids, tannins and phenolic compounds (Tables 1 and 2). Volatile oil and gums were found only in the leaf extracts.

V. Physico-chemical constants – Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values (Table 3) of the leaf and stem powder of *H. enneaspermus* showed higher content of sulphated ash followed by total ash.

The extractive values are primarily useful for the determination of exhausted or adulterated drug. The water soluble extractive (Table 4) was high in both stem and leaf powder of *H. enneaspermus*.

VI. Leaf constants – Leaf constants *viz.* the vein islet number, vein termination number and stomatal index are presented in Table 5.

Conclusion

As there is no pharmacognostical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. Macro and micro morphological standards discussed here can be considered as identifying parameters to authenticate the drug.

Table 3. Ash values of the leaf and stem powder of *H. enneaspermus*

parameters	values % (w/w)
leaf powder	
total ash	5.26
acid insoluble ash	0.59
water soluble ash	1.15
sulphated ash	6.57
stem powder	
total ash	4.60
acid insoluble ash	0.63
water soluble ash	1.99
sulphated ash	6.91

Table 4. Extractive values of the stem and leaf powder of *H. enneaspermus*

parameters	values % (w/w)
stem powder	
a) water soluble extractive	11.56
b) ethanol soluble extractive	11.88
c) ether soluble extractive	3.42
leaf powder	
a) water soluble extractive	12.11
b) ethanol soluble extractive	9.30
c) ether soluble extractive	1.40

Table 5. Leaf constants of *H. enneaspermus*

parameters	values
vein islet number (1 mm^2 leaf surface)	18
vein termination number (1 mm^2 leaf surface)	20
palisade ratio (under 1 epidermal cell)	7.0
stomatal index (on lower epidermis)	5.20

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