

**IN VITRO ANTI-ARTHRITIC ACTIVITY ON TENDER LEAVES OF
ARTOCARPUS HIRSUTUS LAM.**

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ABSTRACT

Artocarpus hirsutus (wild jack) endogenous to Kerala has wide medicinal values which are well documented in the third volume of Hortus Malabaricus, the oldest comprehensive printed book on the natural plant wealth of Asia. The decoction of roots and bark are supposed to cure diarrhea. The leaves when used with white camphor and root of curcuma are believed to treat venereal bubones and chronic hemorrhage respectively. The juice from the cooked unripe fruits are believed to induce appetite and also when applied to the anus relieve the pains of hemorrhage. The ethanol extract of *Artocarpus hirsutus lam* was screened for its anti-arthritis activity *in vitro*. For *in vitro* studies the leaf extract was dissolved in dimethyl sulfoxide prepared in

different concentrations. This compared with standard diclofenac sodium by inhibition of protein denaturation method. Bovine albumin serum used as protein. From the results, it may be concluded that tender leaves of *Artocarpus hirsutus lam* possess significant anti-arthritis activity. The activity may be due to the presence of flavanoids, saponin and coumarin glycosides, and terpenoids.

KEYWORDS: *Artocarpus hirsutus lam*, Bovine albumin serum, Diclofenac sodium, Inhibition of protein denaturation method.

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INTRODUCTION

Rheumatoid arthritis (RA) is a highly variable, chronic inflammatory condition of unknown etiology that affects mostly diarthrodial (hingelike) joints, but often with periarticular and systemic involvement. The word *rheuma* was defined by ancient Greek physicians to mean “flowing,” which fit well with their humoral theory of disease. The term *rheumatism* was used in the 1600s by a French physician as an inexact label for a systemic condition associated with joint ailments. The etiology of rheumatoid arthritis is unknown but is most likely multifactorial, involving endogenous and exogenous factors. Genetic predisposition most likely interacts with endocrinologic, gastrointestinal, infectious, atmospheric, environmental, and other etiologic factors. The autoimmune nature of RA is documented by the presence of immune cell reactivity and the production of antibodies to endogenous elements such as immunoglobulins, collagen, and cellular components. The precise pathophysiology of rheumatoid arthritis remains unknown but appears to involve a complex series of events involving T-lymphocytes, B-lymphocytes, macrophages, and a number of cytokines and enzymes, leading to inflammation and destruction of bone and cartilage. TNF- α and IL-1 appear to be intimately involved in this process, but numerous other cytokines, inflammatory mediators, and enzymes also seem to play an important role^[1].

The plant *Artocarpus hirsutus* Lam, (Moraceae) is a large evergreen tree up to 70m in height, found up to an altitude of 1200M, in evergreen forest of peninsular India. The outer colour of bark is grey and inner colour is brown. The leaves are elliptic rhomboid or ovate and dark green in colour. Male head narrowly cylindrical, female heads simple. Seeds are long ovoid. The wood is light straight or interlocked-grained and even tentured. Its colour is blackish brown. The heartwood is almost as strong; contain all type of flavanoid except nor-artocarpin. The main property and uses of unripe fruits are sour, astringent, sweet, thermogenic, indigestible, an aphrodisiac, constipating and cause flatulence. An infusion of the bark is applied to cure small pimples and cracks on the skin. Powered bark is used to heal sores. Dry leaves are useful in treating bubose and hydrocele^[2]. *Artocarpus hirsutus lam* were widely used in ethnomedicine for the treatment of inflammatory and related disorders, their anti-arthritic properties have not yet been pharmacologically evaluated. Phytoconstituents

such as steroids, terpenoids, flavanoids, phenols and tannins are explored as anti-arthritis agents from various medicinal plants^[3]. These are presented in this plant. Hence, the present study was undertaken to evaluate anti-arthritis activity of ethanol leaf extract by *in-vitro* method.

MATERIALS AND METHODS

Plant material

Collected tender leaves of *Artocarpus hirsutus lam* collected and shade dried and it was converted into moderately coarse powdered and extracted with ethyl alcohol for 27 hours by soxhlet apparatus. Dried extract was used for the Phytochemical screening, and *in vitro* anti arthritis activity.

Drugs and Chemicals

All organic solvents and other reagents were procured from SD Fine chemicals Ltd. Mumbai and were of analytical grade. Diclofenac sodium was obtained as gift sample from Horizon bioceuticals Pvt. Ltd. Kala-amb (Himachal Pradesh).

Preparation of extract

The powdered plant material (300g) was defatted by extracting with ethanol in soxhlet extractor. The drug was macerated with distilled water for 24 hours and then filtered. The marc obtained was again macerated with distilled water and filtered. The filtrates were combined and evaporated to dryness. The dried extracts were kept in dessicator. The percentage yield of extract was 7.8w/w.

Preliminary phytochemical screening

The Phytochemical analysis was done. Major chemical constituents such as flavanoids, terpenes, coumarin and saponin glycosides are presented.

Assessment of *in vitro* anti-arthritis activity by Bovine Serum Protein Denaturation method

Preparation of Reagents

0.5% Bovine Serum Albumin (BSA):

Dissolved 500mg of Bovine Serum Albumin in 100 ml of water.

Phosphate Buffer Saline PH 6.3: Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na₂HPO₄), 0.24 g of

potassium dihydrogen phosphate (KH₂PO₄) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

Method: Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of test solution of various concentrations.

Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of dimethyl sulfoxide.

Product control (0.5ml) consists of 0.45ml of dimethyl sulfoxide and 0.05 ml of test solution.

Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5% w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

Procedure: 0.05 ml various concentrations (10, 50, 100, 200, 400, 800 and 1000 mcg/ml) of test drugs and standard drug diclofenac sodium (10, 50, 100, 200, 400, 800 and 1000 mcg/ml) were taken respectively and 0.45 ml (0.5% w/V BSA) mixed. The samples were incubated at 37°C for 20 minutes and the temperature was increased to keep the samples at 47°C for 30 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solution. The triplicate value of absorbance was measured using UV-Visible spectrophotometer at 660nm. The control represents 100% protein denaturation. The results were compared with diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as:⁴

$$\text{Percent inhibition} = 100 - \frac{(O.D. \text{ of test} - O.D. \text{ of product control})}{O.D. \text{ of test control}} \times 100$$

RESULT

IN VITRO ANTI-ARTHRITIC ACTIVITY BY BOVINE SERUM PROTEIN DENATURATION METHOD

Percentage inhibition of albumin denaturation of leaf extract was performed and the results of different concentrations of extract were showing significant activity by dose dependant manner, which is displayed in fig.3. Different concentrations of leaf extracts exhibited percentage inhibition as mentioned in Mean \pm SEM are 7.5 \pm 0.6928, 14.6 \pm 0.5196, 26.5 \pm 0.4041, 33.9 \pm 0.6351, 41.5 \pm 0.7506, 54.8 \pm 0.8660, 68.3 \pm 0.9238 at concentrations of 10, 50, 100, 200, 400, 800 and 1000 μ g/ml respectively. Percentage inhibition of albumin denaturation was performed by using standard drug diclofenac sodium. Different

concentrations of standard drug exhibited percentage inhibition values are 20.3 ± 0.9815 , 30.4 ± 1.039 , 37.9 ± 0.6351 , 50.5 ± 0.8083 , 60 ± 1.732 , 77.8 ± 1.328 and 81.9 ± 1.443 at different concentrations of 10, 50, 100, 200, 400, 800 and 1000 $\mu\text{g/ml}$ respectively. When the concentrations of leaf extract increases while the percentage inhibition of protein denaturation also increased. The results were displayed in (Table.1).

Table: 1. *In vitro* anti-arthritic activity of *Artocarpus hirsutus lam* by protein denaturation method.

Drug	Conc. ($\mu\text{g/ml}$)	Test absorbance (660nm)	Product control (660nm)	Percentage inhibition of protein denaturation (%)
Control	-	1	-	0
Test(Ethanol extract)	10	0.951	0.026	7.5 ± 0.6928
	50	0.873	0.019	14.6 ± 0.5196
	100	0.752	0.017	26.5 ± 0.4041
	200	0.675	0.014	33.9 ± 0.6351
	400	0.596	0.011	41.5 ± 0.7506
	800	0.454	0.002	54.8 ± 0.8660
	1000	0.318	0.001	68.3 ± 0.9238
Standard (Diclofenac sod.)	10	0.823	0.026	20.3 ± 0.9815
	50	0.715	0.019	30.4 ± 1.039
	100	0.638	0.017	37.9 ± 0.6351
	200	0.509	0.014	50.5 ± 0.8083
	400	0.411	0.011	60 ± 1.732
	800	0.224	0.002	77.8 ± 1.328
	1000	0.184	0.001	81.9 ± 1.443

Number of group $n=4$, mean \pm SEM. Test compared with standard

The IC_{50} value of test was compared with standard. IC_{50} value of standard was found to be 345.78 $\mu\text{g/ml}$.

DISCUSSION

Denaturation of tissue protein is one of the known causes of inflammatory and arthritic diseases and also the production of auto antigen may be due to denaturation of proteins *in vivo*. Phytoconstituents such as steroids, terpenoids, flavanoids, phenols and tannins are explored as anti-arthritic agents from various medicinal plants.^[3] The *in vitro* anti-inflammatory effect of extract was evaluated against denaturation of bovine albumin. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by *Artocarpus hirsutus lam* extract. When the concentrations of leaf extract increases while the percentage inhibition of protein denaturation also increased.

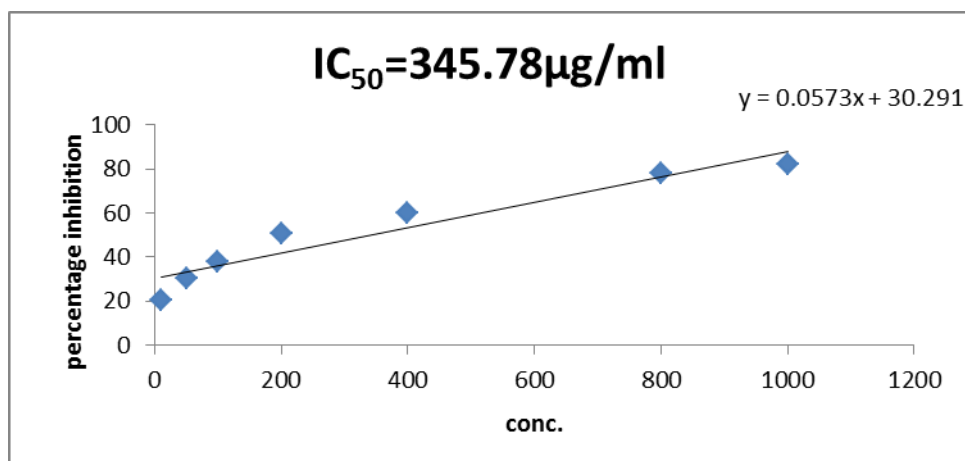


Fig: 1. IC₅₀ value of standard by protein denaturation method

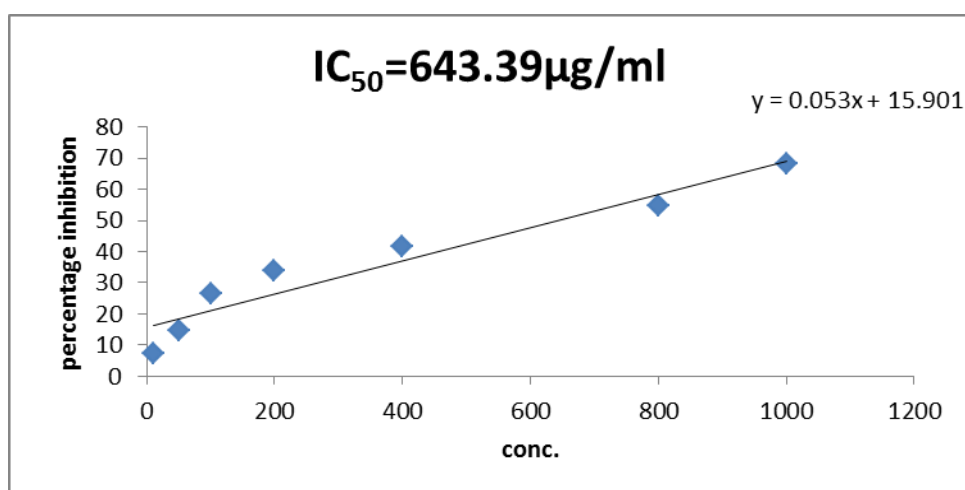


Fig: 2. IC₅₀ value of test by protein denaturation method

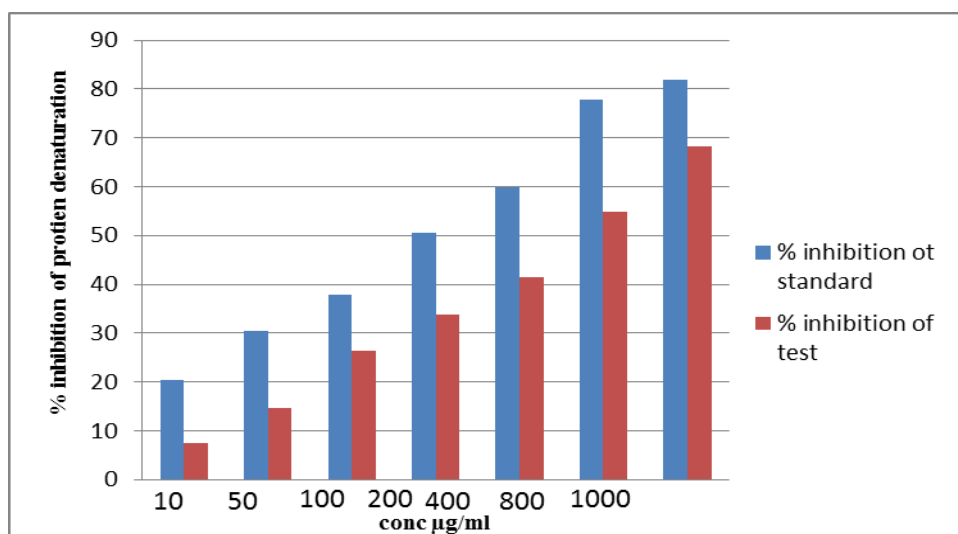


Fig: 3. Comparison of anti-arthritis potential of *Artocarpus hirsutus lam* and standard diclofenac sodium.

CONCLUSION

In the present study, the qualitative phytochemical analysis showed the presence of flavonoids and other active constituents in *Artocarpus hirsutus lam*. From the results, it may be concluded that tender leaves of *Artocarpus hirsutus lam* possess significant anti-arthritis activity. The *in vitro* anti-arthritis effect of the extract was evaluated against denaturation of bovine albumin. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the extract. Phytoconstituents such as steroids, terpenoids, flavanoids, phenols and tannins are explored as *in vitro* anti-arthritis agents from various medicinal plants. The activity may be due to the presence of Steroids, flavanoids, saponin and coumarin glycosides, and terpenoids. Our future aim is to isolate the chemical constituents responsible for that activity. Hence it could be beneficial for further work as active anti-arthritis agent.

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