DEVELOPMENT AND EVALUATION OF TOPICAL THERAPEUTIC SYSTEM FOR THE TREATMENT OF INFLAMMATION

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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, also known as Indian lotus, sacred lotus, bean of India, Egyptian bean or simply lotus, is one of two extant species of aquatic plant in the family Nelumbonaceae. It is often colloquially called a water lily. Under favorable circumstances the seeds of this aquatic perennial may remain viable for many years, with the oldest recorded lotus germination being from that of seeds 1,300 years old recovered from a dry lakebed in northeastern China.

It has a very wide native distribution, ranging from central and northern India (at altitudes up to 1,400 m or 4,600 ft in the southern Himalayas), through northern Indochina and East Asia (north to the Amur region; the Russian populations have sometimes been referred to as "*Nelumbo komarovii*"), with isolated locations at the Caspian Sea.Today the species also occurs in southern India, Sri Lanka, virtually all of Southeast Asia, New Guinea and northern and eastern Australia, but this is probably the result of human translocations. It has a very long history (c. 3,000 years) of being cultivated for its edible seeds,and it is commonly cultivated in water gardens.[2] It is the national flower of India and Vietnam.

Macroscopical evaluation:

Leaves of *Nelumbo nucifera*

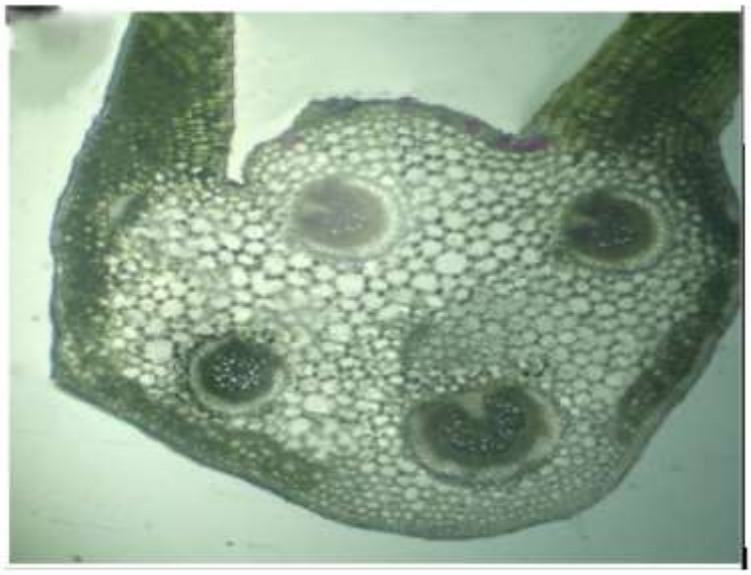
After macroscopical examination and observations *Nelumbo nucifera*leaves, the results shown in table The identity of the collected drug confirm after comparison with published results.

Macroscopical characters of *Nelumbo nucifera*

S.No	Description of the macroscopic characters	Observation
1	External Colour	Pink
2	Size	18-30 cm in length
3	Shape	Pinnate
4	Surface	Smooth
5	Odour	Characteristic
6	Taste	Slightly bitter

Microscopical evaluation:

Transverse section of leaves through the mid rib showed upper epidermis and lower epidermis, single layer epidermal cells with thin cuticle.(Fig 5.2) Some of the epidermal cells are interrupted by stomatal openings. Mesophyll made up of upper 2 to 3 layer compactly arranged palisade parenchyma enriched by. Some of the palisade parenchyma consist oil globules and prismatic crystals of calcium oxalate. Lower layer arranged in to 6 to 8 layers of spongy parenchyma between the palisade spongy parenchyma the vascular strands are passed. Through mid rib shows below the upper epidermis and lower epidermis 1 or 2 layers of collenchymatous layer are present.

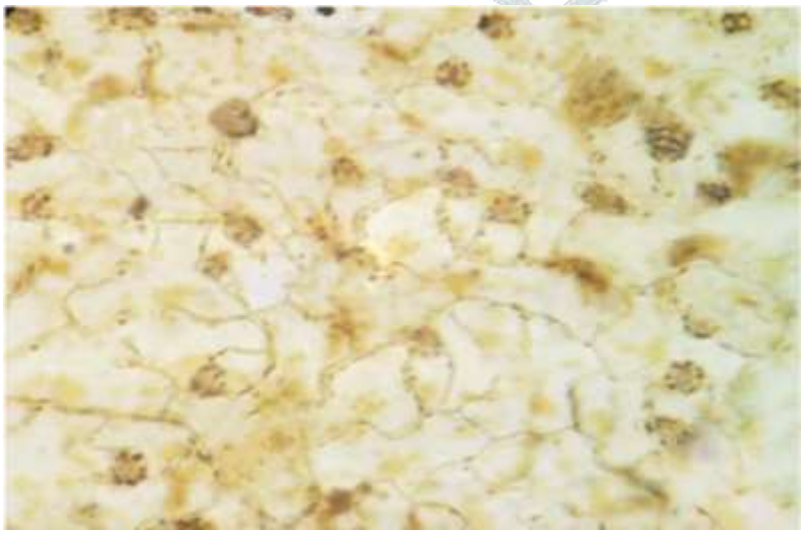


Powder microscopy:

The powder microscopy of powdered material is performed. It identifies and established many microscopic characters which shown differentiation of the substitute of the dried powder. The photomicrographs of the identifying features of the plant material are shown in (Fig 5.3). The leaves powder showed that anomocytic stomata, annular vessels, spiral vessel, prismatic crystal, loosely arranged parenchyma cells, simple trichome without oil, simple fibers, fragments of spongy parenchyma, lignified fibber, oil globule, starch grain and tannin content

Quantitative Microscopy:

The slides of surface preparation of leaf are prepared and subjected to quantitative microscopic examination. The parameters such as vein termination, vein islet and stomatal numbers, stomatal index and palisade ratio of the leaf of ***Nelumbo nucifera***are observed and presented in table (5.4).



view of stomata

Quantitative microscopy of *Nelumbo nucifera*

Drugs	Parameters				
	Stomatal number	Stomatal index	Vein islet	Vein termination	Palisade ratio
Raphanus sativus	7-11 (upper) 8-14 (lower)	20-24 (upper) 22-26 (lower)	7-9	12-14	4-5

Physicochemical evaluation of *Nelumbo nucifera*:

According to WHO guidelines various physicochemical standardization determined. For this air dried, powdered plant materials are subjected.

Extractive value:

Determination of individual extractive values (maceration):

For the maceration process plant materials (air dried, powdered) taken in conical flask at room temperature and extracted with petroleum ether, chloroform, alcohol, hydroalcohol and water separately in a conical flask at a room. The observations are presented in the table .

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract (gm)	Extractwt. (gm)	% Extract
1.	10.00	42.33	54.40	0.057	0.57
2.	10.00	41.33	54.34	0.011	1.29
3.	10.00	40.33	54.42	0.056	0.86
Mean	10.00	41.23	54.38	0.077	0.67

Chloroform extract of *Nelumbo nucifera*

S.No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1.	10.00	53.24	53.25	0.044	0.440
2.	10.00	53.24	53.27	0.039	0.390
3.	10.00	53.24	53.29	0.041	0.410
Mean	10.0000	53.24	53.27	0.0412	0.42

Methanolic extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1.	10.00	51.32	51.39	0.068	0.681
2.	10.00	51.32	51.39	0.062	0.623
3.	10.00	51.32	51.38	0.056	0.559
Mean	10.00	51.32	51.38	0.062	0.621

Hydroalcholic extract of *Nelumbo nucifera*

S.No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1.	10.00	56.40	57.48	0.081	0.810
2.	10.00	56.40	57.47	0.062	0.720
3.	10.00	56.40	57.47	0.073	0.732
Mean	10.00	56.40	57.47	0.074	0.743

Aqueous extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1.	10.00	52.251	52.340	0.089	0.891
2.	10.00	52.251	52.339	0.089	0.886
3.	10.00	52.251	52.331	0.081	0.804
Mean	10.0000	52.251	52.336	0.0856	0.860

Individual extractive values (Soxhlet extraction):

For the soxhlet extraction plant materials (air dried, powdered) taken in conical flask at room temperature and extracted with petroleum ether, chloroform, alcohol, hydroalcohol and water separately in a conical flask at a room. The observations are presented in the table

Petroleum ether extracts of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1.	10.00	51.97	52.214	0.242	2.42
2.	10.00	51.97	52.191	0.219	2.19
3.	10.00	51.97	52.178	0.206	2.06
Mean	10.0000	51.97	52.1943	0.2223	2.223

: Chloroform extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. 2. 3.	10.00 10.00 10.01	46.65 46.65 46.65	47.001 47.102 47.014	0.355 0.452 0.364	3.55 4.52 3.64
Mean	10.0000	46.65	47.05	0.390	3.90

Methanolic extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. 2. 3.	10.00 10.00 10.00	53.25 53.25 53.25	63.77 53.73 63.75	0.524 0.456 0.478	5.25 4.56 4.98
Mean	10.00	53.25	63.75	0.503	6.03

Hydroalcholic extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. 2. 3.	10.00 10.00 10.00	53.25 53.25 53.25	53.86 53.84 53.85	0.624 0.586 0.598	6.25 5.86 5.98
Mean	10.00	53.25	53.86	0.803	8.03

Aqueous extract of *Nelumbo nucifera*

S.No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. **2.** **3.**	10.00 10.00 10.00	48.58 48.58 48.58	38.68 38.64 38.67	0.096 0.083 0.085	0.959 0.831 0.845
Mean	10.00	48.58	38.69	0.088	0.878

Individual Extractive Values (Successive extraction):

For the successive extraction plant materials (air dried, powdered) taken in conical flask at room temperature and extracted with petroleum ether, chloroform, alcohol, hydroalcohol and water separately in a conical flask at a room. The observations are presented in the table .

Petroleum ether extracts of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. 2. 3.	20.00 20.00 20.01	52.60 52.60 52.60	52.909 52.904 52.904	0.309 0.304 0.304	3.086 3.037 3.040
Mean	20.00	52.60	52.905	0.3055	3.054

Chloroform extracts of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt. (gm)	% Extract
1. 2. 3.	18.87 18.87 18.87	57.42 57.42 57.42	57.98 57.99 57.99	0.566 0.565 0.572	5.66 5.65 5.72
Mean	18.77	57.42	57.99	0.562	5.68

Methanolic extract of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	China dish wt. (gm)	China dish wt. + extract(gm)	Extract wt.(gm)	% Extract
1. 2. 3.	17.16 17.16 17.16	52.38 52.38 52.38	54.04 63.94 63.87	0.97 0.91 0.87	9.64 9.04 8.62
Mean	17.26	52.38	63.95	0.909	9.09

Determination of ash values of *Nelumbo nucifera*:

According to WHO guidelines the ash values of *Nelumbo nucifera*determined as the total ash value**,** acid insoluble ash value and water-soluble ash value is also determined as per WHO guide lines. The results and observation are presented in table

Determination of Total ash of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	Crucible wt. (gm)	Crucible wt. + ash wt. (gm)	Ash wt. (gm)	% Total ash
1.	3.00	54.70	55.25	0.540	5.50
2.	3.00	54.70	55.27	0.564	5.44
3.	3.00	54.70	55.26	0.548	5.48
Mean	3.00	54.70	55.26	0.548	5.48

Determination of Acid insoluble ash of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	Crucible wt. (gm)	Crucible wt. + ash wt. (gm)	Ash wt. (gm)	% Total ash
1.	3.00	56.30	56.48	0.172	1.722
2.	3.00	56.30	56.47	0.166	1.856
3.	3.00	56.30	56.47	0.167	1.666
Mean	3.000	56.30	56.47	0.174	1.749

Determination of Water soluble ash of *Nelumbo nucifera*

S. No.	Drug wt. (gm)	Crucible wt. (gm)	Crucible wt. + ash wt. (gm)	Ash wt. (gm)	% Total ash
1.	3.00	45.46	46.387	0.378	3.783
2.	3.00	45.46	46.383	0.383	3.830
3.	3.00	45.46	46.390	0.390	3.903
Mean	3.00	45.46	46.383	0.383	3.838

Fluorescence Analysis of *Nelumbo nucifera:*

The plant materials (air dried) are subjected to lights and different chemicals and .the observation presented in Table

Effect of different chemical reagents on the fluorescence behaviour of crude drug powder

S. No.	Treatment	Day light	UVlight254nm	UV light366 nm
1.	Powder as such	pink	Light pink	Pink
2.	Powder treated with distilled water	pink	Dark pink	Dark brown
3.	Powder treated with 1N NaOH in water	Dark pink	Light pink	Pink
5.	Powder treated with HNO_3	pink	Dark brown	White
6.	Powder treated with H_2SO_4	Light brown	Dark pink	Dark brown
7.	Powder treated with iodine	pink Green	Dark brown	White
8.	Powder treated with conc. HCl	Dark pink	pink	White
9.	Powder treated with ammonia	Light pink	Dark pink	pink
10.	Powder treated with ferric chloride	Dark pink	white	Pinkish brown
11.	Powder treated with Picric acid	Light pink	white	Dark white
12.	Powder treated with Petroleum ether	Dark pink	pink	Dark pink
13.	Powder treated with Chloroform	Dark pink	Brown	Dark pink

Phytochemical screening:

For distinguish the presence and absence of various phytoconstituents the preliminary chemical tests of extracts is subjected. The results of these studies are tabulated in table (5.1.20). Phytochemical evaluation of the plant extracts may provide the information regarding various types of phytoconstituents present. These studies help for analytical profile.

Phytochemical screening of *Nelumbo nucifera*

Constituents	Extract			
	Petroleum ether	Ethyl alcohol	Alcoholic	Aqueous
Carbohydrates	-	-	-	+
Phenolic compounds	-	-	+	-
Alkaloids	+	-	+	+
Flavonoid	-	+	+	-
Lipids	-	-	-	-
Saponins	+	+	+	-
Steroids	-	-	+	+
Amino acid	-	-	-	-
Proteins	-	+	+	+
Terpenoids	-	-	+	+

Determination of pH

S. No.	Sample	pH
1.	pH of 1% solution	6.8
2.	pH of 10%solution	7.3

Moisture Content

Drug	*Nelumbo nucifera,*
Moisture Content	5.6 %

7. Bibliography

1. Sharma Alok (2008). Herbal medicine for market potential in India: An Overview. Academic Journal of Plant Sciences, IDOSI Publications, 1(2): 26-36.
2. Bozzuto Anne (2000). Homeopathy, Herbs and Hypnosis Common Practices, In Complementary and Alternative Medicine, Jacksonville Medicine.
3. Mehrola, M.N., Quality control and equipments of medicinal plants used in traditional medicines ethanobotany, 1990(2), pp 19-20.
4. Farasworth,N.R., Pezzuto, J.M., Rational approaches to the development of plant derived drugs in proceeding of the 2nd national symposium on The Pharmacology and Chemistry of natural products, Joaopessoa, Brazil,1983: 3-5.
5. The Ayurvedic Phrmacopoeia of India.,Government of India., Ministry of Health and Family Welfare., New Delhi.1999, Part I, Vol III, pp 235
6. Kokate C.K., Purohit A.P. and Gokhale S.B. (2005). A Text-book of Pharmacognosy, 31st edition, Nirali Prakashan.
7. Mukherjii P.K. (2001). Quality Control of Herbal Drugs, Business Horizon Publication, 1st Edition 1, 183-219.
8. Singh S. K. (2002). Proceedings of Global Promotion of Tradition Medicine in View of Institute Industry Relationship, Faculty of Ayurveda, Banaras Hindu University, pp. 112–115.
9. Duke J. A. and Bogenschutz-Godwin M. J. (1999). Natural Products from Plants, CRC Press, Boca Raton, FL, USA, 183–205.

10. Ara Tachjian (2010). Use of Herbal Products and Potential Interactions in Patients with Cardiovascular Diseases" Journal of the American College of Cardiology, Vol. 55, No. 6, 2010.

11. Handa S. S. (1992). Medicinal plants based drug industry and emerging plant drugs", Curr. Res. Med. Aromat. Plants, 14, 233–262.

12. G.Ulrich-Merzenich et al. "Drug development from natural products: exploiting synergistic effects", International Journal of experimental biology,48, 2010, 208- 219.

13. Abbas A.B. and Lichtman A.H. (2009). Ch.2 Innate Immunity". Basic Immunology. Functions and disorders of the immune system (3rd ed.). ISBN 978-1-4160-4688-2.

14. Cotran Kumar Collins (1998). Robbins Pathologic Basis of Disease. Philadelphia: W.B Saunders Company. ISBN 0-7216-7335-X.

15. Saxena, R.C., Nath, R., Palit, G., Nigam, S.K., and Bhargava, K.P. 1979, Indian J. Pharmacol., 11, 39.

16. M. Anil kumar (2010). Ethnomedicinal plants as anti-inflammatory and analgesic agents In Ethnomedicine: A Source of Complementary Therapeutics, Editor: Debprasad Chattopadhyay, Research Signpost, Trivandrum, Kerela, India, 267-293 ISBN: 978-81-308-0390-6.