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Investigation of *in vitro* anti-arthritic and membrane stabilizing activity of ethanol extracts of three Bangladeshi plants

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Abstract
Three Bangladeshi medicinal plants *Rhaphidophora glauca*, *Phrynium imbricatum*, *Steudnera colocasiifolia*, have been investigated for their in vitro Arthritic and membrane stabilizing activity. Inhibition proteinase actions were evaluated to assess the antiarthritic effect of the selected plant extracts and membrane stabilizing activity were assessed by using hypotonic solution and heat-induced method. In highest concentration of *Rhaphidophora glauca*, *Phrynium imbricatum*, *Steudnera colocasiifolia*, shows significant antiarthritic activity (53.16%, 69.62%, 62.03%) and membrane stabilizing activity (49.05%, 71.9%, 60.22%) compared with Diclofenac-Na. Further in depth studies on this plant can result in an eco-friendly cost effective antiarthritic herbal drug with anti-inflammatory potential contributing towards the better healthcare of human society.

Keywords: Antiarthritic, Membrane stabilizing, *Rhaphidophora glauca, Phrynium imbricatum, Steudnera colocasiifolia*

1. Introduction
Arthritis is an autoimmune disorder characterized by pain, swelling and inflexibility [1]. Rheumatoid joint inflammation influences more or less 1% of the populace around the world. Its etiology is still obscure [2]. However, advances in understanding the pathogenesis of the disease have fostered the development of new therapeutics; with improved outcomes. Rheumatoid arthritis may rapidly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of mortality [3]. It is an inflammation of synovial joint due to immune mediated response. All anti-inflammatory drugs are not anti-arthritic because it does not suppress T-cell and B-cell mediated response [4]. Recently, it has been reported that microorganism including bacteria, viruses, fungi, parasites, bacterial DNA, and bacterial toxin may exacerbate the inflammatory response in the joint and bone [5]. The component cells of the inflamed rheumatoid synovial membrane are depicted in innate and adaptive predominant compartments of the inflammatory response. Pivotal cytokine pathways are depicted in which activation of dendritic cells (DCs), T cells, B cells and macrophages underpins the dysregulated expression of cytokines that in turn drive activation of effector cells, including neutrophils, mast cells, endothelial cells and synovial fibroblasts. The clinical manifestations of such effects are highlighted [6]. In Indian traditional medicines, Ayurvedic literature describes portions containing parts of certain plants for treating pain and inflammatory conditions like arthritis [7]. A large number of medicinal plants have been tested and found to contain active principles with curative properties against arthritis [8]. Inflammation is a universal host defense mechanism involving a complex network of cell, cell-mediated and tissue interactions. It occurs in response to a variety of harmful stimuli, physical, chemical, traumatic, antigen challenge, infections and ionization, etc. Immune system gets activated, communication and coordination occurs between different classes as well as the actions of immune cells to produce inflammation [9]. Researches in the last few decades have shown that inflammation is regulated by a large number of pro and inflammatory mediators [10, 11].
Rhaphidophora glauca (family: Araceae), an aroid liane native to the subtropical and warm temperate regions of the eastern Himalaya. Which is also distributed In Nepal through NE India to Bangladesh and Myanmar and N Thailand to N Laos and Vietnam.
Phrynium imbricatum (Family: Marantaceae) is a rigid herb. Leaves large, oblong, Spikes oblong, bracts oblong with obtuse, minutely toothed tips. Fruits usually 3-seeded. A paste prepared from leaves of Phrynium imbricatum, Blumea clarkei and an unidentified species (locally called Khedom gas) is applied to affected areas and bandaged for the treatment of

fractures (Chakma). Occurs in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet.
Steudnera colocasiifolia (family: Araceae) is an evergreen herb, which is short Stem, creeping and ascending; persistent cataphylls brown, not netted. Petiole green, cylindric, 30–50cm, slender, base sheathing; leaf blade paler but not glaucous abaxially, green adaxially. It is habitat in dense forests, wet meadows, by streams. Seasonally moist lowland forest. Distributed in Bangladesh, India, Myanmar, Thailand and China. Locally it is used to treat injuries, cuts, snake and insect bites and skin ulcers [12-15].

2. Material and Method

2.1 Plant collection and identification

Leaf of *Rhaphidophora glauca* (Accession No. 1314 CTGUH), leaf of *Phrynium imbricatum* (Accession No. 1315 CTGUH) and whole plants of *Steudnera colocasiifolia* (Accession No. 1316 CTGUH) were collected from Alutila, khagrachari, Chittagong, Bangladesh in the month of September 2014. The plants were authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, and Bangladesh.

2.2 Preparation of Extract

Each of the plant materials was dried and ground (Moulinex Blender AK-241, Moulinex, France) into powder (40-80 mesh, 500 g) and soaked for 7 days with 2–3 days interval in 2.0 L of ethanol at room temperature (23 ± 0.5 °C). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50 °C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 4.4–5.6% W/W) were all placed in glass Petri dishes (90 X 15 mm, Pyrex, Germany).

2.3 *In vitro* anti-arthritic activity

For the evaluation *in vitro* anti-arthritic activities of *R. glauca, P. imbricatum and S. colocasiifolia*, the method used was "inhibition of protein denaturation" [17-20] using diclofenac sodium as 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 ethanolic extract of *R. glauca, P. imbricatum* and *S. colocasiifolia*. 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 (62.5, 125, 250, 500, 1000 µg/ml) of ethanolic extract of *R. glauca* (EERG), *P. imbricatum* (EEPI), *S. colocasiifolia* (EESC) 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 add 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 can be calculated as:

Percentage Inhibition = 100 – [{(absorbance of test solution – absorbance of product control) ÷ (absorbance of test control)} × 100]

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

2.4 *In vitro* Membrane stabilization activity

Anti-inflammatory activity of ethanolic extracts of *R. glauca, P. imbricatum* and *S. colocasiifolia* were evaluated by using *in vitro* human red blood cell stability method. Blood sample was collected from a fresh volunteer, who doesn't have anti-inflammatory or contraceptive drugs at least since a week. The collected blood was mixed with sterilized Alsever solution. Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water. Blood sample was centrifuged at 3000 rpm and packed cell was washed with isosaline and a 10% (V/V) suspension of isosaline was made. Five different concentration of solution of *R. glauca, P. imbricatum* and *S. colocasiifolia* were mixed with 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC suspension. Diclofenac-Na was used as contrastable drug and instead of hyposaline 2 ml water was used as control. The hemoglobin content in supernatant was calculated using Spectrophoto-meter at 560 nm spectrum.
The result was estimated by following equations [21, 22].

% Inhibition of hemolysis = 100 x {OD1-OD2/OD1}

Where:
OD1 = Optical density of control solution
OD2 = Optical density of test sample

2.5 Statistical analysis

The data was analyzed statistically using ANOVA followed by student 't' test with GraphPad Prism Data Editor for Windows, Version 6.0 (GraphPad software Inc., San Diego, CA). Values were expressed as mean ± Standard error for mean (± SEM). $P < 0.05$ - 0.01 were considered as statistically significant.

3. Results

3.1 *In vitro* anti-arthritic activity

The production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. The maximum percentage inhibition of protein denaturation membrane stabilization and proteinase inhibitory action were observed as *R. glauca* (53.16%, 43.04%, 35.44%, 30.38%, 21.52%), *P. imbricatum* (69.62%, 59.69%, 48.83%, 41.44%, 34.05%), *S. colocasiifolia* (62.03%, 50.63%, 39.24%, 36.71%, 29.78%) at 400, 200, 100, 50, 25µg/ml respectively as shown in Table 1. From the results of Figure 1, our study reveals that ethanol extracts are capable of controlling the production of auto antigen and inhibits denaturation of protein, membrane lysis and proteinase action in rheumatic disease.

Table 1.

Concentration	% protein inhibition of *R. glauca*	% protein inhibition of *P. imbricatum*	% protein inhibition of *S. colocasiifolia*	% protein inhibition of Diclofenac-Na
400	53.16	69.62	62.03	94.16
200	43.04	59.69	50.63	92.25
100	35.44	48.83	39.24	88.19
50	30.38	41.44	36.71	81.35
25	21.52	34.05	29.78	76.49

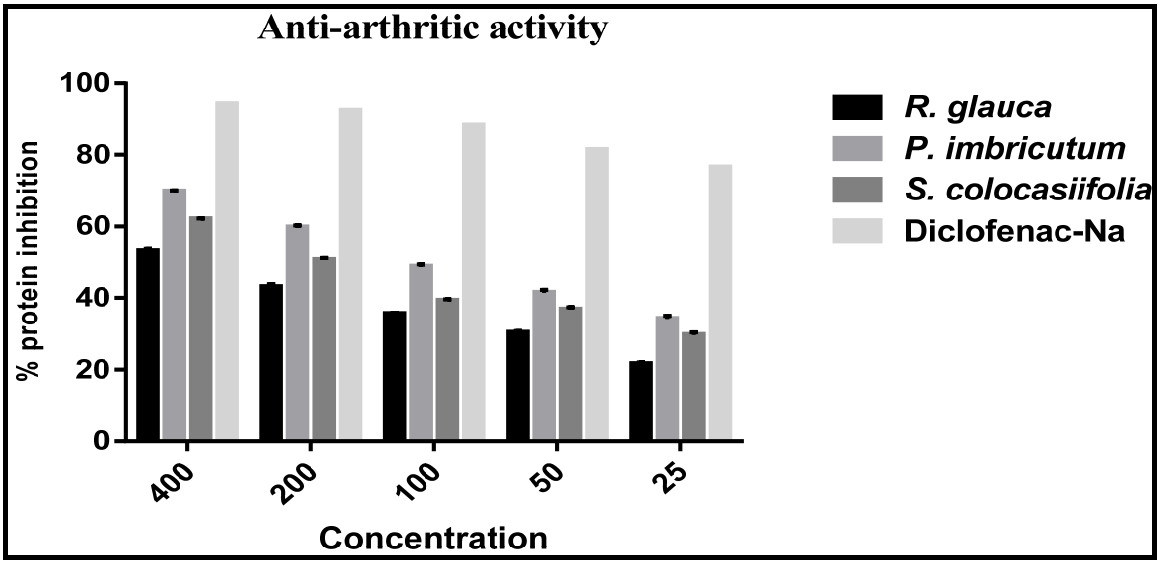

Fig 1: Percentage inhibition of protein denaturation.

3.2 *In vitro* Membrane stabilization activity

The inhibition of hypotonicity induced HRBC membrane lysis i.e., stabilization of HRBC membrane was taken as a measure of the membrane stabilization activity. The percentage of membrane stabilization for ethanolic extracts of *R. glauca, P. imbricatum, S. colocasiifolia* are effective in inhibiting the heat induced hemolysis of HRBC at different concentrations (400, 200, 100, 50, 25 µg/ml) as shown in Table 2. It showed the maximum inhibition *R. glauca* (49.05%), *P. imbricatum* (71.9%), *S. colocasiifolia* (60.22%) at 400 µg/ml. Hence anti-inflammatory activity of the extracts was concentration dependent shown in Figure 2.

Table 2.

Concentration	% membrane tabilization of *R. glauca*	% membrane stabilization of *P. imbricatum*	% membrane stabilization of *S. colocasiifolia*	% membrane stabilization of Diclofenac-Na
400	49.05	71.9	60.22	97.02
200	41.56	57.26	48.8	92.14
100	32.42	46.5	37.4	85.09
50	29.53	39.58	33.34	79.23
25	20.26	32.17	27.67	73.31

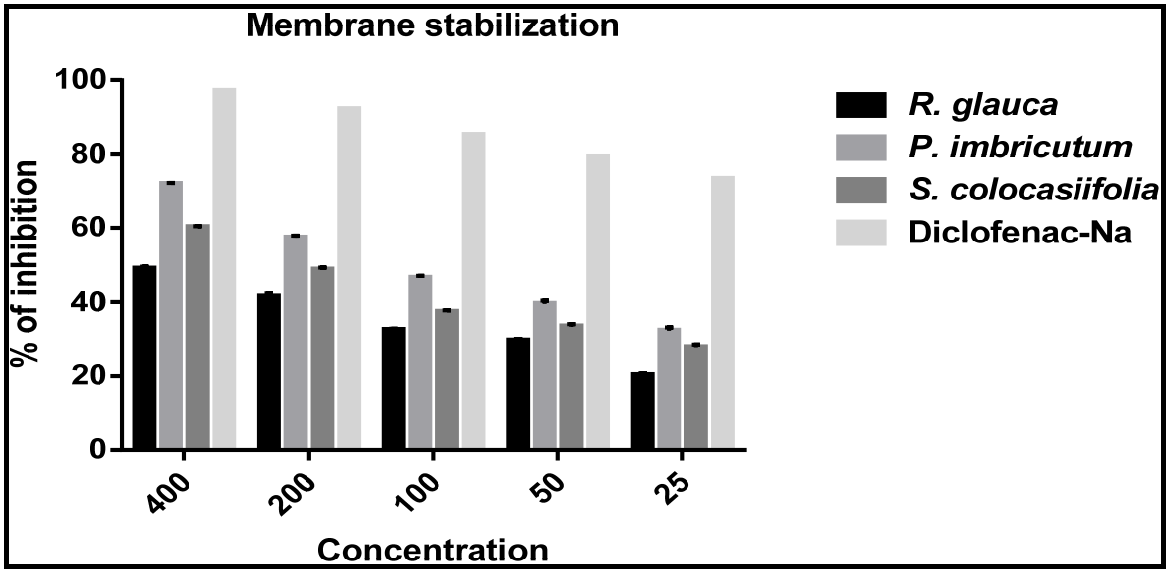

Fig 2: Percentage protection or stabilization of HRBC membrane

4. Discussion

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of auto-antigens in certain arthritic diseases may be due to denaturation of proteins *in vivo*. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. The increments in absorbance of plant extract and reference drug with respect to control indicated the stabilization of albumin protein. This anti-denaturation effect was further supported by the change in viscosities. It has been reported that the viscosities of protein solutions increase on denaturation [23, 24].

HRBC method was selected for the *in vitro* evaluation of Anti-inflammatory property because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [26, 27]. The hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components. The extract may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components [25].

5. Conclusion

In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have various side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against arthritis and inflammation so as to exploit them as herbal anti-arthritic agents.

In Inhibition of protein denaturation method, *R. glauca, P. imbricatum, S. colocasiifolia* extracts exhibited concentration depended inhibition of protein denaturation throughout the concentration range from low to high. This anti-denaturation effect was further supported by increase in the viscosities, while in Human red blood cell membrane stabilization method, the, *R. glauca, P. imbricatum, S. colocasiifolia* exhibited protection of the membrane lysis throughout the conc. range from low to high. The finding of *in vitro* model suggested that EERG, EEPI and EESC has potent anti-inflammatory and anti-arthritic potential. In future isolation of lead molecules responsible for the activity will be carried out which may be beneficial for the development of new anti-inflammatory and anti-arthritic agent.

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