International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

EXTRACTION, PHYTOCHEMICAL ANALYSIS AND *IN VIVO* ANTI ARTHRITIC ACTIVITY OF *FICUS GLOMERATA* EXTRACT

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***Article History:**

Received: 10/02/2026
Revised: 27/02/2026
Accepted: 11/03/2026

ABSTRACT

Arthritis is a chronic inflammatory disorder characterized by pain, swelling, stiffness, and progressive destruction of joints. The present study was designed to investigate the extraction, phytochemical analysis, and in vivo anti-arthritic activity of *Ficus glomerata* extract. The powdered plant material was successively extracted using petroleum ether and ethanol solvents. The extractive values obtained were 0.76% w/w for petroleum ether extract and 9.25% w/w for ethanolic extract. Preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, glycosides, flavonoids, diterpenes, carbohydrates, and saponins. Quantitative estimation showed total flavonoid content of 0.74 mg/100 mg and total alkaloid content of 0.68 mg/100 mg, indicating the presence of significant bioactive constituents. The anti-arthritic activity of the ethanolic extract was evaluated using Freund's adjuvant-induced arthritis model in rats. The extract was administered orally at doses of 100 mg/kg and 200 mg/kg, and paw volume was measured at different time intervals. The ethanolic extract exhibited significant and dose-dependent reduction in paw edema compared to the arthritis control group. The higher dose (200 mg/kg) showed more pronounced anti-arthritic activity, comparable to the standard drug aspirin (200 mg/kg). The observed pharmacological effect may be attributed to the presence of flavonoids, alkaloids, glycosides, and other phytoconstituents possessing anti-inflammatory and antioxidant properties. The study concludes that the ethanolic extract of *Ficus glomerata* possesses significant anti-arthritic activity and supports its traditional use in the management of inflammatory disorders. Further investigations are required to isolate and characterize the active constituents responsible for the observed therapeutic effects.

Keywords: *Ficus glomerata*, Anti-arthritic activity, Freund's adjuvant, Phytochemical screening, Ethanolic extract, Flavonoids, Alkaloids, Medicinal plants.

INTRODUCTION

Arthritis is a chronic inflammatory disorder characterized by pain, swelling, stiffness, and progressive destruction of joints, leading to reduced mobility and impaired quality of life. Among the various forms of arthritis, rheumatoid arthritis is one of the most common autoimmune diseases affecting millions of people worldwide. The disease involves persistent synovial inflammation, cartilage degradation, and bone erosion mediated through inflammatory cytokines, prostaglandins, and reactive oxygen species. Conventional therapies such as non-steroidal

anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDs) are commonly used for the management of arthritis. However, prolonged use of these drugs is often associated with adverse effects including gastrointestinal irritation, hepatotoxicity, nephrotoxicity, and immunosuppression. Therefore, there is an increasing demand for safer and more effective alternatives derived from natural sources.

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Medicinal plants have been widely used in traditional systems of medicine for the treatment of inflammatory and arthritic disorders due to the presence of diverse phytoconstituents such as flavonoids, phenolics, tannins, alkaloids, terpenoids, and glycosides. These bioactive compounds possess antioxidant, anti-inflammatory, immunomodulatory, and analgesic properties that may help in reducing joint inflammation and preventing tissue damage. Scientific evaluation of medicinal plants is essential to validate their traditional claims and identify potential therapeutic agents for arthritis management (Singh *et al.*, 2020).

Ficus glomerata Roxb. (Family: Moraceae), commonly known as Cluster Fig or Gular, is an important medicinal plant extensively distributed throughout India and other tropical regions. Different parts of the plant, including bark, leaves, fruits, and roots, have been traditionally used for the treatment of various ailments such as diabetes, diarrhea, inflammation, ulcers, wounds, liver disorders, and skin diseases. The plant is reported to contain several biologically active phytoconstituents including flavonoids, phenolic compounds, tannins, sterols, triterpenoids, and glycosides, which are known to exhibit significant pharmacological activities. Previous studies have demonstrated antioxidant, antimicrobial, hepatoprotective, antidiabetic, and anti-inflammatory properties of Ficus glomerata. However, limited scientific data are available regarding its anti-arthritic potential (Bhalerao *et al.*, 2014).

Phytochemical analysis plays an essential role in the identification and characterization of secondary metabolites responsible for therapeutic activities. Extraction using hydroalcoholic solvents is considered an effective method for isolating a broad range of

phytoconstituents due to its ability to dissolve both polar and moderately non-polar compounds (Mera *et al.*, 2019).

Preliminary phytochemical screening and quantitative estimation of phenolic and flavonoid contents provide valuable information regarding the chemical composition and biological potential of plant extracts. Since oxidative stress and inflammation are closely associated with the pathogenesis of arthritis, plant extracts rich in antioxidant phytoconstituents may exhibit promising anti-arthritic activity (Jaradat *et al.*, 2015).

Therefore, the present study was designed to investigate the extraction, phytochemical analysis, and in vivo anti-arthritic activity of Ficus glomerata extract. The study aims to evaluate the phytochemical constituents present in the extract and assess its therapeutic efficacy using experimental animal models of arthritis. The findings of the study may provide scientific evidence supporting the traditional use of *Ficus glomerata* and contribute to the development of novel plant-based anti-arthritic agents with improved safety and efficacy.

MATERIALS AND METHODS

Materials

The plant material of *Ficus glomerata* was collected, authenticated, dried, and powdered for extraction. Petroleum ether and ethanol were used as extraction solvents. Various chemicals and reagents used for phytochemical screening included Hager's reagent, Legal's reagent, lead acetate, ferric chloride, Fehling's solution, acetate reagent, and other analytical grade chemicals. Freund's complete adjuvant was used for induction of arthritis in experimental animals, while aspirin was used as standard anti-arthritic drug. Gum acacia was used as a suspending agent for oral administration of extract. All solvents and chemicals used in study were of analytical grade.

Methods

Procurement of plant materials

Leaves of *Ficus glomerata* were collected from ruler area of Bhopal, month of November, 2025. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant.

Extraction by soxhlet extraction techniques

60 gram of shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction techniques. The extraction was continued till the defatting of the material had taken place.

Defatted dried powdered has been extracted with ethanol solvent using soxhlet extraction process for 48 hrs (Khandelwal, 2005). The mixture occasionally stirred to enhance the extraction efficiency by increasing the contact between the plant material and the solvent. After the soaking period, the mixture is filtered to separate the liquid extract from the solid plant residues. The filtrate contains the dissolved phytochemicals. The solvent is then removed, usually by evaporation under reduced pressure or using a rotary evaporator, to concentrate the extract. The concentrated extract can be further processed or analyzed to isolate specific bioactive compounds.

Determination of extractive value (% yield)

The % yield of yield of each extract was calculated by using formula:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Qualitative phytochemical analysis is a fundamental step in the exploration of medicinal plants. By identifying the various bioactive compounds present, researchers can better understand the therapeutic potential of plants, ensure the quality and consistency of herbal products, and pave the way for further detailed studies and drug development (Kokate, 1994).

Quantitative studies of phytoconstituents

Estimation of total alkaloids content

The plant extracts (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract (Shamsa *et al.*, 2008).

6.7.2 Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

***In-vivo* anti-arthritis activity**

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*.

Animas 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.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD, 2001). Ethanolic extract of *Ficus glomerata* (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.

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 (Aloke *et al.,* 2019).

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 *Ficus glomerata* of doses of 100mg/kg p.o.

Group V received extract of ethanolic extract of *Ficus glomerata* 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). 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 phytochemical constituents and anti-arthritic activity of the ethanolic extract of *Ficus glomerata*. The findings obtained from extractive values, phytochemical screening, quantitative estimation, and in vivo anti-arthritic studies provide significant evidence regarding the therapeutic potential of the plant.

The extractive value study revealed that the ethanolic extract showed a considerably higher percentage yield (9.25% w/w) compared to the petroleum ether extract (0.76% w/w). The higher yield obtained with ethanol may be attributed to its ability to extract a broad range of polar and moderately polar phytoconstituents such as flavonoids, alkaloids, glycosides, saponins, and carbohydrates. This suggests that ethanol is an effective solvent for extraction of bioactive compounds from *Ficus glomerata*. The lower yield in petroleum ether extract indicates the comparatively lesser presence of non-polar constituents in the plant material.

Preliminary phytochemical screening of the ethanolic extract demonstrated the presence of several important secondary metabolites including alkaloids, glycosides, flavonoids, diterpenes, carbohydrates, and saponins, whereas phenols, proteins, and tannins were absent. The presence of flavonoids and alkaloids is particularly important because these compounds are well known for their anti-inflammatory, antioxidant, analgesic, and immunomodulatory properties. Glycosides and saponins are also reported to possess anti-inflammatory and membrane-stabilizing effects, which may contribute to the anti-arthritic activity of the extract. The phytochemical diversity observed in the extract supports the traditional medicinal use of *Ficus glomerata* in inflammatory disorders.

Quantitative estimation revealed that the ethanolic extract contained appreciable amounts of total flavonoids (0.74 mg/100 mg) and total alkaloids (0.68 mg/100 mg). The relatively high flavonoid content indicates strong antioxidant potential of the extract. Flavonoids are known to inhibit inflammatory mediators such as prostaglandins, leukotrienes, cytokines, and reactive oxygen

species, which play a major role in the pathogenesis of arthritis. Alkaloids also contribute to anti-inflammatory and analgesic activities through modulation of inflammatory signaling pathways. Therefore, the presence of these bioactive compounds may be responsible for the observed pharmacological activity.

The anti-arthritic activity was evaluated using Freund's adjuvant-induced arthritis in rats, which is a well-established experimental model that closely resembles human rheumatoid arthritis. Administration of Freund's adjuvant produced significant paw edema in the arthritis control group, indicating successful induction of chronic inflammation and arthritis. The paw volume progressively increased from day 7 to day 28 in the arthritis control group, confirming persistent inflammatory response.

Treatment with aspirin (200 mg/kg) significantly reduced paw edema throughout the experimental period, thereby validating the experimental model and serving as a standard reference for comparison. The ethanolic extract of *Ficus glomerata* exhibited significant and dose-dependent anti-arthritic activity. At the dose of 100 mg/kg, the extract produced a moderate reduction in paw volume, with significant inhibition observed from day 14 onwards. In contrast, the higher dose of 200 mg/kg demonstrated a more pronounced reduction in paw edema, showing highly significant activity on days 21 and 28. The reduction in inflammation observed with the higher dose indicates improved therapeutic efficacy with increased concentration of bioactive constituents.

The anti-arthritic effect of the extract may be attributed to the synergistic action of flavonoids, alkaloids, glycosides, diterpenes, and saponins present in the plant. These phytoconstituents are known to suppress inflammatory mediators, inhibit cyclooxygenase and lipoxygenase pathways, stabilize lysosomal membranes, and reduce oxidative stress associated with arthritis. The antioxidant properties of flavonoids may also protect synovial tissues from free radical-mediated damage, thereby reducing progression of inflammatory arthritis.

Although the activity of the extract was slightly lower than that of aspirin, the ethanolic extract showed considerable anti-arthritic potential with significant inhibition of paw edema and inflammatory progression. The findings suggest that *Ficus glomerata* possesses promising therapeutic activity against inflammatory arthritic conditions and may serve as a potential natural alternative for the management of arthritis with fewer side effects compared to synthetic drugs.

Table 1: Extractive values of *Ficus glomerata*

S. No.	Extracts	% Yield* (w/w)
1.	Pet. ether	0.76
2.	Ethanolic	9.25

Table 2: Result of phytochemical screening of extract of *Ficus glomerata*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Hager's Test:	 +ve
2.	**Glycosides** Legal's Test:	 +ve
3.	**Flavonoids** Lead acetate Test: Alkaline test:	 +ve +ve
4.	**Diterpenes** Copper acetate Test:	 +ve
5.	**Phenol** Ferric Chloride Test:	 -ve
6.	**Proteins** Xanthoproteic Test:	 -ve
7.	**Carbohydrate** Fehling's Test:	 +ve
8.	**Saponins** Froth Test:	 +ve
9.	**Tannins** Gelatin test:	 -ve

[+ve= Present, -ve=Negative]

Table 3: Estimation of total alkaloids and flavonoids content of *Ficus glomerata*

S. No.	Extract	Total flavonoids content	Total alkaloids content
1.	Ethanolic	0.74 mg/ 100 mg	0.68 mg/ 100 mg

Table 4: Anti-arthritis activity of ethanolic extract of *Ficus glomerata* against Freund's adjuvant induced arthritis in rats

		Paw volume (mL)			
Group	**Treatment**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	2% Gum acacia	0.26 ± 0.58	0.24 ± 0.48	0.26 ± 0.46	0.24 ± 0.38
Group II	Arthritis control	0.78 ± 0.25	0.88 ± 0.52	0.92 ± 0.28	0.95 ± 0.32
Group III	Aspirin (200 mg/kg p.o)	0.60 ± 0.12	0.53 ± 0.17**	0.50 ± 0.28***	0.33 ± 0.30***
Group IV	Ethanolic extract of *Ficus glomerata* (100 mg/kg p.o)	0.72 ± 0.22	0.66 ± 0.12*	0.62 ± 0.22*	0.28 ± 0.22*
Group V	Ethanolic extract of *Ficus glomerata* (200 mg/kg p.o)	0.67 ± 0.30**	0.60 ± 0.20**	0.56 ± 0.23***	0.48 ± 0.40***

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

CONCLUSION

In conclusion, the ethanolic extract of *Ficus glomerata* demonstrated significant phytochemical richness and notable anti-arthritic activity in Freund's adjuvant-induced arthritic rats. The observed pharmacological activity may be associated with the presence of flavonoids, alkaloids, glycosides, and other secondary metabolites. Further studies involving isolation and characterization of active constituents, as well as detailed mechanistic and clinical investigations, are necessary to establish its efficacy and safety as a potential herbal anti-arthritic agent.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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