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Morpho-Anatomical Studies on Leaf and Root of *Pistia stratiotes* Linn. (Family: Araceae).

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ABSTRACT

The pharmacognostical characters macroscopic, microscopic, physico-chemical analysis, behavior of powder with different chemicals and reagents, fluorescence analysis and phytochemical screening were investigated. Macroscopical study shows that leaves are radical, sessile, between obcordate and triangular; having the exterior margine scalloped, with many elevated ridges running lengthways underneath; down or both sides. Its roots hanging submersed beneath floating leaves. Root consists of numerous, long, tapering, hairy fibers. Transverse section of leaf of *P. stratiotes* reveals the presence of multicellular covering trichomes and paracytic stomata, vascular bundle, rosette type crystals, large aerenchyma and collenchymatous cells. Transverse section of root shows cortex region, acicular calcium oxalate crystals, parenchymatous cells containing rosette crystals, large air space in the cortex region, endodermis covering the pericycle, radial vascular bundles and small pith at the centre. Powder microscopy of leaf shows the presence of uniseriate covering trichomes, crystals, spiral vessels and sclereid. Acid insoluble ash was found to be less than total ash, water soluble ash and sulphated ash. The aqueous extractive value is quantitatively more than that of ether and ethanol. The inorganic element found in the ash of leaf are sodium, iron, sulphate, chloridr and nitrate. The preliminary phytochemical screening revealed presence of alkaloids, carbohydrates, tannins and phenolic substances, steroids and sterols, triterpenoids, flavonoids, saponins and gummy materials.

Keywords: Microscopic, physico-chemical analysis, inorganic element analysis, fluorescence and phyto chemical screening.

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INTRODUCTION

The plant *Pistia stratiotes* Linn. (Family: Araceae). is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water, its roots hanging submersed beneath floating leaves with a peculiar muriatic odor. It is found swimming on pools of stagnant water and ponds in most parts of India, having much the appearance of half-grown Lettuce plants. This plant also found in China, Indochina, Malaya, Brazil. Flowering time May-June and fruits November-December [1, 2]. Leaves radical, sessile, between obcordate and triangular; having the exterior margine scolloped, with many elevated ridges running lengthways underneath; down or both sides. The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures which trap air bubbles, increasing the plant's buoyancy. Root consists of numerous, long, tapering, hairy fibers [3, 4].

The plant is considered antiseptic, antitubercular and antidysenteric. In Gambia, the plant is used as an anodyne for eyewash. Juice of the plant is used by the Mundas in ear complaints [3]**.** The ashes of the plant are applied to the ringworm of the scalp. The leaves are used in eczema, leprosy, ulcers, piles and syphilis [5, 6]. With rose water they are given for cough and asthma [7]. Leaves are said to be anthelmintic [8]. A decoction of the leaves is used in La Reunion as a diuretic and prescribed in diseases of the urinary tract. Juice of the leaves applied externally in chronic skin diseases. The root is laxative and diuretic; good for wounds, inflammation and burns [5, 3]. Hot water extract used as an antifertility agent in New Guinea [9]. Few pharmacological or biological test reports have been reported on this plant in the literature like chloroform methanolic and ethanolic extracts of entire plant shows antibacterial activity [10, 11], methanolic extracts of entire plant possess bronchodilator, neuromuscular blocking activity [12], antioxidant activity [13]. Petroleum ether, methanolic and aqueous extracts of leaves shows anticrustacean activity [14].

Few phytochemical have been reported on this plant in the literature like ascorbic acid [15]. Flavonoid and flavon like chrysanthemin, lucenin, orientin, vicenin, vitexin and luteolin-7-glycoside was isolated from entire plant [16]. The entire plant also yielded steroid namely cholest-22-ene-3-6-dione,5-alpha: 24(s)-ethyl, cholesta-4-22-diene-3-6-dione,24(s)-ethyl [17], sitosterol-3-o-(2'-4'-o-diacetyl-6'-o-stearyl)-beta-d-glucopyranoside [18], stigmasta-4-22-dien-3-one, stigmasterol, stigmasterol stearate [19]. The plant was reported to contain palmitic [19] and linolenic acid [17]. From entire plant two sesquiterpene was isolated viz. stratioside I [20] and stratioside II [21].

MATERIALS AND METHOD

Chemicals

Compound microscope, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for undertaking the study. Microphotographs were taken using a Leica DMLS microscope attached with Leitz MPS -32 camera. Solvents viz. ether, ethanol and reagents viz. phloroglucinol, glycerin, hydrochloric acid, sodium hydroxide, iodine, picric acid, ferric chloride, nitric acid, sulphuric acid, Mercuric chloride, ammonia, silver nitrate and glacial acetic acid were procured from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Plant materials

The fresh plants of *Pistia stratiotes* were collected from ponds of subtropical region of Cuttack district of Odisha during July 2008 and authenticated by the taxonomists of the Botanical Survey of India, Shibpur, Howrah. A voucher specimen [Sp.No: CNH/ I-I / (96)/2008/Tech.II/1396] has been kept in our research laboratory for further reference.After authentication, the plant materials were collected in bulk, washed under running tap water to remove adhering dirt followed by rinsing with distilled water. The plant materials were then shade dried and separately pulverized in a mechanical grinder followed by sieving (sieve no. 40) to obtain coarse powder.

Pharmacognostical evaluation

Macroscopical study

Various macroscopic character of fresh leaves and roots of *Pistia stratiotes* were recorded.

Microscopical study

Pharmacognostical evaluation like microscopical studies are carried out by taking free hand sections [22, 23]. The sections were stained, with toluidine blue as per the method published by O'Brien et al 1964 [24]. Wherever necessary section were stained with safranin and Fast-green. Shade dried plant material were powdered with the help of an electric grinder till a fine powder was obtained. This fine powder was subjected to powder microscopy studies, as per standard procedures mentioned [25, 26]. The quantitative microscopy includes determination of stomatal index; stomatal number, palisade ratio, vein islet number and vein let termination are determined [27, 28].

Physico-chemical analysis

Physico-chemical parameters like ash values (total ash, acid insoluble ash, water soluble ash and sulphated ash), chemical tests for detection of inorganic constituents, moisture content, extractive values (alcohol soluble extractive, water soluble extractive, and ether soluble extractive) were performed on the shade dried powdered [29, 30]. Behavior of powder with different chemical and reagents, fluorescence analysis of the powdered plant material was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents [31, 32].

Extraction

The dried powdered plants materials (500 g each) were separately extracted successively with petroleum ether (60^0–80^0C) and ethanol using a Soxhlet extractor. The period of extraction was fixed at 48 h for every solvent at every stage of the extraction process. The solvents were purified by distillation prior to extraction. After completion of extraction, the extractive value was determined with respect to the dried plant material [33].

Preliminary phytochemical

The plant material are subjected to preliminary phytochemical screening for the detection of various plant constituents [34].

RESULT AND DISCUSSION

Morphological characteristic

The macroscopic characteristics of the plant of *P. stratiotes* were observed. It is a perennial monocotyledon with thick, soft leaves that form a rosette. It floats on the surface of the water. Leaves radical, sessile, between obcordate and triangular; having the exterior margin scalloped, with many elevated ridges running lengthways underneath; down or both sides. The leaves can be up to 14 cm long and have no stem. They are light green, with parallel veins, wavy margins and are covered in short hairs which form basket-like structures which trap air bubbles, increasing the plant's buoyancy. Its roots hanging submersed beneath floating leaves, root consists of numerous, long, tapering, hairy fibers.

Microscopical study

Transverse section of leaf

Upper epidermis layer of *P. stratiotes* leaf consists of single layer, rectangular cells. Epidermis layer consists of multicellular covering trichomes and paracytic stomata. Midrib shows the vascular bundle, which is present in small size. It contain rectangular parenchymatous cell, which measures about from 69.08-32.54µ it

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contains rossete type crystals. Large aerenchyma is present. 6-7 layers of collenchymatous are present at the lower epidermis [Fig -1& Fig-1(a,b,c,d)].

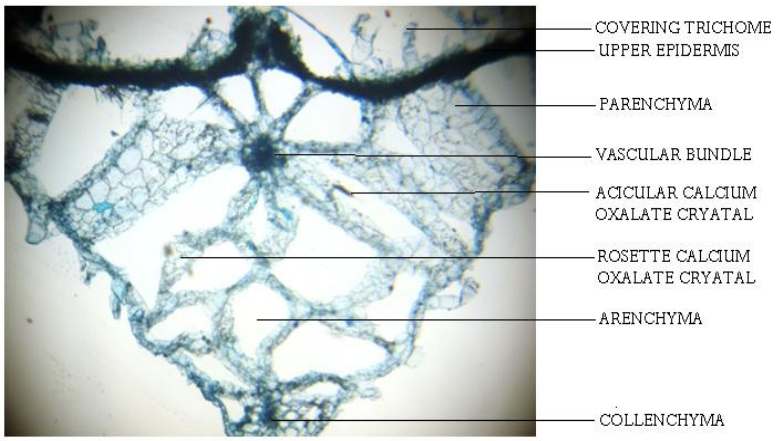

Figure 1: T.S. of leaf of *Pistia stratiotes*.

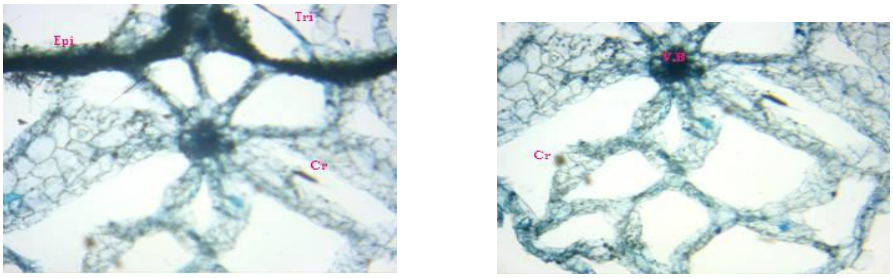

Figure 1(.a,b) : Sectorial T.S. of leaf of *Pistia stratiotes*.
(Epi-Epidermis; Tri-Trichomes; Cr-Crystal; V.B-Vascular bundle)

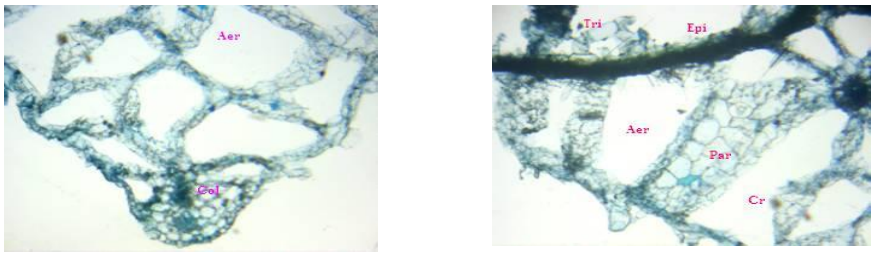

Figure 1 (c,d): Sectorial T.S. of leaf of *Pistia stratiotes.*
(Aer-Arenchyma; Col-Collenchyma; Tri-Trichomes; Epi-Epidermis; Par-Parenchyma; Cr-Crystal.)

Transverse section of lamina

Lamina *P. stratiotes* is dorsiventral in nature. Upper epidermis consists of single layer, rectangular cells. Epidermal layer consist of multicellular covering trichomes and paracytic stomata. In mesophyll palisade cells are arranged in single layer. The cells are small and are closely arranged. Spongy parenchymas are present in 8-9 layers, loosely arranged with intercellular spaces. The cells contain acicular and rossete type of crystals. Vascular bundles are seen. Large air cavity is paresent. Lower epidermis is similar to upper epidermis [Fig -2 (a,b)].

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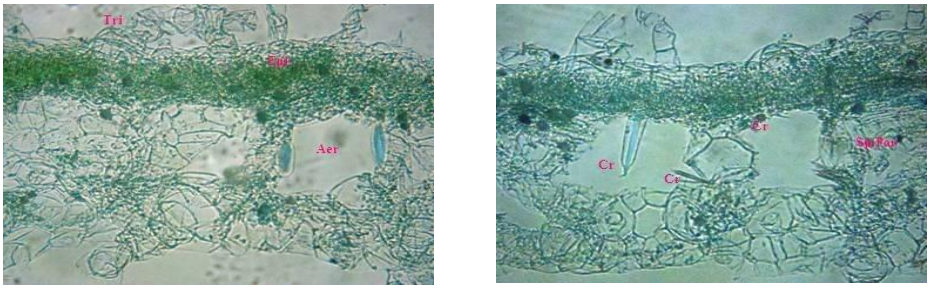

Figure 2 (a,b): Sectorial T.S. of lamina of *Pistia stratiotes.*
(Tri-Trichomes; Epi-Epidermis; Aer-Arenchyma; Cr-Crystal.)

Transverse section of petiol

Transvers section of petiol shows the outer epidermal layer, contain multicellular covering trichomes. Epidermal layer is followed by 6-7 layer of polygonal cell, measures about 32.27-49.08µ. ground tissue shows the presence of groups of vascular bundles. Rossete type of calcium oxalate crystals are found inside the parenchymatous cell. Acicular calcium oxalate crystals are found inside vessels [Fig-3 to Fig-3 (a, b)].

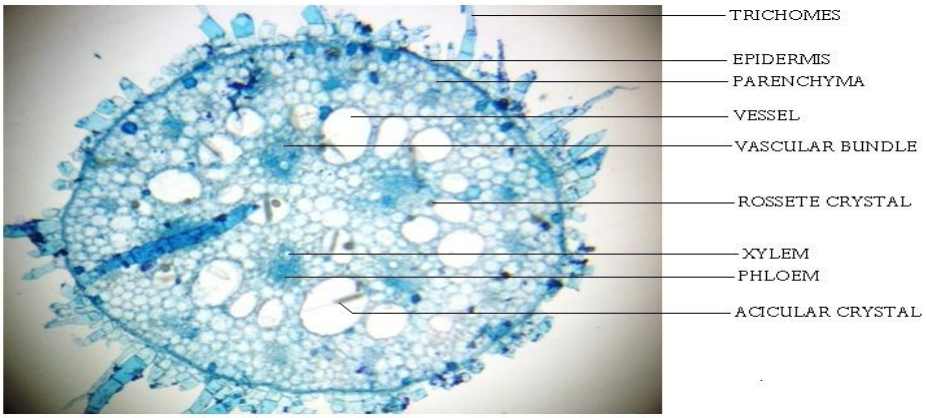

Figure 3: T.S. of Petiol of *Pistia stratiotes.*

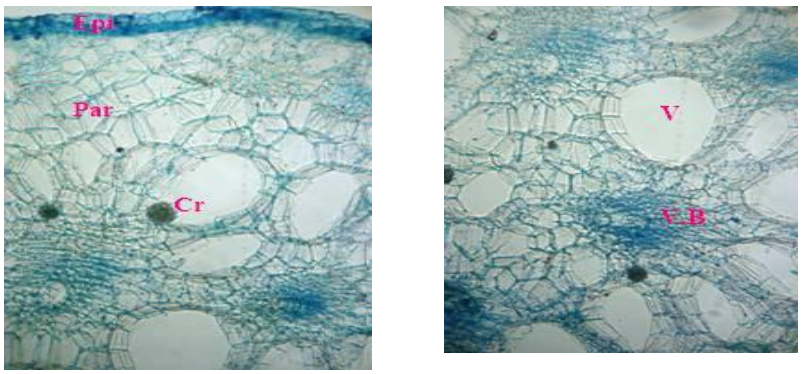

Figure 3(a,b): Sectorial T.S. of Petiol of *Pistia stratiotes*.
(Epi-Epidermis; Par-Parenchymatous cell; Cr-Crystal; V-Vessel; V.B-Vascular bundle.)

Transverse section of root

Transverse section of root shows single layer of compactly arranged elongated parenchymatous cell. Cortex region consists of 4-5 layer of polygonal cells, measures about 32.27-68.29µ and contains acicular calcium oxalate crystals. Chains of rectangular parenchymatous cells are present, containing rossete crystals. Large air space is present in the cortex region. A single layer of endodermis, covering the pericycle, consists of polygonal parenchymatous cells. Endodermis is followed by two layer of pericycle. Pericycle is composed of

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compactly arranged parenchymatous cells. Radial vascular bundles are found at the center. Small pith is present at the centre, having intercellular spaces [Fig-4 & Fig-4 (a, b, c, d)].

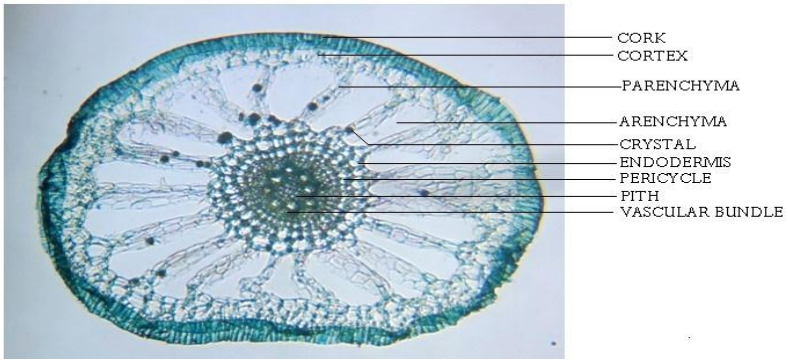
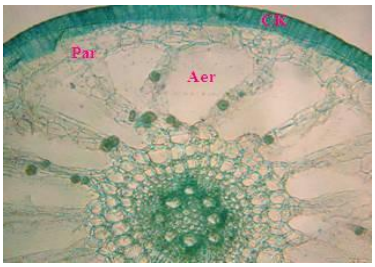


Figure 4: T.S. of root of *Pistia stratiotes.*



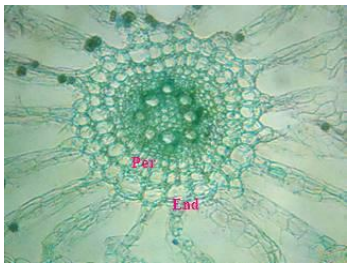
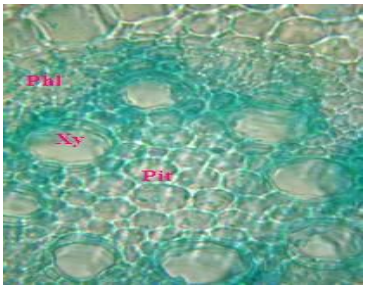


Figure 4(a,b): Sectorial T.S. of root of *Pistia stratiotes*.
(CK-Cork; Par-Parenchymatous cell; Aer- Aerenchymatous cell; Per- Pericycle; End- Endodermis.)



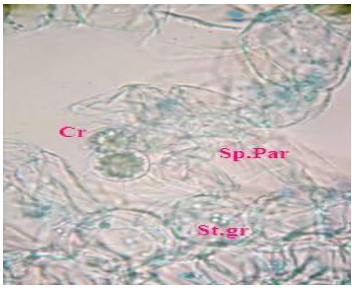


Figure 4(c,d): Sectorial T.S. of root of *Pistia stratiotes*.
(Phl-Phloem; Xy-Xylem; Pit-Pith; Cr-Crystal; Sp.par-Spongy parenchyma; St.gr-Starch grain.)

Powder microscopy

The powder microscopy of *P. stratiotes* leaf consists of the following elements:
Trichomes- These are three to four celled, bent, thick walled, pointed and uniseriate [Fig-5- a,b,c].

Sclereids- It is composed of large no of densely packed cells. Te walls are thick & contain calcium oxalate prism sheath [Fig-5-d].

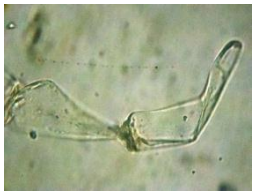


Figure 5-a

Figure b

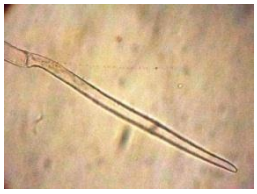


Figure 5-c

Figure 5-d

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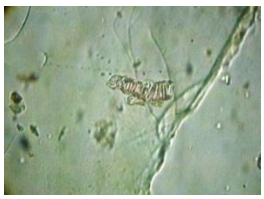

Figure 5-f

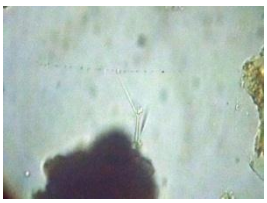

Figure 5-e

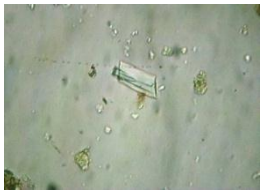

Figure 5-g

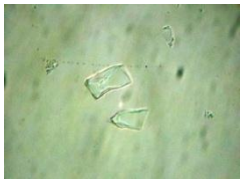

Figure 5-h

Powder characters of leaf of *Pistia stratiotes*.
(a,b,c-covering trichomes; d-Sclereids, e-Spiral vessels; f-Acicular crystals; g, h-Squarish and prismatic crystals.

Vessel-The fragments of lignified fibro vascular tissue with spiral thickening [Fig-5-e].Crystals-The prism of calcium oxalate crystals are found scattered. They vary considerable in size and are occasionally quite large and irregularly shaped. Some times acicular crystals are also found [Fig-5-f, g, h].

Quantitative microscopy

The morphology of leaf epidermis shows that, paracytic stomata are present in both the surface. The epidermal layer consists of straight walled polygonal cells [Fig-6]. The stomatal index was found 32.09/mm^2. The length of stomata vary from 97.62-130.16µ and average length was found 112.26µ. The width of stomata vary from 48.81-65.08µ and average width was found 58.57µ [Table-1]. The venation pattern shows that main vein are more thick than lateral veins, where as veinlets are uniformly thin. Vein islets are found generally rectangular and squarish in outline. Vein termination are long slender [Fig-7].Vein-islet no was found 16.66/mm^2 & vein termination was found 13.33mm^2 [Table-1].

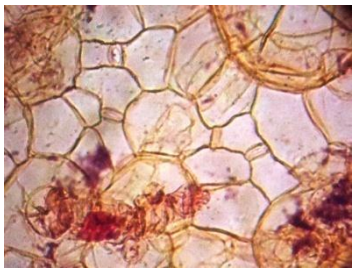

Figure 6
(Epidermal tissue with stomata)

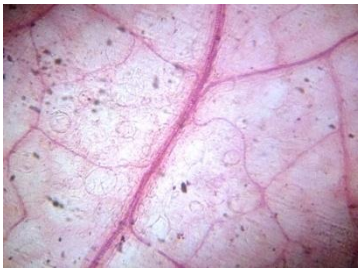

Figure 7
(Vein-islet & Vein termination)

Table-1: Quantitative microscopy

Sl.no	Parameters	Values
1	Stomatal index	39.09/mm^2
2	Vein-islet number	16.66/mm^2
3	Vein termination number	13.33mm^2
4	Palisade ratio	5.6
5	Length of stomata	97.62-130.16µ
6	Average length of stomata	112.26µ
7	Width of stomata	48.81-65.08µ
8	Average width of stomata	58.57µ
9	Length of phloem fibre	325.40-390.49µ
10	Average length of phloem fibre	367.94µ
11	Width of phloem fibre	16.27-32.54µ
12	Average width of phloem fibre	23.59µ
13	Diameter of calcium oxalate crystal	65.08-113.89µ
14	Average diameter of calcium oxalate crystal	87.85µ

Average palisade ratio was found 5.6 [Table-1]. The epidermal cell shows palisade cells. Palisade cells are spherical in shape, distributed through out the epidermal cells. The length of fibre of leaf varies from 325.40-390.49µ and average length was found 367.94µ. The width of fibre of leaf varies from 16.27-32.54µ and average width was found 23.59µ.The diameter of calcium oxalate crystal of leaf varies from 65.08-113.89µ and average diameter was found 87.85µ. The data are given in [Table-1].

Physico chemical analysis

The results obtained for the ash values are total ash 17.9 w/w, acid insoluble ash 3%w/w, water soluble ash 6.5w/w and sulphated ash 25w/w and moisture content is 10.5w/w. Here, acid insoluble ash was found to be less than total ash, water soluble ash and sulphated ash. Sulphated ash was found to more than the others. Ash value is a measure of the quality and purity of the drug. The extractive values were determined to find out the amount of soluble compounds. The ethanol, water and ether soluble extractive values of leaf were found to be 2.3, 6.2, and 1.5w/w. Here, the aqueous extractive value is quantitatively more than that of the others. These are given in [Table-2]. The inorganic element found in the ash of leaf are sodium, iron, sulphate, chloridr and nitrate [Table-3]. The behaviors of the powdered leaf with different chemical reagents are reported in [Table-4]. Behaviors of the powdered drug of leaf with different chemical reagents observed separately under day light and ultraviolet light and the changes in color were also noted and tabulated in [Table-5].

Table 2:Physico-chemical analysis of leaf of *Pistia stratiotes*

Sl.no	Parameter	Values of leaf (% w/w)
1	Total ash	17.9
2	Acid insoluble ash	3
3	Water soluble ash	6.5
4	Sulphated ash	25
5	Moisture content	10.5
6	Ether extractive value	1.5
7	Ethanol extractive value	2.3
8	Water extractive value	6.2

Table 3: Determination of inorganic elements (Leaf)

Sl.no	Test for	Inference
1	Calcium	-
2	Magnesium	-
3	Sodium	+
4	Potassium	-
5	Iron	+++
6	Sulphate	+++
7	Phosphate	-
8	Chloride	++
9	Carbonate	-
10	Nitrate	+

-Absent. +Present, ++Moderate, +++ Frequent.

Table-4: Behaviour of leaf powder with different chemical reagents.

SL.No	Acid/Reagent	Observation
1	Powder as such	Dull green
2	Powder + Picric acid	Yellowish green
3	Powder + Con.Nitric acid	Light yellowish brown
4	Powder + Con.HCL	Yellowish green
5	Powder + Con.H_2SO_4	Brownish black
6	Powder + Glacial acetic acid	Black
7	Powder + 5% $FeCl_3$	Yellowish green
8	Powder + NaOH(5N)	Brown
9	Powder + KOH (5%)	Light brown
10	Powder + Iodine/20	Dark blue

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Table 5: Fluorescence analysis of the leaf powder.

SL.No	Reagent	Day light	Short wave
1	Powder as such	Dull green	Green
2	Powder + 1N NaOH in methanol	Deep green	Green
3	Powder + 1N NaOH	Light green	Light green
4	Powder + Ethanol	Greenish black	Green
5	Powder + HNO_3+NH_3 solution	Brownish red	Light green
6	Powder + 50%HNO_3	Light brown	Light green
7	Powder + 1N HCL	Dull brown	Very light green
8	Powder + HCL	Yellowish green	Deep green
9	Powder + H_2SO_4	Brownish black	Green
10	Powder + 50% H_2SO_4	Dull green	Very light green
11	Powder + Glacial acetic acid	Light green	Light green
12	Powder + HNO_3	Light yellowish brown	Green

Extraction

The color, consistency and extractive values of petroleum ether and ethanol extracts of the plant *Pistia stratiotes* are reported in [Table-6]. Fluorescence characteristics of liquid extracts are depicted in [Table-7].

Table 6: Data showing the colour, consistency and extractive values of petroleum ether and ethanol extracts of the *Pistia stratiotes.*

Sl.no	Parts used	Extract	Colour	Consistency	Yield % w/w
1	Whole plant	Petroleum ether	Pale green	Waxy	1.7
2		Ethanol	Greenish brown	Sticky	6.4

Table 7: Fluorescence characteristics of petroleum ether and ethanol extracts of the *Pistia stratiotes* under daylight and u v light.

Sl.no	Parts used	Extract	Colour		
			Day light	Short UV	Long UV
1	Whole plant	Petroleum ether	Pale green	Pale green	White
2		Ethanol	Greenish brown	Green	Pink

Preliminary phytochemical screening

The preliminary phytochemical screening of *P. stratiotes* revealed presence of alkaloids, carbohydrates, tannins and phenolic substances, steroids and sterols, triterpenoids, flavonoids, saponins and gummy materials [Table-8].

Table 8: Preliminary phytochemical screening of petroleum ether and ethanol extracts of *Pistia stratiotes*.

Test	Extract	
	Petroleum ether	Ethanol
Alkaloids	-	+
Carbohydrates	-	+
Gums and mucilages	+	-
Proteins and amino acids	-	-
Tannins and phenolic compounds	-	+
Steroids and sterols	+	+
Triterpenoids	+	+
Saponins	-	+
Flavonoid	-	+

(+): Present; (-): Absent

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CONCLUSION

The standardization of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in a herbal pharmacopoeia, pharmacognostical parameters and standards must be established. In order to standardize a drug, various macroscopic, microscopic, fluorescence analysis are done. Microscopic methods is one of the cheapest and simplest methods to start with establishing the correct identification of the source materials. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. Stomatal number, stomatal index & palisade ratio, vein-islet, vein termination are equally important in evaluation of crude drug. These values help in the evaluation of purity of drug. Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are primarily useful for determination of exhausted and adulterated drug. The information obtained from preliminary phytochemical screening will be useful in finding out the quality of the drug. From the ongoing studies, it can be concluded that the above macroscopic and microscopic studies together may be used as a tool for identification of *Pistia stratiotes* with its pharmacognostical characteristics, discriminating it from its other species diversity.

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