

IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF ALPINIA CALARATA USING BOVINE ALBUMIN-PROTEIN DENATURATION METHOD AND HRBC-MEMBRANE STABILIZATION METHOD

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ABSTRACT

Objective:The present study aimed at the ethanolic extract of *Alpinia calcarata* can be treated the arthritic medicine as widely used in the folkloric medicine by experimentally proved by Bovine serum albumin protein denaturation method and human red blood cell(HRBC) membrane stabilization method.

Method:The different concentrations (100,200,300,400 and 500 µg/ml) of ethanolic extracts of stem of *Alpinia calcarata* was used for these assays and Diclofenac sodium with different concentration (100,200,300,400 and 500 µg/ml)as standard solution.

Results: The ethanolic extracts of *Alpinia calcarata* showed concentration dependent anti inflammatory activity the percentage of inhibition increased as concentration increased. This result is compared with diclofenac sodium. The percentage inhibition of protein stabilization.

Conclusion: The above results showed that ethanolic extracts of *Alpinia calcarata* have the potential of anti-inflammatory activity. So further studies are related to anti-arthritic activities are carried out from this folkloric medicinal plant.

Keywords: *Alpinia calcarata*, Bovine albumin protein denaturation, HRBC membrane stabilization.

I. INTRODUCTION

Alpinia calcarata (Roscoe) (A. calcarata) belonging to the family Zingiberaceae is a rhizomatous perennial herb, which is commonly used in the traditional medicinal systems in Bangladesh, India, Indonesia, Thailand, Malaysia, Sri Lanka, Taiwan, Cambodia, and Vietnam [1]. *A. calcarata* is used in the oriental part of the world as a food additive, spice and in indigenous system of medicine. It grows in dense forests at high altitudes and is considered as a native of India [2]. *A. calcarata* rhizomes are branched and dense with a light to dark brown colour and known to possess a broad spectrum of medicinal properties. This is a very good source of pinocembrin,

which induces mitochondrial apoptosis in colon cancer cells [3]. Experimentally, rhizomes are shown to possess antibacterial [4], antifungal [5], anthelmintic, antinociceptive [6], antioxidant [7], aphrodisiac [8], antidiabetic activities [9], rheumatism, fever and anticancer activity. It is also widely used to relieve colds and reducing swellings [10, 11]. High blood pressure, diuretic, stomach problems, analgesic [12], anticandidal, antiplatelet, antispasmodic [13] antiulcerous hypotensive [14, 15] insecticidal, muscle relaxant and Uterine stimulant. In the present work was undertaken check to the potential of *A. calarata* rhizome in the treatment of inflammation and arthritis.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Standard and chemicals

Standard Diclofenec was purchased from Sigma-Aldrich, New Delhi. Methanol used in the present research work were of HPLC grade and were procured from E. Merck Mumbai, India.

2.1.2. Collection of plant material

The Rhizomes of *A. calarata* were collected Nammakkal district, Tamil Nadu, India. The plant was identified and rhizomes of *A. calarata* were authenticated and confirmed.

2.1.3 Preparation of methanol extracts

The rhizomes of *A. calarata* were washed in running water, cut into small pieces and then shade dried for a week at 35-40 °C, after which it was grinded to a uniform powder of 40 mesh size. The methanol extracts were prepared by soaking 100 g each of the dried powder plant materials in 1 L of methanol using a Soxhlet extractor continuously for 10 hr. The extracts were filtered through Whatman filter paper No. 42 (125 mm) to remove all unextractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extracts were concentrated to dryness using a rotary evaporator under reduced pressure. The final dried samples were stored in labeled sterile bottles and kept at -20 °C.

2.2. Methods

2.2.1. In-vitro anti-arthritic activity of Protein denaturation method by using Bovine albumin

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation [16]:- Control solution of 5 ml made by 2 ml of Egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffer (pH 6.4) and 2 ml distilled water. Standard drug solution 5 ml made by 2 ml of Egg albumin, 2.8 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) concentration of 100, 200, 300, 400 and 500 µg/ml. Test solution of 5 ml made by 2 ml of Egg albumin, 2.8 ml of phosphate buffer and various concentrations of plant extract (*A. calcarata* rhizome methanolic extract) concentration of 100, 200, 300, 400 and 500 µg/ml. All of the above reaction mixtures were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation can be calculated as using the formula:

$$\text{percentage inhibition} = \left[\frac{V_t}{V_c} - 1 \right] \times 100$$

Where, V_t = absorbance of test sample, V_c = absorbance of control

2.2.2. Human red blood cell membrane stabilization method

2.2.2.1 Preparation of reagents

Hypotonic Saline: 0.36 gm of Sodium chloride in 100 ml of distilled water.

Isotonic Saline: 0.85 gm of sodium chloride in 100 ml of distilled water.

Phosphate buffer (pH 7.4, 0.15 M): 2.38 gm disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8 gm of sodium chloride were dissolved in 100 ml of distilled water [17].

2.2.2.2. Preparation of Suspension (10% v/v) of Human Red Blood cell (HRBC)

The blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment and was mixed with equal volume of sterilized Alsevers solution [18]. This blood solution was centrifuged at 3000 rpm and the packed cells were separated. The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline [19].

2.2.2.3. Assay of Membrane stabilizing activity

The test solution prepared by 1 ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of plant extract of various concentration (100, 200, 300, 400 and 500 µg/ml) and 0.5 ml of 10% w/v human red blood cells. The test control solution prepared by 1 ml of phosphate buffer and 2ml of water and 0.5ml of 10%w/v human red blood cells in isotonic saline. The standard solution was prepared by 1 ml of phosphate buffer, 2 ml of hypotonic saline, 0.5 ml of plant extract of various concentration (100, 200, 300, 400 and 500 µg/ml) and 0.5 ml of 10% w/v human red blood cells.

All the assay mixtures were incubated at 37°C for 30 min. and centrifuged at 3000 rpm. The supernatant liquid was separated and the hemoglobin content was estimated by a spectrophotometer at 560nm. The percentage hemolysis was estimated by assuming the hemolysis produced in content as 100% [20, 21]. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula:

$$\text{Percentage protection} = \left[100 - \left(\frac{\text{optical density sample}}{\text{optical density control}} \right) \right] \times 100.$$

III. RESULTS

The anti-arthritis effect of *A. calcarata* methanolic extract was evaluated against the denaturation of egg albumin *in vitro*. The result is summarized in **Table 1** and **Fig. 1**. This plant extract and Diclofenac sodium were exhibited concentration dependent inhibition of protein (albumin) throughout the concentration range of 100 to 500 µg/ml. HRBC Membrane stabilization method showed the protection percent increased with increase in the concentration (100, 200, 300, 400 and 500 µg/ml). All concentration of plant extract and Diclofenac sodium (standard) absorbance in both assays were closer to each other. The *A. Calcarata* methanolic extract of rhizome showed a concentration dependent anti-inflammatory activity.

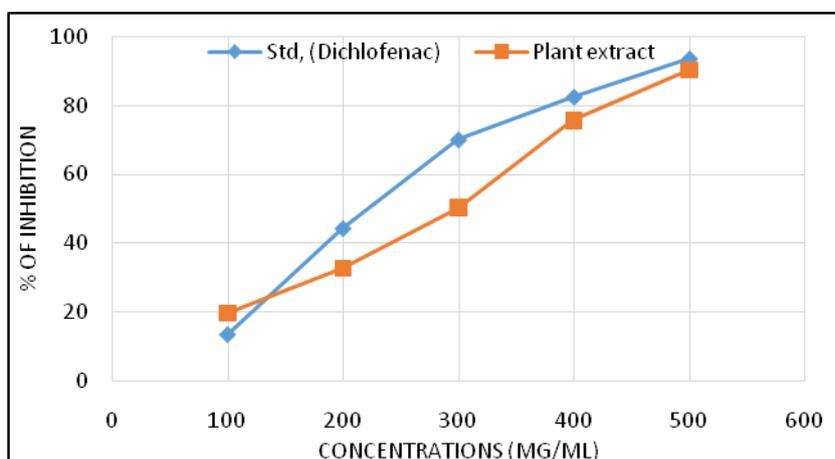
IV. TABLES AND FIGURES

4.1. Table

1.

S.No.	Concentrations ($\mu\text{g/ml}$)	Plant extract	Standard(Diclofenac sodium)
1	100	13.38	19.65 \pm 1.65
2	200	44.16	32.65 \pm 2.02
3	300	69.92	50.23 \pm 3.22
4	400	82.32	75.65 \pm 4.47
5	500	93.46	90.25 \pm 5.89
IC ₅₀ ($\mu\text{g/ml}$)		280.10	258.34

Percentage inhibition of protein denaturation



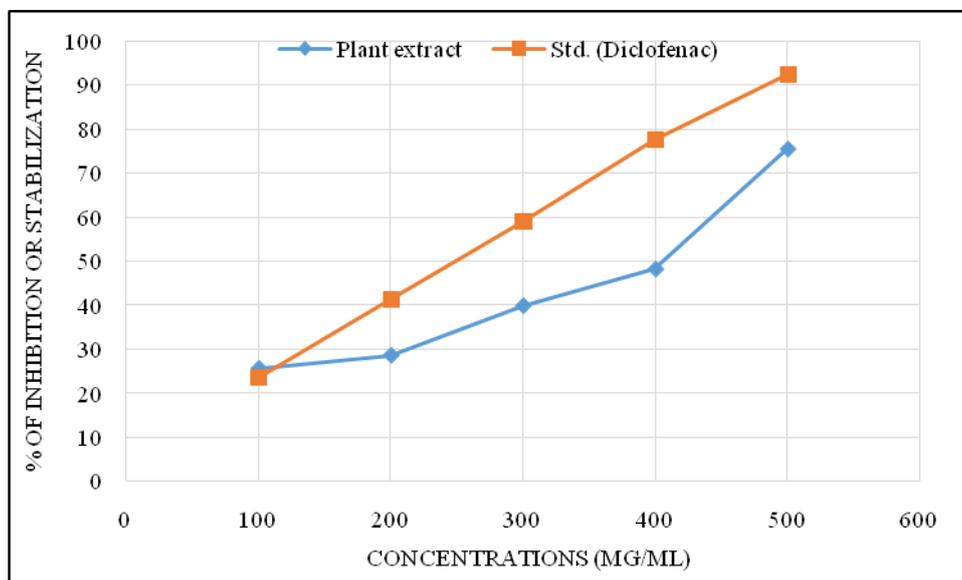
4.2. Fig1:Percentage inhibition of protein denaturation

4.3. Table 2

Percentage protection or stabilization of HRBC membrane

S.No.	Concentrations ($\mu\text{g/ml}$)	Plant extract	Standard (Diclofenac sodium)
1	100	25.59	23.56 \pm 2.14
2	200	28.57	41.23 \pm 2.68
3	300	39.88	58.89 \pm 3.97
4	400	48.21	77.56 \pm 5.12
5	500	75.59	92.32 \pm 6.55
IC ₅₀ ($\mu\text{g/ml}$)		355.71	251.15

4.4. Fig 2 Percentage protection or stabilization of HRBC membrane



V.CONCLUSION

Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases. Production of autoantigens in certain arthritic diseases may be due to denaturation of proteins *in-vitro*. 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. 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 neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [20, 21]. The hypotonicityinduced 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 [18]. 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, *A.Calcaratamethanolic* rhizome extract exhibited concentration depended inhibition of protein denaturation throughout the concentration range from low to high, while in Human red blood cell membrane stabilization method, the *A.Calcaratamethanolic* rhizome extract exhibited protection of the membrane lysis throughout the conc. range from low to high. These finding of *in-vitro* model suggested that *A.Calcaratamethanolic* rhizome extract has potent antiinflammatory 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 [22].

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