



“Antibacterial And Anti Inflammatory Effect Of *Nymphaea Stellata*”

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Abstract:

Medicinal plants are part and parcel of human society to combat diseases. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand because of their wide biological and medicinal activities, higher safety margins and lesser costs ^{3, 4}. The present aim of the study is to prove of Anti-Microbial Activity and anti-inflammatory Activity of *Nymphaea stellata*. To the methanol extract preliminary phytochemical screening was performed by using standard chemical tests. The Methanol extracts of plant for its infectious and/or inflammatory nature were screened for in vitro antibacterial and anti-inflammatory activities. The albino rats were used for acute toxicity studies as per OECD guidelines. The Extracted compounds **MEBM, BME, BMC, BMH, BMQ** has been evaluated for their antimicrobial activity and anti-inflammatory activity. Antibacterial activity was tested using the agar diffusion method while anti-inflammatory activity was tested using the carrageen induced paw edema method. The zone of inhibition and anti-inflammatory was measured as per standard protocol which indicated the test showed significance results with standard drug.

Index terms: *Nymphaea stellata* Albino rats, OECD, agar diffusion, antibacterial, zone of inhibition

Introduction

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization ¹. They usually contain many biologically active ingredients and are used primarily for treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs².

Infectious diseases are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of human immunodeficiency virus (HIV) infections ³. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections⁴. Plant medicines are used on a worldwide scale to prevent and treat infectious diseases ⁸. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found in vitro since they have antimicrobial properties and also have anti inflammatory properties. Medicinal plants have been used to treat inflammation and other health issues since ancient times. Recent reports have revealed that these molecules exhibit an anti-inflammatory effect by suppressing inflammatory mediators involved in inflammatory processes, including cytokines, chemokines, and pro- and neo inflammatory mediators. Plants can reduce inflammation by blocking inflammatory cells, blocking the inflammatory process, blocking the arachidonic acid metabolism and blocking enzymes. Some examples of plants with anti immunity properties like turmeric borage oil daphne etc. Chemical screening of the *Nymphaea stellata* leaves led to the discovery of an alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free aminoacids and triterpenoids which are responsible for its anti-inflammatory and antibacterial activity.

Materials and methods:

1. Antibacterial activity:

Materials:

Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa* were obtained from the bacteria unit Aditya College of pharmacy. All the bacterial strains were maintained as top strains in microbiome cryo wires and kept at minus 80 degrees centigrade until used blank antibiotic disk, Moxifloxacin and streptomycin (as a standard) were purchased from market.

The in-vitro methods used for determining the antibacterial activity

a) Cup plate method/Agar diffusion method:

b) Serial dilution method

a) Disc diffusion method /Cup plate method/Agar diffusion method

Disk diffusion method for anti microbial susceptibility testing was carried out according to the standard method by Bauer et al (1966) to assess the presence of antibacterial activities of the plant extract. A bacteria culture (which has been adjusted to 0.5 mc Farland standard), was used to lawn Muller Hinton Agar plates evenly using sterile swab. The plates were dried for 15 minutes and then used for the sensitivity test. The discs which had been impregnated with a different diluted plant extract were placed on the Muller Hinton Agar surface. Each test plate comprises of six discs. One positive control, which is standard commercial antibiotic disc, one negative control and four treated discs. The Standard antibiotic discs were Moxifloxacin and streptomycin for *Staphylococcus aureus* and *E coli* respectively. The negative control was DMSO (100%). Besides the controls, each plate had four treated discs plated above equidistance to each other. The plate was then incubated at 37⁰ C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation the plates were examined for inhibition zone. The inhibition zone were the measured using calipers and recorded. The test were repeated three times to ensure reliability.

b) Serial dilution method:

The bacterial inoculum uniformly spread using sterile cotton swab on a sterile petri dish MH agar. Nine serial dilutions yielded concentrations of hundred 80, 60,40,20,10, 2.5 and 1.2 for Mg/ ml for extracts and fractions and four serial dilations yielded conc. of 20, 15, 10 and 5 mg/ml for pure substances. 50 uL natural products were added to each of the five wells. The systems were incubated for 24 hours at 36⁰ C +/- 1⁰ C under aerobic conditions. After incubation, confirmed bacterial growth was measured. Inhibition of the bacterial growth was measured in mm.

2. Anti-inflammatory activity:

Wistar rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow. All the experimental procedures are protocols used in this study were reviewed by Institutional Animal Ethics Committee.

Acute Toxicity Studies:

The acute toxicity was performed according to OECD guidelines (NO.423)

The selected albino rats were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 10, 30, 100, & 300 mg/ kg body weight. Immediately, after dosing, the animals were observed continuously for first four hours for behavioral changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhea, lethargy, sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/ kg, body weight) of Methanol leaf extract of *Nymphaea stellata*. Selected animals are used to evaluate anti-inflammatory activity studies.

Anti-inflammatory activity

Animals Albino Wistar rats of either sex with average weight 150–200 g were used. All animals used in the study were housed in standard environmental conditions and fed with standard rodent diet with water ad libitum. All animal procedures were followed in three groups, namely control, test, and standard of six animals each. The Institutional Animal Ethical Committee approved protocol of experiment (CPCSEA/1093), and all the animals used in this work were treated according to the norms established by CPCSEA.

Carrageenan-induced rat paw edema

Animals were fasted for 24 h before the experiment with water ad libitum. Edema was induced by injecting 0.1 ml of 1% w/v carrageenan in saline into the plantar side of right hind paw of rat 1 h before each experiment. Herbal gel 1% was applied to the plantar surface of the hind paw by gentle rubbing 50 times

with the index finger. Rats of the control groups received the plain gel base. 1% valdecoxib gel 0.2 g was applied in the same way as a standard. Drugs or placebo was applied 1 h before the carrageenan injection. Paw volume was measured immediately after carrageen injection and at 1, 2, 3, and 4 h intervals after the administration of the noxious agent using a plethysmometer. Percentage inhibition in paw volume is calculated using the formula.

$$\% \text{inhibition} = [\text{paw volume control} - \text{paw volume test} \div \text{paw volume control}] \times 100$$

Results:

Table: Qualitative phytochemical Evaluation of *Nymphaea stellata*.

Parameters	value
1.Alkaloid	+
2.Carbohydrates	+
3.Glycosides	-
4.Flavonoids	++
5.Tannins&Phenoliccompounds	+
6.Proteins&Aminoacids	+
7.Saponins	+
8.Sterols or Triterpenes	+

++: high content, +: moderate, -: Negative,

From the qualitative phytochemical analysis of ethanolic extract of *Nymphaea stellata*. It contains alkaloids, Tannins, phenolic compounds, sterols, saponins, protein and amino acids and high amount of flavonoids

Table: Zone of inhibition of *Nymphaea stellata* against on *Streptococcus aureus*:

Compounds	50µg/ml	100µg/ml	1000µg/ml
MEBM	5 mm	9 mm	14 mm
BME	10 mm	12 mm	18 mm
BMC	6 mm	10 mm	15 mm
BMH	10 mm	12 mm	14 mm
BMQ	6 mm	10 mm	16 mm
CPN	14 mm	18 mm	22 mm
MXN	16 mm	20 mm	24 mm

Figure: Zone of inhibition of *Nymphaea stellata* against on *Streptococcus aureus*:

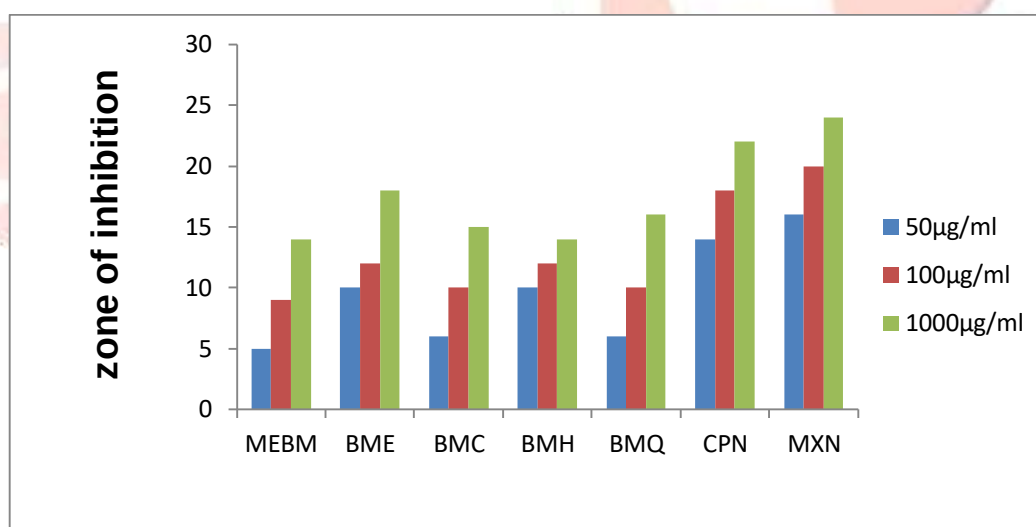
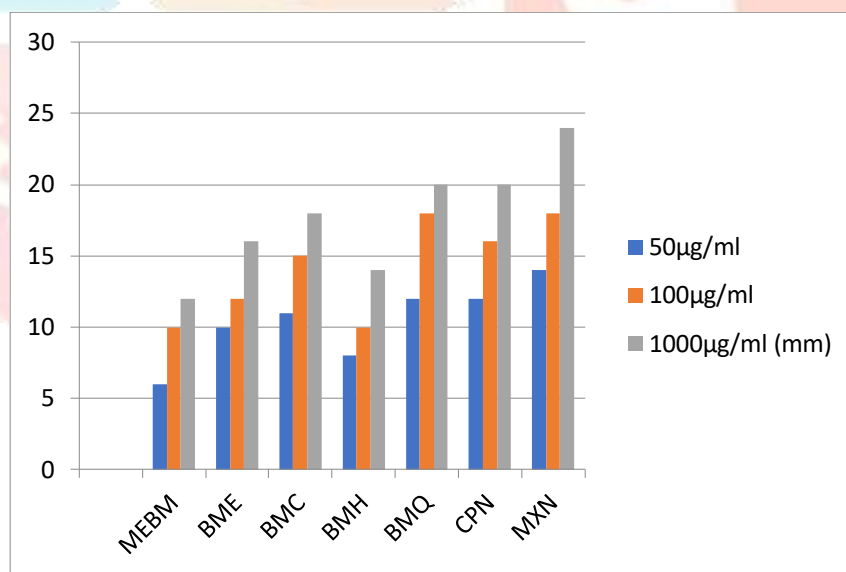


Table: Zone of inhibition of solvent extract of *Nymphaea stellata* against *E.Coli*:

Compounds	50µg/ml (mm)	100µg/ml (mm)	1000µg/ml (mm)
MEBM	6	10	12
BME	10	12	16
BMC	11	15	18
BMH	8	10	14
BMQ	12	18	20
CPN	12	16	20
MXN	14	18	24



ANTI-INFLAMMATORY ACTIVITY

Anti-inflammatory activity of Methanolic extract was investigated by carrageenan-induced paw edema method, and results obtained are shown in Table. Edema inhibition in carrageenan-induced rat paw edema by Methanolic extract and standard 1% valdecoxib is represented in Fig. Whereas Methanolic extract have shown significant percent inhibition. Methanolic extract significantly inhibited the inflammation to the extent

of 58.91%, 62.14% at 3 h and 65.26% 4 h, respectively, while the reference drug reduced the inflammation by 65.92% at 3 h and 75.30% at 4 h. The anti-inflammatory effect of Methanolic extract was comparable to that of valdecoxib at respective time point.

Table 9.8: Effect of various Methanolic extract on carrageenan-induced paw edema in rats

Control	0.29±0.0105	-	0.50±0.081	-	0.61±0.021	-	0.80±0.042	-
Extract	0.20±0.061	23.10	0.22±0.0082	58.91	0.24±0.0010	62.14	0.29±0.0026	65.26
Standard	0.18±0.0015	39.01	0.21±0.0026	53.19	0.2±0.0082	65.92	0.19±0.014	75.30

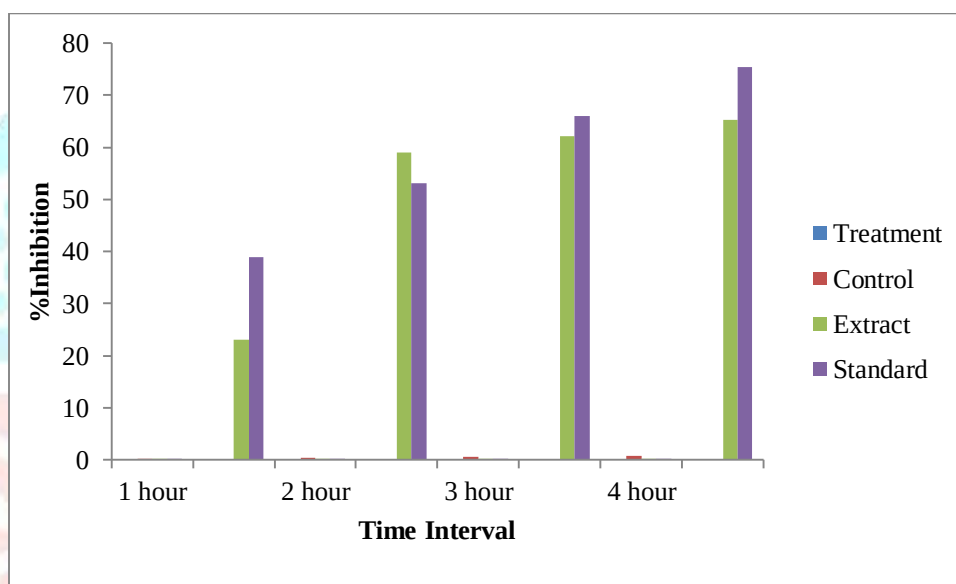


Fig.9.18: Percent edema inhibition in carrageenan-induced rat paw edema by Methanolic extract and standard 1% valdecoxib.

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