

Invitro Anti-arthritic and Anti-inflammatory activities of *Rhynchosia rufescens* (Willd.)DC. Seed extract on Inhibition of Protein Denaturation

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ABSTRACT

The Rheumatic arthritis is an auto immune inflammatory disorder which cause destruction of cartilage and bone damage with risk of infections. Inflammation is the response of living tissues to injury and is a complex pathophysiological process associated with extravasation of fluid and proteins and accumulation of leukocytes at the site of injury. In the present study, the invitro anti-arthritic and anti-inflammatory activity of the ethanolic seed extract of *Rhynchosia rufescens* was evaluated with the inhibition of protein denaturation. Diclofenac sodium was used as control drug. The methanolic seed extract shows high percentage of inhibition of protein denaturation in the concentration of 100µl/ml and significant result of anti-inflammatory activity at 250µl/ml concentration. The results suggested that the *Rhynchosia rufescens* would be a valuable herbal plant for scientific investigations.

Keywords: Anti-arthritic, Anti-inflammatory, Protein denaturation, Diclofenac sodium and *Rhynchosia rufescens*.

I. INTRODUCTION

The use of herbal medicines became popular due to toxicity and side effects of allopathic medicines. This led to rapid increase in the number of herbal drug manufactures [1]. Medicinal plants play an important role in the development of potent therapeutic agents. Today estimate that about 80 % of people in developing countries still using the traditional medicine based largely on species of plants for their primary health care. About 500 plants with medicinal use are mentioned in ancient literature and around 800 in usage in indigenous systems of medicine. India is one of the vast repositories of medicinal plants that are used in traditional medical system [2].

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, which affecting the freely movable joints, such as hand, knee and shoulder joints. The Rheumatic arthritis is an auto immune inflammatory disorder which cause destruction of cartilage and bone damage with risk of infections. Although various modern drugs are used to treat these type of disorders their prolonged usage may cause severe side effects .So there is a need to develop new alternate herbal therapeutic agents with minimum side effects. The plant derived drugs serve as a more effective conventional drugs for the treatment of many forms of arthritis [3].

Rheumatoid arthritis (RA) is an autoimmune disorder characterized by synovial proliferation, inflammation, subsequent destruction like deformity of joints or destruction of cartilage and bone [4, 5]. Inflammation is a very common symptom of many chronic diseases and it is the protective response to injury of tissues caused by chemical or microbial agents [6]. The denaturation of tissue proteins lead to inflammation and arthritic problems[7].

The plant *Rhynchosia rufescens* is one of the tribal pulses belongs to the family Fabaceae is being widely used by tribal people for various ailments and distributed in India, Bangladesh, Sri Lanka, Campodia, Indonesia and Malaysia. The amino acid composition and essential amino acid score of total seed protein in *Rhynchosia rufescens* are found to be higher than those of FAD/WHO (1991) recommended pattern [8]. Though the plant used widely by the tribes for different ailments, there is lack of scientific and documentation. . Since there was not found any scientific and therapeutic records, the present study of “Invitro Anti-arthritic and Anti-inflammatory activities of *Rhynchosia rufescens* (Willd.)DC. Seed extract on Inhibition of Protein Denaturation”.is being reported for the first time and could be very useful criteria for identification and further usage of the species.

II. MATERIALS AND MERTHODS

A. Collection of material

The plant used in this study was collected in the hills of Tiruvannamalai, Tamilnadu, India, identified and authenticated at the Botanical Survey of India (BSI), Coimbatore (No.: BSI/SRC/5/23/2013-14/Tech/1756). The seeds were collected between December and January after the ripening of fruits and dried in shade. Care was taken to select healthy plants and matured seeds. The seeds were washed thoroughly 3 – 4 times with tap water and finally rinsed with double distilled water, shade dried, grounded into coarse powdered form and used for extraction.

B. Preparation of solvent extract

20 grams of coarse powdered seeds of *Rhynchosia rufescens* was filled in the thimble and extracted with 200ml of methanol, acetone, water using soxhlet apparatus. Each of the solvent extract was concentrated separately under reduced pressure using rotary evaporator and stored at - 20°C for further testing.

C. Anti-arthritic activity on inhibition of protein denaturation

Invitro anti-arthritic activity of the methanolic seed extract was tested [9] by using Diclofenac Sodium is taken as a standard drug, against the denaturation of Bovine Serum Albumin (BSA). The procedure to analyze the percentage of protein denaturation inhibition using different concentrations of the sample and diclofenac sodium(20, 40, 60, 80 and 100 µg/ml) is done

Concentration of test substance : 20 to 100 µg/ml

Standard : Diclofenac sodium

Chemicals required : Bovine Serum Albumin (BSA), 1 N HCl, Phosphate buffer (pH 6.3)

Instrument : Incubator, Spectrophotometer – 660 nm

The following 4 solutions were used

1. **Test solution** (0.5 ml) consists of 0.45 ml of BSA (5 % w/v) and 0.05 ml of extracts in various concentration
2. **Test control solution** (0.5 ml) consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of distilled water
3. **Product control** (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of extracts in various concentrations

4. **Standard solution** (0.5 ml) consists of 0.45 ml of BSA (5% w/v) and 0.05 ml of diclofenac sodium solution in various concentrations. The pH of the above solutions was adjusted to 6.3 using small amount of 1N HCl. The samples were incubated at 37oC for 20 min and heated at 57oC for 3 min which was cooled and 2.5 ml of phosphate buffer (pH 6.3) was added to it. Control represents 100 % protein denaturation and the results were compared with standard diclofenac sodium.

The percentage inhibition of protein denaturation was calculated as follows.

Percentage inhibition= $[100 - (\text{optical density of test solution} - \text{optical density of product control}) \div (\text{optical density of test control}) \times 100]$

The control represents the 100% protein denaturation. The results were compared with Diclofenac sodium.

D. Invitro Anti-inflammatory activity on albumin denaturation

Invitro anti-inflammatory activity of the seed extract on albumin denaturation was tested by protein denaturation method as described by Padmanabhan *et al.* with slight modifications [10] Diclofenac sodium a powerful non steroidal anti-inflammatory drug was used as a standard drug. The concentration of both the standard drug and the seed extract were fixed as 50, 100, 150, 200, and 250µg/ml and the percentage of inhibition were calculated.

Test solution consisting of 1ml of different concentrations of Herbal Preparation (HP-4) ranging from 100-500 µg/ml or standard acetylsalicylic acid 100 and 200 µg/ml was mixed with 1ml of egg albumin solution (1mM) and incubated at 27 ±1°C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes .After cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken.

Statistical analysis: Data was represented as mean ±SD, which was statistically analyzed by Student's t-test and p<0.001 vs. control were considered to be significant.

III. RESULTS AND DISCUSSION

A. Invitro anti-arthritic activity

The methanolic seed extract of *Rhynchosia rufescens* shows good anti-arthritic activity and have the capability to control the production of auto antigens by inhibiting the protein denaturation. The results of the study were given below in Table: 1.

The methanolic extract of *Rhynchosia rufescens* seed has significant activity at 55.17% at 100µg/ml by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that methanolic sda

extract is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease [11].

B. Invitro anti-inflammatory activity

The methanolic seed extract of *Rhynchosia rufescens* shows high percentage of albumin denaturation and have the anti-inflammatory activity. The results of the study were given below in Table: 2.

The methanolic extract of *Rhynchosia rufescens* seed has significant activity at 67.42% at 250µg/ml by inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium[10].

Table 1: Invitro anti-arthritic activity on inhibition of protein denaturation

Concentration (µg/ml)	Sample (<i>Rhynchosia rufescens</i>) (% of inhibition)	Diclofenac sodium (% inhibition)
50	27.62±0.32	30.27±0.23
100	32.20±0.36	39.16±0.63
150	45.02±0.23	47.15±0.35
200	51.72±0.35	59.26±0.25
250	67.42±0.27	70.26±0.62

Table 2: Anti-inflammatory activity on albumin denaturation

Concentration (µg/ml)	Sample (<i>Rhynchosia rufescens</i>) (% of inhibition)	Diclofenac sodium (% inhibition)
20	32.12±0.32	34.02±0.03
40	36.01±0.26	40.21±0.21
60	43.16±0.31	59.37±0.31
80	49.26±0.28	55.02±0.53
100	55.17±0.48	65.34±0.32

IV. CONCLUSION

From the results obtained in the present studies, it may be concluded that the herb *Rhynchosia rufescens* (willd.)DC possesses significant anti-arthritic and anti-inflammatory activities which is comparable to synthetic anti-arthritic and anti-inflammatory drugs. Since the plant has various medicinal values and non-availability of scientific records, the present study could be very useful in the further scientific studies and researches in future.

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