

# In vitro Anti-Arthritic activity of *Pseudarthria viscida* against Protein Denaturation and Proteinase enzyme

## Research Article

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### Abstract

Arthritis is an autoimmune with chronic inflammatory, the patient has very painful due deformities and bone erosion which is caused by damage of the joints. The plant *Pseudarthria viscida* was collected from the Thirunelveli district and extracted with aqueous and ethanol solvent. The two method was used for determination of invitro anti-arthritic activity. The Inhibition of Protein Denaturation Method shows the anti-arthritic activity with the value from  $40.46 \pm 0.72$  to  $78.36 \pm 0.64$  for aqueous extract and  $48.62 \pm 0.86$  to  $84.42 \pm 0.86$  for ethanol extract and Inhibition of Proteinase Enzyme Activity shows  $38.62 \pm 0.32$  to  $72.58 \pm 0.58$  in aqueous extract and  $46.28 \pm 0.58$  to  $80.52 \pm 0.56$  in ethanol extract. Diclofenac sodium were used as standard, the concentration is 100, 200, 300, 400, and 500. In both the method the concentration of 500Microgram per milliliters shows maximum inhibition and compare to both extract the ethanol shows better activity than aqueous extract.

**Key Words:** Arthritis, Aqueous, Ethanol, Protein, *Pseudarthria viscida*.

### Introduction

India is widespread of its different geographical and ecological system and also rich in medicinal properties, present in various part of the country. In ancient period of time the medicinal plant is used for various treatment of diseases. WHO state that the primary health care needs of the people depends on traditional medicine in the world. (1,2) Arthritis is an autoimmune with chronic inflammatory, it affects smaller joints, progressing to large joints and along with this skin, eyes, heart, kidneys and lungs also affected. The tendons and ligaments are weakened due to bone and cartilage of joints are destroyed (3) the patient have very painful due deformities and bone erosion which is caused by damage of the joints. The symptoms are referred to stiffness of affected joints, fever, weight loss, joints that are tender, swollen and warm, rheumatoid nodules under the skin and fatigue, the disease start at age between 35 to 60 years with remission and exacerbation. The juvenile RA (JRA) is one affected to the children at the age of 16 years and it is similar to the RA except the rheumatoid factor is not found. (4-7) In the West, the prevalence of RA is believed to be 1-2% (7, 8) and 1% worldwide. (9) osteoarthritis is the most common effect of arthritis the other effects include gout, fibromyalgia, and rheumatoid arthritis (RA). Some forms of arthritis, such as

rheumatoid arthritis and lupus (SLE), can affect multiple organs and cause widespread symptoms. There are four main groups of drugs used to treat arthritis: Pain killers (analgesics), non-steroidal anti-inflammatory drugs (NSAIDs), disease-modifying anti-rheumatic drugs (DMARDs) and corticosteroids (steroids). (10) In invitro denaturation of proteins there is a production of auto antigens in certain arthritic diseases. (11) There is in alteration of electrostatic, hydrogen, hydrophobic and disulphide bonding in the mechanism of denaturation. (12) the anti-arthritic activity is controlled by the production of auto antigen and inhibiting the denaturation of protein and membrane lysis.

Hence, the anti-protein denaturation and membrane lysis are important measure taken in the invitro anti-arthritic activity. (13) In case of inflammatory and arthritic the denaturation of tissue protein plays a vital role in it for its causes. So, in the arthritic disease the denaturation of protein is responsible for the Production of auto antigen in vivo. Agents that can prevent protein denaturation therefore could be worthwhile for anti-arthritis drug development. (14) There is some protein became antigenic due to formation of denaturation and macroglobulin in the immune response and producing biochemical changes in connective tissue, which Ultimately leads to rheumatoid arthritis. (15)

### Materials and Methods

#### Ethnobotanical claims of *Pseudarthria viscida*

India has about 45,000 species of plant and in this species thousands of species have medicinal properties. The some of the medicinal properties are antidiabetic, antioxidant, anticancer and anti-inflammatory etc. these medicinal properties is proved

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by scientifically for their uses. So, it is important for the medicinal plant for its authentication. Research conducted in the last few decades on plants mentioned in ancient literature. Some plants have been demonstrated with animal model for their pharmacological effects, whose active principle have not been isolated and identified.

In some other plants only the biologically active components have been isolated but no pharmacological effects have been described. The use of herbal remedies for arthritis treatment has been gaining momentum in recent years. Roots of medicinal plants are common ingredients of many folk and herbal medicines (16) and extracts of a number of medicinal plants have been reported to possess pharmacological activity, mainly anti-inflammatory activity. Hence an attempt has been made at present work to study the anti-arthritis property, of the *Pseudarthria viscida*. *Pseudarthria viscida* (L) Wight is a shrub of family Fabaceae.

The plant is astringent, sweet, bitter, emollient, digestive, constipating, anthelmintic, cardiotoxic, febrifuge and tonic. They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, diarrhoea, inflammation, cardiopathy, fever, haemorrhoids, gout, diabetes, hyperthermia and general debility. Traditionally this plant is used in the form of decoction or powder for biliousness, excessive heat, intestinal poison, fever, diarrhoea, asthma, heart diseases, worms and piles. This plant is also used in the preparation of ayurvedic medicines namely Dashamoola, Mahanarayana Taila and Dhantara Taila.

### Extraction procedure

#### Collection and Processing of Plant material

The leaf of *Pseudarthria viscida* was collected in and around of Thirunelveli district. The collected plant material was washed with tap water for 3 times and sterilized by spraying with 70% alcohol. The sterilized plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was completely dried it is subjected to prepare fine powder with the help of pestle and motor. The fine powder is collected and used for extraction of crude drug in aqueous, ethanol solvents by Soxhlet extraction method.

#### Extraction by Soxhlet apparatus

The extraction procedure for the isolation of crude drug from plants has been practiced since long time. The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on type of substance that is being isolated. Normally the crude extract is taken from soxhlet apparatus with the aqueous solvent. This apparatus mainly consists of three parts, round bottom flask in which the solvent is taken, main jar in which material from which the compounds to be extracted is kept loaded and condenser in which condensation of vapors of solvents takes place. 200 g of the powder of plant material from which the extract has to be taken is

packed into soxhlet main jar. The solvent is poured into the round bottom flask and extract condensation under reduced pressure and controlled temperature of 60-80°C is set to boil through regulated heating mantle. The vapor of the solvent pass through drive tubes, enter the condenser through the main jar and get condensed where there is continuous flow of water in the condenser. The condensed solvent falls back on the packed material in the main jar before collecting in jar itself.

The collection and extraction of material takes place simultaneously in the main jar as seen by the coloring of the solvent as compound of material get dissolved in the solvent. The marc is obtained, dried and the dried one is used for the ethanol solvent. Thus, the crude extract of the plant material is obtained and normally it takes 7-8 hours for complete each extraction the solvent will be evaporated and finally it yields green extract, this is stored in refrigerator for further usage.

### 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 (17)

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

- **Test solution:** 0.5ml of *Pseudarthria viscida* extract and 0.45ml of bovine serum albumin
- **Test control solution:** 0.45ml of bovine serum albumin and 0.5ml of distilled water
- **Product control solution:** 0.45ml of distilled water and 0.5ml *Pseudarthria viscida* extract
- **Standard solution:** 0.45ml of bovine serum albumin and 0.5ml 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 *Pseudarthria viscida* extract. was calculated using the following formula:

$$\text{Percentage of Inhibition} = 100 - \left\{ \frac{\text{optical density of test solution} - \text{optical density of product control}}{\text{optical density of test control}} \right\} \times 100$$

#### Inhibition of Proteinase Enzyme Activity (18)

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

- **Test solution:** Phosphate buffer+1ml Tris Hydrochloric acid + trypsin+1ml *Pseudarthria viscida* extract

- **Test control solution:** Phosphate buffer+1ml Tris Hydrochloric acid
- **Product control solution:** Phosphate buffer+1ml Tris Hydrochloric acid +1ml *Pseudarthria viscida* extract
- **Standard solution:** Phosphate buffet+1ml Tris Hydrochloric acid + trypsin +1ml Diclofenac sodium.

The reaction mixture contained 0.06mg trypsin. 1.0ml of 25mm Tris Hydrochloric acid buffer (pH 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.

$$\text{Percentage of inhibition} = 100 - \left\{ \frac{\text{Absorbance of test solution} - \text{Absorbance of product control}}{\text{Absorbance of test Control}} \right\} \times 100$$

**Result and discussion**

The inhibition of protein denaturation method for *Pseudarthria viscida* was determined by various concentrations such as 100,200,300,400,500 and the plant percentage inhibition was done with aqueous extract and ethanol extract. The percentage inhibition of protein denaturation with various concentrations shows the different values such as from 40.46±0.72 to 78.36±0.64 and it is compared with the standard diclofenac sodium the comparison shows the percentage inhibition for the concentration of 500 µg/ml shows maximum inhibition in aqueous extract and in standard respectively. In ethanol extract was same as previous one which shows the values from 48.62±0.86 to 84.42±0.86 and also the maximum inhibition was at concentration of 500 µg/ml in table-I. In comparison with both extract the ethanol shows the maximum inhibition activity when compare to aqueous extract.

In inhibition of proteinase enzyme activity method for *Pseudarthria viscida* was determined by various concentrations such as 100,200,300,400,500 and the plant percentage inhibition was done with aqueous extract and ethanol extract. The percentage inhibition of proteinase enzyme activity with various concentrations shows the different values such as from 38.62±0.32 to 72.58±0.58 and it is compared with the standard diclofenac sodium the comparison shows the percentage inhibition for the concentration of 500 µg/ml shows maximum inhibition in aqueous extract and in standard respectively. In ethanol extract was same as previous one which shows the values from 46.28±0.58 to 80.52±0.56 and also the maximum inhibition was at concentration of 500 µg/ml in table-II. In comparison with both extract the ethanol shows the maximum inhibition activity when compare to aqueous extract.

**Table1: Inhibition of Protein Denaturation Method for *Pseudarthria viscida***

Conc (µg/ml)	%inhibition of protein denaturation method for aqueous extract	%inhibition of protein denaturation method for ethanol extract	%inhibition of protein denaturation method for standard (Diclofenac)
100	40.46±0.72	48.62±0.86	80.65±0.36
200	48.28±0.58	56.24±1.24	86.48±0.84
300	56.32±1.08	64.58±0.56	90.08±0.24
400	65.58±0.12	72.68±0.32	94.62±0.76
500	78.36±0.64	84.42±0.86	98.86±0.52

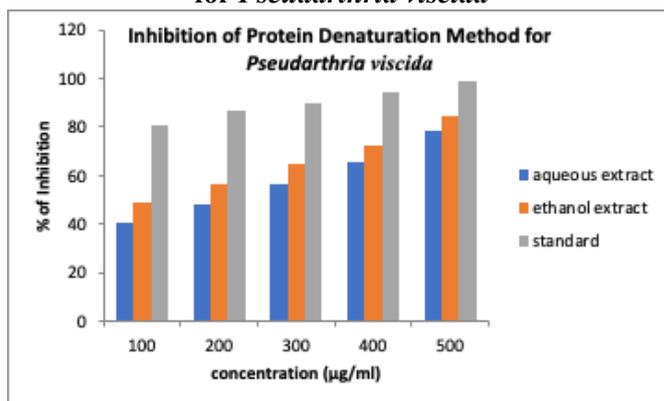
Each value was obtained by calculating the average of three determinants and data are presented as mean± SEM

**Table 2: Inhibition of Proteinase Enzyme Activity for *Pseudarthria viscida***

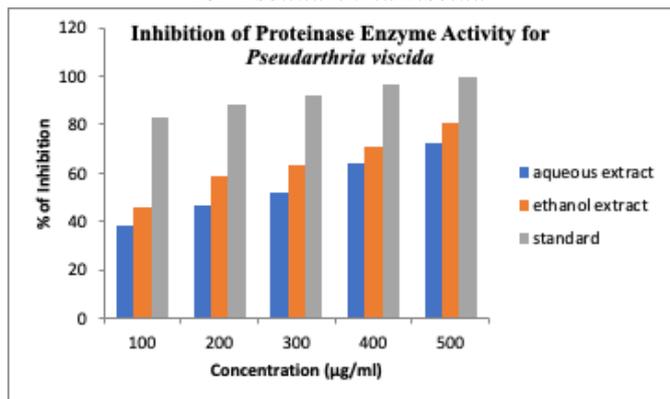
Conc (µg/ml)	% Inhibition of Proteinase Enzyme Activity for aqueous extract	% Inhibition of Proteinase Enzyme Activity for ethanol extract	% Inhibition of Proteinase Enzyme Activity for standard (Diclofenac)
100	38.62±0.32	46.28±0.58	82.64±0.65
200	46.84±0.68	58.68±0.28	88.52±0.58
300	52.36±0.84	63.48±1.38	92.36±0.24
400	64.36±0.24	70.62±0.92	96.32±0.52
500	72.58±0.58	80.52±0.56	99.42±0.68

Each value was obtained by calculating the average of three determinants and data are presented as mean± SEM

**Figure1: Inhibition of Protein Denaturation Method for *Pseudarthria viscida***



**Figure 2 Inhibition of Proteinase Enzyme Activity for *Pseudarthria viscida***



In the arthritis disease, the denaturation of protein, membrane lysis and proteinase action are due to the production of auto antigen the mechanism of denaturation probably involves electrostatic hydrogen, hydrophobic and disulphide bonding. From the results the maximum percentage inhibition of protein denaturation of extract and standard were observed from Table.1 and from Figure 1. In the bovine serum albumin (BSA) there are two interesting binding site namely aromatic tyrosine rich and aliphatic threonine and lysine residue are responsible for the anti-denaturation property of BSA as per the literature. (19) It has been reported that the receptor rich in tyrosine motif which is a therapeutic molecule for activating the threonine signal transduction biological pathway for their overall biological action. (19,20) Compounds interacting with the aliphatic regions around the lysine residue on the BSA could be interesting as anti-oxidant with anticancer activity such as the polyphenols, phenyl propanoids and the disulphides- (19-22) However the extract is phenolic in nature, as per the literature. Hence this may be the region for its possible anti-denaturation activity. It seems that the presence of protease inhibitors at the site of arthritic, first shown by Opie. (23) is important in limiting the destructive activity of proteases that are liberated from polymorphonuclear leucocytes and other necrotizing tissues. At least two such enzymes have been isolated and characterized. (24, 25) It would be interesting to know which proteases are present as inhibitor complexes and which remain uninhibited; for proteases that are not inactivated should be potentially more destructive ones. The inhibition of these proteases by other normally occurring inhibitors as well as synthetic ones could be an important step in preventing tissue damage in arthritis and as per the result the extract and standard shows the maximum Inhibition of Proteinase enzyme activity were observed in table II and figure II The investigation had clearly demonstrated that the *Pseudarthria viscida* possess potent anti-denaturation property and anti-proteinase enzyme activity. The phenolic nature of the *Pseudarthria viscida* may be the region for its possible anti-arthritis activity. Further in-vivo studies have performed to authenticate anti-arthritic activity.

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