



(RESEARCH ARTICLE)



EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF PONGAMIA PINNATA FLOWERS USING ZEBRA FISH (DANIO RERIO) MODEL

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ABSTRACT

Medicinal plants are widely used in traditional medicine because they contain natural compounds with therapeutic benefits and fewer side effects. *Pongamia pinnata* is a well-known medicinal plant reported to possess anti-inflammatory, antioxidant, and antimicrobial properties. In this study, fresh flowers of *Pongamia pinnata* were collected, shade dried, powdered, and extracted using ethanol. Preliminary phytochemical screening of the extract revealed the presence of important bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic compounds, which may contribute to its medicinal activity. Zebrafish were selected as the experimental model due to their sensitivity, easy maintenance, and similarity to human biological systems. Inflammation was induced in the zebrafish, and different concentrations of the ethanolic flower extract were administered. The anti-inflammatory effect was evaluated by observing changes in inflammatory responses.

Keywords: Indian beech tree (*Pongamia pinnata*), Zebra fish, Anti-inflammatory activity, Anesthesia Activity, Aqueous Extract.

1 INTRODUCTION

Inflammation is considered one of the primary physiological defense mechanisms of the human body against harmful stimuli such as microbial infections, burns, toxic chemicals, allergens, and tissue injury. It plays a crucial role in maintaining homeostasis and promoting tissue repair. However, persistent and uncontrolled inflammation may become a major etiological factor in the pathogenesis of various chronic diseases, including arthritis, cardiovascular disorders, diabetes, and cancer.[1]

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for the treatment of pain and inflammation. Although effective, these drugs are often associated with several adverse effects, especially gastrointestinal irritation and ulceration caused by inhibition of cyclooxygenase enzymes in the gastric mucosa. Approximately 34–46% of NSAID

users are reported to experience gastrointestinal complications. Hence, there is a growing need to identify safer anti-inflammatory agents with minimal side effects. [1]

Medicinal plants have served as an important source of therapeutic agents for thousands of years. Herbal medicine remains the primary healthcare system for nearly 75–80% of the population in developing countries because of its affordability, cultural acceptance, better compatibility with the human body, and reduced toxicity. In recent years, the use of herbal medicines has also increased considerably in developed countries. The long-standing traditional use of medicinal plants has demonstrated their safety and therapeutic efficacy in the management of various diseases.[5]

The World Health Organization encourages the incorporation of herbal medicines with proven efficacy and safety into healthcare systems, particularly in developing nations. India possesses a rich ethnobotanical heritage with numerous medicinal plants used traditionally for the treatment and management of pain and inflammatory disorders. However, many of these plants still lack sufficient scientific validation regarding their pharmacological activities. [7]

One such medicinal plant is *Pongamia pinnata*, a medium-sized glabrous tree widely distributed throughout India, Southeast Asia, and Australia. Different parts of the plant have been traditionally used for the treatment of inflammatory and infectious diseases such as leukoderma, leprosy, lumbago, muscular and articular rheumatism, piles, wounds, and ulcers. The leaves possess digestive, laxative, anthelmintic, and wound-healing properties. Leaf extracts are also used in medicated baths for relieving rheumatic pain and cleansing ulcers.[2]

Previous studies have reported anti-inflammatory properties in the root and seed extracts of *Pongamia pinnata*. Therefore, the present study was designed to evaluate the anti-inflammatory activity of methanolic extracts of the plant using the heat-induced protein denaturation technique. [3]

2 PLANT DETAILS

2.1 Introduction

Pongamia pinnata is a medicinal plant belonging to the family Fabaceae. It is commonly known as Indian beech, Karanja, or Pongam tree. The plant is widely distributed in India and other tropical regions of Asia. Different parts of the plant such as leaves, flowers, bark, roots, and seeds are traditionally used in Ayurvedic and folk medicine for the treatment of inflammation, skin disorders, wounds, diabetes, and infections. [6]

2.1.1 Synonyms

Indian beech, Karanja, Onge tree, Anuga chettu.[7]

2.1.2 Geographical Distribution

The plant is commonly found throughout India, especially in coastal regions, riverbanks, roadsides, and tropical climates. It also grows in Sri Lanka, Bangladesh, Malaysia, and Australia.

2.1.3 Major Chemical Constituents

Flavonoids, Tannins, Saponins, Fixed oils, Sterols, Alkaloids, Glycosides. [11]

2.2 Traditional Uses

2.2.1 Different parts of the plant are used traditionally for

Inflammation and pain, Skin diseases, Rheumatism, Wound healing, Diabetes, Fever, Microbial infections.

2.2.2 Pharmacological Activities

Anti-inflammatory activity, Analgesic activity, Antioxidant activity, antimicrobial activity, Antidiabetic activity, Hepatoprotective activity, Anticancer potential. [9]

2.3 Experimental Animals

The zebrafish, *Danio rerio*, is widely used in research studies because it shows many biological and immune responses similar to those found in humans. Due to these similarities, zebrafish has become an effective and reliable model for evaluating anti-inflammatory activity of plant extracts and pharmaceutical compounds. [4]

Zebrafish are also easy to handle and maintain under laboratory conditions. They require less space, smaller quantities of test samples, and lower maintenance costs compared to other animal models. Their small size and rapid growth make them suitable for routine pharmacological screening studies. [6]

Zebrafish is their sensitivity to inflammatory agents. Chemicals such as clove oil or carrageenan can easily induce inflammation in zebrafish, allowing researchers to observe the anti-inflammatory effects of test compounds clearly. In addition, zebrafish respond quickly to treatment, which helps in obtaining experimental results within a short period.[8]



Figure.1: Drying



Figure 2: Live Plant

2.4 Collection Of Plant Materials

- **Plant material:** Flowers of Indian beech tree (*Pongamia pinnata*) were collected and authenticated. The flowers are shade dried for 2-3 days . The dried flowers are grinded into powder.[14]



Figure 3: Grinding



Figure 4: Powder

2.5 Process of extraction

2.5.1 Cold water extraction (Maceration)

- **Collection & Preparation:** Fresh flowers of Indian beech tree (*Pongamia pinnata*) are collected, dried and ground.
- **Soaking (Maceration):** Dried flowers are crushed and taken in a container and distilled water is added within a ratio of 1:10. Covered for 24–48 hours at room temperature.Stirred occasionally.
- **Filtration:** Filter using filter paper.
- **Concentration:** Filtrate is concentrated (if needed) at low temperature.
- **Storage:** Stored in an airtight container, refrigerated.

2.5.2 Hot water extraction (Decoction)

- **Collection and preparation:** Fresh flowers of Indian beech tree (*Pongamia pinnata*) are collected and washed, dried under shade for 2-3.
- **Boiling:** Take 1g of dried flowers are crushed and added in 10ml of water(ratio 1:10) and boiled for 30–60 minutes.
- **Concentration:** Evaporated to get required volume.
- **Cooling & Filtration:** Allowed to cool, filtered with filter paper.
- **Storage:** Stored in a clean, airtight container.



Figure 5: Cold Extraction



Figure 6: Cold Extraction Dose



Figure 7: Hot Extract



Figure 8: Evaporation



Figure 9: Hot Extraction Dose

2.5.3 Ethanolic Extraction (Soxhlet)

- **Collection and preparation:** Fresh flowers were collected, shade dried, and coarsely powdered.
- **Soxhlet apparatus:** About 15-20g of the powdered material was packed into a thimble and placed in a Soxhlet apparatus.
- **Extraction:** Extraction was carried out using 90-100mL of ethanol (95%) as the solvent for 4-6 hours (approximately 6-8 cycles) until the solvent in the siphon tube became colorless.
- **Filtration:** The extract obtained was filtered by filter paper.

- **Concentration:** Concentrated under reduced pressure using a rotary evaporator at 40–45 °C to remove the solvent. The concentrated extract was further dried to obtain a semi-solid mass. The final extract was weighed, transferred into an airtight container, and stored at 4 °C until further use.



FIGURE 10: Soxhlet Apparatus



FIGURE 11: ETHANOL EVAPORATION

Phytochemical Screening

S.N O	TESTS	PROCEDURE	OBSERVATION	INFERENCE
01	Test for Flavonoids Schinoda test:	2 ml extract+ 2–3 small Mg turnings+0.5 ml concentrated HCl (dropwise).	Pink / red / orange color.	Flavonoids present.
02	Test for Tannins- Ferric chloride test:	2 ml extract+2–3 drops of 5% FeCl ₃ solution.	Green.	Condensed tannins.
03	Test for Glycosides Keller-Killiani Test:	2 ml extract+1 ml glacial acetic acid+1–2 drops of 1% FeCl ₃ + carefully 1 ml concentrated H ₂ SO ₄ along the side of test tube.	No Brown ring at junction.	Cardiac glycosides absent.
04	Test for Steroids & Terpenoids – Salkowski Test:	2 ml extract+2 ml chloroform+carefully 1–2 ml concentrated H ₂ SO ₄ along the side.	Reddish-brown layer Yellow-green fluorescence.	Terpenoids Steroids.
05	Test for Phenols – Ferric Chloride Test:	2 ml extract in a test tube+2–3 drops of 5% Ferric Chloride (FeCl ₃) solution.	Deep blue / green / violet color.	Phenols present.
06	Test for Alkaloids Using Mayer's Reagent:	2 ml extract+2–3 drops of Mayer's reagen.	No Cream / pale yellow precipitate.	Alkaloids absent.
	Using Dragendorff's Reagent:	2 ml extract+2–3 drops of Dragendorff's reagent.	No Orange / reddish-brown precipitate.	Alkaloids absent.



Figure 12: Flavonoids



Figure 13: Condensed Tannins

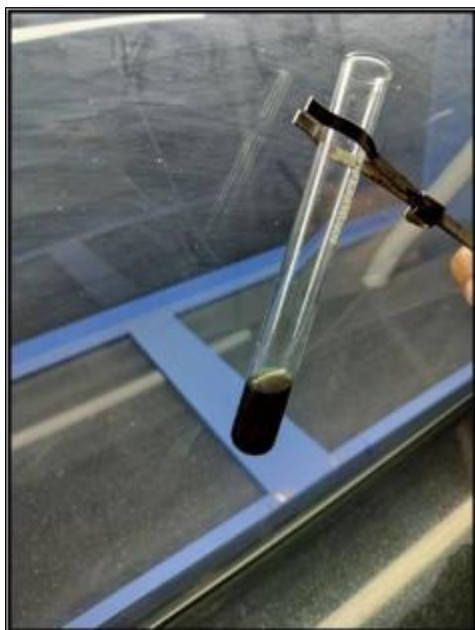


Figure 14: Glycosides



Figure 15: Terpenoids

**Figure 16: Phenols****Figure 17: Dragendroff Test**

3 METHODOLOGY AND EVALUATION OF ANTI INFLAMMATORY ACTIVITY

Healthy adult zebrafish, *Danio rerio*, of either sex were used for this study. The fishes measured about 3–4 cm in length and weight approximately 0.3–0.5 g. Before the experiment, the fishes were acclimatized for one week under laboratory conditions to minimize stress and maintain normal physiological activity. [12]

The zebrafish were maintained in glass tanks containing clean dechlorinated water at a temperature of $28 \pm 2^\circ\text{C}$ with a 12-hour light and 12 hours dark cycle. The fishes were fed one in a day. The water in tank were changed daily to maintain hygiene 30% of water are removed and 30% of fresh water were added. [12]

**Figure 18: Control Group****Figure 19: Test Group**

3.1 Animals Grouping

Fish were divided into 4 groups:

- Control group (no treatment)
- Negative control (clove oil)
- Test group 1(cold extract)
- Test group 2(hot extract)
- EXPERIMENTAL PROTOCOL:

3.2 Grouping of Animals

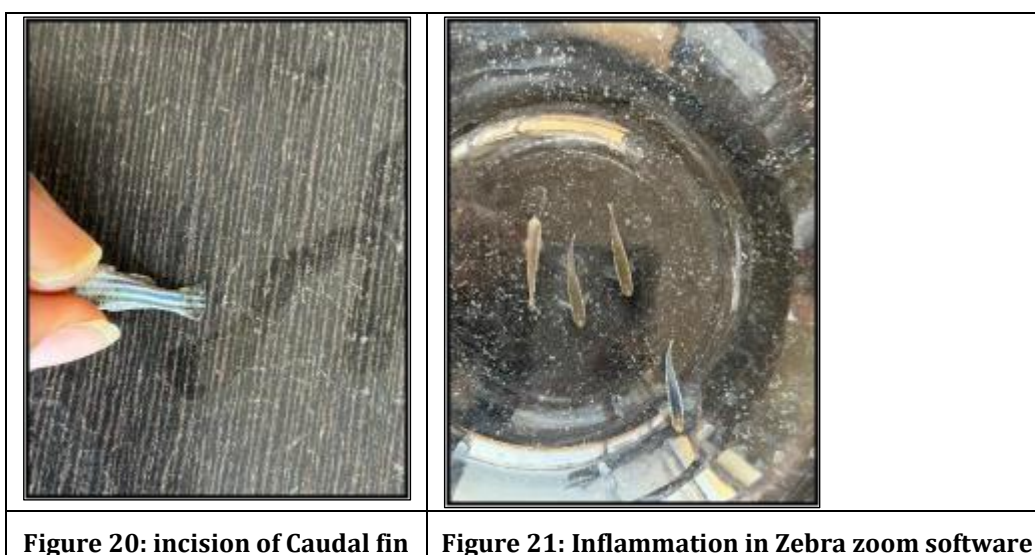
Adult zebrafish were randomly divided into 4 groups (n = 4 per group):

- Group I (Control): Normal water only,
- Group II (Negative Control): 1 ml of clove oil ,
- Group III (Cold extract Dose): Clove oil + extract (5 ml/L) ,
- Group IV : Clove oil + extract (2ml).

3.3 Induction of Inflammation

Inflammation was induced using clove oil solution. The fishes in the negative control group were exposed to 1 mL of diluted clove oil in tank containing 5 liters of water for a specific period. Exposure to clove oil produce anesthesia there may leads to bottom dwelling ,reduced locomotor activity, hypoactivity responses, and also causes sedation and loss of movement. [14]

Later the tail of the fishes were cutter with surgical scissors to observe Inflammation and the fishes were transferred into fresh water immediately after 4-6 hours the Inflammation is show.



3.4 Treatment Procedure

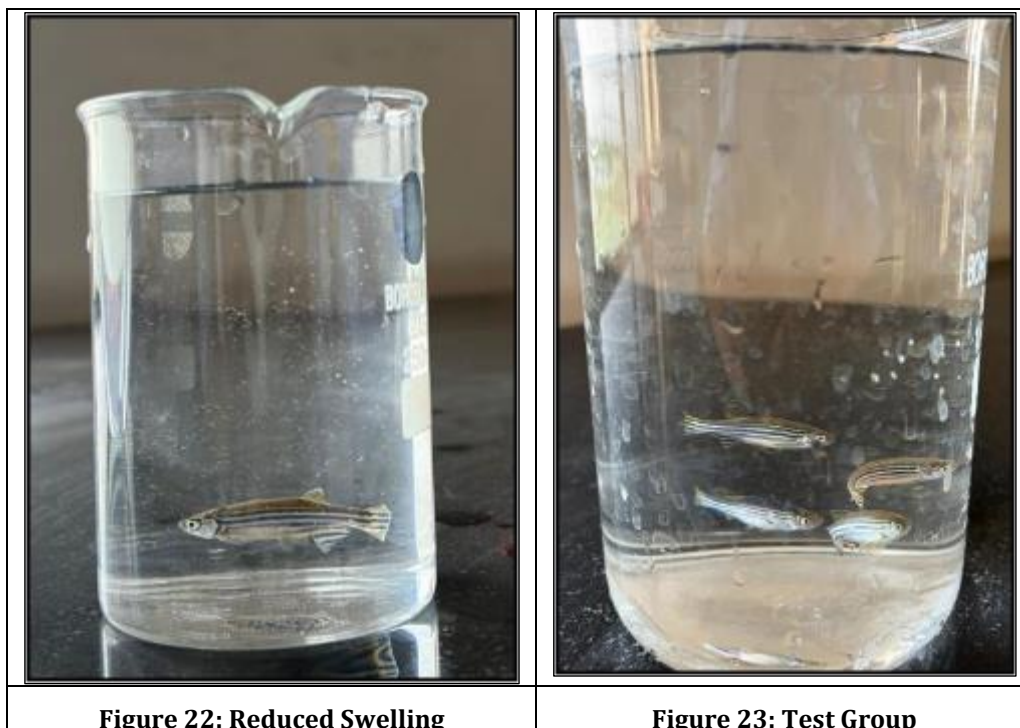
After the Inflammation the fishes were were administered in to respective treatment groups for 15-20 minutes After treatment, the fishes were carefully observed for behavioral and physical changes.

Evaluation of Anti-Inflammatory Activity:

- The anti-inflammatory activity was assessed using the following observations:
- Reduction in Inflammatory Signs.
- The fishes were examined for reduction in swelling, and irritation compared to the negative control group.

3.5 Behavioral Changes

Increases in locomotor activity and swimming rate.



Observation table

S.NO	GROUP	TREATMENT	DOSE	SWELLING	SWIMMING ACTIVITY
01	Group 1	Normal water	-	Absent	Normal
02	Group 2	Negative control [clove oil]	1ml	Severe	Reduced
03	Group 3	Cold extract	5ml	Mild	Improved
04	Group 4	Hot extract	2ml	Absent (or) mild	Normal

4 RESULT

The ethanolic flower extract of *Pongamia pinnata* showed significant anti-inflammatory activity in zebrafish. The treated fishes exhibited reduced swelling, irritation, and improved swimming behavior when compared to the control group. The activity increased with increasing dose of the extract. The anti-inflammatory effect may be due to the presence of flavonoids and phenolic compounds in the plant extract.

Decrease in inflammation = anti-inflammatory activity.

5 CONCLUSION

The present study showed that the flower extract of *Pongamia pinnata* has significant anti-inflammatory activity in zebrafish. The extract reduced swelling and irritation and improved swimming behavior compared to the negative control group. The activity may be due to the presence of flavonoids and phenolic compounds in the plant extract. These findings support the traditional use of *Pongamia pinnata* as a potential natural anti-inflammatory agent.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest regarding this manuscript.

REFERENCES

- [1] Kumar V, Abbas AK, Fausto N. In: Robbins and Cotran Pathological basis of disease. 10th Ed. Philadelphia. Elsevier Saunders. 2004:47-80.
- [2] Sangita K, Shukla G, Sambasivan Rao A. The present status of medicinal plants-Aspects and prospects. Int J Res Pharm Biomed sci 2011;2:19-22.
- [3] Pattari LS, Muchandi VN, Haricharan KN, Himabindu GM, Tejaswi CH, Ramanjaneyulu K, et al. Study of analgesic activity of *Litsea autinosa* (L.) ethanolic extract on swiss albino mice. Int J PharmSci Res 2010; 1:93-7.
- [4] Soorya C, Balamurugan S, Ramya S, Neethirajan K, Kandeepan C, Jayakumararaj R. Physicochemical, ADMET and Druggable properties of Myricetin: A Key Flavonoid in *Syzygium cumini* that regulates metabolic inflammations. Journal of Drug Delivery and Asian Journal of Dental and Health Sciences. 2023; 3(1):8-10 Therapeutics.
- [5] Kirtikar, K.R., Basu, B.D., 1933. Indian Medicinal Plants, vol. I. Lalit Mohan Basu, Allahabad, India, p. 830.
- [6] Singh, R.K., Pandey, B.L., 1996. Anti-inflammatory activity of seed extracts of *Pongamia pinnata* in rats. Indian Journal of Physiology and Pharmacology 40, 355-358.
- [7] Trease, C.E., Evan, V.C., 1959. Pharmacopoeial and Related Drugs of Biological Origin. Part Five: Pharmacognosy. Saunders, London, pp. 161-466.
- [8] Wheeler Aceto, H., Cowan, A., 1991. Neurogenic and tissue mediated components of formalin-induced edema: evidence for supraspinal regulation. Agents Action 34, 264-269.
- [9] Nakamura, H., Motoyoshi, S., Shimizu, M., 1978. Therapeutic effect of anti-inflammatory drugs on hind paw edema induced by kaolin-carrageenin in rats. Japanese Journal of Pharmacology 28, 83.
- [10] Soorya C, Balamurugan S, Ramya S, Neethirajan K, Kandeepan C, Jayakumararaj R. Physicochemical, ADMET and druggable properties of myricetin: A key flavonoid in *Syzygium cumini* that regulates metabolic inflammations. J Drug Deliv Ther. 2021;11(4):66-73.
- [11] Xie Y, Meijer AH, Schaaf MJM. Modeling inflammation in zebrafish for the development of anti-inflammatory drugs. Front Cell Dev Biol. 2020;8:620984. doi:10.3389/fcell.2020.620984.
- [12] Zanandrea R, Bonan CD, Campos MM. Zebrafish as a model for inflammation and drug discovery. Fish Shellfish Immunol. 2020; 108:1-8.
- [13] Belo MAA, Oliveira MF, Oliveira SL, Aracati MF, Rodrigues LF, Costa CC, et al. Zebrafish as a model to study inflammation: A tool for drug discovery. Biomed Pharmacotherapy. 2021; 144:112310. doi: 10.1016/j.biopha.2021.112310.
- [14] Smith C. The potential of zebrafish as drug discovery research tool in immune-mediated inflammatory disease. Inflammopharmacology. 2024; 32:2219-2233. doi:10.1007/s10787-024-01511-1.