

**EVALUATION OF THE IN VITRO ANTI-ARTHRITIC ACTIVITY OF  
TRIBULUS TERRESTRIS L. SEED EXTRACTS**

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**ABSTRACT**

The aqueous and ethanol extracts from that investigation. Following phytochemical testing, the Tribulus terrestris seed showed pharmacological properties, including antarthritic activity. Comparative investigations have demonstrated that aqueous extract is more active than ethanol extract. Extracts from Tribulus terrestris shown efficacy against COC values of 100, 250, and 500 (ug/mL).

**KEYWORDS:** Comparative investigations have demonstrated that aqueous extract is more active than ethanol extract.

**INTRODUCTION**

Symmetrical arthritis is among the most prevalent autoimmune conditions, characterized by progressive synovial joint damage that leads to structural deterioration, deformity, and functional disability (Mishra et al., 2013). Rheumatoid arthritis (RA) is a complex disease marked by cartilage and bone erosion, pannus formation, synovial hyperplasia, and fibrosis, driven by a network of cytokines, protein mediators, and proteolytic enzymes. Patients with erosive RA typically show higher levels of interleukin-1 (IL-1) in blood and synovial fluid compared with those with non-erosive disease. Interleukin-6 (IL-6), a cytokine with pleiotropic activity, plays an essential role in inflammatory signaling, immune regulation, bone metabolism,

endocrine control, and hepatic production of acute-phase reactants (Reddy, n.d.). RA occurrence is highest among women aged 40–50 years and slightly later in men, with symptoms that persist and fluctuate over time, eventually causing joint deformities and disability. Because RA is chronic, spontaneous remission is rare. Although several therapeutic options exist, including nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs), nearly 30% of patients fail to respond adequately to treatment (Mali et al., 2011). Chronic NSAID use can lead to gastrointestinal ulceration and cardiovascular complications (Thillaivanan et al., 2013).

### **IN-VITRO ANTI ARTHRITIS ACTIVITY**

In this study, the anti-arthritic activity of selected plant extracts was assessed using a standard in vitro protein denaturation model, which is widely employed for screening anti-inflammatory and anti-arthritic agents (Mizushima & Kobayashi, 1968; Williams et al., 2008). Diclofenac sodium served as the reference standard due to its well-established anti-inflammatory properties (Kaur et al., 2015).

#### **Preparation of Reagents**

##### **Albumin Reagent (1% Bovine Serum Albumin Solution)**

A 1% BSA solution was prepared by dissolving 1 g of bovine serum albumin in 100 mL of distilled water, as described in standard protein denaturation protocols (Williams et al., 2008).

##### **Preparation of Standard Solution**

A stock solution of diclofenac sodium (1000 µg/mL) was prepared in distilled water. Working concentrations of 100, 250, and 500 µg/mL were prepared fresh for the assay (Kaur et al., 2015).

##### **Preparation of Test Solutions**

The plant leaf extracts were dissolved in an ethanol–water solvent mixture to obtain a stock concentration of 1000 µg/mL, from which working concentrations of 100, 250, and 500 µg/mL were prepared (Singh & Kumar, 2012).

##### **In Vitro Anti-Arthritic Assay (Protein Denaturation Method)**

The anti-arthritic potential of the extracts was evaluated using the protein denaturation method, wherein inhibition of heat-induced albumin denaturation indicates anti-inflammatory or anti-arthritic activity (Mizushima & Kobayashi, 1968). For each test concentration, 1 mL

of the plant extract was mixed with 1 mL of the 1% BSA solution. The mixtures were incubated at 70°C for 15 minutes to induce protein denaturation, followed by cooling at 27°C for 15 minutes.

After cooling, turbidity was measured spectrophotometrically at 660 nm (Williams *et al.*, 2008). Diclofenac sodium, at concentrations ranging from 50–1000 µg/mL, was subjected to identical treatment and served as the standard reference (Kaur *et al.*, 2015). A control sample without any extract or standard was used to determine baseline turbidity. Experiments were conducted in triplicate to ensure reproducibility.

### Calculation of Percentage Inhibition

The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Inhibition of Protein Denaturation (\%)} = 100 \times \frac{(A_1 - A_2)}{A_1}$$

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Where,

$A_1$  is the absorbance of the control, and

$A_2$  is the absorbance of the test or standard sample (Williams *et al.*, 2008). Higher inhibition indicates stronger anti-arthritic activity.

## RESULT

### Antiarthritic Activity of seeds of *Tribulus terrestris* Extract.

Sr. No	Plant Extract	Concentration In µg/ml	Mean Absorbance (Mean ± SEM)	Percentage Inhibition (%)
1	Control	-	0.812	-
2	Standard	100	0.343	52.70
		250	0.362	55.41
		500	0.307	62.19
3	Ethanol extract	100	0.502	38.17
		250	0.484	40.39
		500	0.422	48.02
4	Aqueous extract	100	0.460	43.34
		250	0.412	49.26
		500	0.390	51.97

The present investigation represents the first attempt to evaluate the anti-arthritic activity of *Tribulus terrestris* Linn. seed extracts using the in vitro protein denaturation inhibition assay.

Protein denaturation is a well-established indicator of inflammatory and arthritic processes, and agents capable of inhibiting this process are considered to possess potential anti-arthritic properties.

In the current study, both ethanol and aqueous seed extracts were evaluated across three concentrations: 100, 250, and 500  $\mu\text{g/mL}$ . Diclofenac sodium was used as the reference standard for comparison.

The control sample, containing only bovine serum albumin, showed an absorbance of 0.812, indicating maximal protein denaturation in the absence of any protective compound.

### **Standard Drug (Diclofenac Sodium)**

Diclofenac sodium demonstrated a clear concentration-dependent inhibitory effect.

- At 100  $\mu\text{g/mL}$ , the percentage inhibition was 52.70%.
- This increased to 55.41% at 250  $\mu\text{g/mL}$ ,
- And reached 62.19% at 500  $\mu\text{g/mL}$ , indicating strong anti-arthritic potential, as expected from a clinically established anti-inflammatory drug.

### **Ethanol Extract of Tribulus terrestris Seeds**

The ethanol extract showed moderate inhibition of protein denaturation:

- 38.17% inhibition at 100  $\mu\text{g/mL}$ ,
- 40.39% at 250  $\mu\text{g/mL}$ ,
- 48.02% at 500  $\mu\text{g/mL}$ .

Although the activity increased with concentration, the ethanol extract remained less effective than the standard drug at all tested doses.

### **Aqueous Extract of Tribulus terrestris Seeds**

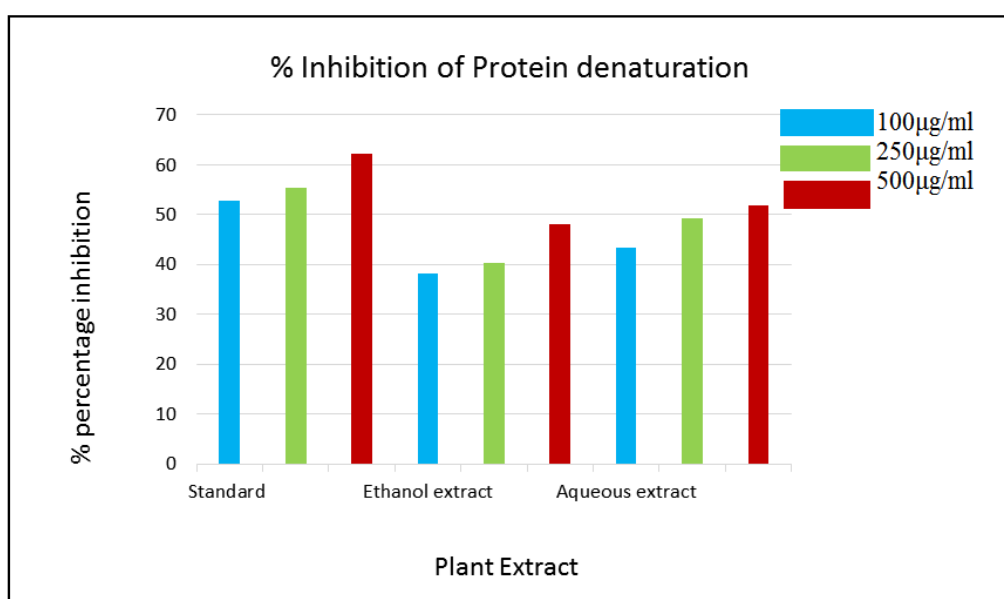
The aqueous extract exhibited comparatively higher inhibitory activity than the ethanol extract.

- At 100  $\mu\text{g/mL}$ , the inhibition was 43.34%,
- Increasing to 49.26% at 250  $\mu\text{g/mL}$ ,
- And achieving 51.97% at 500  $\mu\text{g/mL}$ .

This trend demonstrates a strong dose-dependent response, with the aqueous extract showing the highest activity among the plant extracts tested. At the maximum concentration tested

(500  $\mu\text{g/mL}$ ), the aqueous extract reached 51.97% inhibition, approaching the standard diclofenac sodium value of 62.19%.

Based on the findings, both extracts of *Tribulus terrestris* seeds possess measurable anti-arthritic activity, with the aqueous extract demonstrating superior efficacy compared to the ethanol extract. While neither extract surpassed the inhibition level observed with diclofenac sodium, the aqueous extract showed inhibition values close enough to the standard to suggest meaningful biological activity. These results confirm that *Tribulus terrestris* seeds contain bioactive constituents capable of stabilizing proteins and preventing heat-induced denaturation, thereby supporting their traditional ethnomedicinal use in inflammatory and arthritic conditions. Further studies, including *in vivo* evaluations and phytochemical profiling, are warranted to identify the specific compounds responsible for this activity.



Percentage Inhibition of Protein Denaturation at Different Concentrations of Seeds Extract of *Tribulus Terrestris* Linn.

## CONCLUSION

The present work represents the first known attempt to investigate the anti-arthritic potential of *Tribulus terrestris* Linn. seed extracts using the *in vitro* protein denaturation inhibition assay. Both the ethanol and aqueous extracts were evaluated across increasing concentrations (100, 250, and 500  $\mu\text{g/mL}$ ). The results demonstrated a clear dose-dependent increase in inhibitory activity, indicating that higher concentrations of the extracts were more effective in

preventing protein denaturation—a key mechanism associated with inflammation and arthritis. Among the tested extracts, the aqueous seed extract showed the highest activity, reaching a maximum inhibition of 51.97% at 500 µg/mL. This value was substantially higher than that observed for the ethanol extract at the same concentration, suggesting that water-soluble phytoconstituents of *T. terrestris* seeds may contribute more significantly to anti-arthritic effects. To benchmark the efficacy of the extracts, the results were compared with the standard anti-inflammatory drug diclofenac sodium, which demonstrated 62.19% inhibition at 500 µg/mL. Although the aqueous extract did not surpass the activity of diclofenac, its inhibition value was relatively close, indicating promising biological potential. These findings support the traditional use of *Tribulus terrestris* in managing inflammatory conditions and provide a scientific basis for further pharmacological investigation.

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