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Research Article

Pharmacognostical study of *Hybanthus enneaspermus* (Linn.) F. Muell.

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Abstract

Present paper deals with the pharmacognostical study of leaf, stem and root of ***Hybanthus enneaspermus* (Linn.) F. Muell.**, for its identification and to distinguish it from the co-existing weeds and adulterants. The study includes macroscopic, microscopic and preliminary physico-chemical investigation.

Keywords: *Hybanthus enneaspermus*, Pharmacognostic characterization, Microscopic characters, Fluorescence characters, Physico-chemical characters.

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Introduction

Herbal medicine has been practiced world wide and is now recognized by WHO as an essential building block for primary healthcare[1]. Though the traditional Indian system of medicine has a long history of use, they lacked adequate scientific documentation, particularly in the light of modern scientific knowledge. ***Hybanthus enneaspermus* (Linn.) F. Muell.** (Family: Violaceae) is distributed in plains from the coast to 900m, in exposed places, common in arable, pasture and waste lands[2]. Whole plant is used medicinally in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments. In Ayurveda, it is known as *Sthalakamala*. The plant is reported to possess tonic, diuretic and demulcent properties. The root is diuretic and administered as an infusion in gonorrhoea and urinary affections. The *Sandals* employ the root in bowel complaints of children. The leaves and tender stalks are demulcent and used as a decoction or electuary; mixed with oil, they are employed in preparing a cooling liniment for the headache[3]. An infusion of the plant is given in case of cholera[4].

It grows mixed with many other simulating weeds, viz. ***Ammania baccifera* Linn.**, ***Oldenlandia alata* J. Koenig ex Roxb.**, ***Heliotropium bracteatum* R. Br.** and ***Lindernia oppositifolia* (Retz) Mukerjee**. In the absence of flowers, distinguishing it from co-existing weeds is difficult. Therefore, studies on pharmacognostic characters of this plant would provide an account on correct identification.

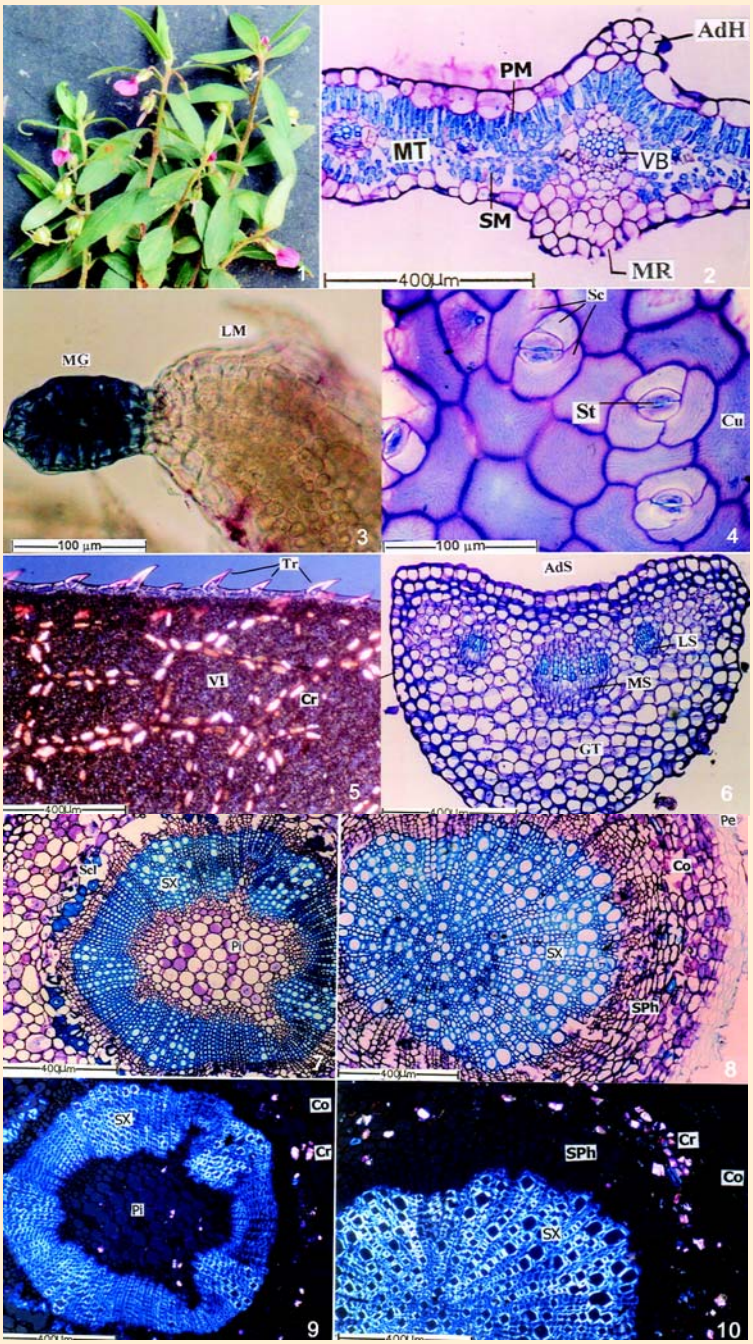
Materials and Methods

Plants were collected from Tirunelveli hills and voucher specimen was deposited in the herbarium of St. Xavier's College, Palayamkottai (Voucher number XCH - 24906). Anatomical studies were carried out as per standard methods[5]. For anatomical studies, sections of about 10 to 12µm thick were prepared and stained with polychromatic stain-toluidine blue[6] and stained with safranin and fast green. Stomatal Index was calculated as per standard methods[7]. Clearing of leaf was done to study the venation pattern[8]. Microphotographs at different magnifications were taken with Nikon Labphot 2 Microscopic Unit to study the anatomical characters. Polarized light was employed to study the crystals. The fluorescence and physico-chemical characters were determined as per standard methods[9, 10]. Powdered plant material was successively extracted with petroleum ether, benzene, chloroform, methanol and water in a Soxhlet's apparatus and was subjected to qualitative test for the identification of various plant constituents[11].

Results and Discussion

Morphological / Macroscopic characters

H. enneaspermus is a small erect herb. Root spindle shaped, cylindrical, rough and light yellow in colour. Stem sparingly branched with woody base and spreading erect branches. Leaves simple, alternate, sub-sessile, linear to lanceolate, 2.5 × 0.7cm, base attenuate, margins serrate with nectariferous glands, apex acute. Flowers 8 to 10 mm across, pink, axillary, solitary



Figs. 1-10 : *Hybanthus enneaspermus* : 1. Habit; 2. T.S. of leaf; 3. Marginal gland; 4. Adaxial stomata; 5. Lamina under polarized light; 6. T.S. of proximal part of the petiole ; 7. T.S. of stem enlarged; 8. T.S. of root; 9. T.S.of stem under polarized light; 10. T.S. of root under polarized light. (AdH-Adaxial hump, MT-Mesophyll tissue, PM-Palisade mesophyll, SM- Spongy mesophyll, VB -Vascular bundle, MR- Mid rib, MG- Marginal gland, LM- Leaf margin, Sc-Subsidiary cells, St - Stomata, Cu-Cuticle, Tr-Trichomes, VI-Vein islet, Cr-Crystals, AdS- Adaxial side, LS-Lateral strand, MS-Median strand, GT-Ground tissue, Scl-Sclerids, SX - Secondary Xylem, Pi-Pith, Pe-Periderm, SPh-Secondary Phloem, Co-Cortex)

and zygomorphic. Sepals five, lanceolate, sub-equal, obovate, upper ones oblong, laterals falcate, lower ones orbicular, clawed, saccate at base. Petals five, pink coloured, unequal, upper ones oblong, laterals falcate long, lower one larger, orbicular, clawed and saccate at base. Stamens five, connate, anterior filaments appendaged, puberulous. Anthers villous. Fruit 5 mm across, capsule, sub-globose with ribbed seeds. Powder is grayish green, slightly odorous and has bland taste (Fig. 1).

Microscopic characters

Leaf

It is mesomorphic, dorsiventral and amphistomatic. The midrib is fairly prominent projecting equally on adaxial and abaxial sides. On the adaxial side the midrib is bluntly conical and on the abaxial side it is hemispherical. Small collateral vascular bundle is present in the middle of the midrib; it is subtended by an abaxial mass of parenchyma. The palisade tissue is transcurrent (running transversely) across the adaxial hump (Fig. 2).

Adaxial epidermis thick, cells rectangular or squarish; some of the cells contain mucilage. The abaxial epidermis thin and small; some of the cells are dilated and posess mucilage. The mesophyll is differentiated into two or three layers of palisade and three to five layers of spongy parenchyma cells (Fig. 2). Some of the mesophyll cells lack chloroplasts and contain brownish contents. Along the margins of the lamina, at the tip of each lateral serration solid, multicellular, elliptic secretory glands present with dark contents (Fig. 3).

a) Stomata

Stomata are 30-33 mm long and 20-28 mm broad. Stomatal frequency is 4 - 6/mm^2 on the adaxial side and 19 -20/mm^2 on the abaxial side. The stomata are anisocytic (Fig. 4) and the stomatal index is 12.5-15.75 on the adaxial and 19.25-22.5 on the abaxial side, the adaxial epidermal cells are straight walled with prominent cuticular striations (Fig. 4). The abaxial epidermal cells are slightly wavy and lack cuticular striations.

b) Venation pattern and distribution

Vein-islets distinct and are rhomboidal or broadly rectangular. The network of veins is marked by the occurrence of calcium oxalate crystals on either side of the veins. The crystals are 'clinorhombic' type and are exclusively restricted to the path of veins. The crystals are 50-60µm long and 10-15µm broad. Epidermal trichomes occur along the margins and veins. The trichomes are either uni or bicellular, thick walled and lopsided (Fig.5).

c) Petiole

In cross sectional view, the petiole is plano-convex in outline. In the proximal part three vascular bundles are present, of which median bundle is larger and other two lateral vascular bundles are smaller (Fig.6). The middle region of the petiole is slightly winged and the three vascular strands tend to fuse to form two strands. In the basal part of the petiole the vascular bundles are fused to form a single shallow arc of xylem and phloem. The distal part of the petiole is spindle shaped with two lateral wings.

Stem

In cross sectional view, the stem is circular with uneven outline. The epidermis is uni-stratose and thin; the cells are squarish with thin cuticle striations. Hypodermis is collenchymatus, 1-2 layered; cortex 5-6 layers of thin walled, parenchymatus cells and contain prismatic crystals of calcium oxalate (Fig.9). The vascular cylinder consists of a single layer of discontinuous patches of perivascular sclerids, a narrow zone of phloem and closed dense cylinder of xylem. The size of sclereids usually vary from 30-45µm in diameter. Xylem cylinder consists of narrow circular, thick walled radialy arranged vessels of 20-30µm diameter and thick walled fibres usually vary from 5-15µm in diameter. Pith is wide and parenchymatous (Fig. 7).

Root

Root has narrow uniform outer zone of phellem (cork) followed internally by cortex. Cortex composed of less compact parenchyma cells and secondary phloem is in small radial masses. Secondary xylem is dense and form compact circular cylinder; it consists of scattered circular vessels of 30-60 µm in diameter (Fig. 8). The fibres are fairly thick walled and wide lumened. Prismatic crystals of calcium oxalate are also present in the cortex (Fig. 10).

Following anatomical features are important for the microscopic characterization of ***H. enneaspermus***:

1. Leaf has equally projecting midrib with adaxial transcurrent palisade tissue and a single strand of collateral vascular bundle.

Table 1: Physico-chemical characteristics of *Hybanthus enneaspermus* plant

S. No.	Particulars	Percentage value
1	Loss of weight on drying	71-73
2	Total ash	9-10.5
3	Acid insoluble ash	3.5- 4.2
4	Water soluble ash	3.8 - 4.4
5	Residue on ignition	1.2 -1.6
6	Sulphated ash	14.5 - 15.6
7	Moisture content	9.5 - 10.5
8	Alcohol soluble extractive	7.5 - 9.5
9	Water soluble extractive	10.5 -12. 4
10	**Extractive values (Successive extraction)**	
	a) Petroleum ether (40-60°C)	2.6 - 3.2
	b) Benzene	5.2 - 6.4
	c) Chloroform	4.2 - 5.1
	d) Methanol	6.6 - 7.1
	e) Water	4.9 - 5.2

2. The lamina has unicellular, thick walled, pointed trichomes along the margins and ellipsoidal darkly staining glands at the tip of each lateral serration.
3. The stomata are mostly anisocytic with distinct cuticular striations and straight anticlinal walls of the epidermal cells.
4. Rectangular prismatic calcium oxalate crystals are abundant and characteristically aligned all along the lateral veins is an interesting phenomenon.
5. The stem shows limited extent of secondary growth and secondary phloem with discontinuous patches of perivascular sclerids.
6. The root has broad zone of periderm, wide solid cylinder of secondary xylem and discontinuous mass of secondary phloem.

Table 2: Fluorescence characters of *Hybanthus enneaspermus* plant powder

S. No.	Particulars of the treatment	Under ordinary light	Under UV light (366nm)
1	Powder as such	Greyish green	Brick red
2	Powder + 1N NaOH (aqueous)	Green	Brick red
3	Powder + 1N NaOH (ethanolic)	Dark green	Reddish green
4	Powder + 1N HCl	Blackish green	Red
5	Powder + H_2SO_4 (1:1)	Green	Dark brown
6	Powder + HNO_3 (1:1)	Yellow	Red
7	**Extracts**		
	a) Petroleum ether (40-60°C)	Golden yellow	Orange
	b) Benzene	Green	Red
	c) Chloroform	Blackish green	Deep red
	d) Methanol	Blackish green	Deep red
	e) Water	Yellowish green	Blackish green

Table 3: Phytochemical analysis of various extracts of *Hybanthus enneaspermus*

Phytochemicals	Petroleum ether extract	Benzene extract	Chloroform extract	Methanol extract	Water extract
Steroids	+	+	+	+	-
Triterpenes	+	-	-	-	-
Sugars	-	-	-	+	+
Alkaloids	-	-	-	+	+
Phenols	-	-	-	+	+
Flavones	-	-	-	+	+
Catachins	-	-	-	+	-
Saponins	-	-	-	-	-
Tannins	-	-	-	+	+
Anthroquinones	-	-	-	+	+
Amino acids	-	-	-	+	+

(+ Present, – absent)

Physico-chemical/ Fluorescence studies

Physico-chemical values and fluorescence characters of the plant powder under ordinary light and Ultra Violet light (UV 366nm) are presented in Tables 1 and 2. Preliminary phytochemical screening of the plant powder was done as per standard methods and results are presented in Table 3. Presence of steroids, triterpenes, sugars, alkaloids, phenols, flavones, catachins, tannins, anthraquinones and amino acids were reported in the plant. The methanol extract showed the presence of steroids, sugars, alkaloids, phenols, flavones, catechins, tannins, anthraquinones and amino acids. Benzene and chloroform extracts showed the presence of steroids. Petroleum ether extracts showed the presence of steroids and triterpenoids and the water extract showed the presence of sugars, alkaloids, phenols, flavones, tannins, anthraquinones and amino acids.

Conclusion

The pharmacognostic characters and phytochemical values reported in this paper could be used as the diagnostic tool for the standardization of this medicinal

plant. Adulterants if any can be easily identified using these parameters. The microscopic features could help in laying down micromorphological standards as per WHO guidelines for authentication of the drug.

References

1 Onayade OA, Scheffer JJC and Svendsen AB, The importance of phytotherapy and screening of plants used medicinally in Africa, *Planta Med*, 1990, **56**, 503-504.

2. Matthew KM, An excursion Flora of Central Tamil Nadu, India, Oxford and IBH Publishing Co., New Delhi, 1995, pp.17-18.

3. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products — Raw Materials Series, Publications and Information Directorate, CSIR, New Delhi, 1959, Vol.V (H-K), p.139.

4. Kirtikar KR and Basu BD, Indian Medicinal Plants, International Book Distributors, Dehra Dun, Reprint Edn, 1975, Vol. I, p. 212.

5. Johansen DA, Plant Microtechnique, McGraw - Hill Book Co. Inc. New York and London, 1940, p. 523.

6. O'Brien TP, Feder N and MCull ME, Polychromatic staining of plant cell walls by toluidine blue – O, *Protoplasm*, 1964, **59**, 364-373.

7. Wallis TE, Text Book of Pharmacognosy, CBS publishers and distributors, New Delhi, India, 1985, p. 652.

8. Sass JE, Elements of Botanical Microtechnique, McGraw-Hill Book Co., Inc, New York and London, 1940, p. 222.

9. Chase CR and Prett RJ, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J Am Pharm Assoc*, 1949, **38**, 324-331.

10. Anonymous, Pharmacopoeia of India, Ministry of Health and Family Welfare, The Controller of Publications, New Delhi, 2nd Edn, 1996, pp. 947-948.

11. Brindha P, Sasikala B and Purushothman KK, Pharmacognostic studies on *Merugan kizhangu*, *Bull Med-Ethno-Bot Res*, 1981, **3** (1), 84-96.