e - ISSN - 2249-7722
Print ISSN - 2249-7730

International Journal of Phytotherapy

www.phytotherapyjournal.com

EVALUATION OF PHARMACOGNOSTICAL, PRELIMINARY PHYTOCHEMICAL STUDIES ON *BLEPHARIS REPENS (VAHL) ROTH.*

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ABSTRACT

Blepharis repens (Vahl) Roth (Acanthaceae*)* is native to South-eastern Asia. This plant species has been of interest to researchers because it is a medicinal plant employed in the Indian indigenous system of medicine. Pharmacognostic standardization, physicochemical evaluation of the Whole plant of *Blepharis repens (Vahl) Roth* was carried out to determine its macro-and micro-scopical characters and also some of its quantitative standards. Microscopical studies were done by using trinocular microscope. Total ash, water-soluble ash, acid-insoluble ash and sulphated ash values, alcohol- and water-soluble extractive values were determined for phytochemical evaluations. Preliminary phytochemical screening was also done to detect different phytoconstituents. Microscopically, root showed collenchyma, secondary phloem, secondary xylem, periderm cells. Powder microscopy showed crystal fibres, parenchyma cells, vessels elements, and with thick pits. Total ash was approximately two times and four times more than acid insoluble and water soluble ash, respectively. Ethanol soluble extractive was approximately two times higher than water soluble extractive. Phytochemically, Whole plant exhibited alkaloids, flavonoids and glycosides, fixed oils, tannins and saponins.

Key words: *Blepharis repens (Vahl) Roth*, Macroscopy, Microscopy, Phytoconstituents.

INTRODUCTION

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicine, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise plants obtain natural substances met can promote health and alleviate illness. In recent times, focus on plant research has increased all over the world and a large body of evidence has collected the show immense potential of medicine plants used in various traditional systems [1].

A through pharmacognostic investigation was carried out on the whole plant of *Blepharis repens (Vahl) Roth* The assignment such as macroscopy, anatomical studies and preliminary phytochemical screening were performed. Since the species was not noted for its pharmacognosy in part. A macroscopical study is a technique of qualitative evaluation based on the study of

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morphological and sensory profiles of Microscopical evaluation of the plant drugs helps to identify the organized drugs by their known histological characters and used to confirm the structural details of the drugs from plant origin [2].

Physic-chemical evaluation of crude drug involves the determination of the identity, purity and quality. Purity depends upon the absence of foreign matter, whether organic or inorganic. While quality refers essentially to the concentration of the active constituents in the drug that makes it valuable to medicine [3].

The pharmacognostical studies of the plant drugs focused on bringing out the diagnostic characters will be immense help in the proper identification and standardization of different botanical species of the plant origin. The pharmacognostical parameters are major and reliable criteria for confirmation of the identity and determination of quality and purity of the crude drugs that plays a major role to establish.

MATERIALS AND METHODS FOR ANATOMICAL STUDIES

Collection of Specimens

The plant specimens for the proposed study were collected from Western Ghats, Erode district. Care was taken to select healthy plants and for normal organs. The required samples of different organs were cut and removed from the plant and fixed in FAA (Farmalin-5ml + Acetic acid -5ml + 70% Ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of Tertiary-Butyl Alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60 C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Microscopical Characters of *Blepharis repens (Vahl) Roth*

Sectioning

The paraffin embedded specimens were section with the help of Rotary Microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure (Johanson, 1940). The sections were stained with Toluidine blue as per the method published by O'Brien et al. (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and Fast-green and IKI (for Starch) [4].For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have briefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the Scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books (Esau, 1964)

Macroscopical characters of *Blepharis repens* (vahl) roth

Colour : green Odour : odourless. Taste : Slightly and characteristic. Size: straight, unbranched. Shape : stem-spindle shape and thick, four angled . leaf-cubical, 1to 2cm length and 1 to 1.5cm breadth. Root-disorganished and compressed, 1mm thick.

Leaf

The leaf consists of their lamina and prominent planoconvex midrib the adaxial part of the lamina is flat and the abaxial part is wide, thick and bowl-shaped. It is 600/ um (micrometer) thick and 450um (micrometer)wide the midrib consist consist of a thin epidermal layer of small cubical, slightly papillate cells. The ground tissue of the midrib is homogeneous and it includes fairly large, compact, thin walled and compact parenchyma cells.

The vascular strand of the midrib is bowl shaped with flat adaxial side and broadly conical adaxial part. It includes wide, circular, thick walled solitary xylem elements which are diffuse in distribution. The xylem elements are 20(micrometer) in diameter. Mixed with xylem elements are wide thick walled fibres along lower part of the vascular strand occurs a thin layer of phloem.

Lamina

The lamina consists of a thick adaxial epidermis; the cells are rectangular to squarish and thin walled; the cuticle is thin and smooth. The abaxial epidermis is thinner comprising of narrowly cyclindrical thin walled cells. The mesophyll tissue consists of a narrow zone of short cyclindrical palisade cells and three layers of lobed and loosely arranged spongy parenchyma cells. The lamina is 100um (micrometer) thick.

Epidermal cells and stomata

The epidermal tissues were studied from the

paradermal sections of the lamina. The epidermal cells have fairly thick highly wavy auticlival walls, so that cells appear annoeboid in outline. The stomata are densely distributed on the epidermis. They are random in distribution. The stomata are diacylic type. These are two subsidiary cells for each stoma. The two subsidiaries occur at opposite poles of the guard cells. The common wall of the two subsidiaries lie at right angle with long axis of the guard cells(3.2) the subsidiary cells have straight walls. The gaurd cells are 20x35 (micrometer) in size, apart from the epidermal cells, these are pairs cells sparsely seen in the epidermis. These cells are epidermal cells dilated into these dilated paired cells are idioblasts for the storage of calcium carbonate crystals.

Venation pattern

In cleaned lamina, the venation system was studied. The veins are thin and straight. They form reticulate system of wide vein- islet. The islet are variable in shape and size and also in orientation the vein terminations are simple and unbranched or more commonly the terminatious are branch or twice assuming dandroid out line (4.2) the terminatious are mostly wavy in outline.

Stem

Both young and fairly old stem were studied. The young stem is slightly four angled. It is 950um (micrometer) thick along the wider dimension the has narrow epidermal layer of small spindle shaped, thick walled cells with thick cuticle. The cortical zone is about 100um (micrometer) thick and includes 6 or 7 layers of angular, compact thin walled parenchyma cells. The vascular cylinder is a four angled cylinder with thin and thick portion alternating with each other. The thin portion consists of three or four layers of thick walled fibres the thick segments. Include a group of wide, circular thick walled vessels and narrow thick walled fibres (5.2) the pith tissue is intact: the cells are angular, thin walled and compact phloan occurs in thick arcs on the outer part of the portions of the vascular cylinder.

The old stem is basically similar to the young stem (6.1: 7).the old stem consists of a thin epidermal layer spindle shaped cells with fairly thick, minutely sechinate cuticle. Two or three layers outer cortical cells of collenchymas and inner zone of four or five layers of large, thin walled compact parenchyma the vascular cylinder includes four thick corners alternating with four thin portion. The thin portion consists of short radial files of secondary xylem fibres: the thick corners have wide, thick walled diffuse clusters of vessels are 10-50um(micrometer) wide. The pith shows lysis of central core of lysis of cells forming wide central pith canal.

Root

The root measuring 1mm thick studied. it exhibits well developed secondary growth. It consists of narrow zone of superficial periderm: the periderm cells are disorganized and compressed. A few inner layer of the periderm cells appear intact (8.2:9.2). The secondary phloem with outer part is crushed into thick dark bands of collapsed phloem and a narrow inner portion consists of non-collapsed (intact) phloem elements. The secondary xylem occupies wide major portion of the root.it includes densely crowded, diffusely distributed wide, circular, thick walled, narrow xylem fibres. The vessels include both wide and narrow elements. They are upto 50 (micrometer) wide [6-7].

Powder microscope observation

Powder preparation of the plant includes the following cell types

Fibres

The fibres are libriform type: they have thick lignified walls they have fairly wide lumen. No pits are evident on the walls. The fibre is uniform in thickness and they become tapering at the ends (fig.10.3) the fibres are upto 250 (micrometer) long and 10 (micrometer) thick.

Vessel Elements

The vessel elements are narrow, long and cylindrical. They have dense, circular, alternate wall pits. The end wall perforation is simple, circular, mostly oblique or horizontal. Some vessel members are tailed at one end. The vessel members are 250-520 (micrometer) long.

Parenchyma

The parenchyma cells are either long, narrow cylindrical or spherical or ovoid. They are thin walled: no pits or cell inclusions are seen in the cells. Elongated parenchyma cells are 150um (micrometer) and 30um (micrometer) wide. The elliptical cells are 30x 50um (micrometer) in size.

PHYTOCHEMICAL STUDIES

A spectrum of natural compounds like alkaloids, glycosides, tannins, and essential oils and similar other secondary metabolites which exert physiological activity are synthesized in the plant, in addition to the carbohydrates, proteins and lipids utilized by man as food articles.

A systematic and complete study of crude drugs should include a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. The different qualitative chemical tests are to be performed for establishing profile of a given extract/fraction for its nature of chemical composition [8-10].

MATERIALS AND METHODS

MATERIALS

The plant *Blepharis repens (vahl) Roth* is widely found in theWestern Ghats, Erode area, Tamilnadu, India.

Collection

For Present work, the whole plant parts from the plant *Blepharis repens (vahl)Roth* were collected in the Western Ghats area, Gobi, Erode district, Tamilnadu.

Treatment

The whole plant were then washed in running tap water, the adhering soil was completely removed. Then these were cut into small pieces by means of stainless steel knife and put on polythene sheets and shade dried. The dried material was powdered by means of wood grinder and the powder was passed through the sieve no. 40 and separated the coarsely powdered for Extraction Process.

PURIFICATION OF SOLVENTS

Petroleum ether

The petroleum ether was distilled and the fraction boiling between 60° to 80°C was collected and used for extraction and chromatographic purposes.

Chloroform

The chloroform was shaken well with equal volume of distilled water twice to remove water soluble impurities and separated using a separating funnel. It was then dried over anhydrous calcium chloride for 24hours, filtered and dried once again with anhydrous potassium permanganate for another 24hours. This was decanted and distilled, the portion boiling at 64°Cwas collected and stored in a dark brown coloured bottle. As a preservative, 1ml of absolute alcohol was added.

Ethyl acetate

It was refluxed for 4hours and distilled. The distillate was shaken and add sufficient amount of anhydrous potassium carbonate, filtered and distilled. The fraction boiling at 77 °C was collected and used.

Ethanol

Rectified spirit was distilled and used for the extraction purpose.

PREPARATION OF EXTRACTS

Procedure for cold extraction

Petroleum Ether Extract

The shade dried material (1000gm) was extracted with petroleum ether (60° - 80°C) for 7days at room temperature. After completion of extraction, the solvent was removed under reduced pressure.

Chloroform extract

The marc left after petroleum ether was extracted with chloroform for another 7days at room temperature. The solvent was removed under reduced pressure

Ethyl acetate extract

The marc left after $CHCl_3$ extraction was dried and then extracted with ethyl acetate for seven days at room temperature. The solvent was removed by distillation under reduced pressure.

Ethanolic extract

The marc left after ethyl acetate extraction was dried and finally extracted with ethanol for 7days at room temperature and concentrated under reduced pressure.

PHYTOCHEMICAL STUDIES

The following tests were carried out by standard methods on the extracts / fractions to detect various phyto constituents present in them [11-15].

P^H DETERMINATION OF POWDERED DRUG

Procedure

1Gms of the accurately weighed powdered drug was dissolved in water & filter. PH of the filtrate was determined by using P^H meter.

PHYSICOCHEMICAL CONSTANTS RESULTS

Table 1. Various Ash Values of Aerial part of *Blepharis repens (vahl) Roth*

S. No	Particulars	*Blepharis repens (vahl) Roth* (%w/w) n=3
1.	Total Ash	4.56
2.	Acid-insoluble Ash	0.39
3.	Water soluble ash	4.08
4.	Sulphated ash	0.26

Table 2. Various Extractive Values of Aerial part of *Blepharis repens (vahl) Roth*

S. No	Particulars	*Blepharis repens (vahl) Roth* (Leaves) (% w/w) n=3
1.	Alcohol soluble extractive	5.2
2.	Water soluble extractive	4.5

Table 3. Vein islet Number and Veinlet termination number

S. No	Species	Range Of Vein-Islet Number	Range of veinlet termination number
1.	***Blepharis repens (vahl) Roth***	9-20	15-20

Table 4. Stomatal Index and stomatal Number

S. No	Species	Range of Stomatal index	Range of Stomatal number
1.	***Blepharis repens (vahl) Roth***	8-16	1-5

Table 5. P^H Determination Of Powdered Drug

Table	1% Solution	2% Solution
P^H	6.0	6.12

Table 6. Flourescence Structure Of Powder Drugs

S.No	Drug	Solvent	Appearance		
			Long	Short	Day/Light
1	Powdered Drug	10 ml of acetone	Red	Green	Green
2	Powdered Drug	10 ml Benzene	Red	Green	Green
3	Powdered Drug	10 ml of Pet. Ether	Light Yellow	Light Green	Light Green
4	Powdered Drug	10 ml of ethanol	Red	Green	Green
5	Powdered Drug	10 ml of Glacial acid acid	Redish brown	Green	Green
6	Powdered Drug	10 ml of Conc. Hcl	Dark brown	Brown	Brown
7	Powdered Drug	10 ml Conc.HNO_3	Light Brown	Brown	Brown
8	Powdered Drug	10 ml of Con. $H2SO_4$	Redish Brown	Brown	Brown
9	Powdered Drug	10 ml of methanol	Red	Green	Green
10	Powdered Drug	10 ml of Iodine	Black	Brown	Brown

Table 7. Nature of phytoconstituents present in whole plant extracts of *Blepharis repens (vahl)roth.*

S.N0	Particulars	Alkaloids	Carbo hydrates	Glycosides	Flavonoids	Proteins & Amino acids	Steroids	Tannins & Phenolics	Saponins	Fi 6xed Oils
1.	***Pet .Ether Blepharis repens(vahl) roth.***	-	-	-	+	-	+	+	+	+
2.	***Chloroform Blepharis repens(vahl) roth.***	-	+	-	+	-	+	+	-	+
3.	**Ethylacetate *Blepharis repens(vahl) roth.***	-	-		+	-	+	+	-	-
4.	**Ethanol *Blepharis repens*(vahl) roth.**	+	+	+	+	-	-	+	+	+
5.	**Aqueous Extract *Blepharis repens*(vahl) roth**	+	+	-	+	-	-	+	+	-

Fig 1. Aerial part and Root of *Blepharis repens (vahl) roth*

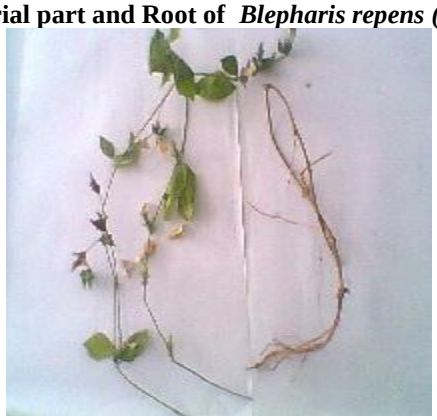
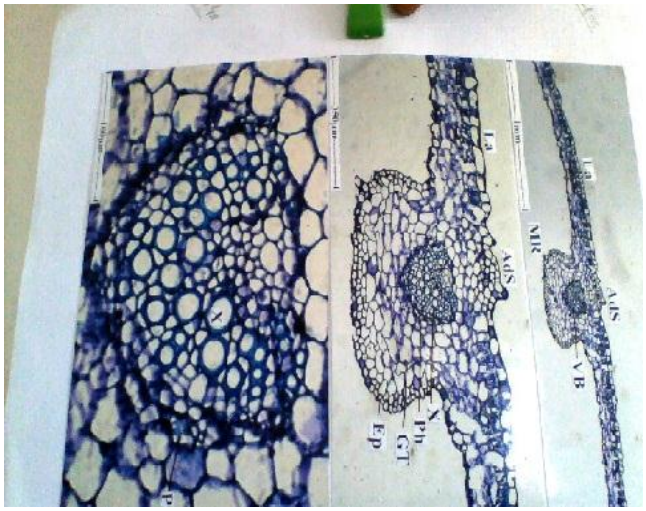
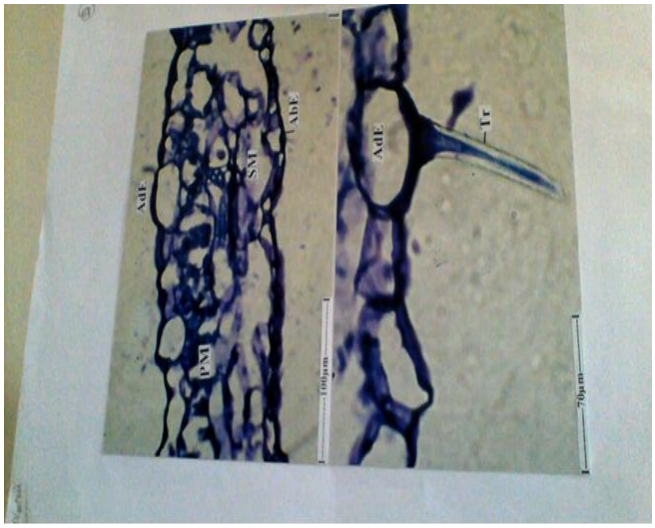


Fig 1.1. Anatomy of the leaf



Ads-Adaxial side, GT- ground tissue. EP-Epidermis, La- lamina, mr-midrib, ph-phloem, vb- vascular bundle, x-xylem1. T.S of leaf through midrib, 2.T.S of midrib, 3. T.S of vascular bundle of the midrib.

Fig.2.1 & 2.2. T.S. of Lamina



Abe:Abaxial Epidermis,Ade:Adaxial Epidermis,PM:Palisode Mesophyll,SM:Spongy Mesophyll,Tr:Trichome

Fig 3.1. Paradermal section of the abaxial Epidermis showing stomata
Fig.3.2. Stomata surface view enlarged
(EC-Epidermal cell, GC-Guard cell, LC-Lithocyst, SC-Subsidary cell, St-Stomata)

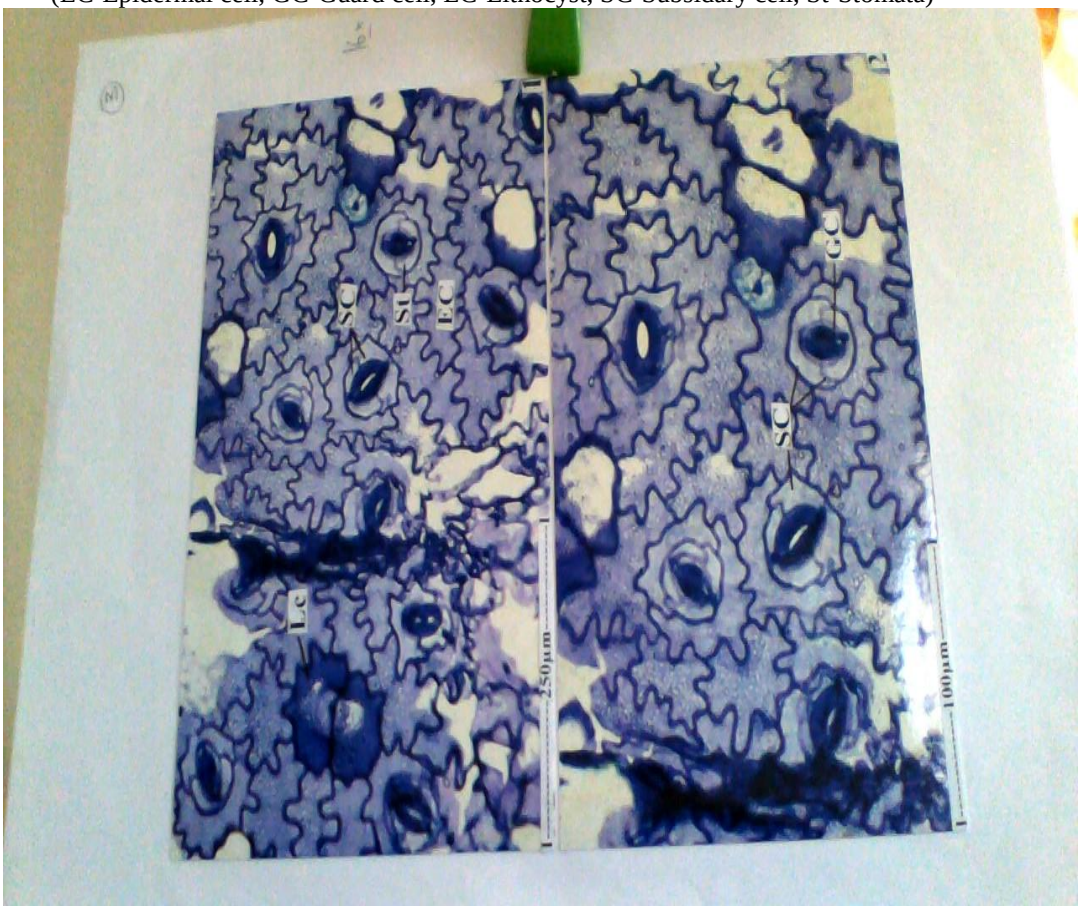
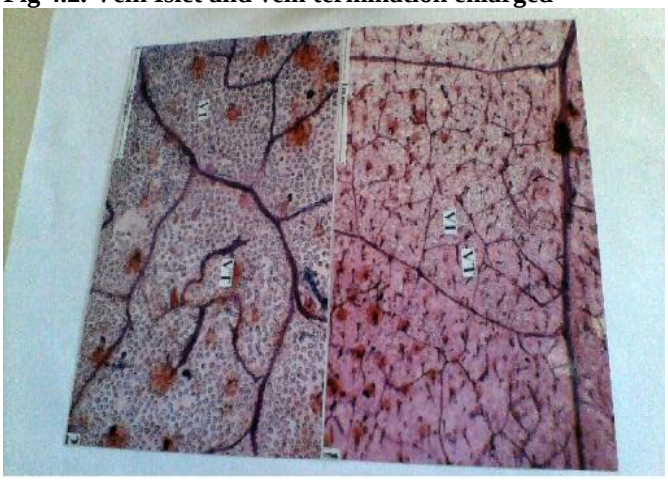
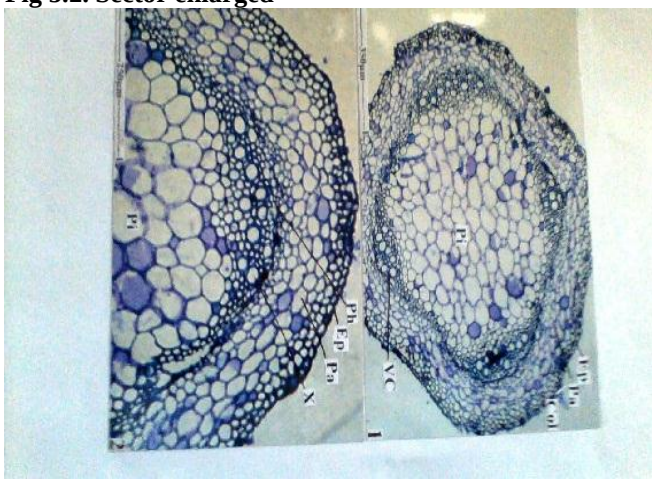


Fig 4.1. Venation pattern of the lamina
Fig 4.2. Vein Islet and vein termination enlarged



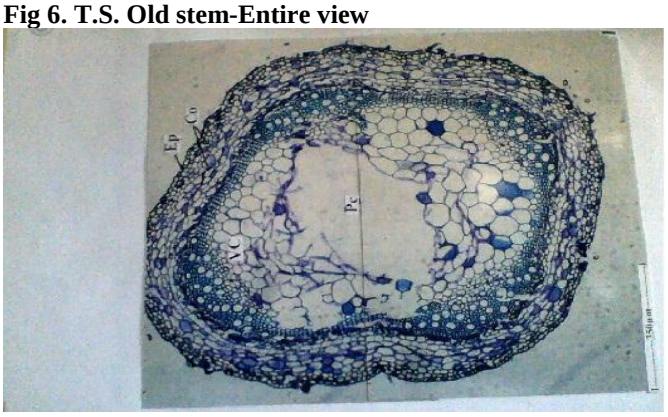
VI:Vein islet(reticulate)
VT:Vein termination(simple,unbranched)

Fig 5.1. T.S.Young stem-Entire view
Fig 5.2. Sector enlarged



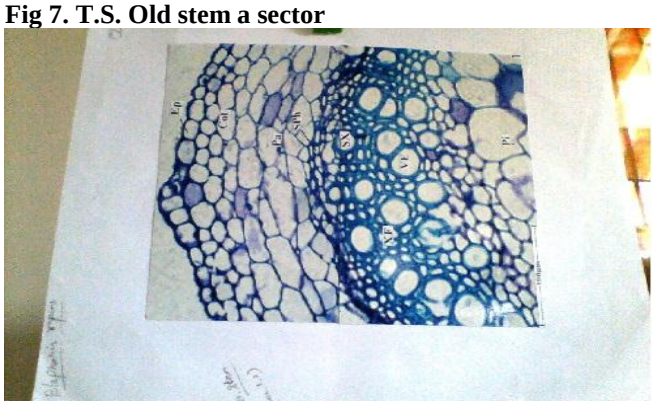
Pi:Pith, Ep:Epidermis cell, Pc:Parenchyma cells, Vc:Vascular cylinder, Ph:Phloem ,X:Xylem

Fig 6. T.S. Old stem-Entire view



Ep:Epidermal layer, Co:Cortex ,V c:Vascular cylinder, Pc: Pith cavity, Vc:Vascular cylinder

Fig 7. T.S. Old stem a sector



Ep:Epidermis cell, Col:Colenchyma, Pa:Parenchyma cells, Sph:Secondary phloem, Xf:Xylem fibres, Ve:Vessels elements, Sx:Secondary xylem

Fig 8.1. T.S. of Root-Entire view
Fig 8.2. T.S. of Root a sector
Fig 8.3. Secondary xylem of the root enlarged

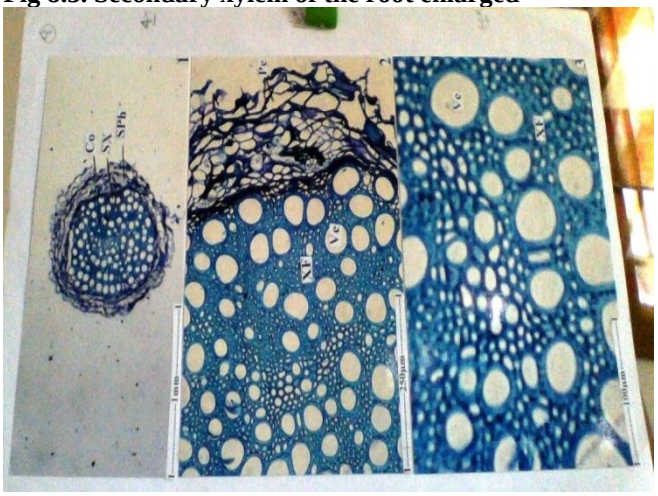


Fig 9.1. T.S. of secondary phloem of the root
Fig 9.2. Secondary phloem elements of root

Sph:Secondary phloem, Xf:Xylem fibres, Ve:Vessels elements, Sx:Secondary xylem

POWDER MICROSCOPY

Fig 10.1. Vessels, Fibres, Parenchyma
Fig 10.2.Vessel elements and parenchyma cells
Fig 10.3&4. Fibres and parenchyma cells

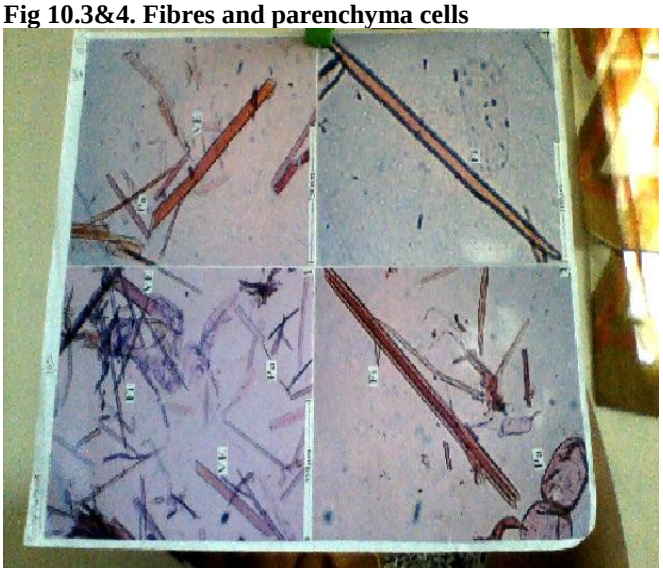
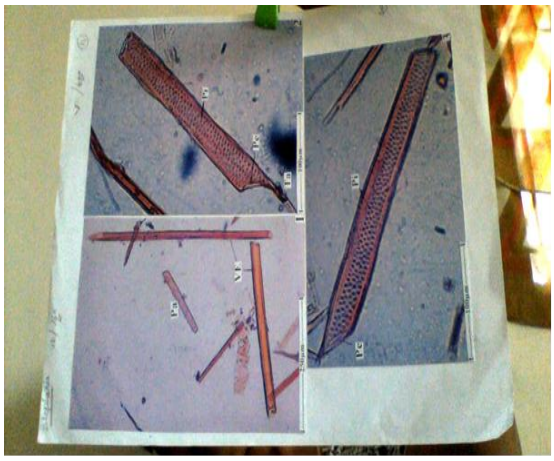


Fig.11.1.Vessel elements and parenchyma cells
Fig.11.2.Vessel elements with tail
Fig.11.3.Vessel elements with lateral wall pits



(Pa-Parenchyma,Pe-Perforation,Pi-Pits,Ta-Tail, Ve-Vessel elements)

RESULTS & DISCUSSION

The plant *Blepharis repens* (vahl) roth. Has been studied to compare give a report on pharmacognostical, preliminary phytochemical, made on it. The pharmacognostical studies made on the whole plant of *Blepharis repens* (vahl) roth. Like macroscopical characters and microscopical characters, behavior of crude drug powder with different chemical reagents, ash values, extractives, loss on drying, fluorescence analysis of crude powder foaming index give valuable information.

The preliminary phytochemical investigation showed the presence of alkaloids, flavonoids, saponin, glycosides, fixed oil and tannins in different extracts.

In macroscopical characters, it is observed that the leaves are green and flower are white colour, odour, taste.

In microscopical studies, it is observed that in plant, the midrib consists of a thin epidermal layer of small cubical, slightly papillate cells. The adaxial part is wide, thick and 450 micrometer wide. The xylem portion is wide, thick walled with 20micrometer in diameter

In transverse section of lamina, the lamina part is about 100micrometer thick. Both adaxial and abaxial epidermal cell are rectangular to squarish and thin walled.

In epidermal morphology, the epidermal cells are thick walled, highly wavy, the cells are appearing like amoeboid in nature. Stomata are diacytic type.

The stomata are densely distributed on epidermis cell. Two subsidiaries occur at opposite poles of the guard cells. The guard cells are 20x35 micrometer in size.

In venation pattern of *Blepharis repens* (vahl) roth. The veins are thin and straight. They form reticulate system of wide vein islets. The islets are variable in shape and size. the vein termination are either simple and un branched or mostly wavy in outline.

In both young and fairly old stem were studied. The young stem is slightly four angled. It is 950micrometer thick the stem has narrow epidermal layer of small spindle shaped, thick walled cells with cuticle. The cortical zone is about 100micrometer thick and includes 6 or 7 layers of angular, compact thin walled parenchyma cells. The phloem occurs in thick arcs on the outer part of the vascular cylinder. In old stem, consists of a thin epidermal layer spindle shaped cells with fairly thick. Two or three layers outer cortical cells of

In root transverse section, the root measuring 1 mm thick was studied. It consists of narrow zone of superficial periderm cell are dis organized and compressed. Secondary phloem, secondary xylem occupies wide major portion of root. It includes densely crowd, diffusely distributed wide, circular, thick walled vessels it includes xylem fibres and vessels.

They are up to 50micrometer wide.

The powder microscopical studies of the plant shows fragments of fibres, vessel elements, parenchyma cells. The fibres are libriform type and have thick lignified wall. The fibres are up to 250micrometer long and 10 micrometer thick. The vessel elements are narrow, long and cylindrical. They are 250-520 micrometer long. The parenchyma cells are long, narrow, cylindrical or spherical or ovoid. Elongated parenchyma cells are 150 micrometer long and 30micrometer in size.

Physicochemical properties are important parameters in detecting adulteration on improper handling

of the drug. In the evaluation of crude drug, ash value, extractive values are important parameters. The estimation of ash value is useful for detecting low-grade products, exhausted drugs and excess of sandy matter. The determination of extractive values with arrange of solvent gives information about extractable polar and non polar as well as total extractable plant constituents.

CONCLUSION

The pharmacognostical studies of the plant were carried out with a focus on bringing out diagnostic character will be of immense help in the proper identification and standardization of botanical species of the plant drug .which play a major role to establish the particulars standard and helps to minimize the adulteration of the plant *Blepharis repens* (vahl) roth. The whole plant ethanol extract shows the presence of constituents such as glycosides, flavonoids, tannins, fixed oil, saponins and alkaloids. The ethanol extract gives high percentage of yield. Hence, ethanol extract will be selected for pharmacological evaluation.

ACKNOWLEDGEMENTS

The authors are very much thankful to Dr. JKK Munirajah, M. Tech Bolton), D. Lit. Chairman, Dr. N. Senthilkumar, Dean and Principal, JKK Munirajaha Medical Research Foundation College of Pharmacy, B.Komarapalayam, TN, for providing the necessary facilities for carrying out this research work.

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