



Short Communication

In vitro anti-arthritic activity of *vitex negundo* and *punica granatum*

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Abstract

The aim of present study is to evaluate the in vitro anti arthritic activity of aqueous and ethanolic extract of *Vitex negundo* and *Punica granatum* by Bovine serum albumin and egg albumin denaturation method. The activity of aqueous and ethanolic extract of *Vitex negundo* and *Punica granatum* was compared with standard Anti -arthritic drug Diclofenac Sodium. Results revealed that the ethanolic extract of *Punica granatum* at a different concentration possessed significant anti- arthrititis activity as compare to std. drug. The current finding exhibited a concentration dependent inhibition of protein (egg albumin and BSA) denaturation by aqueous and Ethanolic extract of *Vitex negundo* and *Punica granatum*. The effect of Diclofenac Sodium was found to be approximate when compared with the *Punica granatum* at (800µg/ml). In denaturation method egg albumin, at concentration of 100, 200, 400 and 800µg/ml, ethanolic extraction of *Vitex negundo* showed 40.11%, 58.05%, 63.43% and 87.52% respectively. Whereas, ethanolic extraction of *Punica granatum* showed 50.28%, 101.71%, 187.75%, 261.08%. In Bovine Serum albumin denaturation method at concentration of 100, 200, 400 and 800µg/ml, ethanolic extraction of *Vitex negundo* showed 20.03%, 42.72%, 59.06%, 72.08% respectively. Whereas, Ethanolic extraction of *Punica granatum* showed 28.54%, 42.88%, 81.39%, 99.18% of inhibition. From the present finding it can be concluded that *Punica granatum* possessed maximum anti-arthritic effect against denaturation of protein invitro. The effect was possibly due to flavonoids, Alkaloids, terpenoids content of *Punica granatum*.

Keywords: *Vitex negundo*, *Punica granatum*, Anti-Arthritic Activity, Denaturation of Protein.

Introduction

Rheumatoid arthritis is an autoimmune disease in which there is inflammation of joint. Destruction of articular cartilage and synovial proliferation¹. Inflammation is a bodily response to injury. Infection or destruction characterized by disturbed physiological functions and heat, redness, pain, Swelling. Rheumatoid arthritis or RA was first described clinically in a 1800 doctoral thesis by Londre-Beauvais, a French medical student, who called the condition "Primary Aesthenic Gout". Sir Alfred Garrod established the distinction between Rheumatoid arthritis and Gout in 1859 and gave the condition its present name². Inflammatory diseases including different types of rheumatoid disease are a major cause of morbidity of the working force throughout world. This has been called the king of human miseries. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents.

It is the body response to inactivate or destroy the invading organism, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells³. *Vitexnegundo* belongs to the family verbenaceae is a large aromatic shrub or a small tree of about three meter in height and it is claimed to possess anti arthritis, anti-leprotic, anti-inflammatory, anti-spasmodicmantic

and promotes the growth of hair and eye disease. *Punica granatum* belongs to the family punicaceae. *Punica granatum* reducing heart disease risk factors inhibit the movement of cancer cells. The seeds and juice are considered as a tonic for heart, throat, eye and for a variety of purposes and like as stopping gum bleeds, nose bleeds, toning skin, firming up sagging breasts and treating hemorrhoids⁴. Current study aimed to find out the possible role of this *Vitex negundo* and *Punica granatum* plant extracts against protein denaturation method that induced arthritis.

Materials and methods

Plant material: The leaves of plant *Vitex negundo* and *Punica granatum* were collected in the month of August, 2016. from Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, Maharashtra, India.

Preparation of plant extract: The leaf of *Vitex negundo* and *Punica granatum* were dried at room temperature in shade and crushed to fine powder with the help of mechanical grinder. 10 grams of powdered material was macerated with 100 ml of distilled water and ethanol. Separately and stored in an airtight conical flask for 48 hours. Plant extract was filtered. Extraction was carried at 40-60°C in soxhlet apparatus. Finally using rotary evaporator sample was stored at 4°C until use.

Evaluation of in vitro anti arthritic activity: Protein denaturation by using egg albumin and Bovine serum albumin⁵: Protein denaturation by using egg albumin was determined by according to the method of Pavithra TK et al. and their absorbance was measured at 660 nm. Diclofenac sodium was used as reference drug. Same procedure was carried out for protein denaturation by Bovine Serum albumin.

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

Percentage inhibition = (Absorbance control – absorbance sample) X 100 / absorbance control.

Results and discussion

The effect of aqueous and ethanolic extract of *Vitex negundo* and *Punica granatum* were evaluated against denaturation of Egg albumin and Bovine serum albumin. The results are summarized in Table-1 and 2. The presenting findings exhibited a concentration dependent inhibition of protein denaturation by *Vitex negundo* and *Punica granatum* throughout the range of 100 to 800µg/ml.

The *Vitex negundo* and *Punica granatum* at different dose levels (100,200,400 and 800µg/ml) provided significant protection against denaturation of protein.

The increments in absorbance of test sample with respective control indicated stabilization of protein i.e. Inhibition of heat

induced protein (albumin) denaturation by *Vitex negundo*, *Punica granatum* and reference drug Diclofenac sodium⁶. *Vitex negundo* and *Punica granatum* contain alkaloids, flavonoid, tannins and a phenolic acid are known to promote anti- arthritic activity⁷. Some literature reported that in rheumatoid arthritis the denaturation of protein is one of the cause^{8,9}. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. Mechanism of denaturation probably involves alteration in hydrogen, hydrophobic electrostatic and disulphide bonding. Various anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation¹⁰. In our present study, Aqueous and ethanolic extract *Vitex negundo* and *Punica granatum* of inhibited heat induced protein denaturation and may be one of the reasons of possessing anti-arthritic activity.

Further studies are needed to elucidate other mechanism of the in-vitro Anti-arthritic activity of the *Vitex negundo* and *Punica granatum* extract and to assess the active constituents responsible for the Anti-arthritic effect.

Conclusion

The result of the study demonstrated that Ethanolic extract of leaves of *Vitex negundo* and *Punica granatum* showed potential Anti-arthritic activity as compared to Aqueous Extract. Result and attributed the presence of active principle such as Flavonoids, Steroids and Terpenoids which are responsible for these activity. Hence it could be beneficial for further work as active anti-arthritic agent.

Table-1: Anti- arthritic activity of *Punica granatum* by protein denaturation method.

% Inhibition (Egg albumin)			% Inhibition (Bovine serum albumin)		Standard Drug
Conc.(µg/ml)	Aqueous	Ethanol	Aqueous	Ethanol	
100	37.91	50.28	13.9	28.54	56.03
200	68.4	101.71	30.6	42.88	106.6
400	172.19	187.7	57.18	81.39	159.19
800	221.03	261.08	71.09	99.18	287.88

Table-2: Anti- arthritic activity of *Vitex negundo* by protein denaturation method

% Inhibition (Egg albumin)			% Inhibition (Bovine serum albumin)		Standard Drug
Conc.(µg/ml)	Aqueous	Ethanol	Aqueous	Ethanol	
100	36.09	40.11	12.22	20.03	56.03
200	47.85	58.05	34.04	42.72	106.6
400	59.21	63.43	43.14	59.06	159.19
800	78.26	87.52	65.06	72.08	287.88

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