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STUDY OF ANTI ARTHRITIC ACTIVITY OF HYDROALCOHOLIC EXTRACT OF HERBAL PLANT

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ABSTRACT

Background: Rheumatoid arthritis is a chronic inflammatory disorder associated with synovial hyperplasia, cartilage degradation, and progressive joint damage. Although conventional drugs provide symptomatic relief, their prolonged use often leads to adverse effects, prompting the need for safer alternatives. Herbal medicines rich in phytochemicals have shown promise in arthritis management. **Objective:** The present study was undertaken to investigate the anti-arthritic activity of the hydroalcoholic extract of *Swertia chirata* in Freund's adjuvant-induced arthritis in rats. **Methods:** The hydroalcoholic extract of *Swertia chirata* was prepared and evaluated for percentage yield, phytochemical composition, and total phenolic and flavonoid content. Anti-arthritic activity was assessed in Wistar rats using the Freund's adjuvant-induced arthritis model, with aspirin (200 mg/kg, p.o.) as the standard reference. Paw volume was measured at regular intervals, and results were expressed as mean ± SEM (n=6). **Results:** The percentage yield of the hydroalcoholic extract was 11.5%. Phytochemical screening confirmed the presence of flavonoids, phenolic compounds, carbohydrates, saponins, diterpenes, and alkaloids. The extract contained total phenolic content of 0.63 mg GAE/100 mg and total flavonoid content of 0.97 mg QE/100 mg. In vivo evaluation revealed that the extract significantly and dose-dependently reduced paw edema in arthritic rats. The 200 mg/kg dose produced marked inhibition of paw swelling ($P<0.001$), comparable to aspirin. **Conclusion:** The hydroalcoholic extract of *Swertia chirata* demonstrated significant anti-arthritic activity, likely due to its flavonoids, phenolics, and diterpenes contributing to anti-inflammatory and antioxidant effects. These findings suggest that *Swertia chirata* may serve as a potential natural therapeutic agent for the management of arthritis.

KEYWORDS: *Swertia chirata*; Hydroalcoholic extract; Anti-arthritic activity; Freund's adjuvant; Phytochemical screening; Flavonoids; Phenolic compounds.

INTRODUCTION

Arthritis is a chronic musculoskeletal disorder characterized by inflammation of the joints, progressive cartilage destruction, stiffness, swelling, and functional disability. Among its various types, rheumatoid arthritis (RA) is the most prevalent autoimmune form, affecting approximately 1% of the global population and significantly impairing quality of life (Smolen et al., 2016). The pathophysiology of arthritis involves complex interactions between genetic, immunological, and environmental factors, leading to synovial hyperplasia, release of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6), as well as increased oxidative stress and cartilage degradation (Aletaha & Smolen, 2018).

Conventional pharmacotherapy for arthritis mainly includes nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying antirheumatic drugs (DMARDs). Although these agents provide symptomatic relief, their long-term use is often associated with serious adverse effects such as gastrointestinal ulcers, hepatotoxicity, nephrotoxicity, and increased susceptibility to infections (Singh et al., 2016). Therefore, there is an urgent need for safer and more effective therapeutic alternatives that can modulate inflammatory processes and provide disease-modifying potential.

Medicinal plants have historically played a vital role in the treatment of inflammatory disorders, and growing evidence highlights their potential in arthritis management. Hydroalcoholic extracts, prepared using a combination of water and alcohol as solvents, are

particularly effective because they solubilize a wide spectrum of bioactive phytoconstituents, including flavonoids, alkaloids, tannins, saponins, and phenolic compounds (Ekor, 2014). These phytochemicals are reported to exert anti-inflammatory, antioxidant, and immunomodulatory activities, which are central to controlling the pathogenesis of arthritis (Gupta et al., 2017).

Several preclinical studies have demonstrated the promising anti-arthritic activity of hydroalcoholic extracts of various herbal plants. For instance, the hydroalcoholic extract of *Moringa oleifera* flowers showed significant inhibition of protein denaturation and membrane stabilization, suggesting potent anti-arthritic effects (Mahajan et al., 2009). Similarly, the hydroalcoholic extract of *Bauhinia purpurea* exhibited protective effects against complete Freund's adjuvant (CFA)-induced arthritis in rats, attributed to its flavonoid and polyphenol content (Ali et al., 2019). The hydroalcoholic root extract of *Hemidesmus indicus* reduced paw swelling, rheumatoid factor, and oxidative stress markers in arthritic models, thereby confirming its therapeutic relevance (Sundaram et al., 2012).

In addition, extracts of *Alchornea cordifolia* and *Ferula persica* have been reported to reduce paw edema, improve hematological parameters, and restore antioxidant enzyme levels in experimental arthritis, supporting the hypothesis that phytoconstituents act synergistically to regulate inflammation and joint damage (Okoye et al., 2014; Karami et al., 2019). These findings strengthen the rationale for investigating hydroalcoholic extracts of herbal plants as potential anti-arthritic agents.

Hence, the present research is aimed at exploring the anti-arthritic activity of hydroalcoholic extracts of selected herbal plants, with an emphasis on their ability to suppress inflammation, oxidative stress, and cartilage degradation. Such studies may provide scientific evidence for their therapeutic use and contribute to the development of novel, plant-based interventions for arthritis.

MATERIAL AND METHODS

MATERIAL

The present study utilized various analytical grade chemicals and reagents procured from reputed suppliers. Potassium mercuric iodide, picric acid, ferric chloride, and chloroform were obtained from Thomas Baker, Mumbai, while iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, ethanol, Folin-Ciocalteu reagent, and pyridine were purchased from Loba Chemie Pvt. Ltd., Mumbai. Potassium bismuth iodide, gelatin, nitric acid, copper acetate, and sodium chloride were supplied by S. D. Fine Chem. Ltd., Mumbai. Methanol, ethanol, and chloroform were procured from Qualigens Fine Chemicals, Mumbai, and Fehling's solution was obtained from Central Drug House Ltd., New Delhi. All chemicals used were of analytical grade to ensure accuracy and reliability of the experimental work.

METHODS

Extraction by maceration process

50 gm dried powdered aerial parts of *Swertia chirata* has been extracted with hydroalcoholic solvent (ethanol: water; 80:20) using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40ºC.

Determination of percentage yield

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

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Phytochemical Screening

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (alkaloids, terpenoids, phenols, flavonoids etc.). The chemical tests were performed for testing different chemical groups present in extracts (Kokate, 1994).

Estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Mishra *et al.*, 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5- 25µg/ml was prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 2 ml (1mg/ml) of this extract was for the estimation of Phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Mishra *et al.*, 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10mg of dried extract of plant material was extracted with 10 ml methanol and filter. 3 ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and

allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

In-vivo anti-arthritis activity of *Swertia chirata* extract

Animals

Albino Wistar rats of either sex (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rat was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (Gothe *et al.*, 2023). Hydroalcoholic extract of *Swertia chirata* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-arthritic effect.

Anti-arthritis activity

Freund's adjuvant induced arthritis in rats: Animals were divided into five groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw (Rajaram *et al.*, 2015).

Group I served as normal and received 2% gum acacia
Group II served as arthritis control-untreated received 2% gum acacia,
Group III received Aspirin (200 mg/kg p.o) served as reference standard
Group IV received extract of hydroalcoholic extract of *Swertia chirata* of doses of 100mg/kg p.o.
Group V received extract of hydroalcoholic extract of *Swertia chirata* of doses of 200mg/kg p.o.

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

Statistical analysis

The values were expressed as mean ± SEM (n=6). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test and $P<0.05$, $P<0.01$, and $P<0.001$ were considered to be statistically significant.

RESULTS AND DISCUSSION

The present study evaluated the anti-arthritic potential of the hydroalcoholic extract of *Swertia chirata* in a Freund's adjuvant-induced arthritis model in rats. The percentage yield of the hydroalcoholic extract was found to be 11.5%, which is in agreement with previous reports suggesting that hydroalcoholic solvents are efficient in extracting diverse phytoconstituents including alkaloids, flavonoids, phenols, saponins, and diterpenes (Table 1 and Table 2). These phytochemicals are well documented for their anti-inflammatory, antioxidant, and immunomodulatory activities, which play a crucial role in arthritis management.

The phytochemical screening revealed the presence of flavonoids, phenolic compounds, proteins, carbohydrates, saponins, and diterpenes in the extract. The positive results in Dragendorff's and Hager's tests indicated the presence of alkaloids, while flavonoids and phenols were confirmed through the lead acetate and $FeCl_3$ tests, respectively. Flavonoids and phenolic compounds are particularly important, as they are known to inhibit cyclooxygenase (COX) and lipoxygenase (LOX) pathways, reduce reactive oxygen species (ROS), and modulate pro-inflammatory cytokines such as TNF-α and IL-1β.

Quantitative estimation revealed that the hydroalcoholic extract contained total phenolic content of 0.63 mg GAE/100 mg and total flavonoid content of 0.97 mg QE/100 mg (Table 3). Although these values appear modest, they may significantly contribute to the observed pharmacological effects due to synergistic interactions among different phytoconstituents.

In the Freund's adjuvant-induced arthritis model, rats in the arthritis control group showed a progressive increase in paw volume throughout the experimental period, confirming the successful induction of arthritis. Treatment with aspirin (200 mg/kg) produced a significant reduction in paw edema from day 14 onwards, serving as the standard reference drug. Importantly, *Swertia chirata* hydroalcoholic extract exhibited a dose-dependent anti-arthritic effect (Table 4; Figure 1). At a dose of 100 mg/kg, the extract significantly reduced paw swelling ($P<0.05$ on day 14; $P<0.01$ on day 21 and day 28), whereas the higher dose of 200 mg/kg showed greater inhibition, comparable to aspirin ($P<0.001$ on day 21 and day 28).

The observed anti-arthritic effect can be attributed to the synergistic action of flavonoids, phenols, and diterpenes present in the extract. Flavonoids have been reported to downregulate NF-κB signaling and reduce inflammatory mediators, while phenolic compounds enhance antioxidant defenses by scavenging free radicals and preventing oxidative stress-mediated joint damage. The

presence of diterpenes further supports anti-inflammatory action through inhibition of cytokine release and suppression of prostaglandin synthesis.

Table 1: % Yield of hydroalcoholic extract of *Swertia chirata*.

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	11.5%

Table 2: Phytochemical screening of hydroalcoholic extract of *Swertia chirata*.

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Dragendroff's test Hager's test	 -ve +ve
2.	**Flavonoids** Lead acetate Alkaline test	 +ve +ve
3.	**Phenol** $FeCl_3$	 +ve
4.	**Proteins and Amino acids** Xanthoproteic test	 +ve
5.	**Carbohydrates** Fehling's test	 +ve
6.	**Saponins** Foam test	 +ve
7.	**Diterpenes** Copper acetate test	 +ve

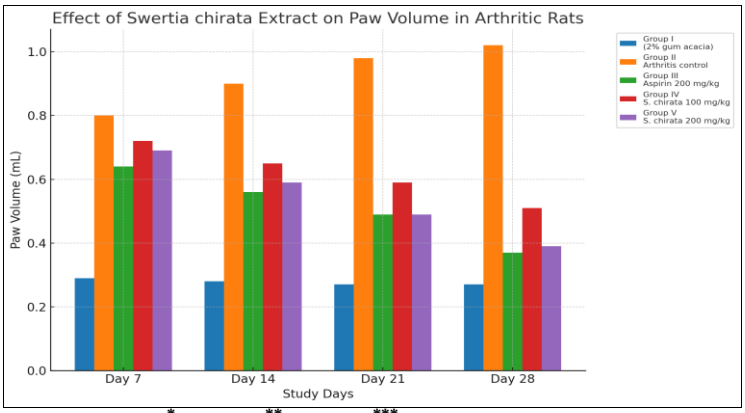
[+ve=Positive; -ve= Negative]

Table 3: Total phenolic and total flavonoid content of *Swertia chirata*.

S. No.	Extract	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydroalcoholic extract	0.63	0.97

Table 4: Anti-arthritis activity of hydroalcoholic extract of *Swertia chirata* against Freund's adjuvant induced arthritis in rats.

Group	Day 7	Day 14	Day 21	Day 28
Group I – 2% gum acacia	0.29 ± 0.05	0.28 ± 0.04	0.27 ± 0.03	0.27 ± 0.02
Group II – Arthritis control	0.80 ± 0.12	0.90 ± 0.15	0.98 ± 0.18	1.02 ± 0.19
Group III – Aspirin (200 mg/kg, p.o.)	0.64 ± 0.08	0.56 ± 0.09 **	0.49 ± 0.11 ***	0.37 ± 0.09 ***
Group IV – *Swertia chirata* extract (100 mg/kg, p.o.)	0.72 ± 0.10	0.65 ± 0.11 *	0.59 ± 0.10 **	0.51 ± 0.07 **
Group V – *Swertia chirata* extract (200 mg/kg, p.o.)	0.69 ± 0.08 *	0.59 ± 0.09 **	0.49 ± 0.08 ***	0.39 ± 0.05 ***



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

Figure 1: Anti-arthritis activity of hydroalcoholic extract of *Swertia chirata* against Freund's adjuvant induced arthritis in rats.

CONCLUSION

The present investigation demonstrated that the hydroalcoholic extract of *Swertia chirata* possesses significant anti-arthritic activity in the Freund's adjuvant-induced arthritis model in rats. The extract showed a yield of 11.5% and was found to contain bioactive phytoconstituents such as flavonoids, phenolic compounds, saponins, diterpenes, and alkaloids. Quantitative analysis revealed the presence of phenols and flavonoids, which are known for their anti-inflammatory and antioxidant properties. In vivo studies confirmed that the extract produced a dose-dependent reduction in paw edema, with the 200 mg/kg dose showing effects comparable to the standard drug aspirin. The anti-arthritic action can be attributed to the synergistic effects of its phytochemicals, which likely act by modulating inflammatory mediators and enhancing antioxidant defense mechanisms. The findings suggest that *Swertia chirata* hydroalcoholic extract could serve as a promising natural therapeutic option for the management of arthritis. However, further studies involving detailed mechanistic investigations, chronic toxicity evaluations, and clinical trials are warranted to validate its efficacy and safety for human use.

REFERENCES

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet*., 2016; 388(10055): 2023–2038.
2. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A review. *JAMA*. 2018; 320(13): 1360–1372.
3. Singh JA, Saag KG, Bridges SL, et al. 2015 American College of Rheumatology Guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol*. 2016; 68(1): 1–26.
4. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front Pharmacol*. 2014; 4: 177.
5. Gupta SC, Tyagi AK, Deshmukh-Taskar P, et al. Downregulation of inflammation by nutraceuticals. *Pharmacol Res*. 2017; 119: 91–116.
6. Mahajan SG, Mehta AA. In vitro evaluation of the anti-arthritic activity of hydroalcoholic extract of *Moringa oleifera* flowers. *Pharmacologyonline*. 2009; 2: 1029–1045.
7. Ali N, Jahan S, Sultana S. Anti-arthritic potential of *Bauhinia purpurea* Linn. bark extract in adjuvant-induced arthritis rat model. *Phcog Mag*. 2019; 15(61): 93–99.
8. Sundaram R, Shanthi P, Sachdanandam P. Effect of *Hemidesmus indicus* on adjuvant-induced arthritis in rats: Biochemical and histopathological studies. *Inflammopharmacology*. 2012; 20(2): 99–107.
9. Okoye TC, Akah PA, Omeje EO, et al. Antiinflammatory and anti-arthritic activities of hydroethanolic extract of *Alchornea cordifolia* leaves. *J Ethnopharmacol*. 2014; 152(3): 471–479.
10. Karami M, Rahimi-Moghaddam P, Zarei M, et al. Anti-arthritic activity of *Ferula persica* extract in experimental models. *Iran J Basic Med Sci*. 2019; 22(5): 571–577.
11. Kokate, C.K. Ed. Practical Pharmacognosy, 4th Edn., Vallabh Prakashan, 1994; 112(20).
12. Mishra AG, Singh R, Meha P, Parkhe G. Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of Gloriosa superba seed extract. Asian J Pharm Educ Res. 2017; 6(2): 12-7.
13. Gothe Shivani Rameshwar, Pawade Uday Venkatrao, Nikam Ashwin Vithalrao, Anjankar Meghsham Pramodrao. OECD guidelines for acute oral toxicity studies: An overview. Int. J. Res. Ayurveda Pharm.,. 2023; 14(4): 137-140.
14. Rajaram C, Ravindra Reddy K, Bonnth Chandra Sekhar K. Evaluation of antiarthritic activity of Caesalpinia pulcherimma in Freund's complete adjuvant induced arthritic rat model. J Young Pharmacists., 2015; 7: 128–132.