



Research Article

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## ***In vitro* Anti arthritic activity of Acacia catechu ethanolic leaf extract**

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### **ABSTRACT**

Rheumatoid arthritis is a chronic systemic disease affecting the population worldwide. Inflammation is a complex biological process and is an initial response to tissue injury. It is mediated by the release of autacoids and usually precedes the development of the immune response. Acacia catechu ethanolic leaf extract is commonly called as Black kutch. It contains catechin, rutin, isorhamnetin, epicatechin, afzelechin. It has antioxidant and free radical scavenging,

Anti microbial, Anti diabetic, Immuno modulatory, Anti cariogenic, Hepato protective activity. The aim of the article is to evaluate the anti arthritic activity of Acacia catechu ethanolic leaf extract invitro by protein denaturation method.

**Keywords:** Inflammation, autacoids, Rheumatoid arthritis, free radical scavenging, protein denaturation

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### **INTRODUCTION**

Rheumatoid arthritis is one of the chronic systemic disease which affects majority of population. It leads to irreversible joint damage and systemic complications [1-3].

It may involve nociceptive and non-nociceptive components, including neuropathic components due to sensitization and peripheral inflammation. Despite the modern wealth of analgesic options available in our country, treating acute to chronic patients still remains clinically challenging. NSAIDS are effective in treating nociceptive arthritis related pain. But because of the side effects and toxicity caused by NSAIDS even after the discontinuation of the drug, the use of NSAIDS has been relatively reduced.

Acacia catechu Willd. belongs to the family Fabaceae and subfamily mimosoideae. It is widely used in Ayurveda for many diseases and mainly skin diseases<sup>4</sup>. People in Kerala use boiled khadira water (karungali water) for drinking purpose. Many Ayurvedic oil preparation use khadira as one of its active ingredient [5]. Acacia catechu has strong astringent and antioxidant activity. It is most commonly known as katha which is an ingredient of Pan, a beetle leaf preparation chewed in India. It is used to reduce the oozing from chronic ulcers and as an astringent In throat, dental and oral infections.

The concentrated aqueous extract known as cutch or khair gum is used as an astringent, cooling and digestive, beneficial in cough and diarrhea, applied externally to ulcer, boils and skin diseases and is extensively used in Ayurvedic preparations<sup>6</sup>. Acacia catechu extracts exhibits various pharmacological effects like antidiarrheal, hypoglycemic, antipyretic, anti inflammatory, hepatoprotective, antioxidant and antimicrobial activities [7,8-19].

## MATERIALS & METHODS

### Plant material

Acacia catechu ethanolic leaf extract is obtained from Green Chem Herbal Extracts & Formulations, Bangalore as a Gift sample.

### EVALUATION OF INVITRO ANTI-ARTHRITIC ACTIVITY

#### Inhibition of Protein Denaturation method [20,21]

Concentration of test substance: 1000 to 200µg/ml

Standard : Diclofenac sodium

Chemicals Required : Bovine serum albumin, 1N HCl, Phosphate buffer (pH 6.3)

Instrument : Incubator, Spectrophotometer - 660nm

The following 4 solutions will be used

1. Test solution (0.5ml) consist of 0.45ml of bovine serum albumin (5% w/v aqueous solution ) and 0.05ml of test solution in various concentration and pH will be adjusted to 6.3 by using a small amount of 1N Hcl .The samples were incubated at 370C for 20 minutes and heated at 570C for 3 minutes. After cooling, to the sample add 2.5ml of Phosphate buffer (pH 6.3).

2. Test control solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5% aqueous solution) and 0.05ml of distilled water and pH will be adjusted to 6.3 by using a small amount of 1N Hcl the samples were incubated at 370C for 20 minutes and heated at 570C for 3 minutes. After cooling, to the sample add 2.5ml of phosphate buffer (pH 6.3)

3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05ml of test solution in various concentration and pH will be adjusted to 6.3 by using a small amount of 1N Hcl. The samples were incubated at 370C for 20 minutes and heated at 570C for 3 minutes. After cooling to the sample add 2.5ml to phosphate buffer (pH6.3)

4. Standard solution (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution and 0.05ml of diclofenac sodium solution in various concentrations and pH will be adjusted to 6.3 by using a small amount of 1N Hcl. The samples were incubated at 370C for 20 minutes and heated at 570C for 3 minutes. After cooling, to the sample add 2.5 ml of phosphate buffer (pH6.3)

The percentage inhibition of Protein denaturation will be calculated as follows.

$$\text{OD of test solution} - \text{OD of product control}$$

$$\% \text{ Inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

$$\text{OD of test control}$$

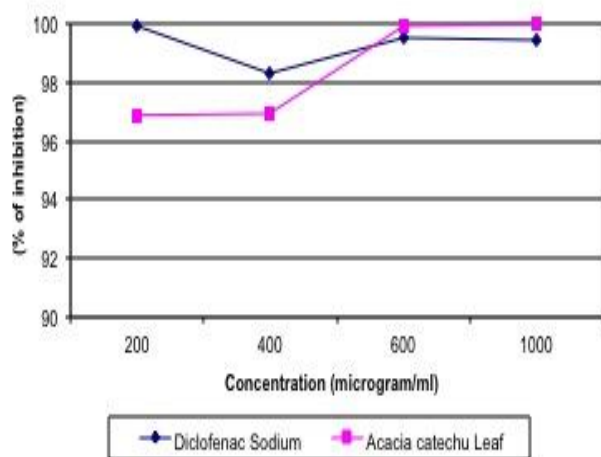
The control represents 100% protein denaturation.

The result is compared with diclofenac sodium treated sample.

## RESULTS AND DISCUSSION

Protein denaturation is one of the important known cause of rheumatoid arthritis. The mechanism of denaturation involves alteration of electrostatic hydrogen and disulphide bonding. Auto antigen production in rheumatoid arthritis probably occurs due to denaturation of protein. Hence Acacia catechu leaf extract is a promising candidate for controlling the production of auto antigen by inhibiting the denaturation of protein and it's effect by comparing with diclofenac.

From the result of present study various fractions of leaves of Acacia catechu were subjected to in vitro anti arthritic activity in various concentrations (i.e) 200,400,800,1000 µg/ml , and the % of inhibition of different concentrations of Acacia catechu leaves by protein denaturation method is depicted in Table 1 and Figure 1.



**Figure 1:** Anti –arthritic activity of Acacia catechu ethanolic leaf extract

Hence, the percentage of inhibition at 200  $\mu\text{g/ml}$  was found to be 96.96 , 400  $\mu\text{g/ml}$  was found to be 98.97 , 800  $\mu\text{g/ml}$  was found to be 99.98 , 1000  $\mu\text{g/ml}$  was found to be 99.99.

**Table 1:** Anti –arthritic activity of Acacia catechu ethanolic leaf extract

S.NO	Concentration $\mu\text{g/ml}$	Diclofenac % of inhibition	<i>Acacia catechu Leaf</i> extract % of inhibition
1	200	99.88	96.88
2	400	98.27	96.96
3	800	99.52	96.88
4	1000	99.45	96.92

## CONCLUSION

Protein denaturation method invitro on leaves of *Acacia catechu* showed the presence of anti-arthritic activity. Hence further research could be targeted on the in vivo Anti-inflammatory /Anti-arthritic activity.

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