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(RESEARCH ARTICLE)

HPLC estimation of curcumin in different plants of zingiberaceae family

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Abstract

The comparative analysis of *Curcuma zanthorrhiza* and *Curcuma zedoaria* extracts reveals distinct medicinal potentials based on their bioactive compound profiles and curcumin concentrations. *Curcuma zedoaria* yielded a slightly higher extraction percentage (6.3%) compared to *Curcuma zanthorrhiza* (5.7%), with both producing dark brown extracts. Qualitative tests indicated that *Curcuma zanthorrhiza* contains carbohydrates, glycosides, saponins, phenols, flavonoids, and diterpenes, whereas *Curcuma zedoaria* has alkaloids and proteins, suggesting differing therapeutic properties. Quantitative analysis showed that *Curcuma zanthorrhiza* has higher total phenol (0.77 mg/100 mg) and flavonoid (0.62 mg/100 mg) contents than *Curcuma zedoaria* (0.65 mg/100 mg phenols and 0.54 mg/100 mg flavonoids), indicating greater antioxidant potential.

Overall, while both species contain valuable medicinal compounds, *Curcuma zanthorrhiza* is likely more effective for antioxidant and anti-inflammatory purposes. In contrast, *Curcuma zedoaria* may provide unique therapeutic benefits due to its alkaloid and protein content. Thus, both species have significant applications in herbal medicine, each serving different medicinal roles based on their distinct bioactive profiles.

Keywords: *Curcuma zanthorrhiza*; *Curcuma zedoaria*; Bioactive compounds; Curcumin HPLC

1. Introduction

1.1. Chromatography

Today, chromatographic techniques have little to do with the separation of color (the technique names evolved from the earliest work of separating dyes or plant pigments on paper), but do involve the separation of compounds in a sample mixture. A number of types of separation methods have developed over the years to accommodate the various physical and chemical states of sample mixtures one may be interested in separating and analyzing. The feature that distinguishes chromatography from most other physical and chemical methods of separation is that, two mutually immiscible phases brought into contact; one phase is stationary and other mobile.

The mobile phase can be gas or a liquid, whereas the stationary phase can only be a liquid or a solid. When the separation involves predominantly a simple portioning between two immiscible liquid phases, one stationary and other mobile, the process is called liquid-liquid chromatography. When physical surface forces are mainly involved in the retentive ability of the stationary phase, the process is denoted as liquid solid chromatography. Liquid chromatography has been performed in a column or on an open bed.

Chromatography is probably the most power full and versatile analytical technique available to modern chemist. Its power arises from its capacity to determine quantitatively many individual components present in the mixture in one, single analytical procedure.

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1.2. Separation Principles

When liquid chromatography was first developed, the most popular method of separation involved the use of nonpolar mobile phase, such as hexane or heptane, with silica gel or alumina as the solid support. The name of this mode "Normal phase chromatography" arose from the fact that the mobile phase was less polar than the solid phase. The major drawback to this technique was that, because the chromatographic character of silica gel changes drastically with its state of hydration, the mobile phase had to be thoroughly dried before use.

1.3. High Performance Liquid Chromatography

Early in the development of liquid chromatography, scientist realized that increase in column efficiency could be brought about by decreasing the partial size of packaging. The technology for producing and using pickings with particle diameter as smalls 3 to10μm was developed. This technology required sophisticated instruments operating at high pressure, which contrasted markedly with the simple glass columns of classic gravity-flow liquid chromatography. The name High Performance Liquid chromatography (HPLC) is employed to distinguish this newer procedure.

HPLC is one of the most popular and wide applied analytical techniques in use today. Among the many reasons for the technique popularity its versatility and wide range of applicability.

Higher separation efficiencies, decreased analysis time, high resolution power, continuous monitoring of the column effluents, accurate quantitative measurement, repetitive and reproducible analysis, using the same column, automation of the analytical procedure and data handling, reduced solvent consumption and increased mass sensitivity are some advantages that have propelled the growth of this technique.

1.4. Phytochemicals

Phytochemistry is the study of chemicals produced by plants, particularly the secondary metabolites. It takes into account their structural compositions, the biosynthetic pathways, functions, and mechanisms of actions in the living system. The study of phytochemicals has been instrumental in the discovery of new plant natural products which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals, and dietary supplement industries. Not left out is the cosmeceuticals industries, clothing and textiles industries, food, wine, and beverage industries, the military among others. Owing to the consistent threat of microorganisms, environmental hazards to public health, the significance of phytochemistry in the medical and pharmaceutical industries for the quest for the discovery of new drugs has overshadowed their essence in other industries.

Phytochemicals have been in existence since time immemorial and are known to be responsible for the organoleptic properties (color, taste, flavor, aroma, and odor) of plants, such as the smell of garlic, ginger, and the deep purple color of blueberries. The ability of plants to exhibit curative potentials and the characteristic difference that exists within them may also have awakened early interests for the knowledge about their chemical compositions. In the plant kingdom, these variations are quite glaring.

1.5. Herbal medicines

Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments. The various indigenous systems such as Siddha, Ayurveda, Unani and Allopathy use several plant species to treat different ailments. The use of herbal medicine becoming popular due to toxicity and side effects of allopathic medicines. Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since antiquity.

The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of world and have made a great contribution towards maintaining human health. In India around 20,000 medicinal plant species have been recorded recently but more than 500 traditional communities use about 800 plant species for curing different diseases. Currently 80% of the world population depends on plant-derived medicine for the first line of primary health care for human alleviation because it has no side effects.

2. Experimental work

2.1. Collection of plant material

Rhizomes of *Curcuma zanthorrhiza* and *Curcuma zedoaria* were collected from local area of Bhopal in month of March 2024.

2.2. Cleaning

After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water.

2.3. Drying

Drying of fresh rhizomes was carried out in sun but under the shade.

2.4. Storage

Dried rhizomes were preserved in plastic bags and closed tightly and powdered as per the requirements.

2.5. Extraction procedure

Extraction is the first step to separating the desired natural products from the raw materials. Maceration is a very simple extraction method with the disadvantage of long extraction time and low extraction efficiency. It could be used for the extraction of thermolabile components. Following procedure was adopted for the preparation of extracts from the shade dried and powdered herbs:

2.6. Defatting of plant material

Rhizomes of *Curcuma zanthorrhiza* and *Curcuma zedoaria* were shade dried at room temperature. 50 grams 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.

2.7. Extraction by maceration process

Defatted rhizomes of *Curcuma zanthorrhiza* and *Curcuma zedoaria* were extracted with hydroalcoholic solvent (ethanol: water: 85:15) using maceration process (12hrs). The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts.

3. Results and discussion

Table 1 Percentage Yield of *Curcuma zanthorrhiza* and *Curcuma zedoaria* extract

Extract	Consistency	Colour	percentage Yield
Curcuma zanthorrhiza	Solid	Dark Brown	5.7%
Curcuma zedoaria	Solid	Dark Brown	6.3%

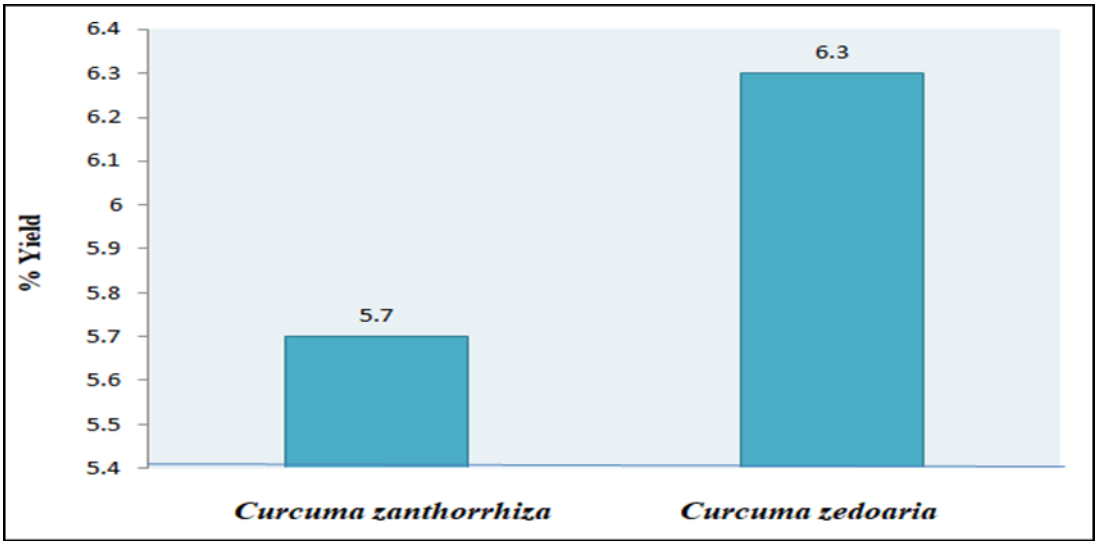

Figure 1 % Yield of *Curcuma zanthorrhiza* and *Curcuma zedoaria* extract

In this study, the extraction yields of *Curcuma zanthorrhiza* and *Curcuma zedoaria* were determined to be 5.7% and 6.3%, respectively, as shown in Table 1. Both extracts presented as solid with a dark brown color, indicating a similar visual appearance. This suggests that *Curcuma zedoaria* might contain a slightly higher concentration of extractable components, such as curcuminoids or essential oils, which are known to vary between species.

3.1. Results of Qualitative chemical test

Table 2 Qualitative chemical tests of extract of *Curcuma zanthorrhiza*

S. No.	Bioactive constituents	Hydroalcoholic extract
1	Alkaloids	-ve
2	Carbohydrates	+ve
3	Glycosides	+ve
4	Saponins	+ve
5	Phenols	+ve
6	Flavonoids	+ve
7	Proteins	-ve
8	Diterpenes	+ve

+ ve – Present, - ve – Absent

The qualitative chemical tests of the *Curcuma zanthorrhiza* hydroalcoholic extract, as presented in Table 2, revealed the presence of several bioactive constituents. Notably, carbohydrates, glycosides, saponins, phenols, flavonoids, and diterpenes were all found to be present, indicated by positive test results. These compounds are often associated with various medicinal properties, such as antioxidant, anti-inflammatory, and antimicrobial effects. The absence of alkaloids and proteins, as indicated by the negative test results, suggests that these constituents do not contribute to the biological activity of the *Curcuma zanthorrhiza* extract.

The presence of flavonoids and phenols, in particular, is significant because these compounds are known for their potent antioxidant properties, which can contribute to the plant's traditional use in treating various health conditions. Saponins are also noteworthy for their potential role in boosting immune function and their known surfactant properties. The detection of diterpenes further supports the plant's therapeutic potential, as these compounds are often linked to anti-inflammatory and antimicrobial activities.

Table 3 Qualitative chemical tests of extract of *Curcuma zedoaria*

S. No.	Bioactive constituents	Hydroalcoholic extract
1	Alkaloids	+ve
2	Carbohydrates	-ve
3	Glycosides	-ve
4	Saponins	-ve
5	Phenols	+ve
6	Flavonoids	+ve
7	Proteins	+ve
8	Diterpenes	+e

+ ve – Present, - ve – Absent

The qualitative chemical tests of the *Curcuma zedoaria* hydroalcoholic extract, as shown in Table 3, indicate the presence of several bioactive constituents, including alkaloids, phenols, flavonoids, proteins, and diterpenes. Interestingly, unlike *Curcuma zanthorrhiza*, *Curcuma zedoaria* tested positive for alkaloids and proteins, which could contribute to its distinct pharmacological profile.

The positive test for proteins in *Curcuma zedoaria* might suggest potential for enzymatic or structural bioactivity, adding another dimension to its medicinal uses. The distinct combination of bioactive constituents in *Curcuma zedoaria*, particularly the presence of alkaloids and proteins, could make it suitable for different therapeutic applications compared to *Curcuma zanthorrhiza*.

3.2. Results of quantitative study

3.2.1. Estimation of total phenol content (TPC)

Total phenol content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: y = 0.016x + 0.002, 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
1	10	0.178
2	20	0.327
3	30	0.502
4	40	0.673
5	50	0.836

*Average of three determination

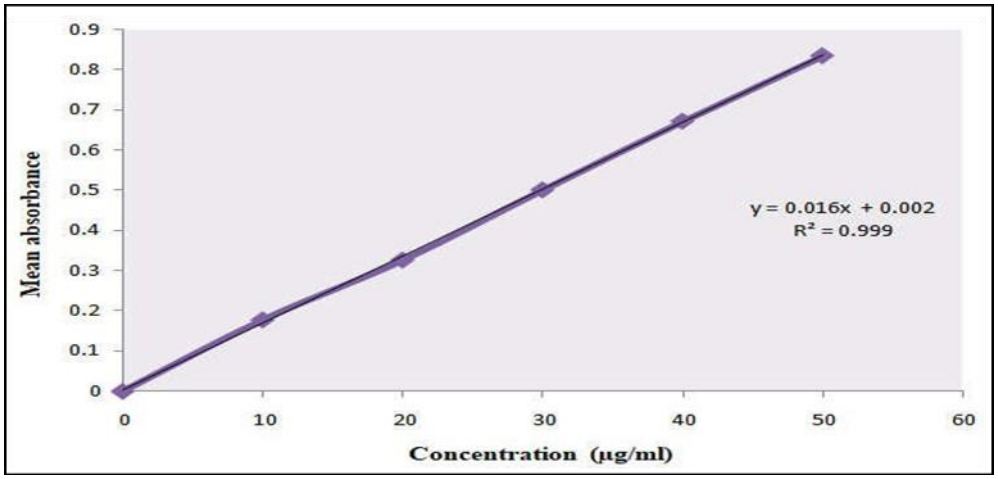

Figure 2 Graph of calibration curve of Gallic acid

3.2.2. Estimation of total flavonoids content (TFC)

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: y = 0.025x - 0.001, R2=0.999, 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
1	5	0.125
2	10	0.246
3	15	0.373
4	20	0.486
5	25	0.632

*Average of three determination

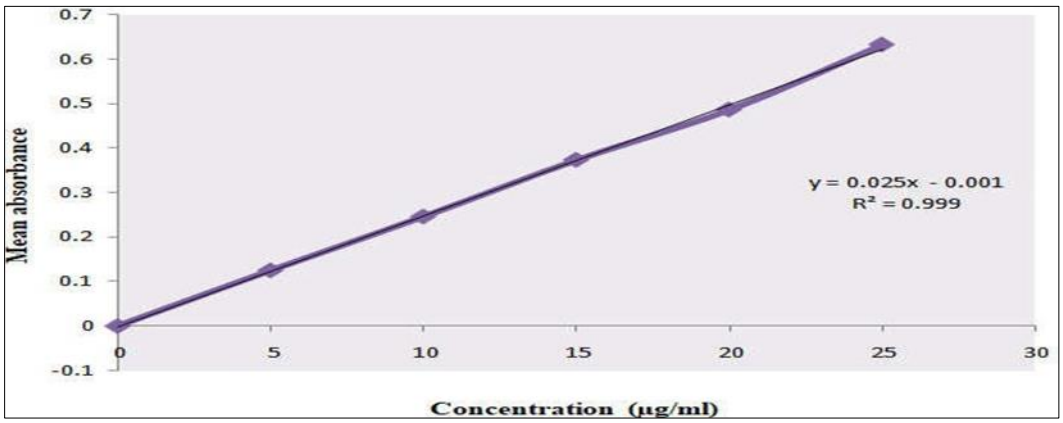

Figure 3 Graph of calibration curve of Quercetin

Table 6 Estimation of total phenol and flavonoids content in *Curcuma zanthorrhiza*

S. No.	Total phenol content	Total flavonoids content
1.	0.77mg/100mg	0.62 mg/100mg

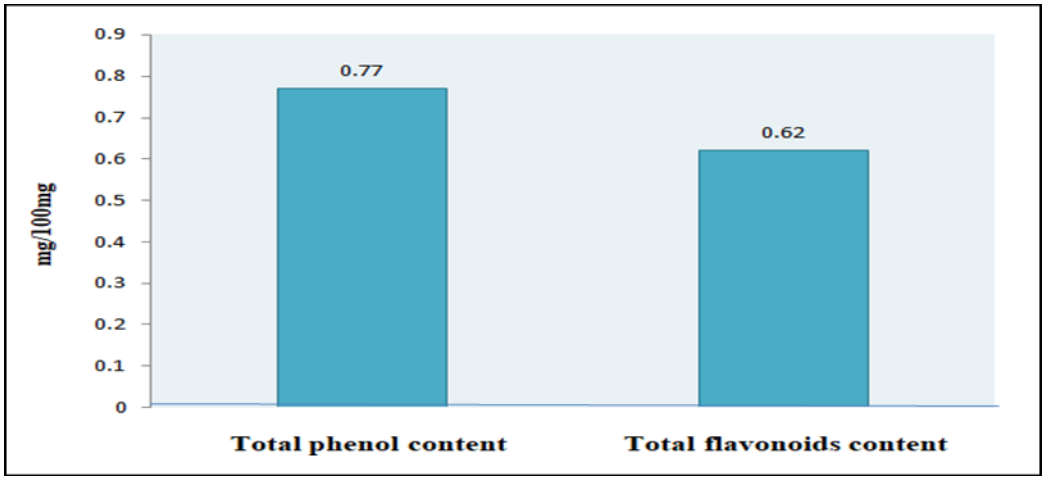

Figure 4 Graph of Total phenol and flavonoids content in *Curcuma zanthorrhiza*

In the estimation of total phenol and flavonoid content in *Curcuma zanthorrhiza* extract, as presented in Table 6, the phenol content was found to be 0.77 mg per 100 mg of extract, while the flavonoid content was slightly lower at 0.62 mg per 100 mg of extract. Both phenols and flavonoids are well-known for their potent antioxidant properties, which play a crucial role in neutralizing free radicals and reducing oxidative stress.

Table 7 Estimation of total phenol and flavonoids content in *Curcuma zedoaria*

S. No.	Total phenol content	Total flavonoids content
1.	0.65 mg/100 mg	0.54 mg/100 mg

In the estimation of total phenol and flavonoid content in *Curcuma zedoaria* extract, as shown in Table 7, the phenol content was measured at 0.65 mg per 100 mg of extract, while the flavonoid content was slightly lower at 0.54 mg per 100 mg. These values indicate that *Curcuma zedoaria* has a somewhat lower concentration of both phenols and flavonoids compared to *Curcuma zanthorrhiza*.

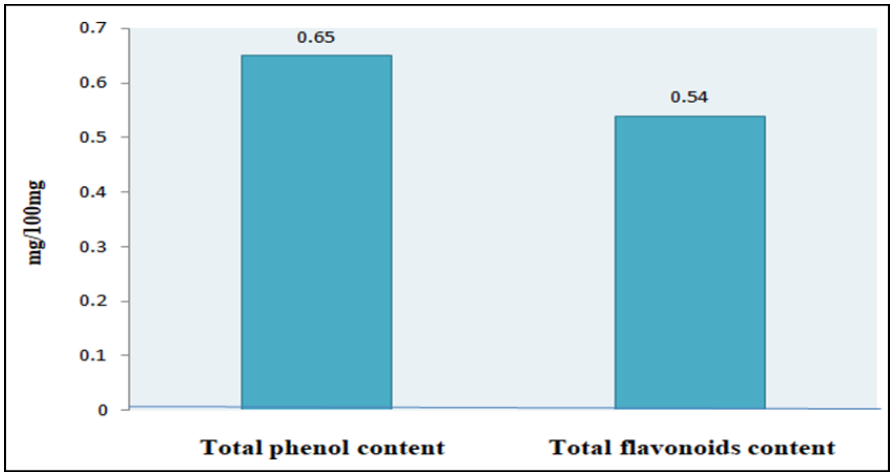

Figure 5 Graph of Total phenol and flavonoids content in *Curcuma zedoaria*

3.3. Identification of marker compound (Curcumin) by HPLC

3.3.1. Calibration curve of Curcumin

Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Table 8 Preparation of calibration curve of Curcumin

S. No.	Concentration (µg/ml)	Mean AUC
1.	5	395.21
2.	10	897.65
3.	15	1334.97
4.	20	1798.14
5.	25	2273.55

*Average of three determination, Mean ± SD

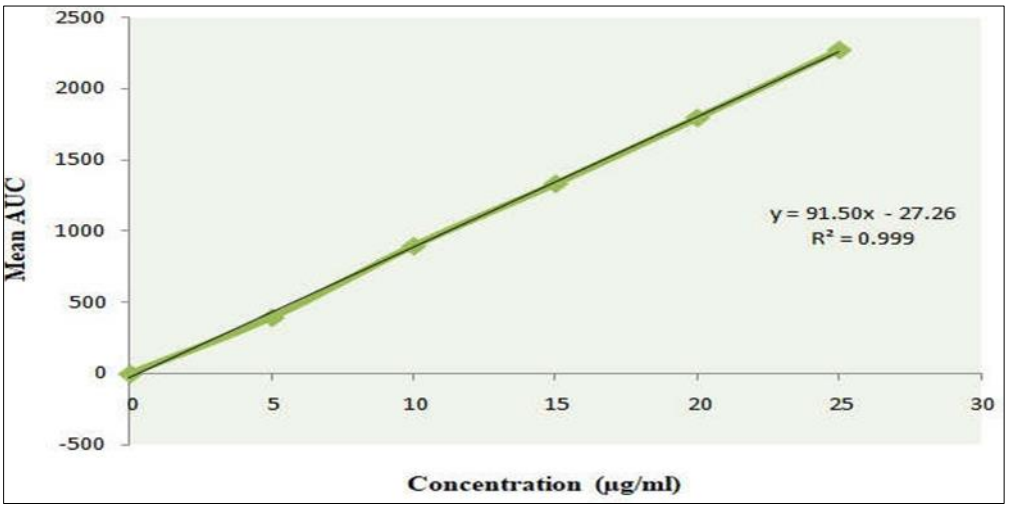

Figure 6 Calibration curve of Curcumin

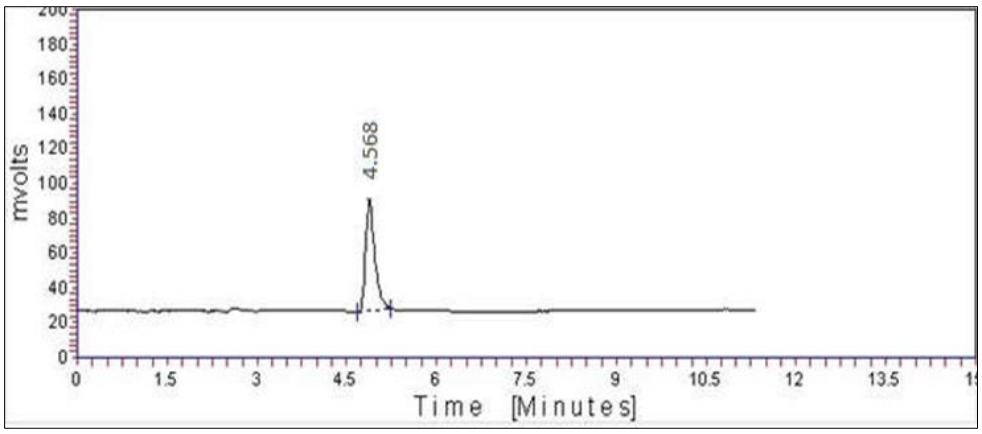

Figure 7 Chromatogram of standard Curcumin

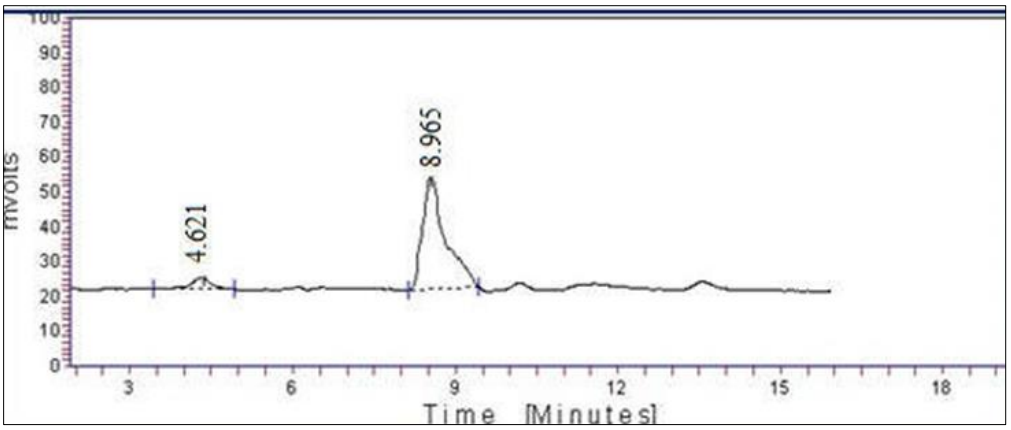

Figure 8 Chromatogram of hydroalcoholic extract of *Curcuma zanthorrhiza*

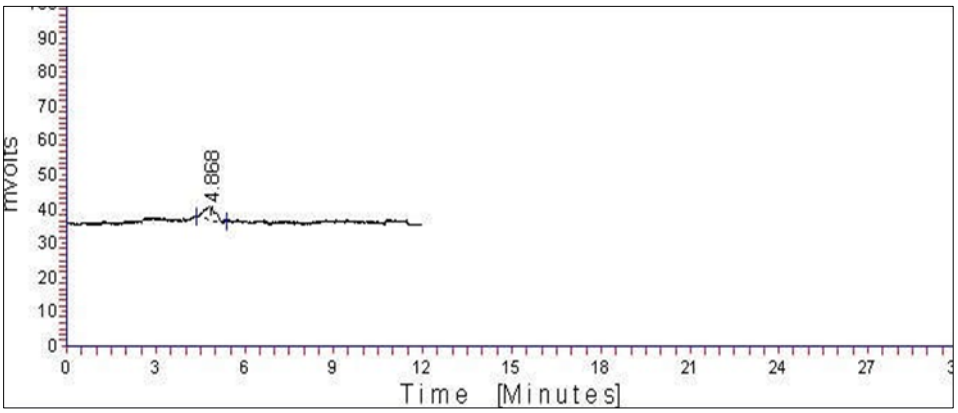

Figure 9 Chromatogram of hydroalcoholic extract of *Curcuma zedoaria*

Table 9 Quantitative estimation of Curcumin in extract

S. No.	Hydroalcoholic extract	RT	percentage Assay
1.	Curcumin	4.568	
2.	*Curcuma zanthorrhiza*	4.621	0.632%
3.	*Curcuma zedoaria*	4.868	0.574%

The quantitative estimation of curcumin content in the hydroalcoholic extracts of *Curcuma zanthorrhiza* and *Curcuma zedoaria*, as presented in Table 9, provides key insights into their curcumin concentrations. Curcumin is a well-known bioactive compound responsible for many of the medicinal properties associated with plants in the Curcuma genus, including anti-inflammatory, antioxidant, and anticancer effects.

The retention times (RT) for curcumin and the extracts of *Curcuma zanthorrhiza* and *Curcuma zedoaria* were 4.568, 4.621, and 4.868 minutes, respectively. These values indicate a close match between the curcumin standard and the curcumin content within both extracts, confirming the presence of curcumin in each. In terms of concentration, *Curcuma zanthorrhiza* had a slightly higher curcumin content, with a % assay of 0.632%, compared to *Curcuma zedoaria*, which had a curcumin concentration of 0.574%. Although the difference in curcumin content is relatively small, this suggests that *Curcuma zanthorrhiza* may be marginally more potent in terms of curcumin-related health benefits.

The higher curcumin content in *Curcuma zanthorrhiza* could explain some of its more pronounced traditional uses for anti-inflammatory and antioxidant purposes. Meanwhile, *Curcuma zedoaria*, despite having a slightly lower curcumin content, still demonstrates valuable therapeutic potential.

4. Conclusion

The comparative analysis of *Curcuma zanthorrhiza* and *Curcuma zedoaria* extracts highlights their medicinal potential based on bioactive compound content and curcumin concentration. The extraction yields were slightly higher for *Curcuma zedoaria* (6.3%) compared to *Curcuma zanthorrhiza* (5.7%), although both species produced solid, dark brown extracts. Qualitative chemical tests showed that *Curcuma zanthorrhiza* contains carbohydrates, glycosides, saponins, phenols, flavonoids, and diterpenes, while *Curcuma zedoaria* lacked these compounds but had alkaloids and proteins, suggesting different medicinal properties. The quantitative estimation of curcumin content further revealed that *Curcuma zanthorrhiza* had a slightly higher curcumin concentration (0.632%) compared to *Curcuma zedoaria* (0.574%). This small difference could contribute to enhanced anti-inflammatory and antioxidant effects in *Curcuma zanthorrhiza*. Overall, while both species contain significant medicinal compounds, *Curcuma zanthorrhiza* appears to be more potent in terms of antioxidant and anti-inflammatory properties due to its higher phenol, flavonoid, and curcumin content. The HPLC estimation of curcumin in different plants from the Zingiberaceae family provides valuable insights into the varying concentrations of this key bioactive compound across species. Curcumin, known for its strong antioxidant, anti-inflammatory, and anticancer properties, is a major contributor to the medicinal value of plants in this family, such as *Curcuma zanthorrhiza*, *Curcuma zedoaria*, and others. The findings suggest that plants from the Zingiberaceae family, particularly those rich in curcumin, like *Curcuma zanthorrhiza*, may be more potent for therapeutic purposes related to curcumin's properties.However, the slight variations in curcumin levels across different species also imply that certain plants may offer additional or complementary medicinal benefits beyond curcumin, as seen with the unique presence of alkaloids or proteins in some species like *Curcuma zedoaria*.

In conclusion, the HPLC estimation underscores the importance of selecting the appropriate plant species within the Zingiberaceae family based on desired curcumin concentration and therapeutic outcomes. While species like *Curcuma zanthorrhiza* may offer enhanced benefits in applications requiring high curcumin levels, other species might be valuable for their diverse bioactive profiles, making them useful for a wider range of medicinal applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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