CODEN: WJAPAC Impact Factor: 5.48 ISSN: 3049-3013

World Journal of Advance Pharmaceutical Sciences

Volume 3, Issue 6, Page: 175-179 Research Article www.wjaps.com

EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITIC POTENTIAL OF ANTIDESMA DIANDRUM EXTRACT

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How to cite this Article: Anupam Gautam*[1], Sunil Shah[1], B. K. Dubey[2], Deepak Basedia[2]. (2026). EXTRACTION, PHYTOCHEMICAL SCREENING AND ANTI-ARTHRITIC POTENTIAL OF ANTIDESMA DIANDRUM EXTRACT. World Journal of Advance Pharmaceutical Sciences, 3(6), 175-179.

Article Info

Article Received: 19 April 2026,

Article Revised: 09 May 2026,

Article Accepted: 29 May 2026.

DOI: https://doi.org/10.5281/zenodo.20581316

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ABSTRACT

The present study was carried out to evaluate the extraction yield, phytochemical constituents, and anti-arthritic potential of the hydroalcoholic extract of *Antidesma diandrum*. The plant material was subjected to hydroalcoholic extraction, and the percentage yield was determined. Preliminary phytochemical screening of the extract revealed the presence of flavonoids, phenols, proteins, carbohydrates, and saponins. Quantitative analysis showed a total phenolic content of 0.263 mg gallic acid equivalent (GAE)/100 mg and a total flavonoid content of 0.742 mg quercetin equivalent (QE)/100 mg of extract. The anti-arthritic activity was evaluated using Freund's adjuvant-induced arthritis model in rats. The hydroalcoholic extract was administered orally at doses of 100 mg/kg and 200 mg/kg and compared with aspirin (200 mg/kg) as a standard drug. The extract produced a significant, dose-dependent reduction in paw edema, particularly at the higher dose, which showed activity comparable to the standard drug. The observed anti-arthritic effect may be attributed to the presence of flavonoids and phenolic compounds with known anti-inflammatory properties. The results of the study scientifically support the traditional use of *Antidesma diandrum* in the management of inflammatory and arthritic conditions.

KEYWORDS: *Antidesma diandrum*; Hydroalcoholic extract; Phytochemical screening; Anti-arthritic activity; Freund's adjuvant-induced arthritis; Flavonoids; Phenolic compounds.

INTRODUCTION

Antidesma diandrum (synonym of *Antidesma acidum*), a member of the Phyllanthaceae family, is a tropical shrub/small tree traditionally used in various rural communities for its medicinal virtues, including treatment of muscular pain, dysentery, and other inflammatory conditions through consumption of tender leaves or decoctions of plant parts, indicating potential ethnomedicinal relevance in pain-related disorders (Nguyen et al., 2024).

Plants of the Antidesma genus have long been valued in traditional medicine across Asia and Africa, wherein different species are employed to manage a wide range of ailments such as rheumatic pains, gastrointestinal disorders, fever, diabetes, and infections (Pakdee and Poowanna; 2025). Phytochemical investigations in several *Antidesma* species have revealed the presence of diverse bioactive constituents including alkaloids, flavonoids, phenolics, terpenoids, sterols, and coumarins, which are known to contribute to anti-inflammatory and therapeutic effects in chronic diseases (Joshi et al., 2023).

Inflammation and immune dysregulation are central to the pathophysiology of arthritis, and natural products rich in secondary metabolites have been investigated extensively for anti-arthritic potential due to their ability to reduce oxidative stress and modulate inflammatory mediators. Studies on related species in the genus have

demonstrated significant anti-inflammatory and analgesic activity in crude extracts, supporting the rationale for further evaluation of *Antidesma diandrum* extract for anti-arthritic activity (Sharma and Goel; 2023).

Despite the promising traditional uses and broad pharmacological profile of the Antidesma genus, specific scientific studies focusing on the extraction, phytochemical screening, and anti-arthritic potential of *Antidesma diandrum* remain limited. Therefore, this study aims to systematically investigate the ethanolic extract of *A. diandrum* for its phytochemical composition and anti-arthritic potential, providing scientific validation for its traditional use and exploring its utility for development of novel anti-arthritic agents.

MATERIAL AND METHODS

Extraction procedure

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs (Mukherjee, 2007):

Extraction by soxhlet extraction process

50 gm dried powdered fruits of *Antidesma diandrum* has been extracted with hydroalcoholic solvent (ethanol: water; 75:25) 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 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

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 (Parkhe, Deepak Bharti, 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/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 *Antidesma diandrum* 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.

Chemicals

Freund's complete adjuvant (Sigma-Aldrich Chemical Co.) was used for experiments.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (Gothe *et al.,* 2023). Hydroalcoholic extract of *Antidesma diandrum* (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.

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 *Antidesma diandrum* of doses of 100mg/kg p.o.
Group V received extract of hydroalcoholic extract of *Antidesma diandrum* 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) (Sanmuga *et al.*, 2010). 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 was undertaken to evaluate the extraction yield, phytochemical composition, and anti-arthritic potential of the hydroalcoholic extract of *Antidesma diandrum*, with the objective of providing scientific support for its traditional use in inflammatory disorders.

The percentage yield of the hydroalcoholic extract was found to be 8.92% w/w**,** indicating that the hydroalcoholic solvent system was effective in extracting a considerable amount of bioactive constituents from the plant material. Hydroalcoholic solvents are well known for their ability to solubilize both polar and moderately non-polar phytoconstituents, which may explain the satisfactory extraction yield obtained.

Preliminary phytochemical screening revealed the presence of several important secondary metabolites. The extract showed positive results for flavonoids, phenols, proteins, carbohydrates, and saponins, while alkaloids, diterpenes, amino acids, and glycosides were absent. The presence of flavonoids and phenolic compounds is particularly significant, as these compounds are widely reported to possess anti-inflammatory, antioxidant, and immunomodulatory properties**,** which play a crucial role in the management of arthritis. Saponins are also known to exhibit anti-inflammatory and membrane-stabilizing effects, which may contribute to the observed pharmacological activity.

Quantitative estimation further supported the phytochemical findings. The total phenolic content was found to be 0.263 mg GAE/100 mg**,** while the total flavonoid content **was** 0.742 mg QE/100 mg of extract. Although the phenolic content was moderate, the comparatively higher flavonoid content suggests that flavonoids may be the major contributors to the biological activity of the extract. Flavonoids are known to inhibit inflammatory mediators such as prostaglandins, cytokines, and nitric oxide, which are implicated in the pathogenesis of rheumatoid arthritis.

The anti-arthritic activity of the hydroalcoholic extract was evaluated using Freund's adjuvant-induced arthritis in rats, a well-established experimental model that closely mimics human rheumatoid arthritis. The arthritis control group showed a progressive increase in paw edema from day 7 to day 28, confirming successful induction of chronic inflammation. In contrast, treatment with the extract at doses of 100 mg/kg and 200 mg/kg resulted in a significant and dose-dependent reduction in paw volume.

The higher dose (200 mg/kg) exhibited a more pronounced inhibitory effect on paw edema, comparable to the standard drug aspirin (200 mg/kg)**,** particularly on days 21 and 28. The statistically significant reduction **(**$P < 0.01$ and $P < 0.001$**)** indicates the strong anti-inflammatory and anti-arthritic potential of the extract. This effect may be attributed to the synergistic action of flavonoids, phenols, and saponins present in the extract, which may suppress inflammatory cell infiltration, stabilize lysosomal membranes, and reduce the release of pro-inflammatory mediators.

The findings of the present study demonstrate that the hydroalcoholic extract of *Antidesma diandrum* possesses significant anti-arthritic activity, supported by its rich phytochemical profile. The results validate its traditional use in inflammatory conditions and suggest that *A. diandrum* could serve as a promising natural source for the development of safer anti-arthritic agents. Further studies focusing on isolation of active constituents and elucidation of the exact mechanism of action are warranted.

Table 1: % Yield of hydroalcoholic extract of *Antidesma diandrum*.

S. No.	Extract	% Yield (w/w)
1.	Hydroalcoholic	8.92%

Table 2: Phytochemical screening of hydroalcoholic extract of *Antidesma diandrum*.

S. No.	Phytochemical Test	Result
1	**Alkaloids** Hager's test	 –ve
2	**Flavonoids** Lead acetate test Alkaline reagent test	 +ve -ve
3	**Phenols** Ferric chloride test	 +ve

4	**Proteins** Biuret's test	+ve
5	**Carbohydrates** Fehling's test	+ve
6	**Saponins** Foam test	+ve
7	**Diterpenes** Copper acetate test	-ve
8	**Amino acids** Ninhydrin test	–ve
9	**Glycosides** Legal's test	–ve

[+ve=Positive; -ve= Negative]

Table 3: Total phenolic and total flavonoid content of *Antidesma diandrum*.

S. No.	Extract	Total phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	Hydroalcoholic extract	0.263	0.742

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

Group	Treatment	Day 7	Day 14	Day 21	Day 28
Group I	2% Gum acacia (Normal control)	0.29 ± 0.55	0.27 ± 0.50	0.28 ± 0.45	0.28 ± 0.35
Group II	Arthritis control	0.79 ± 0.15	0.89 ± 0.20	0.96 ± 0.22	0.98 ± 0.33
Group III	Aspirin (200 mg/kg, p.o.)	0.65 ± 0.10	0.58 ± 0.15**	0.57 ± 0.30***	0.40 ± 0.35***
Group IV	Extract 100 mg/kg, p.o.	0.73 ± 0.15	0.64 ± 0.15*	0.62 ± 0.20*	0.55 ± 0.15*
Group V	Extract 200 mg/kg, p.o.	0.70 ± 0.32**	0.68 ± 0.18**	0.55 ± 0.25***	0.42 ± 0.15***

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

CONCLUSION

The present investigation concludes that the hydroalcoholic extract of *Antidesma diandrum* possesses significant anti-arthritic activity, as evidenced by a dose-dependent reduction in paw edema in Freund's adjuvant-induced arthritic rats. Phytochemical screening and quantitative analysis confirmed the presence of bioactive constituents such as flavonoids, phenolic compounds, saponins, proteins, and carbohydrates, which are known to contribute to anti-inflammatory and antioxidant effects. The extract at a dose of 200 mg/kg demonstrated pronounced efficacy, comparable to the standard drug aspirin, indicating its therapeutic potential. These findings provide scientific validation for the traditional use of *Antidesma diandrum* in inflammatory disorders and suggest that the plant may serve as a promising natural source for the development of safer anti-arthritic agents.

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