INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

PHARMACOGNOSTICAL STUDIES ON LEAVES OF *MARSILEA MINUTA* LINN.

S.Madhu[1]*, V. Kannabirran[2], S.Murugesan[3], M.Charli[3], K.Nithiyaa[3]
[1]Department of Pharmacology, Surya Group of Institutions, School of Pharmacy, Vikravandi, Villupuram, Tamil Nadu, India
[2]Department of Pharmaceutics, Surya Group of Institutions, School of Pharmacy, Vikravandi, Villupuram, Tamil Nadu, India
[3]Department of Pharmacy, Surya Group of Institutions, School of Pharmacy, Vikravandi, Villupuram, Tamil Nadu, India
*Corresponding Author Email: madhupharm2006@gmail.com

Article Received on: 25/01/14 Revised on: 04/02/14 Approved for publication: 07/03/14

DOI: 10.7897/2230-8407.050346

ABSTRACT
Plants have been the basis of many traditional medicines throughout the world for thousands of years and continue to provide new remedies to mankind and one of the richest sources of many bioactive compounds. *Marsilea minuta* Linn (Marsileaceae) found at the edges of ponds and irrigation channels and as a weed in wet rice fields and it is found throughout India. The usefulness of this plant is described in many folk books including Ayurvedic Pharmacopeia. But no reports are available on morph anatomy due to this importance *Marsilea minuta* (L) leaves was analyzed for pharmacognostical studies. The study reveals the leaves with large petiole terminating in to four leaflets, leaflets obovate with entire or dentate margins. The transverse section of leaves shows presence of epidermis, spongy parenchyma cells and a cluster of wide, angular, thick, walled xylem elements and small nest of phloem elements, vascular strand is placed in the median part of the lamina, spongy and palisade mesophyll tissue and paracytic stomata. Physico-chemical parameters such as moisture content, total ash, acid insoluble ash, water soluble ash, sulphated ash and alcohol soluble extractive value, water soluble extractive value are presented. The results of the study could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the plant.
Keywords: *Marsilea minuta* (L), Pharmacognostical studies, Physico-chemical, Morph anatomy

INTRODUCTION

Indian sub-continent is endowed with numerous flora and fauna, which are used for the treatment of various ailments because of their medicinal properties. In spite of spectacular advancements in modern medicine, sizable rural populations of India depend and rely on traditional medicines made from plants and animals. Last decade mark the uprising of branch as the quest for biologically active compounds from natural source had increased its pace. Higher plants have played a vital role as a source of therapeutic agents. Pharmacognostical study is the primary steps in standardization of crude drugs. The complete pharmacognostical evaluation gives valuable information regarding the morphology, microscopically and physical characteristics of the crude drugs. Pharmacognostic studies have been done in many crude drugs and the resulting observations have been incorporated in various official monographs. There are a number of crude drugs where the plant source has not yet been systematically identified. Hence pharmacognostic study gives the systematic information regarding the purity and quality of the crude drugs. *Marsilea minuta* (L) (Marsileaceae) common plant and widely distributed in India, throughout Africa, Madagascar and Comoros. In India it usually grows as a weed in wet rice fields and flooded low lands. Traditionally *Marsilea minuta* (L) is used to stop nose bleeding, used in treatment diarrhoea, bronchitis, diabetes, epilepsy, hepatitis, kidney infection, blood purifier and treatment of piles. The extracts and isolates of *Marsilea minuta* (L) were reported to possess antibacterial activity, anti-inflammatory and analgesic activities, hypocholesterolemic activity, anti anxiety activity[1-9]. Pharmacognostical studies have not been reported for the leaves part of this plant. Therefore the main aim of the present study Pharmacognostical investigation such as organoleptic, morphologic, microscopic and physico-chemical parameters of leaves of *Marsilea minuta* (L) which could be used to prepare a monograph for the proper identification of the plant.

MATERIALS AND METHODS

Collection and Authentication of plant

For the present work the leaves of *Marsilea minuta* (L) was collected from Tamarai Lake, Tiruvannamalai, India. The plant was identified and authenticated by Prof. P. Jayaraman, Plant anatomy research centre, Chennai, India who authenticated the plant from available literature and the voucher specimen no. is PARC/2011/ 865. The leaves of *Marsilea minuta* (L) different organs were cut and fixed in FAA solution (Formalin 5 ml + Acetic acid 5 ml + 70 % Ethyl alcohol 90 ml). After 24 hours of fixing, the specimens were dehydrated with grader series of tertiary butyl alcohol[10]. Infiltration of the specimens was done by gradual addition of paraffin wax (melting point 58-60^{0}C) until super saturation of TBA solution attained. The specimens were cast into paraffin blocks.

Pharmacognostical Studies

Morphological studies were done using simple microscope. The color, odor, size, shape, taste and special features like touch, texture, apex and margin were examined. The macroscopically characters such as color of untreated leaves were examined under a diffuse day light and artificial light source with wavelength similar to those of day light may be used. Odor was examined by taking a small piece of leaf in the palm of hand and also pieces of it slowly and repeatedly inhaled the air over the leaf also crushed the leaf between the thumb and index figure. Size and shape was examined by placing the leaf of various size and shape on graph paper and trace the outline of leaf[10].

Microscopy

Sectioning

The paraffin embedded specimens were sectioned by rotary microtome with the thickness of 10-12 μm. De waxing of the section was customary procedure[11]. The section was stained with toluidine blue[12]. Since toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were obtained. The dye stained pink color to the cellulose walls, blue to the lignified cells and protein bodies, dark green to suberin, violet to the mucilage etc. where ever necessary sections were also stained with safranin and fast green and IKI (for starch).

Photo micrographs

Microscopic descriptions of tissues are supplemented with micrographs where ever necessary. Photographs of different magnifications were taken with the help of Nikon lab photo 2 Microscopic unit. For focusing of normal observations bright field was used and for the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light the observations appears bright against dark background. Magnifications of figures are indicated by the scale-bars and the anatomical feature is as given in the standard plant anatomy books[13,14].

Physico-chemical Parameters

The determination of various physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, water soluble extractive value and alcohol soluble extractive values were determined by employing standard methods as given in Ayurveda Pharmacopoeia of India[15,16].

Total Ash value

The total ash was determined by incinerating 2-3 g of accurately weighed air dried coarsely powdered drug in a tarred silica crucible at a temperature not exceeding 450^0C, which was previously ignited and cooled before weighing. The ignition was repeated and the percentage of ash with reference to air-dried drug was calculated.

Water soluble ash

The total ash was boiled for 5 minutes with 25 ml of water. The residue was washed with hot water, ignited for 15 minutes at a temperature not exceeding 450^0C, cooled and weighed. This weight was subtracted from the weight of ash; the difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to air-dried drug.

Acid insoluble ash

The ash obtained was boiled with 25 ml of dilute hydrochloric acid for 5 minutes and filtered through an ash less filter paper. The residue was washed with hot water, ignited, cooled in a dessiccator and weighed. The percentage of acid insoluble ash was calculated with reference to air dried drug.

Sulphated ash

The sulphated ash was determined by incinerating 1 g of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing at a temperature not exceeding 450^0C. The residue was moistened with 1 ml of concentrated sulphuric acid, ignited at 800 ± 25^0C until all black particles are disappeared. It was then cooled again sulphuric acid was added and ignited. It was cooled and the percentage of sulphated ash was calculated with reference to air dried drug.

Moisture content

5 g of accurately weighed powered sample was kept in IR moisture balance. The loss in weight was recorded as percentage (%) moisture with respect to air dried sample of crude drug[17].

Extractive values

Ethanol soluble extractive value

5 g of dried coarse powder of leaves of *Marsilea minuta* (L) was macerated with 100 ml of 90 % ethanol in a closed flask for 24 h, shaken frequently during 6 hours and allowed to stand for 18 h. Filtered immediately taking precautions against loss of ethanol. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish. The residue was dried at 105^0C and weighed. The percentage of ethanol soluble extractive was calculated with reference to air dried drug.

Water soluble extractive value

5 g of coarse powder of leaves of *Marsilea minuta* (L) was weighed and dissolved in 100 ml of water in a stoppered flask, heated at 80^0C, shaken well and allowed to stand for 10 minutes. It was cooled; 2 g of kieselghur was added and filtered. 5 ml of the filtrate was transferred to a tarred evaporating dish and the solvent was evaporated on a water bath. The percentage of water soluble extractive was calculated with reference to air dried drug.

Table 1: Organoleptic Characters of Leaf of *Marsilea minuta* (L)

Parameters	Organoleptic Parameters
Color	Upper surface green and lower is pale green
Odor	Odor less
Taste	Characteristic
Texture	Thin
Touch	Smooth

Table 2: Macro-morphological Characters of Leaf of *Marsilea minuta* (L)

Parameters	Morphology
Length	2.1 to 2.3 cm
Breadth	1.9 to 2.1 cm
Composition	Compound
Apex	Rounded
Venation	Dichotomously branched veins
Margin	Entire or Dentate margins
Leaf shape	Obovate
Petiole	Large petiole
Sheath	Present

Table 3: Physico-chemical Constituents of Leaves of *Marsilea minuta* (L)

S.NO.	Parameter	Value determined in (% w/w)
1	Alcohol soluble extractive value	13.3
2	Water soluble extractive value	14.4
3	Total ash	5.6
4	Acid insoluble ash	7.6
5	Water soluble ash	5.3
6	Sulphated ash	10
7	Moisture content	1.7

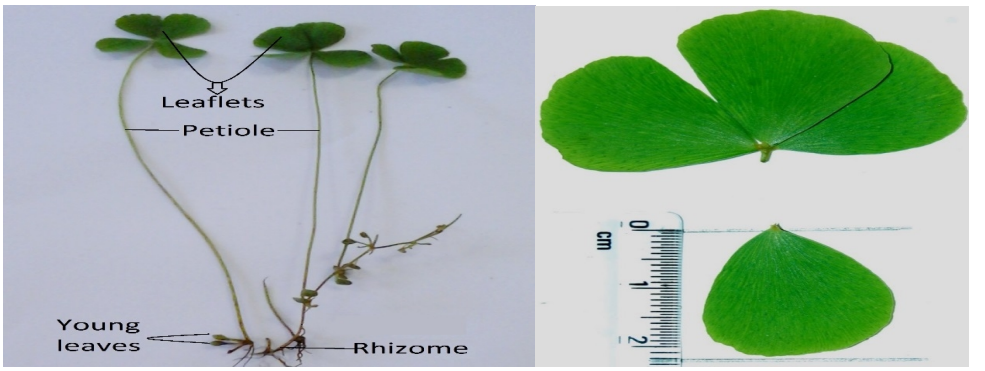

Figure 1: Habitat and Morphology of leaves of *Marsilea minuta* (L)

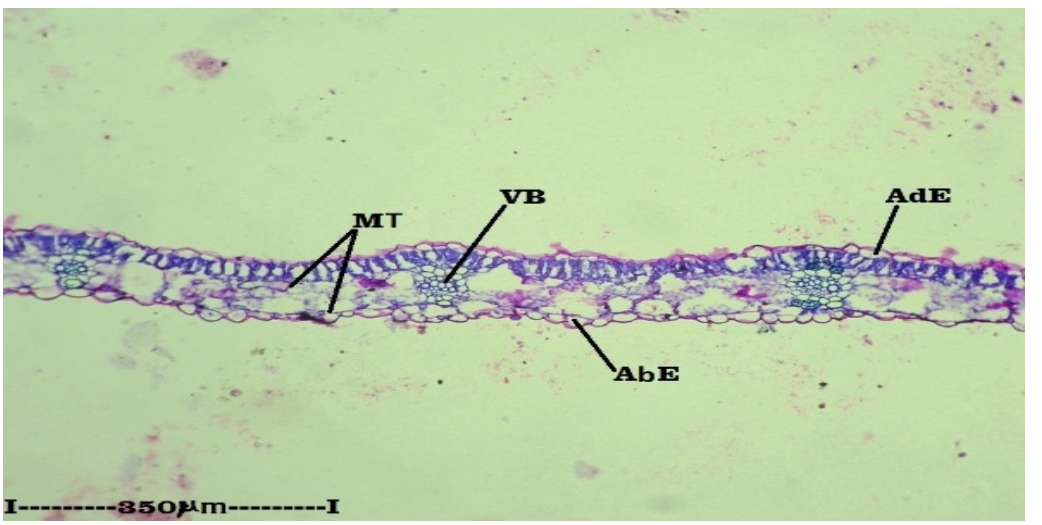

Figure 2: T.S of leaf-middle portion: MT: Mesophyll tissue, VB: Vascular bundle, AbE: Abaxial Epidermis, AdE: Adaxial Epidermis

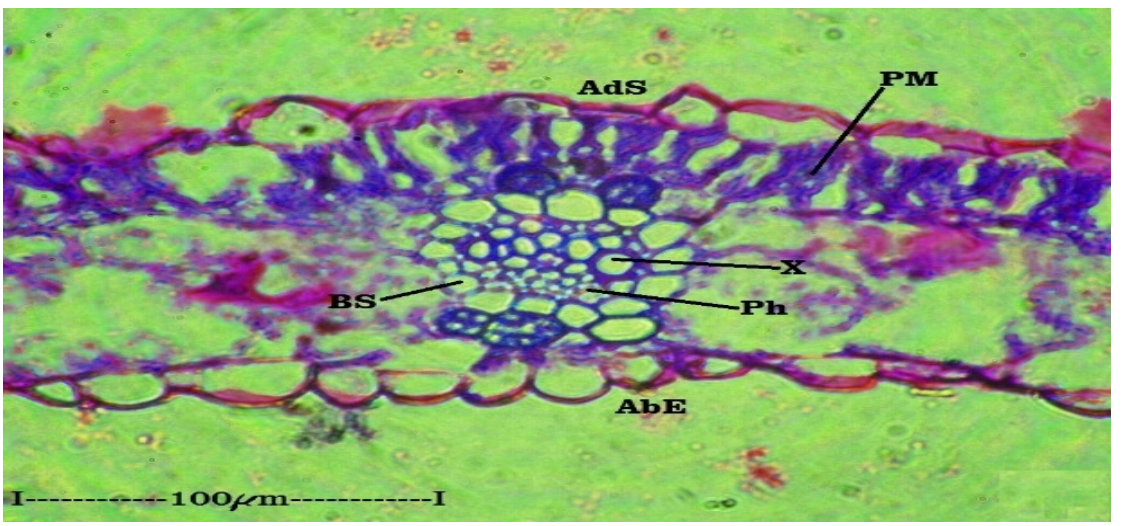

Figure 3: T.S of lamina with vascular sheath enlarged; AbE: Abaxial epidermis, AdE: Adaxial epidermis, PM: Palisade mesophyll, BS: Bundle sheath cells, X: Xylem, Ph: Phloem

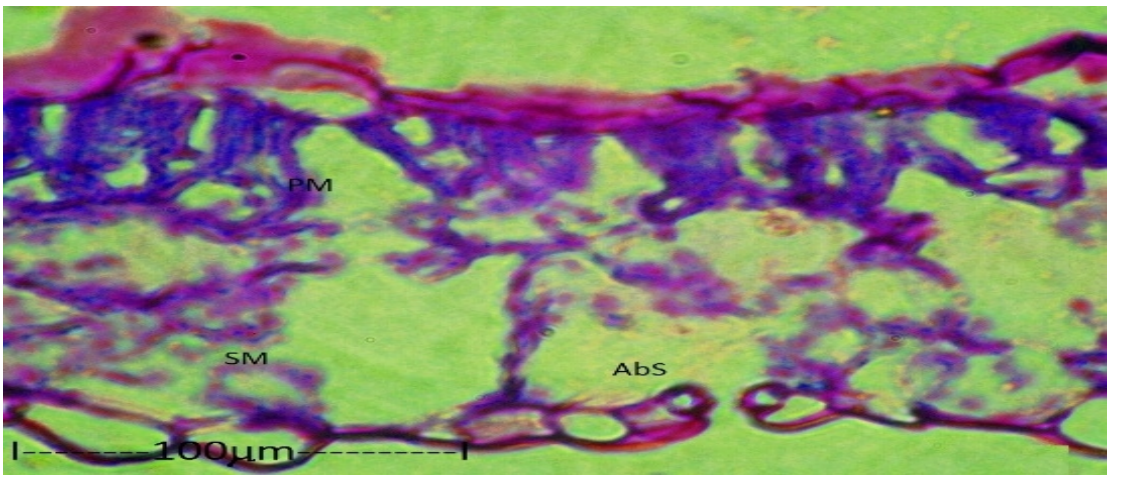

Figure 4: T.S of lamina with abaxial stomata; PM: Palisade mesophyll, SM: Spongy mesophyll, Abs: Abaxial stomata

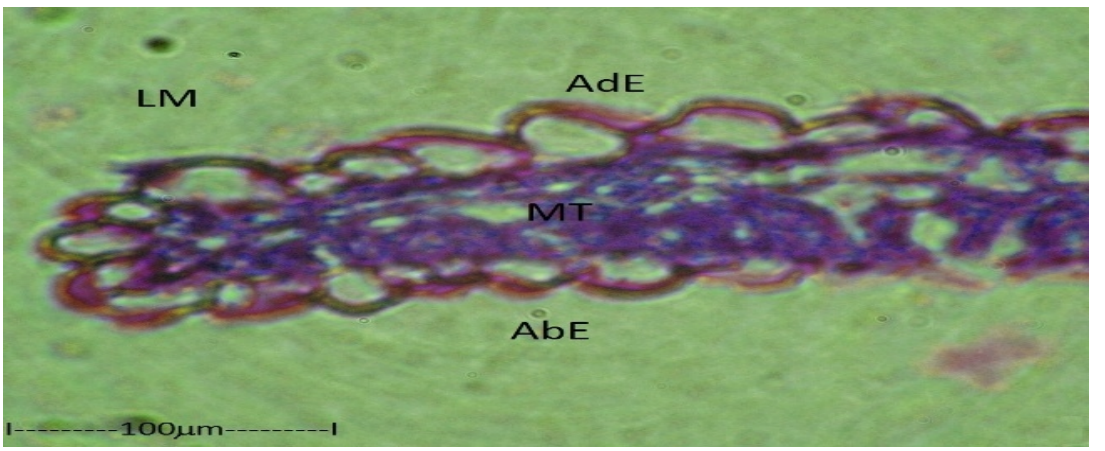

Figure 5: T.S of marginal part of the leaf; LM: Leaf margin, AbE: Abaxial epidermis, AdE: Adaxial epidermis, MT: Mesophyll tissue

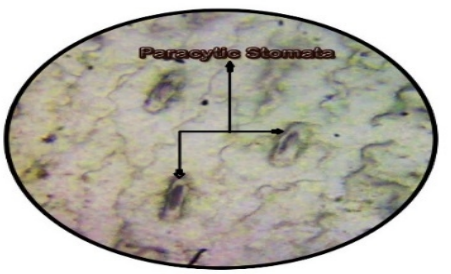

Figure 6: T.S of leaf shows paracytic stomata

RESULT AND DISCUSSION

Present study was focused on characterization, anatomical parameters of *Marsilea minuta* Linn.

Morphological Characters

Macroscopically, the fresh leaves of *Marsilea minuta* (L) is 2.1 to 2.3 cm long, 1.9 to 2.1 cm wide and petiole 24 to 27 cm in length, long-petioled and the leaf of *Marsilea minuta* (L) is pinnately compound, with four pinnules; two pinnules are noticeably higher than the other two and are inserted in alternate fashion, leaflets are obovate, elliptical or wedge-shaped, with entire or dentate margin, many dichotomously branched veins and leaves are green in color. (Figure 1, Table 1 and 2)

Microscopical Characters

Transverse section of leaves of *Marsilea minuta* (L) showed slightly modulated with alternating thick and thin portions. The thick portion contains the vascular bundle (Figure 2). The leaf is 120 µm thick along the veins and 80 µm along the portion in between the veins. The vascular strands are arranged in a horizontal row with equidistance between the strands (Figure 2). The leaf is distinctly dorsiventral hydromorphic and amphistomatic. The lamina has wide, thin

walled tubular cells along the adaxial epidermis; the cells are 15 µm thick. The abaxial epidermis is more prominent with semi-circular cells, the outstangentialwalls of the cells are prominently projecting outward (Figure 3). The abaxial epidermis is 20 µm thick (Figure 3). The vascular strand of the leaf is prominently circular. It is 60 µm in diameter. It consists of a circle of wide parenchymatous bundle sheath, a cluster of wide, angular, thick, walled xylem elements and small nest of phloem elements. The vascular strand is placed in the median part of the lamina, beneath the adaxial palisade zone (Figure 3). The xylem elements are 10 µm wide.

Lamina

The portion in between the vascular strands is the lamina region. It consists of adaxial and abaxial epidermal layers of thin and cylindrical cells or elliptical cells. Paracytic stomata occur on both adaxial and abaxial sides (Figure 4, 6). The mesophyll tissue consists of adaxial, single horizontal row of palisade cells and abaxial zone of three or four spongy parenchyma cells. The palisade cells are less compact and are 20 µm in height. The spongy parenchyma cells are spherical or lobed are loosely arranged with wide air-chambers.

Leaf margin

The leaf margin is as thick as the middle part of the lamina. It is straight with semi-circular end (Figure 5). The extreme margin is 70 µm thick. The epidermal layers of the marginal portion are thick with dilated papilate adaxial cells and semi-circular abaxial cells. The mesophyll tissue of the leaf margin consists of short palisade cells and two rows of compact spongy parenchyma.

Physico-chemical Characters

Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These characters were also used to detect the adulterants if any. Total ash value of *Marsilea minuta* (L) leaves was found to be 5.6 %, acid insoluble ash value 7.6 %, water soluble ash value 5.3 % and sulphated ash value 10 % and Moisture content 1.7 (Table 3). The extractive values of the plant material were 14.4 % for water, 13.3 % for ethanol (Table 3).

CONCLUSION

This study is in line with the quality parameters prescribed in Ayurvedic Pharmacopeia of India and also standards of other international agencies. After the present investigation it can be concluded that the pharmacognostical studies on leaves of *Marsilea minuta* (L) yielded a set of qualitative and quantitative parameters or standards that can serve as an important source of information to ascertain the identity and determine the quality and purity of the plant materials for future studies. These parameters also will serve as standard data for quality control studies of pharmaceutical preparations from the leaves of *Marsilea minuta* (L).

ACKOWLEDGEMENTS

We authors are very much thankful to Dr. P. Gauthama Sigamani, Chairman, Dr. S. Anabazhagan, Dean and Ms. S. Dhanalakshmi, Asst. Professor of Surya Group of Institutions, School of Pharmacy, Villupuram, Tamil Nadu, India for giving the necessary permission and providing the necessary facilities for carrying out this research work in the Institute laboratories.

REFERENCES

1. Sharma SK. Recent approach to herbal formulation development and standardization, pharmainfo.net; 2004.
2. Dhanabal SP, Suresh B, Sheeja E and Edwin E. Pharmacognostical studies on *Passiflora quadrangularis*. Indian Journal of Natural Products 2005; 21(1): 9-11.
3. Balamurugan M, Parthasarathi K, Cooper EL, Ranganathan LS. Anti-inflammatory and anti-pyretic activities of earthworm extract-*Lampito mauritii* (Kinberg). J. Ethnopharmacol 2009; 121(2): 330-332. http://dx .doi.org/10.1016/j.jep.2008.10.021
4. Tiwari OP, Bhattamisra SK, Singh PN, Kumar V. Adaptogenic anti-stress activity of standardized extract of *Marselia minuta* L. Pharmacology online; 2009. p. 290-299.
5. Wallace JW, M Chapman, JE Sullivan and TN Bhardwaja. Polyphenolics of Marsileaceae and their possible phylogenic utility. American J. Botany; 1984. p. 660-665. http://dx.doi.org/10.2307 /2443362
6. Gupta RS, Kumar P, Sharma A, Bhardwaja TN and Dixit VP. Hypocholesterolemic activity of *Marsilea minuta* Linn. in gerbils. Fitoterapia 2000; 71: 113-117. http://dx.doi.org/10.1016/S0367-326X(99)00140-9
7. Parihar Pradeep, Parihar Leena and Bohra Achaleshwar. *In vitro* antibacterial activity of fronds (leaves) of some important pteridophytes. Journal of Microbiology and Antimicrobials 2010; 2: 19-22.
8. Bhattamisra SK, Singh PN, Singh SK, Kumar V. Anxiolytic activity of *Marsilea minuta* Linn. in rodents. J Herbal Medicine Toxicol 2007; 1: 15-20.
9. Sivaranjan VV and I Balachandran. Ayurvedic Drugs and their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi; 1994. p. 455.
10. JE Sass. Elements of Botanical Micro technique. New York and London, McGraw Hill Book Co. Inc; 1940. p. 222.
11. Johansen DA. Plant Micro technique. McGraw Hill Book Co. Inc New York and London; 1940. p. 523.
12. Brien TP, Feder N, Mc Cull ME. Polychromatic Staining of Plant Cell Walls by toluidine Blue-o, Protoplasma, 59. p. 364-373.
13. Easu K. Plant Anatomy John Wiley and sons. New York; 1964. p. 767.
14. Easu K. Anatomy of seed Plants. John Wiley and sons. New York; 1979. p. 550.
15. Brandle JE, Starratt AN, Gijzen M. *Stevia rebaudiana*: its agricultural, biological and chemical properties, Can J Plant Sci 1998; 78(4): 527-536. http://dx.doi.org/10.4141/P97-114
16. Kokate CK. Evaluation of Crude drugs. In: Practical Pharmacognosy. 1st ed. Delhi Vallabh Prakashan; 1986. p. 14.
17. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments Ed 1, Nirali Prakashan, Pune; 2005. p. 27-35.

Cite this article as:

S.Madhu, V. Kannabirran, S.Murugesan, M.Charli, K.Nithiyaa. Pharmacognostical studies on leaves of Marsilea minuta Linn. Int. Res. J. Pharm. 2014;5(3):215-219 http://dx.doi.org/10.7897/2230-8407.050346

Source of support: Nil, Conflict of interest: None Declared