



RESEARCH ARTICLE

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITIC POTENTIAL OF *GARUGA PINNATA* EXTRACT

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and anti-arthritis potential of the ethanolic extract of *Garuga pinnata*. The plant material was subjected to ethanolic extraction, and the percentage yield of the extract was determined. Preliminary phytochemical screening was carried out to identify the presence of various bioactive constituents. Quantitative estimation of total phenolic and total flavonoid content was also performed using standard methods. The anti-arthritis activity of the ethanolic extract was assessed using Freund's adjuvant-induced arthritis in rats, with aspirin used as the standard reference drug. The ethanolic extract showed a percentage yield of 9.78% w/w and was found to contain alkaloids, flavonoids, phenols, proteins, carbohydrates, and saponins. The total phenolic and flavonoid contents were found to be 0.78 mg GAE/100 mg and 0.93 mg QE/100 mg, respectively. In the in vivo study, the extract at doses of 100 and 200 mg/kg significantly reduced paw edema in a dose-dependent manner, with the higher dose showing comparable activity to aspirin. The findings suggest that the ethanolic extract of *Garuga pinnata* possesses significant anti-arthritis activity, which may be attributed to the presence of polyphenolic compounds, supporting its traditional use in the treatment of inflammatory disorders.

INTRODUCTION

Arthritis is a chronic inflammatory disorder characterized by joint pain, swelling, stiffness, and progressive loss of function. Among its various forms, rheumatoid arthritis and osteoarthritis are the most prevalent and are associated with significant morbidity and reduced quality of life. The pathogenesis of arthritis involves immune-mediated inflammation, protein denaturation, lysosomal enzyme release, and oxidative stress-induced tissue damage. Although conventional therapies such as non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs) are effective, their long-term use is often limited due to adverse effects, including gastrointestinal irritation, hepatotoxicity, and immunosuppression (Banik et al., 2020). Medicinal plants have emerged as promising alternatives for the management of inflammatory and arthritic disorders owing to their bioactive phytoconstituents and comparatively better safety profiles. Phytochemicals such as flavonoids, alkaloids, phenolics, terpenoids, and saponins are known to exert anti-inflammatory and anti-arthritis effects by inhibiting pro-inflammatory mediators, stabilizing lysosomal membranes, and preventing protein denaturation (Farooq et al., 2022). These properties justify the scientific validation of traditionally used medicinal

plants for arthritis treatment. *Garuga pinnata* Roxb., belonging to the family Burseraceae, is a deciduous tree widely distributed in India and Southeast Asia. The plant has been traditionally used for the treatment of inflammation, joint pain, wounds, and gastrointestinal disorders. Ethnomedicinal reports indicate the use of *G. pinnata* bark and leaves in inflammatory conditions, suggesting its therapeutic relevance in arthritis-related disorders (Ara et al., 2013). Previous phytochemical investigations have revealed the presence of diarylheptanoids, flavonoids, phenolic compounds, and proteins in *G. pinnata*, which are associated with antioxidant and anti-inflammatory activities (Rahman et al., 2014). Experimental studies have demonstrated significant anti-inflammatory activity of *G. pinnata* extracts using in-vitro and in-vivo models, including inhibition of protein denaturation and reduction of edema formation, supporting its potential role in arthritis management (Islam et al., 2015). Since protein denaturation is one of the well-documented mechanisms involved in the pathogenesis of rheumatoid arthritis, plant extracts capable of inhibiting this process may serve as effective anti-arthritis agents. Therefore, the present study was designed to perform extraction and phytochemical screening of *Garuga pinnata* extract and to evaluate its anti-arthritis potential using in-vitro models. The findings aim to scientifically substantiate the traditional claims and provide a foundation for further pharmacological and formulation-based investigations.

MATERIAL AND METHODS

Material

Fresh plant material of *Garuga pinnata* was collected, authenticated, and shade dried before pulverization. Ethanol was used as the solvent for extraction of the plant material. Standard chemicals and reagents required for phytochemical screening, including Hager's reagent, lead acetate, ferric chloride, Biuret reagent, Fehling's solution, foam test reagents, copper acetate, ninhydrin, and Legal's reagent, were used. Gallic acid and quercetin were employed as reference standards for the estimation of total phenolic and total flavonoid content, respectively. Freund's complete adjuvant was used for induction of arthritis in experimental animals. Aspirin served as the standard anti-arthritis drug, while gum acacia was used as the vehicle. All chemicals and reagents used were of analytical grade.

Methods

Preparation of plant material for study: Plant materials (Leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time. Then these plants materials were shade dried without any contamination for about 3 to 4 weeks. Dried plant materials were grinded using electronic grinder. Dried plant material was packed in air tight container till any further use.

Extraction procedure: Following procedure was adopted for the preparation of ethanolic extract from the shade dried and powdered herbs (Mukherjee, 2007):

Extraction by maceration process: 50 gm dried powdered leaves of *Garuga pinnata* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.

Determination of percentage yield: After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage. The percentage yield of each extract was calculated by using following formula:

Phytochemical Screening: The chemical tests were performed for testing different chemical groups present in extracts (Kokate, 1994).

Estimation of total phenol content: The total phenol content of the extract was determined by the modified folin-ciocalteu method (Parkhe, Deepak Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content: Determination of total flavonoids content was based on aluminium chloride method (Mishra et al., 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were

prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Table 1. % Yield of ethanolic extract of *Garuga pinnata*

S. No.	Extract	Weight of Extract	% Yield (w/w)
1.	Ethanolic	4.89	9.78

Table 2. Phytochemical screening of ethanolic extract of *Garuga pinnata*

S. No.	Phytochemical Test	Method Used	Result
1	Alkaloids	Hager's test	+ve
2	Flavonoids	Lead acetate test	+ve
		Alkaline reagent test	-ve
3	Phenols	Ferric chloride test	+ve
4	Proteins	Biuret's test	+ve
5	Carbohydrates	Fehling's test	+ve
6	Saponins	Foam test	+ve
7	Diterpenes	Copper acetate test	-ve
8	Amino acids	Ninhydrin test	-ve
9	Glycosides	Legal's test	-ve

[+ve=Positive; -ve= Negative]

Table 3. Total phenolic and total flavonoid content of *Garuga pinnata*

S. No.	Extract	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Ethanolic extract	0.78	0.93

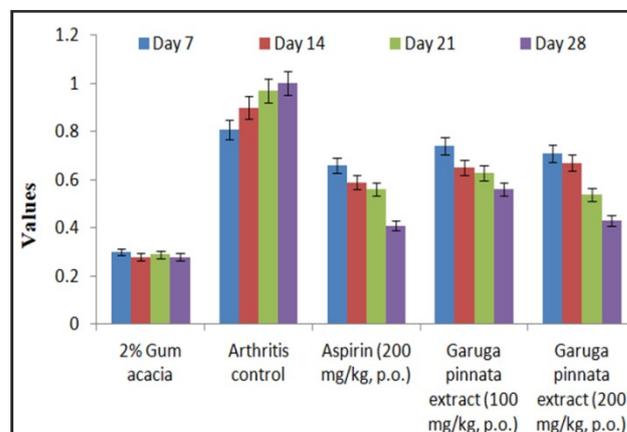


Figure 1. Anti-arthritis activity of ethanolic extract of *Garuga pinnata* against Freund's adjuvant induced arthritis in rats

In-vivo anti-arthritis activity of *Garuga pinnata* extract

Animals: Albino Wistar rats of either sex (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Animals were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rat was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Table 4. Anti-arthritis activity of ethanolic extract of *Garuga pinnata* against Freund's adjuvant induced arthritis in rats

Group	Treatment	Day 7	Day 14	Day 21	Day 28
Group I	2% Gum acacia	0.30 ± 0.06	0.28 ± 0.05	0.29 ± 0.04	0.28 ± 0.04
Group II	Arthritis control	0.81 ± 0.16	0.90 ± 0.19	0.97 ± 0.21	1.00 ± 0.32
Group III	Aspirin (200 mg/kg, p.o.)	0.66 ± 0.11	0.59 ± 0.14**	0.56 ± 0.28***	0.41 ± 0.33***
Group IV	<i>Garuga pinnata</i> extract (100 mg/kg, p.o.)	0.74 ± 0.14	0.65 ± 0.14*	0.63 ± 0.19*	0.56 ± 0.14*
Group V	<i>Garuga pinnata</i> extract (200 mg/kg, p.o.)	0.71 ± 0.30**	0.67 ± 0.17**	0.54 ± 0.24***	0.43 ± 0.14***

Values expressed as mean ± SEM (n=6) *P<0.05, **P<0.01, ***P<0.001 as compared to arthritis Control

Chemicals: Freund's complete adjuvant (Sigma-Aldrich Chemical Co.) was used for experiments.

Acute oral toxicity study: Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (Gothe *et al.*, 2023). Ethanolic extract of *Garuga pinnata* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-arthritic effect.

Anti-arthritis activity: Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw (Rajaram *et al.*, 2015).

Group I served as normal and received 2% gum acacia.

Group II served as arthritis control-untreated received 2% gum acacia.

Group III received Aspirin (200 mg/kg p.o.) served as reference standard.

Group IV received extract of ethanolic extract of *Garuga pinnata* of doses of 100mg/kg p.o.

Group V received extract of ethanolic extract of *Garuga pinnata* of doses of 200mg/kg p.o.

The drug treatment was started from 14th day of adjuvant induction and terminated on 28th day. The changes in paw volume was measured weekly by using Plethysmograph. At the end of experiment histopathology was done to check the inflammation.

Statistical analysis: The values were expressed as mean ± SEM (n=6) (Sanmuga *et al.*, 2010). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and P<0.05, P<0.01, and P<0.001 were considered to be statistically significant.

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the extraction yield, phytochemical composition, and anti-arthritic potential of the ethanolic extract of *Garuga pinnata*. The findings from the experimental work provide scientific support for the traditional use of this plant in inflammatory and arthritic conditions. The ethanolic extraction of *Garuga pinnata* yielded 9.78% w/w (Table 1), indicating efficient extraction of

bioactive constituents using ethanol as the solvent. Ethanol is known to extract both polar and moderately non-polar phytoconstituents, which may explain the satisfactory yield obtained. A good extraction yield is essential as it reflects the abundance of pharmacologically active compounds available for therapeutic evaluation. Preliminary phytochemical screening (Table 2) revealed the presence of alkaloids, flavonoids, phenols, proteins, carbohydrates, and saponins in the ethanolic extract, while diterpenes, amino acids, and glycosides were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these constituents are widely reported to possess potent anti-inflammatory, antioxidant, and immunomodulatory properties. Alkaloids and saponins are also known to contribute to anti-arthritic activity by inhibiting inflammatory mediators and reducing joint swelling. Quantitative estimation of phytoconstituents (Table 3) showed appreciable levels of total phenolic content (0.78 mg GAE/100 mg) and total flavonoid content (0.93 mg QE/100 mg). These values suggest that the extract is rich in polyphenolic compounds, which can scavenge free radicals, inhibit cyclooxygenase and lipoxygenase pathways, and suppress the production of pro-inflammatory cytokines. Such mechanisms play a crucial role in the management of chronic inflammatory diseases like arthritis.

The anti-arthritic activity of the ethanolic extract was evaluated using Freund's adjuvant-induced arthritis in rats, a well-established experimental model that closely resembles human rheumatoid arthritis. As shown in Table 4 and Figure 1, the arthritis control group exhibited a progressive increase in paw edema from day 7 to day 28, confirming successful induction of arthritis. In contrast, treatment with *Garuga pinnata* extract at doses of 100 mg/kg and 200 mg/kg produced a dose-dependent reduction in paw edema. The higher dose (200 mg/kg) showed statistically significant inhibition (P < 0.001) of paw swelling from day 21 onward, with effects comparable to the standard drug aspirin (200 mg/kg).

This reduction in edema indicates suppression of inflammatory processes and stabilization of lysosomal membranes, which are key factors in the pathogenesis of arthritis. The moderate yet consistent activity observed at the lower dose further supports the therapeutic potential of the extract. The anti-arthritic effect of *Garuga pinnata* can be attributed to the synergistic action of its phytoconstituents, particularly phenols and flavonoids, which may act by inhibiting inflammatory mediators, reducing oxidative stress, and modulating immune responses. These findings validate the traditional claims associated with *Garuga pinnata* and highlight its potential as a natural anti-arthritic agent. Further studies focusing on isolation of active compounds and elucidation of precise molecular mechanisms are warranted to strengthen its clinical relevance. Values expressed as mean ± SEM (n=6) *P<0.05, **P<0.01, ***P<0.001 as compared to arthritis Control

CONCLUSION

The present investigation demonstrates that the ethanolic extract of *Garuga pinnata* possesses significant anti-arthritic activity in Freund's adjuvant-induced arthritis in rats. The extract showed a good percentage yield and was rich in bioactive phytoconstituents such as alkaloids, flavonoids, phenols, saponins, carbohydrates, and proteins. The appreciable levels of total phenolic and total flavonoid content suggest a strong antioxidant and anti-inflammatory potential. In vivo evaluation revealed that the extract produced a dose-dependent reduction in paw edema, with the higher dose (200 mg/kg) exhibiting effects comparable to the standard drug aspirin. These findings indicate that the anti-arthritic activity of *Garuga pinnata* may be mediated through modulation of inflammatory pathways and oxidative stress. The study scientifically validates the traditional use of *Garuga pinnata* in inflammatory conditions and highlights its potential as a natural therapeutic agent for the management of arthritis. Further studies are warranted to isolate the active constituents and elucidate the exact mechanism of action.

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