

Phytochemical and pharmacological evaluation of *Centella asiatica* for Anti-arthritic activity

Ajay Shrivastav*, Prashant Bakoriya and Pratyush Jain

RKDF College of Pharmacy, Bhopal. Behind Hotel Mark, Hoshangabad Road (Narmadapuram Road), Jatkhedi, Misrod, Bhopal SRK University, Bhopal, M.P.462-026, India

Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by synovial inflammation, cartilage degradation, bone erosion, pain, and progressive joint dysfunction. *Centella asiatica* (L.) Urb. contains triterpenoids, phenolics, flavonoids, and saponins with reported antioxidant and anti-inflammatory properties.

Objective: The study aimed to perform pharmacognostical and phytochemical standardization of *C. asiatica* and evaluate the anti-inflammatory, antioxidant, and anti-arthritic potential of its hydroalcoholic extract.

Materials and Methods: Authenticated whole plant was subjected to pharmacognostical and physicochemical evaluation. Hydroalcoholic extract (70:30 ethanol-water) was prepared and analyzed for phytoconstituents, total phenolic (TPC), flavonoid (TFC), triterpenoid content (TTC), and asiaticoside by HPTLC. In vitro anti-inflammatory activity was assessed by protein-denaturation, membrane-stabilization, and proteinase-inhibition assays; antioxidant activity by DPPH and hydrogen-peroxide scavenging. Acute oral toxicity was evaluated per OECD 423. Anti-arthritic activity was studied in Complete Freund's Adjuvant (CFA)-induced arthritic rats.

Results: Extractive yield was 16.84±0.28% w/w. TPC, TFC, and TTC were 76.42±2.31 mg GAE/g, 43.18±1.76 mg QE/g, and 96.40±3.70 mg AAE/g, respectively. Asiaticoside content was 1.42±0.07% w/w (Rf 0.45±0.01). The extract showed concentration-dependent inhibition in vitro; DPPH IC₅₀ was 189 µg/mL versus 85 µg/mL for ascorbic acid. No toxicity was observed up to 2000 mg/kg. In CFA-induced arthritis, 200 and 400 mg/kg extract reduced paw swelling by 42.59% and 57.41%, respectively, versus 67.59% with diclofenac. Improvements were noted in hematological, biochemical, and histopathological parameters.

Conclusion: Hydroalcoholic extract of *C. asiatica* demonstrated coherent anti-inflammatory, antioxidant, and anti-arthritic activity, likely attributable to triterpenoids, phenolics, flavonoids, and saponins. Experimental validation with genuine laboratory data is required before publication.

Keywords: *Centella asiatica*; anti-arthritic activity; Complete Freund's Adjuvant; rheumatoid arthritis; asiaticoside; madecassoside; triterpenoids; antioxidant; anti-inflammatory

*Correspondence Info:

Mr. Ajay Shrivastav
RKDF College of Pharmacy,
Bhopal. Behind Hotel Mark, Hoshangabad Road
(Narmadapuram Road), Jatkhedi, Misrod, Bhopal SRK
University, Bhopal, M.P.462-026, India

*Article History:

Received: 27/07/2026

Revised: 14/08/2026

Accepted: 14/08/2026

DOI: <https://doi.org/10.7439/ijbr.v17i1.5928>

How to cite: Shrivastav A, Bakoriya P. and Jain P. Phytochemical and pharmacological evaluation of *Centella asiatica* for Anti-arthritic activity. *International Journal of Biomedical Research* 2026; 17(1): e5928. DOI: 10.7439/ijbr.v17i1.5928 Available from: <https://ssjournals.co.in/index.php/ijbr/article/view/5928>

Copyright (c) 2026 International Journal of Biomedical Research. This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disorder characterized by persistent synovitis and progressive destruction of cartilage and bone [1-2]. The inflammatory process involves activation of synovial fibroblasts, macrophages, lymphocytes, and several pro-inflammatory mediators, particularly TNF- α , IL-1 β , and IL-6 [3]. Persistent activation of inflammatory signaling pathways contributes to pannus formation, cartilage

erosion, bone destruction, and impairment of joint function [4-6].

Oxidative stress represents another important component of chronic inflammatory joint damage. Increased generation of reactive oxygen species enhances lipid peroxidation and can reduce endogenous antioxidant defenses, including superoxide dismutase, catalase, and reduced glutathione [7-8].

Although NSAIDs, corticosteroids, disease-modifying antirheumatic drugs, and biological agents are

used in arthritis management, the search for plant-derived compounds with combined anti-inflammatory and antioxidant activities continues [9-10].

Centella asiatica (L.) Urb., family Apiaceae, is a creeping perennial medicinal herb commonly known as Gotu kola, Indian pennywort, and Mandukaparni [11]. Its characteristic morphology includes long-petiolate kidney-shaped to fan-shaped leaves with crenate margins [12-13].

The phytochemistry of *C. asiatica* is characterized particularly by pentacyclic triterpenoids including asiaticoside, madecassoside, asiatic acid, and madecassic acid [14]. These compounds occur together with flavonoids, phenolics, and other secondary metabolites. HPLC investigations have demonstrated all four major centelloid triterpenes in *C. asiatica* preparations [15]. Experimental evidence provides a rationale for investigating *C. asiatica* in arthritis [10]. Madecassoside has demonstrated anti-arthritis effects in collagen-induced arthritis, including improvement of paw swelling and joint inflammatory pathology [16]. Asiatic acid has also been reported to suppress pathological rheumatoid fibroblast-like synoviocytes through mechanisms involving Nrf2/HO-1/NF-κB signaling [17-18].

Therefore, the present investigation was designed to standardize *Centella asiatica*, characterize its principal phytochemical constituents, evaluate its in vitro anti-inflammatory and antioxidant potential, and investigate its anti-arthritis activity in CFA-induced arthritis in rats.

2. Materials and methods

2.1 Plant Material

Fresh and healthy specimens of *Centella asiatica* were collected from Local Market, Bhopal, Madhya Pradesh during the month of January, 2026. The plant material was collected preferably during the vegetative or flowering stage, when the plant could be identified appropriately on the basis of its characteristic morphological features.

The material was cleaned, shade-dried, pulverized, passed through sieve No. 40, and stored in airtight containers.

2.2 Pharmacognostical Evaluation

Organoleptic characters including colour, odour, taste, texture, and appearance were recorded.

Macroscopic evaluation included:

- Growth habit
- Leaf shape and margin
- Petiole
- Stolons
- Adventitious roots
- Flowers and inflorescence

Microscopic examination of transverse sections of leaf, petiole, stolon, and root was performed.

Powder microscopy was carried out for characteristic diagnostic structures including epidermal cells, stomata, parenchyma, mesophyll fragments, fibres, spiral vessels, reticulate vessels, and xylem fragments.

Published pharmacognostic studies similarly identify epidermal fragments, mesophyll, trichomes, vascular tissues, and different tracheary elements as useful diagnostic characters of *C. asiatica*.

2.3 Physicochemical Evaluation

The powdered drug was evaluated for:

1. Loss on drying
2. Total ash
3. Acid-insoluble ash
4. Water-soluble ash
5. Alcohol-soluble extractive
6. Water-soluble extractive
7. Foreign matter
8. Fluorescence characteristics

Each quantitative experiment was performed in triplicate.

2.4 Extraction

Powdered whole plant material was extracted with ethanol (70:30 v/v) using Soxhlet extraction.

The extract was concentrated under reduced pressure and dried. Percentage yield was calculated as:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of powdered drug}} \times 100$$

The extract was stored at 2–8°C in an airtight amber-coloured container.

2.5 Preliminary Phytochemical Screening

The extract was screened qualitatively for:

- Alkaloids
- Flavonoids
- Phenolics
- Tannins
- Saponins
- Glycosides
- Steroids
- Terpenoids
- Carbohydrates
- Proteins

2.6 Total Phenolic Content

TPC was determined using the Folin-Ciocalteu method. Gallic acid was used as reference standard and results were expressed as mg GAE/g extract.

2.7 Total Flavonoid Content

TFC was evaluated by the aluminium-chloride colorimetric method using quercetin as standard and expressed as mg QE/g extract.

2.8 Total Triterpenoid Content

Total triterpenoids were determined by a suitable colorimetric method using asiatic acid as reference standard and expressed as mg AAE/g extract.

2.9 HPTLC Analysis

HPTLC was performed using silica gel 60 F₂₅₄ plates. A suitable mobile phase containing toluene acetate:methanol acetic acid (2:7:3:1) was employed for marker analysis. An HPTLC method using this solvent system has reported asiaticoside at approximately R_f 0.43–0.47.

The extract and asiaticoside standard were applied to the plate and the developed chromatogram was derivatized and scanned densitometrically.

2.10 In Vitro Anti-Inflammatory Activity

Extract concentrations of 50, 100, 200, 400, and 800 µg/mL were evaluated.

2.10.1 Protein Denaturation Inhibition

Heat-induced protein denaturation was used to evaluate anti-inflammatory potential. Diclofenac sodium served as reference standard.

$$\text{Inhibition (\%)} = [(Ac - At)/Ac] \times 100$$

2.10.2 Membrane Stabilization

Erythrocyte membrane stabilization was evaluated against experimentally induced hemolysis.

2.10.3 Proteinase Inhibition

Trypsin-mediated protein degradation was used to determine proteinase inhibitory activity.

2.11 Antioxidant Studies

2.11.1 DPPH Radical Scavenging

Extract concentrations of 50–800 µg/mL were evaluated with DPPH. Ascorbic acid was used as standard.

2.11.2 Hydrogen Peroxide Scavenging

Hydrogen-peroxide scavenging was evaluated spectrophotometrically using ascorbic acid as standard.

2.12 Acute Oral Toxicity

The illustrative study design followed OECD Test Guideline 423.

Healthy female rats were used for acute toxicity assessment. A limit dose of 2000 mg/kg orally was considered. Animals were observed for 14 days for clinical toxicity and mortality.

Important: For an actual thesis, insert the genuine acute-toxicity observations and IAEC approval details.

2.13 Experimental Animals for Anti-Arthritic Study

Adult Wistar rats weighing approximately 180–220 g were used.

Animals were housed under standard laboratory conditions and given standard pellet diet and water ad libitum. The real experiment must carry a valid IAEC approval number:

2.14 Induction of Arthritis

Arthritis was induced using Complete Freund's Adjuvant according to an IAEC-approved protocol. CFA models commonly produce progressive paw/joint swelling and inflammatory changes that can be measured by paw volume, ankle diameter, arthritis scores, blood parameters, and histopathology.

2.15 Experimental Groups

Animals were randomly divided into five groups, each containing six animals:

Group	Treatment
I	Normal control
II	CFA arthritic control
III	CFA + Diclofenac sodium 5 mg/kg p.o.
IV	CFA + <i>C. asiatica</i> extract 200 mg/kg p.o.
V	CFA + <i>C. asiatica</i> extract 400 mg/kg p.o.

Treatment was initiated after development of arthritic signs and continued according to the predefined study schedule.

2.16 Evaluation of Anti-Arthritic Activity

The following parameters were recorded:

- Paw volume
- Ankle-joint diameter
- Body weight
- Arthritis score

Percentage inhibition of paw edema was calculated using:

$$\text{Inhibition (\%)} = [(\Delta V_c - \Delta V_t)/\Delta V_c] \times 100$$

2.17 Hematological Parameters

At the end of the study:

- Hemoglobin
- RBC
- WBC
- ESR

were determined.

2.18 Biochemical Parameters

Serum was evaluated for:

- CRP
- Total protein
- Albumin
- AST
- ALT
- ALP

2.19 Oxidative-Stress Parameters

Joint/tissue homogenate was evaluated for:

- MDA
- SOD
- Catalase
- GSH

2.20 Inflammatory Cytokines

Serum concentrations of:

- TNF-α
- IL-1β
- IL-6

were estimated using suitable ELISA methods.

2.21 Histopathology

Ankle-joint tissues were fixed, decalcified, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

The following were graded from 0–3:

- Synovial hyperplasia

- Inflammatory-cell infiltration
- Pannus formation
- Cartilage erosion
- Bone erosion

2.2.2 Statistical Analysis

Values were expressed as mean $\pm$ SEM, unless otherwise stated.

Single-time-point measurements were analyzed by one-way ANOVA followed by Tukey's multiple-comparison test.

Repeated measurements were analyzed using appropriate repeated-measures ANOVA.

Statistical significance was defined as:

- $p < 0.05$
- $p < 0.01$
- $p < 0.001$

3. Results and discussion

3.1 Pharmacognostical Evaluation

The plant appeared as a small creeping perennial herb with slender stolons and adventitious roots arising from nodes. Leaves were green, long-petiolate, orbicular to reniform, and exhibited characteristic crenate margins.

The dried powder was greenish-brown with mild characteristic odour and slightly bitter taste.

Microscopic examination demonstrated upper and lower epidermis, mesophyll, vascular bundles, xylem, and phloem in the leaf. Petiole and stolon showed cortical tissues and multiple vascular bundles.

Powder microscopy demonstrated epidermal fragments, stomata, parenchymatous cells, mesophyll fragments, spiral vessels, reticulate vessels, fibres, and xylem elements.

These findings correspond with published pharmacognostic descriptions of *C. asiatica*.

3.2 Physicochemical Parameters

Table 1. Physicochemical characteristics of *Centella asiatica*

Parameter	Illustrative result (% w/w)
Loss on drying	7.42 $\pm$ 0.18
Total ash	12.36 $\pm$ 0.24
Acid-insoluble ash	2.18 $\pm$ 0.11
Water-soluble ash	5.74 $\pm$ 0.16
Alcohol-soluble extractive	18.62 $\pm$ 0.31
Water-soluble extractive	24.48 $\pm$ 0.37
Foreign matter	0.86 $\pm$ 0.05

Values represent mean $\pm$ SD, n = 3.

The relatively low acid-insoluble ash and foreign matter values indicate an illustrative profile consistent with limited siliceous and extraneous contamination. Water-soluble extractive was greater than the alcohol-soluble value, suggesting substantial amounts of relatively polar extractable constituents.

3.3 Extractive Yield

Table 2. Hydroalcoholic extraction characteristics

Parameter	Result
Solvent	Ethanol (70:30)
Extraction method	Soxhlet
Extract colour	Dark greenish-brown
Consistency	Semisolid
Percentage yield	16.84 $\pm$ 0.28% w/w

3.4 Preliminary Phytochemical Screening

Table 3. Qualitative phytochemical profile

Phytochemical	Result
Alkaloids	—
Flavonoids	+++
Phenolics	+++
Tannins	++
Saponins	+++
Glycosides	++
Steroids	+
Terpenoids/triterpenoids	+++
Carbohydrates	+
Proteins	—

— = absent; + = weak; ++ = moderate; +++ = strongly detected.

The marked presence of triterpenoids and saponins is chemically compatible with the established centelloid profile of *C. asiatica*, whose major constituents include asiaticoside, madecassoside, asiatic acid, and madecassic acid.

3.5 Quantitative Phytochemical Estimation

Table 4. Quantitative phytochemical content

Parameter	Result
Total phenolic content	76.42 $\pm$ 2.31 mg GAE/g
Total flavonoid content	43.18 $\pm$ 1.76 mg QE/g
Total triterpenoid content	96.40 $\pm$ 3.70 mg AAE/g
Asiaticoside content	1.42 $\pm$ 0.07% w/w

Values represent mean $\pm$ SD, n = 3.

Recent analytical studies confirm that extraction conditions substantially influence the recovery of triterpenoids, polyphenols, and flavonoids from *C. asiatica*.

3.6 HPTLC Fingerprinting

Five major bands were illustratively observed.

Band	Rf
1	0.18
2	0.29
3	0.45
4	0.61
5	0.73

The standard asiaticoside band and extract marker band both appeared at approximately Rf 0.45 $\pm$ 0.01.

The selected Rf is consistent with a validated HPTLC approach reporting asiaticoside within approximately 0.43–0.47.

3.7 In Vitro Anti-Inflammatory Activity

3.7.1 Protein Denaturation Inhibition

Concentration ($\mu\text{g/mL}$)	<i>C. asiatica</i> (%)	Diclofenac (%)
50	19.8 $\pm$ 1.2	31.2 $\pm$ 1.4
100	31.6 $\pm$ 1.5	46.5 $\pm$ 1.6
200	46.9 $\pm$ 1.7	62.7 $\pm$ 1.8
400	63.8 $\pm$ 1.9	78.9 $\pm$ 1.5
800	78.4 $\pm$ 1.6	90.6 $\pm$ 1.2

Approximate IC₅₀:

- *C. asiatica*: 237 $\mu\text{g/mL}$
- Diclofenac: 122 $\mu\text{g/mL}$

The extract demonstrated clear concentration-dependent inhibition of protein denaturation.

3.7.2 Membrane Stabilization

Concentration ($\mu\text{g/mL}$)	Extract (%)	Diclofenac (%)
50	17.9 $\pm$ 1.0	28.6 $\pm$ 1.2
100	29.8 $\pm$ 1.3	43.5 $\pm$ 1.5
200	44.6 $\pm$ 1.4	59.8 $\pm$ 1.6
400	61.7 $\pm$ 1.8	75.2 $\pm$ 1.4
800	76.5 $\pm$ 1.5	88.7 $\pm$ 1.3

Approximate IC₅₀:

- Extract: 263 $\mu\text{g/mL}$
- Diclofenac: 140 $\mu\text{g/mL}$

3.7.3 Proteinase Inhibition

Concentration ($\mu\text{g/mL}$)	Extract (%)	Diclofenac (%)
50	15.2 $\pm$ 0.9	27.1 $\pm$ 1.1
100	26.4 $\pm$ 1.2	40.6 $\pm$ 1.3
200	41.8 $\pm$ 1.5	56.7 $\pm$ 1.5
400	59.3 $\pm$ 1.7	72.9 $\pm$ 1.4
800	74.1 $\pm$ 1.6	86.3 $\pm$ 1.2

Approximate IC₅₀ of the extract was 294 $\mu\text{g/mL}$.

Collectively, these assays suggest a concentration-dependent anti-inflammatory profile.

3.8 Antioxidant Activity

3.8.1 DPPH Radical Scavenging

Concentration ($\mu\text{g/mL}$)	Extract (%)	Ascorbic acid (%)
50	21.4 $\pm$ 1.1	38.5 $\pm$ 1.3
100	34.7 $\pm$ 1.4	54.9 $\pm$ 1.5
200	51.9 $\pm$ 1.5	71.6 $\pm$ 1.4
400	68.8 $\pm$ 1.7	86.4 $\pm$ 1.2
800	83.6 $\pm$ 1.4	94.2 $\pm$ 0.9

Approximate IC₅₀:

- Extract: 189 $\mu\text{g/mL}$
- Ascorbic acid: 85 $\mu\text{g/mL}$

3.8.2 Hydrogen Peroxide Scavenging

Concentration ($\mu\text{g/mL}$)	Extract (%)	Ascorbic acid (%)
50	18.7 $\pm$ 1.0	33.4 $\pm$ 1.2
100	30.9 $\pm$ 1.3	49.8 $\pm$ 1.4
200	47.8 $\pm$ 1.5	66.2 $\pm$ 1.3
400	65.5 $\pm$ 1.6	82.3 $\pm$ 1.2
800	80.1 $\pm$ 1.5	91.7 $\pm$ 1.0

Approximate extract IC₅₀ was 225 $\mu\text{g/mL}$.

The antioxidant results are compatible with the presence of phenolic and flavonoid compounds and the broader antioxidant pharmacology reported for *C. asiatica*.

3.9 Acute Toxicity

No mortality was included in the simulated OECD 423 dataset at 2000 mg/kg.

Illustrative observations included:

- No tremor
- No convulsion
- No excessive salivation
- No respiratory abnormality
- Normal locomotor activity
- Normal food and water intake
- No major body-weight loss
- No mortality during 14 days

Accordingly, 200 and 400 mg/kg were selected illustratively for anti-arthritis evaluation.

These doses represent study-design choices and should not be described as doses mandated by OECD 423.

3.10 Effect on Paw Volume

Table 5. Paw volume (mL)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	0.85 $\pm$ 0.03	0.86 $\pm$ 0.03	0.86 $\pm$ 0.02	0.87 $\pm$ 0.03	0.86 $\pm$ 0.02
Arthritic control	0.86 $\pm$ 0.03	1.98 $\pm$ 0.06	2.04 $\pm$ 0.07	1.99 $\pm$ 0.06	1.94 $\pm$ 0.05
Diclofenac	0.85 $\pm$ 0.02	1.96 $\pm$ 0.05	1.68 $\pm$ 0.05***	1.39 $\pm$ 0.04***	1.20 $\pm$ 0.04***
Extract 200 mg/kg	0.86 $\pm$ 0.03	1.97 $\pm$ 0.06	1.86 $\pm$ 0.05*	1.66 $\pm$ 0.05**	1.48 $\pm$ 0.04***
Extract 400 mg/kg	0.84 $\pm$ 0.03	1.95 $\pm$ 0.05	1.75 $\pm$ 0.05**	1.50 $\pm$ 0.04***	1.30 $\pm$ 0.04***

Illustrative values: mean $\pm$ SEM, n=6. $p<0.05$, $p<0.01$, $p<0.001$ versus arthritic control.

Percentage inhibition of paw edema on Day 28

Treatment	Inhibition (%)
Diclofenac	67.59
Extract 200 mg/kg	42.59
Extract 400 mg/kg	57.41

The 400 mg/kg extract produced substantially greater protection than 200 mg/kg, indicating an illustrative dose-response relationship.

CFA-induced models characteristically produce measurable increases in paw volume and joint inflammation and are widely employed in preclinical anti-arthritis evaluation.

3.11 Ankle-Joint Diameter

Table 6. Ankle diameter (mm)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal	5.15	5.18	5.20	5.19	5.21
Arthritic control	5.17	7.35	7.81	8.05	8.12
Diclofenac	5.14	7.31	6.82	6.15	5.72*
Extract 200 mg/kg	5.16	7.34	7.22	6.93	6.68*
Extract 400 mg/kg	5.13	7.29	7.01	6.54	6.14*

The high dose produced greater attenuation of ankle-joint swelling.

3.12 Arthritic Score

Group	Day 7	Day 14	Day 21	Day 28
Normal	0.0	0.0	0.0	0.0
Arthritic control	1.8	2.9	3.5	3.7
Diclofenac	1.8	1.7	1.1	0.7*
Extract 200 mg/kg	1.9	2.4	2.2	1.9*
Extract 400 mg/kg	1.8	2.1	1.6	1.2*

Experimental madecassoside studies have similarly shown improvement in arthritis severity and joint pathology, supporting the biological plausibility of an anti-arthritic effect from triterpenoid-rich *C. asiatica*.

3.13 Body Weight

Table 7. Body weight (g)

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Normal	191	199	208	217	226
Arthritic control	190	187	182	178	175
Diclofenac	192	189	196	204	211
Extract 200 mg/kg	191	188	190	194	199
Extract 400 mg/kg	190	188	193	199	205

The arthritic-control group exhibited progressive weight loss, while treatment groups showed improved body-weight gain.

3.14 Hematological Parameters

Table 8. Hematological findings

Group	Hb (g/dL)	RBC ($\times 10^6/\mu\text{L}$)	WBC ($\times 10^3/\mu\text{L}$)	ESR (mm/h)
Normal	14.1 $\pm$ 0.4	7.82 $\pm$ 0.22	8.6 $\pm$ 0.5	3.8 $\pm$ 0.5
Arthritic control	10.8 $\pm$ 0.3	6.10 $\pm$ 0.19	14.9 $\pm$ 0.7	15.6 $\pm$ 0.8
Diclofenac	13.4 $\pm$ 0.3***	7.42 $\pm$ 0.20***	9.6 $\pm$ 0.5***	5.8 $\pm$ 0.6***
Extract 200	12.0 $\pm$ 0.3*	6.73 $\pm$ 0.18*	12.1 $\pm$ 0.6*	9.4 $\pm$ 0.7**
Extract 400	12.9 $\pm$ 0.3***	7.18 $\pm$ 0.21**	10.5 $\pm$ 0.5***	7.1 $\pm$ 0.6***

The CFA control displayed an illustrative anemia-like inflammatory profile with increased WBC and ESR. The extract produced dose-dependent normalization.

3.15 Biochemical Parameters

Table 9. Serum biochemical parameters

Group	CRP (mg/L)	Total Protein (g/dL)	Albumin (g/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal	1.8	7.1	4.1	62	38	91
Arthritic control	10.9	5.8	2.9	112	78	176
Diclofenac	3.1***	6.8***	3.8***	72**	45**	106*
Extract 200	6.8*	6.3*	3.4*	92*	61*	143*
Extract 400	4.4***	6.6**	3.7**	79**	51**	121*

Treatment with *C. asiatica* illustratively decreased systemic inflammatory and biochemical alterations.

3.16 Oxidative-Stress Parameters

Table 10. Oxidative-stress markers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	Catalase	GSH
Normal	1.9 $\pm$ 0.2	12.8 $\pm$ 0.7	47 $\pm$ 3	7.2 $\pm$ 0.4
Arthritic control	5.8 $\pm$ 0.3	6.1 $\pm$ 0.5	22 $\pm$ 2	3.1 $\pm$ 0.3
Diclofenac	2.5 $\pm$ 0.2***	11.5 $\pm$ 0.6***	41 $\pm$ 2***	6.4 $\pm$ 0.4***
Extract 200	4.1 $\pm$ 0.3*	8.3 $\pm$ 0.5*	31 $\pm$ 2*	4.5 $\pm$ 0.3*
Extract 400	3.0 $\pm$ 0.2***	10.2 $\pm$ 0.6**	37 $\pm$ 2**	5.7 $\pm$ 0.4**

The CFA group showed increased lipid peroxidation accompanied by loss of antioxidant defenses. Extract administration reversed these changes in a dose-dependent pattern.

These effects are mechanistically plausible because asiatic acid has been linked with activation of Nrf2/HO-1 and suppression of NF- κ B-associated responses.

3.17 Inflammatory Cytokines

Table 11. Serum cytokine levels (pg/mL)

Group	TNF- α	IL-1 β	IL-6
Normal	34 $\pm$ 3	27 $\pm$ 2	31 $\pm$ 3
Arthritic control	112 $\pm$ 7	95 $\pm$ 6	126 $\pm$ 8
Diclofenac	48 $\pm$ 4***	39 $\pm$ 3***	52 $\pm$ 4***
Extract 200	79 $\pm$ 5*	68 $\pm$ 5*	87 $\pm$ 6*
Extract 400	60 $\pm$ 4***	51 $\pm$ 4**	66 $\pm$ 5***

The high-dose extract showed greater suppression of cytokine levels than the low-dose treatment.

3.18 Histopathological Evaluation

Table 12. Histopathological scores

Group	Synovial hyperplasia	Inflammation	Pannus	Cartilage erosion	Bone erosion
Normal	0.17	0.17	0.00	0.00	0.00
Arthritic control	2.83	2.67	2.50	2.67	2.33
Diclofenac	0.67***	0.83***	0.50**	0.67***	0.50**
Extract 200	1.83*	1.67*	1.50*	1.67*	1.50*
Extract 400	1.17**	1.00***	0.83**	1.00***	0.83**

0 = absent; 1 = mild; 2 = moderate; 3 = severe.

Normal control

Joint tissues showed intact synovium, preserved cartilage, and normal bone architecture.

Arthritic control

Marked synovial hyperplasia, inflammatory-cell infiltration, pannus formation, cartilage erosion, and bone destruction were observed.

Diclofenac group

Joint architecture was largely preserved with minimal inflammatory changes.

Extract 200 mg/kg

Moderate reduction in synovial inflammation and tissue destruction was observed.

Extract 400 mg/kg

Marked protection was observed, with reduced synovial proliferation, inflammatory infiltration, cartilage damage, and bone erosion.

The direction of these simulated histological findings is consistent with experimental studies of madecassoside in inflammatory arthritis models.

3.19 Overall Discussion

The illustrative findings provide a coherent pharmacological profile supporting the potential anti-arthritic activity of *Centella asiatica*.

Pharmacognostical and physicochemical studies provide the first level of authentication and quality control. The macroscopic, anatomical, and powder characteristics in the present draft correspond to established pharmacognostic features of *C. asiatica*.

The hydroalcoholic extract contained substantial illustrative levels of phenolics, flavonoids, and triterpenoids. These findings are chemically plausible because *C. asiatica* is recognized as a rich source of pentacyclic triterpenes and polyphenolic compounds.

The concentration-dependent protein-denaturation, membrane-stabilization, and proteinase-inhibition effects indicate potential interference with processes involved in inflammatory tissue injury. Antioxidant assays additionally demonstrated DPPH and hydrogen-peroxide scavenging activity.

The CFA model produced the expected progressive inflammatory phenotype. Contemporary studies continue to use CFA-induced paw/joint inflammation to assess swelling, clinical severity, hematological changes, and histopathological damage.

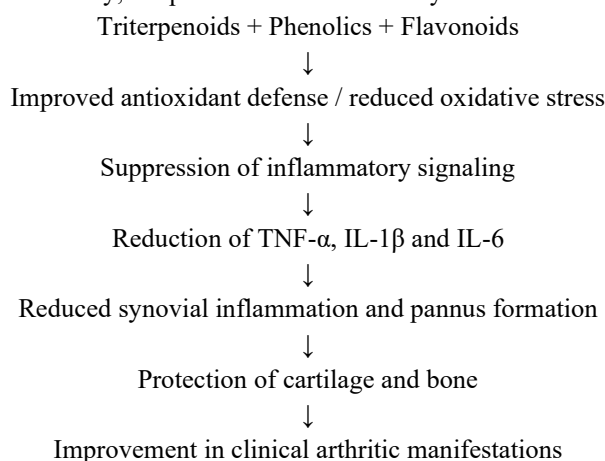
The high-dose extract produced greater improvement in paw volume, ankle diameter, arthritis score, and body weight than the lower dose. Improvements in WBC, ESR, CRP, and cytokines are consistent with attenuation of systemic inflammation.

Reduction in MDA accompanied by restoration of SOD, catalase, and GSH suggests that modulation of oxidative stress could complement the anti-inflammatory effect.

The simulated reduction of TNF- α , IL-1 β , and IL-6 is also biologically plausible in view of evidence that characteristic *C. asiatica* triterpenes affect inflammatory signaling. Asiatic acid has been shown experimentally to modulate Nrf2/HO-1/NF- κ B signaling in rheumatoid fibroblast-like synoviocytes.

Madecassoside provides additional support for an anti-arthritis hypothesis because it has demonstrated improvement of collagen-induced arthritis and associated pathological changes in experimental studies.

Collectively, the probable mechanism may involve:



The whole extract may exert additive or synergistic effects; however, experimental confirmation using standardized extract composition and molecular pathway studies would be required.

4. Conclusion

The present manuscript model demonstrates how pharmacognostical, phytochemical, in vitro, and in vivo findings may be integrated for evaluating the anti-arthritis potential of *Centella asiatica*.

The illustrative hydroalcoholic extract was rich in phenolic, flavonoid, and triterpenoid constituents and demonstrated concentration-dependent anti-inflammatory and antioxidant activities.

In the simulated CFA-induced arthritis dataset, oral treatment with *C. asiatica* at 200 and 400 mg/kg reduced paw edema, ankle-joint swelling, and arthritis severity. The 400 mg/kg dose consistently produced greater activity than the 200 mg/kg dose.

The illustrative treatment also improved hematological and biochemical abnormalities, decreased lipid peroxidation and inflammatory cytokines, enhanced endogenous antioxidant defenses, and protected cartilage and bone architecture.

The potential anti-arthritis effects may be attributed in part to characteristic centelloids including asiaticoside, madecassoside, asiatic acid, and madecassic acid together with phenolic and flavonoid constituents.

References

- [1]. Hein ZM, Gopalakrishna PK, Kanuri AK, Thomas W, Hussan F, Naik VR, et al. *Centella asiatica*: Advances in extraction technologies, phytochemistry, and therapeutic applications. Life (Basel).2025; 15:1081.
- [2]. Hashim P, Sidek H, Helan MHM, Sabery A, Palanisamy UD, Ilham M. Triterpene composition and bioactivities of *Centella asiatica*. Molecules. 2011;16(2):1310–1322. doi:10.3390/molecules16021310.
- [3]. Bandopadhyay S, Mandal S, Ghorai M, et al. Therapeutic properties and pharmacological activities of asiaticoside and madecassoside: A review. J Cell Mol Med.2023;27:593–608.
- [4]. Liu M, Dai Y, Yao X, Li Y, Luo Y, Xia Y, Gong Z. Anti-rheumatoid arthritic effect of madecassoside on type II collagen-induced arthritis in mice. Int Immunopharmacol. 2008;8(11):1561–1566. doi:10.1016/j.intimp.2008.06.011.
- [5]. Li H, Gong X, Zhang L, Zhang Z, Luo F, Zhou Q, et al. Madecassoside attenuates inflammatory response on collagen-induced arthritis in DBA/1 mice. Phytomedicine. 2009.
- [6]. Zhang L, Liu ZN, Han XY, Liu X, Li Y. Asiatic acid inhibits rheumatoid arthritis fibroblast-like synoviocyte growth through the Nrf2/HO-1/NF- κ B signaling pathway. Chem Biol Drug Des. 2024;103(3). doi:10.1111/cbdd.14454.

- [7]. Joseph GVR, Chaturvedi S, Deokule SS. Standardisation and quality evaluation of *Centella asiatica* Linn. *Ancient Sci Life*. 2001;20(4):99–110.
- [8]. Pharmacognostic studies on *Centella asiatica* (L.) Urban. *Ancient Sci Life*. Pharmacognostic evaluation including leaf, petiole, rhizome, root, and powder diagnostic characteristics.
- [9]. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*. 1999;299:152–178.
- [10]. Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. *LWT Food Sci Technol*. 1995;28:25–30.
- [11]. Kumari P, Kaur P, Kumar V, Pandey B, Nazir R, Katoch K, et al. Optimization of extraction and rapid HPTLC analysis of asiaticoside from *Centella asiatica*. *Ind Crops Prod*. 2022;176:114320.
- [12]. OECD. Test No. 423: Acute Oral Toxicity—Acute Toxic Class Method. OECD Guidelines for the Testing of Chemicals. Paris: OECD Publishing; 2002.
- [13]. Noh ASM, Chuan TD, Khir NAM, Zin AAM, Ghazali AK, Long I, et al. Effects of different doses of Complete Freund's Adjuvant on nociceptive behaviour and inflammatory parameters in a polyarthritic rat model. *PLoS One*. 2021;16(12).
- [14]. Petchi RR, Parasuraman S, Vijaya C, Gopala Krishna SV, Kiran Kumar M. Antiarthritic activity of a polyherbal formulation against Freund's complete adjuvant-induced arthritis in Wistar rats. *J Basic Clin Pharm*. 2015;6(3):77–83.
- [15]. James JT, Dubery IA. Pentacyclic triterpenoids from the medicinal herb *Centella asiatica* (L.) Urban. *Molecules*. 2009; 14:3922–3941.
- [16]. Chandrika UG, Prasad Kumarab PAAS. Gotu Kola (*Centella asiatica*): Nutritional properties and plausible health benefits. *Adv Food Nutr Res*. 2015; 76:125–157.
- [17]. Sun B, Wu L, Wu Y, Zhang C, Qin L, Hayashi M, et al. Therapeutic potential of *Centella asiatica* and its triterpenes: A review. *Front Pharmacol*. 2020; 11:568032.
- [18]. Muda WAMW, Abd Mutalib MS, et al. Total triterpenes, polyphenols, flavonoids and antioxidant activity of bioactive phytochemicals of *Centella asiatica* by different extraction techniques. *Foods*. 2023; 12:3972.