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METHOD DEVELOPMENT FOR THE ESTIMATION OF CURCUMIN IN DIFFERENT MEDICINAL PLANTS BY HPLC

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ABSTRACT

Curcumin, a principal bioactive constituent of the Curcuma species, is widely recognized for its therapeutic properties including antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Accurate quantification of curcumin in medicinal plants is essential for quality control, standardization, and pharmacological evaluation. The present study focuses on the development of a simple, precise, and reliable RP-HPLC method for the estimation of curcumin in the ethanolic extracts of *Curcuma caesia* and *Curcuma amada*. The extracts were prepared using ethanol, and their percentage yields were recorded as 8.25% and 7.96%, respectively. Preliminary phytochemical screening revealed the presence of various bioactive constituents such as phenols, flavonoids, diterpenes, and proteins. The total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin–Ciocalteu and aluminium chloride methods. *Curcuma caesia* showed TPC and TFC values of 0.42 mg/100 mg and 0.75 mg/100 mg, respectively, while *Curcuma amada* exhibited 0.23 mg/100 mg and 0.84 mg/100 mg. HPLC analysis using a standard curcumin solution (2 µg/ml) revealed retention times (RT) around 4.38 minutes. The curcumin content in the extracts was quantitatively established, showing 0.315% in *Curcuma caesia* and 0.528% in *Curcuma amada*. The developed method proved to be efficient for the accurate quantification of curcumin in plant extracts and can be applied for routine quality assessment of Curcuma species in herbal formulations.

KEYWORDS: Curcumin, *Curcuma caesia*, *Curcuma amada*, HPLC, Phytochemical screening, Total phenolic content, Total flavonoid content, Quantitative estimation, Standardization, Medicinal plants.

INTRODUCTION

Curcumin is a principal curcuminoid widely recognized for its therapeutic potential, including antioxidant, anti-inflammatory, antimicrobial, anticancer, and neuroprotective effects (Aminnezhad et al., 2023). Although *Curcuma longa* is the primary commercial source of curcumin, several other species of the *Curcuma* genus particularly *Curcuma caesia* (Black turmeric) and *Curcuma amada* (Mango ginger) also contain varying levels of curcuminoids and related phenolic compounds. These plants are used extensively in traditional medicine for treating inflammation, respiratory ailments, gastrointestinal disorders, and microbial infections. However, the curcumin content in these species is significantly less explored, making analytical quantification crucial for their scientific validation and pharmacological standardization (Fuloria et al., 2022).

Given the structural similarity of curcuminoids and the presence of multiple interfering phytochemicals within plant matrices, High-Performance Liquid Chromatography (HPLC) remains the most preferred analytical technique for precise quantification. HPLC offers superior sensitivity, selectivity, and reproducibility compared to other chromatographic methods. A robust method is necessary to ensure accurate quantification of curcumin in these non-conventional species, helping to establish chemotypic variations, evaluate phytoconstituent profiles, and support the development of standardized herbal formulations (Siddique 2021).

Despite the growing interest in *Curcuma caesia* and *Curcuma amada*, limited scientific reports are available on validated HPLC methods specifically optimized for estimating curcumin content in these plants. Therefore, the present study aims to develop a simple, accurate, and reliable HPLC method for the estimation of curcumin in extracts of *Curcuma caesia* and *Curcuma amada*, thereby supporting their quality control, phytochemical profiling, and therapeutic relevance.

MATERIAL AND METHODS

Material

Fresh rhizomes of *Curcuma caesia* and *Curcuma amada* were collected, cleaned, shade-dried, and coarsely powdered for extraction. Analytical grade ethanol, methanol, Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, and gallic acid were procured from standard laboratory suppliers. Curcumin standard (≥98% purity) was used for HPLC calibration. All chemicals and reagents used in the study were of analytical grade. Distilled water was used throughout the experimental work. The entire analysis, including extraction, phytochemical testing, spectrophotometric estimation, and HPLC quantification, was carried out using laboratory glassware, a UV-Visible spectrophotometer, and an RP-HPLC system equipped with a C18 column.

Methods

Extraction procedure

Defatting of plant material

Rhizomes of rhizomes of *Curcuma caesia* and *Curcuma amada* were shade dried at room temperature. 60 gram of each dried rhizomes were coarsely powdered and subjected to extraction with petroleum ether by maceration (Mukherjee, 2007). The extraction was continued till the defatting of the material had taken place.

Qualitative evaluation

Phytochemical tests are conducted to identify and determine the quantity of specific phytochemical compounds present in a plant extract or plant material. These tests employ various chemical, chromatographic, and spectroscopic techniques to isolate, separate, and characterize the phytochemicals. The choice of tests depends on the nature of the phytochemical of interest and the available resources. Phytochemical examinations were carried out for the extract as per the standard methods (Kokate, 1994).

Quantitative studies of bioactive constituents

Estimation of total phenol content

The total phenolic content of the extract was estimated using the modified Folin–Ciocalteu method as described by Parkhe and Bharti (2019). For preparation of the standard, 10 mg of gallic acid was dissolved in 10 ml of methanol, and aliquots in the concentration range of 10–50 µg/ml were prepared. The extract solution was prepared by dissolving 10 mg of the dried extract in 10 ml of methanol, followed by filtration; from this, 2 ml of the extract solution (1 mg/ml) was used for analysis. In the procedure, 2 ml of each standard or extract was mixed with 1 ml of Folin–Ciocalteu reagent (previously diluted 1:10 v/v with distilled water) and 1 ml of sodium carbonate solution (7.5 g/L). The mixture was vortexed for 15 seconds and then allowed to stand for 10 minutes to enable colour development. The absorbance of the resulting blue complex was measured at 765 nm using a UV–Visible spectrophotometer, and the total phenolic content was expressed as gallic acid equivalents.

Estimation of total flavonoids content

The total flavonoid content of the extract was estimated using the aluminium chloride colorimetric method as described by Parkhe and Bharti (2019). For the preparation of the standard, 10 mg of quercetin was dissolved in 10 ml of methanol, and aliquots in the concentration range of 5–25 µg/ml were prepared. The plant extract was prepared by dissolving 10 mg of the dried extract in 10 ml of methanol, followed by filtration; from this, 3 ml of the extract solution (1 mg/ml) was used for analysis. In the procedure, 1 ml of 2% aluminium chloride ($AlCl_3$) solution was added to 3 ml of each standard or extract solution, mixed well, and allowed to stand for 15 minutes at room temperature. The absorbance of the resulting yellow complex was then measured at 420 nm, and the total flavonoid content was expressed in terms of quercetin equivalents.

Identification of marker compound (Curcumin) by HPLC

Methanol and Water were of HPLC grade and purchased from Merck Ltd, New Delhi, India. Water used was of HPLC grade water from Merck Ltd, New Delhi, India.

A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a Data Ace software.

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of methanol: water (77:33 v/v) pH-3 with OPA, and was isocratically eluted at a flow rate of 1 mL min-1. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 254 nm (Patil and Mahajan, 2022).

Table 6.3: Selection of Separation Variable.

Variable	Condition
Column Type	Octadecylsilane (C18), bonded phase
Column Dimensions	250 mm × 4.6 mm
Particle Size	5 µm
Mobile Phase Composition	Methanol: 77% (v/v) ; Water (pH adjusted to 3.0 with OPA): 23% (v/v)
Mobile Phase Preparation	Filter through 0.45 µm membrane and degas for 10 min before use
pH of Mobile Phase	3.0 (adjusted with Orthophosphoric Acid)
Flow Rate	1.0 mL/min
Column Temperature	Room temperature (25 ± 2 °C)
Injection Volume	20 µL
Detector Type	UV/Vis
Detection Wavelength	419 nm
Retention Time	4.384 ± 0.3 min
Run Time	8–10 min
Column Equilibration	Minimum 10 column volumes (~25 min)
System Suitability – %RSD (Peak Area)	≤ 2.0%
System Suitability Tailing Factor	≤ 2.0
System Suitability Theoretical Plates	≥ 2000
Resolution	≥ 2.0
Sample Filtration	0.45 µm membrane filter

Preparation of standard stock solution

10mg of Curcumin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of working standard solution

From stock solutions of Curcumin 1 ml was taken and diluted up to 10 ml. from this solution 0.1, 0.2, 0.3, 0.4, 0.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 1, 2, 3, 4, 5µg/ ml concentration.

Analysis of extract

10 mg extract was taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. The resulting solution was again filtered using 0.45µ membrane filter and then sonicated for 10 min.

RESULTS AND DISCUSSION

The present study involved the extraction, phytochemical screening, and HPLC-based quantification of curcumin in the ethanolic extracts of *Curcuma caesia* and *Curcuma amada*. The percentage yield of the extracts (8.25% for *C. caesia* and 7.96% for *C. amada*) indicates efficient extraction with ethanol as the solvent and aligns with previously reported yields for *Curcuma* species. Both extracts appeared black in colour, consistent with the natural pigmentation of their rhizomes.

Qualitative phytochemical screening revealed notable differences in the chemical profiles of the two plants. *Curcuma caesia* showed the presence of alkaloids, carbohydrates, saponins, phenols, flavonoids, proteins, and diterpenes, whereas glycosides, tannins, and sterols were absent. In contrast, *Curcuma amada* tested positive for glycosides, phenols, flavonoids, diterpenes, and sterols, while alkaloids, carbohydrates, saponins, proteins, and tannins were absent. These variations reflect the distinct phytochemical compositions of both species, which may contribute to their differing medicinal properties.

The total phenolic and flavonoid content further supported this variation. *Curcuma caesia* exhibited higher phenolic content (0.42 mg/100 mg) compared to *Curcuma amada* (0.23 mg/100 mg), while the flavonoid content was slightly higher in *Curcuma amada* (0.84 mg/100 mg) than in *Curcuma caesia* (0.75 mg/100 mg). Since phenolics and flavonoids are major contributors to antioxidant and anti-inflammatory activities, these differences highlight the phytopharmacological uniqueness of each plant.

HPLC analysis confirmed the presence of curcumin in both extracts, with retention times closely matching the standard curcumin peak (RT ≈ 4.38 min), demonstrating good method specificity. Quantitative estimation revealed curcumin content of 0.315% in *Curcuma caesia* and 0.528% in *Curcuma amada*. This indicates that *Curcuma amada* contains comparatively higher curcumin levels despite having a lower phenolic content overall. The observed differences may be attributed to species-specific biosynthetic pathways and

environmental factors influencing curcuminoid accumulation.

The developed HPLC method proved sensitive, accurate, and suitable for curcumin estimation in non-conventional *Curcuma* species. The phytochemical differences and curcumin content variation between *Curcuma caesia* and *Curcuma amada* provide valuable insights for their future use in herbal formulations and pharmacological research.

Table 1: % Yield of *Curcuma caesia* and *Curcuma amada* extract.

Ethanolic Extract	Colour	% Yield
Curcuma caesia	Black	8.25
Curcuma amada	Black	7.96

Table 2: Qualitative chemical tests of extract of *Curcuma caesia.*

S. No.	Bioactive constituents	Test	Ethanolic Extract
1	Alkaloids	Hager's Test	+ve
2	Carbohydrates	Fehling's Test	+ve
3	Glycosides	Legal's Test	-ve
4	Saponins	Froth Test	+ve
5	Phenols	Ferric Chloride Test	+ve
6	Flavonoids	Lead acetate Test	+ve
7	Proteins	Xanthoproteic Test	+ve
8	Diterpenes	Copper acetate Test	+ve
9	Tannins	Gelatin Test	-ve
10	Sterols	Salkowski Test	-ve

+ ve – Present, - ve – Absent

Table 3: Qualitative chemical tests of extract of *Curcuma amada.*

S. No.	Bioactive constituents	Test	Ethanolic Extract
1	Alkaloids	Hager's Test	-ve
2	Carbohydrates	Fehling's Test	-ve
3	Glycosides	Legal's Test	+ve
4	Saponins	Froth Test	-ve
5	Phenols	Ferric Chloride Test	+ve
6	Flavonoids	Lead acetate Test	+ve
7	Proteins	Xanthoproteic Test	-ve
8	Diterpenes	Copper acetate Test	+ve
9	Tannins	Gelatin Test	-ve
10	Sterols	Salkowski Test	+ve

+ ve – Present, - ve – Absent

Table 4: Estimation of total phenol and flavonoids in *Curcuma caesia.*

S. No.	Total phenol content	Total flavonoids content
1.	0.42 mg/100mg	0.75 mg/100mg

Table 5: Estimation of total phenol and flavonoids content in *Curcuma amada.*

S. No.	Total phenol content	Total flavonoids content
1.	0.23 mg/100mg	0.84 mg/100mg

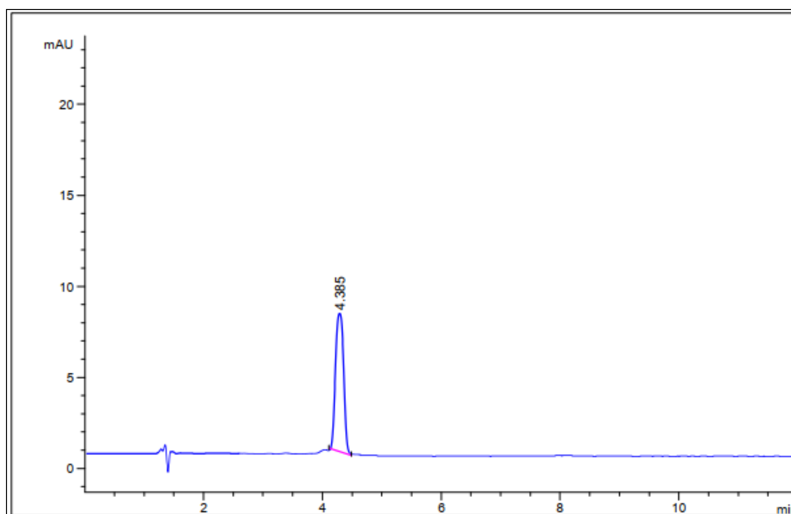

Figure 1: Chromatogram of standard Curcumin (2µg/ml).

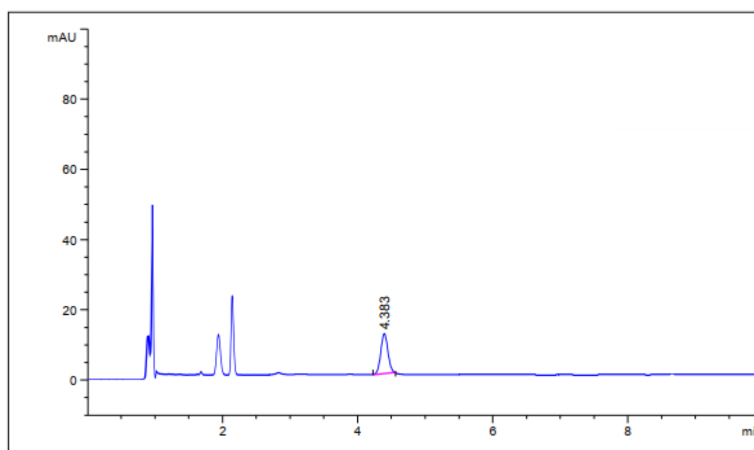

Figure 2: Chromatogram of ethanolic extract of *Curcuma caesia.*

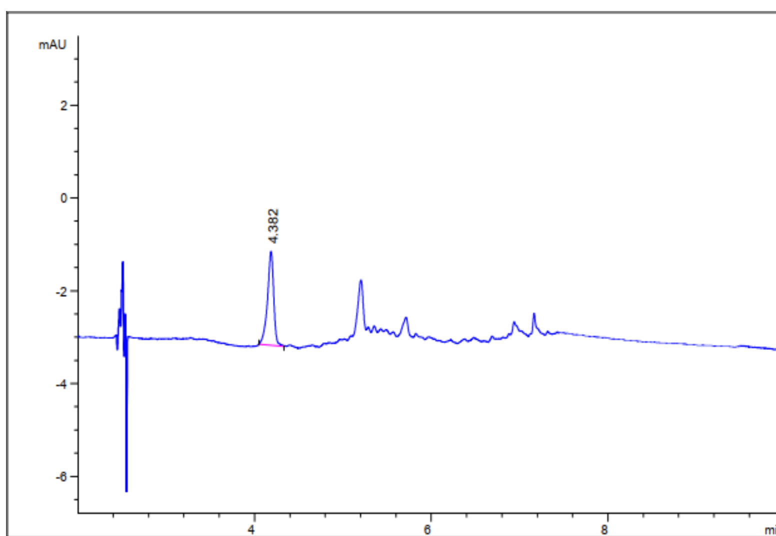

Figure 3: Chromatogram of ethanolic extract of *Curcuma amada*

Table 6: Quantitative estimation of Curcumin in extract.

S. No.	Ethanolic extract	RT	% Assay
1.	Curcumin	4.385	
2.	*Curcuma caesia*	4.383	0.315%
3.	*Curcuma amada*	4.382	0.528%

CONCLUSION

The study successfully developed an HPLC method for the accurate estimation of curcumin in *Curcuma caesia* and *Curcuma amada*. Both extracts showed the presence of important phytochemicals, including phenols and flavonoids. Quantitative evaluation revealed that *Curcuma amada* contained a comparatively higher curcumin content than *Curcuma caesia*. The developed method proved to be precise, reliable, and suitable for routine analysis of curcumin in different medicinal plants. The findings support the use of HPLC as an effective tool for standardization and quality assessment of curcumin-rich herbal materials.

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