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In- vitro comparative study of anti-inflammatory and anti-arthritic effects of the methanol extract of *Cissus pentagona* Roxb and *Thunbergia grandiflora* Roxb leaf

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Abstract
Objective: To evaluate comparative study of anti-inflammatory, anti-arthritic activity of methanol extract of *Cissus pentagona* Roxb. (Vitaceae) and *Thunbergia grandiflora* Roxb. (Acanthaceae) 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-arthritic potential.
Results: The *in vitro* anti-inflammatory activity of the methanol extracts of *C. pentagona* and *T. grandiflora* showed 78.89% and 89.11% of membrane stabilization at 1000 µg/ml conc and 42.22% and 63.37% at 31.25 µg/ml respectively. All the results were compared with standard Diclofenac which showed 94.44% protection at 1000 µg/ml conc. The *in vitro* study on both leaves also showed the presence of significant anti-arthritic activity. Here the extracts of *C. pentagona* and *T. grandiflora* showed 69.35% and 74.19% of protein denaturation at highest conc (1000 ug/ml) and 38.71% and 45.16% at lowest conc (31.25 ug/ml) respectively, in where the standard drug showed the 85.49% at 1000 ug/ml and 51.61% at 31.25 ug/ml conc.
Conclusion: These result suggested that both the methanol extract of *C. pentagona* Roxb. and *T. grandiflora* Roxb. contains anti- inflammatory and anti-arthritic activity.

Keywords: *Cissus pentagona*, *Thunbergia grandiflora*, anti-inflammatory, anti-arthritic, HRBC, inhibition, protein denaturation.

1. Introduction

Cissus pentagona Roxb. is a large woody climber, distributed in southeast Asian country such as- Bangladesh, Bhutan, China, India, Indonesia, Laos, Myanmar, Philippines, Thailand, Vietnam [1]. In Bangladesh, it occurs in Chittagong, Chittagong Hill Tracts and Cox's Bazar. It is known as Sona-tola (in Bangla) and Hajjar ludi (Chakma), poipruchala (Tripura) in local tribes of Chittagong, Bangladesh. The species is applied to affected areas to treat skin disease (Chakma). Roots are used to prepare a paste, which is applied to the affected areas for the treatment of elephantiasis (Tripura) [2]. The roots of this plant in combination with other plants are also used for the treatment of filaria by the tribal in Chittagong Hill Tracts [1]. Another medicinal plant, *Thunbergia grandiflora* Roxb. (Acanthaceae) is a large climbing or twining shrub, which is found widely all over the world such as India, China, Indo-China, Myanmar and many tropical countries of Africa and the New World [3]. It is found throughout the Bangladesh, especially in forests of Gajipur, Chittagong, Chittagong Hill Tracts, Cox's Bazar, Tangail [2]. Generally, it is known as Black Clock Vine, Blue Trumpet Vine. In Bangladesh it is called as Kauathuti, Nallata, and Nillata and in local tribes it is known as Changra Morich, Danludi, Deldi Pata, Deldipata, Del Ladi, Del Ludi, Jeol Ludi, Jheol Ludi, Jhiol Ludi, Lachuney, Lachoainuyee, Lakkali, Lakkani, Sangara Marish (Chakma); Butto Luri, Lachuia-nui, La Soain Nuya, Lawchowanowai, Luck Chuyee-nu, Luck Choai Yee (Marma); Claicloyong (Khumi); Dumangkhong (Tripura) and Botualodi (Tonchonga) [3]. The species is used for the treatment of blood dysentery, cataract, conjunctivitis, diabetes, gout, hydrocele, hysteria, malaria, marasmus, ophthalmia, post eclampsia, pre-eclampsia, rheumatism, spermatorrhoea and stomachache [4]; stomach complaints [5]; eye diseases (opthalmia and conjunctivitis) [6] and elephantiasis, eye diseases, stomach complaints and urinary bladder stone [7].

2. Materials and Methods

2.1 Plant material

C. pentagona Roxb and *T. grandiflora* Roxb leaves were collected from a local area (Bhatiary) of Chittagong district, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Assistant professor, Department of Botany, University of Chittagong, Bangladesh.

2.2 Preparation of extract

The leaves were sun dried and ground. The ground (300 g) 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.

2.3 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, Germany. All chemicals in this investigation were of analytical reagent grade.

3. *In-vitro* anti-inflammatory activity

3.1 The human red blood cell (HRBC) membrane stabilization method

The principle involved in this method is stabilization of human red blood cell membrane by hypotonicity induced membrane lysis. At first, 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 isosaline and a 10% v/v suspension was made and kept at 4 ^{0}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 were incubated at 37 ^{o}C for 30 min and centrifuged at 3000 rpm for 20 min and hemoglobin content of supernatant solution was estimated spectrophotometrically at 560 nm [8]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula

% of membrane stabilization value =

$$\frac{100 - [(\text{Drug test value} - \text{Drug control value}) \times 100]}{\text{Blood control value}}$$

Where, the blood control represented 100% lysis

3.2 *In vitro* anti-arthritic activity

For the evaluation *in vitro* anti-arthritic activity of *C. pentagona* and *T. grandiflora*, the method used was "inhibition of protein denaturation" [9-12] using diclofenac sodium a standard. 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 *C. pentagona* and *T. grandiflora*). 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 *C. pentagona* (CPM), *T. grandiflora* (TGM) and diclofenac sodium (standard) were taken, respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. 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:

% of Inhibition = [100 - (OD of test solution - OD of product control)] × 100

Where OD = optical density

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

4. Results

4.1 Anti- inflammatory Study

The methanol extract of *C. pentagona* and *T. grandiflora* 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 were concentration dependent, with the increasing concentration, the activity is also increased. Here, the methanol extract of *C. pentagona* showed 78.89% of membrane stabilization at 1000µg/ml concentration and 42.22% at 31.25 µg/ml. In case of *T. grandiflora*, it showed 89.11% at higher and 63.37% at lower conc. All the results were compared with standard Diclofenac sodium which showed 94.44% protection at 1000 µg/ml conc.

Table 1: Percent stabilization of membrane of *C. pentagona* Roxb and *T. grandiflora* Roxb

Concentration(µg/ml)	Percent of membrane stabilization		
	CP(Test Solution)	TG(Test Solution)	Diclofenac sodium
31.25	42.22	63.37	65.56
62.5	48.89	68.32	70
125	53.33	72.61	81.1
250	58.33	77.23	86.67
500	70.56	81.19	90.56
1000	78.89	89.11	94.44

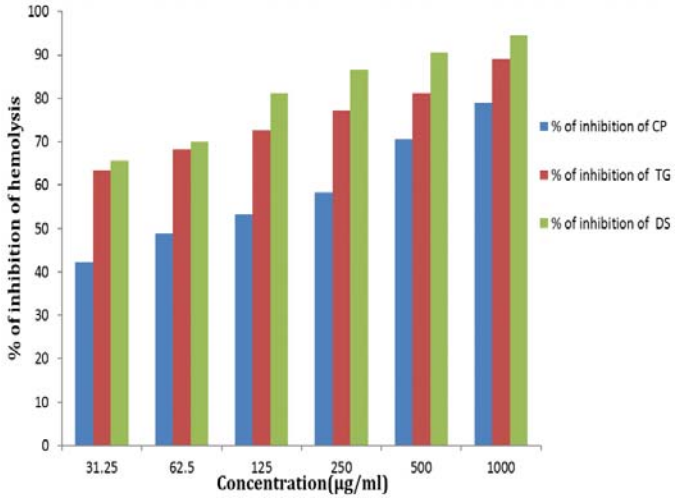

Fig 1: Comparison of the activity of *C. pentagona* Roxb and *T. grandiflora* Roxb leaves extract and the standard diclofenac sodium on hypotonic solution induced hemolysis of erythrocyte membrane.

4.2 Anti- arthritic study

Different concentrations of methanol extract of *C. pentagona* and *T. grandiflora* and diclofenac sodium were tested for anti-arthritic activity and found significant percentage inhibition in protein denaturation (Table 2). Here, in lower concentration the extract of *C. pentagona* and *T. grandiflora* showed 38.71% and 45.16%, where the standard drug diclofenac sodium showed 51.61% of inhibition. And in higher concentration, the extract of *C. pentagona* and *T. grandiflora* exhibited the 69.35% and 74.19% of inhibition, in where the diclofenac sodium exhibited 85.49% of inhibition of protein denaturation.

Table 2: Percent inhibition of protein denaturation of *C. pentagona* (Vitaceae.) and *T. grandiflora* (Acantheceae)

Concentration(µg/ml)	Percent of inhibition in protein denaturation		
	CP(Test Solution)	TG(Test Solution)	Diclofenac sodium
31.25	38.71	45.16	51.61
62.5	46.77	46.77	61.29
125	51.61	48.39	64.52
250	54.84	53.23	74.19
500	62.90	61.29	80.65
1000	69.35	74.19	85.49

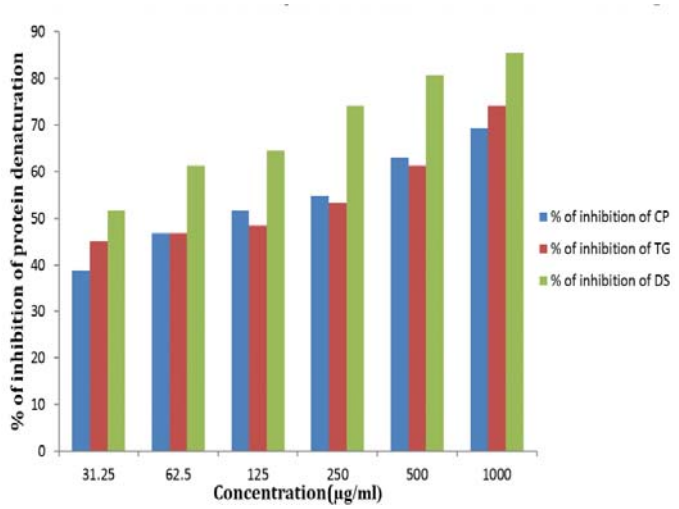

Fig 2: Comparison of the activity of *C. pentagona* Roxb and *T. grandiflora* Roxb leaves extract and the standard diclofenac on protein denaturation.

5. Discussion

C. pentagona and *T. grandiflora* leaves extracts showed membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is similar to the lysosomal membrane [13] and its stabilization suggests that these extracts have the ability to stabilize lysosomal membranes. It is essential to stabilize the lysosomal membrane to limit the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophil such as proteases and bactericidal enzymes, which cause further tissue inflammation and damage upon extra cellular release [14]. A few of the NSAIDs are known to possess membrane stabilization properties which may contribute to the potency of their anti-inflammatory effect. Though the exact mechanism of the membrane stabilization by the extract is unknown yet; Due to osmotic loss of intracellular electrolyte and fluid components, hypotonicity-induced hemolysis may arise from the shrinkage of the cells. This process may enhance the efflux of these intracellular components which can be prevented by the extract [15].

The methanol extracts of *C. pentagona* and *T. grandiflora* leaves showed significant anti-inflammatory activity (78.89% and 89.11%) at the concentration of 1000 µg/ml whereas standard diclofenac sodium exhibited 94.44% at the concentration of 1000 µg/ml. On the basis of the above results it can be concluded that both *C. pentagona* and *T. grandiflora* leaves have an anti-inflammatory activity.

Arthritis is a type of joint disorder that involves inflammation of one or more joints, responsible for pain swelling, stiffness, loss of function in joint. Denaturation of protein is one of the causes of arthritis was documented. Production of auto antigen in certain arthritic disease may occur due to the denaturation of protein. The mechanism of denaturation probably involve alteration I electrostatic hydrogen, hydrophobic and disulphide bonding [16]. Here, both the methanol extracts have shown promising activity at various concentrations and the effects were compared with the standard drug diclofenac sodium. The maximum percentage inhibition of protein denaturation of *C. pentagona* and *T. grandiflora* leaves were observed as 69.35% and 74.19% at 1000 µg/ml which were close to the percentage of inhibition of diclofenac sodium (85.49%). From the result, it can be stated that these extracts are capable of controlling the production of auto antigen to inhibit the denaturation of protein.

From the above studies, it could be concluded that *T. grandiflora* leaves have maximum anti-inflammatory and anti-arthritic activity and it could be natural anti-inflammatory and anti- arthritic source and thus could be useful as therapeutic agents in preventing these diseases. Further studies are needed for their active principle to elucidate.

6. Conflict of interest statement

We declare that we have no conflict of interest.

7. Acknowledgement

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