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In-vitro Evaluation of Anti-arthritic Activity of *Merremia dissecta* by Protein Denaturation Method

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Abstract

The present study aimed to explore anti-arthritic potential of the plant *Merremia dissecta*, a perennial weed and also a shrub like plant which is commonly used as a medicine in folk treatment by *In-vitro* protein denaturation method. The leaves of the plant were harvested, dried and extracted in methanol. Anti-arthritic activity was evaluated by Pharmacological Investigation – Inhibition of protein denaturation using bovine serum albumin with 0.45 ml of bovine serum albumin (5% aq. solution) and 0.05 ml methanolic extract of *Merremia dissecta* in different concentration (100-500 µg/ml), denaturation of bovine/egg albumin with 5ml reaction mixture of test solution consists of 0.2 ml of egg albumin, 2.8 ml of phosphate buffer and 2 ml of different concentrations of extract (100-500 µg/ml) and membrane stabilization 4.5 ml [2 ml hypotonic saline (0.25% NaCl) +1 ml 0.15 M phosphate buffer (pH 7.4) + 1 ml test solution (100-500µg/ml) + 0.5 ml of 10% rat RBC in normal saline]. The different concentrations of methanolic extract were allowed to react with the bovine albumin and egg albumin under controlled experimental conditions and used for the determination of absorbance for the estimation of anti-arthritic activity. Diclofenac sodium was chosen as the reference drug. Protein (albumin) denaturation was found to be inhibited in a concentration dependent manner by Methanolic extract of *Merremia dissecta*. The results indicate *Merremia dissecta* is a good anti-arthritic herb. But further pharmacological research can be done on the isolated active substance components to establish its effectiveness and action.

Keywords: *Merremia dissecta*, Anti-Arthritic, Protein Denaturation

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Introduction

Autoimmune diseases have been caused by an imbalance and abnormal immune system. There are a high prevalence of autoimmune diseases and associated disability and morbidity with more than 5% of the population [1]. According to National Institute of Allergy and Infectious diseases more than 80 diseases can arise due to improper immune responses [2]. Arthritis is one of the major diseases among number of autoimmune diseases. Rheumatoid arthritis and osteoarthritis are most frequent types from more than 100 different types of arthritis [3]. RA is the most prevalent systemic autoimmune disorder linked to inflammatory diseases throughout the world affecting 1% of the population. Females are more prone as compared to males basically, affecting synovial joints which lead to pain, swelling, and ultimately movement restriction may occur [4]. Rheumatoid arthritis is recurrent and advancing disease which leads to cartilage and bone damage.

The release of mediators like MMPs, a disintegrin-like metalloprotease with thrombospondin type 1 motifs 4 and 5 and cathepsins results into joint-space narrowing thus, damages to the cartilage whereas, suppressed osteoblast and activated osteoclast by means of various mediators leads to bone damage. Rheumatoid factors and anti-citrullinated protein antibodies (ACPA) are markers for diagnosis of RA. There are various etiological factors, among which, environmental and genetic factors are more predominant and can even be responsible for the release of ACPA [5]. If not treated according to conservative estimates nearly 40 per cent of patients are unable to work within 5 years of diagnosis, and 50 per cent within 10 years [6]. Currently, conventional (NSAIDs and DMARDs) and novel (TNF monoclonal antibody) therapeutic approaches are available but there is still lacuna in the long-term disease prevention and remission [7].

Again, chronic use of anti-rheumatic drugs comes with its own set of side effects and risks, such as myelosuppression, stomatitis, hepatic fibrosis, nephrotoxicity, cirrhosis, immune reactions, cardiovascular, haematological and pulmonary issues. Looking towards another therapeutic approach herbal medicines can be a great option with less side effect and cost of therapy. Medicinal plants are considered as a priceless gift from the nature and utilized by the mankind since ancient

times. They have long been employed by the populations of different cultures as safe therapeutic measures. According to WHO, about 80% of the world population specially in developing countries are mostly depend on the traditional medicine from plants for their healthcare. The medicinal plants like Alstonia scholaris, Boerhaavia diffusa, Caesalpinia sappan, Cannabis sativum, Curcuma longa, Piper longum, Terminalia chebula, Vitex negundo and Xanthium strumariumet. showing promising antiarthritic activity [8].

Merremia dissecta (Jacquin), perennial climbing weed comes from family convolvulaceae and is found in the United States [9]. The plant is found throughout the world. It is reported in India in the state of Kerala and Uttar Pradesh [10, 11]. Traditionally, the crushed leaves of plant are effective against inflammation also the plant have been used in treatment of urinary tract infection, scabies, herps, skin diseases and giddiness. The plants are frequently used to make an herb garden and for ornamental purposes. *Merremia dissecta* consist of various phytoconstituents like alkaloids, glycosides, proteins, tannins, carbohydrates, saponins, phytosterols, terpenoids, phenols and flavonoids [12]. Alcoholic extract of the leaves has been reported with a remarkable amount of phenolics and Flavonoids. The plant having potent antimicrobial activity [13].

Materials and Methods

Plant material

The uninfected and matured leaves of *Merremia dissecta* was collected from its natural habitat Pusad, Maharashtra, India. The sample was identified and authenticated by Department of Botany, SRTM University Nanded, Maharashtra. A voucher specimen was deposited in the herbarium unit of the Department of Botany, SRTM University Nanded, Nanded.

Preparation of extract

The leaves of *Merremia dissecta* was thoroughly rinsed with water and shade dried at room temperature in hygienic condition. The leaves of *Merremia dissecta* was powdered manually and extracted with methanol using Soxhlet extraction technique. Extraction was performed until the solvent become colourless. The methanolic extract was concentrated using rotary evaporator and stored for further use.

Phytochemical screening

The qualitative phytochemical studies were conducted according to the methods of Jasim et al. [13].

Pharmacological Investigation

Inhibition of protein denaturation using bovine serum albumin [14, 15, 16].

In this method, anti-arthritic activity of methanolic extract of the plant *Merremia dissecta* was evaluated by % inhibition of protein denaturation. In this method bovine serum albumin (BSA) was used. Diclofenac sodium was used as the reference drug. The 0.5ml reaction mixture consist of 0.45 ml of bovine serum albumin (5% aq. solution) and 0.05 ml methanolic extract of *Merremia dissecta* in different concentration (100-500 µg/ml). Test control is made up of 0.5ml of reaction mixture (0.45 ml of BSA and 0.05ml of distilled water). Adjustment of its pH was done by adding 1N Hcl to adjust the pH of the solution to 6.3. The samples were incubated at 37°C for 20 min and heated at 57°C for 30 min. Cool and then add 2.5 ml of phosphate buffer (pH 6.3). Using UV spectrophotometer at 660 nm, we measured the absorbance.

The Percentage inhibition of protein denaturation was calculated using the formula:

%Substitution = [Abs of Test Solution – Abs of Predicted Control / Abs of Test Control] × 100

Inhibition of protein denaturation using egg albumin [15-16].

The anti-arthritic activity of methanolic extract of *Merremia dissecta* was evaluated as % inhibition of denaturation in egg albumin-based model. Reaction mixture used for this test is 5ml in which the egg albumin is 0.2 ml, phosphate buffer is 2.8 ml and the extract is 2 ml with different concentrations (100-500 µg/ml). In test control solution 2ml of distilled water was used instead of test extract. Diclofenac sodium was used as reference standard. The pH of the solutions was adjusted to 6.4 by using 1NHCl. At next step, samples were incubated at 37°C for 20 min then heated at 70°C for 5 min. The absorbance was measured after cooling on spectrophotometer, at 660 nm. The results were compared to the standard Diclofenac sodium

The percentage inhibition of protein denaturation was calculated using the formula:

%Activity = [Abs of control – Abs of test sample / Abs of X test control] × 1000

Effect on Membrane Stabilization [17, 18, 19].

Anti-arthritic activity was evaluated by the percentage membrane stabilization in this method. Diclofenac sodium was used as the reference drug. The reaction mixture contains 4.5 ml which is a mixture of 2 ml of hypotonic saline (0.25% NaCl), 1 ml of 0.15M phosphate buffer (pH 7.4) and 1 ml of test solution containing 100-500 µg of rat RBC per ml, and 0.5ml of rat RBC 10% in normal saline. The mixture was heated for 30 minutes at 56°C. The tubes were allowed to cool for 20 minutes with running tap water. It was centrifuged at 3000rpm for 10min and the absorbance of the supernatant was taken at 560nm. 100% haemolysis is shown in the control.

Percentage membrane stabilizing activity was calculated by formula:

%Activity = [Abs of test – Abs of pretreated control / Abs of control] × 100

Method of preparing 10% rat RBC: Blood was drawn via retro orbital route and put in a heparinised tube. Normal saline was added and then centrifuged at 3000 rpm for 10 min 3-4 times. To 1 ml of the blood 9 ml of normal saline was added after the centrifugation.

Results

The qualitative phytochemical analysis of *Merremia dissecta* reveled presence of rich active constituents such as glycoside, phenols, flavonoids, alkaloids, tannins with basic composition.

Evaluation of anti-arthritic activity using *In-vitro* model:

A) Effect on protein denaturation

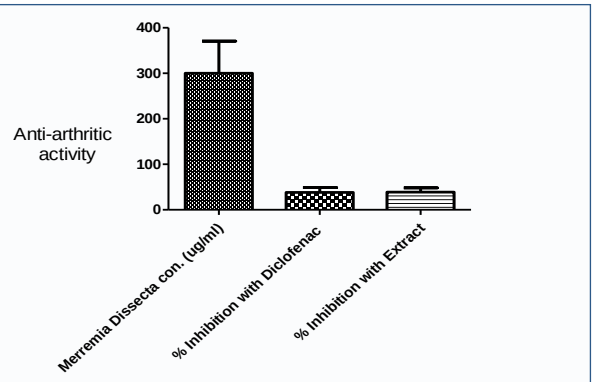
Investigation of anti-arthritic activity of methanolic extract of *Merremia dissecta* using *In-vitro* models for assessing the % inhibition of denaturation was done in 5 different concentrations (100-500 µg/ml). The results are presented in detail below (Tables 1 and 2).

B) Effect on Membrane Stabilization (MS)

The effect of methanolic extract of *Merremia dissecta* and standard diclofenac in concentration 100, 200, 300, 400, 500 µg/ml was found

Table 1: Effect of methanolic extract of *Merremia dissecta* on heat induced protein denaturation using BSA.

Sr. No.	Concentration (µg/mL)	% Inhibition with Standard (Diclofenac)	% Inhibition with *Merremia dissecta* Extract
1	100	14.36 ± 0.72	20.11 ± 0.63
2	200	22.98 ± 0.83	24.13 ± 0.49
3	300	32.18 ± 0.48	35.63 ± 0.82
4	400	44.82 ± 0.96	42.25 ± 0.57
5	500	75.86 ± 0.77	72.41 ± 0.72



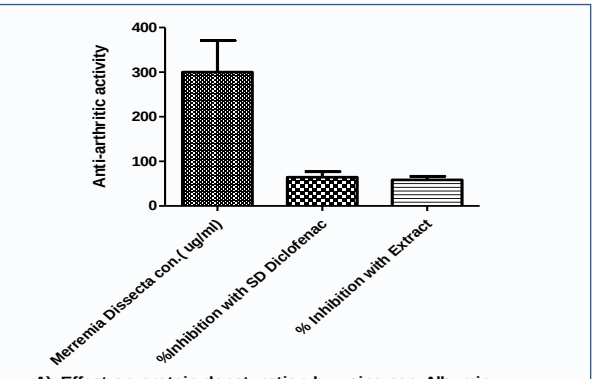
A) Effect on protein denaturation by using Bovin serum albumin

Figure:

All the values are expressed as Mean+ SEM (n=3),
All the values are significant when compared to control (p<0.05)

Table 2: Effect of methanolic extract of *Merremia dissecta* on heat induced protein denaturation using egg albumin.

Sr. No.	Concentration (µg/mL)	% Inhibition with Standard (Diclofenac)	% Inhibition with Extract
1	100	26.98 ± 0.38	34.39 ± 0.46
2	200	47.08 ± 0.67	48.67 ± 0.43
3	300	71.95 ± 0.70	60.37 ± 0.75
4	400	82.01 ± 0.93	69.84 ± 0.69
5	500	95.23 ± 1.03	79.98 ± 0.89



A) Effect on protein denaturation by using egg Albumin

Figure:

All the values are expressed as Mean+ SEM (n=3),
All the values are significant when compared to control (p<0.05)

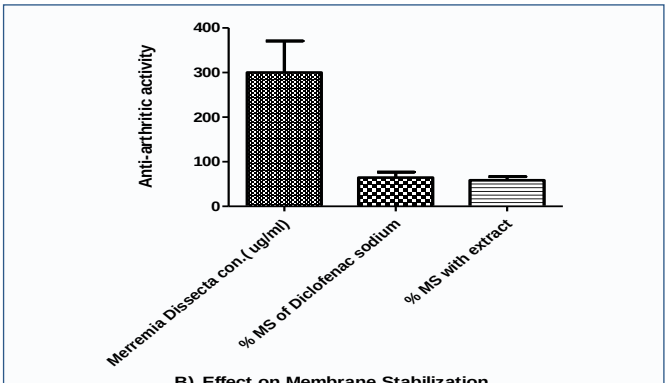
to be significant (p<0.05) when compared to control (Table 3).

Discussion

The present study carried out to evaluate anti-arthritic activity of methanolic extract of Merremia dissect by using *In-vitro* method, inhibition of protein denaturation and membrane stabilization. Protein denaturation involves the distraction of secondary, tertiary

Table 3: Membrane stabilizing effect methanolic extract of *Merremia dissecta* and standard diclofenac.

Sr. No.	Concentration (µg/mL)	% of Membrane Stabilisation (Standard drug)	% of Membrane Stabilisation (Extract)
1	100	26.72 ± 0.87	12.07 ± 0.53
2	200	36.93 ± 0.92	24.31 ± 0.76
3	300	58.74 ± 0.63	32.87 ± 0.92
4	400	75.92 ± 0.87	48.83 ± 1.02
5	500	83.36 ± 0.98	71.08 ± 0.98



B) Effect on Membrane Stabilization

Figure:

All the values are expressed as Mean+ SEM (n=3),
All the values are significant when compared to control (p<0.05)

and quaternary structure of the molecules and finally leads to cell death, it occurs mainly due to stress, high temperature, high level of acidity and high level of salt [17].

When proteins are ruined by physical, chemical and biological agents, protein configuration get disrupted, due to alteration of electrostatic, hydrogen, hydrophobic and disulphide bonds, which results into denaturation, loss of biological function and associated diseases may arise [20]. Denaturation of protein is one of the causative factors may result in production of autoantigens in certain arthritis [21, 22, 23]. The Anti-protein denaturation activity of NSAIDs is very effective in the management of inflammatory diseases. Hence, protein denaturation inhibitory capacity which is a potential anti-inflammatory effect. On heating of Bovine Serum Albumin and egg albumin denaturation occurs and antigens are expressed which are associated with type-III hypersensitivity reaction and disease such as rheumatoid arthritis.

The results of this study show significant (p<0.05) inhibition of protein denaturation in both standard and extract treated groups as compared to control group. The results of the study showed a concentration-dependent inhibition of protein denaturation by *Merremia dissecta* in the concentration range of 100 to 500 µg/ml. Standard drug Diclofenac sodium at concentration 100 and 200 µg/ml exhibited less effect as compared to the 100 and 200 µg/ml Methanolic extract of *Merremia dissecta*.

Based on the present study, it is observed that the production of auto-antigen can be controlled by the methanolic extract of *Merremia dissecta* and this effect is attributed to its ability to prevent *In vivo* denatured proteins in the disease of rheumatism [18]. The results of the research revealed that *Merremia dissecta* may be able to inhibit the denaturation of proteins in rheumatic diseases and consequently, limit the production of autoantigens [24, 25, 26].

The theory of membrane stabilization is that the saline and heat induced lysis of the membrane stabilize the erythrocytes. Lysosomal enzymes with important role in the genesis of autoantigens and thus autoimmune diseases [27]. It is clear that, in the case of arthritis, the membrane of the lysosomes is damaged and the enzymes that cause arthritis get leaked out.

Numerous pathogenesis studies in rheumatoid arthritis have found that lysosomal enzymes such as proteases (Cathepsin K and S) may be involved in its pathogenesis [28]. In order to control inflammation, release of autoantigens and to maintain balanced immune system, normal and stabilized lysosomes are needed.

Earlier studies reported that the *In vitro* method of membrane stabilization by heat and saline via RBC was highly preferred as the membrane of red blood cell is correlated with that of lysosomal membrane. The anti-inflammatory activity was demonstrated as stabilisation or inhibition of lysosomal enzymes by anti-inflammatory drugs like NSAIDS. Damage and lysis of the membrane can be induced by unfavourable environment which includes unfavourable hypotonic media or unfavourable heat. Any agent which protects the RBC membrane can be said to protect the lysosomal membrane, hence hypotonic saline and heat induced RBC lysis can be used as indicator of anti-inflammatory activity, thus anti-arthritic activity [29, 30]. Methanolic extract of *Merremia dissecta* resulted in membrane protection when compared to control, the mechanism of action is yet to be known.

Conclusion

The findings of the present study suggest that methanolic extract of *Merremia dissecta* could to be an effective anti-arthritic drug. We are developing some of the in-vivo models, for confirmation of the anti-arthritic activity. Also, we are working, on isolated active ingredients of *Merremia dissecta* to find exact mode of action of methanolic extract of *Merremia dissecta* as anti-arthritic agent.

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