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Pharmacognostical and phytochemical study of *Dillenia pentagyna* Roxb. with *in- vivo* evaluation of its anti-arthritic and anti-inflammatory activities

Prasanna Habbu[a], Vaishali Maruti Todkar[a,*], Mahalaxmi Hipparagi[b], Smita Madagundi[a], Venkatrao Kulkarni[a] & Chetan Savant[a]

[a]Post-graduate department of Pharmacognosy, S R Nagar, Dharwad 580 002, Karnataka

[b]Shri Sharanbasaveshwar college of pharmacy, SSB Campus, Solapur Road, Vijaypura 586 101, Karnataka

[*]E-mail: vaishalimalabade@gmail.com

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Dillenia pentagyna Roxb. has been an integral part of traditional remedies for cancer, wounds, bone fractures, burns, and rheumatoid arthritis. This study aimed to scientifically evaluate traditional use of this plant focusing on the antioxidant and anti-arthritic potential of its hydroalcoholic bark extract (HBDP). Qualitative phytochemical investigation of the extract unveiled the presence of key secondary metabolites steroids, triterpenoids, tannins together with phenolic and polyphenolic classes of compounds. Quantitative analysis of the overall phenolic and flavonoid concentrations revealed 168.75 milligrams gallic acid equivalents per gram and 52.86 milligrams quercetin per gram, respectively. Oxidant-neutralizing capacity was demonstrated by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging (IC_{50}: 54.18 µg mL^{-1}) and hydroxyl radical inhibition (IC_{50}: 43.93 µg mL^{-1}), indicating significant free radical-neutralizing potential. No prior scientific data on the anti-rheumatic activity of *D. pentagyna* Roxb. was available. Therefore, this study aimed to correlate its effects on inflammation and changes in bone morphology in animal model of arthritis (Complete Freund's Adjuvant (CFA) induced). The extract significantly reduced paw edema and remedied functional parameters such as joint mobility and weight-bearing. It also decreased pro-inflammatory markers and prevented joint deformation. Histopathological evaluation demonstrated decreased cartilage destruction, reduced cellular infiltration, and improved bone marrow status. Additionally, the treatment restored disrupted haematological parameters like RBC count, haemoglobin levels, and WBC count. These findings suggest that *Dillenia pentagyna* Roxb. bark extract exhibits potent reducing power and anti-arthritic properties, endorsing its established medicinal usage.

Keywords: Autoimmune disease, Complete Freund's adjuvant, Cytokines, *Dillenia pentagyna*, Inflammation, Rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory condition characterized by inflammation of synovial joints, progressively leading to cartilage deterioration, osteoporosis, and eventual joint dysfunction[1]. It primarily affects small articulations in limbs, but can also involve other organs, making RA a systemic condition[2]. RA presents in various forms, including seropositive RA, which is allied with the presence of rheumatoid factor (RF) and autoantibodies, often leading to severe joint damage and extra-articular indications[3]. In contrast, seronegative RA lacks these antibodies but presents with similar clinical symptoms, typically with a milder progression[4]. Juvenile idiopathic arthritis (JIA) is another variant that affects children under 16 and can impact growth and development[5]. The disease progresses through several stages, starting with early synovitis (inflammation), progressing to cartilage degradation, and leading to bone erosion and joint deformities in advanced stages[6].

The etiology of RA involved genetic, environmental, and immunological elements that make it intricate to trigger the disease[7]. It involves chronic inflammation of synovial tissues caused by an aberrant immune response[8]. Key marking pathways involved in RA include the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Janus kinase-signal transducers and activators of transcription (JAK-STAT), and mitogen-activated protein kinase (MAPK)[9]. These pathways are activated by inflammatory messengers such as tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), which contribute to synovitis, cartilage damage, and bone resorption[10].

[*]Corresponding author

Lately, it has been noted the potential of phytochemicals as alternatives or adjuncts to conventional therapies for RA[11]. Phytochemicals such as polyphenols, flavonoids, alkaloids, and terpenoids have shown promising anti-inflammatory effects by modulating key molecular targets[12]. These natural compounds inhibit the NF-κB pathway, suppress pro-inflammatory cytokines, and reduce oxidative stress, providing a multi-targeted approach to managing RA[13]. Exploring the therapeutic potential of medicinal plants offers an attractive strategy for reducing the side effects and limitations associated with synthetic drugs[14].

Dillenia pentagyna Roxb., a medicinal plant from the Dilleniaceae family, is widely used in traditional medicine across India and Southeast Asia for treating inflammation, fever, diarrhea, and skin diseases[15]. Pharmacological studies have revealed its rich content of bioactive compounds, including flavonoids, tannins, saponins, and triterpenoids, which are known for their antioxidant, anti-inflammatory, and anti-arthritic properties[16]. *D. pentagyna* has shown the ability to inhibit key inflammatory cytokines, such as TNF-α and IL-6, making it a promising candidate for RA therapy[17].

Phytochemical analyses of *D. pentagyna* bark have previously identified betulinic acid, lupeol, quercetin, β-sitosterol, and dillenetin compounds known for their potent antioxidant and anti-inflammatory effects. These bioactives have been reported in *Dillenia* species (*D. indica*, *D. pentagyna*) to inhibit NF-κB activation, modulate cytokines (TNF-α, IL-1β, IL-6), and scavenge reactive oxygen species, providing a strong phytochemical rationale for exploring its anti-arthritic efficacy.

This research aims to explore the anti-arthritic potential of *D. pentagyna* Roxb. by targeting molecular pathways involved in RA[18]. The specific objectives of the study include identifying and characterizing the bioactive phytochemicals present in *D. pentagyna* that possess anti-rheumatic properties[19], evaluating the effects of these phytochemicals on key signaling pathways such as NF-κB, JAK-STAT, and MAPK in *in vitro* and *in vivo* models of RA[20], and exploring the plant's potential to reduce oxidative stress and pro-inflammatory cytokine production[21]. This investigation seeks to provide scientific data supporting the use of *D. pentagyna* as a plant-based therapeutic agent for RA, potentially leading to the development of safer, more effective treatments[22].

Materials and Methods

Materials

All the chemicals used in this study were of research grade and sourced from reputable suppliers. Ethanol, methanol, mannitol, dimethyl sulfoxide (DMSO), potassium acetate, trichloroacetic acid (TCA), 2-deoxy-D-ribose, ethylenediaminetetraacetic acid (EDTA), thiobarbituric acid (TBA), formaldehyde (formalin), and diethyl ether were obtained from S.D. Fine-Chem Ltd., Mumbai. Complete Freund's Adjuvant (CFA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid (ASC), and Folin-Ciocalteu reagent were supplied by Sigma-Aldrich, Mumbai. Additionally, quercetin, gallic acid, aluminum chloride, mannitol, tragacanth, Tween 80, and sodium carbonate were procured from Hi-Media Laboratories, Mumbai. All materials were used as received without further modification.

Collection and authentication of the plant material

In January 2023, the bark of *D. pentagyna* Roxb. was taken from the botanical garden of Karnataka University, Dharwad. The plant material was attested and documented by Dr. S. S. Hebbar, a botanist at Government P.U.C. College, Dharwad, Karnataka. (Accession no. SETCPD/Ph.cog/herb/77/2023).

Experimental animals

Acute toxicity study was carried out using female albino mice weighing around 25-30 g by up and down method. For main experimental studies male Wistar rats (150-200 g) were used for the main experimental procedures. The animals were collected from Maratha Mandal's Central Research Laboratory, Belgaum, and housed in controlled conditions in the institutional animal house. With the standard provision of water and food, all animals were kept in plastic cages under the 20±2°C temperature and serve a 12-h light-dark cycle. Before experimental study, the animals were subjected to habituation period for 48 h to reduce non-specific stress. Institutional Animal Ethical Committee (IAEC) sanctioned all study protocols in accordance with CPCSEA guidelines (Approval No. SET/CP/IEAC/179/05, dated 25.03.2023).

Pharmacognostical evaluation

The pharmacognostical evaluation of *D. pentagyna* Roxb. involves a detailed examination of both morphological and microscopical features.

Morphologically, the plant specimen was assessed for its perceptual features[23]. For microscopical analysis, fresh bark samples were fixed in a solution of formalin, acetic acid, and ethanol, then dehydrated with tertiary butyl alcohol, and infiltrated with paraffin wax. Sections of the paraffin-embedded specimens were cut using a rotary microtome to a thickness of 10-12 µm, dewaxed, and stained with toluidine blue, safranin, fast green, and IKI for various cell components. The sections were examined under a microscope, and photomicrographs were taken using bright field and polarized light techniques to visualize structures such as crystals and starch grains.

Additionally, powdered plant material was cleared with sodium hydroxide and mounted in glycerin for further microscopic analysis, with chloral hydrate used to prevent crystallization during examination.

Determination of proximate values

The quality, identity and consistent potency of the bark of *D. pentagyna* Roxb. was carried out by evaluating its proximate (physicochemical) parameters. Moisture content, ash values and extractive values were determined as per standard methods. The percentage of all determined values were expressed as percentages relative to the air-dried sample.

Preparation of hydro alcoholic bark extract

The *D. pentagyna* Roxb. bark powder was extracted with 70% ethanol using Soxhlet apparatus at a temperature interval of 60°C to 80°C through continuous hot extraction. After the extraction process, the collected extract was concentrated using a rotary flash evaporator. The condensed extract was then dried in a vacuum desiccator over anhydrous calcium chloride to obtain the hydroalcoholic extract, referred to as HBDP. The percentage yield of the ethanolic extract was calculated based on the initial weight of the bark powder.

Phytochemical investigation

The preliminary qualitative phytochemical investigations of the HBDP were defined by established methods to confirm existence of major phytochemical groups including carbohydrates, proteins and amino acids, lipids, glycosides, saponins, flavonoids, coumarins, tannins, phenolic compounds, alkaloids, steroids, and triterpenoids[24,25].

The results confirmed the presence of major phytochemical groups which are known for their active principles responsible for the known biological activities exhibited by the plants. No individual compounds were identified; only the presence of respective phytochemical classes was confirmed.

For confirmation of phytochemicals in the hydroalcoholic bark extract (HBDP), HPTLC analysis was more precise technique by which we identified quercetin and lupeol as major phytomarkers, confirmed by authentic standards. The chromatography was performed on silica gel 60 F_{254} plates using solvent system of toluene: ethyl acetate: formic acid (7:2.5:0.5 v/v/v), densitometric scanning revealed quercetin content of 12.3 µg/mg extract and lupeol content of 8.6 µg/mg extract. This approach offers both qualitative and quantitative validation of bioactive constituents[26,27], supporting the phytochemical profile *of D. pentagyna* Roxb.'s therapeutic potential.

Quantitative determination of secondary metabolites

Folin-Ciocalteu method was employed to determine concentration of phenolics in HBDP. A standard calibration curve was also generated using gallic acid (10-100 µg/mL) from which the total phenolic concentrations in the extract was derived as gallic acid equivalents (GAE). The absorbance of blue colour developed was measured for different concentrations of HBDP (1 g/100 mL) at 765 nm.

Estimation of total flavonoid content of HBDP was carried out using the aluminium chloride colorimetric assay against a flavonoid standard quercetin (10-100 µg/mL). TFC is quantified from calibration curve and denoted as quercetin equivalents (QUE). The absorbance of yellow colour developed was measured for different concentrations of HBDP (10 - 1000 µg/mL) at an absorbance of 410 nm was derived from this standard graph[28,29].

In-vitro free radical scavenging activity[30,31]

The antioxidant activity of HBDP was evaluated using DPPH free radical scavenging assay. Different concentrations of HBDP (10-100 µg/mL) were mixed with equal volume of 100 µM DPPH solution and kept away from light for 20 min. The absorbance of mixture was measured at 517 nm using ascorbic acid (ASC) as standard. IC_{50} value was calculated using the standard curve of ASC. the percentage DPPH scavenging against ASC concentration gave the standard curve.

Deoxy-D-ribose degradation method was used to measure hydroxyl radicals scavenging activity of HBDP (10-100 μg/ml). After 30 min of mixing deoxy-D-ribose, mannitol, ferric chloride, EDTA, and H_2O_2 in phosphate buffer (pH 7.4) trichloroacetic acid reagent was added, and boiled for 30 min. The absorbance of the reaction mixture was measured at 532 nm using mannitol standard.

HBDP (10-100 μg/mL) in phosphate buffer (pH 6.6) was used for reducing power assay. Each concentration of HBDP was mixed with 1% potassium ferricyanide and incubated for 20 min. at 50 0°C followed by addition of 10% trichloroacetic acid. After centrifugation the upper layer was mixed with distilled water (2.5 mL) and 0.5 mL $FeCl_3$ solution. The optical density was measured at 700 nm using ascorbic acid as standard.

Pharmacological screening

***Determination of acute toxicity*[32]**

Up-and-down/staircase method was used to determine acute toxicity of HBDP in albino mice (25-30 g). All the animals were administered with 4000 mg/kg body weight of HBDP and observed for 2 days for any signs of discomfort, behaviour alterations and mortality. 1/10th and 1/20th of the acute toxicity dose were selected for pharmacological studies.

Experimental design

The anti-arthritic activity of HBDP was studied in Complete Freund’s Adjuvant (CFA) induced arthritic model in Wistar albino rats. Animals were divided into five groups containing 6 rats in each group. Suspension of HBDP in 0.5% tragacanth was freshly prepared and administered orally. Group 1 (normal control) received distilled water (10 mL/kg b.w. p.o.), Group 2 (arthritic control) received CFA (0.1 mL, intradermal), Group 3 received suspension of HBDP (200 mg/kg b.w., p.o. + CFA), Group 4 received suspension of HBDP (400 mg/kg b.w., p.o. + CFA) and Group 5 received diclofenac sodium (15 mg/kg b.w., p.o. + CFA) as a standard treatment. The arthritic syndrome was induced by intradermal injection of 0.1 mL of CFA into the plantar surface of left hind paw of rats under light ether anesthesia on day 1. Treatment with test extract and standard to respective groups was started on the 9th day after CFA injection and continued daily until day 21. Paw size and paw volume were measured on the 4th, 8th, 14th, and 21st days using digital Vernier calipers and a plethysmometer, respectively. Body weight was recorded every 3rd day. The percentage inhibition of arthritis was calculated[33].

Comprehensive assessment of adjuvant induced arthritis: Impact on body weight, visual scoring, hematological parameters, and histopathology

The body weight of rats was measured before and after the induction of CFA induced arthritis during every three days between the treatments. The significant decrease in body weight compared with the normal rats. Results were analyzed statistically by comparing both normal and CFA control groups[34]. Arthritis development and severity were evaluated using a visual analog scale ranging from 0 to 4 per limb, where 0 indicated no change and 4 indicated gross deformity and inability to use the limb. Scores from both hind limbs were combined, with a total score greater than 1 indicating arthritis, and a maximum possible score of 8. Scoring was conducted on day 21. On day 21, post CFA injection blood was withdrawn via cardiac puncture under mild ether anesthesia and placed in heparinized tubes for hematological parameters, including hemoglobin (HGB), total red blood cell (RBC), and total white blood cell (WBC) counts, which were measured using an automated hematology analyzer[35]. Additionally, ankle joints from each group were isolated, fixed in 10% formalin, and sent for histopathological examination at Jeevan Regional Diagnostic, Belgaum, Karnataka.

Statistical analysis

All the data were expressed as Mean ± S.E.M. and statistically analyzed using one-way ANOVA followed by Tukey's post-test conducted with GraphPad Prism version 5.0 software.

Results

Pharmacognostical evaluation of *Dillenia pentagyna* Roxb. Bark

Morphological features and anatomical structure

The pharmacognostical parameters of *Dillenia pentagyna* Roxb. bark revealed distinct morphological and anatomical features. The bark is hard and brittle, usually found in irregular pieces (0.9-1.3 cm thick). The outer surface is grayish in colour mottled with white patches, while the inner surface is reddish-brown with a pinkish-red blaze and white spindled rays. It has a bitter, fibrous taste and a fibrous to granular fracture.

Anatomically, the bark consists of a thick, fissured periderm, a robust cortex, and secondary phloem. The periderm contains several horizontal layers of phellem cells which are tangentially rectangular and thick walled. The phellem is heterocellular having two or three layers of smaller, rectangular thick walled cells which are called phelloid cells. The cortical zone contains parenchymatous, compact and thin walled cells with dense accumulation of tannins. The sclerieds form a thin layer at the end of cortical zone.

Microscopical analysis of Dillenia pentagyna Roxb. bark: Phloem structure and crystal distribution

The microscopical evaluation of *Dillenia pentagyna* Roxb. bark revealed distinct characteristics of the secondary phloem. It is thicker than the cortex and periderm, comprising outer collapsed and inner non-collapsed phloem, with a gradual transition between the two. The secondary phloem contains narrow, darkly stained phloem rays that widen inward, along with sclerides and common secretory cavities in the collapsed zone. In the non-collapsed phloem, the phloem rays are narrow and straight, consisting of sieve elements and companion cells, where sieve elements lack nuclei while companion cells are living and nucleated. Tangential longitudinal sections show phloem rays in vertical parallel lines, characterized by heterocellular structures with short, polygonal cells in the middle and vertically elongated upright cells at the ends. Radial longitudinal sections display rays stacked like bricks, with horizontal prominent cells and vertically oriented upright cells. Calcium oxalate crystals are observed in the parenchyma cells, appearing spherical with a spiny surface and arranged in vertical lines, visible as bright white under polarized light.

Powder microscopy of Dillenia pentagyna Roxb.bark

The powder microscopy of *Dillenia pentagyna* Roxb. bark revealed several key features. The peridermis consists of elongated, polygonal cells with straight, thick-walled anticlinical arrangements, forming a compact flat plate. Parenchyma cells are long and rectangular, measuring 150-200 µm in length and 20-30 µm in width. The fibers are long, tapering at the ends, and highly thick-walled and lignified, with lengths ranging from 350-500 µm and a thickness of about 10 µm. Sclerieds are broad, varying in shape and size, with lignified walls and canal-like pits. Sparse starch grains, which are spherical or elliptical, exhibit polarized marks under polarized light. These microscopic characteristics provide valuable insights into the structural composition of the bark powder.

Physicochemical investigation

Purity and strength of drug various physicochemical parameters, including moisture content, extractive values, total ash value, acid-insoluble ash value, water-soluble ash, and sulfated ash value. The findings for these physicochemical constants and are given in Table 1.

Preliminary qualitative phytochemical analysis

The percentage yield of the hydroalcoholic extract obtained from *D. pentagyna* Roxb. is shown in Table 2. A preliminary qualitative phytochemical screening of the hydroalcoholic bark extract was conducted following established methods to identify various classes of phytoconstituents, including carbohydrates, cardiac glycosides, steroids, triterpenoids, tannins, and phenolic and polyphenolic compounds. The findings from this phytochemical analysis are reported in Table 3.

Quantitative analysis of secondary metabolites

Assessment of total phenolic content (TPC)

According to followed quantitative method the total concentration of phenolics in HBDP extract was found to be 62.20 mg GAE/g using standard plot of Gallic acid ($y = 0.0045x + 0.0005$, $R^2 = 0.9991$). Absorbance was measured at 765 nm.

Table 1 — Proximate analysis of *Dillenia pentagyna* Roxb. Bark

Parameters	Results (n=3)
Moisture content	10.84± 0.02
Alcohol soluble extractive	16.05±0.06
Water soluble extractive	11.03±0.05
Total ash	1.42±0.04
Acid insoluble ash	0.80±0.02
Water soluble ash	0.95±0.08
Sulphated ash	1.12±0.09

Table 2 — Percentage yield and physical characteristics of *Dillenia pentagyna* Roxb. (HBDP)

Colour	Reddish brown
Odour	Characteristic
Consistency	Crystalline
Yield (%w/w)	7.15

Table 3 — Preliminary phytochemical analysis of *Dillenia pentagyna* Roxb. (HBDP)

Phytoconstituents	HBDP
Carbohydrates	+
Tannins and phenolic compounds	+
Flavonoids	+
Steroids	+
Cardiac glycosides	+
Triterpenoids	+

Assessment of total flavonoid content (TFC)

The TFC of HBDP extract was found to be 58.26 mg QUE/g using regression equation ($y = 0.0036x + 0.0068$, $R^2 = 0.9983$) derived from standard plot of quercetin.

Determination of antioxidant activity using DPPH radical scavenging method

The antioxidant activity of HBDP was determined against stable radical DPPH. HBDP shows IC_{50} value of 54.18 µg/mL while IC_{50} value of standard ascorbic acid was 32.35 µg/mL.

Hydroxyl radical scavenging assay

The scavenging activity of HBDP was determined against hydroxyl radicals. formed from the reaction of H_2O_2 and a ferric compound with 2-deoxyribose. HBDP effectively inhibited 2-deoxyribose degradation, indicating strong hydroxyl radical scavenging capacity. The IC_{50} value for HBDP was 43.93 µg/mL, while % of inhibition was found to be highest at 20.00 µg/mL for mannitol.

Reducing power assay

The antioxidant activity of HBDP was evaluated based on its ability to reduce potassium ferricyanide (Fe^{3+}) to intensely blue coloured ferrous (Fe^{2+}) complex in the presence of antioxidants acting as reducing agents was measured at 700 nm. HBDP exhibited a mean IC_{50} value of 64.38 µg/mL, while ascorbic acid showed a stronger reducing power with an IC_{50} of 31.35 µg/mL.

Pharmacological activity

Acute toxicity studies

At highest dose of HBDP (4000 mg/kg b.w.p.o.) no death or any sign and symptom of toxicity was observed in experimental animals throughout observation period. The result imply that oral lethal dose (LD_{50}) of HBDP is greater than 4000 mg/kg.

***In-vivo* anti -rheumatic activity**

The impact of HBDP on paw volume, the percentage reduction of edema, body weight, arthritic index and extend of paw swelling in CFA-induced arthritis is shown in Figure 1 & Figure 2.

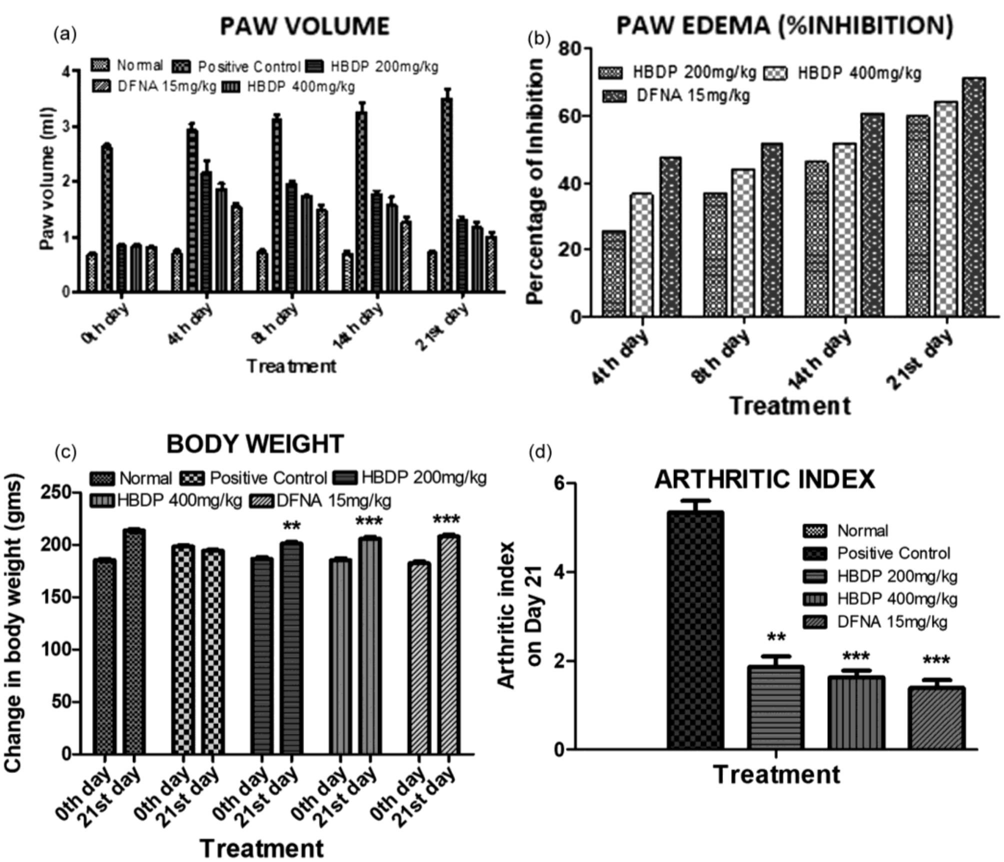

Fig. 1 — Impact of HBDP on paw volume, CFA-induced paw edema (% inhibition), body weight changes in Complete Freund's Adjuvant (CFA)-induced arthritic rats

***Impact of HBDP on arthritic rat* (*injected paw*)**

In studied arthritis model, it was observed that paw edema peaked on day 9 and persisted in the control group, with significant swelling by day 24 (3.494±0.17). The edema on animals of treatment groups of 3 and 4 began to subside gradually on day 24, with a reduction of 57.50% (1.483±0.10) and 66.87% (1.156±0.06), respectively, compared to the control. Diclofenac, the standard drug, showed a 71.26% reduction (1.003±0.08). All reductions were significant (p<0.001) (Table 4).

Impact of the HBDP on body weight induced by CFA

At the onset stage of arthritis model studied, the control group experienced significant weight loss (-4.86 g) from day 7 onward, while the normal group showed consistent weight gain (28.27 g) throughout the study. Diclofenac-treated rats exhibited a weight gain of 25.67 g. HBDP treatment at 400 mg/kg resulted in notable improvement, with a weight gain of 20.48 g, indicating strong efficacy (Table 5).

Impact of the HBDP on arthritic index induced by CFA

In CFA induced animals, the positive control group showed no reduction in arthritic index over 21 days,

Fig. 2 — Impact of HBDP on the extend of paw swelling in CFA induced arthritis. A- Normal, B-Positive control, C- HBDP 200 mg/kg- D- HBDP 400 mg/kg) E- Diclofenac sodium

Table 4 — Impact of HBDP on paw volume induced by CFA in rats

Treatment Days	Dose	Paw volume (mL)				
		0-day	4th day	8th day	14th day	21st day
Group 1 Normal	5 mL/kg	0.669±0.06	0.699±0.06	0.699±0.06	0.693±0.06	0.701±0.05
Group 2 Arthritic control	0.1 mL i.d.	2.614±0.06	2.922±0.12	3.114±0.10	3.253±0.18	3.494±0.17
Group 3 HBDP +CFA	200 mg/kg p.o.	0.832±0.04	2.175±0.19 25.51%	1.962±0.05 36.99%*	1.752±0.08 46.14%**	1.483±0.10 57.50%***
Group 4 HBDP + CFA	400 mg/kg p.o.	0.822±0.04	1.853±0.11 36.54%**	1.743±0.01 44.02%***	1.568±0.15 51.79%**	1.156±0.06 66.87%***
Group 5 Diclofenac sodium + CFA)	15 mg/kg p.o.	0.086±0.03	1.536±0.07 47.39%**	1.496±0.09 51.95%***	1.276±0.1 60.77%***	1.003±0.08 71.26%***

Each group represents mean ± S.E.M. of 6 animals. Significance levels set at *p<0.05, **p<0.01, and ***p<0.001 compared to the control group (Tukey's test).

Table 5 — Impact of HBDP on body weight induced by CFA in rats

Treatment	Dose	Change in body weight (gm)		
		Initial body weight	Final body weight	Mean body weight in g
Group 1 Normal	5 mL/kg	185.53±0.53	213.8±0.48	28.27
Group 2 Arthritic control (CFA)	0.1 mL i.d.	198.4±0.43	194.4±0.39	-4.0
Group 3 HBDP +CFA	200 mg/kg p.o.	186.75±1.51	201.09±1.11**	15.15
Group 4 HBDP+ CFA	400 mg/kg p.o.	185.62±1.54	206.01±1.05***	20.48
Group 5 Diclofenac sodium + CFA)	15 mg/kg p.o.	182.45±1.57	208.12±2.01***	25.67

Each group represents mean ± S.E.M. of 6 animals. Significance levels set at *p<0.05, **p<0.01, and ***p<0.001 compared to the control group (Tukey's test).

while the standard drug (diclofenac) significantly lowered the index by day 21. HBDP treatment also gives significant, dose-dependent decline in the arthritic score. At 400 mg/kg, HBDP showed a marked suppressive effect (1.63±0.15), nearly matching the standard drug (1.39±0.18) (p<0.001) (Table 6).

Impact of HBDP on haematological alterations induced by CFA in rats

Evaluation of all haematological parameters in arthritic control group animals showed marked reduction in RBC count and haemoglobin levels, along with rise in WBC count. Treatment with diclofenac and HBDP improved these parameters, normalizing RBC, haemoglobin, and WBC counts. HBDP at both doses was equally effective in restoring haematological parameters to normal (p<0.05). (Fig. 3 & Table 7)

Table 6 — Impact of HBDP on arthritic index induced by CFA in rats

Treatment	Dose	Arthritic index on Day 21
Group 1 Control	5 mL/kg	0±0
Group 2 Arthritic control (CFA)	0.1 mL i.d.	5.34±0.26
Group 3 HBDP +CFA	200 mg/kg p.o.	1.86±0.24**
Group 4 HBDP + CFA	400 mg/kg p.o.	1.63±0.15***
Group 5 Diclofenac sodium + CFA)	15 mg/kg p.o.	1.39±0.18***

Each group represents mean ± S.E.M. of 6 animals. Significance levels set at **p<0.01, and ***p<0.001 compared to the control group (Tukey's test).

Discussion

Both *in-vitro* and *in-vivo* antioxidant and anti-inflammatory activities of HBDP has been proven. Phytochemical analysis of HBDP showed presence of triterpenoids, tannins, steroids, phenolic and polyphenolic compounds**,** which contributes to its anti-inflammatory and antioxidant activity. The observed *in-vitro* activities in DPPH and hydroxyl assays suggests that these phytochemicals play a major role in alleviating oxidative injury responsible for rheumatism.

The HBDP exerts an antiarthritic activity by significantly altering the pathogenesis during CFA induced arthritis in experimental animals. The antiarthritic potential of HBDP is evidenced by decreased levels of paw volume, improvement in motility and body weight, decreased arthritic score and normalized haematological parameters**,** demonstrating its use in management of chronic inflammatory and autoimmune diseases. The comparison of spotted structural alterations in tissues of ankle joints of all groups revealed that HBDP treatment group showed evidence of normalcy with lesser bone erosion and infiltrating cells, supporting the extract's ability to protect joint architecture from inflammatory damage.

Literature survey indicated no scientific data available on antirheumatic activity activities of HBDP on experimental animals. Hence, antiarthritic activity was evaluated using adjuvant induced arthritis in rats. For instance, *Vitex negundo* and *Calotropis gigantea* extracts have been shown to significantly reduce paw edema and arthritic score in CFA-induced rats by conquering inflammatory cytokines and oxidative mediators[35,36]. Similarly, ethanolic extracts of *Withania somnifera* and *Ricinus*

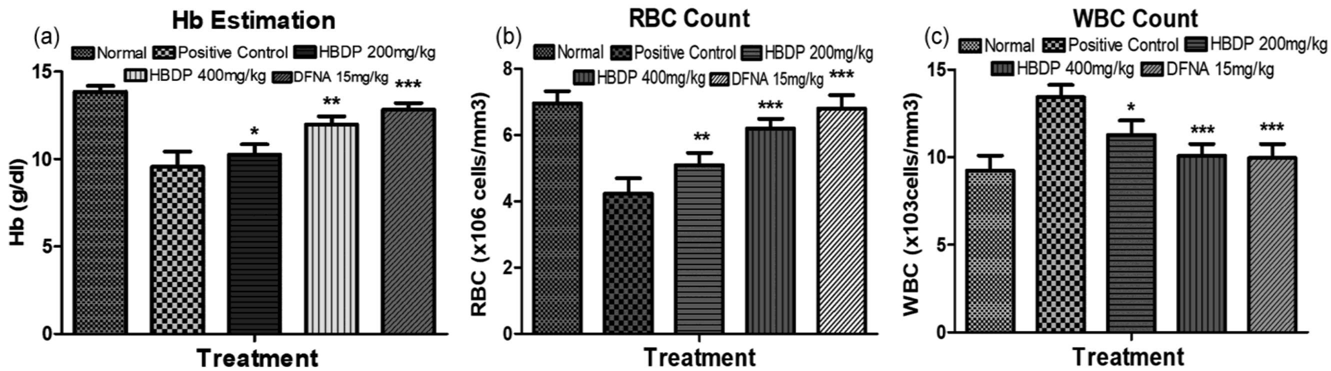

Fig. 3 — Effect of HBDP on hematological parameters in CFA-induced arthritic rats

Table 7 — Effect of the HBDP on haematological alterations induced by CFA in rats

Groups	Dose	Hb (g/dL)	RBC (x10^{6} cells/mm^{3})	WBC (x10^{3} cells/mm^{3})
Group 1 Normal	5 mL/kg	13.86±0.33	6.96±0.37	9.23±0.86
Group 2 Positive control (CFA)	0.1 mL i.d.	9.56±0.87	4.24±0.46	13.43±0.70
Group 3 HBDP +CFA	200 mg/kg p.o.	10.26±0.58*	5.10±0.36**	11.27±0.84*
Group 4 HBDP + CFA	400 mg/kg p.o.	11.98±0.46**	6.20±0.30***	10.08 ±0.68**
Group 5 Diclofenac sodium + CFA)	15 mg/kg p.o.	12.83±0.38***	6.80±0.40***	9.96±0.79***

Each group represents mean ± S.E.M. of 6 animals. Significance levels set at *p<0.05, **p<0.01, and ***p<0.001 compared to the control group (Tukey's test).

communis at 500 mg/kg demonstrated strong arthritic protection by modulating TNF-α and IL-6 levels[37].

The comparable potential of HBDP to diclofenac observed in this study suggests that *Dillenia pentagyna* bark may exert its therapeutic effects through similar mechanisms possibly via inhibition of prostaglandin synthesis and oxidative stress pathways.

Taken together, the present findings establish a pharmacological foundation for the traditional use of *Dillenia pentagyna* in inflammatory and arthritic conditions and justify further mechanistic and phytochemical investigations.

Conclusion

The hydroalcoholic bark extract of *Dillenia pentagyna* Roxb. (HBDP) demonstrated significant antioxidant and anti-arthritic activities, attributed to its rich content of flavonoids, terpenoids, steroids, tannins, and phenolic compounds. The extract effectively reduced oxidative stress, inflammation, paw edema, and alterations in hematological parameters in chronic arthritis model. These results validate its traditional use for inflammatory conditions. Further research should focus on fractionation, isolation of active constituents, and detailed mechanistic studies to fully elucidate its therapeutic potential. Future studies may explore clinical trials to establish *Dillenia pentagyna* as a viable treatment for arthritis and related inflammatory disorders.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

PH: Concept development, study design, overall supervision, and guidance, VMT: methodology, original draft preparation and communication, MH: preparation of extracts and pharmacognostical evaluation, SM: analyzing data, writing support. VK: interpreting results and guidance, CS: assist in experimental study conduction and histopatology. All authors reviewed the manuscript thoroughly and approved the final version for publication.

Data Availability

Data will be available from the corresponding author upon reasonable request.

References

1 Ding Q, Hu W, Wang R, Yang Q, Zhu M, *et al*., Signaling pathways in rheumatoid arthritis: implications for targeted therapy, *Signal Transduct Target Ther*, 8 (1) (2023) 68. doi: 10.1038/s41392-023-01331-9

2 Chando A, Momin M, Quadros M & Lalka S, Topical nanocarriers for management of rheumatoid arthritis: a review, *Biomed Pharmacother*, 141 (2021) 111880. doi: 10.1016/j.biopha.2021.111880

3 Chhikara K, Gupta S, Saharawat S, Sarkar S, Chanda A, *et al*., Design, manufacturing, and trial of a 3D printed customized finger splint for patients with rheumatoid arthritis, *Rheumato*, 3 (1) (2023) 51-62. DOI:10.3390/rheumato3010004

4 Durante A & Bronzato S, The increased cardiovascular risk in patients affected by autoimmune diseases: review of the various manifestations, *J Clin Med Res*, 7 (6) (2015) 379-384. doi: 10.14740/jocmr2122w

5 Patwardhan S K, Bodas K S & Gundewar S, Coping with arthritis using safer herbal options, *Int J Pharm Pharm Sci*, 2 (1) (2010) 1-11.

6 Smolen J S & Steiner G, Therapeutic strategies for rheumatoid arthritis, *Nat Rev Drug Discov*, 2 (6) (2003) 473-488. doi: 10.1038/nrd1109

7 Choy E, Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis, *Rheumatol (Oxford)*, 51 (Suppl 5) (2012) v3-v11. doi: 10.1093/rheumatology/kes113

8 Sweeney S E & Firestein G S, Rheumatoid arthritis: regulation of synovial inflammation, *Int J Biochem Cell Biol*, 36 (3) (2004) 372-378. doi: 10.1016/s1357-2725(03)00259-0

9 Isomäki P & Punnonen J, Pro- and anti-inflammatory cytokines in rheumatoid arthritis, *Ann Med*, 29 (6) (1997) 499-507. doi: 10.3109/07853899709007474

10 Firestein G S, Evolving concepts of rheumatoid arthritis, *Nature*, 423 (2003) 356-361. https://doi.org/10.1038/nature01661

11 Lindstrom T M & Robinson W H, Biomarkers for rheumatoid arthritis: making it personal, *Scand J Clin Lab Investig Suppl*, 242 (2010) 79-84. doi: 10.3109/00365513.2010.493406

12 Mc Ardle A, Flatley B, Pennington S R & FitzGerald O, Early biomarkers of joint damage in rheumatoid and psoriatic arthritis, *Arthritis Res Ther*, 17 (1) (2015) 141. doi: 10.1186/s13075-015-0652-z

13 Eisenstein A, Hilliard B K, Pope S D, Zhang C, Taskar P, *et al*., Activation of the transcription factor NRF2 mediates the anti-inflammatory properties of a subset of over-the-counter and prescription NSAIDs, *Immunity*, 55 (6) (2022) 1082-1095. doi: 10.1016/j.immuni.2022.04.015

14 Ahmed S, Anuntiyo J, Malemud C J & Haqqi T M, Biological basis for the use of botanicals in osteoarthritis and rheumatoid arthritis: a review, *Evid Based Complement Alternat Med*, 2 (3) (2005) 301-308. doi: 10.1093/ecam/neh117

15 Arend W P & Dayer J M, Inhibition of the production and effects of interleukins-1 and tumor necrosis factor α in rheumatoid arthritis, *Arthritis Rheum*, 38 (2) (1995) 151-160. doi: 10.1002/art.1780380202

16 Feldmann M & Maini R N, Anti-TNFα therapy of rheumatoid arthritis: what have we learned?, *Annu Rev Immunol*, 19 (1) (2001) 163-196. doi: 10.1146/annurev.immunol.19.1.163

17 Brown S L, Greene M H, Gershon S K, Edwards E T, Braun M M, *et al*., Tumor necrosis factor antagonist therapy and lymphoma development: twenty-six cases reported to the Food and Drug Administration, *Arthritis Rheum*, 46 (12) (2002) 3151-3158. doi: 10.1002/art.10679

18 Cameron M, Gagnier J J & Chrubasik S, Herbal therapy for treating rheumatoid arthritis, *Cochrane Database Syst Rev*, 2 (2011) CD002948.

19 Jain S, Vaidya A, Gupta P K, Rosenholm J M, Bansal K K, *et al*., Antiarthritic activities of herbal isolates: A comprehensive review, *Coatings*, 11 (2021) 1329. https://doi.org/10.3390/coatings11111329

20 McInnes I B & Schett G, The pathogenesis of rheumatoid arthritis, *N Engl J Med*, 365 (23) (2011) 2205-2219. doi: 10.1056/NEJMra1004965

21 Kinne R W, Stuhlmüller B & Burmester G-R, Cells of the synovium in rheumatoid arthritis. Macrophages, *Arthritis Res Ther*, 9 (6) (2007) 224. doi: 10.1186/ar2333

22 Gonzalez-Gay M A, Gonzalez-Juanatey C & Martin J, Rheumatoid arthritis: a disease associated with accelerated atherogenesis, *Semin Arthritis Rheum*, 35 (1) (2005) 8-17. doi: 10.1016/j.semarthrit.2005.03.004

23 Evans W C & Trease, *Text book of Pharmacognosy*, 15th ed, London: 2002.

24 Khandelwal K R, *Prac Pharmacognosy*, 1st ed., (Nirali Publications, Pune), 1995.

25 Kokate C K, Purohit A P & Gokhale S B, *Practical Pharmacognosy*, 2nd ed., (Nirali Publications, Pune), 1994.

26 Wagner H & Bladt S, *Plant Drug Analysis: A Thin Layer Chromatography Atlas*, (Springer Berlin Heidelberg), 1996.

27 Mahajan R, Li X, Fryman J, Zhang Z, Nekkanty S, *et al*., Co-packaged photonics for high performance computing: status, challenges and opportunities, *J Lightwave Technol*, 40 (2) (2021) 379-392. doi: 10.1109/JLT.2021.3104725

28 Kharel R & Sharma K R, Evaluation of antioxidant potential and quantitative estimation of phenolic and flavonoid content in some selected Nepalese medicinal plants, *Asian J Pharm Clin Res*, 13 (1) (2020) 124-128. DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i1.36182

29 Kabra S & Patel S, Total phenolics & flavonoid content of the leaves of *Carica papaya* & *Syzygium cumini*, *World J Pharm Res*, 7 (14) (2018) 734-741. DOI: 10.20959/wjpr201814-12858

30 Sunday R M, Obuotor E M & Kumar A, Antioxidant and antidiabetic properties of *Mimosa pudica* seeds in streptozotocin-induced diabetic Wistar rats, *Asian J Biotechnol*, 12 (1) (2020) 1-8. DOI: 10.3923/ajbkr.2020.1.8

31 Gupta A, Kumar R & Pandey A K, Antioxidant and antidiabetic activities of *Terminalia bellirica* fruit in alloxan

induced diabetic rats, *S Afr J Bot*, 130 (2020) 308-315. https://doi.org/10.1016/j.sajb.2019.12.010

32 Ghosh M N, *Fundamentals of Experimental Pharmacology*, 2nd ed., (Scientific Publishing Book Agency, Calcutta), 1984, 154-158.

33 Dhikale R, Gulecha V & Zalte A, Amelioration of CFA induced arthritis in rats by *Buchnania lanzan*, *Int J Health Sci*, 6 (Suppl 2) (2022) 2459-2484. https://doi.org/10.53730/ijhs.v6nS2.5565

34 Vijayalaxmi A, Bakshi V, Begum N, Kowmudi V, Naveen K Y, *et al*., Antiarthritic and anti-inflammatory activity of beta-caryophyllene against Freund's complete adjuvant induced arthritis in Wistar rats, *J Bone Rep Rec*, 1 (2) (2015) 9. DOI: 10.4172/2469-6684.10009

35 Ramesh Petchi R, Vijaya C, Parasuraman S, Natchiappan A & Devika G S, Anti-arthritic effect of ethanolic extract of leaves of *Vitex negundo* Linn. (Verbenaceae) in male albino Wistar rats, *Int J Res Pharm Sci*, 2 (2) (2011) 213-218.

36 Gautam R K, Roy K, Thapa G, Arora D, Parashar S, *et al*., Perspective of plant medicine in therapy of rheumatoid arthritis, *Indian J Pharm Sci*, 82 (5) (2020) 741-765. DOI: 10.36468/pharmaceutical-sciences.703

37 Hussain A, Aslam B, Muhammad F, Faisal M N, Kousar S, *et al*., Anti-arthritic activity of *Ricinus communis* L. and *Withania somnifera* L. extracts in adjuvant-induced arthritic rats via modulating inflammatory mediators and subsiding oxidative stress, *Iran J Basic Med Sci*, 24 (7) (2021) 951-961. doi: 10.22038/ijbms.2021.55145.12355