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HPTLC Analysis And Antioxidant Activity Of Indian Medicinal Plants Curcuma Zedoaria, Curcuma Caesia And Curcuma Amada

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ABSTRACT

The present study aimed to evaluate and compare the phytochemical composition, antioxidant activity, and curcumin content of ethanolic extracts of three *Curcuma* species: *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada*. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, alkaloids, and proteins in varying intensities across the species. Antioxidant potential was assessed using DPPH, hydrogen peroxide, and nitric oxide scavenging assays, with *Curcuma caesia* exhibiting the highest activity in all models, followed by *C. amada* and *C. zedoaria*. Curcumin content was quantified using High-Performance Thin Layer Chromatography (HPTLC), and *C. amada* showed the highest curcumin concentration (7.91%), despite not exhibiting the strongest antioxidant activity. The results suggest that antioxidant effects are likely due to a combination of phytoconstituents rather than curcumin alone. These findings support the therapeutic potential of *Curcuma caesia* as a rich source of natural antioxidants and validate the traditional use of these plants in herbal medicine.

Keywords: *Curcuma zedoaria, Curcuma caesia, Curcuma amada, HPTLC, Antioxidant activity, DPPH; Curcumin, Phytochemical screening; Nitric oxide scavenging; Herbal medicine.*

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1. INTRODUCTION

Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is a major contributor to the pathogenesis of numerous chronic diseases, including cancer, cardiovascular disorders, diabetes, and neurodegenerative conditions (Lobo et al., 2010). In recent years, natural antioxidants derived from medicinal plants have gained attention as potential alternatives to synthetic compounds, due to their safety profile, bioactivity, and historical use in traditional medicine (Kaur & Kapoor, 2001).

The **genus** *Curcuma* (family Zingiberaceae) includes several species widely used in traditional Indian medicine for their anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. Among them, *Curcuma zedoaria* (white turmeric), *Curcuma caesia* (black turmeric), and *Curcuma amada* (mango ginger) are known for their ethnomedicinal applications and are rich in secondary metabolites such as curcuminoids, flavonoids, phenolics, and terpenes (Sasikumar, 2005). Despite their traditional significance, comparative scientific evaluation of their phytochemical profiles and antioxidant activities remains limited.

Curcumin, a polyphenolic compound mainly found in *Curcuma longa*, is also present in minor quantities in other *Curcuma* species and is a key contributor to their antioxidant properties (Gupta et al., 2013). Accurate quantification of curcumin in plant extracts is critical for evaluating therapeutic potential. **High-Performance Thin Layer Chromatography (HPTLC)** is a reliable, precise, and cost-effective analytical technique for the qualitative and quantitative assessment of phytoconstituents, especially in complex herbal matrices (Waksmundzka-Hajnos et al., 2008).

The present study aims to compare the phytochemical composition, antioxidant potential, and curcumin content of ethanolic extracts of *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada*. Antioxidant activity was evaluated using DPPH, hydrogen peroxide, and nitric oxide scavenging assays, while HPTLC was employed for curcumin quantification. This investigation provides valuable insight into the pharmacological relevance of these underexplored *Curcuma* species and supports their potential as natural sources of antioxidants.

2. MATERIAL AND METHODS

Material

Ethanolic extracts of *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada* were prepared from authenticated rhizomes collected locally and processed in the laboratory. Analytical grade ethanol was procured from Merck Ltd. (Mumbai, India) for extraction. Chemicals and reagents for phytochemical screening such as Wagner's, Hager's, lead acetate, and Fehling's solutions were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Standard curcumin and HPTLC-grade solvents were supplied by Sigma-Aldrich (St. Louis, USA), while pre-coated silica gel 60 F254 HPTLC plates were purchased from Merck (Darmstadt, Germany). DPPH, hydrogen peroxide, sodium nitroprusside, and other reagents for antioxidant assays were obtained from HiMedia Laboratories (Mumbai, India). All chemicals used were of analytical grade and used without further purification.

Methods

Extraction by maceration process

Maceration is a traditional and widely used extraction technique for isolating bioactive compounds from plant materials. This method is particularly suited for extracting phytochemicals from plants with delicate compounds that may be degraded by more aggressive extraction methods involving heat or solvents. Following procedure was adopted for the preparation of extract from the shade dried and powdered herbs (Khandelwal, 2005; Kokate, 1994).

Defatting with petroleum ether

40 gram of *Curcuma zedoaria*, 50 gram *Curcuma caesia* and *Curcuma amada* shade dried plant material was coarsely powdered and subjected to extraction with hexane by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction with ethanol

Defatted dried powdered has been extracted with ethanol using maceration process for 48 hrs. The mixture occasionally stirred to enhance the extraction efficiency by increasing the contact between the plant material and the solvent. After the soaking period, the mixture is filtered to separate the liquid extract from the solid plant residues. The filtrate contains the dissolved phytochemicals. The solvent is then removed, usually by evaporation under reduced pressure or using a rotary evaporator, to concentrate the extract. The concentrated extract can be further processed or analyzed to isolate specific bioactive compounds.

Determination of extractive value (% yield)

The % yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

Qualitative phytochemical analysis is a important process in the field of natural products Chemistry and Pharmacognosy. It involves the identification of the various bioactive compounds present in plant materials. These compounds, known as phytochemicals, include alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. The Qualitative phytochemical analysis was carried out by standard method.

In-vitro antioxidant activity of ethanolic extract of *Curcuma zedoaria, Curcuma caesia* and *Curcuma amada*

DPPH method

Total free radical scavenging capacity of extract was estimated according to the reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%

Hydrogen peroxide method

In-vitro antioxidant activity of extract using hydrogen peroxide was performed (Wasiullah *et al.,* 2023) proposed. Added 2ml hydrogen peroxide (43 mol) and 1.0 ml ethanolic sample [50-250 µg/ml] accompanied by 2.4 ml 0.1 M phosphate buffer (pH 7.4). The resulting solution was maintained for 10 minutes and the absorbance at 230 nm was recorded. Without adding hydrogen peroxide, blank was ready and control was prepared without sample. It was used as a conventional compound with ascorbic acid. Free radical hydrogen peroxide scavenging activity (percent) has been calculated.

Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Bawane *et al.,* 2020). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. The inhibition proportion was evaluated using the following formula:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times 100$

Where Acontrol is the absorption (without extract) of the control and where Atest is the absorption in the presence of the extract / standard.

HPTLC estimation of curcumin in *Curcuma amada, Curcuma caesia and Curcuma zedoaria*

Estimation of Curcumin using HPTLC method

A CAMAG HPTLC system (Switzerland) comprising CAMAG Linomat 5 applicator, CAMAG TLC scanner 3, CAMAG Wincats software, version 1.44, Hamilton syringe (100µl), CAMAG Reprostar 3, CAMAG TLC plate heater, CAMAG UV Cabinet were used for the study. The VTRL HPTLC facility was used for the present study.

Preparation of the Standard

1mg/ml of the standard, Curcumin was prepared with methanol. From this 100µl was diluted with 950µlof methanol and hence the concentration of the standard was 100 µg/ml.

Preparation of the extract Sample

1mg/ml of the all extract was prepared with methanol seperately. From this 100µl was diluted with 950µlof methanol and hence the concentration of the extract was 100 µg/ml.

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA). 100 µg/ml of the Standard was applied in the form of bands using LINOMAT IV applicator. The volumes applied were 1, 2, 3, 4, 5 and 6µl. The concentration of the sample was 1.0 mg/ml, and the different amounts were 2µl. The mobile step used was Toluene: Acetic acid (9:1v/v). Built the chromatograph For 15 minutes, dried at room temperature and scanned at 425nm. The normal maximum peak area was measured. Average peak area of the standard was calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software (Gangal et al., 2025).

Estimation of Curcumin in herbal extracts

Estimation of Curcumin in ethanolic of *Curcuma amada, Curcuma caesia and Curcuma zedoaria,* the mean peak area of the sample was calculated and the content of Curcumin was quantified using the regression equation obtained from the standard curve.

3. RESULTS AND DISCUSSION

The current study investigated the phytochemical composition, antioxidant potential, and curcumin content of ethanolic extracts from three Indian medicinal plants: *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada*. These plants, traditionally used in Ayurvedic medicine, were selected for their reported therapeutic properties, especially their antioxidant and anti-inflammatory effects.

The extractive values revealed that *Curcuma zedoaria* yielded the highest percentage (1.36% w/w), followed by *C. amada* (1.02%) and *C. caesia* (0.77%). This indicates a higher solubility of bioactive compounds in *C. zedoaria*, potentially due to its unique phytochemical profile. Phytochemical screening further confirmed the presence of key secondary metabolites such as alkaloids, flavonoids, phenolic compounds, and proteins in all three extracts. Flavonoids and phenolics were

particularly abundant, with *C. zedoaria* and *C. caesia* showing strong positive results. These compounds are well-known for their role in free radical scavenging and antioxidant defense mechanisms.

The antioxidant activity was assessed through three in vitro assays: DPPH radical scavenging, hydrogen peroxide neutralization, and nitric oxide inhibition. In all three assays, the extracts demonstrated concentration-dependent activity. Among them, *Curcuma caesia* consistently showed the highest antioxidant potential, with lower IC_{50} values in each method compared to the other two species. For instance, in the DPPH assay, *C. caesia* had an IC_{50} of 50.22 µg/ml, substantially lower than *C. zedoaria* (81.83 µg/ml) and *C. amada* (82.87 µg/ml). Similar trends were observed in hydrogen peroxide and nitric oxide assays, confirming the superior radical scavenging capability of *C. caesia*. Interestingly, while *C. amada* contained the highest curcumin content, its antioxidant activity was not the strongest, suggesting that other phytoconstituents like flavonoids and phenols may be more influential in determining overall antioxidant power.

HPTLC analysis was employed to quantify the curcumin content in each extract. The calibration curve showed excellent linearity with a regression coefficient (R^2) of 0.9953, confirming the reliability of the method. *Curcuma amada* had the highest curcumin concentration (7.91%), followed by *C. caesia* (1.38%) and *C. zedoaria* (0.28%). The relatively low curcumin content in *C. caesia*, despite its strong antioxidant performance, supports the idea that antioxidant activity is not solely dependent on curcumin but also on the synergistic effects of other bioactive compounds.

Table 1: Extractive values of *Curcuma zedoaria*

S. No.	Plants	% Yield* (w/w)
1.	*Curcuma zedoaria*	1.36
2.	*Curcuma caesia*	0.77
3.	*Curcuma amada*	1.02

Table 2: Result of phytochemical screening of extract of

S. No.	Constituents	Ethanolic extract		
		Curcuma zedoaria	***Curcuma caesia***	***Curcuma amada***
1.	**Alkaloids**			
	Wagner's Test:	+ve	+ve	+ve
	Hager's Test:	+ve	+ve	+ve
2.	**Glycosides**			
	Conc. H_2SO_4 Test:	-ve	-ve	-ve
3.	**Flavonoids**			
	Lead acetate Test:	+ve	+ve	+ve
	Alkaline test:	+ve	-ve	-ve
4.	**Diterpenes**			
	Copper acetate Test:	-ve	+ve	+ve
5.	**Phenol**			
	Ferric Chloride Test:	-ve	+ve	+ve
	Folin Ciocalteu Test:	+ve	+ve	+ve
6.	**Proteins**			
	Xanthoproteic Test:	+ve	-ve	-ve
7.	**Carbohydrate**			
	Fehling's Test:	-ve	+ve	-ve

	Benedict's Test	-ve	-ve	-ve
8.	**Saponins** Froth Test:	-ve	-ve	-ve
9.	**Tannins** Gelatin test:	-ve	-ve	-ve
10.	**Sterols** Salkowski Test:	-ve	-ve	-ve

+ve= present, -ve=negative

Table 3: % Inhibition of ascorbic acid and ethanolic extracts using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition			
		Ascorbic acid	*Curcuma zedoaria*	*Curcuma caesia*	*Curcuma amada*
1	10	44.60	14.58	16.45	10.07
2	20	52.94	22.01	39.52	28.38
3	40	60.56	30.50	48.28	35.54
4	60	65.21	37.13	56.23	41.90
5	80	78.62	50.39	70.29	44.82
6	100	91.07	60.21	74.54	47.74
IC 50 value		**18.50**	**81.83**	**50.22**	**82.87**

Table 4: % Inhibition of ascorbic acid and ethanolic extracts using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition			
		Ascorbic acid	*Curcuma zedoaria*	*Curcuma caesia*	*Curcuma amada*
1	50	43.76	22.65	30.25	28.25
2	100	50.83	34.96	48.97	44.97
3	150	72.52	57.14	70.14	65.14
4	200	81.41	62.78	76.92	72.69
5	250	92.67	79.61	90.07	87.25
IC 50 value		**79.25**	**145.20**	**105.11**	**117.04**

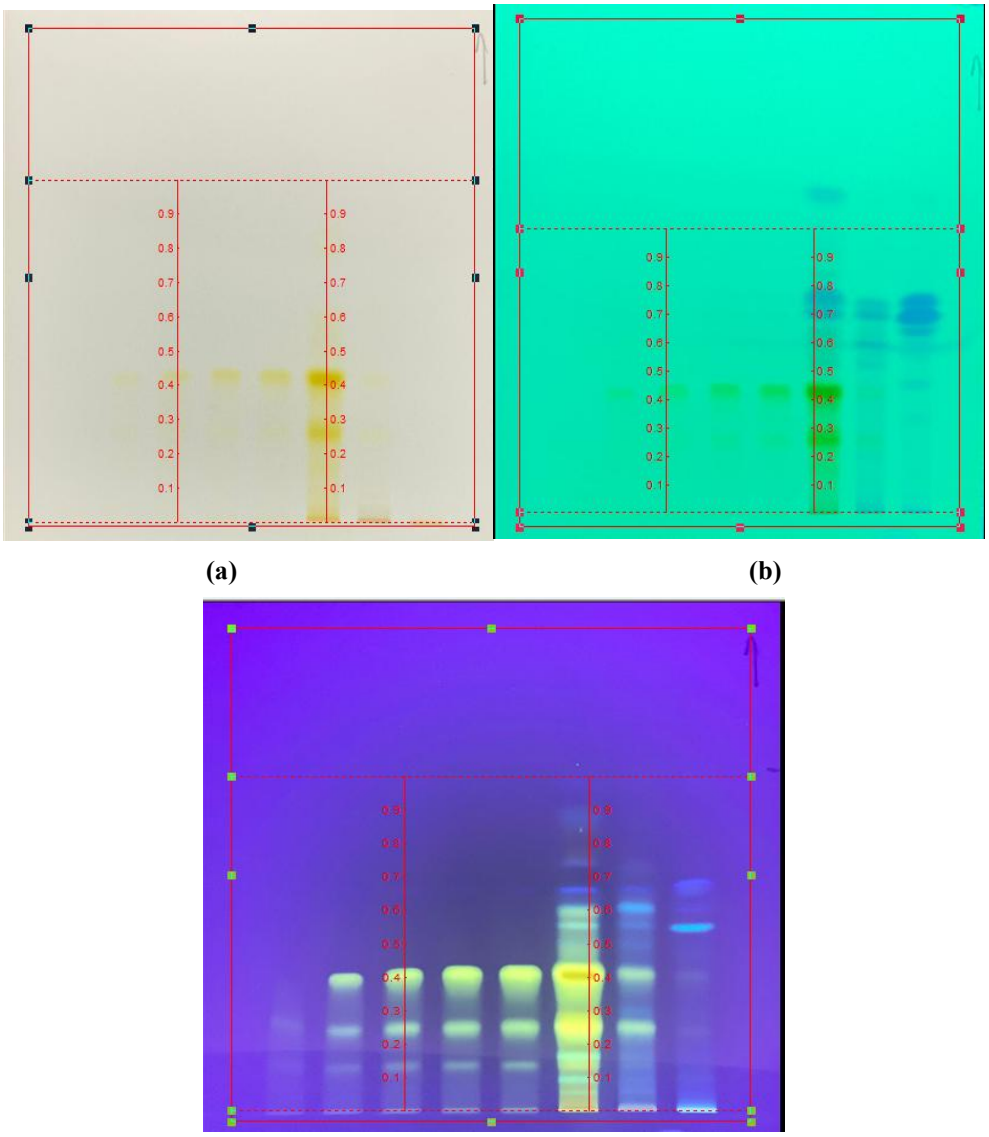
Table 5: % Inhibition of ascorbic acid and ethanolic extracts using Nitric oxide method

S. No.	Concentration (µg/ml)	% Inhibition			
		Ascorbic acid	*Curcuma zedoaria*	*Curcuma caesia*	*Curcuma amada*
1	20	42.03	18.26	27.02	20.41
2	40	67.96	24.93	36.84	26.79
3	60	75.75	32.54	41.78	35.84
4	80	75.24	40.87	57.96	42.65

5	100	85.63	53.92	63.45	57.69
IC 50 value		**19.13**	**96.50**	**69.91**	**89.49**

Table 6: Preparation of calibration Curve of Curcumin by HPTLC

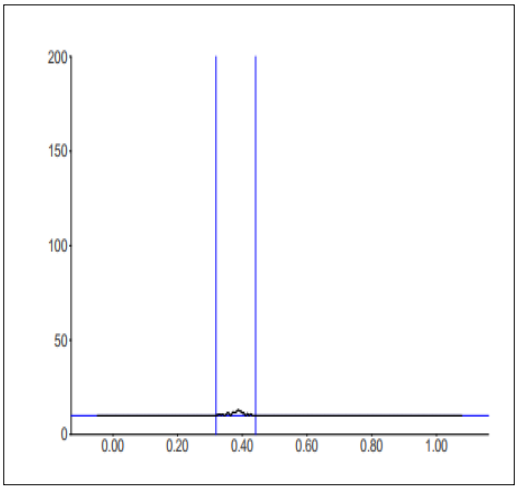
S. No.	Concentration (µg/ml)	Area
1.	0	0
2.	1	1945.8
3.	2	4955.6
4.	3	7785.4
5.	4	11117.9
6.	5	14085.8



(a) **(b)**

(c)

Figure 1: Chromatograms obtained from separation of standards curcumin and plant extracts visualization was under (a) Normal light, (b) UV light of wavelength 254 nm, (c) UV light of wavelength 365 nm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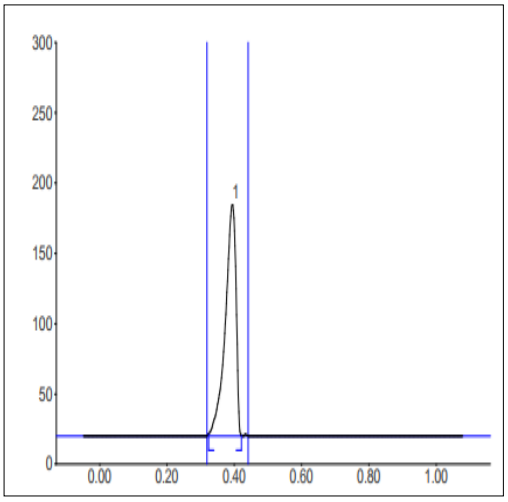
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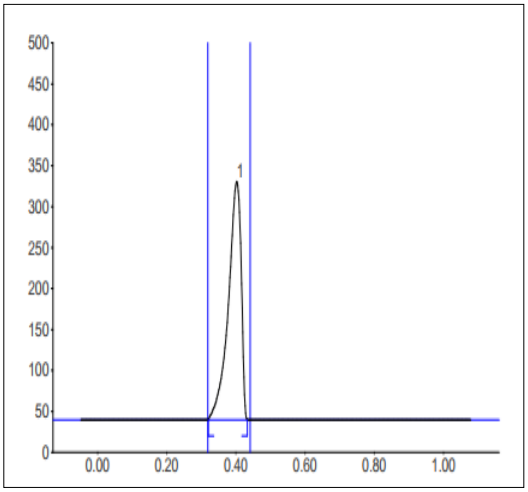
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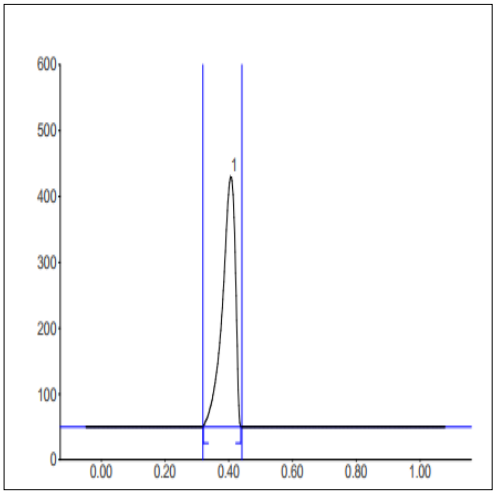
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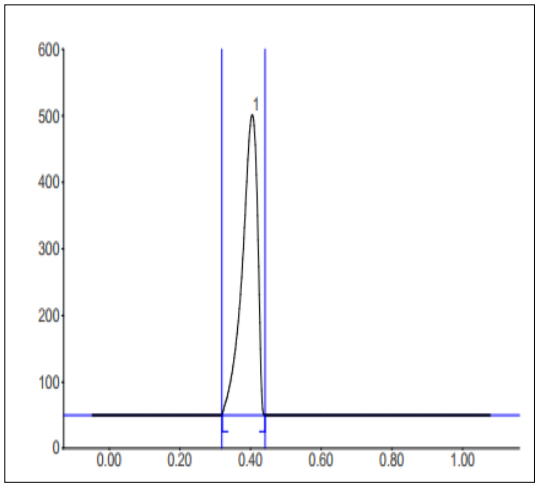
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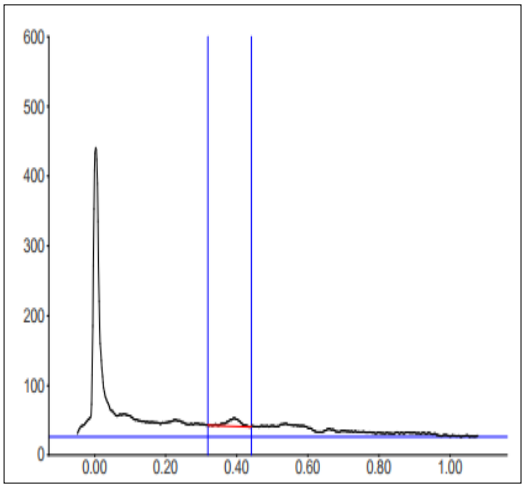
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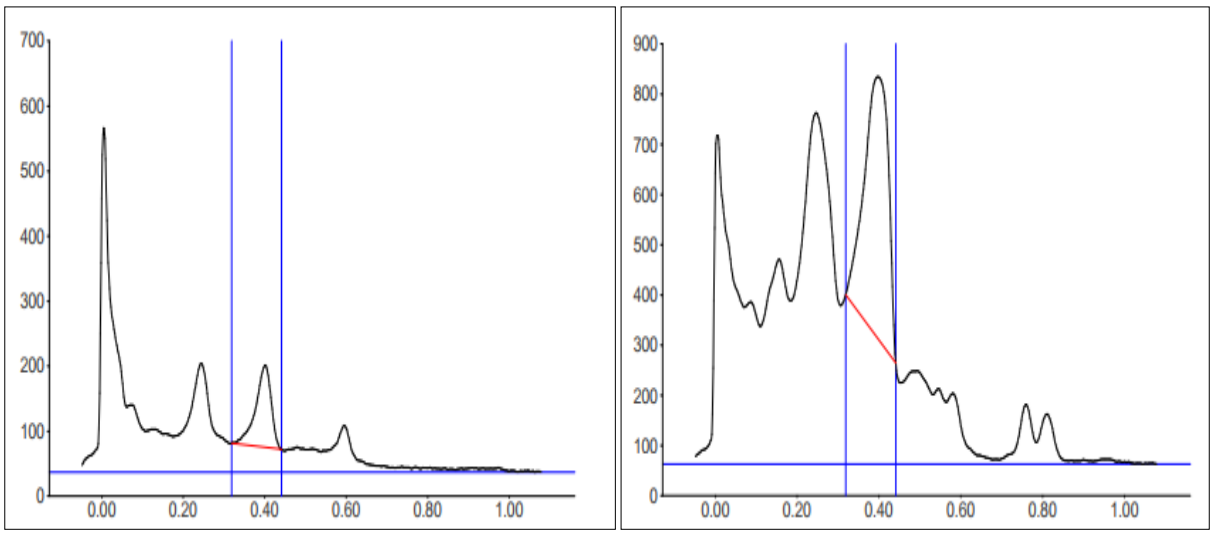

(g) **(h)**

Figure 2: Chromatogram of different standard concentration of Curcumin (a), (b), (c), (d), (e) - 1, 2, 3, 4and 5µg respectively and (f), (g), (h) – 2 µg each concentration of *Curcuma zedoaria, Curcuma caesia* and *Curcuma amada*

Table 7: Results of Statistical analysis

Parameters	**Results**
Regression Equation	y = 2879.3x - 549.8
Regression Coefficient (r^2)	0.9953
Slope	2879.3
Intercept	549.8
Percentage Found	0.82

Table 8: Results of percentage content of curcumin in different extract

S. No.	**Ethanolic extract**	**Area**	**% Found**
1	*Curcuma amada*	22247.4	7.91
2	*Curcuma caesia*	3442.0	1.38
3	*Curcuma zedoaria*	269.1	0.28

4. CONCLUSION

The present investigation highlights the comparative phytochemical composition, antioxidant potential, and curcumin content of ethanolic extracts from *Curcuma zedoaria*, *Curcuma caesia*, and *Curcuma amada*. Phytochemical screening confirmed the presence of key bioactive compounds such as flavonoids, phenolics, and alkaloids, which are associated with antioxidant activity. Among the three species, *Curcuma caesia* demonstrated the most significant antioxidant activity across DPPH, hydrogen peroxide, and nitric oxide scavenging assays, despite not having the highest curcumin content. HPTLC analysis confirmed that *Curcuma amada* contained the highest concentration of curcumin. These findings suggest that antioxidant efficacy may result from the combined effect of various phytoconstituents rather than curcumin alone.

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