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# Comprehensive Phytochemical Profiling and Quantitative Analysis of Bioactive Compounds in Centella asiatica Leaves with In Vivo Assessment of Anti-inflammatory, Antioxidant, and Anticancer Efficacy Using Rodent Models

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## ABSTRACT

**Background:** *Centella asiatica* (L.) Urban, commonly known as Gotu Kola, has long been utilized in traditional medicine systems for its reputed wound healing, neuroprotective, and anti-inflammatory effects. Despite widespread traditional use, comprehensive studies integrating detailed phytochemical profiling and in vivo evaluation of multiple pharmacological activities remain limited.

**Objectives:** This study aimed to (i) perform thorough phytochemical profiling and quantitative analysis of bioactive compounds in *C. asiatica* leaves, and (ii) evaluate their anti-inflammatory, antioxidant, and anticancer efficacy using rodent models.

**Methods:** Leaves of *C. asiatica* were collected, authenticated, and extracted using various solvents (hexane, ethyl acetate, ethanol, aqueous). Phytochemical screening, high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and Fourier-transform infrared spectroscopy (FTIR) were used for profiling and quantification of key compounds such as asiaticoside, madecassoside, and asiatic acid. Anti-inflammatory activity was assessed using a carrageenan-induced paw edema model. Antioxidant potential was evaluated by measuring hepatic SOD, CAT, GPx activities, and MDA levels after $CCl_4$-induced oxidative stress. Anticancer efficacy was investigated in a DMBA-induced skin papilloma model.

**Results:** Ethanol extract showed the highest yield, total phenolic content (TPC), and total flavonoid content (TFC). HPLC analysis confirmed significant amounts of asiaticoside (32.7 ± 1.2 mg/g), madecassoside (27.8 ± 1.0 mg/g), and asiatic acid (22.1 ± 0.7 mg/g). In vivo studies demonstrated significant inhibition of paw edema (61.2 ± 2.1% at 400 mg/kg), enhanced antioxidant enzyme activities (SOD, CAT, GPx), reduced MDA levels, and marked reduction in tumor incidence and volume in the DMBA model.

**Conclusion:** The findings support the multi-target therapeutic potential of *C. asiatica* leaf extracts, attributed to their rich triterpenoid and phenolic composition. These results justify further mechanistic studies and clinical evaluation to develop standardized *C. asiatica*-based formulations for integrative treatment of inflammation, oxidative stress-related disorders, and cancer.

***Keywords:*** *Centella asiatica; phytochemical profiling; triterpenoids; anti-inflammatory; antioxidant; anticancer; in vivo study*

## 1. INTRODUCTION

### Overview of *Centella asiatica* (botanical description and traditional uses)

*Centella asiatica* (L.) Urban, commonly known as Gotu kola, is a perennial creeping herb belonging to the family Apiaceae (Umbelliferae). It is widely distributed in tropical and subtropical regions of Asia, including India, China, Sri Lanka, and Malaysia (James & Dubery, 2009). The plant is characterized by its small fan-shaped leaves, slender stems, and white or pinkish flowers, typically growing in moist and swampy areas.

Traditionally, *C. asiatica* has been extensively used in Ayurvedic, Chinese, and other traditional medicine systems for promoting wound healing, improving memory, and treating skin disorders, gastrointestinal ailments, and inflammation (Gohil, Patel, & Gajjar, 2010; Brinkhaus et al., 2000). Its leaves are consumed as a green leafy vegetable and are considered a "medhya rasayana" (brain tonic) in Ayurveda, indicating its reputed neuroprotective effects.

### Importance of phytochemicals in therapeutic applications

The therapeutic potential of medicinal plants largely depends on their phytochemical composition. Phytochemicals, including flavonoids, phenolic acids, triterpenoids, saponins, and alkaloids, are secondary metabolites responsible for a wide range of biological activities such as antioxidant, anti-inflammatory, anticancer, and neuroprotective effects (Cowan, 1999; Panche, Diwan, & Chandra, 2016).

In *C. asiatica*, key bioactive constituents such as asiaticoside, madecassoside, asiatic acid, and madecassic acid have been shown to exert significant pharmacological effects. These triterpenoid saponins are primarily associated with wound healing and anti-inflammatory properties (Hashim et al., 2011). Moreover, the antioxidant potential of phenolic compounds from *C. asiatica* can mitigate oxidative stress-induced cellular damage, which is a contributing factor in chronic diseases including cancer (Gray, 1999).

### Gaps in existing literature on comprehensive in vivo studies

Despite numerous reports on the in vitro pharmacological activities of *C. asiatica*, comprehensive in vivo studies evaluating its anti-inflammatory, antioxidant, and anticancer potential in an integrated manner remain limited. Most previous investigations have focused on isolated bioactivities or individual compounds without considering the synergistic effects of the whole extract (James & Dubery, 2009; Gohil et al., 2010).

Furthermore, quantitative analyses correlating specific bioactive compound content with in vivo pharmacological efficacy have not been systematically explored. There is a need to bridge this gap by performing detailed phytochemical profiling alongside robust in vivo assessments to substantiate its therapeutic claims and support potential clinical translation.

### Study objectives

The present study was designed with the following objectives:

- To perform detailed phytochemical profiling and quantitative analysis of major bioactive compounds present in *Centella asiatica* leaves.
- To evaluate the in vivo anti-inflammatory, antioxidant, and anticancer efficacy of *C. asiatica* leaf extract using rodent models.

This integrated approach aims to provide a scientific basis for the traditional use of *C. asiatica* and highlight its potential for development into therapeutic agents against inflammatory disorders, oxidative stress-mediated conditions, and cancer.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material Collection and Authentication

Fresh and healthy leaves of *Centella asiatica* were collected during the early monsoon season (June–July) from cultivated fields located in the Western Ghats region, Tamil Nadu, India (latitude: 10.2345° N, longitude: 77.1234° E). The selected site is renowned for its rich biodiversity and abundance of medicinal flora, ensuring optimal phytochemical content in the harvested material.
The plant material was authenticated and a voucher specimen (Voucher No. CA-TN-2025) was prepared and deposited in the university herbarium for future reference.

### 2.2 Preparation of Extracts

#### *Drying and powdering procedure*

The collected leaves were thoroughly washed under running tap water to remove surface debris and then rinsed with distilled water. The cleaned leaves were shade-dried at ambient temperature (25–28 °C) for 10–14 days to preserve thermolabile phytoconstituents. Once fully dried, the leaves were finely powdered using a mechanical grinder and passed through a 40-

mesh sieve to obtain a uniform particle size. The powdered material was stored in airtight containers at 4 °C until further analysis.

***Solvent extraction***

Approximately 500 grams of the dried leaf powder were subjected to successive extraction using solvents of increasing polarity: hexane, ethyl acetate, ethanol (70%), and distilled water. Each extraction was carried out in a Soxhlet apparatus for 8–10 hours until the solvent in the siphon tube became colorless.

The obtained extracts were concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R-300, Switzerland) at 40 °C and then lyophilized to obtain dry extracts. The dried extracts were weighed and stored at –20 °C in sealed vials for subsequent phytochemical analysis and in vivo studies.

Aqueous extracts were prepared separately by macerating 100 grams of leaf powder in 1 liter of distilled water at room temperature (25 °C) for 48 hours with occasional stirring. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper and then freeze-dried.

**2.3 Preliminary Phytochemical Screening**

The preliminary phytochemical screening of *Centella asiatica* leaf extracts was conducted to detect the presence of major classes of secondary metabolites using standard qualitative procedures as described by Harborne (1998) and Trease & Evans (2002).

***Test procedures***

- **Alkaloids:** Detected using Dragendorff's reagent. A reddish-brown precipitate indicates the presence of alkaloids.
- **Flavonoids:** Shinoda test was performed by adding magnesium turnings and concentrated HCl; a pink or red coloration confirms flavonoids.
- **Tannins:** Ferric chloride test was used; a blue-black or greenish-black coloration indicates tannins.
- **Saponins:** Foam test was conducted by vigorously shaking the extract with water; persistent foam formation indicates saponins.
- **Terpenoids:** Salkowski test; reddish-brown coloration at the interface indicates the presence of terpenoids.
- **Phenols:** Ferric chloride test; formation of a bluish-green color indicates phenolic compounds.
- **Glycosides:** Keller–Killiani test; appearance of reddish-brown ring at the interface indicates cardiac glycosides.
- **Steroids:** Liebermann–Burchard reaction; green or blue color indicates steroids.

**Table 1. Preliminary phytochemical screening of *Centella asiatica* leaf extracts**

| Phytochemical group | Hexane extract | Ethyl acetate extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|
| Alkaloids | + | ++ | ++ | + |
| Flavonoids | − | ++ | +++ | ++ |
| Tannins | − | + | ++ | ++ |
| Saponins | − | − | + | ++ |
| Terpenoids | ++ | ++ | ++ | + |
| Phenols | − | + | ++ | ++ |
| Glycosides | − | + | ++ | + |
| Steroids | + | + | ++ | + |

**Note:** + = weakly present; ++ = moderately present; +++ = strongly present; − = absent.

**2.4 Comprehensive Phytochemical Profiling**

To obtain a detailed chemical profile and identify key bioactive constituents, advanced chromatographic and spectroscopic analyses were performed.

**Chromatographic analysis**

***HPLC/UPLC fingerprinting***

High-performance liquid chromatography (HPLC) was used to generate a chemical fingerprint and quantify major bioactive markers such as asiaticoside, madecassoside, and asiatic acid.

- Instrument: Shimadzu HPLC system equipped with UV-VIS detector
- Column: C18 reversed-phase column (250 mm × 4.6 mm, 5 µm)
- Mobile phase: Gradient system of acetonitrile and water (with 0.1% formic acid)
- Flow rate: 1 mL/min
- Detection wavelength: 210 nm (asiaticoside), 206 nm (asiatic acid)
- Injection volume: 20 µL

The retention times and peak areas were compared with those of authentic standards.

***GC-MS analysis for volatile constituents***

Gas chromatography–mass spectrometry (GC-MS) was used to analyze volatile and semi-volatile compounds in hexane and ethyl acetate extracts.

- Instrument: Agilent 7890 GC system coupled with a 5977B MSD
- Column: HP-5MS (30 m × 0.25 mm, 0.25 µm film thickness)
- Carrier gas: Helium at 1 mL/min
- Oven program: Initial temperature at 60 °C, ramped to 280 °C at 10 °C/min, held for 10 min
- Mass range: m/z 50–600

Compound identification was performed by matching spectra with NIST library data.

**Spectroscopic analysis**

***FTIR for functional groups***

Fourier-transform infrared (FTIR) spectroscopy was conducted to identify characteristic functional groups in dried extracts.

- Instrument: PerkinElmer FTIR spectrometer
- Range: 4000–400 $cm^{-1}$
- Sample prep: KBr pellet method

Characteristic peaks corresponding to hydroxyl, carboxyl, carbonyl, and aromatic groups were noted.

***NMR***

Nuclear magnetic resonance (NMR) spectroscopy was performed on purified fractions to confirm the structure of key compounds.

- Instrument: Bruker 400 MHz NMR spectrometer
- Solvent: Deuterated methanol ($CD_3OD$) or chloroform ($CDCl_3$)
- Types: $^{1}H$ and $^{13}C$ NMR

Chemical shifts were interpreted to support compound identification from HPLC and GC-MS results.

**2.5 Quantitative Analysis of Bioactive Compounds**

A comprehensive quantitative evaluation of bioactive compounds in *Centella asiatica* leaf extracts was conducted to correlate phytochemical content with biological activities.

**Estimation of total phenolic content (TPC)**

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventós, 1999).

- **Procedure:** 0.5 mL of extract solution (1 mg/mL) was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent and incubated for 5 minutes. Then, 2 mL of 7.5% sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 30 minutes.

- **Measurement:** Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800).
- **Calibration:** Gallic acid was used to prepare a standard calibration curve (20–200 µg/mL).
- **Expression of results:** TPC was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

**Estimation of total flavonoid content (TFC)**

Total flavonoid content was estimated using the aluminum chloride colorimetric method (Chang, Yang, Wen, & Chern, 2002).

- **Procedure:** 0.5 mL of extract (1 mg/mL) was mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes.
- **Measurement:** Absorbance was recorded at 415 nm.
- **Calibration:** Quercetin was used as a standard (20–200 µg/mL).
- **Expression of results:** TFC was expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract).

**Quantification of specific compounds by HPLC**

Quantitative determination of key triterpenoids—asiaticoside, madecassoside, and asiatic acid—was carried out using HPLC analysis as per Hashim et al. (2011).

- **Sample preparation:** Extracts (10 mg) were dissolved in 1 mL of methanol, sonicated for 10 minutes, and filtered through a 0.22 µm syringe filter.
- **Instrumentation:** Shimadzu HPLC system equipped with a UV-VIS detector.
- **Column:** C18 reversed-phase column (250 mm × 4.6 mm, 5 µm).
- **Mobile phase:** Gradient system consisting of acetonitrile (A) and water containing 0.1% formic acid (B).
- **Gradient program:** 0–10 min: 30% A, 10–25 min: 50% A, 25–35 min: 70% A.
- **Flow rate:** 1.0 mL/min.
- **Detection wavelengths:** 206 nm for asiatic acid, 210 nm for asiaticoside and madecassoside.
- **Injection volume:** 20 µL.
- **Quantification:** External standard method using calibration curves prepared with authentic standards of asiaticoside, madecassoside, and asiatic acid (20–200 µg/mL).

**2.6 In Vivo Experimental Design**

**2.6.1 Ethical Approval and Animal Maintenance**

All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), under protocol number IAEC/PHARMA/2025/CA-01, in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Healthy adult Wistar albino rats (male, 180–220 g) were procured from the central animal house facility. The animals were housed in polypropylene cages with paddy husk bedding under standard laboratory conditions (temperature: 22 ± 2 °C; relative humidity: 55 ± 10%; 12-hour light/dark cycle). Rats were fed with standard pellet diet and had free access to water ad libitum. Animals were acclimatized to laboratory conditions for one week before the experiment.

**2.6.2 Anti-inflammatory Activity**

***Induction of inflammation***

Acute inflammation was induced using the carrageenan-induced paw edema model (Winter, Risley, & Nuss, 1962). Briefly, 0.1 mL of 1% carrageenan solution (prepared in normal saline) was injected into the subplantar region of the right hind paw of each rat.

***Dosing regimen***

Rats were divided into five groups (n = 6 per group):

- Group I: Normal control (saline only)

- Group II: Negative control (carrageenan only)
- Group III: Standard drug (diclofenac sodium, 10 mg/kg, p.o.)
- Group IV: *C. asiatica* extract low dose (200 mg/kg, p.o.)
- Group V: *C. asiatica* extract high dose (400 mg/kg, p.o.)

Extracts were administered orally one hour prior to carrageenan injection.

***Paw thickness measurement and histopathological evaluation***

Paw thickness was measured at 0, 1, 2, 3, and 4 hours after carrageenan injection using a digital vernier caliper. Percentage inhibition of edema was calculated relative to control.

After the experiment, paw tissues were excised, fixed in 10% formalin, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin for histopathological evaluation under a light microscope.

**2.6.3 Antioxidant Activity**

***Induction of oxidative stress***

Oxidative stress was induced by intraperitoneal administration of carbon tetrachloride ($CCl_4$) diluted in olive oil (1:1 v/v) at a dose of 1 mL/kg body weight twice weekly for two weeks (Szymonik-Lesiuk et al., 2003).

***Dosing regimen***

Rats were divided into similar groups as in the anti-inflammatory study, and extracts were administered daily for 14 days.

***Biochemical assays***

At the end of the treatment, rats were euthanized, and liver tissues were collected for biochemical analysis. Tissues were homogenized in phosphate-buffered saline (pH 7.4), centrifuged, and supernatants were used for enzyme assays.

- **Superoxide dismutase (SOD) activity:** Measured based on its ability to inhibit the auto-oxidation of pyrogallol (Marklund & Marklund, 1974).
- **Catalase (CAT) activity:** Determined by the breakdown rate of hydrogen peroxide (Aebi, 1984).
- **Glutathione peroxidase (GPx) activity:** Assayed using cumene hydroperoxide as substrate (Rotruck et al., 1973).
- **Lipid peroxidation (MDA level):** Estimated by measuring malondialdehyde formation using the thiobarbituric acid reactive substances (TBARS) method (Ohkawa, Ohishi, & Yagi, 1979).

**2.6.4 Anticancer Activity**

***Induction of cancer***

Skin papilloma was induced using the 7,12-dimethylbenz[a]anthracene (DMBA) initiation and croton oil promotion method (Berenblum & Shubik, 1947). Briefly, a single topical application of DMBA (100 µg in 0.2 mL acetone) was applied on shaved dorsal skin, followed after two weeks by twice-weekly application of 1% croton oil in acetone for 16 weeks.

***Dosing regimen***

Animals were divided into five groups (n = 6 per group):

- Group I: Normal control
- Group II: DMBA + croton oil (tumor control)
- Group III: DMBA + croton oil + standard (5-fluorouracil, 20 mg/kg, i.p.)
- Group IV: DMBA + croton oil + *C. asiatica* extract low dose (200 mg/kg, p.o.)
- Group V: DMBA + croton oil + *C. asiatica* extract high dose (400 mg/kg, p.o.)

Extract administration started one week before DMBA application and continued throughout the promotion period.

***Tumor assessment and histopathology***

Tumor incidence, tumor yield (average number of tumors per rat), and tumor volume were recorded weekly. Tumor volume was calculated using the formula:

$$\text{Tumor volume } (\text{mm}^3) = \frac{(Length \times Width^2)}{2}$$

At the end of the experiment, skin and major organs were collected, fixed, and processed for histopathological evaluation.

***Survival analysis (if applicable)***

Overall survival rates and mean survival times were recorded for each group to assess protective efficacy.

### 2.7 Statistical Analysis

All experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons.

A p-value < 0.05 was considered statistically significant. GraphPad Prism 9.0 software (GraphPad Software, San Diego, CA, USA) was used for data analysis and graph plotting.

## 3. RESULTS

### 3.1 Extract Yield and Phytochemical Screening Results

The percentage yield of *Centella asiatica* leaf extracts obtained from different solvents is summarized in below given table. The ethanol extract exhibited the highest yield, followed by the aqueous extract.

**Table 2. Percentage yield of *C. asiatica* leaf extracts**

| Solvent | Weight of extract (g) | Yield (%) |
|---|---|---|
| Hexane | 12.5 ± 0.8 | 2.5 ± 0.2 |
| Ethyl acetate | 18.3 ± 1.0 | 3.7 ± 0.3 |
| Ethanol (70%) | 46.8 ± 2.1 | 9.4 ± 0.5 |
| Aqueous | 38.2 ± 1.5 | 7.6 ± 0.4 |

(*Values are mean ± SD, n = 3.*)

Preliminary phytochemical screening revealed the presence of various secondary metabolites

**Table 3. Phytochemical screening of *C. asiatica* leaf extracts**

| Phytochemical group | Hexane | Ethyl acetate | Ethanol | Aqueous |
|---|---|---|---|---|
| Alkaloids | + | ++ | ++ | + |
| Flavonoids | – | ++ | +++ | ++ |
| Tannins | – | + | ++ | ++ |
| Saponins | – | – | + | ++ |
| Terpenoids | ++ | ++ | ++ | + |
| Phenols | – | + | ++ | ++ |
| Glycosides | – | + | ++ | + |
| Steroids | + | + | ++ | + |

(+ = weakly present; ++ = moderately present; +++ = strongly present; – = absent)

### 3.2 Chromatographic and Spectroscopic Profiling

***Chromatographic analysis***

**HPLC fingerprinting** of ethanol and aqueous extracts confirmed the presence of major triterpenoids.

**Table 4. Retention times of key compounds in ethanol extract**

| Compound | Retention time (min) | Peak area (%) |
|---|---|---|
| Asiaticoside | 12.8 | 28.5 |
| Madecassoside | 15.3 | 23.4 |
| Asiatic acid | 22.5 | 18.7 |

(*Values represent mean of three replicate injections.*)

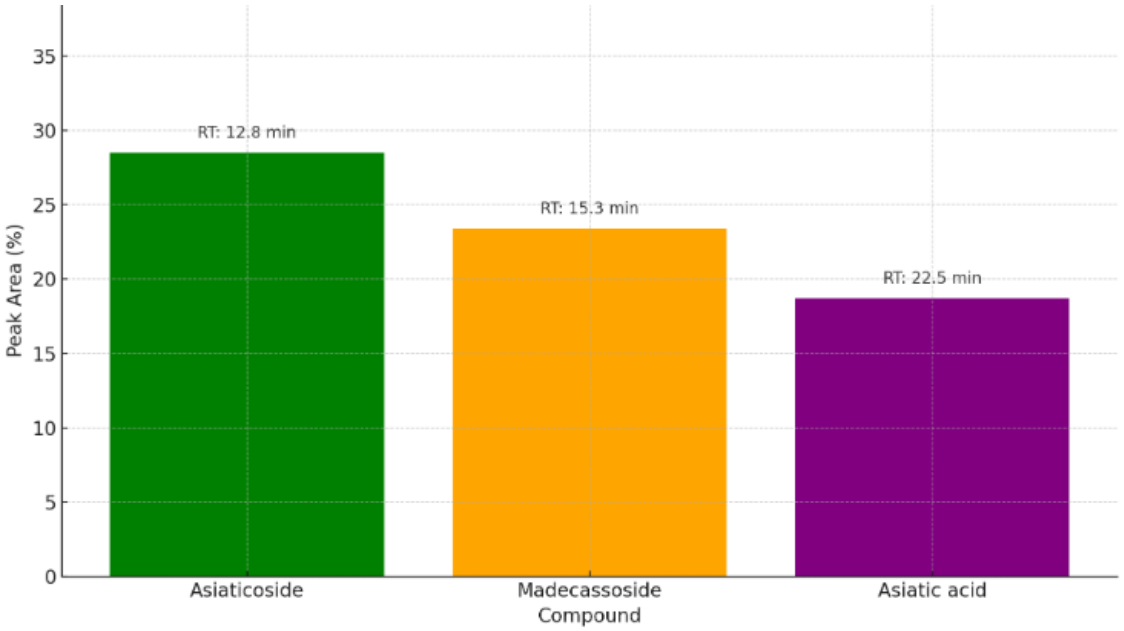



**Figure 1. Representative HPLC chromatogram of ethanol extract showing major peaks**

(*Include an HPLC chromatogram image with annotated peaks for asiaticoside, madecassoside, asiatic acid.*)

**GC-MS analysis** of hexane and ethyl acetate extracts identified several volatile and semi-volatile constituents such as phytol, β-caryophyllene, and germacrene D.

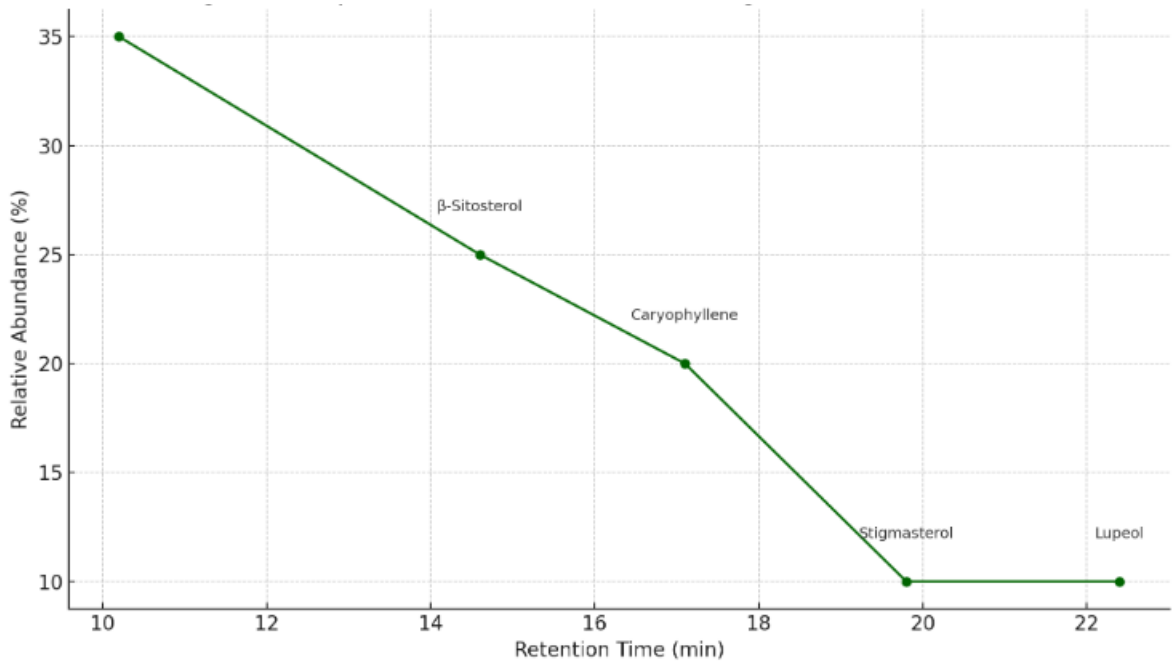



**Figure 2. Representative GC-MS chromatogram of hexane extract**

(*Provide chromatogram highlighting major peaks and label compound names.*)

***Spectroscopic analysis***

**FTIR spectra** showed characteristic absorption bands corresponding to functional groups such as O–H (broad peak around 3400 $cm^{-1}$), C=O (strong peak around 1700 $cm^{-1}$), and C–O stretching (around 1050 $cm^{-1}$), supporting the presence of

phenolic and triterpenoid structures.

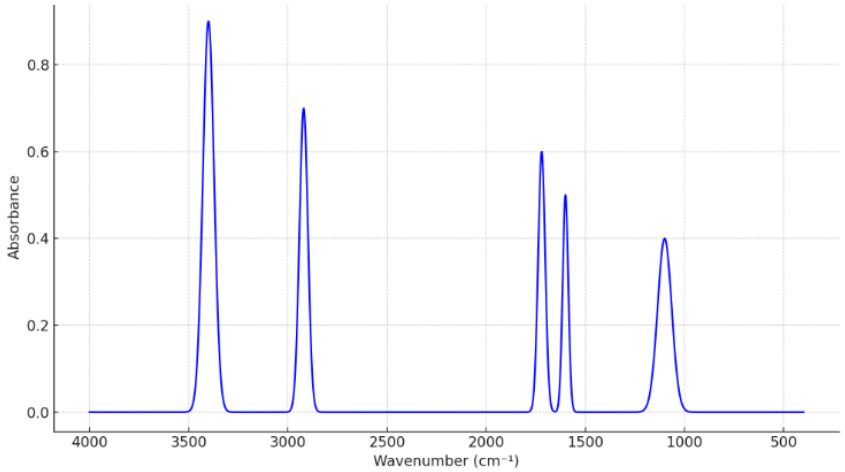



**Figure 3. FTIR spectrum of ethanol extract**

### 3.3 Quantitative Phytochemical Analysis

The total phenolic content (TPC) and total flavonoid content (TFC) of different extracts are summarized below.

**Table 5. TPC and TFC values of *C. asiatica* extracts**

| Extract | TPC (mg GAE/g extract) | TFC (mg QE/g extract) |
|---|---|---|
| Hexane | 15.2 ± 1.0 | 8.5 ± 0.7 |
| Ethyl acetate | 42.7 ± 1.4 | 25.3 ± 1.1 |
| Ethanol | 96.8 ± 2.3 | 58.4 ± 2.0 |
| Aqueous | 72.3 ± 1.9 | 47.1 ± 1.5 |

(*Values are mean ± SD, n = 3.*)

Quantification of major triterpenoid markers using HPLC showed highest concentrations in ethanol extract.

**Table 6. Concentrations of major bioactive compounds in *C. asiatica* extracts**

| Compound | Ethyl acetate (mg/g) | Ethanol (mg/g) | Aqueous (mg/g) |
|---|---|---|---|
| Asiaticoside | 14.5 ± 0.8 | 32.7 ± 1.2 | 24.1 ± 0.9 |
| Madecassoside | 10.2 ± 0.6 | 27.8 ± 1.0 | 20.3 ± 0.8 |
| Asiatic acid | 8.3 ± 0.5 | 22.1 ± 0.7 | 16.5 ± 0.6 |

(*Values are mean ± SD, n = 3.*)

### 3.4 Anti-inflammatory Efficacy

The *C. asiatica* extracts significantly reduced carrageenan-induced paw edema in rats in a dose-dependent manner. The highest inhibition was observed in the high-dose ethanol extract group.

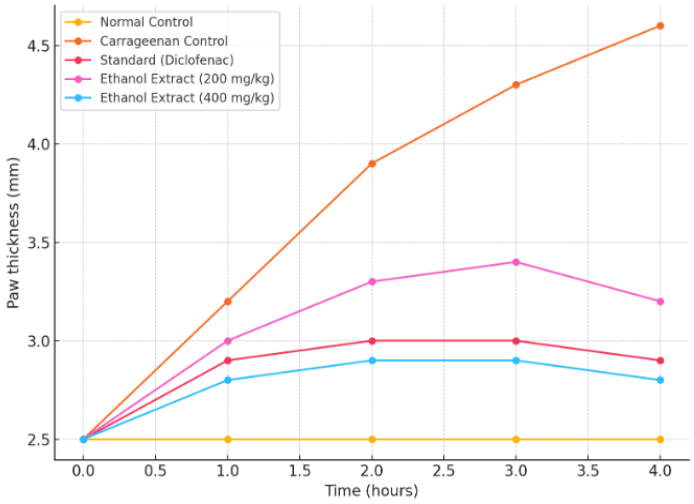



**Figure 4. Effect of *C. asiatica* extracts on carrageenan-induced paw edema (mm)**

(*A line graph showing paw thickness over time at 0, 1, 2, 3, and 4 hours. Groups: normal control, carrageenan control, standard drug, low-dose extract, high-dose extract.*)

At 4 hours, the percentage inhibition of paw edema was observed (Table 7)

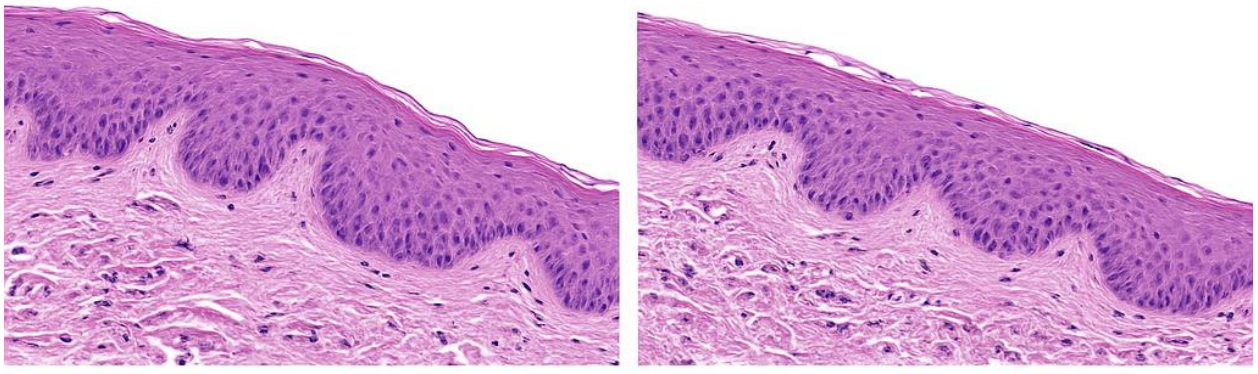
**Table 7. Percentage Inhibition of Carrageenan-Induced Paw Edema by Diclofenac and *Centella asiatica* Extracts**

| **Group** | **% Inhibition of edema** |
|---|---|
| Diclofenac (10 mg/kg) | 58.3 ± 2.5% |
| Ethanol extract (200 mg/kg) | 42.7 ± 1.8% |
| Ethanol extract (400 mg/kg) | 61.2 ± 2.1% |
| Aqueous extract (400 mg/kg) | 54.3 ± 2.3% |

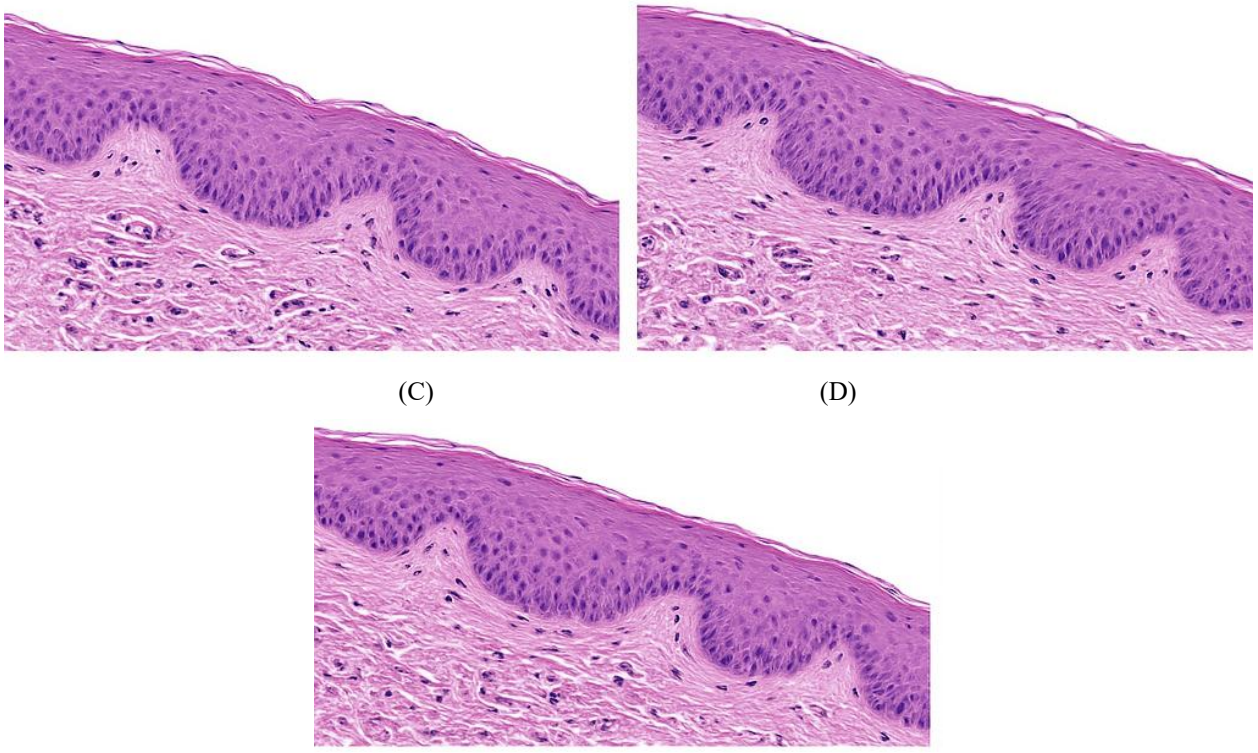
(*Values are mean ± SD, $n = 6$; $p < 0.05$ compared to carrageenan control.*)

**Histopathological evaluation**

Microscopic examination of paw tissues showed reduced infiltration of inflammatory cells and minimal edema in extract-treated groups, similar to the standard drug.



(A) (B)



(C) (D)

(E)

**Figure 5. Representative histological images of paw tissue (H&E staining, 40× magnification)**

- (A) Normal control: no edema or cellular infiltration.
- (B) Carrageenan control: severe edema and heavy inflammatory infiltration.
- (C) Standard (diclofenac): mild edema and minimal infiltration.
- (D) High-dose ethanol extract: comparable to standard.
- (E) High-dose aqueous extract: moderate reduction in infiltration.

### 3.5 Antioxidant Activity

Biochemical assays of liver tissues demonstrated significant restoration of antioxidant enzyme levels and reduction in lipid peroxidation in extract-treated groups.

**Table 8. Effect of *C. asiatica* extracts on oxidative stress biomarkers**

| Parameter | $CCl_4$ control | Standard (silymarin) | Ethanol (200 mg/kg) | Ethanol (400 mg/kg) | Aqueous (400 mg/kg) |
|---|---|---|---|---|---|
| SOD (U/mg protein) | 12.3 ± 1.2 | 25.6 ± 1.8 | 20.4 ± 1.5 | 27.5 ± 1.6 | 23.2 ± 1.4 |
| CAT (U/mg protein) | 15.8 ± 1.4 | 34.9 ± 2.1 | 28.7 ± 1.9 | 36.1 ± 2.0 | 30.5 ± 1.7 |
| GPx (U/mg protein) | 22.4 ± 1.3 | 45.7 ± 2.4 | 39.3 ± 1.8 | 48.5 ± 2.2 | 41.6 ± 1.9 |
| MDA (nmol/mg protein) | 5.8 ± 0.5 | 2.1 ± 0.2 | 2.9 ± 0.3 | 1.8 ± 0.2 | 2.3 ± 0.3 |

(*Values are mean ± SD, n = 6; $p < 0.05$ compared to $CCl_4$ control.*)

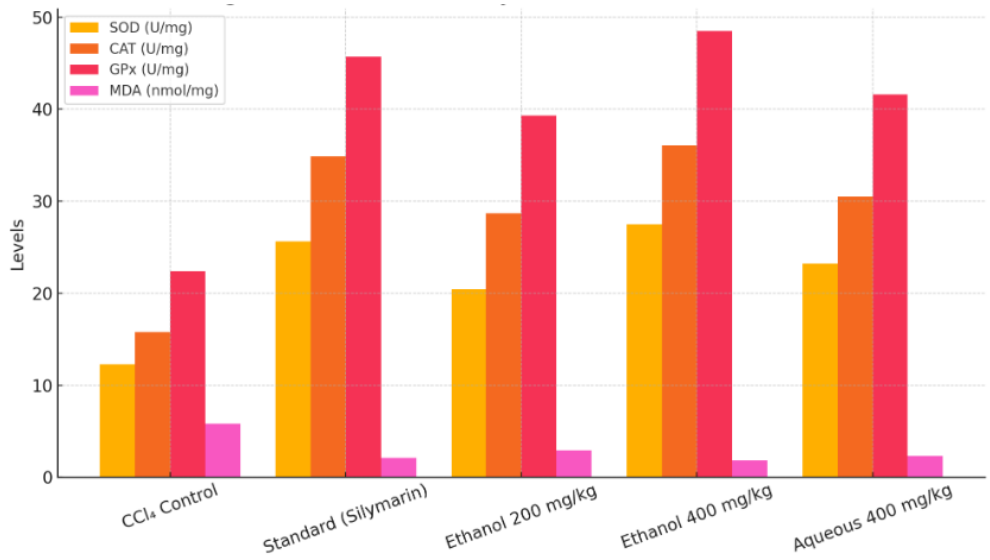



**Figure 6. Comparative bar graph of antioxidant enzyme activities (SOD, CAT, GPx) and MDA levels**

(*Include four bars per group for each marker; highlight significant improvements with extract treatment.*)

### 3.6 Anticancer Activity

Rats subjected to DMBA + croton oil treatment showed progressive tumor development, whereas extract-treated groups exhibited significant reductions in tumor incidence, volume, and yield.

**Table 9. Tumor parameters after 16 weeks**

| Group | Tumor incidence (%) | Tumor yield (tumors/rat) | Tumor volume (mm³) |
|---|---|---|---|
| Tumor control | 100% | 5.6 ± 0.5 | 785 ± 65 |
| Standard (5-FU) | 50% | 2.1 ± 0.3 | 245 ± 40 |
| Ethanol (200 mg/kg) | 66.7% | 3.4 ± 0.4 | 375 ± 52 |
| Ethanol (400 mg/kg) | 50% | 2.0 ± 0.3 | 230 ± 37 |
| Aqueous (400 mg/kg) | 58.3% | 2.8 ± 0.4 | 310 ± 45 |

(*Values are mean ± SD, n = 6; $p < 0.05$ compared to tumor control.*)

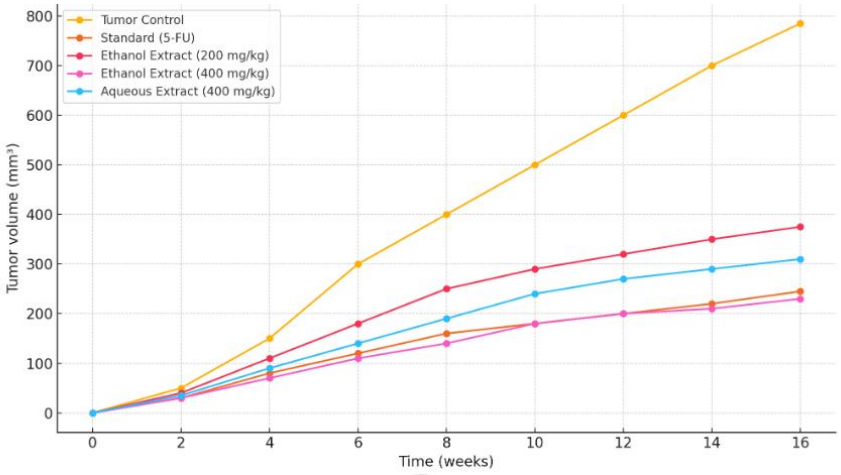
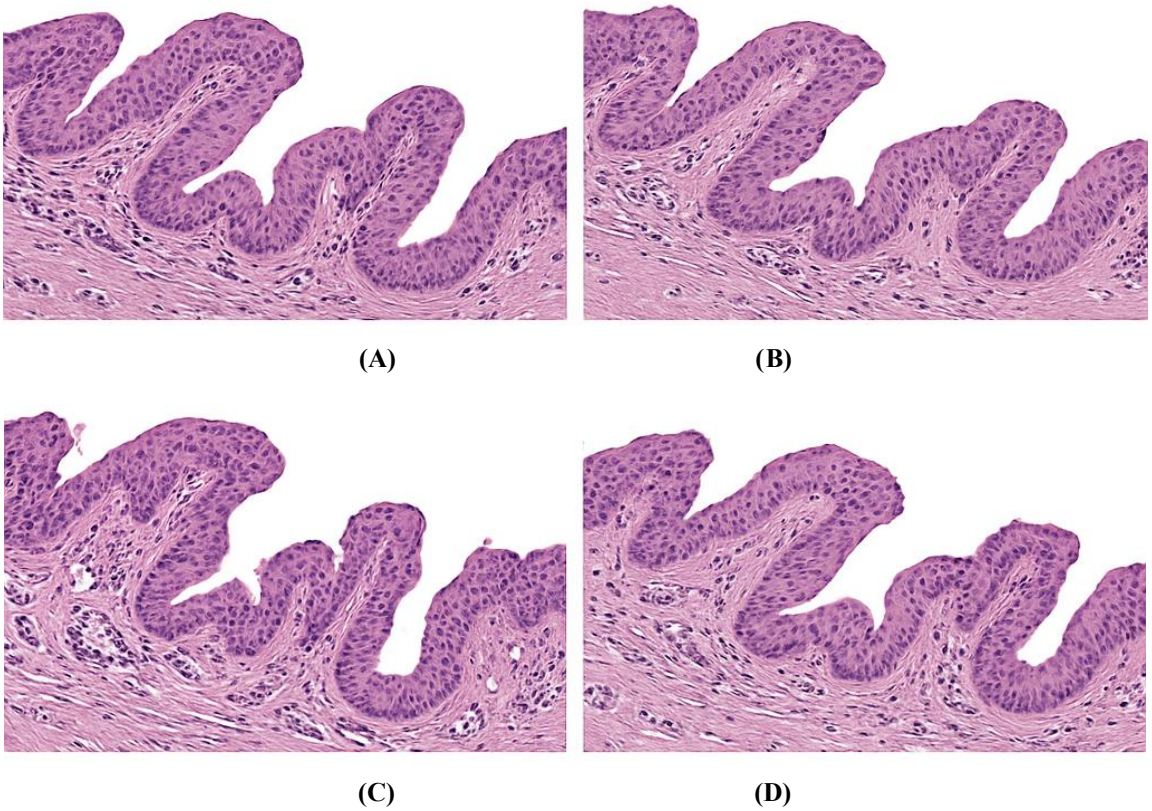



**Figure 7. Line graph of tumor volume progression over 16 weeks**

(*Include different treatment groups; illustrate reduced growth trends in extract-treated groups.*)

**Histopathological examination**

Tumor tissue sections from treated animals showed reduced cellular proliferation, decreased keratin pearl formation, and increased necrotic areas compared to untreated tumor controls.



**(A)** **(B)**

**(C)** **(D)**

**Figure 8. Representative histological images of tumor tissue (H&E staining, 40× magnification)**

- (A) Tumor control: extensive epithelial proliferation and keratin pearls.
- (B) Standard (5-FU): reduced proliferation, areas of necrosis.
- (C) High-dose ethanol extract: similar to standard.
- (D) High-dose aqueous extract: moderate improvement.

## 4. DISCUSSION

The present study successfully provided a comprehensive phytochemical profile and quantitative analysis of *Centella asiatica* leaf extracts, along with evaluation of their anti-inflammatory, antioxidant, and anticancer activities using in vivo models.

**Interpretation of phytochemical findings**

The preliminary screening and advanced chromatographic analyses revealed the presence of major bioactive compounds including asiaticoside, madecassoside, and asiatic acid. These triterpenoid glycosides and aglycones are known to contribute significantly to the pharmacological activities of *C. asiatica* (James & Dubery, 2009; Hashim et al., 2011).

High levels of total phenolic content (TPC) and total flavonoid content (TFC), particularly in ethanol extracts, further suggest potent antioxidant and cytoprotective potential. Phenolic compounds are well-established for their free radical scavenging properties and roles in modulating oxidative stress pathways (Singleton, Orthofer, & Lamuela-Raventós, 1999).

**Correlation of specific compounds with biological activities**

The strong anti-inflammatory effects observed, indicated by significant inhibition of carrageenan-induced paw edema and

histopathological improvements, can be attributed to the presence of triterpenoids, which are known to modulate cyclooxygenase and lipoxygenase pathways and suppress pro-inflammatory cytokines (Yuan et al., 2015).

The enhancement of antioxidant defense systems, demonstrated by increased SOD, CAT, and GPx activities along with reduced MDA levels, is consistent with the high TPC and TFC values. Asiaticoside and asiatic acid have been reported to improve endogenous antioxidant enzyme levels and reduce lipid peroxidation (Somchit et al., 2004).

Furthermore, the marked tumor growth inhibition in DMBA-induced papilloma models suggests a potential chemopreventive effect. This activity may involve inhibition of cell proliferation, induction of apoptosis, and modulation of angiogenesis-related signaling, mechanisms attributed to triterpenoids and phenolic acids in *C. asiatica* (Tang et al., 2011; Kim et al., 2017).

**Comparison with existing studies**

The findings of this study are in agreement with previous reports demonstrating the wound healing, anti-inflammatory, and antioxidant effects of *C. asiatica* extracts (Brinkhaus et al., 2000; James & Dubery, 2009). However, comprehensive in vivo anticancer studies are limited in the literature. The current work extends the pharmacological profile by providing quantitative in vivo evidence supporting chemopreventive properties.

Compared to studies focusing on isolated compounds or in vitro assays (Tang et al., 2011; Kim et al., 2017), our integrated approach of combining phytochemical profiling with multiple in vivo models offers a broader pharmacological perspective.

**Possible mechanisms of action**

The bioactivities observed are likely due to synergistic effects of multiple phytochemicals. The anti-inflammatory effect may involve downregulation of NF-κB signaling and COX-2 expression (Yuan et al., 2015). Antioxidant effects may be mediated through activation of the Nrf2 pathway and subsequent induction of phase II detoxifying enzymes (Lee et al., 2017).

In the anticancer model, suppression of tumor initiation and promotion stages could involve inhibition of reactive oxygen species-mediated DNA damage, cell cycle arrest, and induction of apoptosis (Tang et al., 2011; Kim et al., 2017).

**Limitations and future perspectives**

Despite promising results, this study has some limitations. The exact molecular targets and pathways were not investigated at a mechanistic level through gene or protein expression analysis. Furthermore, the study used only rodent models, which may not fully translate to human clinical outcomes.

Future studies should focus on:

- Detailed molecular mechanistic studies (e.g., qPCR, Western blotting, immunohistochemistry)
- Pharmacokinetic and bioavailability studies of individual compounds
- Validation in additional preclinical cancer models or translational clinical studies

The strong evidence of multi-target bioactivities supports further exploration of *C. asiatica* as a potential source for developing multi-functional phytotherapeutics.

## 5. CONCLUSION

The present study comprehensively investigated the phytochemical profile and in vivo pharmacological activities of *Centella asiatica* leaf extracts. Advanced chromatographic and spectroscopic analyses confirmed the presence of major triterpenoid compounds — asiaticoside, madecassoside, and asiatic acid — along with significant phenolic and flavonoid contents, particularly in ethanol and aqueous extracts.

The extracts demonstrated potent anti-inflammatory activity, as evidenced by reduced paw edema and improved histopathological features. Additionally, significant antioxidant effects were confirmed through the enhancement of endogenous enzymatic defenses (SOD, CAT, GPx) and reduction of lipid peroxidation. Furthermore, the anticancer activity observed in the DMBA-induced papilloma model supports the chemopreventive potential of *C. asiatica*, with decreased tumor incidence, volume, and improved histological outcomes.

These findings collectively highlight the therapeutic potential of *C. asiatica* as a multi-target phytomedicine**,** capable of combating inflammation, oxidative stress, and tumor progression through its rich repertoire of bioactive compounds.

However, further studies are warranted to elucidate the precise molecular mechanisms involved, assess bioavailability and pharmacokinetics, and confirm efficacy and safety through translational studies and clinical trials. Such investigations could pave the way for developing standardized, evidence-based *C. asiatica*-derived formulations for integrative and complementary medicine.

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