



IN-VITRO ANTI- ARTHRITIC ACTIVITY OF METHANOLIC EXTRACTS OF *ALLIUM SATIVUM*

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ABSTRACT

The present study was aimed to evaluate in-vitro anti- arthritic activity of methanolic extract of *Allium sativum* which belongs to the family Alliaceae. The in-vitro anti- arthritic activity was evaluated by using Bovine serum albumin denaturation method and Egg albumin denaturation method. The standard drug is considered as mefenamic acid. The phytochemical screening revealed the presence of alkaloids, saponins, flavonoids and tannins. The experimental results showed that the methanolic extracts of *Allium sativum* possess significant anti-arthritic activity. The in-vitro anti- arthritic of *Allium Sativum* has been reported for the first time.

KEYWORDS: In-vitro, bovine serum albumin, denaturation.

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disease which leads to increase in joint distortion, dysfunction of synovium a specialized tissue which is responsible for maintaining the nutrition and lubrication of the joint.^[1,2] The pathology of rheumatoid arthritis is not easy to analyze and the reason behind this mechanism is still unknown. The standard drugs which were used for the treatment of this disease are like NsAIDS, Inflammatory agents, Immunosuppressant and corticosteroids to newer biological molecules such as monoclonal antibodies and TNF- α, these type of drugs offer temporary relief with side effects like CVS

complications, Ulcer, Nephro and haemato toxicity. So there's a need to develop long acting drugs with less side effects. Even though several studies have been carried out, efficient medicine is not found.^[3,4] Now a days we can see that there are great advances in herbal medicines where this is an alternative treatment for diseases. Plant derived products are more popular because of their natural origin and less side effects. The products derived from the plant have desired pharmacological activity. Larger number of plants have been evaluated for in-vitro anti- arthritic activity. According to W.H.O the use of herbal remedies throughout the world is two to three times more when compared to that of the standard drugs.^[5]

Allium sativum Linn is a bulb forming agent which belongs to the family liliaceae. Its biological activities include anti-bacterial, anti-tumor, and anti-atherosclerosis and prevention of cardiovascular diseases. The oil of garlic is used as anthelmintic and rubefacient. The size of garlic ranges from 1.5 to 2.5cm and their chemical constituents are allin and allicin. It is mostly cultivated in central Asia, southern Europe, USA and India.^[6] It is a perennial plant with narrow leaves and a compound bulb consists of several small bulblets or cloves.^[7] This is the first in-vitro anti-arthritic of methanolic extract of *Allium sativum*. The effect of methanolic extract of *Allium sativum* was performed against denaturation of bovine serum albumin and egg albumin.

MATERIALS AND METHODS

Plant material

Fresh bulbs of garlic were purchased from local market in Gopalpatnam, South Visakhapatnam district.

Chemicals

Bovine serum albumin, sodium chloride, potassium chloride, di- sodium hydrogen phosphate, potassium di- hydrogen phosphate.

All the solvents and chemicals used were analytical grade.

Extraction process

Fresh bulbs of garlic were taken and cloves were separated, peeled and washed. 1000 grams were crushed using mortar and pestle. They were kept under shade for 2-3 days to dry and crumbled with a blender to a fine powder. And then the fine powder was extracted with solvent methanol for 48hrs by maceration and then followed by soxhilation. Solvent was

evaporated under reduced pressure. The obtained extract is taken in petri dishes and allowed for dryness. They were weighed and kept in refrigerator for further study.

In-vitro anti-arthritic activity by protein denaturation method

In-vitro anti-arthritic activity was carried out as per the method of Yogesh Shivhare et.al with major modifications.^[8] The reaction mixture 0.5ml consisted of 0.45ml bovine serum albumin (5% aqueous solution) and 0.05ml of *Allium sativum* extract at different concentrations (100, 150, 200, 250 μ g/ml) pH was adjusted at 6.3 using a small amount of 1N HCL. The samples were incubated at 37°C for 20min and then heated at 57°C for 30min after cooling the samples, 2.5ml phosphate buffer saline solution was added to each tube. Turbidity was measured spectrophotometrically at 660nm for control test 0.05ml distilled water was used instead of extracts while product control test lacks bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

In-vitro anti-arthritic activity by using egg albumin

Mefenamic acid is used as standard drug. The test solution consists of 2ml of various concentrations (100, 150, 200, 250) of extracts, 0.2ml of fresh egg albumin and 2.8ml of phosphate buffer solution of pH 6.4. The control solution consists of 0.2ml of egg albumin and 2.8ml of phosphate buffer solution. The mixtures were incubated at 37°C for 30min and further an increased temperature of 57°C for 10min. the absorbance of sample was measured using UV-spectrophotometer at 660nm. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percentage inhibition} = (\text{Abs control} - \text{Abs sample}) \times 100 / \text{Abs control}$$

RESULTS AND DISCUSSION

The effect of methanolic extract of *Allium sativum* was evaluated against denaturation of bovine serum albumin and egg albumin. The results were tabulated in table 1 and table 2. The motive of this study is to find a potent drug for the treatment of Rheumatoid arthritis. The percent inhibition of methanolic extracts of *allium sativum* exhibited concentration dependent activity throughout the concentrations (100, 150, 200, 250). Mefenamic acid showed less activity when compared to that of the methanolic extracts of *Allium sativum*. The methanolic extract of *Allium sativum* showed a potent anti-arthritic activity. Denaturation of tissue proteins is one of the well documented cause of arthritis. Production of auto antigens in certain arthritic diseases may be due to denaturation of proteins in vivo.^[9] *Allium sativum*

showed better anti-arthritis activity which may be because of the presence of phytochemical constituents saponins, flavonoids, tannins, cardiac glycoside. From the results of the present study it can elucidate that methanolic extract of *Allium sativum* have the potential to control the production of auto antigen by inhibiting the protein denaturation.

Table 1: Effect of *Allium sativum* on protein denaturation (fresh egg albumin).

Test concentration ($\mu\text{g/ml}$)	Test % inhibition (%)	Standard concentration ($\mu\text{g/ml}$)	Standard % inhibition (%)
100	88.5	100	47.6
150	35.2	150	56.4
200	54.1	200	19.4
250	27.0	250	48.2

Table 2: Effect of *Allium sativum* on protein denaturation (bovine serum albumin).

Test concentration ($\mu\text{g/ml}$)	Test % inhibition (%)	Standard concentration ($\mu\text{g/ml}$)	Standard % inhibition (%)
100	61.2	100	48.2
150	56.4	150	38.7
200	59.8	200	45.5
250	55.1	250	41.4

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