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Research Article

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PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION ON THE LEAVES OF *LYGODIUM FLEXUOSUM* LINN.

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ABSTRACT
Establishment of pharmacognostic profile of the leaves will assist in standardization for quality, purity and sample identification. Evaluation of the fresh, powdered and anatomical sections of the *Lygodium flexuosum* leaves were carried out to determine the macromorphological, micromorphological, numerical, fluoroscence analysis and pytochemical profiles. The leaves of *Lygodium flexuosum* is compound, multifoliate, apex is acute, ovate in shape, parallel pinnate type of veniation. In microscopic studies, the leaves showed the presence of covering trichomes, collenchymas, vascular bundles, palisade cells and anomocytic stomata etc. Phytochemical evaluation revealed the presence of alkaloids, flavonoids, Saponins & Cumarin etc. The main constitute of the plant is Lygodinolide which is mainly used in wound healing. Establishment of pharmacognostical and phytochemical profile of the leaves will assist in standardization for quality, purity and sample identification. The investigations also included numerical and quantitative leaf microscopy. The results of the study could be used in establishing some diagnostic indices for the identification and preparation of a monograph of the plant.
KEYWORDS: *Lygodium flexuosum*, Standardization, Pharmacognostic, Monograph.

INTRODUCTION

India has an ancient heritage of traditional medicines; materia medica of India provides lots of information on the folklore practices and traditional aspects of therapeutically important natural products. An Indian traditional medicine is based on various system including ayurveda, siddha, unani. Natural products and especially those derived from higher plants have historically played a pivotal role in the discovery of new pharmaceuticals. In the modern world herbal medicine also known as phytotherapy or alternative therapy. Herbal medicine also taken as modern appearance and are now sold in the form of tablets, capsule, syrups etc. such drug occasionally caused side effect if one is allergic to some ingredients within them and they at times, react with one chemical constituent another drug one in taking[1]. Nature has provided a complete remedy to cure all ailments of mankind. The history of herbal medicines is as old as human civilization.[2] Interest in medicinal plants has increased enormously over the last two decades. *Lygodium flexuosum* (Linn) Sw. is a fern found nearly throughout India up to an elevation of 1500 meter it is commonly known as Kalazha. It belongs to the family Lygodiaceae and widely used in treating various ailments like jaundice, dysmenorrhea, wound healing, eczema. It is an important medicinal plant as some of the scholars of Indian System of Medicine reported that the plant may be 'Rudra Jata', an intermediate drug in classical text of Ayurveda and its medicinal properties have been reported from all the parts of the plant. The rhizome and root is ethnomedicinally useful in the treatment of jaundice. The leaf paste is applied all over the body for 7 days to cure jaundice by Kadar tribes of South Western Ghats of India. From the past decades this plant is used as an expectorant. Fresh roots are boiled with mustard oil and used in external applications for rheumatism, sprains, scabies, eczema and cut wounds, they are reported to be particularly useful for carbuncles. Stems may be used for tying rice sheaves.[3, 4] *Lygodium flexuosum* extract had antiproliferative and apoptotic activity in both cancer cells. *Lygodium flexuosum n*-hexane extracts which is responsible for the possible hepatoprotective action. *Lygodium flexuosum* is a rich source alkaloidal constituent it also constitute of flavanoids, saponins, cumarins. A new triterpene ester, an anthraquinone has been found in this plant. Establishment of pharmacognostical and phytochemical profile of the leaves will assist in standardization for quality, purity and sample identification.[5-7]

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The leaves of *Lygodium flexuosum* were collected in October 2010 from Hathinala region (M.P) near Maharashtra border and authenticated by Tariq Hussain, Scientist-in-charge, Raw material Herbarium and Museum, NBRI Lucknow and a voucher specimen was also deposited (specimen number: 97871) for further references. The leaves were separated, washed under running tap water; air dried under shade, coarsely powdered and kept in airtight container until further use.

MACROSCOPY

The following macroscopic characters for the fresh leaves (Figure 1) were noted: size, shape, colour, surface, venation, petiole, the apex, margin, base, texture, odour and taste.[8-9] Table 1.

Figure 1: Leaf macroscopy

Microscopy of Plant Material

Leaves of *Lygodium flexuosum* was subjected to microscopical study. The study was done by using standard and compendial methods (Brain and turner, 1975a; Johansen, 1940; Wallis, 2002; Dutta, 1995). The micro-powder analysis was done according to the methods (Kokate, 1986a; Khandelwal, 2008).[10-13]

Materials and Method

Simple Microscope, Blades for section cutting, Slide, Cover slip, Dropper, Brush etc. Free hand sectioning was done for fresh leaf and petiole to obtain thin section. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope.

Transverse Section

Structurally the leaves consist of epidermis, endodermis, phloem, xylem, collenchymatous cells. It showed the presence of covering trichomes, vascular bundles, palisade cells and anomocytic stomata.

Epidermis is a single layer of epidermis, which consists of small tangentially elongated rectangular cells with brownish, thick-outer walls. Collenchyma are Spongy cells that are spaciously arranged and irregularly shaped and it is lacunar type of collenchymas. Vascular bundle are Collateral type in which Xylem and phloem aligned along a radius; xylem inside, phloem outside.

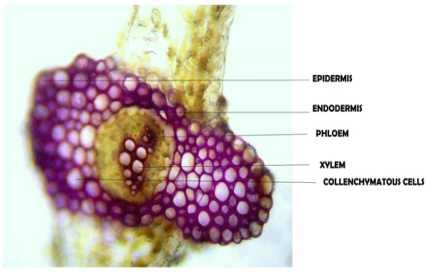

Figure 2: T.S of *Lygodium flexuosum* Leaf

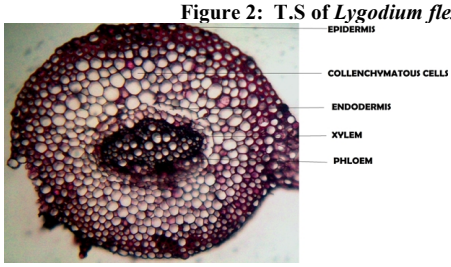

Figure 3: Petiole

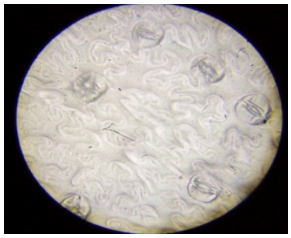

Figure 4: Stomata

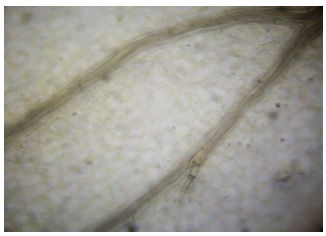

Figure 5: Venation

POWDER MICROSCOPY

The well-dried homogenous, free of dirt and foreign matter drug samples of leaves were subjected to grinding. The powder so obtained was sieved through No. 180, and No. 60 sieves [normal size of aperture was about 0.180 mm and 0.070 mm respectively.[14] The powders were kept in air tight glass containers for further studies. Each time, a small quantity of the powder was taken on the slide and after treatment with respective dehydrating, fixing and staining agents, was observed under the microscope.

Figure 6: Pollen grains

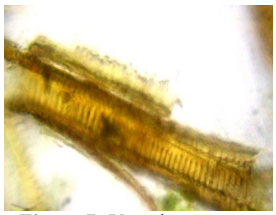

Figure 7: Vessels

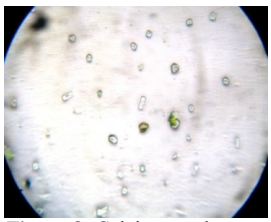

Figure 8: Calcium oxalate crystal

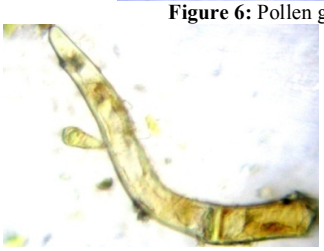

Figure 9: Starch grains

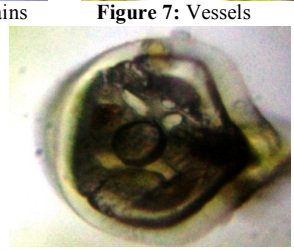

Figure 10: Trichomes

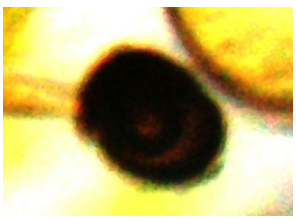

Figure 11: Disc

Figure 12: Cork cells

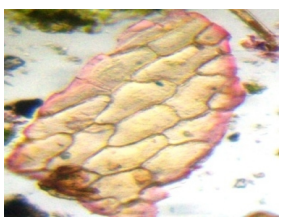

Figure 13: Broken trichomes

Figure 6-13: Powder Microscopy of *Lygodium flexuosum*

Physicochemical Parameters

The leaves of *Lygodium flexuosum* were washed in tap water then mashed into small pieces and air dried. The pieces were then oven dried at 40 ± 2^0C, ground into coarse powder and used for the study of physicochemical parameters. Determination of various physiochemical parameters such total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, chloroform soluble extractive value, pet ether soluble extractive value, ethyl acetate soluble extractive value and moisture content were calculated as per WHO guideline Table 2.

Preliminary Phytochemical Screening

For preliminary phytochemical screening, 200 gm leaves was coarsely powdered and extracted separately with alcohol, water, petroleum ether, chloroform and ethyl acetate by maceration. Each extract was filtered and subjected to preliminary phytochemical screening for the presence of different groups of compounds. The extract obtained from successive solvent extraction is then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides oils, amino acids, flavanoids, saponins etc. Preliminary phytochemical screening for organic and inorganic elements was carried out by using standard procedures described by Khandelwal Table 3 and Table 4.[15-16]

Quantitative fluorescence analysis

Within certain limits of concentration the intensity of fluorescence for a given material is related to its concentration. The instrument employed is a fluorimeter and consists of a suitable UV source and a photoelectric cell to measure the intensity of the emitted fluorescent light. Concentration of a substance in solution is obtained with reference to a standard curve prepared by subjecting standard solutions to the fluorimetric procedure. With plant extracts it is worthwhile to ascertain that the substance being determined is the only one in the solution producing fluorescence at the measured wavelength there are no substances in the solution which absorb light at the given wavelength of the fluorescence. Refined instruments are now available in which the fluorescence spectrum is automatically analyzed and in which the wavelength of the incident radiation can also be varied. To supplement the characterization of powdered drug, particularly in the light of chromatographic separation and identification, the powders were treated with different acids. In most of the cases these were observed definite colour variations under the ordinary and ultraviolet light (365nm and 254nm). Observations are therefore recorded Table 5.

RESULTS

Macroscopically, the leaf of *Lygodium flexuosum* is a compound leaf, ovate, acute apex, serrate, petiole is circular, glabrous and it is dark green in colour with characterstic odour and taste Table 1. Microscopical features revealed that the leaves consist of epidermis, endodermis, phloem, xylem, collenchymatous cells. It showed the presence of covering trichomes, vascular bundles, palisade cells and anomocytic stomata. Epidermis is a single layer of epidermis, which consists of small tangentially elongated rectangular cells with brownish, thick-outer walls. Collenchymas are Spongy cells that are spaciously arranged and irregularly shaped and it is lacunar type of collenchymas. Vascular bundle are Collateral type in which Xylem and phloem aligned along a radius; xylem inside, phloem outside. Powder microscopy showed the presence of pollen grains, vessels, starch grains, calcium oxalate crystals, trichomes, disc and cork cells. For physiochemical parameters coarse powder was used for the study. Determination of various physiochemical parameters such total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, chloroform soluble extractive value, pet ether soluble extractive value, ethyl acetate soluble extractive value and moisture content Table 2. Qualitative chemical tests showed the presence of various phytoconstituents like alkaloids, glycosides oils, amino acids, flavanoids, saponins etc. Preliminary phytochemical screening for organic and inorganic elements and flourscence analysis results are shown in Table 3 to 6.

Table 1: Morphology of the leaf of *Lygodium flexuosum*

Morphological Parameter	Observation
Condition	Fresh
Type	Compound
Size: Length Width	4.5-10cm 3.5-6cm
Shape	Ovate
Apex	Acute
Margin	Serrate
Venation	Parallel
Base	Decurrent
Petiole	Circular
Surface	Glabrous
Colour	Dark green
Odour and Taste	Characterstic and bitter

Table 2: Physicochemical Parameters of *Lygodium flexuosum*

PARAMETERS	VALUES (%w/w)
Alcohol soluble extractive	24
Water soluble extractive	16.5
Chloroform soluble extractive	5.3
Petroleum ether soluble extractive	6.8
Ethyl acetate soluble extractive	0.6
Moisture content (LOD)	10.5
Total ash	16.7
Acid insoluble ash	3.65
Water soluble ash	16.1

Table 3: Preliminary Phytochemical Screening of Different Extracts of *Lygodium flexuosum* (Organic elements)

S.no	Tests	P.ether	Chloroform	Ethyl acetate	Alcohol	Water
1	Alkaloids	–	–	–	+	–
2	Glycoside	–	–	–	+	–
3	Carbohydrates	–	–	+	+	–
4	Fixed oils and fats	+	+	+	+	–
5	Saponins	–	–	–	+	+
6	Phenolic comp. and tannins	–	–	–	–	–
7	Proteins and amino acids	–	–	+	–	–
8	Flavanoids	–	–	–	+	–

Table 4: Inorganic Constituents of Root Powder of *Lygodium flexuosum*.

(-)Notpresent,(+)Present

Elements	Results
Calcium	–
Magnesium	–
Sodium	–
Potassium	+
Iron	+
Sulphate	–
Phosphate	+
Chloride	+
Carbonate	–
Nitrates	–

Table 5: Fluorescence Analysis of the leaves Powder of *Lygodium flexuosum*.

TREATMENT	LIGHT	U.V 254	U.V 366
POWDER AS SUCH	GREEN	DARK GREEN	BLACK
POWDER+ CONC.H_2SO_4	YELLOWISH GREEN	BROWN	BLACK
POWDER+ DIL.HCL	YELLOW	BLUISH BROWN	BLUE
POWDER+ AMMONIA	BROWNISH GREEN	DARK GREEN	BLACK
POWDER+ LEAD ACETATE	DARK GREEN	BLUISH BLACK	BLUE
POWDER+ COBALT CHLORIDE	PINK	BROWN	PURPLE
POWDER+ FERRIC CHLORIDE	YELLOW	LIGTH GREEN	BLACK
POWDER+ COPPER SULFATE	GREEN	PURPLE	LIGHT BLUE
POWDER+ IODINE	YELLOWISH ORANGE	BROWN	BLACK
POWDER+AMMONIUM THIOCYANATE	GREEN	GREENISH BLACK	DARK GREEN
POWDER+ NITRIC ACID	ORANGE	BLUE	BLUE
POWDER+ SODIUM HYDROXIDE	DARK GREEN	GREEN	BROWNISH BLACK
POWDER+AMMONIUM MOLYBADATE	GREEN	PURPLE	BLUISH BLACK
POWDER+MAGNESIUM SULFATE	GREEN	DARK GREEN	PURPLE
POWDER+ WATER	GREEN	BLUE	DARK GREEN

DISCUSSION

Lygodium flexuosum is currently being used in the treatment of various disease conditions without standardization. The standardization of crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in an herbal pharmacopoeia, pharmacognostic parameters and standards must be established. *Lygodium flexuosum* is a fern that has been confused with other species also due to its relative similarities. The result of this investigation could, therefore serve as a basis for proper identification, collection and investigation of the plant. The macro and micro morphological features of the leaf described, distinguishes it from other members of the genera. The various parameters evaluated in this fern is unique and required in the standardization.

CONCLUSION

These parameters which are being reported for the first time and could be useful in the preparation of the herbal section of Herbal Pharmacopoiea.

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