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Original Research Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL INVESTIGATION OF *NELUMBO NUCIFERA* LEAVES

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Abstract: *Nelumbo nucifera* Gaertn. is a monogeneric plant belongs to family *Nelumbonaceae*, commonly known as sacred Indian lotus, rose of India, sacred water lily or East Indian lotus. It has been used throughout Egypt, the Middle East, India, and China since ancient times, primarily as a food, but also as a medicine. The present study was carried out to evaluate the Pharmacognostic and qualitative analysis of various phytochemicals parameters of *Nelumbo nucifera* leaves.

Keywords: *Nelumbo nucifera*, Macroscopy, Microscopy, Phytochemical Screening.

Introduction: Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care[1]. As commercialization of the herbal medicine has happened, assurance of safety, quality and efficacy of medicinal plants and herbal products has become an important issue. The herbal raw material is prone to a lot of variation due to several factors, the important ones being the identity of the plants and seasonal variation (which has a bearing on the time of collection), the ecotypic, genotypic and chemotypic variations, drying and storage conditions and the presence of xenobiotic [2].

Nelumbo nucifera Gaertn. is a monogeneric plant belongs to family *Nelumbonaceae*, commonly known as sacred Indian lotus, rose of India, sacred water lily or East Indian lotus. It has been used throughout Egypt, the Middle East, India, and China since ancient times, primarily as a food, but also as a medicine[3]. The flowers, seeds, leaves, fruit, and rhizomes of the lotus are all edible. The petals of the flower are used as a wrap for foods in Asia, and the rhizome is a common ingredient in soups and stir-fries. Lotus flowers, leaves, seeds, and fruit have been used traditionally to treat a variety of conditions, including diarrhea, abnormal bleeding, poor digestion, fever, and insomnia[3,4].

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In Ayurveda, this plant is used as a diuretic and anthelmintic, as well as in the treatment of strangury, vomiting, leprosy, skin diseases, and nervous exhaustion[6]. All parts of *N. nucifera*, including the leaves, flowers, embryos, and rhizomes, are prescribed as demulcents for hemorrhoids and are beneficial for the treatment of various human diseases[6]. Several studies have shown that *N. nucifera* possesses pharmacologic and physiologic activities, including antioxidant[7], antiviral and immunomodulatory effects[8]. Recently, flavonoid-enriched *N. nucifera* leaf extracts were reported to inhibit the proliferation of breast cancer in vitro and in vivo, improve lipid metabolism, and relieve liver damage resulting from a high fat diet[5]. Moreover, the anti-obesity potential of *N. nucifera* leaves has been demonstrated via increased lipolysis in adipose tissue in mice[9]. The present study was carried out to evaluate the Pharmacognostic and qualitative analysis of various phytochemicals parameters of *Nelumbo nucifera* leaves.

Materials and Methods

Collection and Authentication Of Plant Material: The healthy and disease free leaves of *Nelumbo nucifera* were collected from Surrounding of chota talab , Bhopal (MP) during the month of August to October 2015 and authenticated and voucher specimen deposited at Malhotra College of Pharmacy, Bhopal for future references.

Macroscopic and Microscopic Analysis: The macroscopic and microscopic examinations of plant studied were based on the method of the plant studied were according to the method [7,8]. Transverse sections (T.S.) of Leaf was prepared and stained with saffranin and Fast green as per the procedure [9]. Powder microscopy was performed according to the prescribed procedure [10,11]. The microphotographs were taken by Bright field microscope with digital camera Canon Photo shot G2.

Extraction of Powdered Plant Material: The plant material collected from their natural habitat was cleaned, shade dried at room temperature, coarsely powdered and stored in an air tight glass container. 100 gms of each coarse powder was successively extracted with different solvents viz. Petroleum ether, Chloroform and Methanol (40-60) in Soxhlet extractor for 16-18 hours. Then, the extracts were filtered and concentrated using rotary flash evaporator and residues were dried in desiccators over sodium sulfite below 60°C. Freshly prepared extracts were subjected to phytochemical evaluation for the detection of various constituents using conventional protocol[10].

Results and Discussion

Pharmacognostic Investigations: The detailed and systematic pharmacognostical evaluation would give valuable information for the standardization of drug. The detailed morphology of *Nelumbo nucifera* leaves was carried out to support proper identification of drug.

Taxonomic Classification[3]: Kingdom: Plantae – Plants; Sub Kingdom: Tracheobionta – Vascular Plants; Super Division: Spermatophyta – Seed Plants; Division: Magnoliophyta – Flowering Plants; Class: Magnoliopsida; Subclass: Magnoliidae; Super order: Protaenae; Order: Proteales; Family: Nymphaeaceae– Lotus Family; Genus: Nelumbo Adans – Lotus; Species: *Nelumbo nucifera* Gaen. – Sacred lotus.

Morphology of Leaves: Leaves are large, of both types, aerial as well as floating orbicular 20-90 cm. In diameter, abruptly acute to form a short tip, petiolate, entire glaucous, non-wettable, strong cupped in case of aerial leaves and flat in case of floating ones, radiantly nerved, the fresh leaves are leathery, bout on drying they are nearly membranous and brittle, there is more or less brownish red blotching on the lower surface, petioles of the aerial leaves are erect and stout white those of the floating ones are not strong enough. The usual length varies from 24.00 to 33.00 cm. in case of aerial leaves and 23to 30 cm in case of floating, petioles are smooth, greenish or greenish brown

in colour with small brown dots sometimes rough with very small, but distinct princkees, odour is distinct, fracture is fibrous. When transversely cut, the petiole of leaf stalk always shows four distinct, large cavities in the centre and small cavities in the periphery[11-13].

Microscopy of Leaf (T.S): T.S. section shows a bulged and distinct midrib and wings on its both sides. Epidermis: Both upper and lower epidermis layer are present. Compactly arranged cells. Upper epidermis has many stomata which are lacking from lower epidermis. Mesophylls: It is differentiated into upper palisade and lower spongy Parenchyma. Upper palisade becomes discontinuous near epidermis to form sub- stomata chambers. The lower part of the wing is occupied with large air chambers, numerous trichosclereid are scattered in this region. Elongated sclerotic cells-the trichosclereids commonly called "Internal hairs' often with branched ends are frequently present. Vascular tissue: Vascular bundles occur all along the wings and also in the midrib, there are 3-4 vascular bundles in the midrib these are similar to those present in the wings. Vascular bundle is surrounded by a parenchymatous bundle sheath. Each vascular bundle is conjoint, collateral and closed. These are very much reduced. As usual they are composed of xylem and phloem. The vascular bundle consists of poorly developed xylem and comparatively large phloem. In the laminar region the xylem of the vascular bundle remains oriented towards upper epidermis.

The stomata, however, become obliterated by the readjustment of neighboring epidermal cells. During initial stages of degeneration the guard cells show irregularly thickened walls, disintegrated nuclei, and highly vacuolated cytoplasm. Such abnormal features finally lead to the disappearance of stomata from the lower surface of leaves. The ontogeny, structure and distribution of stomata on leaves, perianth lobes, stamens, receptacles and carpels are described. The stomata are haplocheilic in development and are anomocytic (ranunculaceous) at maturity.

Powder Microscopy: Powder microscopy was performed according to the prescribed procedure[10].Abundant trichosclereids of either entire or broken pieces, the surface of the sclereid are warty due to the deposition of minute prismatic crystals large masses calcium, oxalate crystals seen in powder of *Nelumbo nucifera*.

Phytochemical Screening: Qualitative assay for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, triterpenoids, polyphenols and steroids were carried out on freshly prepared extract and recorded in Table -4.

Conclusion: In present investigation various standardized parameters such as macroscopic, microscopic, pharmacognostic and phytochemical screening was carried out and which could be helpful in authentification of *Nelumbo nucifera*. The presence of characteristic Upper epidermis has many stomata which are lacking from lower epidermis, Elongated sclerotic cells-the trichosclereids commonly called "Internal hairs' often with branched ends are frequently present are the salient features of diagnostic value in the pharmacognostic determination of the drug. The results of present study will also serve as reference material in the preparation of monograph for its proper identification and detection of adulteration/ substitution.

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TABLE -1:PHYSICO-CHEMICAL CONSTANTS (%w/w)

I: ASH VALUE	
a) Total ash	4.45 %
b) Water soluble ash	2.23%
c) Alkalinity of water soluble ash	0.77%
d) Acid insoluble ash	1.02%
II: SOLUBILITY	
a) Alcohol	10.65%
b) Water	09.43%
III EXTRACTIVE VALUES	
a) Alcohol	17.33%
b) Hexane	3.45%
c) Benzene	0.876%
d) Chloroform	8.453%
e) Water	16.478%
IV. QUALITATIVE INORGANIC TESTS	
a) Acid radicals	Sodium, Potassium, Iron, Calcium
b) Basic radicals	Carbonate, Sulphate, Chloride, Phosphate
V. MOISTURE CONTENT	
a) Moisture Content	6.02+ 0.76 % W/W

TABLE -2: FLUORESCENCE ANALYSIS OF DRUG POWDER

Material	**Day Light**	**UV Light**
Drug Powder	Light green	Green
Drug Powder + 1N NaOH (aqu)	Reddish Brown	Red
Drug Powder + 1N NaOH (alc)	Reddish Black	Red
Drug Powder + 1N HCI	Light Green	Pale green
Drug Powder +50% H_2SO_4	Green	Brownish green

TABLE -3: FLUORESCENCE ANALYSIS OF EXTRACTS

Extracts	**Day Light**	**UV Light**
Hexane	Pale green	Parrot green
Benezene	Green	Olive green
Chloroform	Yellowish green	Dark green
Alcohol (Methanol)	Bluish green	Dark green
Water	Pale green	Pale green
Acetone	Bluish green	Green

TABLE -4: PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *Nelumbo nucifera*

Phytoconstituents	**Petroleum ether**	**Chloroform extract**	**Ethanol extract**
Carbohydrates	-	+	+
Alkaloids	-	-	+
Phytosterols	+	-	+
Saponins	-	+	-
Fixed oils	+	+	+
Tannins	-	-	-
Flavonoids	+	+	+
Phenol	-	-	+
Glycosides	-	+	+

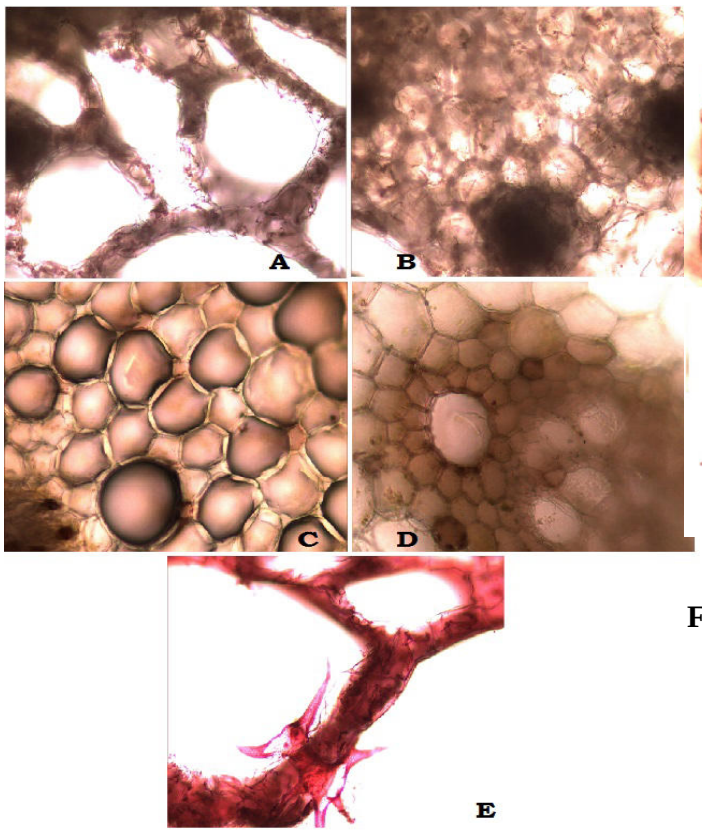

Fig. 1: -T.S. of Young Leaf, a) Air Chamber Region, b) Corner View with Stomata and Upper Epidermis, c) Petiole with Arenchyma Cells, d) Vascular Region, e) Richo Sclereids

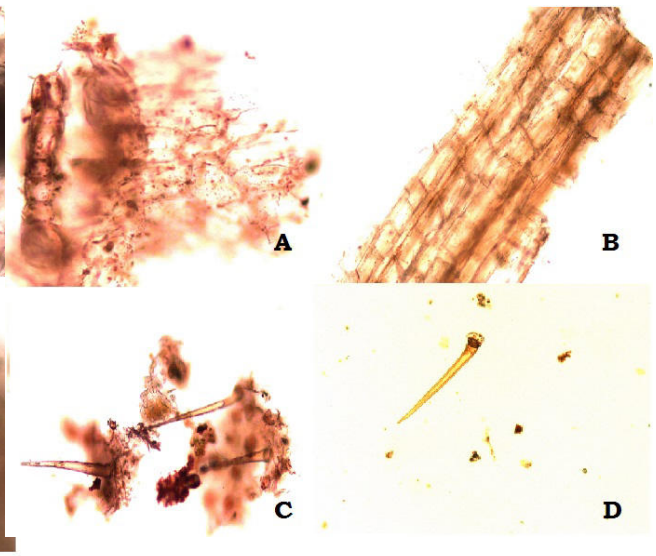

Fig. 2: -Powder Microscopy, a) General View of Powder Sample, B) Lamellar Cells, c) Trichome and Stomata, d) Trichome