INNOVARE JOURNAL OF LIFE SCIENCE

Vol 14, 2026

ISSN - 2321-550X

Research Article

STUDY OF ANTI-ARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *EUGENIA HEYNEANA*

KM. SHIVANI GUPTA*, VIVEK THAKUR, B. K. DUBEY, SUNIL SHAH, DEEPAK BASEDIA

Department of Pharmacy, TIT-College of Pharmacy, Bhopal, Madhya Pradesh, India. Email: sg87066@gmail.com

Received: 10 October 2025, Revised and Accepted: 25 November 2025

ABSTRACT

Objectives: The present study aimed to evaluate the anti-arthritic activity of the hydroalcoholic extract of *Eugenia heyneana* using the Freund's adjuvant-induced arthritis model in rats. In addition, the study assessed the phytochemical profile, total phenolic content (TPC), and total flavonoid content (TFC) of the extract to understand its possible role in mediating anti-inflammatory effects.

Materials and Methods: Preliminary phytochemical screening was performed to identify major secondary metabolites. TPC and TFC were estimated as mg gallic acid equivalents (GAE)/100 mg and mg quercetin equivalents (QE)/100 mg, respectively. Arthritis was induced in Wistar rats using Freund's complete adjuvant (FCA). The animals were divided into groups and treated orally with Aspirin (200 mg/kg) as the standard, and *E. heyneana* extract at doses of 100 mg/kg and 200 mg/kg. Paw volume was measured at regular intervals to assess anti-arthritic efficacy.

Results: Leaves of *E. heyneana* were subjected to hydroalcoholic extraction, yielding 7.16% (w/w). Phytochemical analysis revealed the presence of flavonoids, phenols, carbohydrates, proteins, diterpenes, and saponins. The TPC and TFC were found to be 0.31 mg GAE/100 mg and 0.97 mg QE/100 mg, respectively. The FCA control group showed a significant increase in paw volume, confirming the induction of arthritis. Treatment with *E. heyneana* extract produced a dose-dependent reduction in paw edema, with the 200 mg/kg dose showing significant activity comparable to Aspirin ($p<0.001$).

Conclusion: The hydroalcoholic extract of *E. heyneana* exhibited significant anti-arthritic activity in FCA-induced arthritic rats. The therapeutic potential may be attributed to the synergistic effects of its phytoconstituents, particularly flavonoids and phenolic compounds, which possess known anti-inflammatory and antioxidant properties. These findings support the traditional use of *E. heyneana* in managing inflammatory joint disorders and highlight its potential for further pharmacological development.

Keywords: *Eugenia heyneana,* Anti-arthritic activity, Freund's adjuvant, Flavonoids, Phenolic compounds, Inflammation, Phytochemical screening.

 DOI: http://dx.doi.org/10.22159/ijls.2026v14.57493. Journal homepage: https://innovareacademics.in/journals/index.php/ijls

INTRODUCTION

Arthritis, particularly inflammatory forms such as rheumatoid arthritis, is a chronic, immune-mediated disorder characterized by synovial inflammation, cartilage destruction, and progressive joint dysfunction [1]. It remains a major cause of pain, disability, and reduced quality of life worldwide. Current pharmacotherapies such as non-steroidal anti-inflammatory drugs, corticosteroids, and disease-modifying antirheumatic drugs are effective but often associated with adverse effects, incomplete efficacy, and high cost, thereby limiting long-term use. This limitation has driven increasing interest in plant-derived therapeutics possessing anti-inflammatory, antioxidant, and immunomodulatory properties as safer or adjunctive options for managing arthritic disorders [2].

The genus *Eugenia* (family Myrtaceae) is large and chemically diverse. Many *Eugenia* and allied *Syzygium* species are used in traditional medicine and have demonstrated antioxidant, antimicrobial, and anti-inflammatory activities in various experimental studies. Previous reviews highlight the recurring presence of phenolics, flavonoids, tannins, and essential-oil components in *Eugenia* species, which plausibly mediate these biological effects and support the genus as a promising source of anti-arthritic leads [3].

Several species of *Eugenia* and closely related *Syzygium* have shown *in vitro* and *in vivo* anti-inflammatory effects, supporting their pharmacological potential [4]. *Eugenia heyneana* Duthie (sometimes synonymized with *Syzygium salicifolium* or *Syzygium heyneanum*) is a small tree or shrub native to parts of India. Ethnobotanical records report its traditional use for medicinal purposes, particularly in the treatment of inflammatory and infectious diseases. However, peer-reviewed pharmacological and phytochemical data specific to *E. heyneana* remain limited.

Given the ethnomedicinal relevance of the plant and the documented anti-inflammatory potential across the *Eugenia/Syzygium* group, scientific exploration of the hydroalcoholic extract of *E. heyneana* for anti-arthritic activity is both rational and timely. Demonstrating *in vivo* efficacy and identifying the active phytoconstituents and mechanisms, such as inhibition of pro-inflammatory mediators or antioxidant activity, would help validate traditional claims and provide a basis for further phytochemical isolation, mechanistic evaluation, and preclinical development.

The aim of the present study was to prepare a hydroalcoholic extract of *E. heyneana* and to evaluate its *in vivo* anti-arthritic efficacy using an established Freund's complete adjuvant-induced arthritis model in rats, along with preliminary phytochemical screening and assessment of extract yield and safety profile.

MATERIALS AND METHODS

Materials

The materials used in this study included various analytical-grade chemicals and reagents obtained from reputed suppliers. Ferric chloride, picric acid, and potassium mercuric iodide were procured

from Thomas Baker, Mumbai, while sodium hydroxide, lead acetate, sodium nitroprusside, and Folin–Ciocalteu reagent were obtained from Loba Chemie Pvt. Ltd., Mumbai. Pyridine, gelatin, potassium bismuth iodide, nitric acid, copper acetate, and sodium chloride were supplied by S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform were purchased from Qualigens Fine Chemicals, Mumbai, and Fehling's solution was obtained from Central Drug House Ltd., New Delhi. All chemicals used were of analytical grade and used as received without further purification.

Methods

Selection and collection of plant material

Ethnobotanical surveys were conducted in different tribal localities of Madhya Pradesh. The method adopted for the collection of data was interview with tribals, local medicine men, and one-to-one discussion about therapeutic use of local plants in the treatment of various diseases. The present work carried out on plant species *E. heyneana*. Leaves of *E. heyneana* were collected from rural area of Bhopal (M.P), in the month of February 2025. The leaves of *E. heyneana* were authenticated by J. Mehta, Career College (Bhopal).

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 3–4 weeks. Dried plant materials were grinded using electronic grinder. Dried plant material was packed in air tight container till any further use.

Extraction by maceration process

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade-dried and powdered herbs [5]. 50 g dried powdered leaves of *E. heyneana* have been extracted with ethanol solvent using maceration process for 48 h, filtered, and dried using vaccum evaporator at 40°C.

Determination of percentage yield

The extraction yield is evaluate of the solvent's efficiency to extracts bioactive components from the selected natural plant samples, and it was defined as the quantity of plant extracts recovered in mass after solvent extraction compared with the initial quantity of plant samples. After extraction, the yield of the plant extracts obtained was calculated in grams and then converted it into a percentage. The percentage yield of extract was calculated by using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}} \times 100$$

Phytochemical Screening

Phytochemical screening serves as a foundation for pharmacological investigations, standardization of herbal formulations, and discovery of new lead compounds for drug development. The chemical tests were performed for testing different chemical groups present in extracts [6].

Estimation of total phenolic content (TPC)

The TPC of the extract was determined by the modified Folin-Ciocalteu method [7]. 10 mg Gallic acid was dissolved in 10 mL methanol, and various aliquots of 5–25 µg/mL were prepared in methanol. 10 mg of dried extract of plant material was extracted with 10 mL methanol and filter. 2 mL (1 mg/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 15 min at 40°C for color development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content (TFC)

Determination of TFC was based on aluminum chloride method [8]. 10 mg quercetin was dissolved in 10 mL methanol, and various aliquots of 5–25 µg/mL were prepared in methanol. 10 mg of dried extract of plant material was extracted with 10 mL methanol and filter. 3 mL (1 mg/mL) of this extract was for the estimation of flavonoid. 1 mL of 2% $AlCl_3$ 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.

***In vivo* anti-arthritis activity of *E. heyneana* 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 h and 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) (Approval No. TIT/IAEC/2025/86), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

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 Organization for Economic Co-operation and Development [9]. Hydroalcoholic extract of *E. heyneana* (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.

Table 1: % Yield of hydroalcoholic extract of *Eugenia heyneana*

S. No.	Extract	Weight of extract	% Yield (w/w)
1.	Hydroalcoholic	3.58	7.16

Table 2: Phytochemical screening of hydroalcoholic extract of *Eugenia heyneana*

S. No.	Phytochemical test	Tests	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

Table 3: Total phenolic and total flavonoid content of *Eugenia heyneana*

S. No.	Extract	Total phenol (GAE) (mg/100 mg)	Total flavonoid (QE) (mg/100 mg)
1.	Hydroalcoholic extract	0.31	0.97

GAE: Gallic acid equivalents, QE: Quercetin equivalents

Table 4: Anti-arthritis activity of hydroalcoholic extract of *Eugenia heyneana* against Freund's adjuvant induced arthritis in rats

Group	Treatment	Day 7	Day 14	Day 21	Day 28
Group I	2% Gum acacia	0.28±0.50	0.27±0.45	0.27±0.40	0.26±0.30
Group II	Arthritis control	0.78±0.18	0.87±0.22	0.95±0.25	0.97±0.30
Group III	Aspirin (200 mg/kg, p.o.)	0.64±0.12	0.56±0.14**	0.52±0.22***	0.38±0.28***
Group IV	Hydroalcoholic extract of *Eugenia heyneana* (100 mg/kg, p.o.)	0.72±0.14	0.63±0.13*	0.60±0.18*	0.53±0.12*
Group V	Hydroalcoholic extract of *Eugenia heyneana* (200 mg/kg, p.o.)	0.69±0.20**	0.61±0.15**	0.53±0.20***	0.40±0.12***

Values expressed as mean±standard error of the mean (n=6) *p<0.05, **p<0.01, ***p<0.001 as compared to arthritis control

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 0.1 mL of complete Freund's adjuvant (10 mg of heat-killed mycobacterium tuberculosis per mL of paraffin oil) into the plantar surface of the left hind paw [10].

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 hydroalcoholic extract of *E. heyneana* of doses of 100 mg/kg p.o.

Group V received extract of hydroalcoholic extract of *E. heyneana* of doses of 200 mg/kg p.o.

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

Statistical analysis

The values were expressed as mean±standard error of the mean (n=6) [11]. The statistical significance was assessed using one-way analysis of variance, followed by Tukey's test and p<0.05, p<0.01, and p<0.001 were considered to be statistically significant.

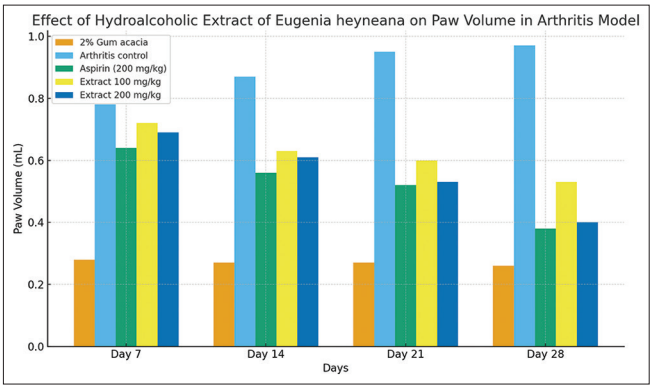

Fig. 1: Anti-arthritis activity of hydroalcoholic extract of *Eugenia heyneana* against Freund's adjuvant-induced arthritis in rats

DISCUSSION

The present study evaluated the anti-arthritic potential of the hydroalcoholic extract of *E. heyneana* using the Freund's adjuvant-induced arthritis model in rats. The percentage yield of the hydroalcoholic extract was found to be 7.16% (Table 1), indicating an efficient extraction process. Phytochemical screening (Table 2) revealed the presence of flavonoids, phenols, proteins, carbohydrates, saponins, and diterpenes, which are known to exhibit anti-inflammatory and antioxidant properties. The total phenolic (0.31 mg gallic acid equivalents [GAE]/100 mg) and flavonoid (0.97 mg quercetin equivalents [QE]/100 mg) contents (Table 3) further support the extract's potential antioxidant activity, suggesting its capacity to neutralize free radicals and mitigate oxidative stress associated with arthritis.

The Freund's adjuvant-induced arthritis model mimics the pathophysiological features of human rheumatoid arthritis, characterized by joint inflammation, edema, and tissue damage. In the present investigation, the arthritic control group exhibited a continuous increase in paw volume from day 7 to day 28, indicating progressive inflammation. Treatment with Aspirin (200 mg/kg, p.o.), used as a standard reference, significantly reduced paw edema (p<0.001), validating the sensitivity of the model.

Notably, the groups treated with the hydroalcoholic extract of *E. heyneana* at 100 mg/kg and 200 mg/kg doses demonstrated a dose-dependent reduction in paw volume compared to the arthritic control (Table 4, Fig. 1). The higher dose (200 mg/kg) showed a more pronounced anti-arthritic effect, nearly comparable to that of Aspirin by the 28th day (p<0.001). This suggests that the extract effectively alleviated inflammation and joint swelling, possibly through inhibition of pro-inflammatory mediators such as prostaglandins, cytokines, and reactive oxygen species.

The observed pharmacological effects can be attributed to the bioactive phytoconstituents detected in the extract. Flavonoids and phenolic compounds are known to inhibit key enzymes involved in the inflammatory cascade, including cyclooxygenase and lipoxygenase, thereby reducing prostaglandin synthesis. Furthermore, their antioxidant properties help prevent oxidative damage to joint tissues, which is a major contributor to arthritis progression.

The hydroalcoholic extract of *E. heyneana* demonstrated significant anti-arthritic activity in experimental rats, supporting its traditional use in inflammatory disorders. The activity may be mediated through both anti-inflammatory and antioxidant mechanisms, primarily due to the presence of flavonoids, phenols, and diterpenes.

CONCLUSION

The present investigation demonstrated that the hydroalcoholic extract of *E. heyneana* exhibits significant anti-arthritic activity in rats induced with Freund's complete adjuvant arthritis**.** The extract showed a dose-dependent reduction in paw edema and joint inflammation**,** with the 200 mg/kg dose producing effects comparable to the standard drug Aspirin (200 mg/kg, p.o.)**.** The phytochemical analysis confirmed the presence of flavonoids, phenols, saponins, diterpenes, and carbohydrates**,** which are known to possess anti-inflammatory and antioxidant properties**.** The total phenolic (0.31 mg GAE/100 mg) and flavonoid (0.97 mg QE/100 mg) content further support the extract's potential to modulate oxidative stress and inflammatory responses. These findings suggest that the anti-arthritic effect of *E. heyneana* may be mediated through the inhibition of pro-inflammatory mediators and antioxidant defense mechanisms**.** Thus, the plant possesses therapeutic potential in the management of rheumatoid arthritis and related inflammatory disorders**.** However, further studies involving isolation of active constituents**,** mechanistic evaluation**,** and clinical validation are

warranted to confirm its efficacy and establish its pharmacological and toxicological profile for future therapeutic development.

ACKNOWLEDGMENT

I express my deep sense of gratitude to TIT-College of Pharmacy, Bhopal (M.P.), and the Technocrats Institute of Technology–Pharmacy, Bhopal (M.P.), for providing the necessary facilities, guidance, and support required to carry out this research work.

AUTHORS' CONTRIBUTIONS

All the authors have equally contributed to the manuscript.

CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

FUNDING

Nil.

REFERENCES

1. Jahid M, Khan KU, Ul-Haq R, Ahmed RS. Overview of rheumatoid arthritis and scientific understanding of the disease. Mediterr J Rheumatol 2023;34:284-91.
2. Kciuk M, Garg A, Rohilla M, Chaudhary R, Dhankhar S, Dhiman S, *et al*. Therapeutic potential of plant-derived compounds and plant extracts in rheumatoid arthritis: Comprehensive review. Antioxidants (Basel) 2024;13:775.
3. El-Saber Batiha G, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. *Syzygium aromaticum* L. (Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules 2020;10:202.
4. Amir Rawa MS, Mazlan MK, Ahmad R, Nogawa T, Wahab HA. Roles of *Syzygium* in anti-cholinesterase, anti-diabetic, anti-inflammatory, and antioxidant: From Alzheimer's perspective. Plants (Basel) 2022;11:1476.
5. Mukherjee PK. Quality control of herbal drugs. 2nd ed. New Delhi: Business Horizons; 2007. p. 2-14.
6. Kokate CK. Practical pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994. p. 112-20.
7. Parkhe G, Bharti D. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of *Vitex trifolia* Linn. J Drug Deliv Ther 2019;9:705-7.
8. Mishra AG, Singh R, Patil N, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superb* seed extract. Asian J Pharm Educ Res 2017;6:12-7.
9. Gothe SR, Pawade UV, Nikam AV, Anjankar MP. OECD guidelines for acute oral toxicity studies: An overview. Int J Res Ayurveda Pharm 2023;14:137-40.
10. Rajaram C, Ravindra Reddy K, Bonnth Chandra Sekhar K. Evaluation of antiarthritic activity of *Caesalpinia pulcherrima* in Freund's complete adjuvant induced arthritic rat model. J Young Pharm 2015;7:128-32.
11. Sanmuga Priya E, Senthamil Selvan P, Venkataraman S. Evaluation of antiarthritic activity of *Strychnos potatorum* Linn seeds in Freund's adjuvant induced arthritic rat model. BMC Complement Altern Med 2010;10:56.