

Original Article

**IN-VITRO COMPARATIVE STUDY OF ANTI-INFLAMMATORY AND ANTI-ARTHRITIC EFFECTS OF FLEMINGIA STRICTA ROXB AND NYMPHAEA NOUCHALI LEAF**

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ABSTRACT

**Objective:** To evaluate the comparative study of anti-inflammatory, anti-arthritis activity of methanol extract of *Flemingia stricta* and *Nymphaea nouchali* leaf.

**Methods:** Human Red Blood Cell (HRBC) membrane stabilization method was evaluated for anti-inflammatory activity. Anti-denaturation method was performed by using Bovine Serum Albumin (BSA) to evaluate the anti-arthritis potential.

**Results:** The *in vitro* anti-inflammatory activity of the methanol extracts of *Flemingia stricta* and *Nymphaea nouchali* showed 81.85±0.67% (P<0.01) and 85.59±0.58% (P<0.01) of membrane stabilization at 1000µg/ml conc. and 51.85±0.49% (P<0.01) and 70.63±0.50% (P<0.01) at 31.25µg/ml respectively. All the results were compared with standard Diclofenac which showed 93.15±1.03% protection at 1000µg/ml conc. The *in vitro* study on both leaves also showed the presence of significant anti-arthritis activity. Here the extracts showed 70.43±1.42% (P<0.01) and 83.33±0.54% of protein denaturation at the highest conc. (1000 µg/ml) and 39.25±1.08% (P<0.01) and 38.71±0.93% (P<0.01) at the lowest conc. (31.25µg/ml), in where the standard drug displayed the 86.56±2.15% at 1000µg/ml and 51.08±1.42% at 31.25 µg/ml.

**Conclusion:** These results suggest that both the methanol extract of *Flemingia stricta* and *Nymphaea nouchali* possess significant anti-inflammatory and anti-arthritis activity.

**Keywords:** *Flemingia stricta*, *Nymphaea nouchali*, Anti-inflammatory, Anti-arthritis, HRBC, Protein denaturation.

INTRODUCTION

*Flemingia stricta* (Fabaceae) Roxb. is an erect subshrub, distributed in the Southeast Asian country such as-Bangladesh, Bhutan, China, India, Indonesia, Laos, Myanmar, Philippines, Thailand and Vietnam [1, 2]. In Bangladesh, it is available in Chittagong, Chittagong Hill Tracts and Sylhet. It is known as Charchara (in Bangla) and Krangdunaduepay, Sai Kheu (Marma), Keramkana (Tripura), Tamatamaking (Khumi) and Harsanga, Khaskura, Uskura (Chakma) in local tribes of Chittagong, Bangladesh [3]. *Flemingia stricta* is used by Chakma healers for treatment of polio. The species is also used to treat rheumatism followed by bone fracture, cough, asthma, goiter, urinary problems, snake bite, insect bite, leprosy, tumor and cancer, caries, hysteria, tuberculosis, insomnia and intestinal worms [4-6].

*Nymphaea nouchali* (Nymphaeaceae) is another important medicinal plant which is also known as water lily, distributed in Bangladesh, Australia, Afghanistan, China, Egypt, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Philippines, South Africa, Sri Lanka, Thailand, Vietnam, Zimbabwe [7, 8]. In Bangladesh, it is known as Sapla, Sada Sapla, and Shaluk. Traditionally it is also known as Bibalchak (Mandi), Gekhak-afluk (Mandi), Aphlak (Garo) in local tribes of Bangladesh [9]. Roots and rhizomes of this plant contain protein, tannic and gallic acids, starch, gum, resin, glucosides and the alkaloids, nupharine and nymphaeine. Leaves contain flavone, glucoside, myricitrin, tannic acid, phytosterin, steroids and flavonoids. Flowers contain a cardiac glycoside, nymphalin having digitalis-like action. Flowers and rhizome also yield two alkaloids, both showing sedative action in small doses [10].

Powdered rhizomes are demulcent and diuretic, used in piles, dysentery and dyspepsia. Flowers are astringent, cardiotoxic and refrigerant, alleviative of the cough, bile, vomiting, giddiness, and worms and burning of the skin. Filaments are astringent and cooling, useful in burning of the body, bleeding piles and menorrhagia. Seeds are used as a cooling medicine in cutaneous diseases [11]. Rhizome paste is used for menstruation. The dried rhizome powder is used

for dyspepsia (Garo) [12]. Rhizome paste is used to treat menstruation problem (Mandi) [13]. The literature survey revealed that there are no scientific studies carried out regarding the anti-inflammatory, anti-arthritis activity. Thus the experiment was designed to evaluate the comparative *in vitro* anti-inflammatory, anti-arthritis activity of *F. stricta* and *N. nouchali* leaf extracts.

MATERIALS AND METHODS

Plant material

*F. stricta* and *N. nouchali* leaves were collected from a local area (Bhatary and Anowara) of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Assistant Professor, Department of Botany, University of Chittagong, Bangladesh.

Preparation of extract

The leaves were dried and ground. The ground leaves (300g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

Chemicals and drugs

The chemicals used were Bovine serum albumin (BSA), Diclofenac Sodium, Sodium Dihydrogen phosphate, Disodium hydrogen phosphate, Sodium Chloride, Dextrose, sodium citrate, citric acid, were purchased from Sigma-Aldrich. All chemicals in this investigation were of analytical reagent grade.

*In-vitro* anti-inflammatory activity

The human red blood cell (HRBC) membrane stabilization method

The principle concerned in this method is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. The blood was collected (2 ml) from healthy human volunteer who had

not taken any NSAIDs for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with iso saline and a 10% v/v suspension was made and kept at 4 °C undistributed before use. Various concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) of extracts were prepared in normal saline, diclofenac sodium as standard with different concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) and blood control (distilled water instead of hyposaline to produce 100% hemolysis) were separately mixed with 1 ml (0.15M) of sodium phosphate buffer, 2 ml of hyposaline and 0.5 ml of 10% HRBC suspension was added to prepared. Erythrocyte suspension was absent in drug control while drugs were omitted in blood control. All the assay mixture was incubated at 37°C for 30 min and centrifuged at 3000rpm for 20 min and hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm [14]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\% \text{ of membrane stabilization value} = 100 - [(D_{tv} - D_{cv}) / B_{cv}] \times 100$$

Where, Dtv was the Drug test value, Dcv was drug control value and Bcv was the blood control value.

Here the blood control represented 100% lysis.

#### In vitro anti-arthritis activity

For the evaluation *in vitro* anti-arthritis activity of *F. stricta* and *N. nouchali*, the method used was "inhibition of protein denaturation" with diclofenac sodium as a standard [15-18]. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (methanol extract of *F. stricta*). The test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) of methanol extract of *F. stricta* and diclofenac sodium (standard) were taken respectively. All the solutions were

adjusted to pH 6.3 using 1 N HCl. The samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with diclofenac sodium.

The percentage inhibition of protein denaturation of different concentrations is tabulated in table 1. The percentage inhibition of protein denaturation can be calculated as:

$$\% \text{ of inhibition} =$$

$$[100 - (\text{OD of test solution} - \text{OD of product control})] \times 100$$

Where OD = optical density.

Control represents 100% protein denaturation. The results were compared with diclofenac sodium.

#### Statistical analysis

The results were expressed as mean of the three repetitions and standard deviations were calculated. Statistical comparisons were made using the Independent t-test and p<0.05 was considered as significant.

## RESULTS

#### Anti-inflammatory study

Methanol extract of *F. stricta* and *N. nouchali* was studied for *in vitro* anti-inflammatory activity by HRBC membrane stabilization method which is reported in table 1. The *in vitro* anti-inflammatory activity of the extracts was concentration dependent, with the increasing concentration, the activity is also increased. Here, the methanol extract of *F. stricta* showed 81.85±0.67% (P<0.01) of membrane stabilization at 1000µg/ml conc. and 51.85±0.49% (P<0.01) at 31.25µg/ml and in case of *N. nouchali*, it showed 85.59±0.58 % (P<0.01) at higher and 70.63±0.50 % (P<0.01) at the lower conc. All the results were compared with standard Diclofenac which showed 93.15±1.03 % protection at 1000µg/ml conc.

Table 1: It shows the stabilization of membrane (%) of *F. stricta* and *N. nouchali*. FS: *Flemingia stricta*, NN: *Nymphaea nouchali*

Concentration(µg/ml)	Stabilization of membrane (%)		
	FS (%)	NN (%)	Diclofenac sodium (%)
31.25	51.85±0.49**	70.63±0.50**	65.19±0.37
62.5	57.59±0.67**	73.27±0.33	70.56±0.85
125	62.96±0.19**	75.03±1.08*	81.67±0.32
250	67.04±0.37**	79.87±0.69*	85.00±1.16
500	75.19±0.49**	84.82±0.50**	90.74±0.81
1000	81.85±0.67**	85.59±0.58**	93.15±1.03

Values are expressed as mean±SEM of three replicate (n=3), \*\*P<0.01, \*P<0.05

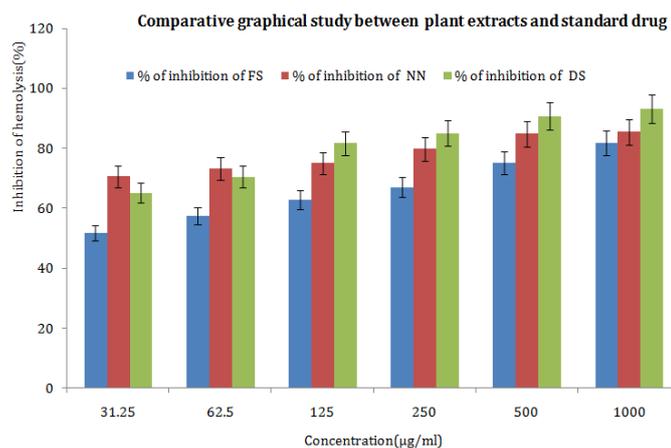


Fig. 1: It shows the inhibition of hemolysis (%) of *Flemingia stricta*, *Nymphaea nouchali* and Diclofenac sodium

### Anti-arthritis study

Different concentrations of methanol extract of *F. stricta*, *N. nouchali* and diclofenac sodium was tested for anti-arthritis activity and found significant percentage inhibition in protein denaturation (table 2). Here, in lower concentration (31.25 µg/ml) the extract of *F. stricta* and *N. nouchali* showed 39.25±1.08 % (P<0.01) and

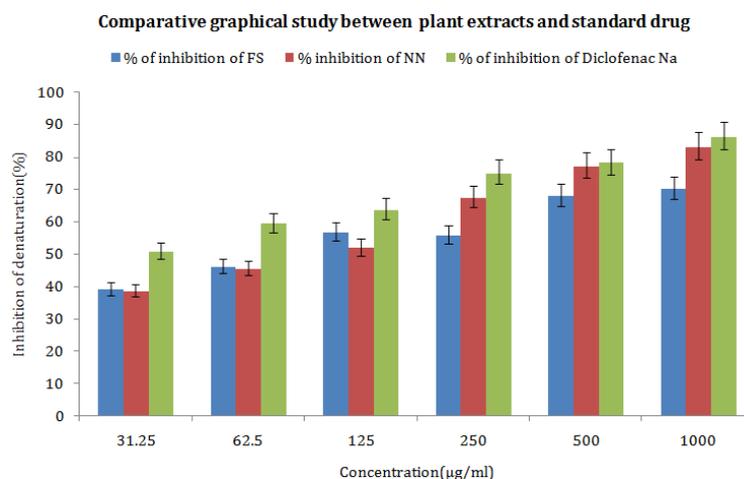
38.71±0.93% (P<0.01), where the standard drug diclofenac sodium showed 51.08±1.42% of inhibition.

And in higher concentration (1000 µg/ml), the extract of *F. stricta* and *N. nouchali* exhibited the 70.43±1.42% (P<0.01) and 83.33±0.54% of inhibition, in where the diclofenac sodium exhibited 86.56±2.15% of inhibition of protein denaturation.

**Table 2: It shows the inhibition of protein denaturation (%) of *Flemingia stricta* and *Nymphaea nouchali*. FS: *Flemingia stricta*, NN: *Nymphaea nouchali***

Concentration(µg/ml)	Inhibition in protein denaturation (%)		
	FS (%)	NN (%)	Diclofenac sodium (%)
31.25	39.25±1.08**	38.71±0.93**	51.08±1.42
62.5	46.24±1.42**	45.70±0.54**	59.68±1.61
125	56.99±2.34	52.15±2.69*	63.98±0.54
250	56.08±2.46**	67.74±1.86*	75.27±1.07
500	68.28±0.54*	77.42±0.93	78.49±1.42
1000	70.43±1.42**	83.33±0.54	86.56±2.15

Values are expressed as mean±SEM of three replicate (n=3). \*\*P<0.01, \*P<0.05



**Fig. 2: It shows the inhibition of denaturation (%) of *Flemingia stricta*, *Nymphaea nouchali* and Diclofenac sodium**

### DISCUSSION

*F. stricta* and *N. nouchali* leaves extracts exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of the erythrocyte membrane. The erythrocyte membrane is similar to the lysosomal membrane [19] and its stabilization implies that the extract may as well stabilize lysosomal membranes. It is important to stabilize the lysosomal membrane to limit the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release [20]. Some of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potential of their anti-inflammatory effect. Though the exact mechanism of membrane stabilization by the extract is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The process may stimulate or enhance the efflux of these intracellular components which can be prevented by the extract [21]. Methanol extract of *F. stricta* and *N. nouchali* showed significant (P<0.01) anti-inflammatory activity (81.85±0.67% and 85.59±0.58%) at the conc. of 1000 µg/ml. On the basis of the above results it can be concluded that *N. nouchali* has significant anti-inflammatory activity than *F. stricta*.

Arthritis is a form of joint disorder that involves inflammation of one or more joints, responsible for painful swelling, stiffness, loss of

function in the joint. Denaturation of protein is one of the causes of arthritis as documented. Production of autoantigen in certain arthritic disease may be due to denaturation of protein. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding [22]. Here, both the methanol extract have shown significant (P<0.01) activity at various concentrations and the effects were compared with the standard drug diclofenac sodium. The maximum percentage inhibition of protein denaturation of *F. stricta* and *N. nouchali* were observed as 70.43±1.42% and 83.33±0.54% at 1000 µg/ml which were close to the percentage of inhibition of diclofenac sodium (86.56±2.15%). According to the result, it can be stated that *N. nouchali* extract has the capability of controlling the production of autoantigen to inhibit the denaturation of protein significantly.

### CONCLUSION

From the above studies, it can be concluded that *Nymphaea nouchali* have maximum anti-inflammatory and anti-arthritis activity and it could be a natural anti-inflammatory and anti-arthritis source and thus could be useful as therapeutic agents in preventing the diseases. Further studies are needed for their active principle to elucidate.

### CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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