RESEARCH PAPER

Estimation and Quantification of Curcumin in Different Curcuma Species Using HPTLC and UV Spectrophotometric Methods

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Abstract
Curcuma longa (Turmeric) species is used successfully in Ayurvedic formulations from ancient times. Itis a rich source of bioactive phytoconstituents like curcuminoids, turmerone and many more. Curcuminoids is the group of chief dynamic components and has number of medicinal uses such as anti-inflammatory, anti–HIV, antitumour, antiviral, anticancer, antifungal and antiparasitic. Different analytical methods have been developed in recent year for the quality control analysis of curcuminoids in *Curcuma longa* extract including HPTLC and UV Spectrophotometry. While the primary component curcumin species from curcuminoids is still lacking for its analytical method development along with validation. Therefore, in the present study, a simple UV and HPLTLC method was developed and validated according to international conference harmonization (ICH) guidelines for the quantitative estimation of curcumin in Curcuma longa extract.
Keywords: Curcuminoids, curcumin, curcuma longa, analytical methods, HPTLC, UV spectroscopy
How to cite this article: Goyal S, Nayak G, Namdeo P. Estimation and Quantification of Curcumin in Different Curcuma Species Using HPTLC and UV Spectrophotometric Methods. Int J Drug Deliv Technol. 2026;16(69s): 1965-1973. DOI: 10.25258/ijddt.16.69s.193

INTRODUTION

Phytochemistry is the study of the investigation of phytochemicals produced by plants, their structural compositions, biosynthetic pathways, functions and mechanism of actions in the living systems (Egbuna et al., 2019). The chemical substances present in the plants often called phytochemicals are categorized on the basis of their structure and biosynthetic pathways as primary and secondary metabolites (Velu et al., 2018). Primary metabolites are highly cognate in their structural features. They are also vital for growth, metabolism, development and survival of plants. Proteins, carbohydrates, lipids and nucleic acid are the examples of primary metabolites (Erb and Kliebenstein, 2020; Eastwood, 2001; Bentley, 1997) Secondary metabolites are not fundamental for the survival of plants but these components determine their interaction with surroundings and hence their existence in the ecosystem. Polyphenols, flavanoids, alkaloids, saponins, cardiac glycosides and anthocyanins are the examples of secondary metabolites.

Plant based conventional medications are preferred over synthetic medicines due to its environment friendly properties and they are restraint of side effects. Phytochemicals are plant substances consisting varied bioactivities with abundant health advantages and have also been employed in conventional medicine system to treat a variety of ailments and diseases (Konappa et al., 2020; Sharma et al., 2019). Plants comprising of various bioactive phytochemicals possess diverse range of bioactivities like antioxidant, anticancer, analgesic, anti-microbial, anti-diarrheal, and so on (Ganesan and Xu, 2017).

Ultraviolet (UV) spectroscopy is a powerful and widely utilized analytical technique for characterizing bioactive compounds, particularly in the field of natural product research. The wavelength and intensity of the absorption peaks are indicative of the compound's structural and electronic properties, making UV spectroscopy a useful tool for qualitative and quantitative analysis. In phytochemical studies, UV spectroscopy is often employed to identify and characterize plant-derived compounds, such as flavonoids, phenolics, and other secondary metabolites, contributing to the understanding of their bioactivity and therapeutic potential.

High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits. Many plants utilized in Indian medical systems have undergone HPTLC investigation for a variety of pharmacological properties like CNS, hepatoprotective, etc. The Micheliachampaca (leaves and stembark) quercetin was detected and quantified using the HPTLC method, and the estimated values show that the leaves constitute the plant's highest source of quercetin. With strong reliability and reproducibility, the HPTLC method can be used regularly to estimate the amount of curcumin in commercial turmeric powder (Hitesh et al., 2012).

Curcumin is the major bioactive constituent of Curcuma species and is responsible for their wide range of therapeutic activities, including anti-inflammatory, antioxidant, and anticancer effects. The concentration of Curcumin varies significantly among different Curcuma species due to botanical and environmental factors. Quantification of Curcumin is therefore essential for evaluating the

quality, potency, and suitability of Curcuma species for pharmaceutical and nutraceutical applications. HPTLC allows simultaneous analysis of multiple herbal samples with good resolution, making it useful for comparative quantification. UV-Visible spectrophotometry is a simple and economical technique suitable for routine estimation. 4.4 Research Envisaged The proposed study involves extraction of Curcumin from different Curcuma species followed by quantitative estimation using HPTLC and UV-Visible spectrophotometric techniques. Standard Curcumin will be used for comparative quantification, and Curcumin content in each species will be calculated and expressed as percentage or mg/g of dried extract. The results obtained from both methods will be compared to assess variations in Curcumin levels among the selected Curcuma species.

MATERIALS AND METHODS

Collection of plant materials

Plants can be collected from either wild woods or herbariums. Rhizomes of *Curcuma amada* and *Curcuma zedoaria* were collected from local area of Bhopal, Madhya Pradesh in the month of December, 2025.

The plant components are dried to eliminate the water content and so can be preserved after being dry. This operation should be carried out as soon as the plants are harvested in order to prevent spoiling. The plants are kept in the shade and air dried in sheds in this method. This method takes several weeks to completely dry out the dampness. This time is determined by the temperature and humidity. Further the dried plant was coarsely powdered & subjected to extraction. Dried rhizomes of *Curcuma amada* and *Curcuma zedoaria* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Extraction by maceration process

Extraction from plant materials is an important step in phytochemical processing for discovering bioactive secondary metabolite. Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs. Rhizomes of *Curcuma amada* and *Curcuma zedoaria* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction Dried powdered rhizomes (50 gram) of *Curcuma amada* and *Curcuma zedoaria* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40º C (Mukherjee, 2007; Khandelwal, 2005).

Determination of percentage yield

Percentage yield measures the effectiveness of the entire extraction process. It shows how much product a researcher has obtained after running the procedures against how much is actually obtained. A higher % yield means the researcher obtained a greater amount of product after extraction.

The % yield was calculated by using formula.

Phytochemical screening

Plants generate compounds known as phytochemicals. These are created by the primary and secondary metabolisms of the plant. Phytochemicals examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Estimation of total phenolic content

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly. The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (10-50µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl3 solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of Curcumin in ethanolic extract of Curcuma amada and Curcuma zedoaria using HPTLC

The chromatographic separation was carried out in a CAMAG twin-trough development chamber pre-saturated with the mobile phase consisting of n-Hexane: ethyl acetate (80:20, v/v). The solvent front was allowed to migrate up to a distance of 70.0 mm using 10.0 mL of the mobile phase. After development, the plates were dried in an oven at 60°C for 5 min. Sample bands were applied with the first track positioned at 15.0 mm from the plate edge, maintaining a distance of 11.6 mm between adjacent tracks. Densitometric scanning was performed using a CAMAG TLC Scanner III controlled by WinCATS software (version 1.4.3.6336). Scanning was carried out in absorption mode using a deuterium–tungsten (D_2 & W) lamp at a wavelength of 366 nm. The scanning parameters included slit dimensions of 5.00 × 0.30 mm (micro), scanning speed of 20 mm/s, data resolution of 100 µm/step, scan start position at 5.0 mm, and scan end position at 75.0 mm. The optical system was optimized for light, with second-order

optical filtering, automatic detector mode, remission measurement type, and a photomultiplier tube (PMT) voltage set at 443 V.

Pure Standards

Reference standards of Curcumin (99.0% purity) were procured from Himedia Pvt. Ltd., India.

Standard Solution

Preparation Standard stock solutions were prepared by accurately weighing 10 mg each of Curcumin transferring them into separate 10 mL volumetric flasks. The volume was adjusted with methanol to obtain a concentration of 1 mg/mL. The solutions were stored in tightly closed containers under refrigerated conditions. From this stock solution, 1 mL was further diluted to 10 mL with methanol to obtain stock solution A. Calibration curves were prepared in accordance with the International Conference on Harmonization (ICH) guidelines.

Sample Preparation

A stock solution of the both the extracts was prepared by dissolving 10 mg of the dried extract in methanol and making up the volume to 10 mL in a volumetric flask. The solution was stored under refrigerated conditions until analysis.

Calibration Curve

Calibration curves were constructed in accordance with ICH guidelines. Standard Curcumin solutions in the concentration range of 1–5 µL were applied in triplicate as bands of 6.0 mm width on aluminum-backed HPTLC plates precoated with silica gel 60 F_{254} (10.0 × 10.0 cm). The bands were applied using a CAMAG Linomat V applicator, maintaining a distance of 11.7 mm between adjacent tracks, 12 mm from the plate edge, and 8 mm from the bottom of the plate. The application rate was kept constant at 15µL/s (Majumder and Saha, 2025). Chromatographic development was carried out in a CAMAG twin-trough chamber presaturated for 20 min with the mobile phase consisting of Toluene: Acetic acid (9:1 v/v). The plates were developed to a distance of 70 mm, followed by air-drying. Densitometric scanning was performed using a CAMAG TLC Scanner III at a wavelength of 425 nm in absorption mode. Curcumin was well resolved with an Rf value of 0.46. Peak areas were recorded, and calibration curves were generated by plotting peak area against applied concentration. Linear regression analysis was employed to establish the calibration equation and assess linearity (Gantait et al., 2011).

Sample Assay

Sample (Extract of Curcuma amada and Curcuma zedoaria) and standard solutions were applied to the TLC plates and developed under identical chromatographic conditions. After development and drying, the analytes were well resolved from other constituents. Densitometric scanning was performed at 366nm, and peak areas corresponding to Curcumin were recorded for quantitative estimation.

RESULTS AND DISCUSSION

The percentage yield values obtained from the extraction process provide insights into the efficiency of extracting bioactive constituents from Curcuma amada and Curcuma zedoaria using ethanol as solvents.

Table 1: % Yield of *Curcuma amada and Curcuma zedoaria*

S. No.	Ethanolic extract	Weight of extract	% Yield (w/w)
1.	*Curcuma amada*	3.51	7.02
2.	*Curcuma zedoaria*	4.94	9.88

Curcuma zedoaria exhibited a higher yield (9.88% w/w) with an extract weight of 4.94 g, compared to Curcuma amada, which showed a yield of 7.02% w/w with 3.51 g of extract. This difference in yield may be attributed to variations in the phytochemical composition, polarity of constituents, and solubility of bioactive compounds in ethanol. The higher yield of Curcuma zedoaria suggests a greater abundance of ethanol-soluble constituents such as flavonoids, phenolics, and saponins.

Results of phytochemical screening of extract

Table 2: Phytochemical screening of ethanolic extract of Curcuma amada

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Wagner's test Hager's test	 **+ve** **-ve**
2.	**Glycosides** Legal's test	 **+ve**
3.	**Flavonoids** Lead acetate Alkaline reagent test	 **+ve** **-ve**
4.	**Phenol** Ferric chloride test	 **+ve**
5.	**Proteins** Xanthoproteic test	***+ve***
6.	**Carbohydrates** Fehling's test	***+ve***
7.	**Saponins** Foam test	***-ve***
8.	**Diterpenes** Copper acetate test	***+ve***

Table 3: Phytochemical screening of ethanolic

+ve =Positive; -ve= Negative

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids** Wagner's test Hager's test	 **-ve** **-ve**
2.	**Glycosides** Legal's test	 **+ve**
3.	**Flavonoids** Lead acetate Alkaline reagent test	 **+ve** **-ve**
4.	**Phenol** Ferric chloride test	 **+ve**
5.	**Proteins** Xanthoproteic test	***+ve***
6.	**Carbohydrates** Fehling's test	***+ve***
7.	**Saponins** Foam test	***+ve***
8.	**Diterpenes** Copper acetate test	***+ve***

The Phytochemical screening of the ethanolic extract of *Curcuma amada* and *Curcuma zedoaria* revealed the presence of several important bioactive constituents. The extract tested positive for glycosides, flavonoids (in lead acetate test), phenolic compounds, proteins, carbohydrates, saponins, and diterpenes, indicating a rich and diverse phytochemical profile.

Results of estimation of total phenol and flavonoids content

Estimation of total phenolic content (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.012x - 0.003, R2 = 0.999, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

Table 4: Preparation of calibration curve of Gallic acid

S. No.	Concentration(µg/ml)	Mean Absorbance
0	0	0
1	10	0.114
2	20	0.239
3	30	0.357
4	40	0.487
5	50	0.603

*Average of three determination, Mean ± SD

Total flavonoids content estimation (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.033x + 0.012, R2 =0.998, where X is the quercetin equivalent (QE) and Y is the absorbance.

Table 5: Preparation of Calibration curve of Quercetin

S. No.	Concentration(µg/ml)	Mean Absorbance
0	0	0
1	5	0.187
2	10	0.354
3	15	0.529
4	20	0.693
5	25	0.842

*Average of three determination, Mean ± SD

The calibration curve of quercetin was prepared using concentrations ranging from 5 to 25 µg/mL, and the corresponding mean absorbance values were recorded. It was observed that the absorbance increased proportionally with an increase in concentration, indicating a direct linear relationship between concentration and absorbance. The absorbance values ranged from 0.187 at 5 µg/mL to 0.842 at 25 µg/Ml.

Table 6: Estimation of total flavonoids and phenol content of Curcuma amada and Curcuma zedoaria

S.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/100mgofdried extract)
1.	*Curcumaamada*	0.73	0.85

2.	*Curcumazedoaria*	0.65	0.92

The estimation of total flavonoid and phenolic content in the ethanolic extracts of Curcuma amada and Curcuma zedoaria revealed slight variations in their phytochemical composition. Curcuma amada showed a higher flavonoid content (0.73 mg/100 mg of dried extract) compared to Curcuma zedoaria (0.65 mg/100 mg), indicating a relatively better contribution to antioxidant activity through flavonoid compounds.

Curcuma zedoaria exhibited a higher total phenolic content (0.92 mg/100 mg) than Curcuma amada (0.85 mg/100 mg), suggesting a stronger presence of phenolic constituents, which are also known for their potent antioxidant and free radical scavenging properties. These results indicate that while both extracts are rich in bioactive compounds, Curcuma amada may be comparatively richer in flavonoids, whereas Curcuma zedoaria contains a higher amount of phenolic compounds. Both plants demonstrate significant potential for antioxidant activity due to the presence of these important phytoconstituents.

Results of estimation of Curcumin using HPTLC

Table 7: Preparation of calibration curve of Curcumin

S. No.	Concentration(µg/ml)	Area
1.	1	2705.6
2.	2	4734.1
3.	3	8234.8
4.	4	10852.3
5.	5	13037.2
7.	6	14490.5

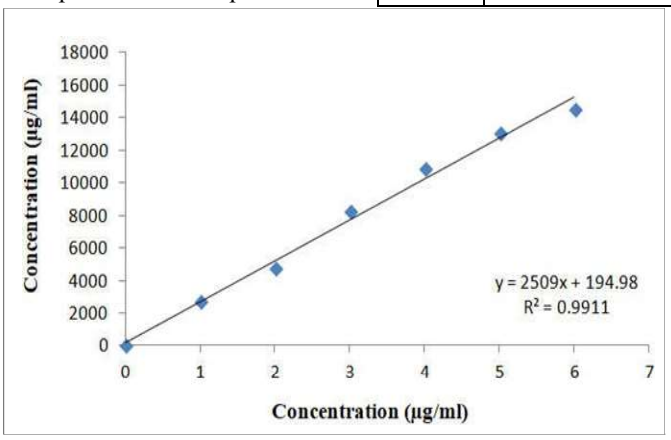

Figure 1: Calibration curve of Curcumin by HPTLC

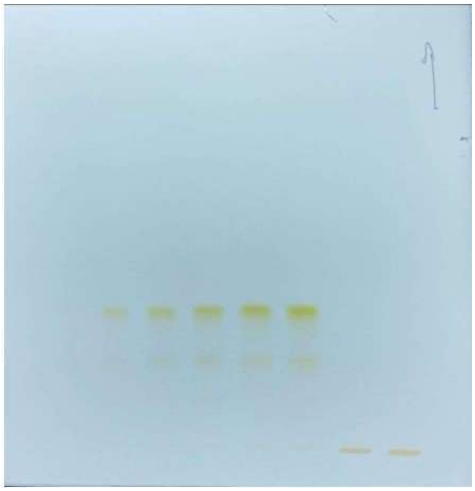

Figure 2: Thin layer chromatography in Normal light

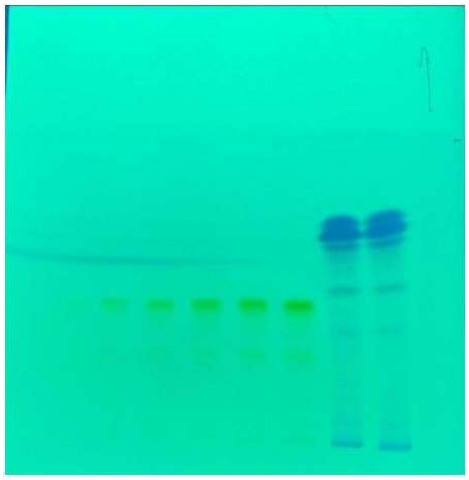

Figure 3: Thin layer chromatography in Short U.V

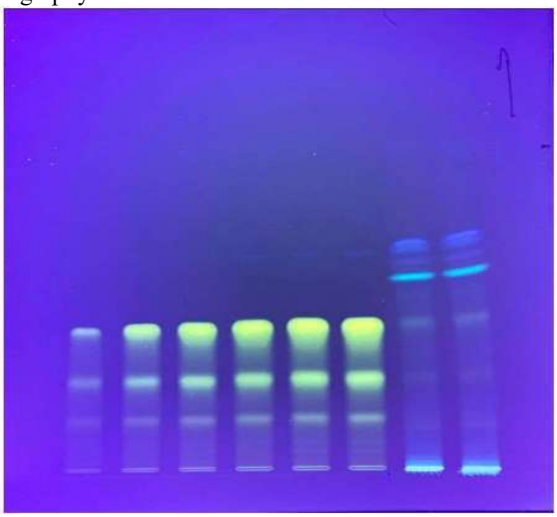

Figure 4: Thin layer chromatography in long U.V.

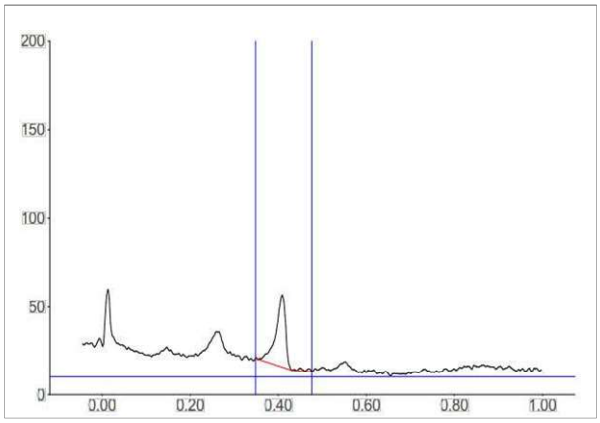

Figure 5: Chromatogram of standard Curcumin 1.0µg/ml

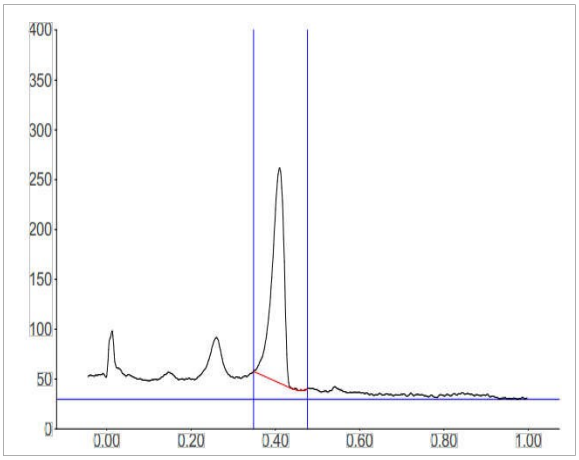

Figure 6: Chromatogram of standard Curcumin 2.0µg/ml

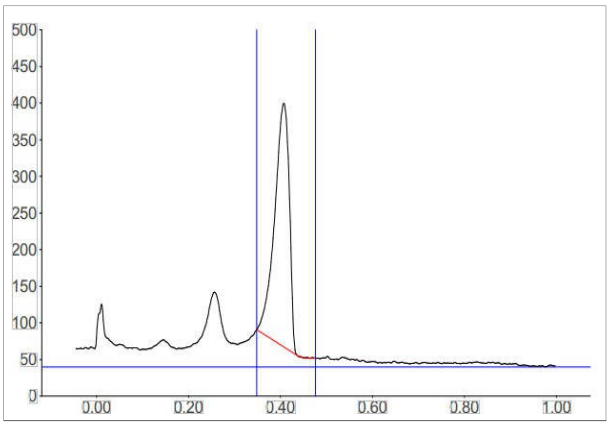

Figure 7: Chromatogram of standard Curcumin 3.0µg/ml

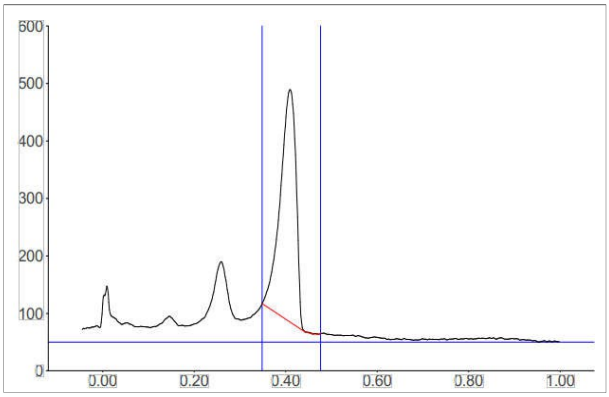

Figure 8: Chromatogram of standard Curcumin 4.0µg/ml

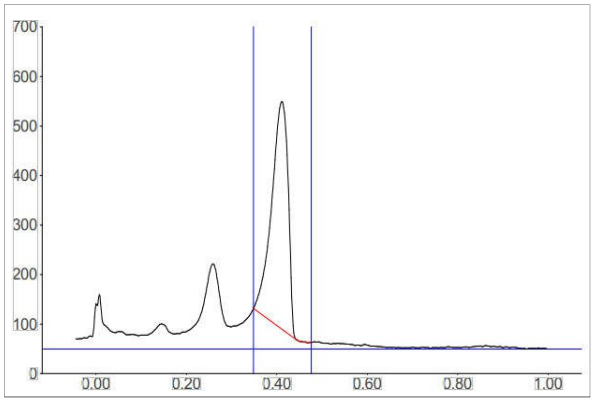

Figure 9: Chromatogram of standard Curcumin 5.0µg/ml

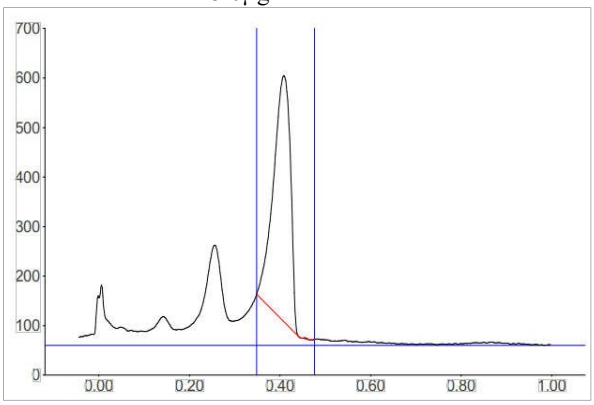

Figure 10: Chromatogram of standard Curcumin 6.0µg/ml

Figure 11: HPTLC chromatogram of hydroalcoholic extract of Curcuma amada

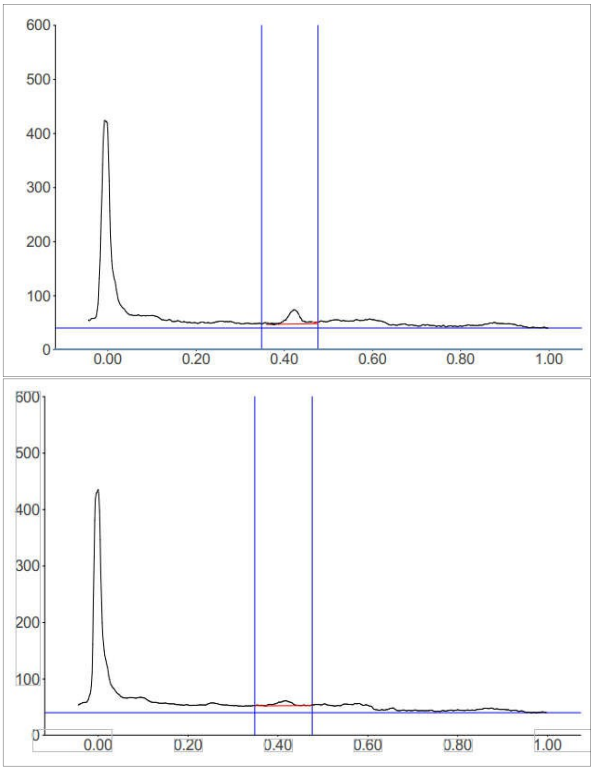

Figure 12: HPTLC chromatogram of hydroalcoholic extract of Curcuma zedoaria

Table 8:
Results of % assay of Curcumin in Curcuma amada and Curcuma zedoaria

S.	Extract	% Assay
1.	*Curcumaamada*	0.40
2.	*Curcumazedoaria*	0.15

The quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) further supported the phytochemical screening results. The total flavonoid content was found to be 0.73 mg/100 mg of dried extract for Curcuma amada and 0.65 mg/100 mg for Curcuma zedoaria, indicating that Curcuma amada contains a relatively higher concentration of flavonoid compounds. Similarly, estimation of total phenolic content showed that Curcuma zedoaria contained 0.92 mg/100 mg of dried extract, whereas Curcuma amada contained 0.85 mg/100 mg. The higher phenolic content observed in Curcuma zedoaria indicates a comparatively greater abundance of phenolic constituents, which are recognized for their excellent free radical scavenging, antioxidant, anti-inflammatory, and protective biological activities.

HPTLC chromatograms recorded under normal light, short UV, and long UV conditions clearly identified the curcumin bands in both plant extracts. The assay results revealed that Curcuma amada contained 0.40% curcumin, whereas Curcuma zedoaria contained 0.15% curcumin. The significantly higher curcumin content in Curcuma amada suggests that it may possess greater therapeutic potential, particularly with respect to antioxidant, anti-inflammatory, antimicrobial, and anticancer activities associated with curcumin.

Conclusion

The findings of the present investigation demonstrate that both Curcuma amada and Curcuma zedoaria are valuable medicinal plants containing diverse classes of bioactive phytoconstituents. While Curcuma zedoaria exhibited a higher extraction yield and greater total phenolic content, Curcuma amada showed higher flavonoid content and a substantially greater concentration of curcumin. These differences indicate that each plant possesses distinct phytochemical characteristics that may influence its pharmacological properties. The combined results of extraction yield, phytochemical screening, spectrophotometric estimation, and HPTLC analysis provide scientific evidence supporting the traditional medicinal use of these species and highlight their potential as natural sources of therapeutic phytochemicals.

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