PHYSIOCHEMICAL SCREENING OF NELUMBO NUCIFERA LEAVES WITH SPECIFIC REFERENCE TO THEIR PHARMACOGNOSTICAL EVALUATION TEST

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***Abstract*: In this proposal research work the development of two plants prepared extracts of with different solvents and to evaluate. In vitro and In vivo inflammation activity topical therapeutics system for selective drugs. Prepared gel formulation would be evaluated for various physical parameters, In-vitro drug release kinetics and in-vivo acute dermal toxicity study The present work was aimed on herbal drug preparations and plants used in the treatment of inflammation. The medicinal property of plant could be based on the anti-oxidant, antimicrobial, antipyretic effect of the photochemical present. Traditionally, herbs have been considered to be non-toxic and have been used for treating various problems by the general public and/or traditional medicine doctors worldwide. Formulated gel was evaluated and compared with gel for pH, viscosity, spreadability, extrudability, drug content, in vitro drug diffusion, ex-vivo bio-adhesive test and skin irritation test. Topical gel having best drug releasing profile was evaluated for anti-inflammatory and analgesic potency by animal paradigms.**

***Keywords*: Nelumbo nucifera, Pharmacognostical study, physicochemical identification, leaves extraction and Pharmacological Screening, Preparation of Topical gel, Anti- inflammatory and analgesic activity, in-vitro Activity.**

INTRODUCTION:

Nelumbo nucifera known as lotus in belongs to the family Nelumbonaceae. It is distributed throughout the tropics and subtropics where it is extensively cultivated1. It is a perennial, herbaceous plant, with copious milky latex reaching to 2-3 meters tall2. Its erect stem is about 30 cm thick and roughened with leaf scars3. Leaves contain alkaloids carpain, pseudocarpain, and dehydrocarpaineI and II, choline, carposide, vitamin C, A and E4. Phytochemical screening of the leaves revealed the presence of bioactive compound saponins, cardiac glycoside, alkaloids, vitamins and mineral constituents5. diarrhea, cholera, fever, and hyperdipsia.[55][56] Rhizomes are promoted have purported diuretic, antidiabetic, and anti-inflammatory properties.. It showed the phytochemicals, vitamins and minerals composition of green, pink of leave6. Fresh, green papaya leaf is an antiseptic, whilst the brown, dried Nelumbo nucifera is the best as a tonic and blood purifier7.

MATERIAL AND METHODS:

Collection and authentication of plant material:

Samples of Nelumbo nucifera leaves was collected from GGSIPU university campus,New Delhi, India(2018) and samples were identified by Taxonomist The specimen was studied in Pharmacognosy and Phytochemistry laboratory, Vivek College of Technical Education, Bijnor,U.P.

Macroscopical and Microscopic study: The fresh leaves were examined to macroscopical and microscopical study. The dried leaves were examined for powder microscopy using with different staining reagents for different types of microscopical characters.

Physicochemical Evaluation of Drug:

Determination of individual extractive values: The amount of soluble components extracted with different solvents from the powder plant material.

Maceration: The air-dried coarse drug powdered was macerated with different solvents like pet.ether, chloroform, water and methanol of in a closed flask and place for 24 hours, shaking frequently during 6 hours and allowing standing for 24 hours. After the filtration, evaporated to dryness in a dish and dried at 105oC, to constant weight and get percentage yield.

Soxhlet extraction: The dried coarse powdered drug was packed in a Soxhlet apparatus separately for different solvents like pet.ether, chloroform, water and methanol. The each extract was evaporated till to dryness and extractive value was noted.

Successive Extraction: The dried coarse powdered drug was subjected for successive extraction in Soxhlet apparatus with different solvents as pet.ether, chloroform and methanol. The extract was evaporated till to dryness and extractive values were noted.

Determination of ash values:

Ash value is an essential parameter of a drug for the extent of adulteration and also establishes the quality and purity of the drug.

Determination of total ash values: After ignition of medicinal plant yield total ash constituting in which both physiological and non-physiological ash was present. The drug was incinerated in a silica crucible at temperature which not more than 450oC. Then was cooled and weighed to get the total ash content.

Determination of Acid insoluble ash values: Sand and siliceous earth both forming acid insoluble ash represents. Ash is boiled with dil. HCl (6N) for 5 minutes. After that the insoluble matter collected on an ash less filter paper, rinsed with hot water and ignited at a temperature which not more than 450oC to a constant weight.

Determination of Water-soluble ash values: The ash was in dissolved distilled water after that the insoluble part of ash collected on an ash less filter paper which ignited at 450oC to a constant weight. The weight of soluble part of ash is noted by subtracting the weight of insoluble part from the ash.

Florescence analysis: Florescence analysis of the powder drug was exposed to in daylight and UV light (254 and 366 nm) and treated with different reagents like sodium hydroxide, picric acid, iodine, hydrochloric acid, nitric acid, pet.ether, ferric chloride, chloroform etc.

Phytochemical screening: The different extracts of the selected drugs like Petroleum ether extract, Chloroform extract, Methanolic extract, aqueous extract were reported to preliminary phytochemical investigation for the detection of secondary metabolites. The plant extracts may provide the information regarding various types of phytoconstituents present such as Alkaloids, Carbohydrates, Flavonoids, Protein, Saponins, mucilage, resins, fat and lipids etc8.

Determination of PH:

pH 1% solution: Drug was dissolved in distilled water, filtered this and noted pH of the filtrate with a standardized glass electrode.

pH 10% solution: Drug was dissolved in distilled water, filtered this and noted pH of the filtrate with a standardized glass electrode.

Determination of moisture content: Excess of water in medicinal plant will encourage the microbial growth and also presence of fungi and insect resulting in deterioration and hydrolysis. Weighed drug and dried in oven at 1050C temp. For 1 hour, then cool in desicator and weight.

Loss on Drying = Wt. before drying – wt. after drying X 100 / wt. sample taken.

Heavy metals residue: Heavy metals were determined such as lead, arsenic, mercury and cadmium in the leaf extract of the plant by using Atomic Absorption Spectrophotometer9.

Pesticide residue: According to American Organisation of Analytical Chemist (AOAC) by using GC-MS method pesticides residue were determined such as pyrethroids, organochlorines, organophosphates in the leaf extract of the plant9.

Aflatoxin analysis: According to American Organisation of Analytical Chemist (AOAC) by using HPLC method Aflatoxin were analysed in leaf extract of the plant9.

RESULT AND DISCUSSION:

Macroscopical study: The leaves of Nelumbo nucifera green in colour, simple lobed shape, smooth surface, 50-70cm in diameter size, bitter in taste and characteristic odour as shown in (**Table 1).**

Table 1: Macroscopic Character of leaves of Nelumbo nucifera

S.no.	Parameters	Observation
1.	Colour	Pink
2.	Odour	Characteristic
3.	Taste	Bitter
4.	Size	50-70 cm in diameter
5.	Shape	Simple, Lobed
6.	Surface	Smooth

Microscopic study:

Transverse section of leaves: Transverse section of leaves through the mid rib showed upper epidermis and lower epidermis surrounded by well-defined 5-7 layer of collenchyma and scellerenchyma. The endodermis is composed of parenchymatous cells. The pith is found to be absent as the stalk is hollow from inside. A middle portion is covered with xylem and phloem surrounded by parenchymatous cell that in turn surrounded by scellerenchyma cells. Numerous fibers are present with cluster crystals. Some xylem vessels (pitted vessels) are also visible which are lignified. Cells of palisade and spongy parenchyma are also visible. (**Fig. 1)**

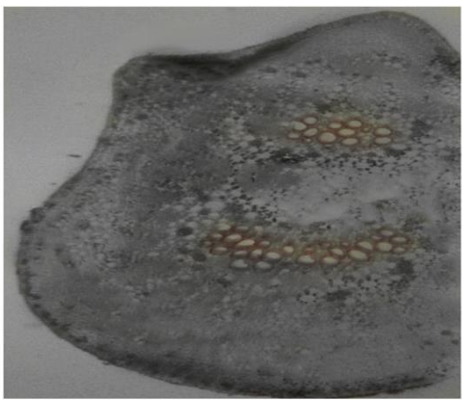

Fig. 1 view of vascular bundle

Quantitative Microscopy: The slides of surface preparation of leaf are prepared and subjected to quantitative microscopic examination.**Fig.2.** The parameters such as vein termination, vein islet and stomatal numbers, stomatal index and palisade ratio of the leaf as shown in (**Table 2).**

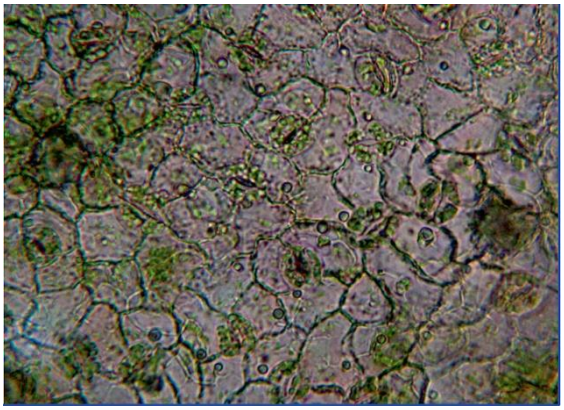

Fig. 2 view of stamata

Table 2: Quantitative microscopy of leaves of Nelumbo nucifera

S.no.	Parameters	Observation
1.	Stomatal no.	
	Upper surface	3 ± 4
	Lower surface	6 ± 8
2.	Stomatal index	
	Upper surface	24 ± 28
	Lower surface	26 ± 30
3.	Vein termination	5 ± 6
4.	Palisade ratio	12 ± 13

Physicochemical evaluation: The various physicochemical parameters were determined by using air dried powder plant material as shown in **(Table 3).** Graphical representation shown in **Fig. 3, 4, 5, 7.**

Table 3: Physicochemical evaluation of powder drug of leaves of Nelumbo nucifera

S.no.	Parameters	Result %w/w
1.	**Maceration extraction**	
	Petroleum ether	1.46
	Chloroform	1.05
	Methanol	1.45
	Hydroalchohal	1.06
2.	**Soxhlet extraction**	
	Petroleum ether	0.24
	Chloroform	0.49
	Methanol	1.13
	Hydroalchohal	0.89
	Aqueous	0.76
3.	**Successive extraction**	
	Petroleum ether	0.48
	Chloroform	0.55
	Methanol	0.78
4.	**Ash values**	
	Total ash	1.63
	Acid insoluble ash	1.39
	Water soluble ash	0.16
5.	pH of 1% solution	6.87
	pHof 10% solution	5.26
6.	Moisture content	6.40

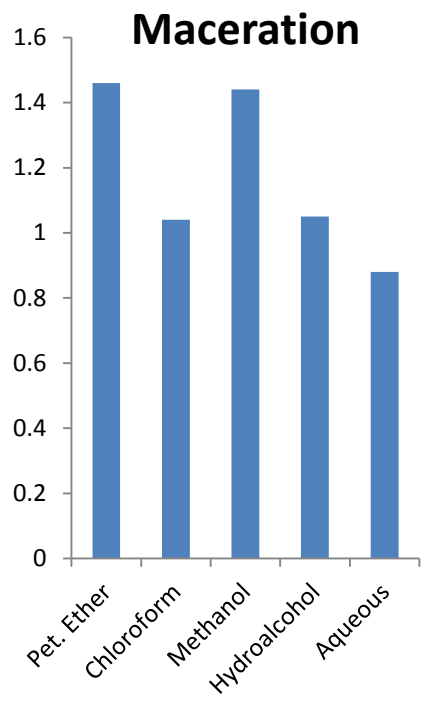

Fig 3 : %w/w of Maceration Extraction

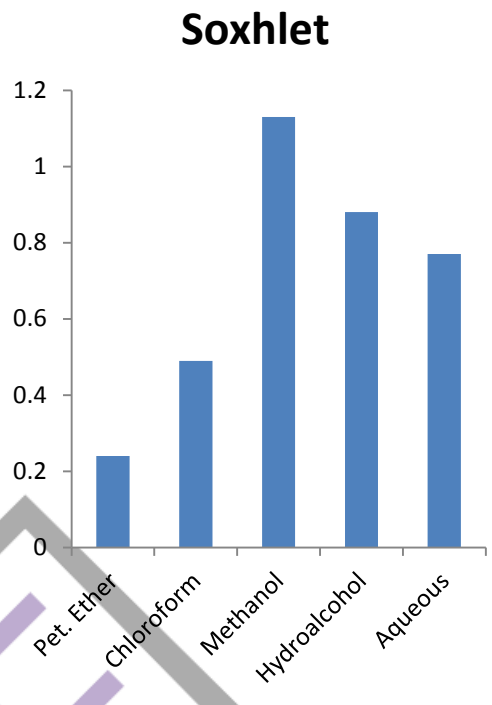

Fig 4 : %w/w of Soxhlet Extraction

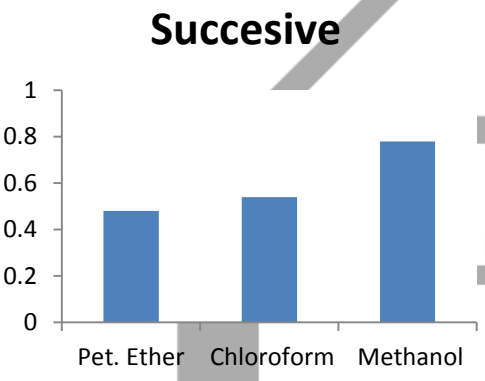

Fig 5 : %w/w of Successive Extraction

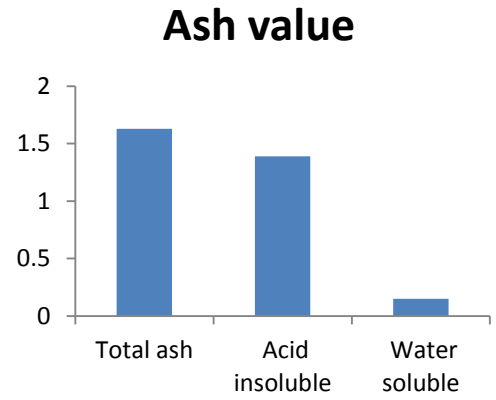

Fig : %w/w of Ash value

Determination of Heavy metal residue: As per WHO, the determination of heavy metals was carried out in the extract of Nelumbo nucifera leaves by using Atomic absorption spectrophotometer as shown in **(Table 4).** Graphical representation shown in **Fig. 7.**

Table 4: Heavy metals residue analysis of leaves of *Carica papaya*

S.no.	Heavy metals	Concentration
1.	Cadmimum(Cd)	0.20 ± 0.04
2.	Arsenic (As)	0.37 ± 0.08
3.	Mercury (Hg)	0.45 ± 0.06
4.	Lead (Pb)	0.38 ± 0.09

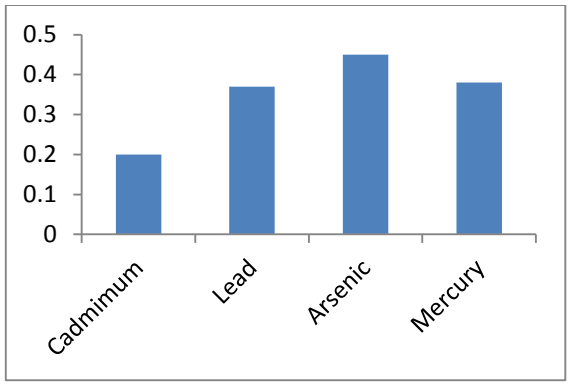

Fig 7: Concentration of Heavy Metals

Determination of Aflatoxin residue:
The detection of aflatoxin such as B1, B2, G1, G2 was carried out in the extract of Nelumbo nucifera leaves as shown in **(Table 5).**

Table 5: Aflatoxin residue analysis of leaves of Nelumbo nucifera

S.no.	Parameters	Method	Results	MDL
1.	Aflatoxin B1	AOAC 990.332	Not detected	1.0µg/kg
2.	Aflatoxin B2	AOAC 990.332	Not detected	1.0µg/kg
3.	Aflatoxin G1	AOAC 990.332	Not detected	1.0µg/kg
4.	Aflatoxin G2	AOAC 990.332	Not detected	1.0µg/kg

Determination of Pesticide residue: According to AOAC guidelines pesticide residue was carried out in the extract of *Carica papaya* leaves as shown in **(Table 6).**

Table 6: Pesticide residue analysis of leaves of Nelumbo nucifera

Sno.	Pesticide	Test method	Results	MDL
1	α-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
2	β-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
3	γ-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
4	δ-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
5	α-Chlordane	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
6	β-Chlordane	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
7	Heptachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
8	Heptachlor_Epoxide	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
9	α-Endoulfan	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
10	β-Endoulfan	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
11	Endrin	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
12	Endrin_Aldehyde	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
13	Total DDE	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
14	Total DDD	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
15	Total DDT	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
16	Alachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg

17	Butachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
18	Monochlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
19	Malathoin	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
20	Methyl – parathion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
21	Chlorpyrifos	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
22	Ethion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
23	Diazinone	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
24	Phosphamidon	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
25	Simazine	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
26	Atrazine	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
27	Fenitrothion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
28	Mevinphos	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
29	Dimethoate	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
30	Phorate	AOAC790.52/EPA525.5	Not detected	0.01mg/kg

PHYTOCHEMICAL ANALYSIS: The presence and absence of various phytoconstituents to the preliminary chemical test of extracts is subjected as shown in**(Table 7).**

Table 7: Phytochemical evaluation of the leaves extract of Nelumbo nucifera

S.no.	Constituents	Extracts Petrolium ether	Chloroform	Alcoholic	Aqueous
1.	Carbohydrate	-	-	+	+
2.	Phenolic compound	+	+	+	+
3.	Alkaloids	-	+	+	+
4.	Flavonoids	-	-	+	-
5.	Lipids	+	-	-	-
6.	Saponins	-	-	+	-
7.	Steroidas	+	+	+	-
8.	Amino acids	-	-	+	-
9.	Proteins	-	-	+	-

(-: Absent, +: Present)

Fluorescence Analysis:

The air dried powder of the leaves was subjected in lights and UV light with different chemical reagents to be observed as shown in **(Table 8).**

Table 8: Fluorescence analysis of powder of leaves of Nelumbo nucifera

S.no	Reagent	Day light	UV light 254nm	UV light 366nm
1.	Powder such as	Light Green	Dark green	Light Green
2.	Powder treated with dist. water	Green	Dark green	Brown
3.	Powder treated with 1N NaOH	Light green	Dark green	Green
4.	Powder treated with HNO3	Brown	Black	Dark green
5.	Powder treated with H2SO4	Light brown	Dark green	Dark brown

6.	Powder treated with Iodine	Brown	Dark brown	Green
7.	Powder treated with conc. HCL	Green	Brown	Black
8.	Powder treated with ammonia	Green	Green	Brown
9.	Powder treated with ferric chloride	Yellowish green	Black	Light brown
10.	Powder treated with picric acid	Light green	Green	Dark green
11.	Powder treated with pet. Ether	Dark green	Black	Dark green
12.	Powder treated with chloroforrn	Dark green	Brown	Dark green

CONCLUSION:

The present study is an attempt to develop the phamacognostic, physicochmemical and phtytochemical standards parameters to be used for identification, purity, quality and extracts of the leaves of Nelumbo nucifera. Clinical evaluation of these plants in human beings may be carried out for the above promising pharmacological activities.

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