

Research Article**Comparative evaluation of anti-arthritic activity of *Pongamia pinnata* (L.) Pierre seeds and *Bryophyllum pinnata* (L.) leaves: *In-vitro* study**Divya Singh^{1*}, Jai Singh Vaghela¹, Pushpendra K. Saini², Narendra Sharma²¹Bhupal Nobles College of Pharmacy, Udaipur, Rajasthan, India²Sri Balaji College of Pharmacy, Benad Road, Jaipur, Rajasthan, India

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Abstract

Objective: Comparative Anti-arthritic Activity of seed extract of *Pongamia pinnata* (L.) Pierre and *Bryophyllum pinnata* leaves by in vitro model. **Materials and methods:** *Pongamia pinnata* (L.) Pierre (Family: Leguminosae) and *Bryophyllum pinnata* (Family: *Crassulaceae*) are a medicinal plant which is indicated for the treatment of arthritis in folklore medicine. The anti-arthritic activity of *Pongamia pinnata* seed and *Bryophyllum pinnata* leaves ethanolic extract were done by Inhibition of protein denaturation in vitro methods. The ethanolic extract of *Pongamia pinnata* seeds and *Bryophyllum pinnata* leaves were subjected to in vitro Inhibition of protein denaturation in various concentrations i.e. 50, 100, 200, 400, 800, 1000 and 2000µg/ml. **Results:** *P. pinnata* and *B. pinnata* both extracts exhibited a concentration dependent inhibition of protein (albumin) denaturation. The prepared extracts showed better anti-Arthritic activity than the standard drug; when compared with each other, *P. pinnata* extracts showed better anti-arthritic activity compared to *B. pinnata* extracts. **Conclusion:** In the present study, *Pongamia pinnata* seed extract showed better activity comparative to *Bryophyllum pinnata* leaves and standard drug.

Keywords: *Pongamia pinnata*, *Bryophyllum pinnta*, anti-arthritic activity

Introduction

Remedies are made from single or multiple herbs and minerals for various medical conditions like asthma, flu, diabetes, arthritis, heart disease, digestive problems, mental health and skin problems. Herbal medicines yielding about 25% of currently used crude drugs with another 25% derived from chemically altered natural products.

In recent years, there is increased research on traditional Ayurvedic herbal medicines on the basis of their known effectiveness in the treatment of ailments for which they have been traditionally applied (Agrawal and Paridhavi, 2007; Chitme and Patel, 2009; Gautam et al., 2013; Rajput et al., 2011).

Rheumatoid arthritis is a systemic autoimmune disease with chronic inflammation characterized by hyperplasia of synovial cells and angiogenesis in affected joints, which ultimately leads

to the destruction of cartilage and bone (Gautam et al., 2013). The common signs & symptoms often affects the wrist joints and the finger joints closest to the hand, also other parts of the body besides the joints and causes pain, swelling, stiffness, and loss of function in the joints (Mohan, 2005; Patwardhan et al., 2010).

Pongamia pinnata seeds have several chemical constituents such as Karangin, pongamol, pongagalabrone, and pongapin, pinnatin and kanjone. *Pongamia pinnata* seeds used in hypertension, skin ailments and rheumatic arthritis. Seed powder valued as a febrifuge, tonic and in bronchitis and whooping cough (Nadkarni, 1998; Yadav et al., 2011). *Bryophyllum pinnatum* leaf contains P-coumaric acid, Ferulic acid, Syringic acid, Bufadienolides- Bryophyllin A (bryotoxin) chemical constituents. *Bryophyllum pinnatum* use in Neuropharmacological, CNS depressant effects, Anti-inflammatory, analgesic, antinociceptive and wound healing effect (Bhattacharjee, 2004).

Materials and methods**Collection and authentication of plant**

The seeds of *Pongamia pinnata* and *Bryophyllum pinnatum*

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leaves were collected from local vendors of Jaipur. Identified and authenticated of the plants in the Department of Botany, University of Rajasthan, Jaipur.

Preparation of Ethanolic extract of seeds of *P. pinnata* and *B. pinnatum* leaves

Dried Seeds and leaves were powdered mechanically through mesh sieve. The powdered were first defatted with petroleum ether (40–60°C) and extracted with ethanol by continuous hot percolation method using Soxhlet apparatus. The filtrate of the extract was concentrated to dryness.

Inhibition of protein denaturation method

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation:

Standard solution was prepared by addition of 2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) conc. of 50, 100, 200, 400, 800, 1000 and 2000 µg/ml.

Control solution was prepared by addition of 2 ml of Egg albumin (from fresh hen's egg), 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

Test solution containing 2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of plant extracts (PPEE and BPEE) conc. of 50, 100, 200, 400, 800, 1000 and 2000 µg/ml.

All of the above solutions were adjusted to pH 6.4 using a small amount of 1N HCl. The samples were incubated at 37°C for 15 minutes and heated at 70°C for 5 minutes. After cooling the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 660nm their viscosity was determined by using Ostwald viscometer (Chandra et al., 2012).

The percentage inhibition of protein denaturation was calculated using the following formula (Sangeetha et al., 2011):

$$\text{Percentage inhibition} = \left[\frac{V_t}{V_c} - 1 \right] \times 100$$

Where, V_t = absorbance of test sample, V_c = absorbance of control

Statistical analysis

Three replicates of each sample were used for each test to statistical analysis and the data were represented as Mean ± Standard Error Mean.

Results and discussion

The anti-arthritis effect of *Pongamia pinnata* ethanolic extract (PPEE), *Bryophyllum pinnatum* ethanolic extract (BPEE) leaf and diclofenac sodium were evaluated against the denaturation of egg albumin *in vitro*.

The result is summarized in (Table 1 and Figure 1). PPEE, BPEE and Diclofenac sodium were exhibited concentration dependent inhibition of protein (albumin) throughout the concentration range of 50 to 2000 µg/ml.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by *Pongamia pinnata* ethanolic extract (PPEE) seed and *Bryophyllum pinnatum* ethanolic extract (BPEE) leaf throughout the concentration range of 50 to 2000 µg/ml. Diclofenac sodium (at the concentration range of 50 to 2000 µg/ml) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation; however, the effect of diclofenac sodium was found to be less as compared with PPEE and BPEE. Inflammatory and arthritic disease well documented was caused by denaturation of tissue proteins.

Protein denaturation is a process in which protein lose their tertiary and secondary structure by application of external stress or compound such as strong acid or base, an organic solvent or heat most biological protein lose their biological function when denaturated (Megha et al., 2013; Gambhire et al., 2009; Marliyah and Ananthi, 2015). Production of auto-antigens in certain arthritic diseases may be due to

Table 1. *In-vitro* activity of PPEE, BPEE and diclofenac sodium (std drug) by inhibition of protein denaturation method

Conc. (µg/ml)	PPEE (%Inhibition) (±SEM)	BPEE (% Inhibition) (± SEM)	Diclofenac sodium (%Inhibition) (± SEM)
50	30±2.88	24.52±0.011	107.66±0.005
100	53.67±1.85	52.83±0.006	112.99±0.010
200	98.67±0.88	98.08±0.012	116.98±0.004
400	154.33±2.33	147.07±0.002	143.07±0.002
800	366±1.154	363.25±0.030	238.99±0.003
1000	601.67±1.20	580.50±0.005	382.38±0.003
2000	727.67±1.45	710.37±0.013	472.63±0.012

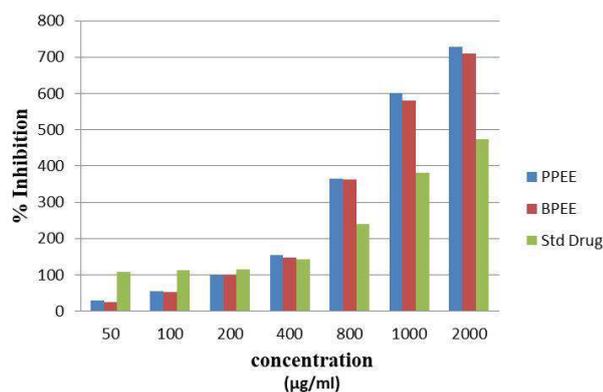


Figure 1. % inhibition v/s concentration of protein denaturation of PPEE, BPEE and Standard drug

denaturation of proteins *in vivo*. The mechanism of denaturation probably involves alteration I electrostatic hydrogen, hydrophobic and disulphide bonding. The increments in absorbance of plant extracts and standard drug to indicated the stabilization of albumin protein (Chandra et al., 2012; Sangeetha et al., 2011).

Conclusion

The present study was comparative evaluation of anti-arthritis activity of ethanolic Extract of *Pongamia Pinnata* (L.) Pierre seed and *Bryophyllum pinnata* leaves with standard drug. The higher percentage inhibition of albumin denaturation at the highest concentration of ethanolic extract of *Pongamia Pinnata* (L.) was 2000µg/ml.

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Conflicts of interest: Not declared.

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