



ANTIARTHRITIC ACTIVITY OF THE LEAVES OF *SPHAGNETICOLA TRILOBATA* (L.)

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ABSTRACT

The aim of the present study is to carry out the *in vitro* antiarthritic activity of the methanolic extract of the leaves of *Sphagneticola trilobata*. The activity was carried out using protein denaturation method. The leaf extract of the plant produced remarkable antiarthritic activity and the activity produced was comparable to activity produced by the reference standard used.

KEYWORDS: *Sphagneticola trilobata*, Anti arthritic activity, Aspirin, Egg albumin, Bovine albumin.

INTRODUCTION

Arthritis is a kind of rheumatism characterized by swollen, stiff, and painful joints.^[1] Rheumatoid arthritis is a chronic autoimmune inflammatory disorder that causes inflammation of the joints and synovia, swelling, heat and pain which may result in deformities of the joints, bones and cartilages. From the researches and investigatory studies so far conducted, it is evident that many natural medicinal plants have the ability to treat arthritis and its associated inflammation.^[2] Anti-arthritic activity can be estimated using both *in-vivo* and *in-vitro* techniques. *In-vivo* methods of anti-arthritic study includes formaldehyde induced arthritis in rats, Complete Freund's Adjuvant (CFA)-induced arthritis in rats.^[3] *In-vitro* anti-arthritic study is commonly performed using protein denaturation assay. Protein denaturation assay is carried out using Bovine Serum Albumin and egg albumin.^[4]

The process of losing the secondary, tertiary or quaternary structure of protein due to the application of some external compounds or external stress is defined as protein denaturation. The external stress or compounds applied can be heat, strong acids or bases, concentrated salts, organic solvents etc.^[3] It is evident that protein denaturation is the cause of inflammation^[3,5] and NSAIDs that are used for treating rheumatoid arthritis works by inhibiting the denaturation of proteins, thereby preventing the inflammation.^[3]

Sphagneticola trilobata is a creeper belonging to the family Asteraceae. The plant is widely distributed in India, North and South America, Mexico, Brazil, Florida, South Africa etc. The plant is commonly found in wet

and waste lands. The plant has both medicinal and ornamental value. The synonym of the plant is *Wedelia trilobata*.^[6]

MATERIALS AND METHODS

Plant material

The leaves of the plant were collected from the waste lands of Cheruthazham village of Kannur district, Kerala state in the month of July 2019. Its botanical identity was confirmed and then shade dried and the specimen bearing voucher has been deposited in the department of Pharmacognosy, College of Pharmaceutical Sciences, Government Medical College, Kannur, Kerala state.

Preparation of methanolic extract

The dried leaves of the plant were powdered and were extracted with 95% methanol in soxhlet apparatus for 6 hours. The total methanolic extract was then concentrated to a syrupy consistency and dried in vacuum desiccator.

EVALUATION OF ANTI ARTHRITIC ACTIVITY

Denaturation of proteins by egg albumin

The 5ml of reaction mixture consists of 0.2ml of egg albumin obtained from the fresh hen's egg, 2.8ml of phosphate buffered saline of pH 6.4 and 2ml of varying concentrations of methanolic extract of the leaves of *Sphagneticola trilobata* so that the final concentration become 100, 200, 400, 600, 800 and 1000 µg/ml. Similar volumes of distilled water served as control. Then the mixtures were incubated at 37±2 °C in a BOD incubator for 15 minutes and then heated at 70°C for 15 minutes. After cooling, their absorbance was measured at 660nm. Aspirin was used as reference standard.^[7] The percentage

of inhibition of protein denaturation was calculated by using the following formula;

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Denaturation of proteins by bovine albumin

The reaction mixture was consisting of methanolic extract of the *Sphagneticola trilobata* at different concentrations and 1% of aqueous solution of bovine

albumin. The samples were incubated at 37°C for 20 minutes and then heated at 57°C for 20 minutes after cooling the samples. Absorbance of turbidity was measured at 660nm.^[8] The percentage of inhibition of protein denaturation was calculated by using the following formula;

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

Table 1: Percentage inhibition of protein denaturation of the leaves of *Sphagneticola trilobata* using egg albumin.

Treatment Regimen	Concentration (µg/ml)	Percentage of Inhibition (%)
<i>Sphagneticola trilobata</i> (Methanolic extract)	100	10.8
	200	29.6
	400	47.3
	600	66.7
	800	89.2
	1000	109.6
Aspirin (Reference standard drug)	Control - 50	77.9

Table 2: Percentage inhibition of protein denaturation of the leaves of *Sphagneticola trilobata* using bovine albumin.

Treatment Regimen	Concentration (µg/ml)	Percentage of Inhibition (%)
<i>Sphagneticola trilobata</i> (Methanolic extract)	100	9.5
	200	19.8
	400	36.4
	600	53.2
	800	79.5
	1000	99.8
Aspirin (Reference standard drug)	Control - 50	84.3

The methanolic extract of the leaves of *Sphagneticola trilobata* and aspirin were carried out for antiarthritic activity by protein denaturation methods like denaturation of proteins by egg albumin and denaturation of proteins by bovine albumin. From the results obtained, the antiarthritic activity was maximum with 1000 µg/ml concentration, and the same was minimum with 100

µg/ml concentration. In the *invitro* antiarthritic activity of the methanolic extract of the leaves of the plant by egg albumin method, the percentage of arthritic protection was found to be 99.8 in 1000ml concentration and that of aspirin was 84.3. The inhibition of protein denaturation by bovine albumin was found to be 109.6 and that of aspirin was found to be 77.9.

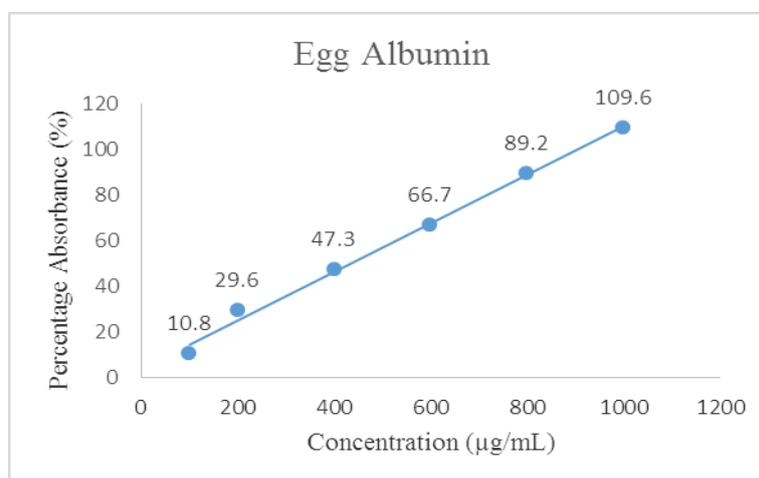


Fig. 1: Percentage inhibition of protein denaturation of the leaves of *Sphagneticola trilobata* using egg albumin.

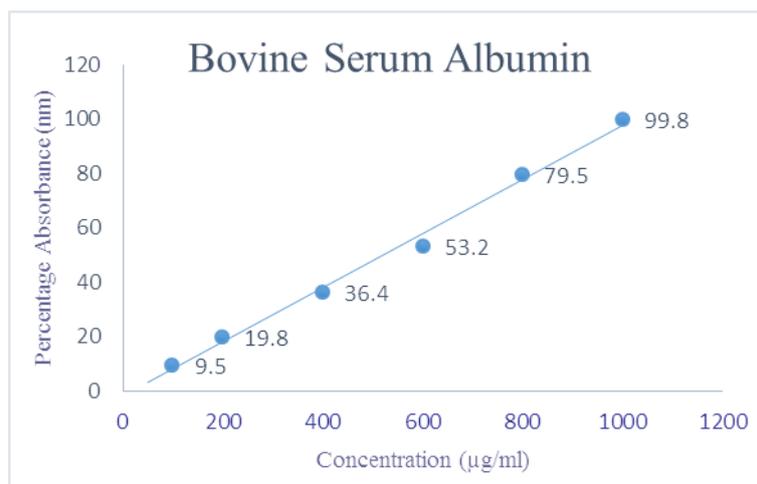


Fig. 2: Percentage inhibition of protein denaturation of the leaves of *Sphagneticola trilobata* using bovine albumin.

The result shows that the methanolic extract of the leaves of *Sphagneticola trilobata* exhibited a dose dependent response. The antiarthritic activity of the methanolic extract of the leaves were comparable with aspirin which was used as the reference standard.

The phytochemical screening of the plant shows the presence of phenolic compounds, flavonoids etc.^[9,10] The activity shown by the leaf extract of the plant could be due to the presence of flavonoids and phenolic compounds. Anti-inflammatory activity of the plant have been already identified and confirmed by various studies.^[6,11,12] Denaturation of tissue protein is one of the established reasons for inflammatory and arthritic diseases. Auto antigens are produced in certain arthritic diseases may be due to *in vivo* denaturation of protein.^[13,14] So the drugs which are capable to prevent the protein denaturation can be used as an anti arthritic agent.

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