Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 4, 2012

Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF *HYBANTHUS ENNEASPERMUS* LINN.

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Received: 25 Mar 2012, Revised and Accepted: 13 May 2012

ABSTRACT

The plant *Hybanthus enneaspermus* Linn. (Violaceae), commonly known as spade flower and pink lady's slipper, is growing in the warmer parts from Uttar Pradesh southward to the Deccan Penninsula. In Ayurveda, it is known as 'Sthalakamala'.The whole plant is used in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments. *H. enneaspermus* grows mixed with many other simulating weeds like *Ammania baccifera*, *Oldenlandia alata* etc. In the absence of flowers it is very difficult to distinguish this plant from other co-existing weeds. In the present study morphological and anatomical features of vegetative parts and physico-chemical characters of plant powder have been analysed to support the proper identification of the plant. The results of the study reveals morphological characters like bisexual, pink flowers, small endosperm with an elaiosome in the form of an appendage of raphe in Seeds, entomophyllous pollination and anisocytic stomata. Physicochemical parameters like ash value, extractive value and phytochemical screening with different reagents showed the presence of steroids, triterpenoids, phenols, tannins and flavonoids. These observed characters will help in the Pharmacognostical evaluation of *H.enneaspermus.*

Keywords: *Hybanthus enneaspermus,* Pharmacognostical evaluation, Phytochemical screening, Physico-chemical characters.

INTRODUCTION

Hybanthus enneaspermus Linn. is one of the three important genera of the family Violaceae. In India it is found in the warmer parts, from Uttar Pradesh southward to the Deccan Peninsula. It has common names like spade flower and pink lady's slipper. The plant is a perennial herb or small herb. In Ayurveda, it is known as 'Sthalakamala'. The whole plant is used in Ayurveda, Siddha and other traditional systems of medicine for curing various ailments. The plant is reported to possess tonic, diuretic and demulcent properties. The root sandals are employed for the bowel complaints of children. The leaves and tender stalks are demulcent and used as a decoction or electuary. They are employed in preparing a cooling liniment for head ache. An infusion of the plant is given in the case of cholera and decoction or powder of the whole plant is taken to improve memory, vitality and as a remedy for tuberculosis, asthma, fever and leprosy. Its infusion is good for all diseases of eyes. Fruit is used to treat scorpion sting[1].

Hybanthus enneaspermus grows mixed with many other simulating weeds like *Ammania baccifera*, *Oldenlandia alata* etc. So it is very difficult to distinguish this plant from the co-existing weeds in the absence of flowers. Therefore, studies on pharmacognostic characters of this plant would provide an account on correct identification. During the last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical research all over the world in an effort to discover more potent plant drugs. The present study is focussed on the pharmacognostic characters of the plant and its bioactive compounds. Pharmacognostical evaluation include examination of morphological and microscopical characters, physio-chemical and fluorescence properties. There are no previous reports on the preliminary phytochemical analysis on *H.enneaspermus*

MATERIALS AND METHODS

Collection of Plant material

The whole plants of *H. enneaspermus* was collected from the plain areas of Kariavattom campus, University of Kerala, Thiruvananthapuram, Kerala. The plant was authenticated by Dr. G.Valsaladevi, Curator, Department of Botany, and preserved.

Morphology studies

The fresh plant is collected and observed thoroughly with the help of a dissection microscope. The characters were observed and photographs of various plant parts were taken with the help of a stereo microscope. The characters observed were verified by referring the flora of Agasthyamala and flora of Karnataka.

Microscopic studies

Fresh materials of *H.enneaspermus* were collected for microscopic studies. Microscopic sections were cut by free hand sectioning. Numerous temporary mounts of the microscopical sections of the stem, leaf and root were prepared, stained with saffranin and observed under the microscope. Microphotographs at different magnifications were taken with an image analysing system (Olympus-BX51TF, Japan) to study the anatomical characters.

Stomatal number

Peel out upper and lower epidermis separately by means of forceps. Thin transparent region of epidermis is mounted in glycerine on a clean slide. Draw a square of 1mm by means of a stage micrometer. Place the slide with cleared leaf on the stage. Trace the epidermal cell and stomata. Count the number of stomata present in the area of one square mm; include the cell if at least half of its area lies within the square. Record the results for each ten fields and calculate the average no. of stomata per square mm.

Stomatal index

Stomatal index is the percentage, which the no. of stomata / unit area and the total no. of epidermal cells were counted, each stomata being counted as one cell. Stomatal index can be calculated by the following equation.

$$I = \frac{S}{E+S}$$

I = Stomal index.

S = No. of stomata per unit area.

E = No. of epidermal cells in the same unit area.

Qualitative analysis

Physico-chemical analysis

Analysis of physico-chemical parameters such as total ash value, acid-insoluble ash, water soluble ash, extractive values, alcohol soluble extractive, water soluble extractive, cold water extractive and hot water extractive were determined according to the standard procedure[2]. Analysis of crude fiber content, fluorescence analysis and organoleptic analysis of powder was also done.

Phytochemical screening

The extracts of various solvents such as petroleum ether, ethyl acetate, acetone, methanol and distilled water were subjected to preliminary phytochemical screening for the identification of various classes of active chemical constituents using the methodology of [3, 4].

RESULTS AND DISCUSSION

Morphology

Hybanthus enneaspermus is a small perennial herb reaching the height of 15-60 cm. Leaves are simple, stipulate, sessile to subsessile and alternate. Leaf variable, linear to lanceolate, elliptic or oblong in shape, sub entire to crenate-serrate or dentate-serrate at margin, acute or sub obtuse and often mucronate at apex, 3.9 x 0.7 cm size, glabrous to densely pubescent, lateral nerves 4-7pairs. Stem is simple or moderately branched, often woody at base, ridged, sub glabrous to densely pubescent often becoming glabrescent. Flowers are solitary, pedicellate, bracteate, bracteolate, axillary, zygomorphic, hypogynous, pentamerous, and complete. Pedicel is slender, jointed with a pair of subulate bracteoles at the joint. Petals are five, unequal, pink coloured, upper two symmetric, elliptic, acuminate 3-5 mm long (Fig. 1), Stamens are also five, 2-3 mm long, connective grown beyond anther as a membraneous appendage, alternate to petals, introrse, dithecous and persistant.

Fig. 1: ***Hybanthus enneaspermus***

Ovary globose, pubescent, tricarpellary, syncarpous and superior. Style is short, thick, fleshy, terminal and persistant. Stigma is often hooded (capitate) and persistant. Ovules are many and are arranged in parietal placentation. Fruit is a capsule which is often split into three boat shaped valves, and sub-globose with 5mm size (Fig. 2). It consists of persistant calyx, corolla, stamen, style and stigma. Seeds are small, hard, shiny, ovoid-ellipsoid in shape(Fig. 3). Endosperm is copious and fleshy. The plant produce flowers and fruits throughout the year. The plant exhibits entomophyllous pollination.

Fig. 2: Fruit of ***H. enneaspermus***

Fig. 3: Seed of ***H. enneaspermus***

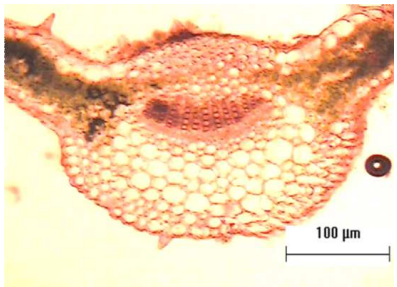

Fig. 4: C.S. of Leaf

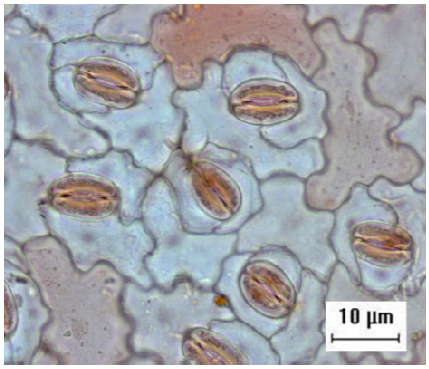

Fig. 5: Stomata

Microscopic studies of leaf, Stem and root

Leaf is simple and its midrib is bluntly conical on the abaxial side and it is hemispherical on the adaxial side. Adaxial epidermis is single layered, thick and cells are barrel shaped. Abaxial epidermis is thin. The mesophyll tissue consists of two or three layered palisade tissue and it is transcurrent (running transversly). Mesophyll tissue is of 2-3 layered. Small collateral vascular bundle is present in the middle of the midrib. Xylem rays are rounded in shape (Fig. 4). The stomata is anisocytic. Stomatal frequency is 4-6/mm^2 on the adaxial side and 19-20/ mm^2 on the abaxial side. The stomatal index is 12.5% on adaxial side and 24.1% on the abaxial side (Fig. 5).

In cross sectional view, the stem is triangular with three ridges. The epidermis is single layered with barrel shaped cells. Unicellular hairs are present on the epidermal cells (Fig. 6). The epidermis possess thin cuticle striations. Hypodermis consist of 1-2 layered collenchymatous cells. Cortex is composed of 1-2 layered chlorenchymatous cells in the outer layer and 3-4 layered parenchymatous cells in the inner layer. The vascular cylinder consists of single layer of discontinuous patches of perivascular sclerids, a narrow zone of phloem and closed dense cylinder of xylem. There is three secondary vascular bundles with sclerenchymatous cap at three corner. Pith is wide, homogenous and composed of parenchymatous cells.

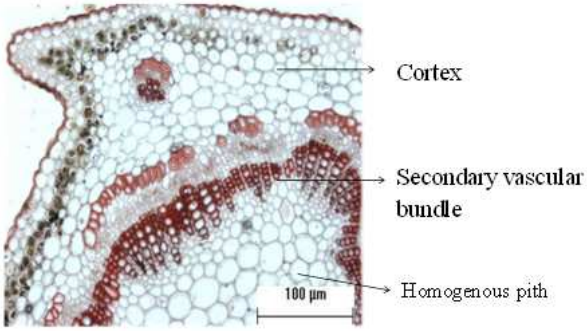

Fig. 6: C.S. of Stem

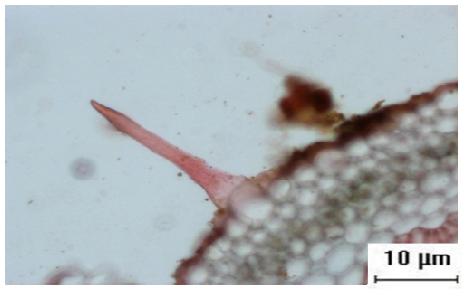

Fig. 7: Unicellular hair

Physico-chemical analysis

Moisture content of the drug was found to be 9.5-10.1%. Total ash of any drug is the residue obtained on its complete incineration in an electric Bunsen. This mainly represents the inorganic salts present in the drug. Thus ash value is a general criteria to ascertain the purity of any drug. In the present study, the total ash was found to be 8.18-9%. Acid insoluble ash is mainly give the percentage of sand and impurities that remains insoluble in 6N HCl and it was found to be 0.29-0.31%, low value of acid insoluble ash indicate high purity of the sample. Water insoluble ash mainly gives the percentage of organic matter present in the ash and this was found to be 3.98-4.2%. Extractive values mainly represents the percentage of organic constituents. These values are specific to each drug. For *H.enneaspermus*, the cold water and hot water extractive values were found to be 10.5-12.2 and 9.3-9.8 % respectively. The alcohol extractive value and crude fibre were found to be 7.5-8.4 **and** 19.5-22.3% respectively (Table 1).

Table 1: Physico-chemical parameters of *H. enneaspermus*

Parameters	Results (%)
Moisture content	9.5-10.1
Alcohol soluble extractives	7.5 - 8.4
Water soluble extractives	
a)cold water soluble extractives	10.5 -12.2
b) hot water soluble extractives	9.3 -9.8
Fibre content	19.5 -22.3
Total ash	8.18-9
Acid –insoluble ash	0.285-0.31
Water soluble ash	3.72-4.2

For the isolation of active constituents from crude drug, extraction of the plant powder with organic solvents of increasing polarity was done. The extracts obtained by exhausting crude drugs are indicative of approximate measures of their chemical constituents[5]. The benzene extract of the plant have action on central nervous system anti-depression activities[6] (Table 2).

Table 2: Yield (%) of successive extracts from various solvents

Solvents	% yield
Petroleum ether	1.72
Ethyl acetate	2.4
Acetone	1.3
Methanol	9.4
D.H2O	4.6

Fluorescence analysis

The fine plant powder was boiled with different solvents according to their increasing polarity. The boiled powder with solvents were examined under UV (short-254 nm and long-365 nm)and visible light (Table 3).

Organoleptic analysis

The plant powder was treated with various chemicals and the reaction colour was noted in the Table 4.

Table 3: Fluorescent analysis

Treatment	Visible light	Short UV	Long UV
Powder as such	Greyish Green	Brown	Brick red
Powder+ 50%NaOH	Light Brown	Light Green	DarkBrown
Powder+ Ab.Ethanol	Light Green	Green	Brick red
Powder+n-Butanol	Light Green	Green	Brown
Powder+n-Hexane	Yellowish Green	Light Green	DarkBrown
Powder+ 1NNaOH	Light yellow	Green	Brick red
Extracts			
a)Petroleum ether	Golden yellow	Light orange	Orange
b)Ethyl acetate	Green	Brown	Red
c)Acetone	Dark Green	Brown	Brick red
d)Methanol	Blackish green	Brown	Brick red
	Yellowish green	Green	Dark Green

Table 4: Results of organoleptic analysis

S. No.	Solvents	Colour Obtained
1	5%FeCl3	Dark Brown
2	50%NaOH	Light Brown
3	NH4 Solution	Yellow
4	Glacial Acetic acid	Yellowish Green
5	50% HNO3	Brown
6	Conc.HNO3	Brick Red
7	50% HCl	Greyish Green
8	Conc. HCl	Dark Green
9	50% H2SO4	Green
10	Conc. H2SO4	Dark Brown

Table 5: Results of preliminary phytochemical analysis

Chemical constituents	Pet. Ether	Ethyl acetate	Acetone	Methanol	Water
Tannins	+	+	+	+	–
Saponins	–	–	–	+	–
Flavonoids	–	–	–	+	+
Steroids	–	–	–	+	+
Terpenoids	–	–	–	+	+
Antharaquinone	–	–	–	+	+
Alkaloid	–	–	–	+	+
Sugar	–	–	–	+	+
Phenols	–	–	–	+	+

Phytochemistry

The qualitative analysis of the plant powder was carried out as per standard methods. The screening of plant extract and plant products for secondary metabolites have shown that higher plants represent a potential source of novel anti-biotic prototypes[7]. Preliminary phytochemical analysis helpful for the screening of secondary metabolites. The methanolic and water extract showed the presence of maximum number of compounds, in which steroid was found to be the predominant compound (Table 5).

Today natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action. Recent studies involved the identification and isolation of new therapeutic compounds of medicinal importance from the higher plants for specific diseases.[8,9] The curative properties of plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavanoids, phenols, saponins and steroids. They were known to show medicinal as well as physiological activity[10,11].

CONCLUSION

The pharmacognostical study is one of the major criteria for the identification of plant drug as well as drug standardization. In the present study morphological and anatomical features of vegetative parts and physico-chemical characters of plant powder was analysed to support the proper identification of the plant, *H.enneaspermus*. Standardization of a crude drug is an integral part of establishing its correct identification. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostic parameters and standards must be established. The specific and unique morphological characters such as persistent nature of calyx, corolla, stamen style and stigma helps to distinguish the plant from co-existing weeds. The anatomical characters like anisocytic stomata, supporting the unique identification of the plant. Physico-chemical analysis is the major criteria for drug identification. The fluorescence natures of powder and ash value have great importance in physico-chemical analysis. The results of these investigations could, therefore serve as a basis for proper identification, collection and investigation of the plant. The macro and micro-morphological features of the plant described are enabling to distinguish it from the co-existing and similar looking weeds. In conclusion the parameters which are reported here can be considered as distinctive enough to identify and decide the authenticity of this drug in herbal industry/trade and this can be included as microscopic standards in Indian Herbal Pharmacopeia.

ACKNOWLEDGMENT

We are thankful to Professor and Head, Department of Botany, University of Kerala, Kariavattom, for providing facilities for doing this work.

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