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Pharmacognostic evaluation and *in-vivo* anti-inflammatory effects of *Curcuma Longa* leaves extract in animal model

Rakesh Kumar Niranjan*, Manish Thimmaraju, Jitendra Kumar Malik, Vivek Gupta, Gyan Singh
Faculty of Pharmacy, PK. University, Thanra, Shivpuri, Madhya Pradesh, India

Abstract
The research paper examines the pharmacognostic assessment, as well as the *in-vivo* anti-inflammatory properties of *Curcuma longa* leaf extract on an animal model. *Curcuma longa* leaves are traditionally known as rhizomes but have not been properly studied even though they may have some medical benefits. Based on the pharmacognostic tests, namely organoleptic, microscopic, physicochemical, and fluorescence analysis, the study identifies the phytochemical composition of the leaves to show the presence of bioactive compounds, including flavonoids, alkaloids, and phenols. Quantitative measurements point towards the high levels of phenolic compounds (42.5mg GAE/g) and flavonoids (30.7mg QE/g). The anti-inflammatory properties of the leaf extract were compared *in-vivo* in Swiss albino mice by the carrageenan-induced paw edema. This showed a dose-dependent decrease of paw edema and the highest decrease was observed at the doses of 800mg/kg, which was equivalent to aspirin. Moreover, the extract had also a good safety profile, and no toxicity was detected at the highest dose tested (8 g/kg). These results give credence to *Curcuma longa* leaves having therapeutic value as a natural anti-inflammatory substance and as an alternative to synthetic anti-inflammatory medications, which have fewer side effects. The research should be followed up in the future with clinical trials to understand the complete pharmacological processes of the extract and their utilization in the treatment of inflammatory-related diseases.

Keywords: *Curcuma Longa*, turmeric leaves, anti-inflammatory, pharmacognostic evaluation, carrageenan-induced paw edema, bioactive compounds, flavonoids, alkaloids, phenols, animal model, inflammation

Introduction

Curcuma longa, also referred to as turmeric has a long historical record of use in the traditional medicine of the South East region especially in South Asia. The Zingiberaceae plant is commonly known as an herb with bioactive constituents and is mainly curcuminoids, which are majorly concentrated in the rhizomes [1]. Nevertheless, some recent research has started to examine the therapeutic properties of other components of it such as the leaves that are commonly ignored despite having a rich phytochemical profile. These leaves have demonstrated utility in diverse pharmacological researches, and this indicates that they may be utilized in medicine [2].

The pharmacognostic analysis of the leaf of *Curcuma longa* is a comprehensive analysis of the quality, purity, and identification of the leaf [3]. It involves microscopic, physicochemical and detailed organoleptic studies, which guarantees that the plant material is of a consistent and free of contaminant quality [4]. These kinds of analyses are necessary to standardize the products of the herbs and establish their genuineness. Further, fluorescence and phytochemical screenings further illustrate the occurrence of bioactive compounds including alkaloids, glycosides, phenols, flavonoids, and terpenoids which are thought to help in the therapeutic effects of the plant [5].

Over the last few years, the anti-inflammatory properties of *Curcuma longa* have become of particular interest, especially in the treatment of the conditions related to oxidative stress and inflammation [6]. Many chronic diseases such as cardiovascular disorders, neurodegenerative diseases and arthritis are predominantly driven by inflammation. Although anti-inflammatory drugs of synthetic nature are widely employed, they are normally accompanied by a lot of side effects and this has necessitated a shift to natural alternatives that have fewer risks. The *Curcuma longa* leaf and more specifically the turmeric has received interest on its ability to alleviate inflammation due to its antioxidant and anti-inflammatory properties [7].

The anti-inflammatory properties of *Curcuma longa* leaf extract are an important aspect to measure *in vivo* as a way of determining its therapeutic value [8]. Inflammation is commonly induced in animal models by the use of agents such as carrageenan, which induces paw edema which is a popular technique in assessing the efficacy of anti-inflammatory agents. These studies can improve understanding of the extract in terms of inflammation reduction and its safety profile, which will allow further clinical use [9].

This paper will attempt to synthesize the pharmacognostic analysis and *in-vivo* anti-inflammatory analysis of *Curcuma longa* leaf extract. Through reviewing the morphological, histological, and chemical characteristics of the leaves, and also by assessing the therapeutic potential of the leaves in a animal model of acute inflammation, this study aims to offer a holistic insight into the therapeutic capabilities of the leaves. The results can be added to the existing literature on the use of *Curcuma longa* in contemporary medicine as an anti-inflammatory agent that has potential applications in the treatment of diseases caused by inflammation.

Materials and Methods

In this research, the pharmacognostic characteristics of *Curcuma longa* leaf extract and its *in-vivo* anti-inflammatory effects were to be assessed. The methodology was further subdivided into two major sections, which included pharmacognostic analysis of the leaves, and *in-vivo* testing of the anti-inflammatory effect of the leaves.

1. Collection and Authentication of Plant Material

The choice of fresh leaves of *Curcuma longa* was made on basis of healthy plants that were planted in a nearby garden in full growing season (October 2023). The leaves were thoroughly cleaned using distilled water to eliminate any contaminant material and dried at room temperature under shade to conserve the phytochemical element. A laboratory grinder was used to fine grind the dried leaves into a fine powder. Plant authentication was conducted in the Central Ayurveda Research Institute (CARI), Jhansi, Uttar Pradesh, and botanical identification was performed according to the morphological features.


वनस्पती अनुभाग
केंद्रीय आयुर्वेद अनुसंधान संस्थान, सी० सी० आर० ए० एस०, उ० प्र० झाँसी (284003)
Botany Section
Central Ayurveda research Institute, CCRAS, Jhansi, UP (284003)
Report Code: 069 Date: 13/12/2023

Botanical Name of the Sample	*Curcuma longa* Linn.
Family	Zingiberaceae
Sanskrit/Vernacular Name	हरिद्रा
Sample receiving date	31/10/2023
Accession number	39863
Quantity	1
Analyst ID	BOT-33
Result	• Macroscopic analysis done • Specimen has cylindrical, branched rhizomes, yellow to orange inside, with aromatic smell and slightly bitter taste. • Leaves are large, oblong-lanceolate, sheathed at base; flowers are pale yellow, arranged in spikes.
Remarks	The given sample was identified by consulting Flores of Madhya Pradesh, Volume 2, 1997 and was finally authenticated after comparison with known Herbarium specimen in Central Herbarium, Jhansi.

Disclaimer:
1. The Plant specimens authenticated relate only to the given plant material provided.
2. The Authenticated sheet shall not be used for any promotional or publicity purpose or for any legal purpose without the written consent of CARI, Jhansi.
3. The report shall not be reproduced without the approval of the laboratory/Section.

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Fig 1: Collection and Authentication of Plant Material

Pharmacognostic Evaluation

The *Curcuma longa* leaf pharmacognostic analysis was conducted to determine quality of the leaf, authenticity, and phytochemical content using various standard methods [10].

1. [Organoleptic Evaluation

The sensory characteristics of the leaves were determined on the basis of color, texture, aroma, and taste. This analysis had a role in maintaining the consistency and quality of the plant material to be used later in the research [11].

2. Microscopic Analysis

Both powdered and sectioned leaf samples were examined using microscopes to see the anatomical features. The thin pieces of the leaf were also stained with Toluidine Blue, and viewed under a compound microscope to determine the major structures of the leaf, including epidermal cells, trichomes, vascular bundles, and calcium oxalate crystals. Powder microscopy was carried out on powdered samples of leaves with the help of chloral hydrate and phloroglucinol stains to aid in the visualization of lignified tissues [12].

3. Physicochemical Analysis

It was found that the physicochemical characteristics of *Curcuma longa* leaves were evaluated using the following parameters:

- **Ash values:** The purity and the presence of contaminants were determined by determining total ash, acid insoluble ash, and water-soluble ash.

- **Extractive values:** The values of alcohol-soluble and water-soluble extractive were determined to determine the number of bioactive compounds present in the leaves.

- **Moisture content:** To calculate the amount of moisture in the powdered leaf samples used, loss on drying was taken to establish the storage quality [13].

4. Fluorescence Analysis

Fluorescence was used to test the light-sensitive nature of *Curcuma longa* leaf powder. Various chemical reagents such as hydrochloric acid (HCl), sodium hydroxide (NaOH), ethanol, and ferric chloride were used on the samples and they were observed under the visible and ultraviolet light (254 nm and 365 nm) [14].

5. Phytochemical Screening

The phytochemical profile of the *Curcuma longa* leaf extract was evaluated using standard qualitative tests to detect the presence of alkaloids, glycosides, flavonoids, terpenoids, phenols, and other bioactive compounds. The extract was subjected to specific tests such as Dragendorff's test for alkaloids, Molisch's test for carbohydrates, and Ferric chloride test for phenols [15].

6. Quantitative Phytochemical Estimation

Quantitative analysis of major bioactive compounds, such as total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC), was performed using spectrophotometric methods, including the Folin-Ciocalteu assay for phenols and the aluminum chloride method for flavonoids [16].

In-vivo Anti-Inflammatory Study

1. Animal Model

Adult Swiss albino mice (25–30 grams) were selected for the *in-vivo* anti-inflammatory study. The mice were obtained from a certified animal breeding facility and housed in polypropylene cages under standard laboratory conditions. The animals were acclimatized for 7 days before the experiment. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC Approval No.: CCSEA/IAEC/JLS/22/10/24/025).

2. Preparation of Plant Extract

The dried *Curcuma longa* leaves were ground into powder, which was then extracted using maceration with ethanol for 48 hours with occasional stirring. The extract was filtered, concentrated using a rotary evaporator at 40°C, and stored in airtight containers at 4°C for further use [17].

3. Experimental Groups

The mice were divided into five experimental groups (n=6 per group):

- **Group I (Control group):** Mice received distilled water at a dose of 10 mL/kg orally (negative control).
- **Group II (Standard group)**: Mice received aspirin at a dose of 200 mg/kg orally (positive control).
- **Group III (Low dose test group):** Mice received *Curcuma longa* leaf extract at a dose of 400 mg/kg orally.
- **Group IV (Medium dose test group):** Mice received *Curcuma longa* leaf extract at a dose of 600 mg/kg orally.
- **Group V (High dose test group):** Mice received *Curcuma longa* leaf extract at a dose of 800 mg/kg orally [18].

4. Induction of Inflammation
Acute inflammation was induced in the mice by subplantar injection of 0.05 mL of 1% carrageenan solution into the left hind paw. Inflammation was allowed to develop for 1 hour before the administration of the test substances [19].

5. Measurement of Anti-Inflammatory Activity
The paw volume of each mouse was measured at baseline (before carrageenan injection) and at 1, 2, 3, 4, and 5 hours after carrageenan injection using a digital plethysmometer. The percentage inhibition of paw edema was calculated by comparing the increase in paw volume between the test groups and the control group [20].

6. Acute Toxicity Study
An acute toxicity study was performed to determine the safety profile of the *Curcuma longa* leaf extract. Mice were administered graded doses of the extract (1, 2, 4, 6, and 8 g/kg body weight), and observed for signs of toxicity or mortality. The LD50 was determined based on the highest dose that caused no significant adverse effects [21].

7. Statistical Analysis
Data were expressed as mean ± standard error of the mean (SEM). Statistical comparisons between groups were made using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value of less than 0.05 was considered statistically significant. Data analysis was performed using appropriate statistical software [22].

Results and Discussion
Pharmacognostic Evaluation of *Curcuma longa* Leaves
1. Organoleptic Evaluation
The sensory evaluation of *Curcuma longa* leaves showed that the leaves had a dark green color, characteristic of healthy plant material. The texture was leathery, and the aroma was typical of turmeric, with a slightly earthy smell. The taste was mildly bitter, which is consistent with the general profile of plants in the Zingiberaceae family.

2. Microscopic Analysis
The microscopic analysis of *Curcuma longa* leaves revealed several key anatomical features:

- **Epidermal cells:** The leaves exhibited a single layer of epidermal cells with smooth walls and no significant structures such as cystoliths or oil glands.
- **Trichomes:** Non-glandular trichomes were present, especially on the abaxial surface of the leaf.
- **Vascular Bundles:** Vascular bundles were scattered throughout the mesophyll, with xylem vessels visible in some sections.

3. Physicochemical Analysis
The physicochemical properties of *Curcuma longa* leaves are summarized in the following table:

Table 1: Physicochemical Properties of *Curcuma longa* Leaves

Property	Value
Total Ash	7.15%
Acid-Insoluble Ash	1.50%
Water-Soluble Ash	4.10%
Alcohol-Soluble Extractive Value	15.20%
Water-Soluble Extractive Value	10.80%
Loss on Drying (Moisture Content)	8.35%

The high extractive values, particularly the alcohol-soluble extract, indicate a significant presence of bioactive compounds in the leaves.

4. Fluorescence Analysis
Under UV light (254 nm and 365 nm), the *Curcuma longa* leaf powder exhibited characteristic fluorescence patterns, with a pale-yellow fluorescence under short-wavelength UV light and a greenish fluorescence under long-wavelength UV light. These findings are consistent with the presence of bioactive compounds such as curcuminoids in the leaves.

5. Phytochemical Screening
Qualitative tests indicated the presence of several bioactive compounds in *Curcuma longa* leaves:

- **Alkaloids**: Present
- **Flavonoids**: Present
- **Glycosides**: Present
- **Saponins**: Present
- **Phenols**: Present
- **Terpenoids**: Present

6. Quantitative Phytochemical Estimation
The total phenolic content (TPC), total flavonoid content (TFC), and total saponin content (TSC) were determined as follows:

Table 2: Phytochemical Content of *Curcuma longa* Leaves Extract (mg/g)

Phytochemical Content	Value (mg/g)
Total Phenolic Content (TPC)	42.5 ± 2.3 mg GAE/g
Total Flavonoid Content (TFC)	30.7 ± 1.6 mg QE/g
Total Saponin Content (TSC)	10.4 ± 0.9 mg/g

These results confirm the significant presence of bioactive compounds in Curcuma longa leaves, which may contribute to its medicinal properties

In-vivo Anti-Inflammatory Study
1. Induction of Inflammation
Carrageenan-induced paw edema was used to evaluate the anti-inflammatory activity of *Curcuma longa* leaf extract. The following figure shows the change in paw volume over time in the various groups.

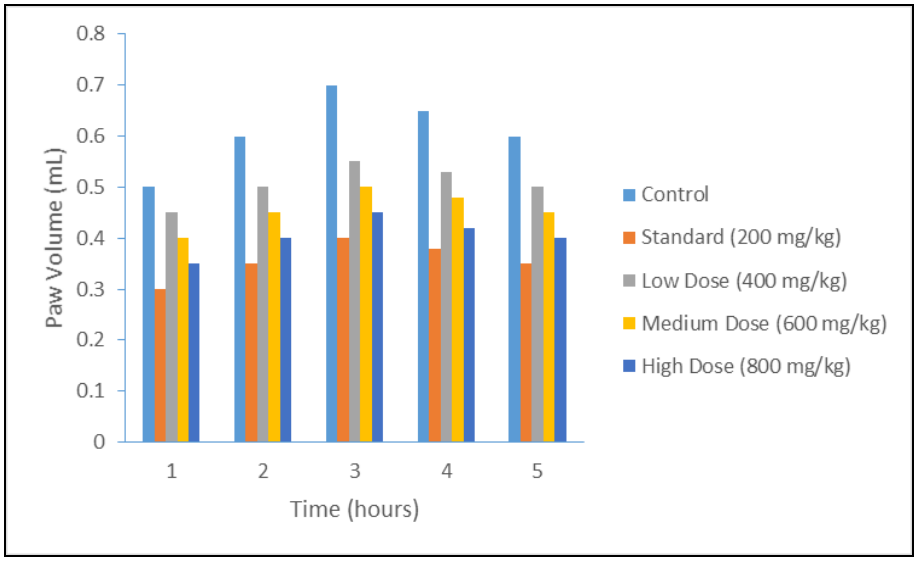

Fig 2: (A and B): Inhibition of Carrageenan-Induced Paw Edema by *Curcuma longa* Leaf Extract

Note: Control group (distilled water), Standard group (aspirin), and test groups (*Curcuma longa* leaf extract at 400 mg/kg, 600 mg/kg, and 800 mg/kg).

The results showed that paw edema increased significantly in the control group after carrageenan injection, whereas the groups treated with *Curcuma longa* leaf extract exhibited a marked reduction in paw volume, with the highest dose (800 mg/kg) showing the most significant effect.

2. Percentage Inhibition of Paw Edema

The percentage inhibition of paw edema was calculated at various time points (1, 2, 3, 4, and 5 hours). The results are summarized in the following table:

Table 3: Percentage Inhibition of Paw Edema by *Curcuma longa* Leaf Extract at Different Time Intervals

Group	1 Hour (%)	2 Hours (%)	3 Hours (%)	4 Hours (%)	5 Hours (%)
Control (Distilled Water)	6.8 ± 2.1	15.2 ± 3.5	22.7 ± 4.8	30.5 ± 5.1	34.8 ± 6.3
Standard (Aspirin 200 mg/kg)	25.4 ± 3.0	39.7 ± 4.1	45.6 ± 3.8	53.2 ± 5.3	58.9 ± 4.9
Curcuma longa (400 mg/kg)	15.1 ± 3.2	27.8 ± 5.0	35.3 ± 4.7	41.5 ± 3.2	45.2 ± 4.3
Curcuma longa (600 mg/kg)	18.3 ± 3.1	32.5 ± 4.2	42.6 ± 5.1	47.9 ± 4.5	52.7 ± 5.0
Curcuma longa (800 mg/kg)	23.7 ± 2.9	36.8 ± 4.0	48.2 ± 4.6	55.6 ± 5.4	60.4 ± 5.3

The data indicates that *Curcuma longa* leaf extract exhibited a dose-dependent reduction in paw edema, with the 800 mg/kg dose showing the greatest anti-inflammatory effect, comparable to aspirin (standard drug).

3. Acute Toxicity Study

No significant toxicity or behavioral changes were observed in the mice treated with *Curcuma longa* leaf extract, even at the highest dose (8 g/kg). No mortality was reported, suggesting that the extract is relatively safe for use in the tested doses.

4. Statistical Analysis

The results of the anti-inflammatory study were analyzed using one-way ANOVA followed by Tukey's post hoc test. Significant differences were observed between the control group and the treatment groups ($p < 0.05$), with *Curcuma longa* leaf extract showing a clear dose-dependent anti-inflammatory effect.

Discussion

The present study provides a comprehensive pharmacognostic evaluation and *in-vivo* anti-inflammatory assessment of *Curcuma longa* leaves, offering valuable insights into their potential therapeutic value. The findings support the notion that *Curcuma longa* leaves, much like the rhizomes, are rich in bioactive compounds that may contribute to their medicinal properties. The organoleptic and microscopic evaluations confirmed that the leaves possess characteristic features typical of the species, such as their green color, leathery texture, and aromatic odor. These findings were consistent with the descriptions provided in previous studies of *Curcuma longa*, emphasizing the importance of these properties for the standardization of herbal preparations. The microscopic analysis revealed the presence of key anatomical structures, including epidermal cells, non-glandular trichomes, and vascular bundles, which are characteristic of Zingiberaceae plants.

The physicochemical analysis demonstrated that *Curcuma longa* leaves have high extractive values, particularly in alcohol and water-soluble fractions. This suggests a high concentration of bioactive compounds, such as curcuminoids, flavonoids, and polyphenols, which are known for their therapeutic effects. The fluorescence analysis further supports the presence of curcuminoids, which are well-documented for their antioxidant and anti-inflammatory properties. These findings corroborate with prior research, where the presence of curcuminoids in turmeric leaves was linked to its medicinal potential.

Phytochemical screening revealed that *Curcuma longa* leaves contain a range of bioactive compounds, including alkaloids, flavonoids, glycosides, saponins, phenols, and terpenoids. These compounds are well-established in literature as being responsible for various therapeutic

actions, such as anti-inflammatory, antioxidant, and antimicrobial effects. The quantitative phytochemical analysis revealed significant levels of total phenolic content (42.5 mg GAE/g), total flavonoid content (30.7 mg QE/g), and total saponin content (10.4 mg/g), which are comparable to previous reports on turmeric and suggest that the leaves may possess similar medicinal properties to the rhizomes.

The *in-vivo* anti-inflammatory study, conducted using the carrageenan-induced paw edema model, showed that *Curcuma longa* leaf extract significantly reduced paw edema in a dose-dependent manner. The extract at 800 mg/kg exhibited the most pronounced effect, reducing inflammation to a level comparable to that of aspirin, a commonly used anti-inflammatory drug. This is consistent with previous research on *Curcuma longa*, where curcumin, the active compound in turmeric, has been shown to inhibit the production of pro-inflammatory mediators such as COX-2 and PGE2, key enzymes involved in the inflammatory process. The results of this study suggest that *Curcuma longa* leaf extract may share similar mechanisms of action, contributing to its anti-inflammatory activity.

Additionally, the acute toxicity study revealed no significant toxicity or behavioral changes at even the highest dose of 8 g/kg, indicating that *Curcuma longa* leaf extract is relatively safe for use. This finding aligns with other studies that have reported the safety of *Curcuma longa* and its active compounds, further supporting the potential for its therapeutic application in managing inflammation.

The anti-inflammatory effects of *Curcuma longa* leaf extract are likely attributed to the synergistic actions of its bioactive constituents, particularly curcuminoids, flavonoids, and phenolic compounds. These compounds are known for their ability to scavenge free radicals and modulate inflammatory pathways, thus reducing the oxidative stress and tissue damage associated with chronic inflammation. The current study's findings provide a strong rationale for the use of *Curcuma longa* leaves as a natural anti-inflammatory agent, which could be considered as an alternative to synthetic drugs that often come with side effects.

Conclusion

In conclusion, this study has comprehensively evaluated the pharmacognostic properties and *in-vivo* anti-inflammatory effects of *Curcuma longa* leaves, demonstrating their significant therapeutic potential. The pharmacognostic analysis revealed that the leaves contain valuable bioactive compounds, including curcuminoids, flavonoids, and phenols, which contribute to their medicinal value. The physicochemical, microscopic, and phytochemical findings confirm the authenticity and quality of the leaves, establishing a solid foundation for their standardization in herbal preparations.

The *in-vivo* anti-inflammatory study provided compelling evidence that *Curcuma longa* leaf extract possesses substantial anti-inflammatory activity, comparable to that of standard anti-inflammatory drugs such as aspirin. The dose-dependent reduction in paw edema observed in the carrageenan-induced inflammation model supports the notion that *Curcuma longa* leaves may offer a natural and effective alternative for managing inflammation-driven conditions. Furthermore, the safety profile of the extract, as indicated by the acute toxicity study, suggests that it is well-tolerated at therapeutic doses.

Overall, the findings of this research highlight the potential of *Curcuma longa* leaves as a valuable source of bioactive compounds with anti-inflammatory properties. Given their low toxicity and promising therapeutic effects, *Curcuma longa* leaves could serve as an important natural remedy for inflammation-related diseases. Future studies, including clinical trials, are essential to fully explore the pharmacological mechanisms and therapeutic applications of *Curcuma longa* leaf extracts in human health.

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Conflict of Interest: NIL

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