World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Phytochemical investigation and assessment of anti-arthritic activity of *Dicanthium annulatum* herbal plant extract in Wistar rats

Naudishtha Khandare [1,*], Manju Prajapati [2] and Akhlesh Kumar Singhai [3]

[1] *Research Scholar, School of Pharmacy, LNCT University, Bhopal (M.P), India.*
[2] *Professor, School of Pharmacy, LNCT University, Bhopal (M.P), India.*
[3] *Director, School of Pharmacy, LNCT University, Bhopal (M.P), India.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 22(03), 201-210

Publication history: Received on 01 April 2025; revised on 01 June 2025; accepted on 04 June 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.3.0496

Abstract

This study focuses on the phytochemical screening, antioxidant evaluation, and anti-arthritic activity of the methanolic extract of *Dichanthium annulatum* (whole plant). The extraction process yielded 4.16%, and preliminary phytochemical analysis confirmed the presence of alkaloids, glycosides, flavonoids, tannins, saponins, and carbohydrates. Quantitative estimation revealed a total phenolic content of 36.88 mg/g gallic acid equivalent and a total flavonoid content of 23.3 mg/g rutin equivalent. Antioxidant potential was assessed via the DPPH free radical scavenging assay, indicating moderate activity with an IC_{50} value of 50.10 µg/mL. The anti-arthritic effect was evaluated in Wistar rats using the Freund's Complete Adjuvant (FCA)-induced arthritis model. Oral administration of the extract (200 mg/kg and 400 mg/kg) significantly reduced paw edema and improved hematological (RBC, WBC, Hb) and biochemical (ALP, AST, ALT, CRP) parameters. The results were comparable to the standard drug diclofenac sodium. These findings suggest that *Dichanthium annulatum* possesses significant anti-inflammatory and anti-arthritic potential due to its bioactive constituents.

Keywords: *Dichanthium annulatum*; Phytochemical Analysis; Antioxidant Activity; Anti-Arthritic Effect; Wistar Rats; FCA Model; Flavonoids; Phenolic Compounds

1. Introduction

The traditional herbal medicines (HM) and their preparations have been widely used for thousands of years in many oriental countries, like in China, Korea, Japan, etc. Nonetheless, a feature of oriental herbal medicine preparations is that, during the decoction process, boiling water is used to extract all herbal medicines, whether they appear as individual herbs or as mixtures of herbs in composite formulas. This could be the primary cause of the difficulty in ensuring the quality of oriental herbal medications compared to western medications. as stated in the World Health Organization's 2000 "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines." Traditional medicine has not received formal recognition in the majority of nations, despite its long history and continuous usage over several centuries, as well as its recent popularity and widespread use. [1] Arthritis is inflammation of one or more joints. The degeneration of cartilage is a feature of arthritis. A joint is often protected by cartilage, which permits smooth motion. Additionally, cartilage absorbs shock when the joint is under pressure, such when you walk. When there is insufficient cartilage, the bones grind against one another, resulting in stiffness, pain, and inflammation. The individuals of any age can be affected with Arthritis; the usual age of onset is between 25 and 50 with a peak in the 40s and 50s [4].The key risk factors of arthritis include age, gender, excess weight, injury, dietary pattern, consumption of excess alcohol, life style, heredity, hormonal factors, environmental factors and lack of physical activity. Four major categories of medications are used to treat arthritis: Corticosteroids (steroids), disease-modifying anti-rheumatic

*Corresponding author: Naudishtha Khandare

medications (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and painkillers (analgesics). [2] *Dichanthium annulatum* (family: Poaceae) is a perennial grass widely distributed in tropical and subtropical regions. Traditionally, it has been used as a fodder plant and in soil conservation, but emerging ethnobotanical knowledge suggests potential medicinal applications. While not commonly used in modern herbal medicine, local communities have attributed anti-inflammatory and wound-healing properties to the plant. Phytochemicals such as flavonoids, particularly quercetin, and phenolic compounds present in *D. annulatum* are known to exhibit strong antioxidant and anti-inflammatory effects in the management of arthritis. Despite its promising profile, the plant remains scientifically underexplored for its pharmacological activities. [3]

2. Material and methods

2.1. Chemicals

All chemicals and reagents used in this study were of analytical grade. Glacial acetic acid, sodium hydroxide, ammonia, formalin, and magnesium were procured from Merck. Concentrated sulfuric acid was obtained from Fizmerck, while ethanol was sourced from Molychem. Diclofenac sodium, used as the standard anti-arthritic drug, was supplied by Reddy's Laboratories. Chloroform, concentrated hydrochloric acid (HCl), and 95% alcohol were purchased from Clorofiltind. Magnesium was acquired from Himedia. The Folin-Ciocalteu reagent, essential for phenolic content determination, was obtained from Sigma-Aldrich, and sodium carbonate was procured from GHCL Limited.

2.2. Plant collection

Dichanthium annulatum (300 g) was collected from a specific region in Bhopal and authenticated by a botanist. The whole plant was washed, shade-dried for three days, and then oven-dried at 45 °C. The dried material was coarsely powdered and stored in airtight containers for further use.

2.3. Extraction

Soxhlet extraction was employed to phytoconstituents from *Dichanthium annulatum* . A known quantity of dried, powdered plant material was placed in a thimble and extracted using methanol as the solvent. The continuous reflux process allowed repeated washing of the plant material, facilitating efficient extraction. The process continued until complete extraction was achieved. The percentage yield was calculated using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

After evaluating the organoleptic characteristics such as color, odor, and percentage yield, the prepared extracts were labelled and stored in airtight containers for subsequent use.[4]

2.4. Phytochemical investigation

Phytochemical investigation of *Dichanthium annulatum* was carried out to identify the bioactive compounds present in the plant. The methanolic extract was subjected to various qualitative tests, revealing the presence of alkaloids, glycosides, flavonoids, tannins, and saponins. These compounds are known for their medicinal properties, including anti-inflammatory and antioxidant effects. The results suggest that *Dichanthium annulatum* contains a range of phytochemicals that may contribute to its therapeutic potential.**[5]**

2.5. Quantitative Phytochemical Estimation

2.5.1. TPC

To ascertain the *Dichanthium annulatum* methanolic extract's overall phenolic content. In a test tube, 200 µL of Folin-Ciocalteu reagent, 3.16 mL of distilled water, and 40 µL (1 mg per 1 mL of methanol) of the plant extract (or standard gallic acid solution) were combined and gently shaken. After an incubation of 8 min, 600 µL sodium carbonate solutions were combined and added. The blend was incubated for 40 °C for 30 min before recording its absorbance in a spectrophotometer at 760 nm against a blank. The curve for calibration was prepared with standard solution of gallic acid equivalent using 10, 30, 50, 70, 90 µg/mLsolutions. [6]

2.5.2. TFC

The flavonoid content of *Dichanthium annulatum* extract was quantified using a colorimetric method with aluminium chloride. A 0.5 mL extract was mixed with distilled water, followed by the addition of sodium nitrite, aluminium chloride, and sodium hydroxide. After a brief incubation, the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was expressed as rutin equivalents (mg/g) using a standard calibration curve with rutin concentrations of 10, 30, 50, 70, and 90 µg/mL.[7]

2.6. DPPH

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay measures the antioxidant activity of plant extracts by evaluating their ability to scavenge the DPPH radical, which is purple in color. Antioxidants reduce the DPPH radical, leading to a decrease in absorbance at 517 nm. The results are expressed as IC50, the concentration required to inhibit 50% of the DPPH radical, providing a simple method to assess antioxidant potential. [8]

The percentage (I%) of inhibition of free radical DPPH was computed as follows:

$$\% \text{ Scavenging Activity} = 100[(Ac - As)/Ac]$$

Where, Ac and as are absorbances of negative control and sample, respectively

2.7. FT-IR

FTIR spectroscopy is a technique used to obtain the absorption or emission infrared spectrum of a *Dichanthium annulatum* extract. FTIR examination to create translucent sample discs, 10 mg of KBr pellet was used to enclose dried powder (methanolic extract). The pellet's powdered sample was placed onto an FTIR spectroscope with a resolution of 4 cm-1 and a scan range of 400 to 4000 cm-1. [9]

2.8. Acute Toxicity Study

In the acute oral toxicity study, three animals were used in each phase, following the OECD 423 guideline. The study received approval from the Faculty Ethical Committee. Each treatment group consisted of five rats (n=3), which were allowed access to food and tap water. Rats were randomly assigned to their respective groups. The treatment groups were administered varying doses of methanol extract of *Dichanthium annulatum* , while the control group was given distilled water as the vehicle. A starting dose of one of four preset dose levels—5, 50, 300, or 2000 mg/kg body weight—must bechosen. [10]

2.9. Freund's Adjuvant-Induced Arthritic Model:

Rats were injected with 0.2 mL of Freund's adjuvant and PBS (1:1) into the left hind and forepaws. Paw volumes were measured using a plethysmometer on days 0, 7, 14, 21, and 28. The percentage inhibition of paw volume was calculated using the formula:

$$(Vc - Vt) / Vc \times 100$$

where Vc is the control paw volume and Vt is the treated paw volume. [11]

2.10. Experimental work

2.10.1. Animals required

Animals were randomly selected from the Pinnacle Biomedical Research Institute (PBRI), Bhopal, India, and assigned to treatment groups. They were housed in propylene cages with sterile husk bedding, maintained at 22±2°C, 30.7% humidity, and a 12:12 light-dark cycle. The rats were fed standard pellets (Golden Feeds, New Delhi) and had unlimited access to water. They acclimatized to the lab environment for seven days before testing. Each experiment used a different group of six rats, and the study was approved by the Institutional Animal Ethics Committee (IAEC) of PBRI.

2.10.2. Experimental protocol

Five groups of male Wistar albino rats (n = 6) were used in this study

- **Group I:** served as control (without treatment)

- **Group II:** Negative control (rats with arthritis that receive no therapy)
- **Group III:** Treated with Diclofenac sodium 20 mg/kg (positive control) the standard anti - arthritic drug
- **Group IV:** Treated with *Dichanthium annulatum* received 200 mg/kg orally
- **Group V:** Treated with *Dichanthium annulatum* received 400 mg/kg orally

At the conclusion of the experiment, all animals were killed by cervical decapitation, and blood was drawn for plasma/serum separation in tubes containing EDTA and plain. The plasma/serum and homogenized samples were subjected to biochemical examination like total protein and albumin, globulin. [12]

2.11. Analysis of general parameters

- Paw Volume: The left hind paw volume of each rat was measured using a paleothermometer on day 0 before FCA injection and at intervals until day 28. The change in paw volume was calculated by subtracting the initial volume from the final measurement. [13]
- Biochemical Analysis: On day 28, blood was collected via retro-orbital puncture, and serum was analyzed for acid phosphatase and alkaline phosphatase levels. [14]
- Haematological Analysis: Complete blood parameters, including red blood cell count, haemoglobin, platelet count, white blood cell count (total and differential), neutrophils, MCHC, MCV, MCH, and PCV, were measured using Erba test kits and the Star 21 Automated Analyzer. [15]
- Body Weight Assessment**:** Body weight was recorded on days 0, 5, 15, and 25 and expressed as the percentage change relative to the baseline weight on day 0. [16]

3. Results and discussion

3.1. Percentage Yield

Table 1 Percentage Yield of crude extracts of *Dichanthium annulatum* extract

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1.	*Dichanthium annulatum*	Methanol	300	12.5	4.16%

3.2. Preliminary Phytochemical study

Table 2 Phytochemical testing of extract

S. No.	Experiment	Absence or Presence of phytochemical test
		Methanolic extract
1.	Alkaloids	
1.1	Dragendroff's test	Present (+ ve)
1.2	Mayer's reagent test	Present (+ ve)
1.3	Wagner's reagent test	Present (+ ve)
1.4	Hager's reagent test	Present (+ ve)
2.	Glycoside	
2.1	Borntrager test	Present (+ ve)
2.2	Legal's test	Present (+ ve)
2.3	Killer-Killiani test	Present (+ ve)
3.	Carbohydrates	
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)

3.4	Barfoed's test	Present (+ ve)
4.	Proteins and Amino Acids	
4.1	Biuret test	Absent (- ve)
4.2	Ninhydrin test	Absent (- ve)
5.	Flavonoids	
5.1	Alkaline reagent test	Present (+ ve)
5.2	Lead Acetate test	Present (+ ve)
6.	Tannin and Phenolic Compounds	
6.1	Ferric Chloride test	Present (+ ve)
7.	Saponin	
7.1	Foam test	Present (+ ve)
8.	Test for Triterpenoids and Steroids	
8.1	Salkowski's test	Absent (- ve)
8.2	Libbermann-Burchard's test	Absent (- ve)

3.3. Quantitative Analysis

3.3.1. Total Phenolic content (TPC) and Total Flavonoids content (TFC) estimation

Table 3 Standard table for Gallic acid and Rutin

S. No.	**Concentration (µg/ml)**	**Absorbance**
1.	10	0.089
2.	30	0.115
3.	50	0.130
4.	70	0.145
5.	90	0.160
S. No.	Concentration (µg/ml)	Absorbance
1.	10	0.110
2.	30	0.135
3.	50	0.158
4.	70	0.175
5.	90	0.193

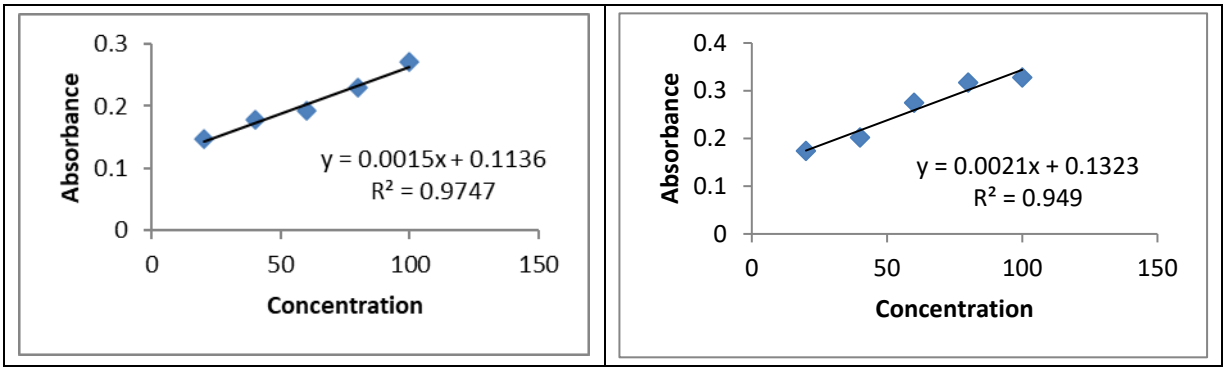

Figure 1 Represent standard curve of Gallic acid and Rutin

3.4. *In vitro* Antioxidant Assays

3.4.1. DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

Table 4 DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Concentration (µg/ml)	Concentration (µg/ml)
20	20	20
40	40	40
60	60	60
80	80	80
100	100	100
Control	0.996	
IC50	40.10	

Table 5 DPPH radical scavenging activity of methanol extract of *Dichanthium annulatum*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.558	40.064
40	0.492	47.153
60	0.454	51.235
80	0.386	58.539
100	0.336	63.909
Control	0.931	
IC50	52.68	

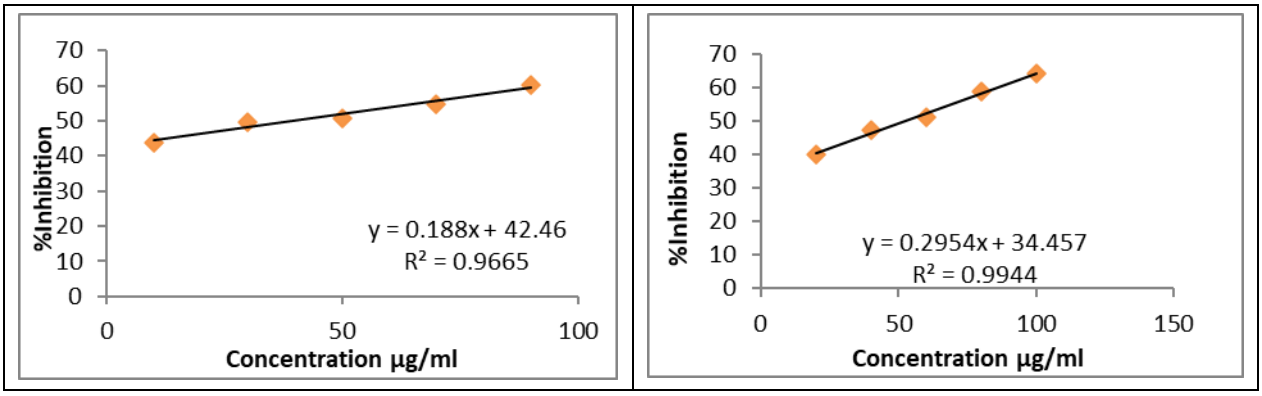

Figure 2 DPPH radical scavenging activity of Std. Ascorbic acid and extract of *Dichanthium annulatum*

3.5. Functional group identified by FTIR Study

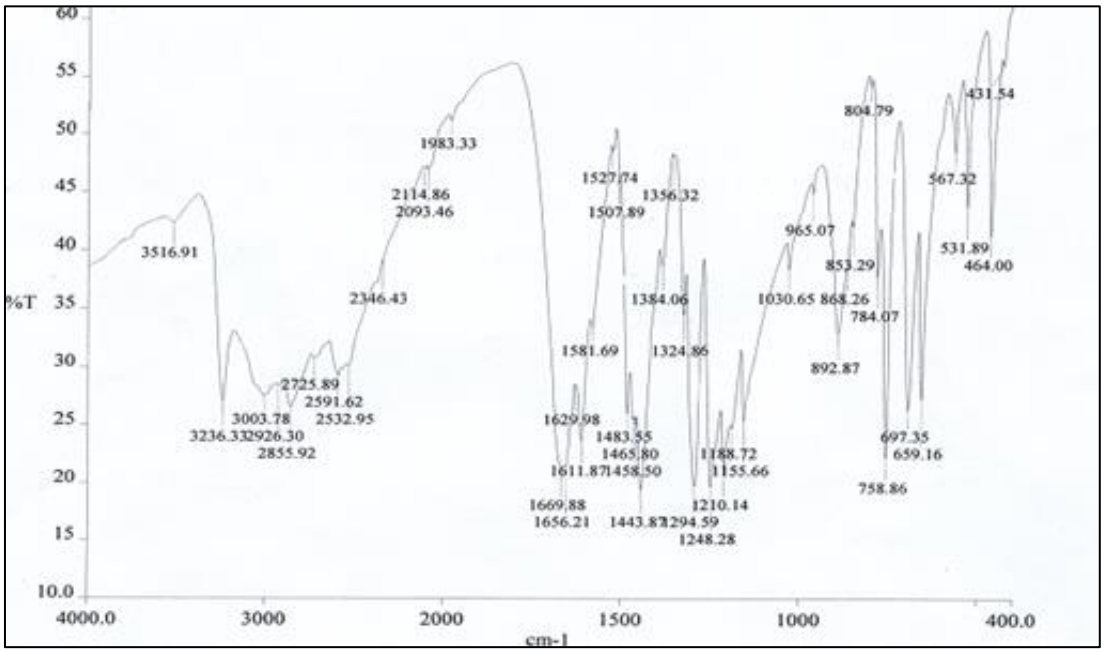

Figure 3 *FTIR of Dichanthium annulatum*

3.6. Paw volume

Table 6 Effect of *Dichanthium annulatum* paw volume of Freund's adjuvant induced arthritic rats

	Paw volume of the rats in mm Mean ± SD (% inhibition)				
Groups	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	3.83 ± 0.05	3.85 ± 0.08	3.84 ± 0.09	3.85 ± 0.15	3.85 ± 0.17
Group II	4.12 ± 0.07	4.44± 0.011	4.65± 0.03	4.79± 0.013	5.12 ± 0.07
Group III	3.92 ± 0.08	4.34 ± 0.011	4.51 ± 0.09	4.38 ± 0.06	4.11 ± 0.05
Group IV	4.15 ± 0.09	4.54 ± 0.08	5.12 ± 0.06	4.91 ± 0.10	4.78± 0.09
Group V	4.21 ± 0.07	4.68 ± 0.06	5.01 ± 0.04	4.55 ± 0.06	4.16± 0.09

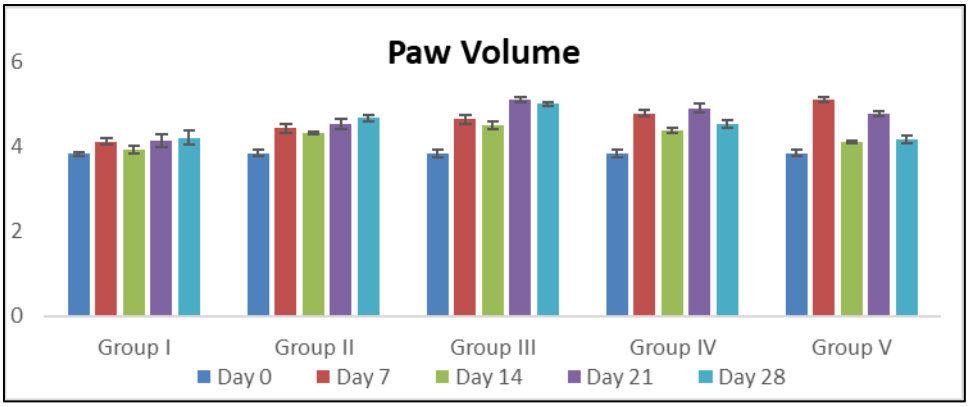

Figure 4 Paw volume in rats with *Dichanthium annulatum*

3.7. Haematological parameters

Table 7 Effect of *Dichanthium annulatum* haematological characteristics of rats with adjuvant-induced arthritis and control

Groups	RBC (millions/mm3)	WBC (Thousands/mm3)	Hb (gm/dL)
Group I	7.23 ± 0.051	9.89 ± 0.021	14.21 ± 0.041
Group II	3.41 ± 0.046	19.91 ± 0.153	9.08 ± 0.042
Group III	6.08 ± 0.019	9.89 ± 0.011	13.15 ± 0.071
Group IV	5.48 ± 0.688	11.37 ± 0.059	10.99 ± 0.152
Group V	5.98 ± 0.023	9.101 ± 0.211	11.12 ± 0.051

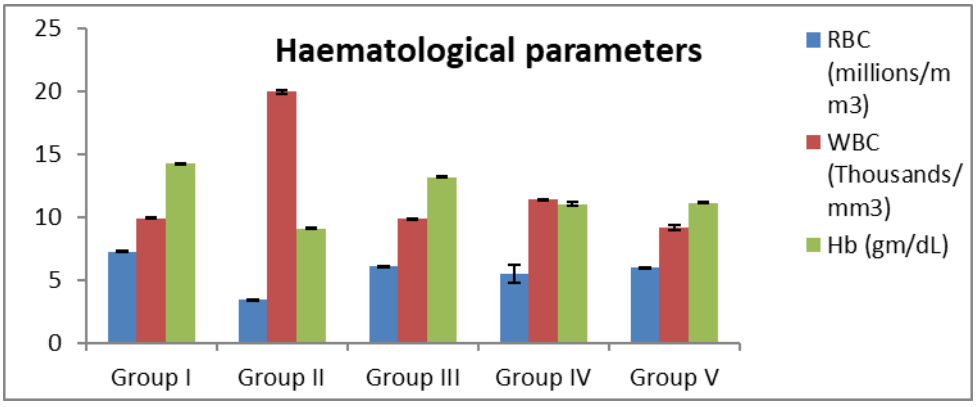

Figure 5 Haematological parameters

3.8. Biochemical parameters

Table 8 Effect of *Dichanthium annulatum* Adjuvant-induced arthritis and control rats' biochemical characteristics

Groups	ALP (Alkaline phosphatise)	AST (Aspartate transaminase)	ALT (Alanine transaminase)	CRP (C-reactive protein)
Group I	151.11 ± 0.015	60.47 ± 0.181	67.59 ± 0.249	106.7 ± 0.191
Group II	250.13 ± 1.378	118.04 ± 0.111	99.28 ± 0.080	137.08 ± 0.143

Group III	136.08 ± 0.473	69.44 ± 0.201	75.64 ± 0.161	110.06 ± 0.299
Group IV	192.07 ± 0.891	94.53 ± 2.059	87.23 ± 0.148	122.04 ± 0.145
Group V	183.09± 0.411	80.35 ± 0.196	81.03 ± 0.264	112.09 ± 0.214

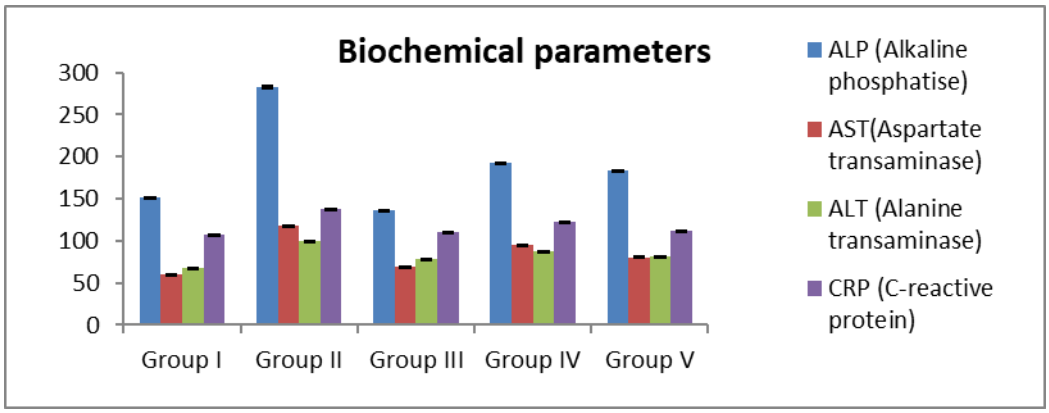

Figure 6 Biochemical parameters

The *Dichanthium annulatum* extract, when assessed for its therapeutic potential, exhibited significant results across various parameters. The methanol extract showed a 4.16% yield and contained bioactive compounds such as alkaloids, glycosides, flavonoids, and saponins, which are known for their medicinal properties. The antioxidant activity, measured by the DPPH assay, displayed a moderate IC50 value of 52.68 µg/mL, indicating the extract's capacity to scavenge free radicals.

In the *Freund's adjuvant-induced arthritis* model, the extract significantly reduced paw volume, especially after 28 days, suggesting its anti-inflammatory properties. This reduction was confirmed by the calculation of paw volume inhibition, which was higher in treated groups than the control. Haematological analysis revealed that the extract positively influenced the red blood cell (RBC) count and haemoglobin levels, which were reduced in the untreated arthritic rats, indicating a recovery in blood parameters. Additionally, the biochemical markers such as alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), and C-reactive protein (CRP) were found to be reduced in the treatment groups, further supporting the extract's anti-inflammatory and tissue-protective effects.

These promising results suggest that *Dichanthium annulatum* possesses anti-arthritic and antioxidant potential, making it a valuable candidate for the development of natural anti-inflammatory therapies. However, further research into its active compounds, dosage optimization, and long-term effects is necessary to fully validate its therapeutic applications.

4. Conclusion

Strong evidence for the anti-arthritic properties of *Dichanthium annulatum* (whole plant) extract is presented in this study. The extract's notable anti-inflammatory properties were revealed by the arthritic rat model's decreased paw volume. Important bioactive substances with anti-inflammatory and antioxidant qualities, like flavonoids and phenolics, were found during the phytochemical analysis. These substances probably have a part in the anti-arthritic benefits that have been noted. The extract was also found to be safe, with no significant toxicity observed in acute oral toxicity studies and no adverse effects on haematological or biochemical parameters in rats.

These results suggest that *Dichanthium annulatum* holds promise as a safe, natural, and efficient treatment for inflammatory diseases like arthritis. To investigate its methods of action, ideal dosage, and possible clinical uses, more research is necessary. With its rich phytochemical content and demonstrated pharmacological activity, *Dichanthium annulatum* might be a useful supplement to the treatment options available for arthritis, offering a natural alternative or adjunct to conventional therapies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

All experimental procedures involving animals were conducted in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute, Bhopal (CPCSEA Registration No.: 1824/PO/RcBi/S/2015/CCSEA, Approval No.: PBRI/IAEC/30-09-24/11. All efforts were made to minimize animal suffering and to reduce the number of animals used in the study.

References

[1] Mosihuzzaman M. Herbal medicine in healthcare-an overview. Natural product communications. 2012 Jun;7(6):193.

[2] Shivanand P. Arthritis an autoimmune disorder: Demonstration of In-vivo anti-arthritic Activity. Int J Pharm Life Sci. 2010;1(1):38-43.

[3] Chaudhuri, A. B. (2007). Endangered medicinal plants. Daya Books.

[4] Patwardhan SK, Bodas KS, Gundewar S. Coping with arthritis using safer herbal options. Int J Pharm Sci. 2010;2(1):1-1.

[5] Azmir J, Zaidul IS, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MH, Ghafoor K, Norulaini NA, Omar AK. Techniques for extraction of bioactive compounds from plant materials: A review. Journal of food engineering. 2013 Aug 1;117(4):426-36.

[6] Velavan S. Phytochemical techniques-a review. World Journal of Science and Research. 2015;1(2):80-91.

[7] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965 Jan 1;16(3):144-58.

[8] Lin JY, Tang CY. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food chemistry. 2007 Jan 1;101(1):140-7.

[9] Tepe B, Sokmen M, Akpulat HA, Sokmen A. In vitro antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chemistry. 2005 May 1;90(4):685-9.

[10] Dhivya SM, Kalaichelvi K. UV-Vis spectroscopic and FTIR analysis of Sarcostemmabrevistigma, wight. and arn. International Journal of Herbal Medicine. 2017;9(3):46-9.

[11] P'ng XW, Akowuah GA, Chin JH. Acute oral toxicity study of Clinacanthusnutans in mice. International Journal of Pharmaceutical Sciences and Research. 2012 Nov 1;3(11):4202.

[12] Du F, Lü LJ, Fu Q, Dai M, Teng JL, Fan W, Chen SL, Ye P, Shen N, Huang XF, Qian J. T-614, a novel immunomodulator, attenuates joint inflammation and articular damage in collagen-induced arthritis. Arthritis research & therapy. 2008 Dec; 10:1-1.

[13] Ekambaram S, Perumal SS, Subramanian V. Evaluation of antiarthritic activity of Strychnospotatorum Linn seeds in Freund's adjuvant induced arthritic rat model. BMC complementary and alternative medicine. 2010 Dec; 10:1-9.

[14] Barbier A, Navarro J, Breliere JC, Roncucci R. Biochemical and clinical changes in rats with developing adjuvant arthritis. Agents and Actions. 1984 Aug;15(1):103-5.

[15] Gomes A, Bhattacharya S, Chakraborty M, Bhattacharjee P, Mishra R, Gomes A. Anti-arthritic activity of Indian monocellate cobra (Najakaouthia) venom on adjuvant induced arthritis. Toxicon. 2010 Feb 1;55(2-3):670-3.

[16] Adeneye AA, Oreagba AI, Ishola IO, Kalejaiye HA. Evaluation of the anti-arthritic activity of the hydroethanolic leaf extract of Alchorneacordifolia in rats. African journal of traditional, complementary and alternative medicines. 2014 Jul 4;11(2):402-10.