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Research Article

IN VITRO ANTI-ARTHRITIC ACTIVITY OF *CISSUS QUADRANGULARIS* STEM EXTRACT

VAIJAYANTHIMALA P*, SAKTHIPRIYA M, SANGAMESWARAN B

Department of Pharmaceutical Chemistry, SSM College of Pharmacy, Erode - 638 312, Tamil Nadu, India. Email: vaijphd2014@gmail.com

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ABSTRACT

Objective: The present investigation deals with the study of *in vitro* anti-arthritic activity by inhibition of protein denaturation method by bovine serum albumin method and egg albumin method. *Cissus quadrangularis* Linn plant is a perennial tendril climber with quadrangular stem. It is used in the treatment of gout, syphilis, stomach ache, regularized the menstrual cycle, antimicrobial activity, and piles in Ayurvedic medicine, and traditionally used for the bone fracture.

Method: The air-dried powder of *C. quadrangularis* Linn (stem parts) was extracted using a Soxhlet apparatus with methanol *C. quadrangularis* (MECQ) and aqueous *C. quadrangularis* water (AECQ) as solvent. The extracts were concentrated under reduced pressure. The activities were carried out using the following concentration (100, 200, 300, 400, and 500 µg/ml) and compared with diclofenac as standard drug. It has significant *in vitro* anti-arthritic in both the methods.

Result: The extract of *C. quadrangularis* possessed significant anti-arthritic property in MECQ than compared to AECQ.

Conclusion: Activity may be due to the presence of the chemical profile such as phenolic acid, flavonoid (leuteotin), and β-sitosterol. The results of the study have suggested in the use of *C. quadrangularis* Linn as a potent anti-arthritic in several applications.

Keywords: *Cissus quadrangularis* Linn, Anti-arthritic, Bovine serum albumin, Methanol, Diclofenac.

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INTRODUCTION

In the very last few decades, there is a tremendous growth in the region of herbal medicine. It is coming popularized in both developing and developed countries due to its natural origin because of its lesser side effects. Herbal remedies provide a lot of drugs for the treatment of internal diseases which are considered to be stubborn and incurable by other system of medicines [1].

Arthritis is an autoimmune disorder characterized by pain, swelling, and inflexibility [2]. Rheumatoid joint inflammation influences more or less 1% of the populace around the world. Its etiology is still obscure [3]. However, advances in understanding the pathogenesis of the disease have raised the development of new therapeutics, with improved outcomes. Rheumatoid arthritis may quickly progress into a multisystem inflammation with irreversible joint destruction and increase the risk of humanity [4].

Rheumatoid arthritis was defined as "The condition in which Primary Aesthenic Gout. Alfred Garrod established the difference between Rheumatoid arthritis and Gout". Inflammatory diseases which include different types of rheumatoid disease are a major cause of morbidity of the working force all over the world. This also knew as the king of human miseries. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical, or microbial agents [5,6].

Cissus quadrangularis L. is a plant belonging to the family Vitaceae commonly known as Asthisamhari found in tropical and subtropical xeric wood. It can be found throughout the hotter parts of India alongside hedges and neighboring countries such as Pakistan, Bangladesh, Sri Lanka, and Malaysia [7,8].

MATERIALS AND METHODS

Materials

Drugs and chemicals

All reagents procured were analytical grade.

Plant collection

Fresh stem of *C. quadrangularis* Linn was collected from field of Thalavadi near Erode and authenticated by Dr. M.Palanisamy, Scientist D and Head office in charge, Southern Regional Centre, TNAU campus, Coimbatore. (BSI/SRC/5/23/2017/Tech–2844). A voucher specimen (No: SSMCOP/106/25) has been deposited in the Department of Pharmacognosy, SSM College of Pharmacy, Jambai village, Tamil Nadu, India.

The stem of *C. quadrangularis* Linn was dried and then crushed into fine powder using laboratory homogenizer, then stored for further use.

Preparation of plant extracts

The crude drugs were extracted with methanol *C. quadrangularis* MECQ and Aqueous *C. quadrangularis* waterAECQ as a solvent using the Soxhlet apparatus for continuous hot extraction. The extract was filtered and evaporated to separate solvent and residue. The semisolid residue thus obtained was stored in desiccator until further use.

In vitro anti-arthritic activity

Inhibition of protein denaturation (bovine serum albumin)

Denaturation of tissue protein is one of the well-documented causes of inflammatory and arthritic diseases. Production of the autoantigen in certain arthritic diseases may be due to denaturation of protein *in vitro.*

Table 1: Anti-arthritic activity of *Cissus quadrangularis* by bovine and egg serum albumin method

Effect of herbal extracts in different concentration	(%) Inhibition by bovine serum method	(%) Inhibition by egg albumin method
Control		
Diclofenac		
100 µg/ml	89.41	90.81
Aqueous extract of *C. quadrangularis*		
100 µg/ml	16.47	8.27
200 µg/ml	37.64	14.11
300 µg/ml	43.52	20.52
400 µg/ml	60.00	36.74
500 µg/ml	74.11	62.35
Methanol extract of *C. quadrangularis*		
100 µg/ml	36.76	16.47
200 µg/ml	56.47	21.17
300 µg/ml	69.41	34.11
400 µg/ml	67.05	40.29
500 µg/ml	82.35	82.35

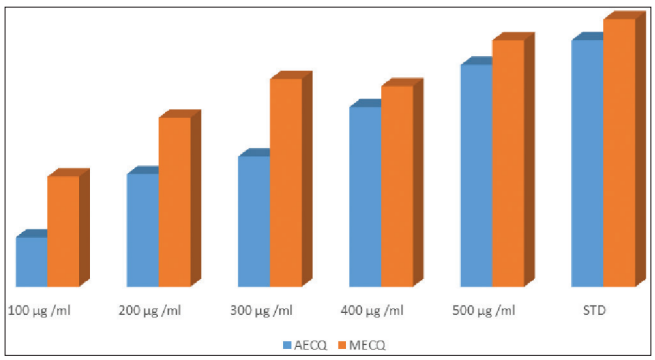

Fig. 1: Anti-arthritic activity of *Cissus quadrangularis* by bovine serum method

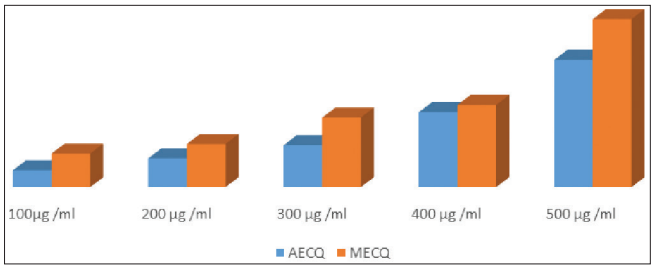

Fig. 2: Anti-arthritic activity of *Cissus quadrangularis* by egg albumin method

Agents that can prevent protein denaturation, therefore, could be worthwhile for anti-arthritis drug development [9]. Some literatures stated that protein denaturation and macroglobulin formation cause the proteins to become antigenic, thus initiating the immune response and producing biochemical changes in connective tissue, which ultimately leads to rheumatoid arthritis [10,11].

Methods

The following three solutions were used.

Test solution

0.5 ml of test solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of extracts in various concentrations (100, 200, 300, 400, and 500 µg/ml).

Test control solution

0.5 ml of test control solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of distilled water.

Standard solution

0.5 ml of standard solution consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of diclofenac sodium solution (100 µg/ml).

The pH of the above solutions was adjusted to 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 57°C for 3 min which were cooled, and 2.5 ml of phosphate buffer (pH 6.3) was added to it. Control represents 100% proteins. After cooling, their absorbance was measured at 660 nm using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{OD control - OD sample}}{\text{OD control}}$$

Inhibition of albumin denaturation (egg albumin) [12,13]

Methodology

The following three solutions were used.

Test solution

5 ml of test solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and 2 ml of in various concentrations of extracts (100, 200, 300, 400, and 500 µg/ml).

Test control solution

5 ml of test control solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffered saline and 2 ml of distilled water.

Standard solution

5 ml of standard solution consists of 0.2 ml of egg albumin and 2.8 ml of phosphate buffer saline and diclofenac 100 µg/ml.

The pH of the above solutions was adjusted to 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 20 min and heated at 70°C for 5 min denaturations, and the results were compared with standard diclofenac sodium.

After cooling, their absorbance was measured at 660 nm using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for the determination of absorbance. The percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{\text{OD control} - \text{OD sample}}{\text{OD control}}$$

RESULTS AND DISCUSSIONS

In vitro anti-arthritic activity

Hence, the results of our study reveal that extracts of C. quadrangularis were capable of controlling the production of autoantigens and inhibit denaturation of protein especially denaturation of albumin. [14]. The present studies indicate that extracts of *C. quadrangularis* exhibit strong anti-arthritic property which could be a potential source of anti-arthritic property.

The inhibition of protein denaturation, albumin denaturation, and membrane stabilization was studied to establish the mechanism of anti-arthritic activity of *C. quadrangularis*. Therefore, our *in vitro* studies on the extract of *C. quadrangularis* demonstrate the significant anti-arthritic activity. Hence, this mangrove plant can be used as a potent natural anti-arthritic agent. The results show that the extracts of *C. quadrangularis* exhibiting anti-arthritic activities might be due to the presence of active principles such as polyphenolic content, triterpenoids, alkaloids, and flavonoids. From the results of the study, it can be concluded that the extract of *C. quadrangularis* possessed significant antiarthritic property in MECQ than compared to AECQ. The present study revealed the potential of plant extract in the management of inflammation and arthritis confirming the folk core use of medicinal plants. However, one should try to further figure out extract more as having much better activity in the quest of active candidate or chemical molecule that is mainly responsible for this activity through detailed experimentation (Table 1 and Fig.1 and 2).

Statistical analysis

All the results were expressed as mean ± standard deviation, and all the grouped data were statistically evaluated with GraphPad prism. Hypothesis testing methods included one-way analysis of variance followed by least significant difference test.

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AUTHORS' CONTRIBUTIONS

All the authors contributed equally to conductance of the study, writing, and editing the article.

CONFLICTS OF INTEREST

The manuscript represents valid work,neither this manuscript nor one with substantially similar content under my authership has been published or being consider to publication elsewhere.I confirm that work is an accurate representation of the trial results.

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