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PHARMACOGNOSTICAL AND PHYTO-CHEMICAL ANALYSIS OF PADM – KESHAR (Nelumbo nucifera)

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ABSTRACT Nelumbo nucifera is a large good looking aquatic herb for treating various diseases . In the current study the used part of Nelumbo Nucifera i.e. Stamen is assessed for its Pharmacognostical and Phytochemical findings. In the Phamacognostical study, the sample material is evaluated for both morphologically and microscopically. In phytochemical study the presence of different organic materials like carbohydrate , tannin , Glycoside, Saponin , phenol, steroids, flavonoids , fixed oil and fats are found. Where as test for protein, starch, readucing sugars are found negative . The data obtained are discussed critically to lay out the possible way of raw drug standardization for herbal material . Hope this scientific write up will be a step ahead for drug standardization in Ayurvedic system of treatment

KEYWORDS : Nelumbo nucifera, Padm – Keshar, lotus

INTRODUCTION-

Lotus (Nelumbo nucifera) is one of the world's most celebrated flower. It is a large good looking aquatic herb with slender , elongate, branched, creeping, rhizome, sending out roots at the nodes, leaves peltate, 60-90cm or more in diameter much raised out of water, flowers solitary , Large, fragrant white or rosy with a centrally located yellow, conical torus, in which carpels are shrunking fruits ovoid nut like achene's. Properties as per Ayurvedic text it has madhur, Kasaya-rasa, Vipaka-Madhur, Veerya-Sheeta, Guna-Sheeta, Ruksha. It is extensively used in Ayurvedic medicines. In charak samhita it is mentioned in Agraya varga as a hemostatic agent (Padm- Keshar). Due to its applicability in many ayurvedic formulations and its substantial use in Ayurvedic drug industry for the authentication of the raw material. Now a day's different Physico-chemical Parameters are used for its quality assessment . To assess the drug Pharmacognostical and phytochemical findings are the basic tools. These tools are also facilitating the raw drug (herbal) standardization . Though the indentifying and authenticating features of the study material i.e. Nelumbo nucifera (Padm- keshar) has been defined in the ancient texts, but for facilitating the cross disciplinary debate and for global acceptance , honest efforts have been made to assess it on the above said Parameters and for establishing the data obtained .

Materials and methods-

The sample material i.e. Nelumbo nucifera (Padm-kasher) is assessed for its pharmacognostical and phytochemical value to establish the possible fingerprints for its authentication.

Materials:

Following materials are required for pharmacognostical and phyto-chemical analysis.

Drug:

Sample material i.e. Padm -Keshar (Stamen of Nelumbo nucifera) is collected form Jaipur market and water extract is prepared in the laboratory of Department of Dravyaguna, NIA (National Institute of Ayurveda).

Apparatus/ Equipments:

Electronic Microscope, petridish, Slides with Cover slip, Microtome, Butter paper, Filter paper , Crucible, Electric Muffle furnace, Distillation Apparatus, Beaker (200ml, 500ml), Test tube, Burette and Pipette.

Chemicals:

Sulphuric acid (H_2SO_4), Hydrochloric acid (HCL), Potassium lodide (KI), Mayer's reagent, Dragon droff;s reagent ferric chloride ($FeCL_3$), Sodium hydroxide (NaOH), Ninhydrin solution, alcoholic KOH, Molich's reagent and vanillin solution.

Method:-

Pharmacognostical study of Padm-Keshar (Nelumbo nucifera) :

Pharmacognosy is the study of physical chemical, bio-chemical and biological properties of the drugs and drug substances of natural origin . This study is performed in two steps i.e. Firstly identification and preparation of the sample and in the second step microscopically examination of the sample.

Identification and preparation of the sample :

The sample is idenified as Padm -keshar (Nelumbo nucifera) by the expert committee for drug identification of NIA (National Institute of Ayurveda), Jaipur, following the API (Ayurvedic Pharmacopoeia of India) guidenlines, is considered for study. Padm Kesher (stamen) of the plant is considered for the study sample .

Sample is taken and some fine transverse section are prepared with microtome and were kept in petridish containing water. Then, the most possible uniformly fine section is chosen, kept on a clean and dry slide and drop of glycerine is added to it. Then it is covered with the cover slip and taken for microscopic analysis.

Analysis of Sample :

Padm- keshar (stamen of the plant Nelumbo Nucifera) is subjected to macroscopic (organoleptic) and microscopic identification for establishing the data in the following scientific method-

Macroscopic identification:-

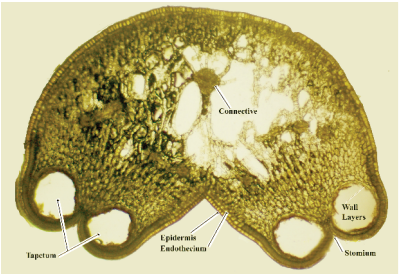
Freshly collected stamen of the said plant is taken, washed carefully , air dried and naked eye observations are noted.

Macroscopic observation:

Solitary, large 10-25 cm in diameter, white pink of pinkish white, fragrant, peduncles arising from the nodes of the rhizome, sheathing at the base, 1-2 cm, long, green or blackish green, hard and stout, smooth or rough due to the presence of numerous small scattered prickles. Sepals, petals and stamens are spirally arranged passing gradually one into another. Sepals 4-5 caducous, petals, numerous. hypogynous, many seriate, elliptic, obtuse, concave, 5-12.5 cm long and 3-4 cm, broad, smooth, stamens free, numerous, many seriate, hypogynous, anthers with an elevate appendage at the end, yellow in white flowered and orange in rose colored forms, Pistils, many sunk in the flat top of an obconic fleshy, 2 cm. high torus, flat top of which is 1.5 to 2.6 cm. in diameter. Styles, excreted, stigma is terminal and dilated, ovary contains a pendulous anatropous ovule on the side towards the axis.

Microscopic Observation:
Slide prepared with transverse section of Padm -Keshar is kept under the electronic microscope and following findings are observed while watching the sample by moving the slide backward and forward and focusing at different places.

An organized anther is bilobed tetrasporangiate or dithecous in T.S, The anther wall is derived from the parietal layer and consists of an outer epidemis, an endothecium, 2 to 3 middle layers and single layers the, innermost tapetum (nutritive layer), It has a column of sterile tissue, called the connective, an either side of which is an anther lobe which usually shows a concentric vascular bundle. The cells of tapetum at maturity are multinucleate, contain dense cytoplasm and show compactness, the cells of the endothecium are radially cytoplasm and show compactness; the cells of the endothecium are radially elongated and exhibit characteristic inner tangential fibrous thickenings. The pollengrains are, at first, arranged in tetrads later, these separate and occur as individual pollen grains, dispersed throughout the chamber; each microspore shows characteristic shape, size, structure and even colour.



T.S. of Anther of *Nelumbo nucifera*

Phytochemical analysis of Padm-Keshar
Phyto-chemicals are the chemical substances present in the plant materials responsible for the colour and organoleptic features of the material. For preparing the fingerprint of the sample selected, the phyto-chemical analysis is also performed along with pharamcognostical analysis.

For the phyto-chemical analysis the aqueous extract of the sample is taken and analysis for different phytochemicals is performed with the use of suitable reagents and following the standard procedure for analysis of organic materials.

Carbohydrates:
2 ml of the aqueous extract of the sample is taken in a test tuble and 2 ml of the Molich's reagent is added, shaken carefully, followed by pouring of 1 ml. of conc. H_2SO_4 from side of the test tube slowly. After some time a red brown ring at the junction of the two layers is observed, indicating the presence of carbohydrate.

Alkaloids:
In MeOH extract of the sample gives any white or pale yellow colour precipitation with Mayer's reagent indicating presence of alkaloids. Also appearance of orange colour precipitation in Dragon Droff's reagent indicating presence of alkaloids.

Tannins:
Aqueous extract of the drug is treated with Vanillin HCI Alcohol reagent (Vanillin 1 gm + 10 ml conc. HCI +10 ml Alcohol) brick or red colour is formed showing the presence of tannin.

Glycoside:
To an aqueous extract of the sample Glacial Acetic Acid, a few drops of $FeCl_3$ and conc. H_2SO_4 are added. A reddish brown colour at the junction of two layers and changing of the upper layer into Bluish Green indicated presence of Glycoside.

Phenols:
2 ml of aqueous extract of padm-keshar is taken in a test tube and 2 ml of $FeCl_3$ solution is added. Blue or Deep Green colour of the solution is suggestive to presence of Phenols.

Steroids:
Add 1ml of conc sulphuric acid to 2ml of chloroform extract of the drug carefully, from the side of the test tube. A red colour is produced in the chloroform layer indicates the presence of steroids.

Sapponin:
About 1ml of aqueous extract is diluted by distilled water upto 10ml and shaken in a graduated cylinder for 15min. formation of 1cm layer of froth indicates presence of sapponin

Flavonoids:
In a test tube containing 0.5 ml of alcoholic extract of the drug, add 5-10 drops of dilute hydrochloric acid followed by a small piece of Zinc or magnesium. Boil the solution for few minutes. In the presence of flavonoids a pink, reddish pink or brown color is produced.

Fixed oil and fats:
Add few drops of acetonitrile in chloroform extract of the drug. Presence of ppt indicates the fixed oil and fats.

Observation of Qualitative analysis of Organic matter in stamens of Nelumbo nucifera:

Sl.No.	Chemical constituent	Test Applied	Result
1.	Carbohydrates	Molich's reagent	+
2.	Reducing sugars	Fehling solution	-
3.	Starch	Iodine solution	-
4.	Alkaloids	Dragondroff's reagent	+in MeOH ext.
5.	Protein	Ninhydrin solution	-
6.	Tannin	Vanillin solution	+
7.	Glycoside	Killer Killiani test	+
8.	Saponin	Shaking with water	+
9.	Phenol	$FeCl_3$ solution	+
10.	Steroids		+
11.	Flavonoids		+
12.	Fixed oil and Fats		+

Discussion:-
Padm Keshar (Nelumbo nucifera) is a Potent drug as a hemostatic agent in therapeutics.In this analytical study it is tried to establish the pharma-cognostical and phytochemical findings of Padm- keshar (Nelumbo nucifera) to identify the raw sample for the preparation of different genuine Ayurvedic medicnes and develop the fingerprint for the crude Padm keshar. The sample drug contains glycosides, carbohdrates , tannins and phenols, steroids . Proteins, Reducing sugars, starch are absent. The colour of the sample, pale yellow. Due to presence of tannins a little bitter taste is observed in oral route administration. In microscopic study it is found , the anther wall is derived from the parietal layer and consists of an outer epidermic and endothecium, 2 to 3 middle layers and single layers the innermost tapetum (nutritive layer) it has a column of sterile tissue, called the connective, on either side of which is an anther lobe which usually shows a concentric vascular bundle. The cells of tapetum at maturity are multinucleate, contain dense cytoploasm and show compactness, The pollengrains are, at first arranged in tetrads later, these separate and occur as individual pollan grains, dispersed through out the chamber, each microspore shows characterstic shape, size, structure and even colour.

Conclusion:
In current era to find out the quality and standard raw material is a challenge to the manufacturer for traditional system. Padm Keshar is used as a sing drug for treating DUB in current scientific study it is tried to establish the crude Padm Keshar in terms of its phytochemical and pharmacognositical findings. It is concluded that the Padm Keshar does not contains protein reducing sugars and starch . carbohydrates and tannin flavonoids, Sapoin, Fixed oil fats are present . It is bilobed , tetra sporangiate or dithecous in T.S. The Pollengrains are at fist arranged in tetrads are found in microscopically are the pharamacognostic finger print of padm- keshar.

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