



Research Article

IN VITRO ANTI-ARTHRITIC ACTIVITY OF KASHAYA OF *SIDA CORDIFOLIA* LINN.

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ABSTRACT

Arthritis is a form of joint disorder that involves inflammation of one or more joints. It is very common condition especially in women and older people. Ayurveda has contributed a lot for the management of arthritic conditions. The plant *Bala*, identified as *Sida cordifolia* Linn. is a widely used drug in many of the Ayurvedic formulations especially in those for arthritic conditions. *Kashaya* (decoction) is one of the commonly prescribed preparations in Ayurveda. Present study was aimed to assess the anti-arthritic activity of *Kashaya* (decoction) of root of *Sida cordifolia* Linn. by inhibition of protein denaturation method and inhibition of proteinase enzyme activity. *Kashaya* of roots of *Bala* was prepared as per standard procedure and was used to induce protein denaturation in Bovine serum albumin and to inhibit the activity of proteinase enzyme, trypsin. The absorbance was read by spectrophotometer to evaluate the percentage of inhibition in both the procedures. Each experiment was done in triplicates. The results were compared with standard drug Diclofenac sodium. *Sida cordifolia* Linn. showed dose dependent inhibitory activity and highest activity was seen in 500µg/ml concentration in both the experiments. The result showed that root of *Sida cordifolia* Linn. is having anti-arthritic property. Further studies can be carried out with other formulations of *Bala* like *Choorna* (powder), *Swarasa* (juice) etc. to compare their anti-arthritic activity. The study supports the classical use of plant *Bala* in various formulations in the treatment of arthritis.

KEYWORDS: Anti-arthritic activity, *Sida cordifolia*, *Bala*, Protein denaturation method, Proteinase enzyme activity.

INTRODUCTION

Arthritis affects about 15% of people in India. Arthritis is more common in women than men in all age groups. Common symptoms include swelling, pain, stiffness and decreased range of motion^[1]. It may be caused by any infectious agent, immunological and genetic factors, association with primary diseases of cartilage or bone, joint or soft tissue disorders etc. Non-Steroidal Anti-Inflammatory drugs are the contemporary medical treatment advised. Ayurveda offers effective treatment for arthritic conditions. *Bala*, identified as *Sida cordifolia* Linn. is widely used in many Ayurvedic formulations like *Balapunarnavadi kashayam*, *Balasaahacharadi kashayam*, *Bala tailam*, *Ksheerabala tailam* etc, which are indicated for conditions similar to arthritis. Anti-arthritic activity of various extracts of *Sida cordifolia* Linn. have already been proven in-vitro methods.^[2] Analgesic, anti-inflammatory and hypoglycaemic activities of aerial and root extracts of *Sida cordifolia* Linn. have also been studied and proven in-vivo^[3]. Antioxidant potential of ethanol extracts of leaf, stem, root and whole plant of *Sida cordifolia* Linn. has also

been proved in-vivo^[4]. An Ayurvedic formulation, *Balapunarnavadi choornam*, containing *Bala* as an ingredient was also evaluated and proved to have anti-arthritic activity in-vitro^[5]. In Ayurveda many medicines are prescribed as *Kashaya* (decoction) where the complete plant part is made use in the preparation of *Kashaya*. The present study was done to evaluate the anti-arthritic activity of *Kashaya* of root of *Sida cordifolia* Linn.

MATERIALS AND METHODS

Plant Material

The fresh roots of *Sida cordifolia* Linn. were collected from their natural habitat at Thiruvananthapuram during their flowering season. Roots were washed well and shade dried. Dried roots were crushed into coarse powder to prepare *kashaya*.

Preparation of *Kashaya*^[6]

48gm of coarsely powdered drug was taken, washed well and boiled in 8 times of water (384ml). It was reduced to 1/4th part (96ml). It was filtered through a cotton cloth and the filtrate was collected as

Kashaya. This freshly prepared *Kashaya* was used for evaluating the anti-arthritic activity.

Evaluation of anti-arthritic activity

Anti-arthritic activity was evaluated through Inhibition of protein denaturation method and Inhibition of proteinase enzyme activity. Diclofenac sodium was the standard drug used. Each experiment was done in triplicates and the average percentage of inhibition was calculated from the three results.

Inhibition of Protein Denaturation Method [7]

Protein Denaturation Method was carried out as per standard procedure using Bovine serum albumin.

Test solution (0.5ml): 0.05ml of decoction of root of *Bala (Sida cordifolia Linn.)* and 0.45ml of bovine serum albumin

Test control solution (0.5ml): 0.45ml of bovine serum albumin and 0.05ml of distilled water

Product control solution (0.5ml): 0.45ml of distilled water and 0.05ml of decoction of root of *Bala (Sida cordifolia Linn.)*

Standard solution (0.5ml): 0.45ml of bovine serum albumin and 0.05ml of Diclofenac Sodium

All the sample solutions were incubated at 37°C for 20 minutes. Temperature was increased to 57°C for 3 minutes. It was then allowed to cool for some time and 2.5ml of phosphate buffer was added to all the above solutions. The absorbance of resulting solution was measured at 416nm using UV visible spectrophotometer. The percentage of inhibition by the root decoction of *Sida cordifolia Linn.* was calculated using the following formula;

$$\text{Percentage of Inhibition} = 100 - \{(\text{optical density of test solution} - \text{optical density of product control}) / \text{optical density of test control}\} \times 100$$

Inhibition of Proteinase Enzyme Activity^[8]

As per standard procedure, the proteinase enzyme used was trypsin.

Test solution: Phosphate buffer+1ml TrisHCl + trypsin+1ml decoction of root of *Bala (Sida cordifolia Linn.)*

Test control solution: Phosphate buffer+1ml TrisHCl

Product control solution: Phosphate buffer+1ml Tris HCl+1ml decoction of root of *Bala (Sida cordifolia Linn.)*

Standard solution: Phosphate buffet+1ml Tris HCl + trypsin +1ml Diclofenac sodium.

The reaction mixture contained 0.06mg trypsin. 1.0ml of 25mm Tris -HCl buffer (p^H 7.4) and 1.0ml aqueous solution of test sample were incubated at 37°C for 5 minutes. Then 1.0ml of 0.8% (w/v) Casein was added and incubated for 20 minutes. 2.0ml of 70% (v/v) Perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Optical density of supernatant was read at 280nm against buffer as blank. The percentage of inhibition was calculated using the formula.

$$\text{Percentage of inhibition} = 100 - \{(\text{Absorbance of test solution} - \text{Absorbance of product control}) / \text{Absorbance of test Control}\} \times 100$$

RESULTS

The anti-arthritic activity of *Kashaya* of root of *Sida cordifolia* Linn. was evaluated by analyzing inhibition of bovine serum albumin denaturation and inhibition of trypsin enzyme activity. The results are summarized in Table 1 and Table 2.

Table 1: In Vitro Anti-Arthritic Activity of *Kashaya* of *Sida Cordifolia* Linn. By Inhibition of Protein Denaturation Method

Concentrations (µg/mL)	Percentage of inhibition			Average Percentage of inhibition	Percentage of Inhibition of Diclofenac Sodium (%)
	Triplicate I	Triplicate II	Triplicate III		
62.5	6.59	13.41	3.18	7.73	50.77
125	29.77	27.50	31.14	29.47	74.27
250	42.27	39.77	38.41	40.15	81.13
500	64.77	57.27	64.32	62.12	89.36

Table 2: In vitro Anti-Arthritic Activity of Kashaya of *Sida Cordifolia* Linn. by Inhibition of Proteinase Enzyme Activity

Concentrations (µg/mL)	Percentage of inhibition			Average Percentage of inhibition	Percentage of Inhibition of Diclofenac Sodium (%)
	Triplicate I	Triplicate II	Triplicate III		
62.5	7.26	15.32	13.98	12.19	74.67
125	25.00	38.98	27.15	30.38	81.26
250	35.22	55.11	33.33	41.22	85.98
500	68.55	80.65	64.78	71.33	90.66



Fig. 1: *Sida cordifolia* Linn.



Fig. 2: Root of *Sida cordifolia* Linn.



Fig.3: Kashaya of root of *Sida cordifolia* Linn.

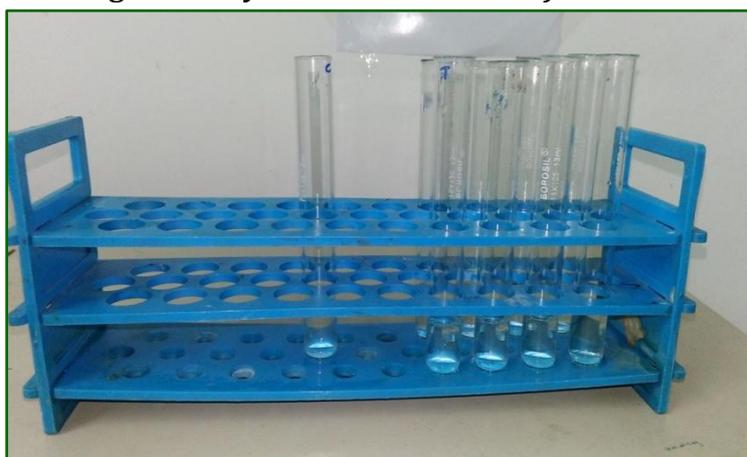


Fig. 4: Solutions prepared for Inhibition of Protein Denaturation Method



Fig. 5: Solutions prepared for Inhibition of Proteinase Enzyme Activity

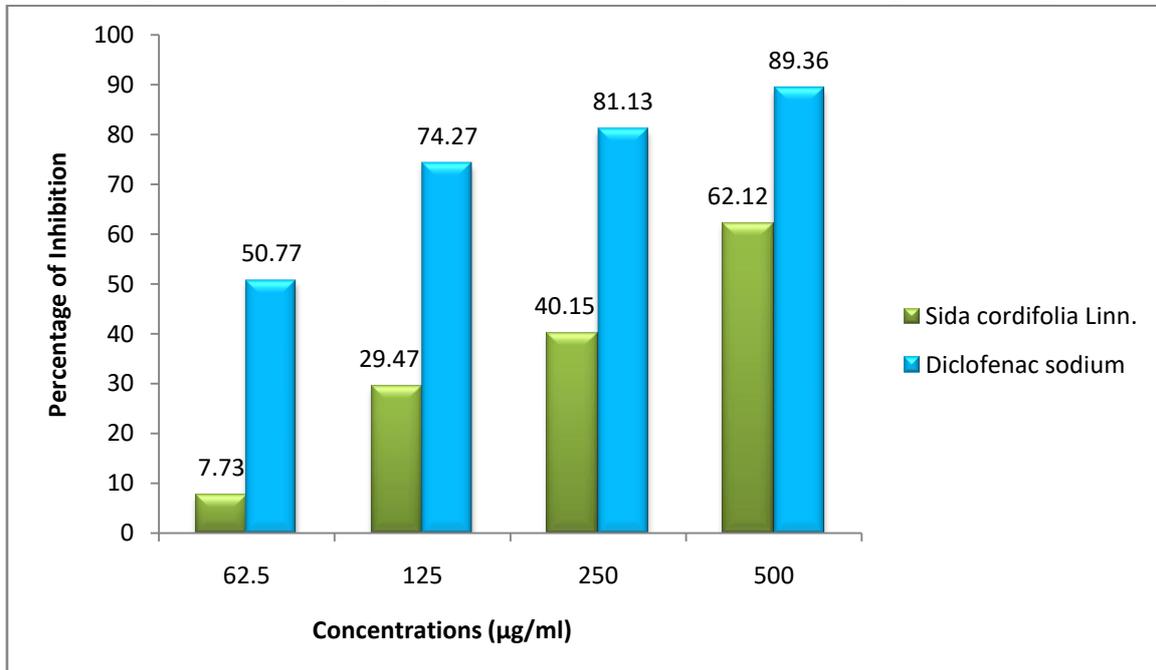


Fig. 6: Anti-arthritic activity of root of *Sida cordifolia* Linn. compared to Diclofenac sodium by Inhibition of Protein Denaturation Method

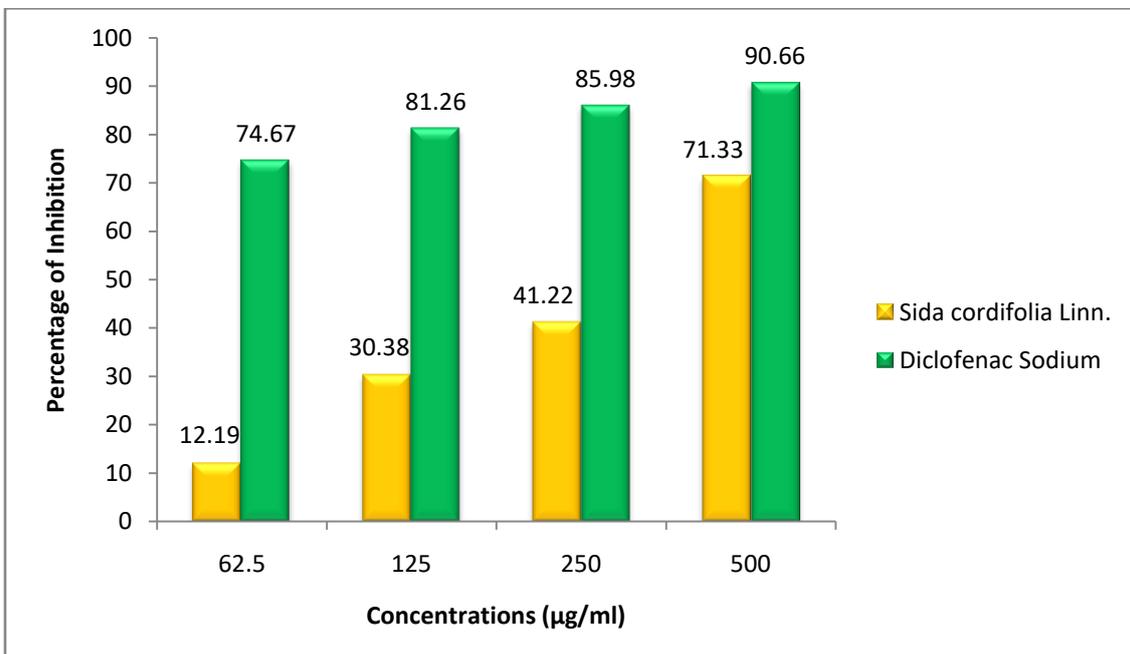


Fig. 7: Anti-arthritic activity of root of *Sida cordifolia* Linn. compared to Diclofenac sodium by Inhibition of Proteinase Enzyme Activity

DISCUSSION

The results show that *Sida cordifolia* Linn. root *Kashaya* has dose dependent anti-arthritic activity. In both the experiments, as the concentration was increased from 62.5µg/ml to 500µg/ml, the ability of the drug to inhibit the denaturation of protein and proteinase enzyme activity increased respectively. Maximum activity was shown in 500µg/ml. Literature shows that denaturation of proteins is associated with the pathology of arthritis^[4]. The antigenic property acquired by some cells due to protein denaturation may initiate immune response in the body and result in biochemical changes in connective tissue. This may ultimately end up in rheumatoid arthritis. The study shows that the *Kashaya* of *Sida cordifolia* root has the ability to inhibit denaturation of protein and also to inhibit the activity of proteinase enzyme.

CONCLUSION

The evaluation of anti-arthritic activity through Inhibition of Protein denaturation method and Inhibition of proteinase enzyme activity proved that the *Kashaya* of *Sida cordifolia* root has the ability to resist arthritis. Though various extracts of *Sida cordifolia* Linn. have showed anti-arthritic activity, the study assured the efficacy of *Kashaya* formulation of the drug, justifying its use in various Ayurvedic formulations. The study also supports the use of *Sida cordifolia* Linn. as *Bala* in Ayurvedic medicines for treating arthritic conditions. Further studies can be carried out with other varieties of *Bala* mentioned in Ayurvedic classical text books and also with various Ayurvedic formulations containing *Bala*, for their anti-arthritic activity.

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Cite this article as:

Anuja Varghese, Jollykutty Eapen. In Vitro Anti-Arthritic Activity of *Kashaya* of *Sida Cordifolia* Linn. International Journal of Ayurveda and Pharma Research. 2018;6 (7):60-64.

Source of support: Nil, Conflict of interest: None Declared

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