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EXTRACTION PHYTOCHEMICAL SCREENING AND ANTI-INFLLAMATRY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *NYMPHAEA CAERULEA*

Saket Saurav*, Kalpana Prajapati, Dr. Satkar Prasad, Dr. Sailesh Kumar Ghatuary

RKDF School of Pharmaceutical Sciences, Bhopal (M.P.)

*Corresponding Author's Email ID: saketyadav983@gmail.com

Abstract

Nymphaea caerulea, known in English as Egyptian lotus, blue lotus, blue water lily (RSA), Cape water lily (RSA), frog's pulpit (RSA), blue lotus of the Nile blue waterlily, blue Egyptian lotus, blue Egyptian water lily (India), sacred blue lily of the Nile (India), Cape blue waterlily (USA) and sacred blue lily, is a water lily in the genus Nymphaea, a botanical variety *of Nymphaea caerulea*. The aim of present work extraction, phytochemical screening and anti-infllamatry activity of hydroalcoholic extract of *Nymphaea caerulea* The present finding reveals that Hydroalcoholic extract *Nymphaea caerulea* leaves efficiently inhibits alpha amylase enzymes in vitro in a dose dependent manner. The Hydroalcoholic extract *Nymphaea caerulea* leaves showed a dose dependent inhibitory effect on alpha-amylase activity. The antidiabetic action of Hydroalcoholic extracts *Nymphaea caerulea* leaves can also be attributed to the intestinal alpha-amylase inhibitory activity. Further studies are required to elucidate whether Hydroalcoholic extract *Nymphaea caerulea* leaves have antidiabetic potential by in vivo for validating the traditional claim of the plant.

Key words: *Nymphaea caerulea,* Extraction, Phytochemical screening, Anti-infllamatry activity, Hydroalcoholic extract

Introduction

Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic. Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes (Kumar *et al.,* 2013). Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmic fluid and blood cells. The complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. However, many studies have been continuing an inflammatory disease and the side effects of the currently available anti-inflammatory drugs are the major problem during their clinical uses. The most commonly used drug for management of inflammatory conditions are non steroidal anti-Inflammatory drugs (nsaids), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers.

Inflammation is a protective strategy evolved in higher organisms in response to detrimental insults such as microbial infection, tissue injury and other noxious conditions. It is an essential immune response by the host that enables the removal of harmful stimuli as well as the healing of damaged tissue. Acute inflammation has therefore been considered as a part of innate immunity, the first line of host defense against foreign invaders and danger molecules. Mankind has known the classical symptoms of inflammation for hundreds of years, which include redness, pain, swelling and heat (Medzhitov, 2008). However, emerging literature suggests that inflammation operates as a much-sophisticated system than ever thought at the molecular level. The entire course of inflammation comes with many different processes involved in its initiation, regulation and resolution. Nowadays a diverse range of inflammations have been identified, with many different forms initiated by numerous stimuli and governed by various regulatory mechanisms. Due to its extensive and widespread nature,

inflammation is believed to have an impact on every aspect of normal human physiology and pathology.

Nymphaea caerulea, known in English as Egyptian lotus, blue lotus, blue water lily (RSA), Cape water lily (RSA), frog's pulpit (RSA), blue lotus of the Nile blue waterlily, blue Egyptian lotus, blue Egyptian water lily (India), sacred blue lily of the Nile (India), Cape blue waterlily (USA) and sacred blue lily, is a water lily in the genus Nymphaea, a botanical variety *of Nymphaea caerulea*. The aim of present work extraction, phytochemical screening and anti-infllamatry activity of hydroalcoholic extract of *Nymphaea caerulea*

Material and Methods

Collection of plant materials

Organoleptic characters, morphological characters, and microscopical examination would help in identifying crude drug. For identification of unknown drugs herbariums and leading botanical gardens are of great help. The leaves of selected plant namely *Nymphaea caerulea* were identified and collected from various areas of Madhya Pradesh on the basis of geographical availability. The entire plant drug was authenticated by expert botanist of Department of Botany Geetanjali College Bhopal. All collected plant drug were cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of plant drugs

All Collected plant drugs were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant drugs were weighed (250 gm of each plant drug namely leaves of *Nymphaea caerulea* and packed in Soxhlet apparatus. Each plant drug was defatted with petroleum ether (40°-60°C) for about 12 hrs separately & complete defatting was censured by placing a drop from the thimble on a filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove last traces of petroleum ether. The defatted plant drugs were subjected to extraction by methanol and water as solvent. The process was carried out for about different timings for different solvents. The liquid

extracts were collected in a tarred conical flask. The solvent removed by distillation. Last traces of solvent being removed under vacuum. The extracts obtained with each solvent were weighed to a constant weight and percentage w/w basis was calculated.

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of leaves of *Nymphaea caerulea*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Khandelwal KR 2002, Ansari HS 2001, and Gafar Kemi 2012).

Quantitative estimation of phenols and flavonoids

Estimation of total phenolic content

Folin-Ciocalteu (FC) colorimetric method is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color that exhibits a broad light absorption with a maximum at 725 nm. In the present investigation, Folin-Ciocalteu (FC) colorimetric method is employed for the quantitative estimation of total phenolic content present in different solvent extract of leaves of *Nymphaea caerulea*.

Procedure

The total phenolic content was estimated according to the method of Makkar et al. 1993. The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5ml of sodium carbonate solution (20%) were added. After mixing, solution was incubated at 90oC for one minute and the absorbance was recorded at 725 nm against the reagent blank. Using catechol, a standard curve was prepared. Using the standard curve, the total phenolic content was calculated and expressed as Catechol equivalent in µg/mg of extract.

Estimation of total flavonoids content

In the present investigation aluminum chloride colorimetric method is employed for the quantitative estimation of flavonoids present in different solvent extract of leaves of

Nymphaea caerulea. The principle of aluminum chloride colorimetric method is that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols.

Procedure

Total flavonoid contents of all the extracts were determined by the method of Zhishen (1999) and expressed as catechol equivalent in µg/mg of extract. An aliquot (1ml) of extracts or standard solution of catechol (20, 40, 60, 80 and 100mg/ml) was added with 0.3ml of 5% $NaNO_2$, 0.3 ml of 10% AlCl3. The mixture was incubated for 5 min at room temperature then it was added with 2ml NaOH. The total volume was made up to 10ml by adding distilled water. The solution was mixed well and the absorbance was measured at 510 nm. Using the standard curve, the total flavonoid content was calculated.

***In-vivo* anti-inflammatory activity**

Animals and experimental protocol

Albino rats of both sexes weighing between 200-250 gm were used for experiment. They were housed in standard environmental condition. Animals were given standard pellet and water in free access. All procedures and protocols were approved by the Institution of Animal Ethics Committee (IAEC). All animal experiments were carried out strictly in accordance with the guidelines of CPCSEA.

Determination of anti-inflammatory activity

In present study formalin induced rat hind paw edema method was used for determination of anti-inflammatory activity. Analgin was used as standard drug.

Formalin induced rat hind paw edema method

Albino Wister rats (200-250 gm) were used for the study. The animals were divided into five groups of six rats in each group. Inflammation was produced in animals by injection of 0.1 ml of 1%w/v formalin into the sub plantar region of left hind paw (Mohan et al., 2009).

Grouping of Animals

Group I served as normal control animals administered distilled water.

Group II formalin control animals administered formalin (0.1 ml).

Group III standard animal administered formalin (0.1 ml) and analgin (30 mg/kg).

Group IV animal administered formalin (0.1 ml) and Hydroalcoholic extract of *Nymphaea caerulea* (Leaves) (200 mg/kg).

Group V animal administered formalin (0.1 ml) and Hydroalcoholic extract of *Nymphaea caerulea* (Leaves) (300 mg/kg).

The paw edema volume was measured mercury displacement technique using plethysmograph at 0, 15, 30 and 60 min after formalin injection. The difference between the initial and subsequent values gave the actual edema volume which was compared with control

The paw volume was measured after formalin injection. The average paw inflammations in the group of extract treated rats were compared with control. The inhibition of inflammation was calculated using the formula

$$\% \text{ Inhibition } = \frac{D0 - Dt}{D0} x\ 100$$

Where D0 was the average inflammation of the group of rats after 15 min treatment of formalin Dt was the average inflammation of the drug treated rats after 30 and 60 min.

Statistical analysis

All values of experiment are expressed as Mean ± SEM. The data obtained from pharmacological screening were statistically analysed by one way analysis of variance (ANOVA) followed by Dunnett's comparison test using Graph pad Instat. $P < 0.01$ was considered to be statistically significant.

Results and Discussion

From the results obtained it is clear that the *Nymphaea caerulea* Wight leaves shows the presence of saponins, flavonoids and amino acid were found present in whole parts when extracted with different solvents using maceration extraction procedure. The phytochemical analysis of *Nymphaea caerulea* leaves indicates the presence of phenols and flavonoids present in sufficiently enough quantity according to preliminary

phytochemical analysis. Phenolic and flavonoids are the phytochemical that are present in Hydroalcoholic extract of *Nymphaea caerulea* leaves.

The yields were found to be (10.25 % w/w of crude drug) of petroleum ether extract with semisolid mass of brown colour, (16.27 % w/w of crude drug) of hydroalcoholic extract with orange black colour semisolid mass for *Nymphaea caerulea* leaves.

Total phenols and flavonoids contents in Hydroalcoholic extract obtained from *Nymphaea caerulea* leaves, was evaluated. The total phenol and flavonoids content of the Hydroalcoholic extract *Nymphaea caerulea* leaves present in the excellent amount.

The present finding reveals that Hydroalcoholic extract *Nymphaea caerulea* leaves efficiently inhibits alpha amylase enzymes in vitro in a dose dependent manner. The Hydroalcoholic extract *Nymphaea caerulea* leaves showed a dose dependent inhibitory effect on alpha-amylase activity. The antidiabetic action of Hydroalcoholic extracts *Nymphaea caerulea* leaves can also be attributed to the intestinal alpha-amylase inhibitory activity. Further studies are required to elucidate whether Hydroalcoholic extract *Nymphaea caerulea* leaves have antidiabetic potential by in vivo for validating the traditional claim of the plant.

Table 1: Extractive values obtained from *Nymphaea caerulea* leaves using different solvents

S.N.	Solvent	Time of extraction (Hours)	Color of extract	% Yield
1	Petroleum ether	12	Brown	10.25%
2	Ethanol: Water	28	Orange-Black	16.27 %

Table 2: Preliminary phytochemical screening of *Nymphaea caerulea* leaves

S.N.	**Phytoconstituents**	**Test Name**	**Hydroalcoholic Extract**
1	Alkaloids	Mayer's Test	Absent
		Dragendorff's Test	Present

2	Glycosides	Raymond's Test	Present
		Killer Killani Test	Present
3	Carbohydrates	Molisch's Test	Absent
		Fehling's Test	Absent
4	Tannins	Vanillin- HCl Test	Present
		Gelatin Test	Absent
5	Flavonoids	Lead acetate	Present
		Shinoda Test	Present
6	Resins	Color detection with ferric chloride	Absent
		Turbidity Test	Absent
7	Steroids	Libermann- Bur chard Test	Present
		Salkowski Reaction	Present
8	Proteins & Amino acids	Biuret Test	Present
		Precipitation test	Absent
		Ninhydrin Test	Present
9.	Phenols	Ellagic Acid Test	Present

Table 3: Total Flavonoid content of Hydroalcoholic extract of leaves of *Nymphaea caerulea*

S. No.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	Hydroalcoholic extract (100µg/ml)	23.33 ± 0.014

n=3, values are given in SEM

Table 4: Anti-inflammatory activity Hydroalcoholic extract of *Nymphaea caerulea* (leaves)

Group and Dose	0min (before treatment of formalin)	15min after Formalin treatment of paw volume	30 min after drug administration of paw volume	% Inhibition after 30 min	60min after drug administration of paw volume	% Inhibition after 60 min
Control (distal water)	0.414±0.014	0.414±.004**	0.416±0.001**	---	0.421±0.003**	----
Formalin (1%) (0.1ml)	0.471±0.012	0.922±.0025**	0.93±0.0021**	---	0.913±0.001**	----
Formalin + Standard (Analgin 30mg)	0.433±0.080	0.984±0.006**	0.836±0.003**	16.07%	0.67±0.001**	34.66%
Formalin + *Nymphaea caerulea* extract (200 mg)	0.459±0.016	0.895±0.001**	0.853±0.008**	5.87%	0.829±0.008**	7.01%
Formalin + *Nymphaea caerulea* extract (300 mg)	0.463±0.008	0.922±0.0008**	0.896±0.40**	3.77%	0.754±0.004**	18.12%

Result expressed as Mean ± SEM from six observations ** indicate P<0.01 significant.

Conclusion

The present finding of Phytochemical screening of the plant extract confirmed the presence of several bioactive compounds like alkaloids, flavones, tannins and phenols which could be responsible for the versatile medicinal properties of this plant. The present study also revealed that Hydroalcoholic extract *Nymphaea caerulea* leaves exhibit considerable α-amylase inhibitory activities. Further, this study supports that the concerned plant can be used as an ethnomedicinal for management of diabetes.

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