



***In-vitro* Anti Arthritic Activity potentials of *Erythroxylum monogynum* leaves Hydro-alcoholic Extract**

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ABSTRACT

Rheumatoid arthritis (RA) is an evolving, enduring, and a crippling disorder categorized by puffiness, agony, and synovial joints arduousness. The exact reason of this devastating illness is mysterious. However, it is strongly linked to self-protection reaction triggered by various genetic and external factors. The current research work was designed to explore the anti-arthritic probable of the plant *Erythroxylum monogynum*. The plant leaves was collected, shade dried, blended and extracted (continuous hot percolation) with Hydro-alcoholic solvent. Preliminary phytochemical evaluation were carried out. Entirely the *in-vitro* models i.e. inhibition of protein denaturation, membrane stabilization and proteinase inhibition were carried out by using standard reference drug diclofenac sodium. In the present study there was significant ($p < 0.05$) inhibition of protein denaturation in both standard and extract treated group and % inhibition of protein denaturation produced by extract at concentrations 100 and 300 $\mu\text{g/ml}$ exhibited improved activity than standard (Diclofenac sodium). From the results of the present study it can be stated that *Erythroxylum monogynum* may control the production of auto-antigens by preventing *in-vivo* denaturation of proteins in rheumatic diseases. Dose based and significant ($p < 0.05$) of anti arthritic activity in *in-vitro* designs were found. The results disclose hopeful anti arthritic probables of the herb. However further pharmacological investigation using isolated active ingredients can be carried out to confirm its usefulness and effectiveness.

Keywords: Anti-arthritic activity, *Erythroxylum monogynum*, *in-vitro* studies, inhibition of protein denaturation, membrane stabilization, proteinase inhibition,

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INTRODUCTION

The plants offer foodstuff, attire, shelter and medicine. Most of the herbal assistances will known to be established through opinion of wild animals and by experimental and error methods. As time goes on, people ongoing to bargain and to consume more herbs having medicinal power. They scientifically brought together evidence on herbs and elaborated to well-defined herbal pharmacopoeias i.e. traditional medicinal system¹. Traditional use of medicine is documented as a method to study about potential forthcoming medicines. Because of extensive biological and medicinal values, high safety boundaries and lesser cost of herbal medicine, it has inordinate demand and used as source of basic health care in together of developed and emergent republics^{2,3}. WHO notes that around 200 pharmaceutical medicines are derived from the plant, in modern medicinal system nearly 74% of which are used and that can directly correlated with ancient medicinal uses⁴. Arthritis is one of the most common chronic inflammatory complaints, leading reason of frailty in world wide. There are additional 100 different categories of arthritis and related conditions. Out of which rheumatoid arthritis and osteoarthritis are the major ones. Most of the diseases of joints affect synovial joints. Indications of one type arthritis are unlike other type. Some people may show mild but some are with strong symptoms. Some of the common symptoms are: Pain, Edema of Joints, Rigidity, Sensitivity, Tenderness, Warmth, Loss of Elasticity, Limping, Bone Spurs, Discomfort when Standing or Walking, Fatigue (feeling tired)^{5,6,7}.

Rheumatoid arthritis is an usual autoimmune disease that is connected with progressive disability, systemic problems, primary death and socioeconomic costs. Rheumatoid arthritis is characterized by synovial inflammation and hyperplasia (“swelling”), autoantibody production [rheumatoid factor and anti-citrullinated protein antibody (ACPA)], cartilage and bone annihilation (deformity), systemic features, including cardiovascular, pulmonary, psychological, and skeletal disorders^{6,7}.

MATERIALS AND METHOD

Collection of Plant Material and Extraction

The aerial parts of *Erythroxylum monogynum* was collected from the local areas in and around kalasapadu, kadapa district, Andhra Pradesh. The plant was authenticated by Dr. A. Madhusudhanreddy, professor, department of botany, Yogi Vemana University, kadapa.

Table1: Plant details

S.No	Name of the plant and parts used	Family
1	<i>Erythroxylum monogynum</i> -Leaves	Erythroxylaceae



Figure 1: Erythroxylum monogynum

Preparation of Extract

The leaves of the plant of *Erythroxylum monogynum* was separated, washed and shade dried. The dried plant material was blended with food grade blender and separated as moderately coarse powder. This moderately coarse powder was extracted by using Soxhlet apparatus by using Hydro-alcoholic solvent at 70-30 portion (70 Ethanol-30 water) and concentrated.

Assessment of *In-vitro* anti-arthritic activity

Inhibition of protein denaturation^{14, 15}

In this model valuation of anti-arthritic activity of Hydro-alcoholic extract of the plant *Erythroxylum monogynum*, was done for measuring the % inhibition of denaturation. The trial was carried out by taking both bovine serum albumin and egg albumin.

The reaction mixture 0.5ml consists of (0.45ml bovine serum albumin/egg albumin of 5% aqueous solution + 0.05ml of 100-500µg/ml of extract). The pH (6.3) was adjusted by using 1N HCl. Samples were incubated at 37oC for 20min and then heated at 57oC for 3min. After cooling the samples, 2.5ml of phosphate buffer saline (pH 6.3) was added to each tube. The pH of the buffer saline was attuned to 6.3 using 1N HCl. Absorbance was measured spectrophotometrically at 660nm. Percentage inhibition of protein denaturation was calculated using the following formula

$$\% \text{ Inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample}) \times 100}{\text{Abs control}}$$

IC50 value of extract and standard were also calculated.

Effect on Membrane Stabilization^{14, 16}

In this model percentage membrane stabilizing activity was used to assess the anti inflammatory activity. The standard drug used was Diclofenac sodium.

The reaction mixtures 4.5ml consists of 2ml hypotonic saline (0.25% Nacl) + 1ml 0.15M phosphate buffer (pH 7.4) + 1ml test solution (100-500µg/ml) in normal saline + 0.5ml of 10% rat RBC in normal saline. The mixture was incubated at 56°C for 30 minutes. The tubes were cooled beneath running tap water for 20 minutes. The mixture was centrifuged at 3000rpm for 10min and the absorbance of the supernatant was measured at 560nm.

Percentage membrane stabilizing activity was calculated based on the following formula¹⁵

$$\% \text{ Membrane stabilization} = \frac{(\text{Abs of Control} - \text{Abs of Sample}) \times 100}{\text{Abs of control}}$$

RESULTS AND DISCUSSION

Evaluation of anti arthritic activity using *in-vitro* model

Effect on protein denaturation

Scrutiny of anti-arthritic activity of Hydro-alcoholic extract of *Erythroxyllum monogynum* using this model was done in 5 different concentrations (100-500µg/ml). The detailed results are tabulated below

Table 2: % Inhibition Hydro-alcoholic extract of *Erythroxyllum monogynum* on heat induced protein denaturation

Concentration (µg/ml)	% Inhibition			
	Diclofenac+BSA	Diclofenac+EGG	Extract+BSA	Extract+EGG
100	14.10±0.85	28.27±1.37	20.25±0.66	37.49±1.49
200	25.23±0.75	49.20±1.08	24.23±1.9	55.99±2.70
300	33±1.07	74.02±1.61	38.17±0.67	67.16±1.66
400	45.43±0.6	84.77±2.48	45.36±0.6	77.85±1.63
500	74.33±1.7	92.73±0.641	65.50±1.32	84.27±1.50

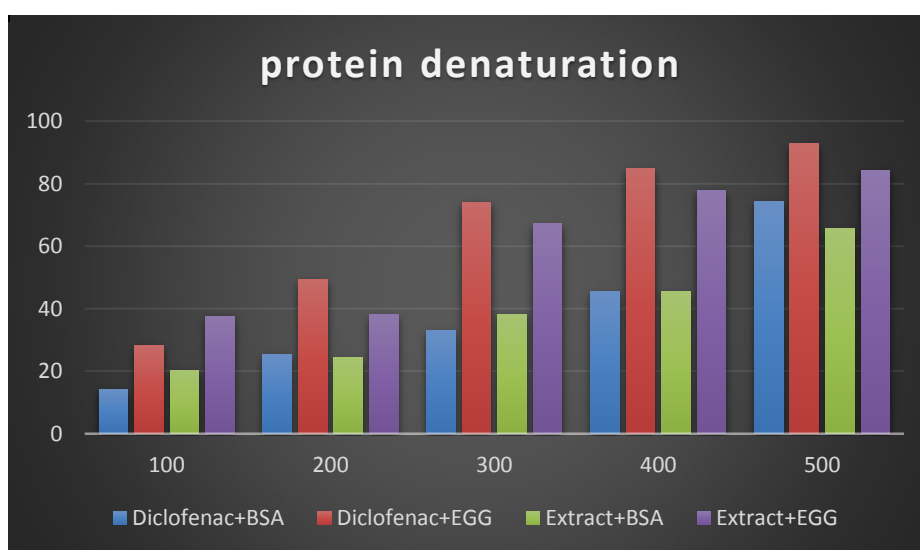


Figure 2: Protein denaturation by *Erythroxyllum monogynum* leaf extract

Table 3: % of membrane stabilization Hydro-alcoholic extract of *Erythroxylum monogynum*

Treatment	Concentration ($\mu\text{g/ml}$)	% of membrane stabilization
Standard	100	28
	200	38
	300	60
	400	75
	500	82
Extract	100	11.3
	200	23.36
	300	35
	400	51
	500	73

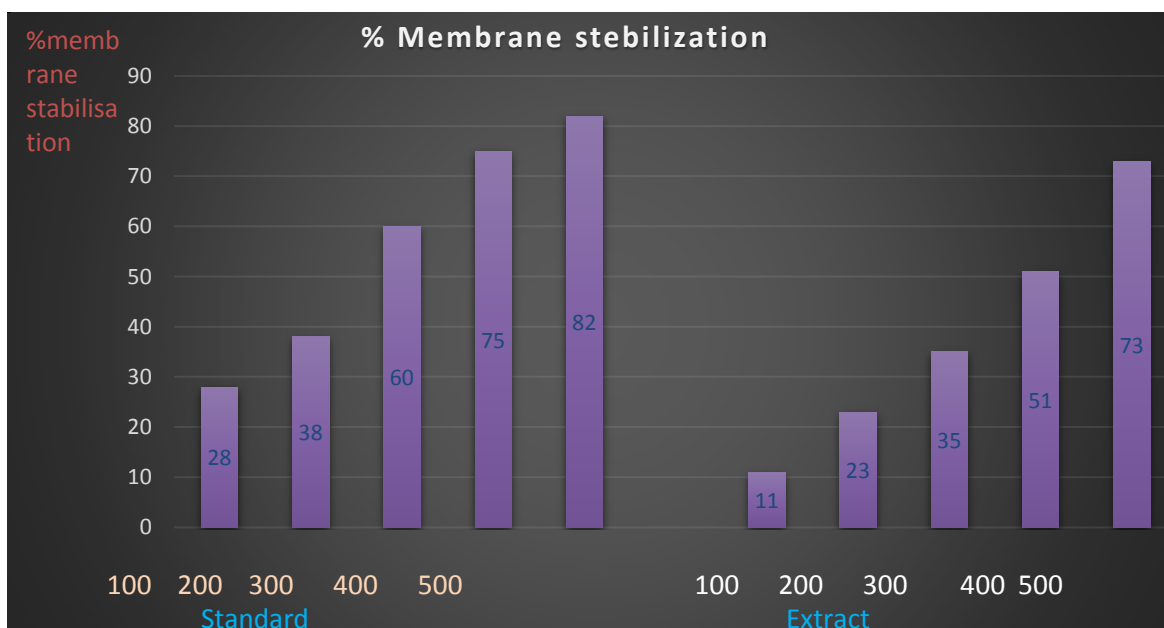


Figure 3: % Membrane stabilization by *Erythroxylum monogynum* leaf extract

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