



Evaluation of *In-Vitro* Anti-Inflammatory Activity of Leaves of *Pongamia pinnata*

Jyoti Bairagi *, Vivekanand Katare, Bharti Chourey, Abhilasha Delouri, Shivam Nema

Vivekanand College of Pharmacy, Shikandrabad, Bhopal, MP, 462044, India

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*Address for Correspondence:

Jyoti Bairagi, Vivekanand College of Pharmacy, Shikandrabad, Bhopal, MP, 462044, India

Mail id: bairagijyoti01@gmail.com

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infections, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as etiological factor for many chronic illnesses¹. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain and inflammation. Currently available NSAIDs are associated with unwanted side effects and have their own limitations. About 34-46% of the users of NSAIDs usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa². Hence there is a need for anti-inflammatory drugs with fewer side effects. Plants have been an important source of medicine for 1000's of years. Herbal medicine is still the mainstay of therapy for about 75-80% of the whole population in developing countries for primary health care³. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries⁴. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine⁵. World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases⁶. Many Indian ethno botanic

Abstract

Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. So, number of herbal medicines is recommended for the treatment of inflammation that has no side effects. *Pongamia pinnata*, Pierre (Family- Fabaceae) commonly known as karanja. The seed and seed oil of the plant have been reported to be effective in various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism, cutaneous affection including herpes and scabies etc. in folk medicine and Ayurveda, a traditional system of healing. Anti-inflammatory activities of this plant for various parts have been reported in various models. This study examined the anti-inflammatory potential of a methanolic leaves extract of *Pongamia pinnata* and reference drug Diclofenac sodium, invitro using heat-induced protein denaturation technique. The concentrations of the leave extract used were 50-250µg/ml. While diclofenac sodium were 50, 100, 150, 200 and 250µg/ml. Both the Diclofenac sodium and extract showed the IC₅₀ values 18.36 and 82.24µg/ml respectively. It is concluded that the methanolic leaves extract of *Pongamia pinnata* possess marked in vitro anti-inflammatory activity which is mediated possibly by flavonoids, tannins and alkaloids. This is a novel finding. Further the results scientifically justify the use of leaves of *Pongamia pinnata* in India traditional medicine to treat inflammation.

Keywords: Inflammation, *Pongamia pinnata*, Fabaceae, heat-induced protein denaturation technique

traditions propose a rich repertory of medicinal plants used by the population for the treatment, management and/or control of different types of pain⁷. However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. One such plant from Indian flora *Pongamia pinnata* (Fabaceae), a medium-sized glabrous tree, found throughout India and further distributed eastwards, mainly in the littoral regions of south eastern Asia and Australia. In the literature of India, different parts of this plant have been recommended as a remedy for various ailments. The seed and seed oil of this plant have been used for treating various inflammatory and infectious diseases such as leucoderma, leprosy, lumbago, muscular and articular rheumatism. The leaves are spicy, digestive, laxative, anthelmintic and cure piles, wounds and other inflammations. A liquid solution of the leaves is used as a medicated bath for relieving rheumatic pains and for cleaning ulcers in gonorrhoea and scrofulous enlargement. Root and seed extracts of *Pongamia pinnata* have been reported to have anti-inflammatory activity⁸. Hence, in the present study, anti-inflammatory activities of methanolic extracts of *Pongamia pinnata* were evaluated using heat-induced protein denaturation technique.

MATERIALS AND METHODS

Plant material

The leaves of plant *Pongamia pinnata* were collected from local area of Bhopal (M.P), India.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction of plant material

Cold maceration method

Leaves of *Pongamia pinnata* were collected, washed and rinsed properly. They were dried in shade and powdered mechanically. About 250gm of the leaves powder was macerated with petroleum ether and methanol and stored for 72 hours in ice cold condition for the extraction of phytochemicals. At the end of the third day extract was filtered using whatman No. 1 filter paper to remove all un-extractable matter, including cellular materials and other constituents that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts⁹.

Assessment of in vitro anti-inflammatory activity by protein denaturation technique

The reaction mixture (5ml) consisted of 0.2ml of egg albumin (from fresh hen's egg), 2.8ml of phosphate buffer saline (PBS, pH 6.4) and 2ml of varying concentrations of plant extract

so that final concentrations become 50, 100, 150, 200, 250 µg/ml. Similar volume of double distilled water served as control. Then the mixture were incubated at (37±2)°C in incubator (Universal) for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660nm (Systronic 2202) by using vehicle as blank. Diclofenac -sodium at the final concentration of 50, 100, 150, 200, 250 µ/ml was used as reference drug and treated similarly for determination of absorbance and viscosity¹⁰. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

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

RESULTS AND DISCUSSIONS

The crude extracts so obtained after cold maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The percentage yield of extraction is very important in phytochemical extraction in order to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from the leaves of the plants using petroleum ether and methanol as solvents are depicted in the Table 1. Results of In vitro Anti-inflammatory activity of diclofenac sodium standard and methanolic extract was given in Table 2 using protein denaturation methods.

Table 1: Results of percentage yield of leaves extracts

S. No.	Solvent	Color of extract	Weight of plant material (gm)	Weight of extract (gm)	% yield
1.	Petroleum ether	Dark Green	180	4.32	2.4
2.	Methanol	Brown	160.71	10.68	1.29

Table 2: Effect of Diclofenac sodium and *Pongamia pinnata* methanolic extract on Protein Denaturation

S. No.	Concentration (µg/ml)	% Inhibition	
		Diclofenac sodium	Methanolic extract
1	50	50.33738192	42.37516869
2	100	59.51417004	54.38596491
3	150	71.25506073	64.1025641
4	200	78.54251012	72.73954116
5	250	87.44939271	80.02699055
IC 50		18.36	82.24

CONCLUSION

In conclusion, this study shows, for the first time, in vitro anti-inflammatory activity of methanolic leaf extract of Indian, *Pongamia pinnata* and scientifically justify its use in India traditional medicine to treat inflammatory conditions. Its anti-inflammatory action is mediated by the synergistic action of flavonoids, tannins and purine and pseudo-alkaloids. Furthermore, some studies have shown that the heat-induced protein denaturation test also indicated anti-rheumatoid arthritis activity in which case the results also suggest that *Pongamia pinnata* may be useful to treat rheumatoid arthritis as well.

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