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Research article

Pharmacognostic studies of the leaf and stolon of *Pistia stratiotes* Linn.

K.K.Omprakash*, R.Rathinamala[1], R.Rajasekaran[2], & Sasikala Ethirajulu[3]

1. Ph.D Scholar, National Institute of Siddha, Chennai.
2. Research Officer (Siddha), National Institute of Indian Medical Heritage, Hyderabad.
3. Asst. Director (Pharmacognosy) Siddha Central Research Institute, Chennai.

Abstract

Pistia stratiotes Linn. is found in ponds and streams throughout India. The plant has demulcent, refrigerant activities and useful in the treatment of dysuria. It is considered as antiseptic, antitubercular and antidysenteric. It is used in the preparation of inorganic Ayurveda and Siddha medicaments. The leaves are used in eczema, leprosy, ulcer, piles, and syphilis. The present study deals with the various pharmacognostical examinations include the morphological, microscopical characters and physiochemical characters like ash values and extractive values. The preliminary phytochemical screening is also carried out and it is revealed the presence of various phytoconstituents like Steroid, Triterpenoid, Phenol, Flavanoid, Tanin, Alkaloid, Glycoside and Saponin. These data's could be used to standardize the plant which is essential and is need of the hour.

Keywords:

Pistia stratiotes L., Pharmacognosy, Phytochemistry

Introduction:

Pistia is a monotypic genus of floating aquatic stoloniferous herb (Fig 1), represented as *Pistia stratiotes* L.(Fam: Araceae). It is distributed in the tropical and subtropical Asia, Africa and America. Four varieties are recognised. The Indian variety is known as *var.cuneata* Engl(1). It is commonly known as Water lettuce in English, Akayathamarai or Antharathamarai in Tamil and Kumbhika or Jalakumbhi in Sanskrit.

The plant is considered as antiseptic, antitubercular, antidysenteric, anthelmintic (1) and used to destroy bugs (2). The leaves are having refrigerant, demulcent, laxative (3) activities. They are also used in eczema, leprosy, ulcers, piles and syphilis (1). The leaves are made into poultice and externally applied for piles. Mixed with rice and coconut milk it is given for dysentery. The leaves mixed with rosewater and sugar is given for asthma and cough. The ash of the plant is applied to the ringworm of the scalp. Juice of the leaves boiled in coconut oil is applied externally in chronic skin diseases (4). The plant is one of the important ingredients in Siddha formulations viz. Karuvanga parpam used for vadha diseases (5), Sangu parpam (6) and Uppu chenduram (7) etc, which are prescribed for gastric ulcer.

The plant is reported to contain stigmasta-4, 22-dien-3-one, stigmasterol, stigmasteryl stearate, palmitic acid (8), a new stigmastane 11α-hydroxy-24(s) ethyl-5α-cholest-22-ene-3,6dione, three new sitosterol acylglycosides viz. sitosterol-3-0(2'4'-0-diacetyl - 6' – 0 - stearyl) – β - D glucopyranoside;sitosterol – 3 – 0 - (2'-0-

***** Corresponding Author:
Dr. K.K.Omprakash
Siddha Physician,
Chennai
Email: kkomprakash@gmail.com
Phone: 09444216007

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stearyl) – β -D-0-xylopyranoside and sitosterol-3-0(4'-0-steryl)- β –D-xylopyranoside(9), vicenin, lucenin, cyanidin-3-glucoside, Vitamins A, B and C (10). Linoleic acid, γ linolenic acid, α asarone and 12-hydroxy-9,13,15 octadecatrienoic acid. 9 hydroxy-10,12,15-octadecatrienoic acid, 24(s) ethyl-4,22-cholestadiene-3,6-dione have also been identified from the leaves and these compounds inhibited growth of some microalgae in solid medium (9).The leaves exhibited antidermatophytic activity(11) and anthelmintic activity(12). The methanolic extract of the whole plant also exhibited dose related bronchodilating activity on isolated trachea of guinea pig. The extract also caused a decrease in blood pressure in anaesthetised rats (13).

Despite such various uses of the leaf, there is no report on the Pharmacognostic standardisation. Hence an attempt has been made to standardise this herb using botanical, chemical and analytical techniques.

Materials and Methods:

The plant was collected from Thuraipakkam, Chennai, Tamilnadu and identified at Botanical survey of India, Coimbatore. Herbarium specimen of the plant was deposited in the Survey of Medicinal Plants Unit (Siddha) CCRAS, Palayamkottai (Acc No 1615). Fragments of leaf and stolon were fixed in FAA.(Formalin 5 ml + acetic acid 5ml + 70% Ethyl alcohol 90ml) Free hand as well as microtome sections of leaf and stolon were taken and standard micro techniques (14) were followed for anatomical study. Sections of 10-12 µm thickness were taken, stained with fastgreen (0.25% alcoholic) and safranin(0.5% aqueous). Clearing of leaves was done by using 5% sodium hydroxide along with chlorinated soda solution supplemented with gentle heat.Photographs were made at different magnifications. This was done on the Leitz research microscope using Asahi pentax, 35 mm SLR spotmatic 11 Camera and Kodak gold super 200 colour films. Quantitative values such as stomatal number stomatal index, vein islet number and palisade ratio were determined (15,16). Physiochemical standards were determined according to the standard procedures of Indian Pharmacopoeia (17). Preliminary phytochemical tests were also performed (18).For thin layer chromatography the powdered leaves (5gm) were extracted with petroleum ether and then with ethanol in a Soxhlet apparatus. The petroleum ether and ethanol extracts were subjected to TLC (19) over silica gel 60F254 pre coated aluminium plate (Merck, layer thickness 0.2mm). The solvents used for TLC were of analytical grade.

Results and Discussion:

Macroscopic characters:

Leaf:

Leaves are close spiral, sessile, obovate to cuneate, apical margin rounded or retuse or shallowly lobulate and undulate, densely pubescent on both faces, size very variable 2 to 10 cm long and half as broad, the largest the outer and the central smaller.

Stolon:

It is the subaerial modification of stem. It orginates in the axil of a leaf as a short, more or less thickened horizontal branch. It elongates to a certain extent and produces at the apex a tuft of leaves above and a cluster of roots below.

Microscopic characters:

Leaf:

Transverse section of leaf shows grooves on the adaxial surface, while on the abaxial side the vein is raised (Fig. 2A).The epidermis is single layered. Some of the cells in the upper epidermis become large and bulbiform supporting uniseriate unbranched trichomes, about 400-700 µm in length and 35-50 µm wide. The epidermal cells are cuticularised. The cells

of the adaxial epidermis in this section are 50-65 × 30-45 µm in dimension; whereas the cells of the abaxial epidermis are 50-65 × 20-35. The bulbiform cells are 75-85 µm × 85-95 µm in dimension.The stomata measure 25-30 µm along the guard cells and 8-13 µm across the guard cells. In the lamina portion, stomata are present on both the surfaces. The upper epidermis is followed by two layers of closely arranged columnar palisade tissue. Below this, huge air spaces are seen which are encircled by a network of uniseriate parenchymatous cells. The vascular bundle of the vein is situated in the centre and surrounded by bundle sheath made up of one layer of large parenchyma cells (Fig. 2C). In the rib region, two vascular bundles are seen. The smaller one is situated in the centre and the larger one towards the abaxial side (Fig. 2B). The vascular bundles have prominent sclerenchyma patches on the upper side. Each vascular bundle consists of a strand of phloem and 2 or 3 metaxylem elementss. In between those fibres are also present. The vascular bundles are surrounded by a layer of bundle sheath composed of parenchyma cells. The palisade tissue runs along the rib region. The ground tissue consists of large air spaces encircled by a network of uniseriate elongated parenchyma cells.

Epidermis in surface view:

The adaxial foliar epidermis is composed of penta or hexagonal cells with straight walls and perforated by a few paracytic stomata (Fig. 2D).The abaxial foliar epidermis is composed of penta or octagonal cells with straight walls. It is perforated by numerous paracytic stomata (Fig. 2E).

Transverse Section of Stolon:

Transverse section of stolon is oval in shape with numerous small ridges (Fig. 3F).The epidermis is single layered. Most of the epidermal cells elongate to form uniseriate multi cellular trichomes (Fig. 3I). The ground tissue is aerenchymatous with numerous large airspaces. Numerous collateral vascular bundles are scattered in the ground tissue. The larger vascular bundles are situated towards the periphery and occur below the ridges. Smaller vascular bundles are situated in the inner region. The vascular bundles have prominent sclerenchyma patches on the phloem (Fig. 3G, H).Some of the parenchyma cells contain acicular crystals of raphides. (Fig. 3G, J).The examination of the powdered leaves showed the presence of uniseriate multicellular unbranched trichomes, fragments of leaf with paracytic stomata, parenchyma cells, fibres and acicular crystals.

Quantitative Microscopy (15, 16)**:**

Quantitative values such as stomatal number stomatal index, vein islet number and palisade ratio were determined as per the standard methods (15, 16).

Stomatal Number:

Adaxial epidermis = 8 – 10 – 12 / mm^2
Abaxial epidermis = 36 – 40 – 46 / mm^2

Stomatal index:

Stomatal Index = S/E+ S × 100

S = Number of stomata per unit area

E = Number of ordinary epidermal cells in the same unit area**.**

Upper epidermis = 20 – 25- 28
Lower epidermis = 42 – 45 – 47

Vein islet Number: 10 - 12

The number of vein islets per square mm in the central part of the lamina, midway between the midrib and the margin is calculated.

Palisade Ratio: 3 – 4

The average number of palisade cells beneath the epidermal cell are calculated and recorded.

Physio chemical analysis (17)**:**

Ash values were used to determine the quality and purity of the crude drugs. Procedure given in Indian Pharmacopoeia

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was used to determine the different ash values such as total ash and acid insoluble ash. Alcohol soluble and water soluble extractive value were also determined as per procedure given in Indian Pharmacopoeia.

Preliminary phytochemical analysis (18):

The dried powder material was extracted with Petroleum ether, ethanol and chloroform successively in a Soxhlet apparatus. The extracts were filtered while hot and concentrated under reduced pressure. The practical and % yields of the extracts were calculated. The concentrated extracts of the leaves were subjected to qualitative chemical test for the identification of various active constituents. The Investigation revealed presence of Steroid, Triterpenoid, Phenol, Flavanoid, Tanin, Alkaloid, Glycoside and Saponin.

Thin layer Chromatography (19):

The petroleum ether and ethanol extracts were subjected to TLC. Developing solvents were toluene: ethyl acetate 9:1, chloroform: methanol 9:1 respectively. Plates were viewed under 366nm. Rf values and colour of the various spots were noted. The petroleum ether extract showed three spots at Rf 0.42(dark blue) 0.52(pink) and 0.58(dark blue). The ethanol extract showed 4 spots at Rf 0.51(purple), 0.58(pale grey), 0.66(purple) and 0.82 (pale grey). TLC profile of the petroleum ether and ethanol extracts are given in **Plate I & II** respectively.

Table I

No	Parameters	Results
1.	Foreign matter	Nil
2.	Total ash	17.5%
3.	Water soluble ash	3.0%
4.	Acid insoluble ash	1.5%
5.	Alcohol soluble extractive	5.0%
6.	Water soluble extractive	2.0%

Table II

Type of constituent	Extract		
	Petroleum ether	Chloroform	Ethanol
Steroid	+	+	-
Triterpenoid	-	+	+
Phenol	-	+	+
Flavanoid	-	-	+
Coumarin	-	+	+
Quinones	-	-	-
Tannin	-	-	+
Alkaloid	-	-	+
Glycoside	-	-	+
Saponin	-	-	+

Conclusion

The pharmacognostical, physiochemical and preliminary phytochemical analysis evolved from the present investigation provide useful information and authentication of the plant. Phytochemical study is also useful to isolate the pharmacologically active principles present in the drug. The result of the present study will also serve as

reference material in the preparation of herbal monograph.

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Figure 1 *Pistia stratiotes* Linn.

Figure 2A: Transverse Section of leaf

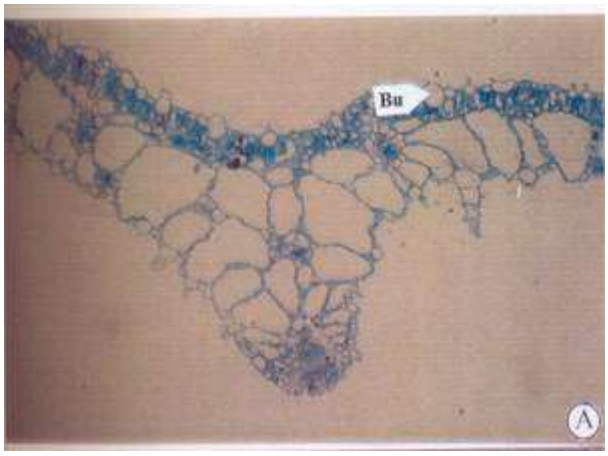


Figure 2B: Transverse section of rib region

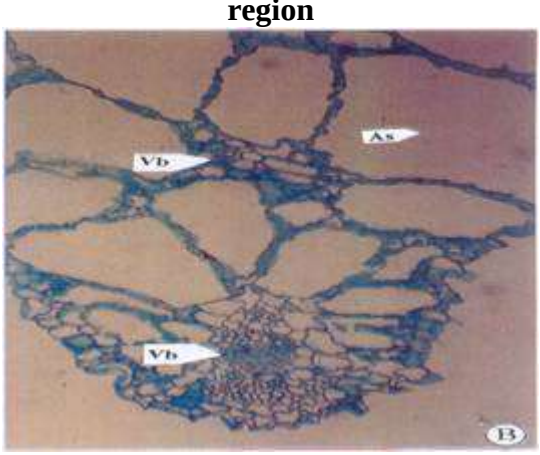
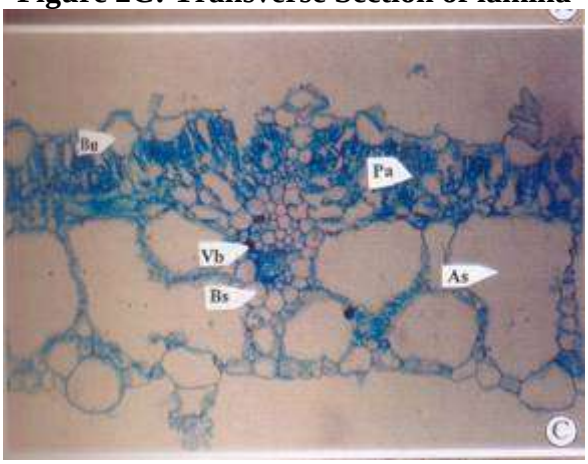


Figure 2C: Transverse Section of lamina



Vb - Vascular bundle; As - Air space; Bu - Bulbiform cell; Bs-Bundle sheath; Pa - Palisade tissue

Figure 3D: Adaxial foliar epidermis

Figure 3E: Abaxial foliar epidermis

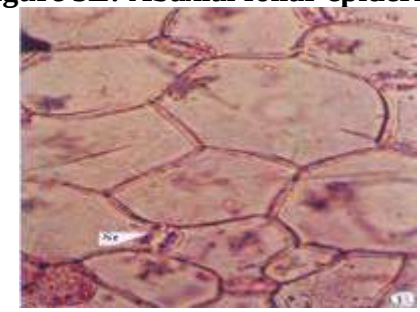


Figure 3F: T.S. of stolon (Scale L)

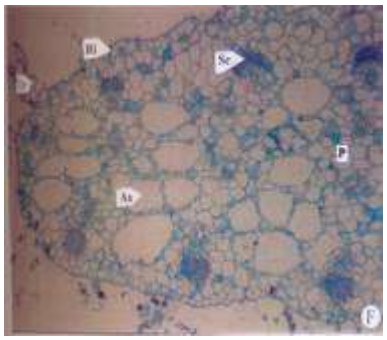


Figure 3G: T.S. of stolon – A Portion enlarged (Scale - K)

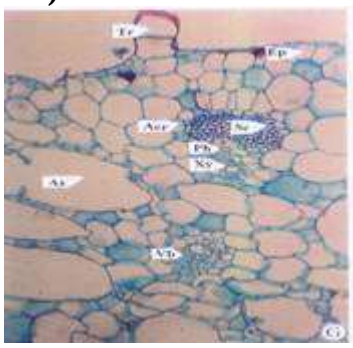


Figure 3H: T.S. of stolon showing vascular bundle

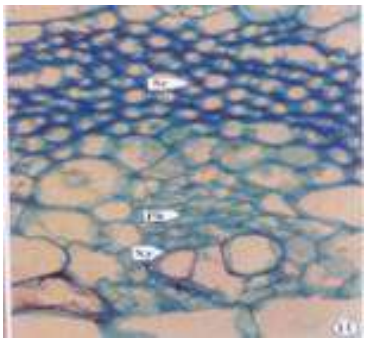


Figure 3I: Uniseriate multi cellular trichomes (Scale - K)

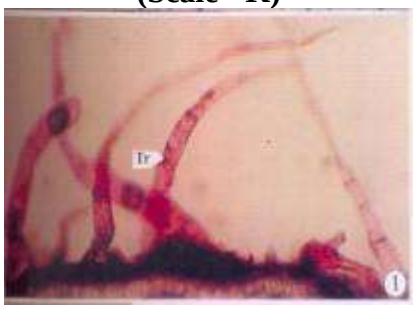


Figure 3J: Acicular crystals (Scale - M)

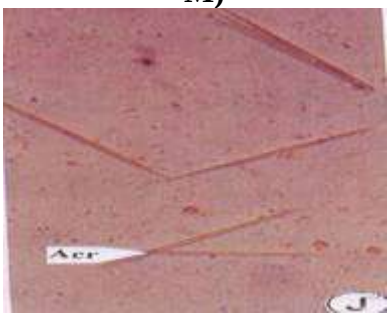
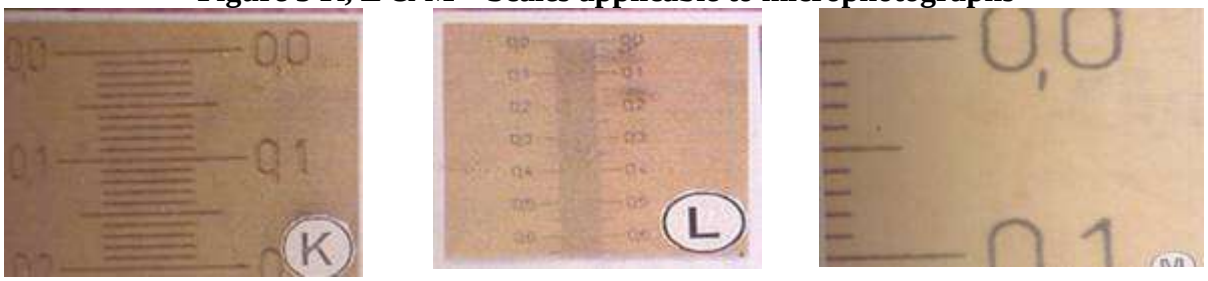


Figure 3 K, L & M – Scales applicable to microphotographs



Tr – Trichomes; Ri – Ridges; As - Air space: Sc –Sclerenchyma; P -Parenchyma
Acr - Acicular crystal; Ep – Epidermis; P -Parenchyma; Pa - Palisade tissue; Ph – Phloem; Ri – Ridges; Sc -Sclerenchyma; St - Stomata; Tr - Trichomes; Vb - Vascular bundle; Xy – Xylem

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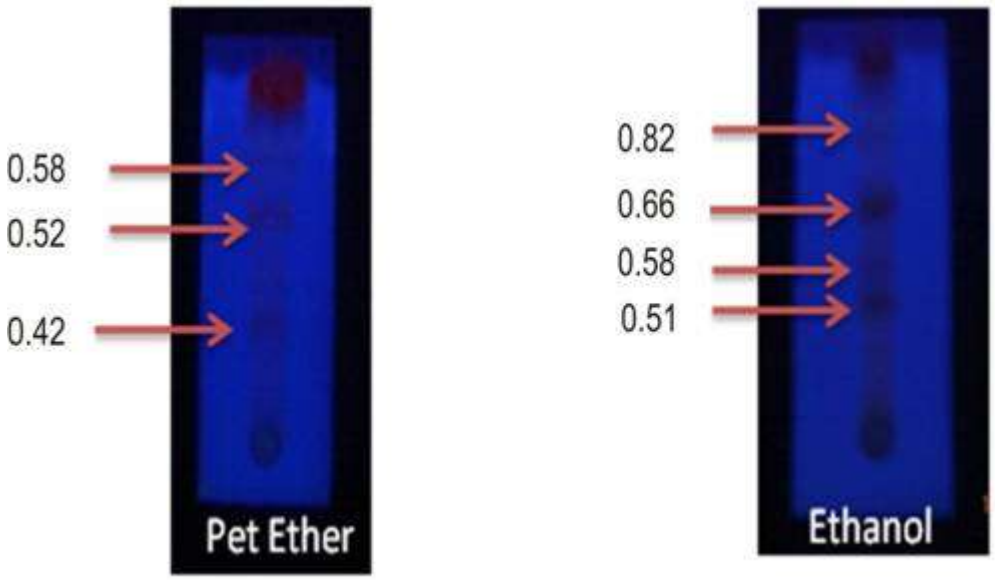

Plate - I Plate - II

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