



Anti-inflammatory activity and phytochemical analysis of *Macaranga peltata* Boiv. Ex Baill. leaves and *Pongamia pinnata* (L.) Pierre. seeds

[Actividad antiinflamatoria y análisis fitoquímico de hojas de *Macaranga peltata* Boiv. Ex Baill. y semillas de *Pongamia pinnata* (L.) Pierre.]

Honnesh N. Honnappa¹, Basavaraj Metikurki², V. Kusum Devi³, Santanu Saha^{4*}

¹Department of Pharmacognosy, Nitte College of Pharmaceutical Sciences, 6429, NITTE Campus, Govindapura, Gollahalli, Yelahanka, Bangalore - 560064, Karnataka, India.

²Department of Pharmaceutical Chemistry, Nitte College of Pharmaceutical Sciences, 6429, NITTE Campus, Govindapura, Gollahalli, Yelahanka, Bangalore - 560064, Karnataka, India.

³Department of Pharmaceutics, Nitte College of Pharmaceutical Sciences, 6429, NITTE Campus, Govindapura, Gollahalli, Yelahanka, Bangalore - 560064, Karnataka, India.

⁴Department of Pharmacognosy, NGSIM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Panner, Deralakatte, Mangaluru-575018, Karnataka, India.

*E-mail: santanusaha@nitte.edu.in

Abstract

Context: Traditional medicinal plant knowledge represents one of the oldest and most valuable contributions to the health sector of humanity. It serves as an essential guide to humans to develop better and healthier well-being. Medicinal plants possess phytoconstituents which exhibit enormous therapeutic effects.

Aims: To evaluate the *in vitro* and *in vivo* anti-inflammatory activity of *Macaranga peltata* (MPCF) and *Pongamia pinnata* (PPEAF) fractions and estimate the phytochemicals responsible for their therapeutic action.

Methods: Plant extracts and fractions were tested for their *in vitro* anti-inflammatory properties using the bovine serum albumin method and *in vivo* activity using the carrageenan-induced rat paw edema model. The selected fragments were analyzed for phytochemicals such as flavonoids, tannins, and total phenolics.

Results: The extract yields indicate that *P. pinnata* contains non-polar compounds and *M. peltata* contains polar phytochemicals. Both the MPCF and PPEAF were found to contain flavonoids. However, MPCF also contained tannins and phenolic compounds that were found in lesser percentages in PPEAF.

Conclusions: The chloroform fraction of *M. peltata* and ethyl acetate fraction of *P. pinnata* showed significant anti-inflammatory activity compared to the positive control group.

Keywords: bovine serum albumin; carrageenan; inflammation; *Macaranga peltata*; *Pongamia pinnata*.

Resumen

Contexto: El conocimiento de las plantas medicinales tradicionales es el mayor y más antiguo servicio al sector de la salud de la humanidad. El conocimiento herbario tradicional es la mejor guía para que los seres humanos desarrollen un bienestar mejor y más saludable. Las plantas medicinales poseen fitoconstituyentes que muestran enormes efectos terapéuticos.

Objetivos: Evaluar la actividad antiinflamatoria *in vitro* e *in vivo* de las fracciones de *Macaranga peltata* (MPCF) y *Pongamia pinnata* (PPEAF) y estimación de los fitoquímicos responsables de la acción terapéutica.

Métodos: Se probaron extractos y fracciones de plantas para determinar sus propiedades antiinflamatorias *in vitro* utilizando el método de albúmina sérica bovina y su actividad *in vivo* utilizando un modelo de edema de pata de rata inducido por carragenina. La estimación de fitoquímicos como flavonoides, taninos y fenólicos totales se realizó para fracciones seleccionadas.

Resultados: Los rendimientos de los extractos mostraron que *P. pinnata* contiene compuestos no polares y *M. peltata* contiene fitocompuestos polares. Se encontró que MPCF y PPEAF contienen flavonoides, pero también se encontró que MPCF contiene taninos y compuestos fenólicos, pero menos en PPEAF.

Conclusiones: La fracción de cloroformo de *M. peltata* y la fracción de acetato de etilo de *P. pinnata* mostraron una actividad antiinflamatoria significativa en comparación con el grupo de control positivo.

Palabras Clave: albúmina sérica bovina; carragenano; inflamación; *Macaranga peltata*; *Pongamia pinnata*.

ARTICLE INFO

Received: February 26, 2024.

Accepted: November 5, 2024.

Available Online: November 29, 2024.

AUTHOR INFO

ORCID:

[0000-0002-7569-6810](https://orcid.org/0000-0002-7569-6810) (HNH)

[0000-0002-5112-2468](https://orcid.org/0000-0002-5112-2468) (BM)

[0000-0002-3265-8929](https://orcid.org/0000-0002-3265-8929) (KD)

[0000-0002-2715-1082](https://orcid.org/0000-0002-2715-1082) (SS)

Abbreviations: MPCF: *Macaranga peltata* chloroform fraction; MPEE: *Macaranga peltata* ethyl acetate fraction; PPEAF: *Pongamia pinnata* ethyl acetate fraction; PPPEF: *Pongamia pinnata* petroleum ether fraction.

INTRODUCTION

Macaranga peltata Müll.Arg. (family *Euphorbiaceae*), a medicinal plant grown in the Western Ghats, is also known as Chandana and Uppalige. It is used in traditional medicines to treat wounds, swellings, boils, bruises, and sores (Joseph, 2014; Rekha et al., 2014). The crude extracts and isolated compounds of this plant have demonstrated a wide range of pharmacological effects, including anti-microbial, anti-inflammatory, antioxidant, and anti-plasmodial properties (Joseph, 2014; Rekha et al., 2014). The phyto-compounds isolated from this plant are bergenin, 8,10-di-O-methylether, tri-O-methyl ether, siarasinolic acid, lupeol, sitosterol, stigmaterol, and oleanolic acid (Joseph, 2014; Rekha et al., 2014). *Macaranga* genus plants are used to cleanse wounds in northeast India (Changkija et al., 2023), so an attempt was made to know anti-inflammatory activity and the responsible phytochemical group.

Pongamia pinnata (L.) Pierre (family *Fabaceae*) is a traditional plant known as Honge and Karanj. According to reports, the seeds of this plant have an oil content of 28% to 34% with a significant amount of polyunsaturated fatty acids (Akram et al., 2021). The seed oil contains pinnatin, kanjone, pongapin, pongalabrone, and karangin, while the plant leaves and stem contain flavones and chalcone-derived substances such as galbone, pongone, pongalbol, and pongagallone A and B (Akram et al., 2021). The fruit of this plant contains pongamoside D and three new pongamosides A–C, furnoflavanoid and glucosides. The root extract of *P. pinnata* contains anticancer agents and derivatives of etoposide, paclitaxel, teniposide, and fluoxetine. The powdered seeds possess anthelmintic and antibacterial properties and are used to treat bronchial inflammation, fever, hemorrhoids, congestion, whooping cough, and irritations (Akram et al., 2021). Therefore, the present study has been designed to standardize the extracts and fractions for *in vitro* and *in vivo* anti-inflammatory activity.

MATERIAL AND METHODS

Chemicals

The reagents and substances utilized in the present investigation were all of laboratory analysis grade. The source of bovine serum albumin was Hi-Media Research Laboratory Pvt. Ltd. located in Mumbai. Chemicals were acquired from Vasa Scientific Com-

pany, and carrageenan was procured from HMRL Pvt. Ltd., Mumbai. Tween 80 was obtained from SDFC Pvt. Ltd., Mumbai.

Collection and authentication of plants

The *Macaranga peltata* leaves were gathered in Mangalore's Paneer and Deralakatte districts. (Latitude: 12.91723 Longitude: 74.85603) The *Pongamia pinnata* seeds were supplied by Pioneer Agro Industries in Coimbatore, Tamil Nadu (Latitude: 11.0555 Longitude: 76.96612). The gathered plants were authenticated at the Foundation for Revitalization of Local Health Traditions (FRLHT), Bangalore, and a voucher specimen (4717-18/28.0/.2021) was deposited.

Extraction

The authenticated seeds and leaves were dried in a hot air oven and ground to powder, which was passed through 22 mesh to obtain a coarse powder. The powders underwent ethanol maceration (1:8 ratio), followed by fractionation using petroleum ether, chloroform, ethylene acetate, and methanol sequentially. The extracts were filtered and solvent recovered by rota evaporator, dried on a magnetic stirrer, and preserved in the desiccator.

Fractionation of ethanolic extract

About 1200 g and 540 g ethanol extracts of *Macaranga peltata* and *Pongamia pinnata* were fractionated using petroleum ether at 60–80°C (8 × 500 mL), followed by fractionation with chloroform (8 × 500 mL), ethyl acetate (8 × 500 mL), and methanol (8 × 500 mL). The fractions were dried over anhydrous sodium sulfate, cleaned with 30 ml of distilled water, and the solvents were removed by distillation. The *M. peltata* ethanol extract was fractionated into petroleum ether (99.42 g), chloroform (267 g), ethyl acetate (265 g), and methanol (433.71 g) fractions. Similarly, for *P. pinnata*, the petroleum ether (92 g), chloroform soluble (68 g), ethyl acetate (54 g), and methanol (36 g) fractions were obtained from the ethanolic extract.

To screen for biological activity, a 1% solution of Tween 80 in water was utilized to suspend the ethanol extract and chloroform fraction of *M. peltata* leaves, as well as the petroleum ether and ethyl acetate fractions of *P. pinnata*. The same solution was also used to prepare a suspension of the standard medication, diclofenac. In these studies, 1% Tween 80 solution, diluted with water, was used as the vehicle.

In vitro anti-inflammatory activity

In this study, the bovine serum albumin denaturation inhibition method (Aggarwal and Saha, 2020) was used. Separate solutions containing 1% bovine serum albumin were combined with the test extracts, fractions, and the reference medication, diclofenac sodium, at varying concentrations (10–50 µg/mL). After adding 1 N HCl to bring down the pH to 6.3 ± 0.5 , the mixture was incubated for 20 minutes at 37°C. Each mixture containing the test extract and the reference medication was then independently heated for 3 minutes at 57°C and allowed to cool. Using the vehicle as a blank, the absorbance was measured at 416 nm after mixing the reaction solutions with 2.5 mL of phosphate buffer. The investigation was completed in triplicate. The percentage of protein denaturation inhibition was calculated using the formula [1].

$$\text{Anti-denaturation activity (\%)} = \frac{Ab_c - Ab_s}{Ab_c} \times 100 \quad [1]$$

Where Ab_c : Control absorbance and Ab_s : Sample absorbance; Anti-denaturation activity Percentage = Percentage inhibition of protein denaturation = Anti-inflammatory activity percentage.

In vivo studies

Animals

The studies employed albino Wistar rats (200–250 g) of both sexes. All the animals were acquired from the Nitte Gulabi Shetty Institute of Pharmaceutical Sciences' animal house in Mangaluru. The rats were fed a standard diet and had unlimited access to water. The creatures were housed in polyacrylic cages, maintained at a relative humidity of $60 \pm 5\%$ and an ambient temperature of $25 \pm 2^\circ\text{C}$. In the institutional animal house, the animals were acclimated to a 12-hour light/dark cycle. The study procedures were approved by the Institutional Animal Ethical Committee of the Nitte Gulabi Shetty Institute of Pharmaceutical Sciences in Mangaluru (NGSMIPS/IAEC/JAN-2023/341).

Acute toxicity study

The acute toxicity study was carried out using the up-and-down method of OECD guideline no. 425 (OECD, 2001) on adult female albino Wistar rats (3 rats per group) that were neither pregnant nor nulliparous to calculate the median fatal dosage (LD_{50}) of test material: *M. peltata* chloroform fraction (MPCF); *M. peltata* ethyl acetate fraction (MPEE); *P. pinnata* ethyl acetate fraction (PPEAF); *P. pinnata* petroleum ether fraction (PPPEF). The extract and fraction preparations were administered orally to the animals, and they were observed at half-hour intervals for four hours, followed by a 24-hour observation period for

general behavioral, neurological, and autonomic profiles, and finally, for mortality.

The test materials, MPEE, MPCF, PPPEF, and PPEAF, were found to be safe up to an oral dose of 2000 mg/kg and 5000 mg/kg. Based on the study, three extract dose levels were selected for all test materials used to conduct the in vivo biological activity studies. The low dose was half of the middle dose, the high dose was twice the middle dose, and the intermediate dose was roughly one-tenth of the LD_{50} . The selected doses were 100 mg/kg, 200 mg/kg, and 400 mg/kg.

In vivo anti-inflammatory activity study

The anti-inflammatory efficacy of the test substances was examined using the carrageenan-induced rat paw edema model. Using the previously mentioned approach, the acute anti-inflammatory activity of MPCF and PPEAF was evaluated using the carrageenan-induced rat paw edema model (Ghosh, 2005). To induce paw edema in the plantar region, 0.1 mL of a 1% (w/v) carrageenan suspension in 0.9% (w/v) sterile saline was injected into the left hind paws of all the animals. One hour before the carrageenan injection, eight distinct groups of six rats ($n = 6$) received oral treatments of the vehicle (sterile saline solution), the test medicines (MPCF and PPEAF, 100, 200, and 400 mg/kg body weight), and diclofenac (10 mg/kg body weight). The level of inflammation in the left paw was measured using the right paw as a reference. The increase in the paw volume was measured using a Digital Plethysmograph Model No PLM 02, Orchid Scientific, India, at one-, two-, three-, and four-hour intervals following carrageenan injection.

The percentage inhibition of edema volume was calculated using the formula [2].

$$\text{Inhibition (\%)} = 100 \times (1 - V_t / V_c) \quad [2]$$

where, V_t represents the average paw edema volume of the groups treated with diclofenac and extracts, and V_c represents the average paw edema volume of the control group.

Group details

Group 1: Carrageenan (1% w/v) 0.1 ml + Negative control (0.9% w/v NaCl).

Group 2: Carrageenan (1% w/v) 0.1 ml + Positive control (diclofenac sodium 10 mg/kg body weight).

Group 3: Carrageenan (1% w/v) 0.1 mL + MPCF (100 mg/kg body weight).

Group 4: Carrageenan (1% w/v) 0.1 mL + MPCF (200 mg/kg body weight).

Group 5: Carrageenan (1% w/v) 0.1 ml + MPCF (400 mg/kg body weight).

Group 6: Carrageenan (1% w/v) 0.1 mL + PPEAF (100 mg/kg body weight).

Group 7: Carrageenan (1% w/v) 0.1 mL + PPEAF (200 mg/kg body weight).

Group 8: Carrageenan (1% w/v) 0.1 mL + PPEAF (400 mg/kg body weight).

PPEAF- *Pongamia pinnata* ethyl acetate fraction, MPCF- *Macaranga peltata* chloroform fraction

Phytochemical evaluation

Determination of total flavonoid content

The total flavonoid content was measured by a colorimetric assay (Fattahi et al., 2014; Zhishen et al., 1999). Initially, 0.5 mL or 500 μ L (0.5 g) of the extract was added to 4 mL of distilled water, and then 0.3 mL of 5% sodium nitrite was added to this. After 5 minutes, 0.3 mL of 10% aluminum chloride was added, and in the 6th min, 2 mL of 1 M sodium hydroxide was added to the mixture. Following this, the mixture was immediately diluted by adding 3.3 mL of distilled water and mixed thoroughly. Immediately, the absorbance was measured at 510 nm against a blank using quercetin as a standard for the calibration curve using a UV-visible spectrophotometer (Shimadzu 1780. Japan). The total flavonoid content of the extract was expressed as mg quercetin equivalents (QE) per gram of sample (mg/g). The results were represented as a replica of three readings.

Determination of total phenolic content

The Folin Ciocalteu reagent was used for the analysis of total phenolic content (Chun et al., 2003; Fattahi et al., 2014). Briefly, 0.5 mL or 500 μ L (0.5 g) of the extract was mixed with 0.5 mL of Folin-Ciocalteu reagent. The F-C reagent purchased was a dark green color diluted with an equal quantity of water as specified on the label used for estimation. The solution was kept at 25°C for 5-8 min before adding 2 mL of 7.5% sodium carbonate solution and adjusting the volume to 8 mL with water. After 2 hours, the absorbance was measured at 725 nm by using gallic acid as the standard for the calibration curve using a U-visible spectrophotometer (Shimadzu 1780. Japan). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of sample (mg/g dry extract). The results were represented as a replica of three readings.

Total condensed tannin content

The tannin contents or proanthocyanidin were determined using catechin as a reference compound for

the calibration curve (Rebaya et al., 2015). A volume of 0.5 mL or 500 μ L (0.5 g) of extract was added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation, the absorbance was read at 500 nm using a UV-visible spectrophotometer (Shimadzu 1780. Japan), and the total tannin content was expressed as mg catechin equivalent (CE) per gram of dry extract. The results were represented as a replica of three readings.

Statistical analysis

The results were expressed as mean $\pm$ the standard error mean (S.E.M) of six observation readings. Dunnet's t-test is utilized to assess statistical significance. Statistically, significance at $p < 0.05$ and $p < 0.001$ were considered to assess the significance of the activity.

RESULTS

Extraction

The extraction and fractionation are presented in Table 1, which is sequentially elaborated below as follows:

Description of *Macaranga peltata* ethanol extract

Nature: Solid amorphous powder. Color: Dark brownish green color. Odor: Characteristic. Taste: Bitter. Yield: 24.16%.

Description of *P. pinnata* ethanol extract

Nature: Semisolid paste. Color: Yellowish brown color oily extract. Odor: Characteristic. Taste: Extremely bitter. Yield: 10.8%.

Fractionation of *M. peltata* ethanol extract

Description of *M. peltata* petroleum ether fraction

Nature: Amorphous paste. Color: Light brown. Odor: Characteristic. Taste: Bitter. Yield: 8.28%.

Description of *M. peltata* chloroform fraction

Nature: Amorphous powder. Color: Dark brown. Odor: Characteristic. Taste: Bitter. Yield: 22.28%.

Description of *M. peltata* ethyl acetate fraction

Nature: Amorphous paste. Color: Dark brown. Odor: Characteristic. Taste: Bitter. Yield: 22.14%.

Description of *M. peltata* methanol fraction

Nature: Amorphous powder. Color: Dark brown. Odor: Characteristic. Taste: Bitter. Yield: 36.14%.

Table 1. Extraction and fractionation.

No.	Solvent used for extraction	Extract (g)	
		<i>Pongamia pinnata</i> seed powder (5 kg)	<i>Macaranga peltata</i> leaf powder (5 kg)
1	Ethanol	10.8% (540 g)	24.16% (1.2 kg)
Solvent used for fractionation		%Fraction (g)	
01	Petroleum ether fraction	17% (92 g)	8.28% (99.42 g)
02	Chloroform fraction	12.59% (68 g)	22.28% (267 g)
03	Ethyl acetate fraction	10% (54 g)	22.14% (265 g)
04	Methanol fraction	6.66% (36 g)	36.14% (433.71 g)

Fractionation of *P. pinnata* ethanol extract

Description of P. pinnata petroleum ether fraction

Nature: Semisolid oily nature. Color: Light yellow color sticky fraction. Odor: Characteristic. Taste: Extremely bitter. Yield: 17%.

Description of P. pinnata chloroform fraction

Nature: Semisolid oily nature. Color: Yellow color. Odor: Characteristic. Taste: Extremely bitter. Yield: 12.59%.

Description of P. pinnata ethyl acetate fraction

Nature: Semisolid oily nature. Color: Yellowish cream color. Odor: Characteristic. Taste: Extremely bitter. Yield: 10%.

Description of P. pinnata methanol fraction

Nature: Semisolid oily nature. Color: Reddish brown color. Odor: Characteristic. Taste: Bitter. Yield: 6.66%.

In vitro anti-inflammatory activity

The ethanol extracts from *M. peltata* and *P. pinnata* were found to have respective IC₅₀ values of 38.81 and 50.19 µg/mL (Table 2). *M. peltata* and *P. pinnata* petroleum ether fractions were found to have IC₅₀ values of 46.00 and 40.76 µg/mL, respectively. The *M. peltata* and *P. pinnata* chloroform fractions were found to have respective IC₅₀ values of 38.26 and 46.42 µg/mL. The ethyl acetate fractions from *M. peltata* and *P. pinnata* were found to have respective IC₅₀ values of 48.16 and 38.49 µg/mL. The methanol fractions from *M. peltata* and *P. pinnata* were found to have respective IC₅₀ values of 47.03 and 51.64 µg/mL.

In vivo studies

Acute toxicity study

The acute toxicity study results showed that the rats did not die or exhibit any appreciable behavioral

alterations in response to the test samples, MPCF and PPEAF, even at doses up to 2000 mg/kg. Given the demonstrated safety of the test materials at an oral dosage of up to 2000 mg/kg of body weight, oral doses of 100, 200, and 400 mg/kg of body weight were chosen for further study (Table 3).

Anti-inflammatory activity

The anti-inflammatory activity was tested on rats using a carrageenan-induced inflammatory model. Paw volumes (in mL) of the animals in the negative control and positive control (diclofenac sodium) group, as well as their % of edema inhibition, were compared with those of the test samples after the administration of carrageenan.

The result showed significant anti-inflammatory activity in *M. peltata* chloroform fraction at 400 mg/kg (60.76% edema inhibition) and *P. pinnata* ethyl acetate fraction at 400 mg/kg (61.94% edema inhibition), compared with positive control (diclofenac 10 mg/kg) group (66.37% edema inhibition) after 4 hours of treatment with carrageenan. In contrast, there was no anti-inflammatory activity observed in the control group treated with 1% v/v Tween 80 (Table 4).

Estimation of total flavonoids

In flavonoids estimation, after preparation of dilutions, yellow color precipitate was obtained. The blank solution was transparent, and the standard quercetin solution produced a dark yellow precipitate. The PPEAF solution showed a light yellow color, while a dark reddish brown color was obtained for the MPCF solution, respectively. The flavonoid content in *M. peltata* chloroform fraction and *P. pinnata* ethyl acetate fraction was found to be 930.72 mg and 600.29 mg QE/g dry extract (Table 5).

Estimation of total phenolic content

In the estimation of phenolic compounds, the F-C reagent was diluted as specified, which gave it a light

Table 2. *Pongamia pinnata* and *Macaranga peltata* ethanol extracts and fractions' *in vitro* anti-inflammatory efficacy as measured by the bovine serum albumin method.

No.	Treatment	Concentration (µg/mL)	Inhibition (%)	
			<i>Pongamia pinnata</i>	<i>Macaranga peltata</i>
1	Diclofenac sodium	10	25.33 ± 0.03	20.64 ± 0.02
		20	34.24 ± 0.01	32.20 ± 0.06
		30	49.33 ± 0.07	46.43 ± 0.08
		40	58.57 ± 0.02	56.24 ± 0.05
		50	65.82 ± 0.04	64.12 ± 0.03
		IC ₅₀	33.17**	35.47**
2	Ethanol extract	10	7.45 ± 0.02	9.42 ± 0.04
		20	25.00 ± 0.05	21.66 ± 0.02
		30	36.66 ± 0.06	33.25 ± 0.03
		40	40.00 ± 0.01	45.32 ± 0.01
		50	46.62 ± 0.02	58.33 ± 0.06
		IC ₅₀	50.19**	38.81**
3	Chloroform fraction	10	11.26 ± 0.04	11.24 ± 0.08
		20	25.10 ± 0.01	23.34 ± 0.05
		30	35.41 ± 0.02	32.58 ± 0.03
		40	41.46 ± 0.06	43.00 ± 0.01
		50	53.68 ± 0.01	52.62 ± 0.04
		IC ₅₀	46.42*	38.26**
4	Petroleum ether fraction	10	9.66 ± 0.07	8.64 ± 0.02
		20	17.57 ± 0.01	13.33 ± 0.07
		30	26.35 ± 0.06	26.66 ± 0.04
		40	41.43 ± 0.08	36.32 ± 0.06
		50	56.32 ± 0.02	47.46 ± 0.02
		IC ₅₀	40.76*	46.00*
5	Ethyl acetate fraction	10	8.16 ± 0.01	8.30 ± 0.05
		20	16.66 ± 0.05	16.54 ± 0.03
		30	25.32 ± 0.04	23.36 ± 0.02
		40	43.52 ± 0.02	33.58 ± 0.04
		50	50.42 ± 0.04	48.42 ± 0.16
		IC ₅₀	38.49*	48.16*
6	Methanol fraction	10	8.59 ± 0.02	10.52 ± 0.04
		20	22.65 ± 0.06	22.42 ± 0.02
		30	34.33 ± 0.05	34.33 ± 0.03
		40	40.00 ± 0.01	46.54 ± 0.07
		50	45.58 ± 0.04	60.64 ± 0.01
		IC ₅₀	51.64**	47.03**

Dunnet's t-test was utilized to assess statistical significance. For each value, the three-reading mean ± S.E.M was used. Statistically significant at *p<0.05 and **p<0.001.

Table 3: Acute Toxicity study of *Pongamia pinnata* ethyl acetate Fraction and *Macaranga peltata* chloroform fraction

Signs monitored	Treatment			
	Negative control	Positive control (diclofenac sodium)	PPEAF (Dose-2 g/kg bw) and (Dose-5 g/kg bw)	MPCF (Dose-2 g/kg bw) and (Dose-5 g/kg bw)
Increased motor activity	No change	No change	No change	No change
Decreased motor activity	No change	No change	No change	No change
Tremors and ataxia	No change	No change	No change	No change
Tonic convulsions	No change	No change	No change	No change
Sedation	No change	No change	No change	No change
Diarrhoea	No change	No change	No change	No change
Salivation	No change	No change	No change	No change
Respiration	No change	No change	No change	No change
Skin colour change	No change	No change	No change	No change

No deaths were observed in rats. PPEAF: *Pongamia pinnata* ethyl acetate fraction; MPCF: *Macaranga peltata* chloroform fraction; bw: body weight.

Table 4. Results of *in vivo* anti-inflammatory activity of ethyl acetate fraction of *Pongamia pinnata* and *Macaranga peltata* chloroform fraction by carrageenan-induced rat paw oedema method.

Group	Treatment	Dose/kg bw, p.o	Paw volume (mL)							
			1 h	Inhibition (%)	2 h	Inhibition (%)	3 h	Inhibition (%)	4 h	Inhibition (%)
I	Negative control	0.9% w/v NaCl	1.210 ± 0.1120	---	1.176 ± 0.489	---	0.9895 ± 0.125	---	0.8973 ± 0.621	---
II	Positive control	Diclofenac 10 mg/kg	0.8521 ± 0.03035	29.58	0.6120 ± 0.02316	47.95	0.3350 ± 0.0375	66.14	0.3017 ± 0.0467**	66.37
III	PPEAF	100 mg/kg	0.9625 ± 0.08502	20.45	0.7763 ± 0.0644	33.99	0.6217 ± 0.1345	37.17	0.5021 ± 0.1154*	44.04
IV	PPEAF	200 mg/kg	0.9424 ± 0.09153	22.12	0.6950 ± 0.0927*	40.90	0.5543 ± 0.0432**	43.98	0.4050 ± 0.0278**	54.86
V	PPEAF	400 mg/kg	0.9244 ± 0.02143	23.60	0.5670 ± 0.0632*	51.79	0.4443 ± 0.0371**	55.10	0.3415 ± 0.0356**	61.94
VI	MPCF	100 mg/kg	0.9524 ± 0.10211	21.29	0.7513 ± 0.09575	36.11	0.6117 ± 0.0378*	38.18	0.5458 ± 0.0421*	39.17
VII	MPCF	200 mg/kg	0.9611 ± 0.08315	20.57	0.7352 ± 0.07854	37.48	0.5678 ± 0.125*	42.61	0.4315 ± 0.1263**	51.91
VIII	MPCF	400 mg/kg	0.9124 ± 0.09132	24.60	0.7321 ± 0.09512	37.74	0.5053 ± 0.0278**	48.93	0.3521 ± 0.0164**	60.76

Dunnet's t-test is utilized to assess statistical significance, and the data are presented as mean ± S.E.M. for each group of six animals. Statistically significant at *p<0.05 and **p<0.001. MPCF: *Macaranga peltata* chloroform fraction, PPEAF: *Pongamia pinnata* ethyl acetate fraction; bw: body weight; p.o: per os.

Table 5. Estimation of total flavonoids, total phenolic compounds, and total tannins.

Phytochemical evaluation	Standard	<i>Pongamia pinnata</i> ethyl acetate fraction	<i>Macaranga peltata</i> chloroform fraction
Total flavonoid content	≥98% quercetin	600.29 ± 2.4* mg QE/g LE	930.72 ± 1.5** mg QE/g DE
Total phenolic content	≥98% gallic acid	90.65 ± 0.8* mg GAE/g LE	720.48 ± 2.2** mg GAE/g DE
Total tannin content	≥95% catechin	240.7 ± 0.5** mg CE/g LE	910.0 ± 0.2** mg CE/g DE

Dunnet's t-test is utilized to assess statistical significance. For each value, the three-reading mean ± S.E.M is used. Statistically significant at *p<0.05 and **p<0.001. MPCF: *Macaranga peltata* chloroform fraction, PPEAF: *Pongamia pinnata* ethyl acetate fraction, GAE: gallic acid equivalents; QE: quercetin equivalent, CE: catechin equivalent, DE: dry extract, LE: Liquid extract.

green color. The phenolic content in *M. peltata* chloroform fraction and *P. pinnata* ethyl acetate fraction was found to be 720.48 mg and 90.65 mg GAE/g dry extract (Table 5).

Estimation of total tannins estimation

The catechin ($\geq 95.0\%$) was used as a standard for estimation of total tannin content. MPCF has shown 910 mg CE/g dry extract, whereas PPEAF was found to contain 240.7 mg CE/g dry extract (Table 5).

DISCUSSION

The *M. peltata* and *P. pinnata* plants extracts and fractions were investigated for their anti-inflammatory activity. In the previous research, it has shown that 47% extract was obtained by using methanol as solvent (Verma et al., 2012) whereas in our study, 25% ethanol extract yield was noticed by extraction from the leaf. So far, no research has been done on these plant fractions, so fractionation was done to know the therapeutically active fraction. Previous researchers have reported *M. peltata* fruits acetone extract has shown better antibacterial activity than petroleum ether extract 10% (Badarudheen et al., 2015), *M. barteri* stem bark ethanol extract for anti-inflammatory activity and has shown significant activity of 64% edema inhibition at dose of 300 mg per kg body weight (Asante-Kwatia et al., 2019). In contrast, the present study reported 60.76 at a dose of 400 mg/kg body weight. The previous study reported *M. peltata* leaf extract for *in vitro* anti-inflammatory activity, antioxidant and anticancer activity on lung cancer cell lines (Anil and Mohandas, 2023) and concluded as a valuable herbal for antioxidants and anticancer phytochemicals. A recent study reported the anticancer activity of ethanol extract of *M. peltata*, which has shown significant activity on human oral squamous carcinoma cell lines (Nizar et al., 2024). In the earlier study, the ethanol extract from *P. pinnata* seeds was tested *in vitro* for anti-inflammatory and antioxidant activities, and significant results were reported (Yasothkumar et al., 2023). *P. pinnata* stem bark extract ointment (5% and 10%) and wound healing activity were performed, and the result showed significant activity in albino Wistar rats (Bhandirge et al., 2015). However, no additional fractionation was carried out to identify a relevant fraction. The carrageenan-induced method was selected to know the anti-inflammatory activity since inflammation can be induced within one hour; it is a valid model that gives highly sensitive and reproducible results (Kute, 2017). In this study, a decrease in edema with respect to time is considered since not only does the sample and standard compound decrease inflammation simultaneously, but the body's normal immune response

also controls and decreases inflammation. And up to 4 hours, inflammation can be clearly seen as red edema later, redness decreases, so it is difficult to differentiate between inflammatory paw and non-inflammatory paw.

In the present study, using the denaturation inhibition technique of bovine serum albumin, the *in vitro* anti-inflammatory efficacy was observed. The previous *in vitro* protein denaturation method performed for 50 $\mu\text{g/mL}$ leaf extract of *P. pinnata* methanol extract has shown 42.37% inflammation inhibition (Bairagi et al., 2023) compared to the present result of 45.58% inhibition for methanol fraction of seed ethanol extract at a dose concentration of 50 $\mu\text{g/mL}$. The *P. pinnata* petroleum ether fraction's IC_{50} value of 40.76 $\mu\text{g/mL}$ and the *P. pinnata* ethyl acetate fraction's IC_{50} value of 38.49 $\mu\text{g/mL}$ demonstrated superior IC_{50} values over the other fractions, and notable activity was observed in comparison to the reference diclofenac sodium in the present study. The IC_{50} value represents the average result of all percentage inhibitions at different concentrations. The chloroform fraction of *M. peltata* has reported an IC_{50} value of 38.26 $\mu\text{g/mL}$, and the IC_{50} value for the methanol fraction as 47.03 $\mu\text{g/mL}$ showed better IC_{50} values when compared to other fractions, with significant activity being noted in contrast to the standard diclofenac sodium. Thus, these fractions were further used to perform *in vivo* anti-inflammatory activity, and this study showed that they possessed promising activity.

So far, there is no proper phytochemical evaluation done for the major group of phytochemicals for *M. peltata*. So, in the present study, phytochemical evaluation was conducted by measuring the total flavonoid content, total phenolic content, and total tannin content in the prepared plant fractions. The overall results indicated that the MPCF has a high percentage of flavonoids (930.72 mg QE/g) compared to PPEAF (600.29 mg QE/g). The phenolic content in MPCF was found to be 720.48 mg GAE/g, which is GAE, whereas PPEAF showed a very low phenolic content of 90.65 mg GAE/g. The total tannin content was also evaluated. MPCF contained 910.0 mg of CE/g, while PPEAF showed 240.7 mg of CE/g.

These phytochemical results demonstrate that MPCF was a rich source of flavonoids, phenolics, and tannins, which are the supportive phytochemicals known for their therapeutic activities such as antibacterial, antioxidant, anti-inflammatory and skin protective and wound healing properties (Anil and Mohandas, 2023; Badarudheen et al., 2015; Rosamah et al., 2023; Yasothkumar et al., 2023). PPEAF also contained flavonoids in an optimum quantity when compared to its tannins and phenolic content, which might be responsible for its potent activity in this study.

CONCLUSION

Macaranga peltata and *Pongamia pinnata* ethanol extracts have demonstrated promising anti-inflammatory effects when compared to positive diclofenac in the in vitro model. Additionally, the in vitro model demonstrated superior efficacy for the *M. peltata* chloroform fraction and the *P. pinnata* ethyl acetate fraction. Using a carrageenan-induced model, an *in vivo* anti-inflammatory study was also conducted, and this demonstrated that significant activity was shown by both plant components at 400 mg/kg of body weight. Based on these investigations, the study shows that flavonoids may be the phytochemicals that are responsible for the anti-inflammatory activity of both plants.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

The authors thank Dr. C.S. Shastry, Principal of the NGSM Institute of Pharmaceutical Sciences, and Dr. Murali Badanthadka, Head of the Pharmacology Department at NGSMIPS, for providing the facility to carry out the anti-inflammatory activity. They also extend my thanks to Dr. Nimmy Varghese, Assistant Professor at the Pharmacology department at NGSMIPS, for their guidance while testing the activity. Also, authors cordially thank Dr. K.C. Gangadhar, Ashwatha T, Pratibha Goudar, and Narendranath K.N. Nitte Meenakshi Institute of Technology Library staff for their support of the plagiarism check. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

- Aggarwal NN, Saha S (2020) Evaluation of anti-inflammatory and nitric oxide scavenging activity of *bauhinia variegata* leaves by *in vitro* method. *Plant Arch* 20(2): 4234–4238.
- Akram M, Nimesh S, Chishti MA, Ahmad MI, Dhama S, Lal M (2021) *Pongamia pinnata*: An updated review on its phytochemistry, & pharmacological uses. *Pharmacol Int J* 9(5): 194–199. <https://doi.org/10.15406/ppij.2021.09.00344>
- Anil L, Mohandas S (2023) *In vitro* antioxidant and anticancer activity of *Macaranga peltata* leaf extracts on lung cancer cell lines. *Int J Curr Pharm Res* 15(4): 26–32.
- Asante-Kwatia E, Jibira Y, Mensah AY, Osei-Sarfoh D (2019) *Macaranga barteri* stem bark extract exerts anti-inflammatory and anti-hyperalgesia activity in murine models. *Discov Phytomed* 6(3):130–137. <https://doi.org/10.15562/phytomedicine.2019.104>
- Badaruddeen R, Lubna N, Dufaida KM, Mashhoor K (2015) Antibacterial activity of *Macaranga peltata*. *Int J Adv Res Emerging Disc* 3(2): 77–83.
- Bairagi J, Katare V, Chourey B, Delouri A, Nema S (2023) Evaluation of *in-vitro* anti-inflammatory activity of leaves of *Pongamia pinnata*. *Asian J Dent Health Sci* 3(1): 8–10. <https://doi.org/10.22270/ajdhs.v3i1.35>
- Bhandirge SK, Tripathi AS, Bhandirge RK, Chinchmalatpure TP, Desai HG, Chandewar AV (2015) Evaluation of wound healing activity of ethanolic extract of *Pongamia pinnata* bark. *Drug Res* 65(6): 296–299. <https://doi.org/10.1055/s-0034-1384537>
- Changkija S, Thakuria D, Cynthia A (2023) Traditional use of *Macaranga* trees for soil fertility: By Naga shifting cultivators in northeast India. In *Farmer Innovations and Best Practices by Shifting Cultivators in Asia-Pacific*. GB: CABI, pp. 557–570. <https://doi.org/10.1079/9781800620117.0026>
- Chun OK, Kim DO, Lee CY (2003) Superoxide radical scavenging activity of the major polyphenols in fresh plums. *J Agri Food Chem* 51(27): 8067–8072. <https://doi.org/10.1021/jf034740d>
- Fattahi S, Zabihi E, Abedian Z, Pourbagher R, Ardekani AM, Mostafazadeh A, Akhavan-Niaki H (2014) Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on HeLa and BT-474 cell lines. *Int J Mol Cell Med* 3(2): 102–107. <https://pmc.ncbi.nlm.nih.gov/articles/PMC4082812/>
- Ghosh MN (2005) *Fundamentals of experimental Pharmacology*. Calcutta, India: Scientific Publishing Book Agency.
- Joseph JM (2014) Phytochemistry and pharmacology of the genus *Macaranga*: A review. *J Med Plants Res* 8(12): 489–503. <https://doi.org/10.5897/JMPR2014.5396>
- Kuete V (2017) *Medicinal species and vegetables from Africa. Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic Diseases*. Dschang, Cameroon: Academic Press.
- Nizar A, Ravindran R, Palani J, Nripan T, Asha SD, Pynadath MK (2024) Anticancer effects of ethanolic extracts of *Macaranga peltata* leaves on human oral squamous carcinoma cell lines: An *in vitro* study. *J Pharm Bioallied Sci* 16(2):1833–1837. <https://doi.org/10.4103/jpbs.jpbs.28.24>
- OECD (2001) OECD guidelines for testing of chemicals. Guideline 425. acute oral toxicity – UP and Down Procedure (Adopted, December 17). Organization for Economic Cooperation and Development (OECD). <https://doi.org/10.1787/9789264071049-en>
- Rebaya A, Belghith SI, Baghdikian B, Leddet VM, Mabrouki F, Olivier E, Kalthoum Cherif J, Ayadi MT (2015) Total phenolic, total flavonoid, tannin content, and antioxidant capacity of *Halimium halimifolium* (Cistaceae). *J Appl Pharm Sci* 5(1): 52–57. <https://doi.org/10.7324/JAPS.2015.50110>
- Rekha RP, Jain N, Supriya B, Babu KS, Rao MJ (2014) Chemical constituents from *Macaranga peltata* roots and screening of their cytotoxic activity. *Int J Pharm Chem Sci* 3(2): 415–419.
- Rosamah E, Haqiqi MT, Putri AS, Kuspradini H, Kusuma IW, Amirta R, Yuliansyah Y, Suwinarti W, Paramita S, RamadhanR, Tarmadi D, Ismayati M, Ariyanta HA, Fatriasari W, Aswandi A, Kholibrina CR, Azelee NIW, Kim YU, Arung ET (2023) The potential of *Macaranga* plants as skincare cosmetic ingredients: A review. *J Appl Pharma Sci* 13(7): 1–12. <https://dx.doi.org/10.7324/JAPS.2023.77745>
- Verma M, Kamath N, Kumar N, Venkata Rao J, Dhanaraj SA, Vasanth Raj P (2012) Screening of plant *Macaranga peltata* for *in vitro* antioxidant activity. *Int J Med Pharm Sci* 3(1): 31–39.
- Yasothkumar D, Jayaraman S, Ramalingam K, Ramani P (2023) *In vitro* anti-inflammatory and antioxidant activity of seed ethanolic extract of *Pongamia pinnata*. *Biomed Pharmacol J* 16(4): 2187–2193. <https://dx.doi.org/10.13005/bpj/2795>
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64(4): 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

AUTHOR CONTRIBUTION:

Contribution	Honnesh NH	Basavaraj M	Devi KV	Saha S
Concepts or ideas	x			x
Design	x			x
Definition of intellectual content	x	x	x	x
Literature search	x	x	x	x
Experimental studies	x	x		x
Data acquisition	x			x
Data analysis	x		x	
Statistical analysis	x		x	
Manuscript preparation	x		x	x
Manuscript editing	x		x	x
Manuscript review	x	x	x	x

Citation Format: Honnesh NH, Basavaraj M, Devi KV, Saha S (2025) Anti-inflammatory activity and phytochemical analysis of *Macaranga peltata* Boiv. Ex Baill. leaves and *Pongamia pinnata* (L.) Pierre. seeds. J Pharm Pharmacogn Res 13(2): 662–671.
https://doi.org/10.56499/jppres24.1997_13.2.662

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Open Access: This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits use, duplication, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.