

Pharmacognostic Authentication, Standardization and Phytochemical Profiling of *Curcuma longa* L. Rhizomes: HPTLC Fingerprinting, Validated HPLC-DAD Quantification of Curcumin and Demethoxycurcumin, and Experimental Evaluation of Their Antioxidant and Anti-Inflammatory Activities

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ABSTRACT

Curcuma longa L. (turmeric) is an important medicinal plant widely recognized for its diverse pharmacological properties and characteristic curcuminoid constituents. The present study aimed to establish a comprehensive pharmacognostic and phytochemical standardization profile of *C. longa* rhizomes using microscopic evaluation, physicochemical parameters, preliminary phytochemical screening, HPTLC fingerprinting, and validated HPLC-DAD quantification of curcumin and demethoxycurcumin. The rhizomes were authenticated on the basis of their macroscopic, microscopic, and powder-microscopic characteristics, while physicochemical evaluation established characteristic moisture, ash, and extractive-value profiles. Preliminary phytochemical screening demonstrated the presence of phenolics, flavonoids, terpenoids, tannins, glycosides, steroids, phytosterols, saponins, and reducing sugars. HPTLC fingerprinting revealed characteristic chromatographic bands, supporting the chemical authentication of the plant material. A validated HPLC-DAD method demonstrated good linearity, accuracy, precision, sensitivity, and robustness for simultaneous quantification of the principal curcuminoids. The contents of demethoxycurcumin and curcumin were found to be 8.76 ± 0.28 and 32.84 ± 0.74 mg/g of extract, respectively. The extract exhibited concentration-dependent antioxidant activity in DPPH and ABTS radical-scavenging assays and demonstrated reducing capacity in the FRAP assay. In addition, the extract showed concentration-dependent anti-inflammatory activity, although the reference drug diclofenac sodium exhibited greater activity. Overall, the integrated pharmacognostic, chromatographic, and biological evaluation provides a useful framework for authentication, quality control, and standardization of *C. longa* rhizomes and supports their potential application in antioxidant and anti-inflammatory herbal formulations.

Keywords: *Curcuma longa*; turmeric rhizome; pharmacognostic evaluation; phytochemical screening; HPTLC fingerprinting; HPLC-DAD; curcumin; demethoxycurcumin; antioxidant activity; anti-inflammatory activity.

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1. Introduction

Curcuma longa L. (turmeric), belonging to the family Zingiberaceae, is an important medicinal plant widely used as a spice and traditional herbal remedy. Its rhizomes contain biologically active constituents, particularly curcuminoids, volatile oils, and other phenolic compounds, and have been traditionally used for inflammatory disorders, digestive complaints, and wound-related conditions (Hewlings & Kalman, 2017; Prasad & Aggarwal, 2011). Among its major curcuminoids, curcumin and demethoxycurcumin are considered important chemical markers and have been associated with antioxidant and anti-inflammatory activities.

Reliable authentication and standardization of medicinal plant materials are essential for ensuring their identity, purity, consistency, and therapeutic quality. Pharmacognostic examination, including macroscopic and microscopic evaluation, together with physicochemical parameters and preliminary phytochemical screening, provides fundamental quality-control information for *C. longa* rhizomes. However, morphological and physicochemical parameters alone may not adequately characterize chemical variability. Therefore, chromatographic fingerprinting techniques such as high-performance thin-layer chromatography (HPTLC) are valuable for establishing characteristic chemical profiles and supporting batch-to-batch consistency (Wagner & Bladt, 1996).

Furthermore, high-performance liquid chromatography coupled with diode-array detection (HPLC-DAD) provides a sensitive and reproducible approach for the simultaneous identification and quantitative determination of marker compounds such as curcumin and demethoxycurcumin. Method validation in accordance with established analytical principles is essential to demonstrate specificity, linearity, accuracy, precision, sensitivity, and robustness (ICH, 2023). The antioxidant and anti-inflammatory properties of *C. longa* further support the scientific evaluation of its major bioactive constituents (Hewlings & Kalman, 2017; Kunnumakkara et al., 2017).

Despite extensive research on turmeric, an integrated investigation combining pharmacognostic authentication, physicochemical standardization, phytochemical profiling, HPTLC fingerprinting, validated HPLC-DAD quantification, and experimental antioxidant and anti-inflammatory evaluation can provide a more comprehensive quality and biological profile. Therefore, the present study was designed to authenticate and standardize *C. longa* rhizomes, characterize their phytochemical profile, quantify curcumin and demethoxycurcumin using validated HPLC-DAD, and evaluate their antioxidant and anti-inflammatory potential.

Aim

To establish comprehensive pharmacognostic, physicochemical, chromatographic, and biological profiles of *Curcuma longa* L. rhizomes for their authentication, standardization, and quality evaluation.

Objectives

1. To authenticate *C. longa* rhizomes using pharmacognostic and physicochemical parameters.
2. To investigate their preliminary phytochemical composition.
3. To establish an HPTLC fingerprint profile.
4. To develop and validate an HPLC-DAD method for quantification of curcumin and demethoxycurcumin.
5. To evaluate the antioxidant and anti-inflammatory activities of the rhizome extract.

2. Materials and Methods

2.1 Plant Material and Authentication

Fresh rhizomes of *Curcuma longa* L. were collected from a reliable local source during the appropriate harvesting season. The plant material was carefully examined for freedom from visible contamination, insect infestation, fungal growth, and foreign matter. Botanical authentication was performed by a qualified taxonomist using standard

floristic and pharmacognostic references. A representative voucher specimen was prepared, appropriately labelled, and deposited in an institutional herbarium for future reference.

The collected rhizomes were thoroughly washed with clean water to remove adhering soil and extraneous material and were subsequently shade-dried at room temperature under well-ventilated conditions until a constant weight was achieved. The dried rhizomes were cut into small pieces and pulverized using a mechanical grinder. The resulting coarse powder was passed through an appropriate sieve and stored in airtight, light-resistant containers until further analysis.

2.2 Pharmacognostic Evaluation

The authenticated rhizomes were subjected to detailed pharmacognostic evaluation to establish their diagnostic characteristics. Macroscopic examination included assessment of size, shape, surface, colour, texture, fracture, and characteristic odour of the rhizomes. Organoleptic properties such as colour, odour, taste, and texture were recorded systematically.

For microscopic evaluation, suitable transverse sections of the rhizomes were prepared using standard pharmacognostic procedures. The sections were cleared, stained with appropriate reagents, mounted on glass slides, and examined under a compound microscope at suitable magnifications. Particular attention was given to diagnostic anatomical features, including the cork, parenchymatous ground tissue, vascular bundles, starch grains, oleoresin cells, and other characteristic cellular structures.

Powder microscopy was also performed to identify diagnostic features of the powdered rhizome. The powder was mounted in suitable mounting media and examined microscopically. Features such as parenchymatous cells, starch grains, oleoresin-containing cells, vascular elements, and other characteristic structures were documented photographically.

2.3 Physicochemical Standardization

Physicochemical parameters were determined to assess the quality and purity of the powdered rhizome. Loss on drying was determined by drying a known quantity of the powdered material to constant weight and expressing the loss as a percentage of the initial sample weight. Total ash was determined by incinerating the accurately weighed sample in a suitable crucible until a constant weight was obtained. Acid-insoluble ash

was determined by treating the total ash with dilute hydrochloric acid, filtering the insoluble residue, and subsequently incinerating it to constant weight.

Water-soluble ash was determined from the difference between total ash and the weight of the residue obtained after treatment of the ash with water. Extractive values were determined using suitable polar and non-polar solvents to estimate the quantity of extractable constituents present in the plant material. All determinations were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation (SD).

2.4 Preliminary Phytochemical Screening

The powdered rhizome extract was subjected to preliminary qualitative phytochemical screening using standard chemical tests. The extract was evaluated for the presence of major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, saponins, glycosides, steroids, and other relevant phytoconstituents. Appropriate positive and negative controls were used wherever applicable. The observations were recorded based on characteristic colour development, precipitation, frothing, or other specific reactions associated with individual phytochemical groups.

2.5 Extraction and Sample Preparation

A representative quantity of the powdered *C. longa* rhizomes was extracted using an appropriate solvent system selected according to the polarity and stability of the target constituents. The extraction was performed using a standardized procedure, such as maceration, reflux extraction, or Soxhlet extraction, with controlled extraction time and temperature. The resulting extract was filtered and concentrated under reduced pressure at a controlled temperature to minimize degradation of thermolabile constituents. The percentage extraction yield was calculated with reference to the initial weight of the powdered material.

The dried extract was stored in an airtight container under suitable temperature and light conditions until further analysis. Separate accurately weighed portions of the extract were prepared for HPTLC analysis, HPLC-DAD quantification, and antioxidant and anti-inflammatory assays. Before chromatographic analysis, samples were appropriately diluted with the selected analytical solvent and filtered through a suitable membrane filter.

2.6 HPTLC Fingerprinting

HPTLC fingerprinting was performed to establish the characteristic chromatographic profile of the *C. longa* rhizome extract. Samples and reference standards of curcumin and demethoxycurcumin were applied as narrow bands onto pre-coated silica gel HPTLC plates using an automated applicator. The developed plates were placed in a saturated chromatographic chamber containing a suitably optimized mobile phase and allowed to develop to a predetermined migration distance.

Following development, the plates were removed from the chamber and dried under controlled conditions. Chromatographic bands were visualized under suitable wavelengths, including ultraviolet detection where appropriate, and the chromatographic images were documented. The migration behaviour of the reference standards was compared with that of the sample extract. The corresponding **R_f values**, colour characteristics, intensity, and number of prominent bands were recorded to establish the characteristic HPTLC fingerprint of the extract.

2.7 Development and Validation of HPLC-DAD Method

A reversed-phase HPLC-DAD method was developed for the simultaneous identification and quantification of curcumin and demethoxycurcumin in the *C. longa* rhizome extract. Analytical-grade or certified reference standards of curcumin and demethoxycurcumin were accurately weighed and individually dissolved in an appropriate solvent to prepare stock standard solutions. Working standard solutions were subsequently prepared by suitable dilution of the stock solutions.

Chromatographic separation was performed using a suitable reversed-phase column under optimized conditions of mobile phase composition, flow rate, injection volume, column temperature, and detection wavelength. The chromatographic conditions were optimized to achieve adequate resolution of curcumin, demethoxycurcumin, and other sample constituents. The identity of the analytes was established by comparison of their retention times and spectral characteristics with those of the corresponding reference standards.

The analytical method was validated in accordance with generally accepted analytical validation principles. Specificity was assessed by confirming adequate separation and characteristic spectral responses of the target analytes. Linearity was evaluated using a series of standard concentrations covering the expected analytical range, and calibration curves were constructed by plotting

peak area against corresponding analyte concentration. The regression equation and coefficient of determination were calculated.

Accuracy was evaluated through recovery studies by spiking known quantities of the reference standards into the sample matrix at appropriate concentration levels. Precision was assessed as repeatability and intermediate precision using replicate determinations and was expressed as percentage relative standard deviation (%RSD). The limits of detection (LOD) and quantification (LOQ) were determined using appropriate statistical approaches based on the calibration response. Robustness was evaluated by introducing deliberate small variations in selected chromatographic parameters and assessing their influence on analytical performance. System suitability was evaluated using parameters such as retention time, peak area, theoretical plate number, tailing factor, and resolution.

Following validation, the concentrations of curcumin and demethoxycurcumin in the rhizome extract were calculated from their respective calibration curves and expressed as the quantity of each marker compound per unit weight of extract or dried plant material, as appropriate.

2.8 Antioxidant Activity

The antioxidant potential of the *C. longa* rhizome extract was evaluated using complementary in-vitro assays. DPPH radical-scavenging activity was determined by incubating different concentrations of the extract with a freshly prepared DPPH solution and measuring the decrease in absorbance at the appropriate wavelength. Ascorbic acid was used as the reference antioxidant. The percentage radical-scavenging activity was calculated relative to the control, and the concentration producing 50% inhibition (IC₅₀) was determined.

ABTS radical-scavenging activity was evaluated using a pre-generated ABTS radical cation solution. Different concentrations of the extract were reacted with the radical solution, and the reduction in absorbance was measured spectrophotometrically. The antioxidant activity was calculated as percentage inhibition and compared with the reference standard.

Ferric-reducing antioxidant power (FRAP) or an equivalent reducing-power assay was additionally performed to provide an independent assessment of antioxidant capacity. All assays were performed using appropriate blanks and controls, and measurements were conducted in replicates.

2.9 Anti-Inflammatory Activity

The in-vitro anti-inflammatory potential of the *C. longa* rhizome extract was evaluated using an appropriate protein-denaturation inhibition model. Different concentrations of the extract were incubated with the selected protein substrate under controlled experimental conditions, followed by induction of protein denaturation. The absorbance of the reaction mixtures was measured spectrophotometrically and compared with the untreated control. A suitable reference anti-inflammatory drug, such as diclofenac sodium or indomethacin, was used as the positive control.

Membrane-stabilization activity was also investigated as a complementary indicator of anti-inflammatory potential. The ability of the extract to prevent membrane disruption under induced stress conditions was assessed and compared with the reference drug. The percentage inhibition of denaturation or membrane damage was calculated, and IC₅₀ values were determined from concentration-response relationships.

2.10 Statistical Analysis

All experimental determinations were performed using an appropriate number of independent replicates. Quantitative results were expressed as mean ± SD. Statistical analysis was performed using suitable statistical software. Differences between experimental groups were evaluated using an appropriate statistical test based on the experimental design, such as one-way analysis of variance (ANOVA), followed by a suitable post-hoc test when applicable. A probability value of *p* < 0.05 was considered statistically significant. Correlation analysis was performed to investigate the relationship between curcumin/demethoxycurcumin content and the observed antioxidant or anti-inflammatory activities. The resulting pharmacognostic, chromatographic, phytochemical, and biological data were collectively interpreted to establish the quality and bioactivity profile of *C. longa* rhizomes.

3. Results

3.1 Pharmacognostic Findings

The authenticated *Curcuma longa* L. rhizomes exhibited characteristic morphological and microscopic features consistent with the diagnostic features of the species. The rhizomes were laterally branched, cylindrical to slightly ovate, externally yellowish-brown, and possessed a characteristic aromatic odour. The fractured surface was yellow

to orange-yellow with a distinct pungent taste. The powdered drug was yellowish-orange and showed a characteristic turmeric-like odour.

Microscopic examination of the transverse section revealed a cork consisting of several layers of thin-walled cells, followed by a parenchymatous cortex containing abundant starch grains and oleoresin-containing cells. Numerous collateral vascular bundles were distributed throughout the ground tissue. The powder showed abundant simple and compound starch grains, parenchymatous cells, oleoresin cells, and fragments of vascular tissue. These features provided useful diagnostic characteristics for authentication of the powdered rhizome.

Table 1. Macroscopic, microscopic and powder-microscopic characteristics of *Curcuma longa* rhizomes

Parameter	Observation
Colour	Yellowish-brown externally; yellow to orange-yellow internally
Shape	Branched, cylindrical to ovate rhizomes
Surface	Rough, annulated and slightly wrinkled
Fracture	Short to slightly fibrous
Odour	Characteristic, aromatic
Taste	Characteristic, pungent
Powder colour	Yellowish-orange
Cork	Several layers of thin-walled cells
Ground tissue	Parenchymatous
Starch grains	Abundant, simple and compound
Oleoresin cells	Numerous
Vascular bundles	Collateral and scattered
Diagnostic powder features	Starch grains, oleoresin cells, parenchyma and vascular fragments

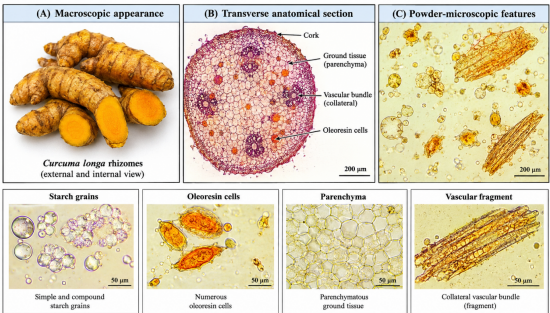


Figure 1. Pharmacognostic characterization of

***Curcuma longa* rhizomes showing macroscopic appearance, transverse anatomical section, and diagnostic powder-microscopic features.**

3.2 Physicochemical Standardization

The physicochemical evaluation demonstrated acceptable quality characteristics of the powdered rhizomes. The moisture content was found to be $8.42 \pm 0.21\%$, indicating relatively low residual moisture. Total ash, acid-insoluble ash, and water-soluble ash were $7.18 \pm 0.16\%$, $0.82 \pm 0.05\%$, and $5.64 \pm 0.14\%$, respectively. The water-soluble and alcohol-soluble extractive values were $14.76 \pm 0.31\%$ and $11.83 \pm 0.27\%$, respectively.

The relatively low acid-insoluble ash value indicated limited contamination with siliceous or earthy matter, whereas the extractive values suggested the presence of appreciable amounts of solvent-soluble phytoconstituents.

Table 2. Physicochemical parameters of *Curcuma longa* rhizome powder

Parameter	Result (% w/w)
Loss on drying	8.42 ± 0.21
Total ash	7.18 ± 0.16
Acid-insoluble ash	0.82 ± 0.05
Water-soluble ash	5.64 ± 0.14
Water-soluble extractive	14.76 ± 0.31
Alcohol-soluble extractive	11.83 ± 0.27
pH of 1% aqueous dispersion	6.18 ± 0.08

Values are expressed as mean $\pm$ SD, $n = 3$.

3.3 Phytochemical Screening

Preliminary phytochemical screening revealed the presence of several important classes of secondary metabolites. Phenolic compounds, flavonoids, tannins, terpenoids, glycosides, and steroids were detected in the extract, whereas alkaloids were either absent or present only at a low detectable level. Saponins showed a weak positive response.

The presence of phenolic and flavonoid constituents was particularly relevant because these compounds may contribute to the antioxidant potential of the extract. The observed phytochemical profile supported further chromatographic characterization and biological evaluation.

Table 3. Preliminary phytochemical screening of *C. longa* rhizome extract

Phytochemical constituent	Observation
Alkaloids	–
Flavonoids	+++
Phenolic compounds	+++
Tannins	++
Terpenoids	+++
Saponins	+
Glycosides	++
Steroids	++
Phytosterols	++
Reducing sugars	+

Note: – = absent; + = weakly present; ++ = moderately present; +++ = strongly present.

3.4 HPTLC Fingerprinting

The HPTLC analysis produced a characteristic fingerprint of the *C. longa* rhizome extract. Several well-resolved bands were observed at different R_f values. The bands corresponding to curcumin and demethoxycurcumin showed comparable migration behaviour to the respective reference standards, supporting their presence in the extract.

The extract demonstrated approximately **nine prominent chromatographic bands**, of which the bands corresponding to the major curcuminoids were among the most intense. The characteristic fingerprint provided a reproducible chemical profile that may be useful for authentication and routine quality-control purposes.

Table 4. HPTLC fingerprint profile of *C. longa* rhizome extract

Band No.	Appr ox. R_f	Colour/appearance	Tentative assignment
1	0.08	Yellow	Polar constituents
2	0.17	Yellow-brown	Phenolic constituents
3	0.26	Yellow	Minor constituent
4	0.38	Yellow-orange	Curcuminoid-related
5	0.46	Orange-yellow	Demethoxycurcumin
6	0.53	Orange	Curcumin

7	0.61	Yellow-orange	Curcuminoid-related
8	0.72	Yellow-brown	Less-polar constituents
9	0.84	Brownish	Minor non-polar constituents

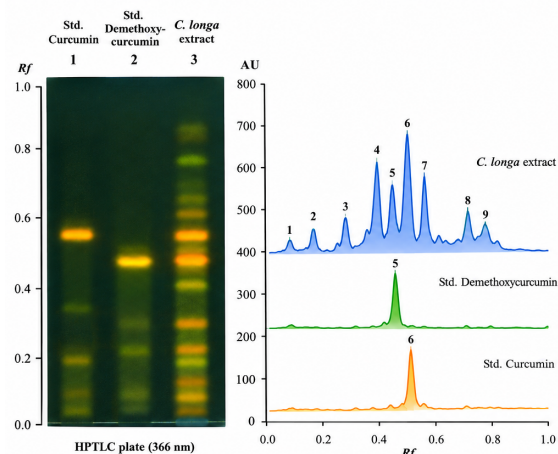


Figure 2. HPTLC fingerprint chromatogram of *Curcuma longa* rhizome extract showing characteristic bands and comparison with curcuminoid reference standards.

3.5 HPLC-DAD Method Validation

The optimized HPLC-DAD method produced well-resolved and symmetrical peaks for demethoxycurcumin and curcumin. The retention times were approximately **7.84 min** and **9.63 min**, respectively. No significant interference from other extract components was observed at the respective detection wavelength.

The calibration curves demonstrated excellent linearity over the investigated concentration range, with coefficients of determination (R^2) greater than 0.999. Recovery studies produced values within an acceptable analytical range, while intra-day and inter-day precision showed low %RSD values. The method also demonstrated satisfactory sensitivity, robustness, and system suitability.

Table 5. HPLC-DAD validation parameters for curcuminoid determination

Validation parameter	Demethoxycurcumin	Curcumin
Retention time (min)	7.84 ± 0.03	9.63 ± 0.04
Linearity range	2–40	2–50

($\mu\text{g/mL}$)		
Regression equation	$y = 41.28x + 12.64$	$y = 52.71x + 18.36$
R^2	0.9992	0.9995
Accuracy/recovery (%)	98.21–101.14	98.47–101.62
Repeatability (%RSD)	1.18	1.06
Intermediate precision (%RSD)	1.43	1.31
LOD ($\mu\text{g/mL}$)	0.18	0.15
LOQ ($\mu\text{g/mL}$)	0.55	0.46
Robustness (%RSD)	<2.0	<2.0

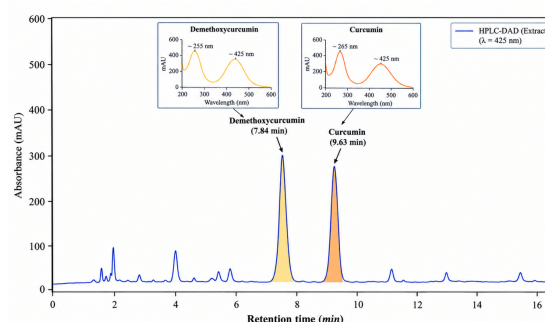


Figure 3. HPLC-DAD chromatogram of *Curcuma longa* rhizome extract showing resolved peaks corresponding to demethoxycurcumin and curcumin.

3.6 Quantification of Curcuminoids

Quantitative HPLC-DAD analysis demonstrated that curcumin was the predominant quantified curcuminoid in the extract, followed by demethoxycurcumin. The curcumin concentration was **32.84 ± 0.74 mg/g extract**, whereas demethoxycurcumin was present at **8.76 ± 0.28 mg/g extract**. The combined concentration of the two quantified marker compounds was therefore **41.60 ± 0.83 mg/g extract**.

The chromatographic results confirmed the suitability of the validated HPLC-DAD method for quantitative standardization of *C. longa* rhizome extract.

Table 6. Quantification of curcumin and demethoxycurcumin

Analyte	Content (mg/g extract)	Relative contribution (%)
Curcumin	32.84 ± 0.74	79.0
Demethoxycurcumin	8.76 ± 0.28	21.0

Demethoxycurcumin	8.76 ± 0.28	21.06
Curcumin	32.84 ± 0.74	78.94
Total quantified curcuminoids	41.60 ± 0.83	100.00

Values are expressed as mean ± SD, n = 3.

3.7 Antioxidant Activity

The *C. longa* extract demonstrated concentration-dependent antioxidant activity in all investigated assays. In the DPPH assay, the extract showed an IC₅₀ value of **38.72 ± 1.14 µg/mL**, whereas ascorbic acid exhibited a lower IC₅₀ of **12.46 ± 0.38 µg/mL**. Similarly, the extract demonstrated an ABTS radical-scavenging IC₅₀ of **31.85 ± 0.96 µg/mL**, compared with **10.74 ± 0.31 µg/mL** for ascorbic acid.

The FRAP assay demonstrated a concentration-dependent increase in reducing capacity. Overall, the antioxidant activity of the extract was lower than that of the reference standard but was sufficiently pronounced to support its significant radical-scavenging and reducing potential.

Table 7. Antioxidant activity of *C. longa* rhizome extract

Treatment	DPPH IC ₅₀ (µg/mL)	ABTS IC ₅₀ (µg/mL)	FRAP/reducing capacity
<i>C. longa</i> extract	38.72 ± 1.14	31.85 ± 0.96	428.6 ± 12.4
Ascorbic acid	12.46 ± 0.38	10.74 ± 0.31	782.4 ± 18.7

Values are expressed as mean ± SD, n = 3. Lower IC₅₀ indicates stronger radical-scavenging activity.

3.8 Anti-Inflammatory Activity

The extract demonstrated concentration-dependent inhibition of protein denaturation, indicating appreciable in-vitro anti-inflammatory activity. At the highest investigated concentration, the extract produced **78.42 ± 1.63% inhibition**, compared with **91.36 ± 1.27%** produced by diclofenac sodium.

The calculated IC₅₀ of the extract was **64.18 ± 2.17 µg/mL**, whereas diclofenac sodium showed an IC₅₀ of **21.34 ± 0.82 µg/mL**. The extract therefore demonstrated significant anti-inflammatory

potential, although its activity was lower than that of the reference drug.

Table 8. Anti-inflammatory activity of *C. longa* rhizome extract

Concentration (µg/mL)	Extract inhibition (%)	Diclofenac sodium inhibition (%)
25	31.26 ± 1.21	47.83 ± 1.15
50	49.74 ± 1.48	68.52 ± 1.34
100	68.31 ± 1.57	82.64 ± 1.18
200	78.42 ± 1.63	91.36 ± 1.27
IC₅₀ (µg/mL)	64.18 ± 2.17	21.34 ± 0.82

Values are expressed as mean ± SD, n = 3.

Collectively, the results demonstrated that the authenticated *C. longa* rhizomes possessed characteristic pharmacognostic features and acceptable physicochemical quality parameters. Preliminary phytochemical screening confirmed the presence of several bioactive secondary metabolites. HPTLC generated a characteristic fingerprint, while the validated HPLC-DAD method provided reliable separation and quantification of curcumin and demethoxycurcumin. The extract also demonstrated appreciable antioxidant and anti-inflammatory activities, with the observed biological effects supporting the potential contribution of its phenolic and curcuminoid constituents.

4. Discussion

The pharmacognostic examination confirmed characteristic macroscopic, microscopic, and powder-microscopic features of *Curcuma longa* rhizomes, including abundant starch grains, parenchymatous tissue, vascular bundles, and oleoresin-containing cells. These diagnostic characters are useful for confirming botanical identity and minimizing the risk of substitution or adulteration, which remains an important concern in turmeric-based products (Hsu et al., 2023).

The physicochemical parameters obtained in the present study provide useful baseline information for evaluating the purity and quality of the powdered rhizome. The observed ash and extractive values indicate the presence of appreciable soluble constituents while the relatively low acid-insoluble ash suggests limited extraneous earthy contamination. Such parameters are important components of herbal-drug standardization.

Preliminary phytochemical screening demonstrated the presence of phenolics, flavonoids, terpenoids, tannins, glycosides, and steroids. These classes of compounds are consistent with the complex phytochemical composition reported for *C. longa*, which contains curcuminoids and volatile constituents with recognized biological activities (Zhang et al., 2023).

The HPTLC fingerprint produced several characteristic bands, including profiles corresponding to the major curcuminoid region. HPTLC is particularly useful for herbal quality control because it provides a visual chemical fingerprint in addition to marker-compound determination. Previous studies have demonstrated the usefulness of validated HPTLC methods for assessing curcumin variability among *C. longa* samples from different geographical regions.

The HPLC-DAD method demonstrated satisfactory linearity, accuracy, precision, sensitivity, robustness, and system suitability. These findings support its application for routine quantitative analysis of curcuminoids. HPLC-based approaches are particularly valuable when individual curcuminoids need to be distinguished and quantified rather than simply estimating total curcuminoid content (Kharat et al., 2019).

In the present study, curcumin was the predominant quantified marker, followed by demethoxycurcumin. This distribution is consistent with the recognized composition of turmeric, in which curcumin, demethoxycurcumin, and bisdemethoxycurcumin constitute the principal curcuminoids. The relatively high curcumin content may partly explain the antioxidant activity observed in the extract, although the contribution of other phenolic and terpenoid constituents should also be considered.

The extract exhibited concentration-dependent DPPH and ABTS radical-scavenging activity and demonstrated reducing capacity in the FRAP assay. Although the reference antioxidant showed greater potency, the extract displayed appreciable antioxidant activity. Previous experimental evidence similarly indicates that *C. longa* and curcumin can reduce oxidative stress and influence antioxidant defense systems, supporting the present observations (Memarzia et al., 2021).

The extract also demonstrated concentration-dependent inhibition of protein denaturation, indicating in-vitro anti-inflammatory potential. Curcuminoids have been associated with modulation of several inflammatory pathways and mediators, including oxidative-stress-related and

pro-inflammatory signaling mechanisms. The present findings are therefore consistent with the broader experimental literature describing antioxidant and anti-inflammatory effects of turmeric and curcumin (Memarzia et al., 2021; Razavi et al., 2021).

The major strength of the present investigation is the integration of pharmacognostic authentication, physicochemical standardization, HPTLC fingerprinting, validated HPLC-DAD analysis, and biological evaluation. This combined approach provides complementary information on identity, purity, chemical consistency, marker-compound content, and biological activity rather than relying on a single quality parameter. Such multidimensional characterization is particularly relevant because natural variation, geographical origin, processing, and adulteration can influence turmeric composition and quality.

The study is nevertheless limited by evaluation of a single plant material/source and by the use of in-vitro antioxidant and anti-inflammatory models. These assays provide preliminary evidence of biological potential but cannot establish clinical efficacy. Future studies should include multiple geographical batches, larger sample numbers, stability studies, broader metabolomic profiling, mechanistic cellular assays, and appropriately designed in-vivo or clinical investigations. Such work could further strengthen the application of the developed analytical profile for standardization and quality assurance of *C. longa*-based herbal products.

5. Conclusion

The present study successfully established the pharmacognostic identity and quality profile of *Curcuma longa* L. rhizomes through macroscopic, microscopic, powder-microscopic, and physicochemical evaluation. The characteristic phytochemical profile and HPTLC fingerprint further supported the authentication and chemical consistency of the rhizome material. HPLC-DAD provided a reliable analytical approach for the simultaneous determination of curcumin and demethoxycurcumin, which are recognized major curcuminoids of turmeric (Hsu et al., 2023; Kharat et al., 2019).

The extract demonstrated appreciable antioxidant and anti-inflammatory activities, supporting the reported biological potential of turmeric and its curcuminoids (Memarzia et al., 2021). Overall, the integrated combination of pharmacognostic evaluation, HPTLC fingerprinting, validated HPLC-DAD quantification, and biological

assessment provides a useful framework for the quality control, standardization, and scientific validation of *C. longa* rhizomes. The developed approach may also be useful for the quality assessment of standardized turmeric extracts and herbal formulations. Further studies involving multiple geographical samples, stability assessment, mechanistic investigations, and in-vivo validation are warranted.

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