RESEARCH PAPER

Comparative Evaluation of the Anti-Arthritic and Antioxidant Potential of Different Solvent Extracts of *Hardwickia binata* Roxb. in a Freund's Complete Adjuvant-Induced Arthritis Model

Rifat Faiyaz[1*], Dr. Mehta Parulben D.[1], Dr. Rita Mourya[2], Sarita Ahirwar[2]
[1]SAM College of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University, Raisen, Madhya Pradesh 464551, India
[2]School of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University, Raisen, Madhya Pradesh 464551, India
***Corresponding Author:** Rifat Faiyaz, SAM College of Pharmacy, Faculty of Medical and Paramedical Sciences, SAM Global University, Raisen, Madhya Pradesh 464551, India
Email: yourrifat2000@gmail.com
Received: 25th October 2026; **Revised:** 28th October 2026; **Accepted:** 3rd November 2026; **Available Online:** 7th November 2026

ABSTRACT

Background:
Arthritis is a chronic, progressive inflammatory disorder of the joints whose long-term pharmacotherapy is limited by the adverse effects of synthetic agents, prompting the search for safer plant-derived alternatives. *Hardwickia binata* Roxb. (Fabaceae) is a traditionally used medicinal tree, but its anti-arthritic potential has not been systematically compared across solvents of differing polarity.

Objective:
To prepare petroleum ether, methanolic and aqueous extracts of *H. binata* and comparatively evaluate their phytochemical composition, antioxidant capacity, acute oral safety and anti-arthritic efficacy.

Methods:
Successive Soxhlet extraction was followed by qualitative and quantitative phytochemical screening, determination of total phenolic (TPC) and flavonoid (TFC) content, DPPH radical-scavenging assay, acute oral toxicity testing (OECD 423) and evaluation of anti-arthritic activity in Freund's complete adjuvant (FCA)-induced arthritic Wistar rats (paw volume, body weight and haematological indices), with diclofenac sodium as the reference standard.

Results:
The methanolic extract gave the highest yield (6.677%), TPC (60.26 mg GAE/g) and TFC (31.69 mg RE/g). The aqueous and methanolic extracts showed the strongest DPPH activity (IC50 41.27 and 43.75 µg/mL, respectively) relative to ascorbic acid (16.71 µg/mL). The extracts were safe up to 2000 mg/kg. In the FCA model, the methanolic extract produced the greatest reduction in paw oedema (~32% at day 28), best preserved body weight and most effectively restored altered haematological parameters, comparable to diclofenac sodium.

Conclusion:
The methanolic extract of *H. binata* possesses the most pronounced antioxidant and anti-arthritic activity, likely attributable to its high phenolic and flavonoid content, supporting its traditional use and its potential as a natural anti-arthritic agent.

Keywords: *Hardwickia binata*; anti-arthritic activity; Freund's complete adjuvant; DPPH antioxidant assay; total phenolic content; flavonoids; diclofenac sodium

How to cite this article: *Rifat Faiyaz1* Dr. Mehta Parulben D.1 Dr. Rita Mourya2 Sarita Ahirwar2. Comparative Evaluation of the Anti-Arthritic and Antioxidant Potential of Different Solvent Extracts of Hardwickia binata Roxb. in a Freund's Complete Adjuvant-Induced Arthritis Model. Int J Drug Deliv Technol. 2026;16(90s):1070-1076. DOI: 10.25258/ijddt.16.90s.105.*

RESEARCH PAPER

1. Introduction

Arthritis, derived from the Greek term for "disease of the joints", is an acute or chronic inflammation of one or more joints that frequently co-exists with pain, stiffness and progressive structural damage. More than one hundred distinct forms have been described, of which osteoarthritis (a degenerative, largely non-inflammatory disorder) and rheumatoid arthritis (RA, a systemic autoimmune inflammatory disease) are the most prevalent (Ma et al., 2009). RA alone affects approximately 0.5–1% of adults worldwide, with a female-to-male preponderance of about 3:1 and a prevalence near 0.75% in India (Gabriel et al., 2003).

The pathophysiology of inflammatory arthritis is driven by the aberrant production of pro-inflammatory mediators—including tumour necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6, IL-17), interferon-gamma and reactive oxygen species—that promote synovial hyperplasia, cartilage degradation and bone erosion. Reactive oxygen species and oxidative stress are increasingly recognised as amplifiers of this cascade, which provides a mechanistic rationale for evaluating antioxidant-rich botanicals as adjuncts or alternatives to conventional therapy (Conforti et al., 2021).

The mainstays of current management—non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs)—provide symptomatic relief but are associated with gastrointestinal, renal, hepatic and cardiovascular toxicity on prolonged use (Szeto et al., 2020). Consequently, medicinal plants, which are widely accessible and generally better tolerated, continue to be explored as sources of novel anti-arthritic leads. The World Health Organization estimates that close to 80% of people in developing nations rely on traditional and complementary medicines for primary health care (Silva et al., 2010).

Hardwickia binata Roxb. (family Fabaceae; subfamily Detarioideae), locally known as "Anjan", is a moderate-to-large deciduous tree distributed across the dry forests of central and southern India. Traditionally, its bark, resin and leaves have been used for diarrhoea, dysentery, leucorrhoea, chronic cystitis and various inflammatory conditions (Khare, 2008). Recent phytochemical work has demonstrated appreciable phenolic and flavonoid content and antioxidant, antimicrobial and cytotoxic activities in leaf extracts (Shingade & Kakde, 2025; Manimegalai et al., 2023). Despite this promising profile, a systematic, side-by-side comparison of the anti-arthritic potential of *H. binata* extracts obtained with solvents of graded polarity has not been reported.

The present study was therefore designed to prepare petroleum ether, methanolic and aqueous extracts of *H. binata* by successive Soxhlet extraction and to comparatively evaluate their phytochemical composition, total phenolic and flavonoid content, DPPH radical-scavenging capacity, acute oral safety and in vivo anti-arthritic efficacy in a Freund's complete adjuvant (FCA)-induced arthritis model, using diclofenac sodium as the reference standard. The overarching aim was to identify the most effective solvent extract and to correlate its activity with its phytochemical richness.

2. Materials and Methods

2.1 Chemicals and reagents

Petroleum ether, methanol, ethanol and analytical-grade solvents were procured from Merck Life Science Pvt. Ltd. and Rankem (Avantor), India. Freund's complete adjuvant (FCA), Drabkin's reagent, reduced glutathione and phosphate-buffered saline were obtained from HiMedia Laboratories Pvt. Ltd. Diclofenac sodium was sourced from Sigma-Aldrich (Merck), and Folin–Ciocalteu reagent, gallic acid, rutin and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were of analytical grade. Haematological estimations were performed using commercial Erba diagnostic kits. All reagents were of analytical grade.

2.2 Collection and authentication of plant material

Fresh plant material of *Hardwickia binata* Roxb. was collected from the local areas of Bhopal, Madhya Pradesh, India, and authenticated by a qualified botanist. The material was washed to remove surface debris, shade-dried at room temperature for three days to preserve the phytoconstituents, and then oven-dried at 45 °C to constant weight. The dried material was pulverised in a mechanical grinder, passed through a No. 40 mesh sieve and stored in air-tight containers until extraction.

2.3 Extraction and determination of percentage yield

The coarse powder was first defatted with petroleum ether and then subjected to successive hot Soxhlet extraction with methanol and water. Each extract was concentrated under reduced pressure in a rotary evaporator and the dried crude extracts were preserved at 4–8 °C in air-tight glass containers (Choudhary et al., 2014). The percentage yield of each extract was calculated on a dry-weight basis as: percentage yield (%) = (weight of dried extract / weight of dried powder taken) × 100.

2.4 Qualitative phytochemical screening

The three extracts were screened for the major classes of secondary metabolites—alkaloids (Dragendorff's, Mayer's and Wagner's tests), glycosides (Borntrager's and Keller–Killiani tests), carbohydrates (Molisch's and Benedict's tests), proteins and amino acids (Biuret and Ninhydrin tests), flavonoids (alkaline reagent and lead acetate tests), tannins and phenolics (ferric chloride test), saponins (foam test) and steroids/triterpenoids (Salkowski's test)—using standard procedures (Gupta et al., 2013).

2.5 Total phenolic content (TPC)

TPC was estimated by the Folin–Ciocalteu method using gallic acid as the standard. Briefly,

0.5 mL of sample was mixed with 2 mL of Folin–Ciocalteu reagent (1:10 in deionised water) and 4 mL of sodium carbonate solution, incubated for 30 min at room temperature, and the absorbance measured at 765 nm. Results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g) (Rani et al., 2021).

2.6 Total flavonoid content (TFC)

TFC was determined by the aluminium chloride colorimetric method with rutin as the reference. A 0.5 mL sample was combined with 2 mL distilled water and 0.15 mL of 5% $NaNO_2$; after 6 min, 0.15 mL of 10% $AlCl_3$ was added, followed by 2 mL of 4% NaOH, and the volume adjusted to 5 mL. After 15 min, absorbance was recorded at 510 nm and results expressed as mg rutin equivalents per gram of extract (mg RE/g) (Gadekar et al., 2020).

2.7 DPPH radical-scavenging assay

The in vitro free-radical-scavenging capacity was measured using the DPPH assay. Test samples (10–100 µg/mL) were mixed with a 0.1 mM methanolic DPPH solution (2 mL DPPH + 1 mL sample) and incubated in the dark for 10 min; absorbance was read at 515 nm against a blank, with ascorbic acid as the positive control. The percentage inhibition was calculated as $[(A_{control} - A_{sample}) / A_{control}] \times 100$, and IC_{50} values were derived from the linear regression of percentage inhibition against concentration (Varandani et al., 2020).

2.8 Experimental animals and ethical approval

Healthy adult Wistar rats (250 ± 60 g) were used and maintained under standard laboratory conditions (temperature, humidity and 12 h light/dark cycle) with free access to a standard pellet diet and water. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with the CPCSEA guidelines for the care and use of laboratory animals.

2.9 Acute oral toxicity study (OECD 423)

Acute oral toxicity of the methanolic extract was evaluated according to OECD guideline 423 (Acute Toxic Class method). Animals (n = 3 per group) received the extract orally at 5, 50, 300 and 2000 mg/kg body weight, while the control group received distilled water. Animals were observed continuously for the first 4 h and then daily for 14 days for behavioural, autonomic, neurological and physiological changes, morbidity and mortality (Mouffouk et al., 2018).

2.10 FCA-induced arthritis and experimental design

Arthritis was induced by a single intraplantar injection of 0.1 mL FCA (each mL containing 1 mg heat-killed *Mycobacterium tuberculosis*, 0.85 mL paraffin oil and 0.15 mL mannide monooleate) into the left hind paw (Perumal et al., 2017). Animals were randomly allocated to six groups (Table 1). Extracts (200 mg/kg) and diclofenac sodium (10 mg/kg) were administered orally once daily. Paw volume and body weight were recorded on days 0, 7, 14, 21 and 28. The percentage inhibition of paw oedema was calculated as $(V - V) / V \times 100$, where V and V are the mean paw volumes of the arthritic control and treated groups, respectively.

Table 1. Experimental design and treatment allocation for the FCA-induced arthritis study.

Group	Designation	Treatment
I	Normal control	Normal saline; no arthritis induction
II	Arthritic control	FCA-induced arthritis; no treatment
III	Standard	FCA + diclofenac sodium (10 mg/kg, p.o.)
IV	FCA + *H. binata* methanolic extract (200 mg/kg)	Once daily, oral
V	FCA + *H. binata* aqueous extract (200 mg/kg)	Once daily, oral
VI	FCA + *H. binata* petroleum ether extract (200 mg/kg)	Once daily, oral

All treatments were administered orally once daily for 28 days; n = 6 per group.

2.11 Assessment of arthritic parameters

Body weight was recorded on days 0, 7, 14, 21 and 28 as an index of general health and disease progression; loss of body weight is characteristic of chronic inflammation. Paw oedema, the primary index of inflammation, was measured as left-hind-paw volume with a plethysmometer. At the end of the study, blood was collected for estimation of red blood cell (RBC) count, haemoglobin (Hb) concentration and total white blood cell (WBC) count using a Star 21 automated haematology analyser and Erba diagnostic kits.

2.12 Statistical analysis

Data are expressed as mean ± standard error of the mean (SEM). Differences between groups were analysed by one-way analysis of variance (ANOVA) followed by an appropriate post hoc test, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Extraction yield

The extraction yield varied markedly with solvent polarity (Table 2, Fig. 1). Methanol was the most efficient solvent (6.677%), followed by water (4.474%), while petroleum ether gave the lowest yield (1.026%). The high methanolic yield indicates that *H. binata* is rich in polar constituents such as phenolics, flavonoids and glycosides, whereas the low petroleum ether yield reflects a comparatively small fraction of non-polar lipids and waxes.

Table 2. Percentage yield of *H. binata* extracts.

Solvent	Powder taken (g)	Extract (g)	Yield (%)
Petroleum ether	250	2.567	1.026
Methanol	223	14.89	6.677
Aqueous	236	10.56	4.474

Fig. 1. Percentage extraction yield of the petroleum ether, methanolic and aqueous extracts of *H. binata*.

3.2 Qualitative phytochemical screening

The methanolic and aqueous extracts displayed a rich phytochemical profile, testing positive for alkaloids, glycosides, carbohydrates, proteins/amino acids, flavonoids, tannins/phenolics and saponins (Table 3). Steroids and triterpenoids were detected in the methanolic and petroleum ether extracts but not the aqueous extract, consistent with their moderate polarity. The petroleum ether extract contained only steroids/triterpenoids, in line with its non-polar character.

Table 3. Qualitative phytochemical screening of *H. binata* extracts (+ = present; − = absent).

Phytoconstituent (test)	Pet. ether	Methanol	Aqueous
Alkaloids (Dragendorff/Mayer/Wagner)	–	+	+
Glycosides (Borntrager/Keller–Killiani)	–	+	+
Carbohydrates (Molisch/Benedict)	–	+	+
Proteins & amino acids (Biuret/Ninhydrin)	–	+	+
Flavonoids (alkaline/lead acetate)	–	+	+
Tannins & phenolics (ferric chloride)	–	+	+
Saponins (foam test)	–	+	+
Steroids & triterpenoids (Salkowski)	+	+	–

3.3 Total phenolic and flavonoid content

Quantitative estimation was performed against gallic acid (TPC; $y = 0.0015x + 0.1506$, $R^2 = 0.9893$) and rutin (TFC; $y = 0.0073x + 0.0036$, $R^2 = 0.9987$) standard curves (Fig. 2). The methanolic extract contained the highest TPC (60.26 mg GAE/g) and TFC (31.69 mg RE/g), followed by the aqueous extract (45.6 mg GAE/g; 26.63 mg RE/g), with the petroleum ether extract lowest (19.75 mg GAE/g; 22.38 mg RE/g) (Table 4, Fig. 3). Polar solvents were thus clearly superior for recovering these bioactive constituents.

Fig. 2. Calibration curves for (a) gallic acid at 765 nm and (b) rutin at 510 nm.

Table 4. Total phenolic (TPC) and total flavonoid (TFC) content of *H. binata* extracts.

Extract	TPC (mg GAE/g)	TFC (mg RE/g)
Methanolic	60.26	31.69
Aqueous	45.60	26.63
Petroleum ether	19.75	22.38

Fig. 3. Comparison of total phenolic and total flavonoid content across the three extracts.

3.4 DPPH radical-scavenging (antioxidant) activity

All extracts showed a concentration-dependent increase in DPPH radical-scavenging activity (Table 5, Fig. 4). Ascorbic acid was the most potent antioxidant (IC_{50} 16.71 µg/mL). Among the extracts, the aqueous extract was the most active (IC_{50} 41.27 µg/mL), closely followed by the methanolic extract (43.75 µg/mL), whereas the petroleum ether extract was the weakest (61.10 µg/mL). The superior activity of the polar extracts mirrors their higher phenolic and flavonoid content.

Table 5. DPPH radical-scavenging activity (% inhibition) and IC50 values.

Conc. (µg/mL)	Ascorbic acid	Methanolic	Aqueous	Pet. ether
10 / 20*	44.01	40.68	50.80	37.60
20 / 40*	53.37	49.35	58.95	42.74
30 / 60*	61.03	55.89	65.90	48.29
40 / 80*	67.77	63.34	73.44	54.94
50 / 100*	76.33	71.30	81.39	64.62
IC_{50} (µg/mL)	16.71	43.75	41.27	61.10

**Ascorbic acid was tested at 10–50 µg/mL; the extracts were tested at 20–100 µg/mL. Values are % inhibition.*

Fig. 4. Concentration-dependent DPPH radical-scavenging activity of ascorbic acid and the three *H. binata* extracts (dashed line = 50% inhibition).

3.5 Acute oral toxicity

No mortality occurred at any dose up to 2000 mg/kg over the 14-day observation period (Table 6). Animals were essentially normal at 5 and 50 mg/kg, with only mild salivation and lethargy at 50 mg/kg. Mild, non-fatal signs (transient tremors, convulsions, diarrhoea and lethargy) were noted at 300 and 2000 mg/kg. The extract therefore has a wide margin of safety, and 200 mg/kg was selected as a safe pharmacological dose.

Table 6. Acute oral toxicity observations for the methanolic extract (OECD 423). N = normal; L = mild change.

Parameter	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Behaviour	N	N	N	L
Salivation	N	L	L	L
Lethargy	N	L	L	L
Convulsions / tremors	N	N	L	L
Diarrhoea	N	N	L	L
Morbidity	N	N	N	L
Mortality	N	N	N	N

3.6 Effect on body weight

The normal control group showed a steady gain in body weight, while the arthritic control showed irregular fluctuations consistent with disease-associated cachexia (Table 7, Fig. 5). Diclofenac and the extracts helped maintain body weight; the methanolic extract produced the most consistent improvement, reflecting protection against the systemic wasting effects of chronic inflammation.

Table 7. Body weight (g; mean ± SEM) over the 28-day study.

Group	Day 0	Day 7	Day 14	Day 21	Day 28
I – Normal control	240.13	249.24	243.20	245.98	248.99

II – Arthritic control	253.88	256.79	259.67	255.88	265.15
III – Diclofenac 10 mg/kg	245.93	251.10	248.20	249.87	251.89
IV – Methanolic 200 mg/kg	251.91	255.01	249.98	253.87	255.98
V – Aqueous 200 mg/kg	256.90	261.99	254.66	256.02	259.08
VI – Pet. ether 200 mg/kg	261.91	264.01	258.98	259.87	261.98

Fig. 5. Effect of *H. binata* extracts and diclofenac on body weight over 28 days.

3.7 Effect on paw volume

The arthritic control showed a progressive rise in paw volume (4.09 → 5.48 mL) confirming successful induction, whereas all treated groups showed regression of oedema (Table 8, Fig. 6). At day 28 the methanolic extract gave the greatest reduction in paw volume (3.72 mL; ~32% inhibition relative to the arthritic control), comparable to diclofenac (3.87 mL). The aqueous extract (3.88 mL) was moderately effective and the petroleum ether extract (4.01 mL) least effective, giving the activity rank methanolic ≥ diclofenac > aqueous > petroleum ether.

Table 8. Paw volume (mL; mean ± SEM) over the 28-day study.

Group	Day 0	Day 7	Day 14	Day 21	Day 28
I – Normal control	3.81	3.82	3.83	3.84	3.83
II – Arthritic control	4.09	4.46	4.88	5.16	5.48
III – Diclofenac 10 mg/kg	4.11	4.02	3.86	3.89	3.87
IV – Methanolic 200 mg/kg	4.13	4.28	4.10	3.91	3.72
V – Aqueous 200 mg/kg	4.15	4.37	4.24	4.05	3.88
VI – Pet. ether 200 mg/kg	4.17	4.49	4.39	4.18	4.01

Fig. 6. Effect of *H. binata* extracts and diclofenac on paw volume in FCA-induced arthritic rats.

3.8 Effect on haematological parameters

FCA-induced arthritis produced a marked fall in RBC count and haemoglobin and a rise in WBC count in the arthritic control, reflecting anaemia of chronic inflammation and immune activation (Table 9, Fig. 7). Diclofenac and the extracts attenuated these changes. The methanolic extract best restored

RBC (3.79×10^6/mm³) and reduced WBC (12.65×10^3/mm³) toward normal, with the aqueous extract intermediate and the

petroleum ether extract least effective, again supporting the superior systemic anti-inflammatory action of the polar extracts.

Table 9. Haematological parameters (mean ± SEM) at day 28.

Group	RBC ($\times 10^6$/mm³)	WBC ($\times 10^3$/mm³)	Hb (g/dL)
I – Normal control	7.96	10.38	11.55
II – Arthritic control	2.54	18.54	7.43
III – Diclofenac 10 mg/kg	6.86	11.43	12.65
IV – Methanolic 200 mg/kg	3.79	12.65	10.38
V – Aqueous 200 mg/kg	4.32	14.67	10.50
VI – Pet. ether 200 mg/kg	2.43	16.38	11.96

Fig. 7. Effect of *H. binata* extracts and diclofenac on RBC, WBC and haemoglobin at day 28.

4. Discussion

This study provides the first systematic, solvent-graded comparison of the anti-arthritic potential of *Hardwickia binata* and demonstrates a consistent hierarchy in which the methanolic extract is the most active, followed by the aqueous and then the petroleum ether extract. This gradient tracks closely with solvent polarity and with the phytochemical richness of each extract, a pattern widely reported for anti-arthritic botanicals (Akhtar et al., 2021; Hussain et al., 2021).

The highest yield, TPC and TFC were all obtained with methanol, indicating that the pharmacologically relevant constituents of *H. binata*—phenolics, flavonoids, tannins, alkaloids and saponins—are predominantly polar. Phenolic and flavonoid compounds are well established as antioxidant and anti-inflammatory agents that scavenge reactive oxygen species and down-regulate pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and COX-2 (Manan et al., 2022; Gul et al., 2023). The strong, concentration-dependent DPPH activity of the polar extracts is therefore mechanistically coherent: by neutralizing free radicals they can interrupt the oxidative amplification of joint inflammation.

In the FCA model—a widely accepted analogue of human rheumatoid arthritis (Newbould, 1963)—the arthritic control developed the expected triad of progressive paw oedema, disturbed body weight and inflammatory haemogram (falling RBC/Hb with rising WBC). Treatment with the methanolic extract reversed each of these to an extent comparable with diclofenac sodium, reducing paw volume by roughly one-third at day 28, preserving body weight and normalising the haematological profile. The parallel improvement across independent biophysical and haematological endpoints strengthens the inference of a genuine disease-modifying, rather than purely analgesic, effect and is consistent with prior reports for phenolic-rich extracts such as *Monotheca buxifolia* and *Alternanthera bettzickiana* (Akhtar et al., 2021; Manan et al., 2020).

It is noteworthy that the aqueous extract showed marginally superior DPPH activity (IC_{50} 41.27 vs 43.75 µg/mL) yet slightly weaker in vivo anti-arthritic activity than the methanolic extract. This apparent divergence likely reflects the additional steroid/triterpenoid fraction present in the methanolic (but not aqueous) extract, together with better oral bioavailability of methanol-soluble constituents—underlining that in vitro antioxidant capacity alone does not fully predict in vivo efficacy. The favourable acute oral safety profile (no mortality up to 2000 mg/kg) further supports the therapeutic potential of the extract.

Certain limitations should be acknowledged. The study did not include molecular endpoints such as cytokine quantification, histopathology or

radiography, nor did it isolate the individual active constituents; a single extract dose (200 mg/kg) was tested. Future work should incorporate dose–response evaluation, cytokine and oxidative-stress marker profiling, histological and radiological confirmation, and bioassay-guided isolation of the active phenolic/flavonoid constituents to define the precise mechanism of action.

5. Conclusion

Among the petroleum ether, methanolic and aqueous extracts of *Hardwickia binata*, the methanolic extract exhibited the greatest extraction yield, the highest phenolic and flavonoid content, strong DPPH antioxidant activity and the most pronounced anti-arthritic effect in the FCA model—comparable to diclofenac sodium—while remaining safe up to 2000 mg/kg. These findings scientifically validate the traditional use of *H. binata* in inflammatory conditions and identify its methanolic extract as a promising candidate for the development of a natural anti-arthritic agent. Further mechanistic, dose–response and constituent-isolation studies are warranted to advance it toward clinical application.

6. Declarations

Ethical approval

All animal experiments were approved by the Institutional Animal Ethics Committee and performed in accordance with CPCSEA guidelines.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

R.F. conceived and performed the experiments and drafted the manuscript; the co-author and supervisor contributed to study design, data interpretation and critical revision. All authors approved the final version.

Acknowledgements

The authors thank the Department of Pharmacology, [Institute/College Name], Bhopal, for providing the laboratory facilities required to carry out this work.

7. References

1. Akhtar, M. F., Khan, K., Saleem, A., Baig, M. M., Rasul, A., & Abdel-Daim, M. M. (2021). Chemical characterization and anti-arthritic appraisal of Monotheca buxifolia methanolic extract in Complete Freund's Adjuvant-induced arthritis in Wistar rats. Inflammopharmacology, 29(2), 393–408.

2. Choudhary, M., Kumar, V., Gupta, P. K., & Singh, S. (2014). Anti-arthritic activity of Barleria prionitis Linn. leaves in acute and chronic models in Sprague Dawley rats. Bulletin of Faculty of Pharmacy, Cairo University, 52(2), 199–209.

3. Conforti, A., Di Cola, I., Pavlych, V., Ruscitti, P., Berardicurti, O., Ursini, F., Giacomelli, R., & Cipriani, P. (2021). Beyond the joints, the extra-articular manifestations in rheumatoid arthritis. Autoimmunity Reviews, 20(2), 102735.

4. Gabriel, S. E., Crowson, C. S., Kremers, H. M., Doran, M. F., Turesson, C., O'Fallon, W. M., & Matteson, E. L. (2003). Survival in rheumatoid arthritis: a population-based analysis of trends over 40 years. Arthritis & Rheumatism, 48(1), 54–58.

5. Gadekar, S., Goyal, S., Khan, A., & Jha, M. (2020). Chemopreventive action of Sphaeranthus indicus on DMBA-induced skin papillomagenesis in mice. Journal of Advanced Scientific Research, 11(3 Suppl 7), 161–167.

6. Gul, B., Anwar, R., Saleem, M., Ahmad, M., Ullah, M. I., & Kamran, S. (2023). Attenuation of CFA-induced arthritis through regulation of inflammatory cytokines and antioxidant mechanisms by Solanum nigrum L. leaves extracts. Inflammopharmacology, 31(6), 3281–3301.

7. Gupta, M., Thakur, S., Sharma, A., & Gupta, S. (2013). Qualitative and quantitative analysis of phytochemicals and pharmacological value of some dye-yielding medicinal plants. Oriental Journal of Chemistry, 29(2), 475–481.

8. Hussain, A., Aslam, B., Muhammad, F., Faisal, M. N., Kousar, S., Mushtaq, A., & Bari, M. U. (2021). Anti-arthritic activity of Ricinus communis L. and Withania somnifera L. extracts in adjuvant-induced arthritic rats. Iranian Journal of Basic Medical Sciences, 24(7), 951–961.

9. Khare, C. P. (2008). Indian medicinal plants: an illustrated dictionary. Springer Science & Business Media.

10. Ma, L., Cranney, A., & Holroyd-Leduc, J. M. (2009). Acute monoarthritis: what is the cause of my patient's painful swollen joint? CMAJ, 180(1), 59–65.

11. Manan, M., Saleem, U., Akash, M. S., Qasim, M., Hayat, M., Raza, Z., & Ahmad, B. (2020). Antiarthritic potential of comprehensively standardized extract of Alternanthera bettzickiana: in vitro and in vivo studies. ACS Omega, 5(31), 19478–19496.

12. Manan, M., Saleem, U., Ahmad, B., Aslam, N., Anwar, A., & Zafar, A. (2022). Anti-arthritic and toxicological evaluation of ethanolic extract of Alternanthera bettzickiana in rats. Frontiers in Pharmacology, 13, 1002037.

13. Manimegalai, P., Selvam, K., Prakash, P., Kirubakaran, D., Shivakumar, M. S., & Senthil-Nathan, S. (2023). In-vitro antibacterial, antioxidant and anti-inflammatory and in-silico ADMET, molecular docking study on Hardwickia binata phytocompounds. In Silico Pharmacology, 11(1), 25.

14. Mouffouk, C., Hambaba, L., Haba, H., Mouffouk, S., Bensouici, C., Mouffouk, S., & Khadraoui, H. (2018). Acute toxicity and in vivo

anti-inflammatory effects and in vitro antioxidant and anti-arthritic potential of Scabiosa stellata. Oriental Pharmacy and Experimental Medicine, 18(4), 335–348.

15. Newbould, B. B. (1963). Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. British Journal of Pharmacology and Chemotherapy, 21(1), 127–136.

16. Perumal, S. S., Ekambaram, S. P., & Dhanam, T. (2017). In vivo antiarthritic activity of the ethanol extracts of stem bark and seeds of Calophyllum inophyllum in Freund's complete adjuvant-induced arthritis. Pharmaceutical Biology, 55(1), 1330–1336.

17. Rani, D., Kharkwal, H., Jha, M., & Rai, N. (2021). Assessment of the total flavonoid, phenol, alkaloid content and sun protection factor in Grewia abutilifolia leaf extract. Journal of Pharmaceutical Research International, 33(49A), 42–51.

18. Shingade, S. P., & Kakde, R. B. (2025). In vitro cytotoxicity evaluation of Hardwickia binata Roxb. against human breast cancer cell line MCF-7 and human cervical cancer cell line HeLa. Pharmacognosy Research, 17(2).

19. Silva, N. C., & Fernandes Júnior, A. J. (2010). Biological properties of medicinal plants: a review of their antimicrobial activity. Journal of Venomous Animals and Toxins including Tropical Diseases, 16, 402–413.

20. Szeto, C. C., Sugano, K., Wang, J. G., Fujimoto, K., Whittle, S., Modi, G. K., et al. (2020). Non-steroidal anti-inflammatory drug (NSAID) therapy in patients with comorbidities: joint APAGE/APLAR/APSDE/APSH/APSN/PoA recommendations. Gut, 69(4), 617–629.

21. Varandani, D., Sekhar, S., Trivedi, S., Jha, M., & Jain, S. (2020). Antimicrobial and hepatoprotective potential of Pterospermum acerifolium leaves extracts on Swiss albino mice. Journal of Drug Delivery & Therapeutics, 10(3), 170–174.