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SHORT COMMUNICATION

In Vitro Anti-arthritis Activity of Asparagus Racemosus Linn Seed Extract Against Albumin Denaturation

Anubhav Dubey[1*], Sribatsa Lanchhana Dash[1], Chandan Mukherjee[2], Tankadhar Mishra[3], Mamta Kumari[4] and Sanyogita Shahi[5]

[1]Department of Pharmacy, Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur - 209217, Uttar Pradesh, India; anubhavdwivedi803@gmail.com

[2]Department of Life Sciences, School of Biosciences and Technology, Galgotias University, Greater Noida - 203201, India

[3]The Pharmaceutical College, Bargarh - 768028, Odisha, India

[4]School of Pharmaceutical and Biological Sciences, HBTU, Kanpur - 208002, Uttar Pradesh, India

[5]Department of Science (Chemistry), Kalinga University, Raipur - 492101, Chhattisgarh, India

Abstract:

Background: "Arthritis" is an inflammatory disorder of the joints. Elbows and knees are examples of joints since they are the points where two bones connect. Various forms of arthritis have diverse etiologies and therapeutic approaches. Some varieties can spread to other parts of the body, affecting things like the eyes, heart, or skin. **Aim:** To assess the anti-arthritis efficacy of a chloroform extract of *Asparagus racemosus* seed against albumin denaturation. **Method:** Investigating anti arthritis activity *in vitro* using albumin denaturation method. **Results:** In controlled experimental conditions, egg albumin was incubated with various concentrations of chloroform extract, and absorbance was measured to determine the percentage of protein inhibition for anti-arthritis action, with indomethacin as the reference medication. A dose-dependent rise in percentage inhibition was discovered as a consequence. The chloroform extract *of A. racemosus* seed inhibited albumin denaturation at all concentrations tested. **Conclusions:** According to the findings of this study, *A. racemosus* seed has a significant anti-arthritis effect *in vitro* against albumin denaturation. The impact was caused by the presence of xanthone, a phytochemical component found in *A. racemosus* seed.

Major Findings: Traditional or folk medicine uses a wide variety of plant species to treat inflammatory diseases. This study found that *A. racemosus* seed inhibits albumin denaturation and arthritis *in vitro*.

Keywords: Albumin Denaturation, Anti-arthritis, *Asparagus racemosus*, Indomethacin, Phytochemical

1. Introduction

Inflammation, synovial proliferation, and articular cartilage degradation are hallmarks of Rheumatoid Arthritis (RA), an autoimmune illness[1]. To activate and produce cytokines, which are chemotactic for neutrophils, antigen-antibody immune complexes include IgM[2]. Lysosomal enzymes that cause cartilage destruction and bone erosion are produced when cells become inflamed. The immune system has been linked to oxidative stress, which causes inflammation and tissue damage in conditions like arthritis[3]. Protein denaturation may have a role in the formation of autoantigens in RA. It is possible to block the denaturation of tissue proteins and the lysis of membranes in rheumatic disorders, which in turn has anti-arthritic or anti-inflammatory effects, and to reduce the production of autoantigens in some

*Author for correspondence

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arthritic diseases[4]. There are a number of unwanted side effects linked to the various non-steroidal and immunosuppressive medications used today to manage pain and inflammatory symptoms. Research into herbal remedies for arthritic pain has advanced at an exponential rate in recent years, thanks in large part to the widespread belief that these treatments are both safe and effective. There are many different compounds found in herbal medicine, and some of these have biological effects[5]. Due to its broad range of biological activities, better perceived efficacy, lower frequency of significant side effects, lower cost, and widespread availability, herbal medicine is in high demand in both developed and developing nations, such as India. Using plants and plant extracts as a treatment for illness is the focus of herbal medicine. Brain illnesses, dyspepsia, tumours, inflammation, neuropathy, hepatopathy, and enhanced longevity are just a few of the many maladies that *A. racemosus* is used for in Ayurvedic medicine[6]. The root extract of *A. racemosus* has a number of pharmacological actions, including those that are anti-inflammatory, anti-ulcer, anti-diabetic, and immunomodulatory. According to an analysis of ancient classical Ayurvedic texts, the *A. racemosus* root has been recommended for usage as a galactogogue and in cases of imminent abortion[7]. The root of *A. racemosus* is known by several names, including cooling, emollient, bittersweet, nervine tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antibacterial, and tonic. Potential uses for *A. recemosus* root include treatment of hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, acidity, and certain viral diseases[8,9]. Alkaloids, tannins, phenolic flavonoids, carbs, and proteins were all detected in the phytochemical analysis. Benzophenones, xanthones, and bioflavonoids are the most prominent secondary metabolites found in *A. racemosus*. Xanthones, one of the primary components found in plants belonging to the *Asparagaceae* family, were validated by phytochemical testing. The pharmacological and biological actions of naturally occurring xanthone are rather impressive, including anti-arthritis, antibacterial, anti-inflammatory, analgesic, and anti-viral properties. One of the primary causes of inflammation and rheumatoid arthritis is denaturation of proteins[10,11]. *A. racemosus* seed has not been studied for its potential anti-arthritis effects *in vitro*, according to the reviewed literature. Therefore, the purpose of this research was to determine whether or not a chloroform extract of *A. racemosus* seed had any anti-arthritis effects by inhibiting albumin denaturation *in vitro*.

2. Materials and Methods

2.1 Collection of Seed

The Vatika Agro shops in Jaipur, Rajasthan, India, was where we got our hands on a few *A. racemosus* seeds. After being submitted in the university's herbarium house with the number J/Bot/2022APS-019 as a voucher specimen, the plant's authenticity was confirmed at Janta Postgraduate College, A.P.S. University, Rewa, M.P., India. The seeds of the plant were extracted and then dried at 45°C in an independent oven.

2.2 Drugs and Chemicals

Sigma Aldrich of Mumbai, India, supplied the indomethacin. Commercially procured analytical grade chemicals were used for all other purposes. All of the water used in the study was double-distilled using an all-glass still.

2.3 Preparation of the Crude Extract

To make the basic extract, the dried seeds were ground to a powder using a mechanical grinder. The 52 grams of powdered seeds were extracted using Soxhlet apparatus and 500 millilitres of chloroform, a solvent. The next step was to use a water bath to evaporate the filtrate and concentrate it. By weighing the drained extracts, we were able to calculate the yield of the soluble constituents using the computation[12].

$$Yield\ \% = \text{Weight of dry extract} / \text{Weight of dry seeds powder} \times 100$$

In preparation for further study, the dried extracts were stored at 40°C.

2.4 Phytochemical Test for Xanthone

1 millilitre of *A. racemosus* extract and one millilitre of alcoholic ferric chloride were combined. The presence of xanthones is indicated by the production of brown colour.

2.5 Evaluation of *In Vitro* Anti-arthritis Activity

Each solution included less than 2.5% dimethyl formamide after dissolving the *A. racemosus* chloroform extract in a minimum quantity of dimethyl formamide (0.2M, pH 6.4). The test solution was prepared by mixing 1 milliliter of mM albumin solution in phosphate buffer saline with 1 milliliter of *A. racemosus* chloroform extract, which had varying concentrations ranging from 31.25, 62.5, 125 microgram per millilitre, 250 microgram per millilitre, 500 microgram per millilitre, and 1000 microgram per millilitre. The mixture was then incubated in a BOD incubator for 15 minutes. For denaturation, the reaction mixture was maintained in a water bath at 70°C for 10 minutes. Using dimethyl formamide (DMF) as a blank, the absorbance of turbidity was measured at 660 nm (Shimadzu, UV 1800) after cooling[13,14]. The absorbance was determined using indomethacin in the standard drug range of concentrations (31.25, 62.5, 125 µg, 250, 500, 1000 microgram per millilitre), which was handled in the same way. Determined the percentage of denaturation inhibition relative to the control group without medication addition. We averaged the results from three separate experiments[15]. It was also used to determine the absorbance of indomethacin, a standard. Using the following formula, we were able to determine the percentage inhibition of albumin denaturation:

$$\%\text{inhibition} = 100x\ (Vt/\ Vc\ 1)$$

where Vt = absorbance of test sample, Vc= absorbance of control.

3. Results

This study found that the chloroform extract of *A. racemosus* seed had an *in vitro* anti-arthritic effect by preventing the denaturation of egg albumin. A synopsis of the data presented in Table 1. The suppression of albumin (protein) denaturation by *A. racemosus* chloroform fruit extract varied with concentration, from 31.25 to 1000 microgram per millilitre, according to the current results. An indomethacin reference medication was utilised with a concentration range of 31.25 to 1000 microgram per milliliter. The results are summarised in Table 1, which also showed that the suppression of protein denaturation varied with concentration. With respect to standard and *A. racemosus* seed extract concentrations.

Table 3.1. Action of *A. racemosus* and Indomethacin on denaturation of albumin

Concentrations (microgram per milliliter)	*A. racemosus* percentage inhibitions	Indomethacin Percentage inhibitions
Control		
62.5	0.72	20.67
125	1.07	29.18
250	4.27	41.42
500	14.92	70.60
1000	19.02	84.23

4. Discussion

Using animals in pharmaceutical research isn't without its challenges, such as problems with animal ethics committees and hard-to-justify aspects[16]. Therefore, the denaturation of protein bioassay was chosen for the purpose of *in vitro* assessment of anti-arthritis activity in the chloroform extract of *A. racemosus* seed in the present investigation. Protein denaturation is a known inflammatory and arthritic disease culprit. For rheumatoid arthritis and medication development, it might be worthwhile to file this case since autoantigens are created in inflammatory arthritic illness, which may be caused by *in vivo* protein denaturation. The biological characteristics of a protein molecule are lost as it denatures[17]. Researchers looked at the plant's anti-inflammatory and anti-arthritis properties in relation to its ability to prevent protein denaturation. Rheumatoid arthritis and inflammation are both caused by proteins denaturing[18]. Inflammation and RA can be largely prevented by mechanisms that prevent protein denaturation. An increase in absorbance relative to the control sample suggested that the protein was stabilising, or at least inhibiting heat-induced protein (albumin) formation. Reportedly, the capacity to stabilise heat-treated albumin at physiological concentrations (i.e., avoid denaturation) is a property of several NSAIDs. The pH ranges from 6.2 to 6.5. Thus, our study's research shows that

A. racemosus seed chloroform extract strongly inhibits albumin denaturation, an indicator of arthritis, in an *in vitro* setting. Hence, anti-inflammatory medications inhibited albumin denaturation to varying degrees.

5. Conclusion

A return to natural practices is on the rise as a result of widespread concern about the negative consequences of allopathic medicine. Traditional or folk medicine uses a wide variety of plant species to treat inflammatory diseases[19]. The purpose of this study is to investigate the efficacy of *A. racemosus* seed in alleviating inflammation and arthritis through controlled laboratory trials. So, *A. racemosus* seed has powerful anti-inflammatory and anti-arthritic properties. Possible xanthones, a kind of secondary metabolite, are responsible for the anti-inflammatory and anti-arthritic effects. To determine the mechanism behind the anti-arthritis and anti-inflammatory action, more xanthone isolation is required. The necessity for effective, safe, and naturally occurring anti-inflammatory and anti-arthritis medicines has prompted this investigation. It is a fruitful and rationally evaluated research technique to derive novel anti-arthritis and anti-inflammatory medications from herbal medicine.

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7. Author Contribution

A.D. Writing – review and editing, D.SL. Writing – original draft, M.C. Investigation, M.T. Supervision, K.M reviewing Manuscript, S.S.- Revision. All authors have read and agreed to the published version of the manuscript.

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