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Research Article

Pharmacognostic and Physico-Chemical Investigation of Hemionitis Arifolia (Burm.) Moore.

Jeethu Joy*, Bindu A R

University College of Pharmacy, Mahatma Gandhi University, Cheruvandoor, Ettumanoor-686631, Kottayam, Kerala, India

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ABSTRACT

Hemionitis arifolia (Family-Adiantaceae)is an attractive and unusual dwarf fern.It is used folkloric to treat ear ache, migraine, haemorrhoids, arthritis, intestinal worms and wounds.It has been medically evaluated for its hypoglycaemic and anti-diabeticproperties in rats.The present investigation deals with macroscopic and microscopic evaluation of the leaf material and establishment of its quality parameters, including physicochemical and phytochemical evaluation.Macroscopy revealed lamina as dimorphous, simple; sterile fronds deeply cordate, margin entire, apex rounded; lower surface light green with scattered, appressed scales and hairs, densely along the midrib sparsely on surface. In the microscopic studies, the lamina is isobilateral and has no distinction of adaxial and abaxial sides.The vascular strand consists of a wide, bow shaped row of xylem elements; the two margins of the xylem strand are thin; the middle part being thick. Phloem occurs in thin band both on the lower and upper portions of the xylem arc. Chief characters of powder include epidermal cells, elliptical stomata, multicellular, uniseriate trichomes, xylem elements. Physicochemical parameters such as moisture content, chlorophyll estimation, fluorescence analysis, ash values and extractive values were done. Phytochemical screening revealed the presence of many therapeutically important classes of phytoconstituents such as glycosides, phenolics, flavonoids, carbohydrate, terpenoids, sterols and saponins. Thus this study can serve as a valuable source of information and provide suitable standards for identification of this plant material in future investigations and applications.

Keywords: pharmacognostic, Hemionitis, Fern

INTRODUCTION

We in India are fortunate to have systems of medicines which date back more than 3000 years and have deep rooted social acceptance. There has been a resurgence of interest on plants and plant derived products as a source of medicine in the last few decades. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained. When compared with economically valuable higher plants, pharmacognostical and phytochemical studies on pteridophytes and angiosperms have been unfortunately ignored[1]. Number of Pteridophytic species having potential active compound and elaborative future prospects have been determined of immense economic value for the benefit of human society. *Hemionitis arifolia* syn. *Parahemionitis cordata* (Family – Adiantaceae), is also known as Elicheviyan or Mathilpanna (Malayalam) is an attractive fern with dark green leaves and can be cultivated in ports. A small terrestrial rhizomatous fern, rhizomes short 5-10cm long, sub erect covered with persistent leaf bases, scales lanceolate acuminate, leaves dimorphic sterile ones deeply cordate,fertile ones sagittate, stiped, shining green, coriaceous reticulately veined, sori covering entire surface[2]. Tribally fronds juice used in burns, Root is mixed with turmeric ground into paste and applied over the affected places to cure cuts, wounds, body pains and swellings[3]. It is used in menstrual disorder[4]. Fronds are used as antifertility and antiflatulence agent. Rhizome used as antibacterial agent. It pacifies vitiated vata, pitta, earache, intestinal worms, haemorrhoids, arthritis, in fever& cold in dogs.Its antidiabetic property has been scientifically proved[5]. The objective of present study is to evaluate various pharmacognostical parameters such as macroscopy, microscopy, physicochemical, fluorescence and phytochemical studies of the plant.This thorough evaluation would be useful in standardization of the leaf material.

MATERIALS AND METHODS

Plant material collection and authentication

H.arifolia was collected from Kottayam in the month of September and authentified by the botanist, Mr. Joby Paul, School of Environmental sciences, M.G University, Kottayam. A voucher specimen (SES.M.G.UTY NO.

Table 1: Morphology.

Properties	Observation
Colour	Upper surface-dark green lower surface-light green
Odour	Characteristic
Taste	Bland

*Author for Correspondence: jeethujoy7@gmail.com


Figure 1: *Hemionitis arifolia* leaves –upper and lower surface.

Table 2: Physicochemical screening.

Parameters	Result
Total ash	6.03±0.03%w/w
Acid insoluble ash	1.11±0.05%w/w
Water soluble ash	3.99±0.14%w/w
Water soluble extractive value	19.56±0.24%w/w
Alcohol soluble extractive value	6.42±0.11%w/w
Chlorophyll a	7.74 µg/ml
Chlorophyll b	3.09 µg/ml.
Total chlorophyll	10.805 µg/ml.
Total carotenoids	2.437µg/ml

Table 3: Percentage yield of the extracts.

Extracts	Percentage yield on dry weight basis (%W/W)
Total Ethanolic Extract	15%
Pet.Ether extract	6.54%
Chloroform extract	2.88%
Ethyl acetate extract	1.23%
Aqueous extract	19.53%

Table 4: Fluorescence analysis of leaf powder of *H.arifolia*..

Treatment	Colour of powder under UV(365nm)
Powder as such	Light green
Powder + Picric acid	Dark brown
Powder+ 1N HCl	Dark brown
Powder+1N NaOH	Pale green
Powder+1N NaOH in methanol	Dark green
Powder+50% HNO_3	Pale green
Powder+50%H_2SO_4	Dark green
Powder+$FeCl_3$	Dark violet
Powder+ Iodine solution	Faint Black

1507) is preserved at the Herbarium of School of Environmental science, M.G University, Kottayam for future references. The fresh leaves were used for the study of macroscopic and microscopic characters, whereas the dried leaf powder was used for the extraction of active constituents and phytochemical investigation.

Pharmacognostic studies

Macroscopic studies

Morphological studies were done using simple microscope. The shape, apex, base, margin, taste and odour of leaves were determined.

Microscopic studies: For microscopical study the leaf samples were cut and fixed in FAA (formalin-5ml+ acetic acid-5ml+705 ethyl alcohol-90ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary butyl alcohol[6]. Infiltration of the specimen was carried out by gradual addition of paraffin wax (melting point-58-60^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was by customary procedure 80. The sections were stained with toluidine blue[7]. The dye imparted pink color to the cellulose walls, blue to the lignified cells, dark green to the suberin, violet to the mucilage, blue to the protein bodies etc.

Powder microscopy: Powdered materials of different parts were cleared with 5% sodium hydroxide and mounted in glycerine medium after staining. Different cell components were studied and measured. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was used. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars.

Physiochemical studies: The moisture content, total ash, water- soluble ash, acid- insoluble ash, alcohol and water soluble extractive values were determined as a part of its physiochemical parameters[8]. The chlorophyll estimation was also carried out on fresh leaves[9].

Fluorescence analysis: Powdered leaf parts were subjected to analysis under day/visible light and ultra violet light after treatment with various chemical as a part of Fluorescence analysis[10].

Phytochemical studies: Fresh leaves were collected and shade dried at room temperature to remove moisture, and size reduced. Extraction of the shade dried leaves of

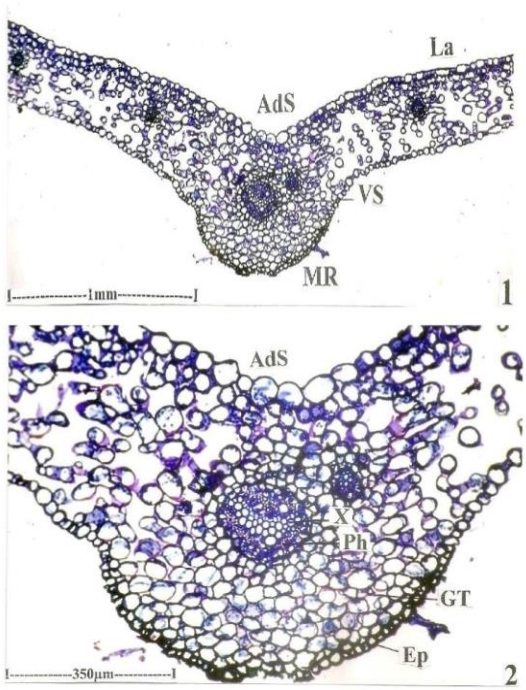

Figure 2: 1.Transverse section of Leaf. 2. Transverse section of Midrib.

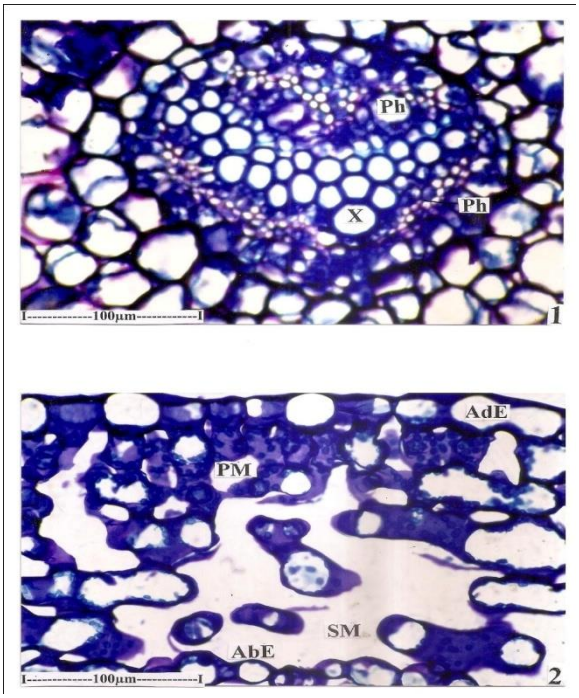

Figure 3: 1. Vascular strand of the Midrib. 2. Transverse section of Lamina.

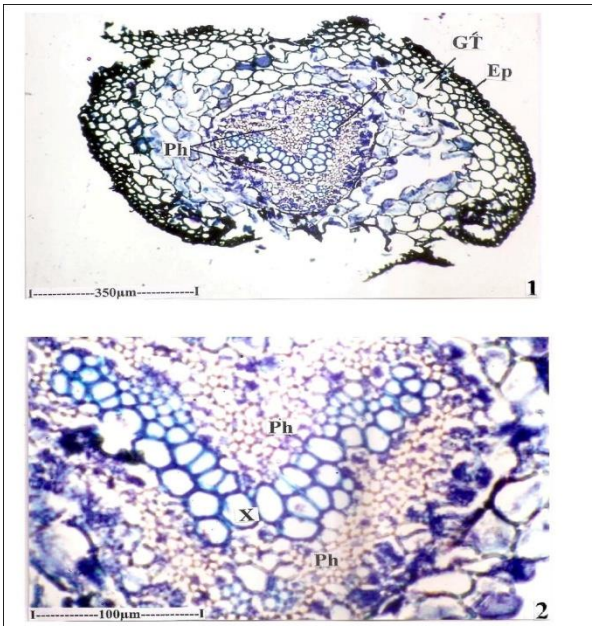

Figure 4: 1. Transverse section of Radius. 2. Stele of the Radius.

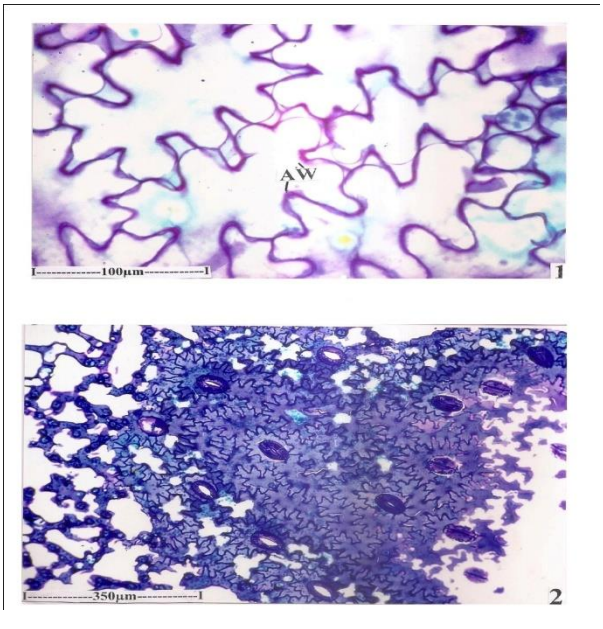

Figure 5: 1. Paradermal section- Adaxial Epidermis. 2. Paradermal section- Abaxial Epidermis.

Hemionitis arifolia was carried out by soxhlation using ethanol, thus total ethanolic extract is obtained. This total ethanolic extract is fractionated using solvents in the increasing order of polarity, petroleum ether, chloroform, ethyl acetate. Aqueous extract is also prepares by boiling with water.The extract obtained was collected and concentrated. The concentrated extract was then weighed and stored for further studies. The percentage yield of the extracts were calculated and tabulated. Qualitative chemical tests were carried out in various extracts[11,12].

RESULT

Pharmacognostic studies

Macroscopic studies

Lamina dimorphous, simple; sterile fronds up to 8×5.7cm deeply cordate, margin entire, apex rounded; lower surface light green with scattered, appressed scales and hairs, densely along the midrib sparsely on surface, venation reticulate with basal half of the midrib feebly grooved in the basal half of the midrib protruded on the lower surface, feebly grooved in the basal half on the upper, bearing a pair of prominent branches at the extreme base, fertile lamina sagittate, apex obtuse or acute, base forming an inverted 'V'; texture coriaceous; costule dark and raised

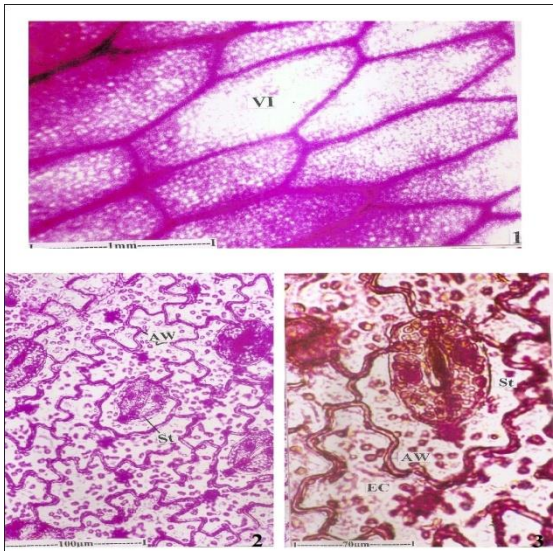

Figure 6: 1. Venation of the lamina. 2. Stomal type and Epidermal cells. 3. One stoma- Enlarged.

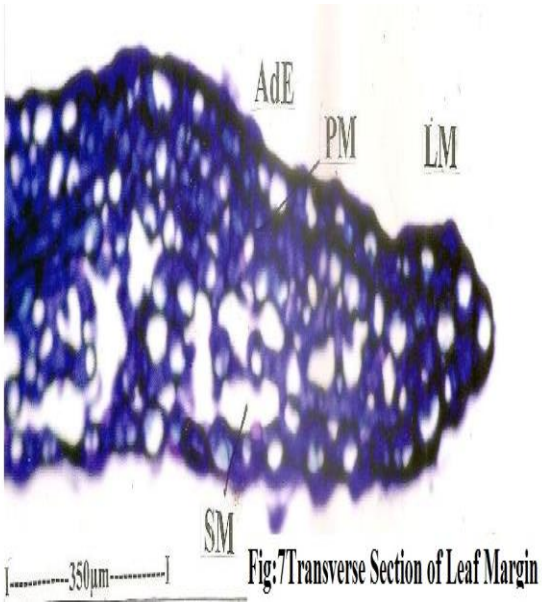

Figure 7: Transverse section of leaf Midrib.

Figure 8: Uniseriate Unbranched Trichomes.

Figure 9: 1. Scales- Multi seriate Type. 2. Scales -Bi seriate Type.

below , grooved and concolorous with lamina above, veins obscure, anastomoasing closely, areoles seen as depressions in dry fronds (Fig: 1). Sori continuous along the veins, filling the entire surface of lamina when mature, intermixed with copious hairs and scales. Rhizome erect or sub erect, unbranched, 1-2 cm long, 2-4mm thick; scales lanceolate, acuminate, entire, gland –tipped, up to 2.5× 0.5mm, dark brown. Stipes tufted, sterile ones up to 19cm long, fertile up to 31cm long, 1.5mm thick, faintly grooved on the abaxial surface, dark, glossy with short scales at base, long deciduous scales and multiseriate spreading hairs higher up.The leaves of *Hemionitis arifolia* were found to have characteristic odour and bland taste (Table: 1).

Microscopic studies

In the microscopic studies, the leaf was found to be isobilateral, and shows all the typical characteristics of leaf,

T.S of leaf through Midrib: The leaf exhibits prominent midrib and uniformly thick lamina (Fig: 2.1) the midrib has shallow depression on the adaxial side and fairly thick semicircular midrib on the abaxial side .The midrib is 600µm thick and 850µm wide. The adaxial epidermal layer of the midrib is made up of dilated circular and thin walled cells. The abaxial epidermis consists of small, hemispherical, thick walled cells (Fig: 2.2).The ground tissue is homogenous, parenchymatous and less compact. The vascular strand is single, more or less circular and centrally placed. It is 250µm in horizontal plane. The vascular strand consists of a wide, bow shaped row of xylem elements; the two margins of the xylem strand are

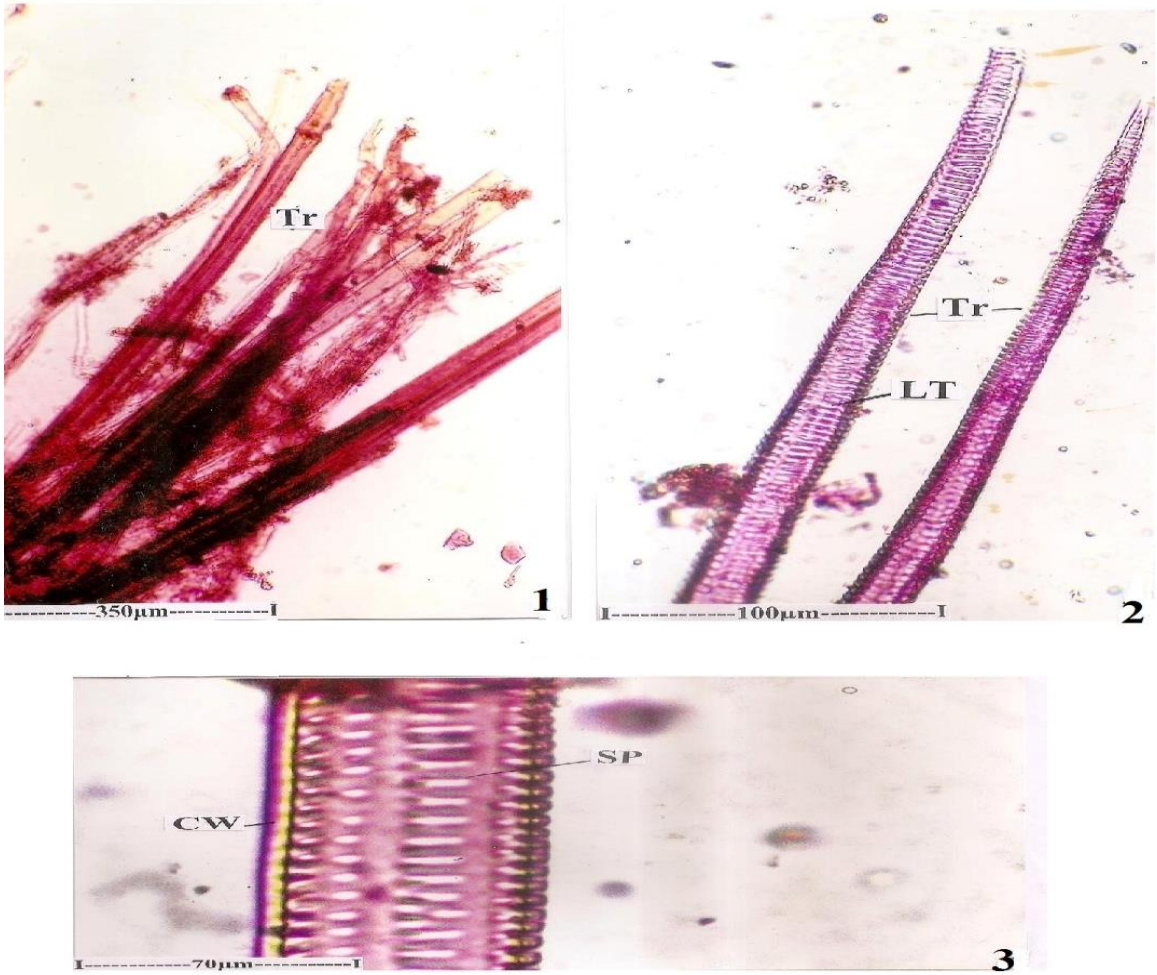

Figure 10: 1. Bundle of xylem elements. 2.two tracheids. 3. Scalariform pits.

thin; the middle part being thick. Xylem elements are tracheids; they are angular, thick walled and wide (Fig: 2.2; 3.1); they measure up to 20µm in diameter .Phloem occurs in thin band both on the lower and upper portions of the xylem arc.

Lamina (Fig: 3.2): The lamina is isobilateral and has no distinction of adaxial and abaxial sides. The upper epidermis is thicker with wide circular or rectangular cells. The lower epidermis is width cells are circular to cylindrical. The mesophyll tissue is undifferentiated into palisade and spongy parenchyma. It consists of five or six layers of large, lobed loosely arranged cells.

Leaf margin (Fig: 7)

The marginal part of the lamina is conical and is 200µm of thick walled cells. The mesophyll is similar to the middle part of the lamina and lacks the palisade spongy parenchyma differentiation. The epidermal cells and stomata were studied from the paradermal sections. The adaxial epidermis is apostomatic (lacking stomata); the epidermis consists of large highly lobed thin walled cells (Fig: 5.1). The abaxial epidermis is densely stomatiferous. The epidermal cells are large and deeply locked (Fig: 5.2); they appear stellate in outline in surface view. The stomata are large, elliptical with wide stomatal pore (Fig: 6.2, 3).The stomata are said to the hanging type; they are attached at one and with the anticlinal wall of the epidermal cell .The guard cells are 50×70µm.

Radius (Fig: 4.1, 2)

The radius is semicircular in sectional view with shallow concavity on the adaxial side. The epidermis consists of small, thick walled and echinate on the surface.v Inner to the epidermis is a narrow zone of two layers of thick walled cells. The remaining portion of the ground tissue consists of circular compact thin walled cells. The vascular strand is a solitary meristele. It is similar to the vascular strand of the midrib of the lamina. The xylem strand is V-shaped with divergent arms .The xylem elements (tracheids) are wide, angular, thick walled and compact (Fig: 4.2).They are 20µm wide. Phloem occurs in thick bands on the lower and upper portions of the xylem strand.

Powder Microscopy

Fragments of Lamina are often seen in the powder. The fragments of lamina exhibits the venation pattern .The venation is reticulate; the veins are straight and thick .Vein-islets are uniformly hexagonal with four long sides and two short ends. The islets are parallel to each other. The vein-terminations are totally lacking. Fragments of the epidermal peeling shows small pieces of epidermis are occasionally seen in powder. The peelings consist of epidermal cells and stomata. The epidermal trichomes seen

Table 5: Preliminary phytochemical screening of Extracts.

Phytoconstituents	Pet. Ether Extract (PEE)	Chloroform extract(CE)	Ethyl acetate extract(EAE)	Total Ethanolic Extract(TEE)	Aqueous extract(AE)
Alkaloids	-	-	-	-	-
Glycosides	-	+	-	-	+
Phenolics	-	-	+++	++	+
Flavones and Flavonoids	-	-	++	++	+
Carbohydrates	-			+	+
Proteins and aminoacids	-	-	-	-	-
Terpenoids	++	+	++	++	-
Sterols	++	+	++	++	-
Saponins	-	-	-	-	+++

in abundance on the marginal portion of the lamina. The trichomes are two or three celled, uni seriate and unbranched .Some of the trichomes have somewhat dilated, bottle shaped terminal cell; others have gradually tapering pointed terminal cell and others may have cylindrical terminal cell (Fig.8), the trichomes have protoplast. They are 280-600µm long.

Scales (Fig.9.1, 2)

These are long, multicellular, uniseriate, unbranched scales with dilated basal cell (Fig: 9.1) .A second type of scales includes partly bi seriate (Fig: 9.2).The cells of the trichomes are elongated narrow and thick walled. The basal part is wider and the scale becomes gradually narrow into slender tapering tip. The scales are 1.6-2.6mm long and 10-20µm wide at the base.

Xylem elements (Fig.10 1, 2, 3)

Macerated xylem elements are seen in loose bundle and isolated elements. The isolated xylem elements are tracheids .The tracheids are long, narrow thick walled cells without end wall perforations. So, the tracheids are called imperforate tracheary elements. The end walls are tapering and pointed (Plate No.9.2) scalariform pits. The pits may be horizontally recta ngular or elliptical and are arranged one below the other in the form ladder or scalariform (Plate No.9.3).The tracheids are 20µm thick.

Physicochemical Evaluation

Moisture content (Loss on drying): Moisture content of *H.arifolia* leaf powder was found to be 3.39±0.4% w/w. Ash values, extractive values, and chlorophyll content of the drug were studied and tabulated in table: 3.Percentage yield of the extracts were tabulated in table :4 flourescence studies were depicted in table: 5

Preliminary phytochemical evaluation

Qualitative chemical tests were carried out in various extracts (Petroleum ether (PEE), Chloroform (CHE), Ethyl acetate (EAE), Total ethanolic extract (TEE) and Aqueous (AQE) extracts. The results of the chemical tests for each extract are tabulated in the following table: 5. (++) indicate active constituents in high amount, (+) indicate active constituents in low amount, (-) indicates the absence of active constituents

DISCUSSION

The pteridophytes which dominated the earth during carboniferous are survived today by about 12,000 species comprising 305 genera. Even to this day the primitive tribal societies that exist depend on the plant life in their surroundings. When compared with higher plants, pharmacognostical and phytochemical studies on pteridophytes have been unfortunately ignored**.** So it becomes necessary to study the pharmacognostic characteristic of the ptredophytes before its use in the field of research and also in pharmaceutical formulation. Moreover it also helps in distinction from other allied species and adulterants. In this connection, the pharmacognostical characteristics of the entire plant H. arifolia (Burm) moore. were done.

The present studies revealed that pharmacognostic screening can serve as a basis for preparation of the herbal monograph for proper identification, authentification and standardization of drugs. The present study on H. arifolia will help to identify the correct species of the plant, since no such scientific data are available. The qualitative and quantitative analysis of various extracts of H. arifolia were carried out and extracts showed the presence of bioactive components such as phenolics, flavonoids, saponins, tannins.This shows high level of its possible medicinal value. Ethyl acetate and Total ethanolic extracts showed the presence of most of these photochemical, possessing anti-inflammatory, analgesic and related activities.

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