BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, March 2025. Vol. **22**(1), p. 165-172

Comparative Analysis of Phytochemicals and Antioxidant Potential in Various Rhizome extracts of *Curcuma zedoaria* Roscoe

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http://dx.doi.org/10.13005/bbra/3350

(Received: 18 November 2024; accepted: 13 February 2025)

Plants rich in bioactive metabolites play a significant role in food and pharmaceutical industries. *Curcuma zedoaria* Roscoe is a popular medicinal plant, used for treating respiratory ailments. The present study characterizes the phytochemicals and antioxidant activity in methanol and n-Hexane extracts of *C. zedoaria* rhizomes collected from North-east and North-west region of India. Results revealed highest phenolics, flavonoids and tannins in methanolic extracts of rhizomes collected from North-west. Potent antioxidant activity of *C. zedoaria* rhizomes can be ascribed to the presence of high content of different phytochemicals. Hence, *Curcum*a rhizomes are potential source of natural bioactive molecules for promoting human health.

Keywords: *Curcuma zedoaria*, secondary metabolites, antioxidant activity, phytochemicals.

Medicinal plants are accustomed to treat diseases and solving various health problems since time immemorial. Due to incredible expansion of herbal treatments there is a growing interest in the use of traditional medicines [1]. As per WHO (World Health Organization), around 80% of global population depends on plants as they are endowed with secondary metabolites with medicinal properties to treat various diseases [2]. Large progress made in pharmaceutical industries is due to the use of plants as resources because of the presence of these metabolites [3,4].

Curcuma zedoaria Roscoe belonging to family *Zingiberaceae* is an important species of genus *Curcuma.* It grows widely in many parts of world including China, Indonesia, Sri Lanka and Taiwan. In India, the plant is found in North-eastern parts and some places of Himachal Pradesh. The plant is a herb with green leaves and underground storage stems called rhizomes. The plant is also called "white turmeric" because the interior of rhizomes is creamish white. The rhizomes have campherous aroma and are bitter in taste. The rhizomes have aromatic, anti-cancer and stimulant properties [5]. Besides their use as spice, they have anti-inflammatory, spasmolytic, antitumor, and neuroprotective effects [6,7].The main active components found in rhizomes of *C. zedoaria* are the non-volatile curcuminoids and terpenoids [8].

Production of bioactive substances in plants largely depends on their association with the surrounding environment. Therefore, survival of the plant depends mostly on various secondary metabolites produced by them, which act as defense molecules and are specific to them. Thus, quantification of these metabolites is important

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as they will result in the synthesis of novel and effective drugs and can also scientifically validate the existing traditional practices [9]. Till date there are no reports that reveal comparison in metabolites in *C. zedoaria* growing in North-east and North-west regions of India and accounts for the natural compounds present in them that elucidate their bio-functional health-promoting qualities. Thus the aim of this study was to evaluate the antioxidant activity and phytochemicals in *C. zedoaria* rhizomes collected from North-east and North-west India.

MATERIALS AND METHODS

Collection of Plant material

The rhizomes of *Curcuma zedoaria* from North-west were collected from Banog region (Sirmaur) of Himachal Pradesh and from North-east, from Shillong, from their natural habitat. The rhizomes were washed under tap water for 2-3 times followed by single washing with autoclaved water. The collected rhizomes were cut into pieces, dried in shade, fine powdered and used for phytochemical analysis. Ten grams of fine powder was taken from each sample and was extracted with 100ml of two different solvents: one polar (methanol) and one non polar (n-Hexane) in the flasks. The flasks were kept at room temperature overnight and the solvent was filtered and the filtrate was collected and air dried in a rotary evaporator at 40 ^{o}C. All the four extracts (one each from methanol and n-Hexane of rhizomes from North-east and North-west) were stored in sterile dark bottles and kept at 4 ^{o}C till use.

Phytochemical analysis

In accordance with standard methods preliminary phytochemical analysis was conducted for the four extracts [10,11].

Test for terpenoids

To 1ml of extract, chloroform (1ml) was added followed by addition of 1.5 ml concentrated sulphuric acid along the side walls of test tube. Formation of red brown colour indicated the presence of terpenoids.

Test for saponins

To 1 ml of extract, with continuous shaking 2.5 ml of distilled water was added followed by few drops of olive oil. Formation of foam indicated the presence of saponins.

Test for steroids

To 1 ml of extract, 3 ml of chloroform was added followed by addition of 2 ml of concentrated sulphuric acid slowly from the side of the test tube. Red colored ring at the junction of two liquids indicated the presence of steroids.

Test for flavonoids

Appearance of yellow/red/pink colour on addition of few drops of concentrated hydrochloric acid to 1 ml of extract indicated the presence of flavonoids.

Test for tannins

To 1ml of extract, few drops of 5% ferric chloride were added. Blue/black precipitates indicated the presence of tannins.

Test for proteins

To 1 ml of extract, 1 ml of 4% NaOH solution and few drops of 1% $CuSO_4$ solution were added. Violet-pink colour formation indicated the presence of proteins.

Test for carbohydrates

Few drops of Molisch's reagent were added to 1 ml of extract followed by addition of concentrated sulphuric acid along the sides of the test tube. was then added. The presence of carbohydrates in the extract was indicated by violet ring formation at the junction of two liquid layers.

Test for phenolics

To 1 ml of extract, few drops of $FeCl_3$ were added. Blue or green coloration indicated the presence of phenols in the extract.

Test for coumarins

To 1 ml of extract, 10% of sodium hydroxide (1 ml) of was added. Emergence of yellow colour indicated the presence of coumarins.

Quantitative phytochemical analysis

Total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu reagent [12]. To each of 50 µl extract in tube, 950 µl of water was added followed by addition of 0.5 ml of 1N FC reagent. Mixture was kept for 5 min at room temperature followed by addition of 7.5% sodium carbonate (2ml) to it. The tubes were incubated for 1 hour in the dark. O.D was taken at 750 nm and gallic acid was used as the reference. The results were expressed as µg Gallic acid equivalent per gram of extract.

Total flavonoid content

Total flavonoid content was measured using Aluminium chloride colorimetric method [13]. To 100 µl of each extract, 150 µl of $NaNO_2$ was added and incubated for 5 minutes at room temperature, followed by addition of 300µl of 10% $AlCl_3$. After incubating at room temperature for 6 min, 300 ìl of 1 M NaOH and 550 ìl of distilled water was added and the absorbance was measured at 510 nm. Quercetin was used as standard and the total flavonoid content was expressed as milligram Quercetin equivalents per gram of extract.

Total tannin content

Total tannins were studied by the method given by Elfalleh [14]. To 1 ml of each of the diluted extracts 2.5% KIO_3 (5ml) was added and vortexed for 10-12 s. The tubes were incubated for 2 minutes at room temperature. Red colored mixture was formed and its absorbance was measured at 550 nm. Tannic acid was used as reference and the results were expressed as mg tannic acid equivalent per g of extract.

***In vitro* antioxidant activity**

The four extracts of *C. zedoaria* were analyzed for their antioxidant activity.

Ferric ion reducing antioxidant power (FRAP assay)

FRAP assay was used for assessing the total antioxidant activity using protocol given by Benzie and Strain [15]. To 100 ìl of each extract in tube, 3 ml of FRAP reagent was added which was prepared by mixing 300 mmol/l acetate buffer at pH 3.6 with 10mmol/l TPTZ (dissolved in 40 mmol/l HCl and 20 mmol/l $FeCl_3$). Tubes were incubated for 5 minutes at 37°C and the absorbance was taken at 593nm. $FeSO_4$ was used as reference. The results are expressed as ìmol/g dry weight Fe^{2+} reduced.

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH assay)

DPPH assay was determined using the protocol given by Brand-Williams [16]. To 10 ìl of each extract, 100 ìl of DPPH (18mg of DPPH was dissolved in 100 ml methanol) was added and then 2 ml of acetate buffer (0.410g of sodium acetate was dissolved in 50 ml distilled water and 115 µl of glacial acetic acid was dissolved in 50ml distilled water and mixing both the solutions) was added. The tubes were incubated in dark (30 min) at room temperature and the absorbance was calculated at 517 nm. Butylated hydroxyl toluene (BHT) was used as a standard. The results were expressed using the following equation:

$\%\ RSA = [(A_0-A_s)/A_0] \times 100$ where A_0 is the control absorbance and A_s is the test sample absorbance.

RESULTS

Preliminary phytochemical analysis

The phytochemical characteristics of methanolic and n-Hexane extracts of *Curcuma zedoaria* from North-east and North-west are given in table 1. Phytochemicals like terpenoids, phenolics, flavonoids, saponins, steroids and

Table 1. Phytochemical constituents in methanol and n-Hexane extracts in rhizomes from North-west and North-east

S. No.	Plant Metabolites	Methanol extract (North-west)	n-Hexane extract (North-west)	Methanol extract (North-east)	n-Hexane extract (North-east)
1.	Phenolics	+	+	++	++
2.	Saponins	+++	++	+++	+++
3.	Steroids	+++	++	+++	+
4.	Terpenoids	+++	+++	+++	+
5.	Flavonoids	+	+	++	+
6.	Coumarins	+	-	++	++
7.	Tannins	+	-	++	-
8.	Carbohydrates	+++	+++	+++	+++
9.	Proteins	+	+	++	++

(+++ Present in large amount; ++ present in less amount; + present in traces; - absent)

carbohydrates are present in large amount while coumarins, tannins, proteins are present in traces in the studied rhizome extracts.

Total phenolic content

Phenolic compounds with redox properties are present in the plants and allow them to act as antioxidants [18]. High phenolic content was observed in the methanolic extract from rhizomes of North-west location (23.60 ± 0.01 mg GAE/g extract) as compared to North-east location which was 22.37 ± 0.015mg GAE/g extract. Similarly n-Hexane extracts of *C. zedoaria* rhizomes from both the locations North-west and North-east exhibited lowest amount of total phenolics (Table 2).

Total flavonoid content

Flavonoids are the low-molecular-weight secondary metabolites, rich in antioxidants and this activity is based on the number and position of free hydroxyl groups [19]. Highest flavonoid content was recorded in methanolic extract of rhizomes from North-west (63.81 ± 0.24 mg QE/g dry wt.) as compared to North-east (57.87± 0.40 mg QE/g dry wt.). However, n-Hexane extracts of North and North-east locations exhibited lowest flavonoid content (15.0 3± 0.51 mg QE/g dry wt. and 17.0 9 ± 0.57mg QE/g dry wt. respectively) (Table 2).

Total tannin content

Tannins are polyphenolic secondary metabolites present in higher plants that protect the cellular structures from oxidative effects by their enhanced synthesis [20]. The rhizomes of *Curcuma zedoaria* were recorded to have low content of tannins. The total tannins were high in the methanolic extract of rhizomes collected from North-west location (22.37 ± 0.015mg/g dry wt. extract) as compared to n-Hexane extract (21.61 ± 0.049 mg/g dry wt). However, tannin content was low both in methanolic and n-Hexane extracts of rhizomes from North-east location (Table 2).

***In vitro* antioxidant activity**

Radical scavenging activity in methanol and n-Hexane extracts of *Curcuma zedoaria* from North-west and North-east locations were studied for antioxidant activity using DPPH assay. Results revealed significant difference in antioxidant activity among the methanolic and n-Hexane extracts. Highest scavenging activity was recorded in the methanolic extract from rhizomes of North-west (236.5 ± 0.2 IC_{50}µg/ml), followed by rhizomes from North-east location. The scavenging activity in n-Hexane extracts from North-west and North-east location was 364.2±0.5 IC_{50}µg/ml and 330.1 ± 0.5 IC_{50}µg/ml respectively.

Table 2. Qualitative analysis of phytochemicals in methanol and n-Hexane extracts of *Curcuma zedoaria*

S. No	Extracts	Total Phenolics Content (µg GAE/g extract)	Total Flavonoid Content (mg QE/g extract)	Total Tanin Content (mg TAE/g extract)
1.	Methanol extract (North-west)	23.60±0.01	63.81±0.24	22.37±0.15
2.	n-Hexane extract (North-west)	21.61±0.04	15.0 3± 0.51	21.61±0.49
3.	Methanol extract (North-east)	22.37 ±0.05	57.87± 0.40	10.5 0 ±0.48
4.	n-Hexane extract (North-east)	21.60 ± 0.02	17.0 9 ±0.57	7.08 ± 0.52

Table 3. Comparison of antioxidant activity in the rhizome extracts of *Curcuma zedoaria* collected from North-west and North-east

Extracts	DPPH Assay (IC_{50}µg/ml)	FRAP Assay (µmol/g dry wt)
Methanol extract (North-west)	236.5 ± 0.21	51.3 ± 0.25
n-Hexane extract (North-west)	364.2 ± 0.53	18.2 ± 0.58
Methanol extract (North-east)	250.4 ± 0.24	60.7 ± 0.51
n-Hexane extract (North-east)	330.1 ± 0.56	31.1 ± 0.50

A strong antioxidant activity was observed in the methanolic extracts from both the locations which could be due to high content of flavonoids, phenolics and terpenoids. Rhizomes from North-east showed maximum Ferric reducing antioxidant potential (60.7 ± 0.5µmol/g dry wt. Fe^{2} reduced) as compared to rhizomes from North-west location (51.3 ± 0.2 µmol/g dry wt. Fe^{2} reduced). Also, in both the extracts, Fe^{2} reduced potential was more in methanolic extracts as compared to n-Hexane extracts (Table 3).

DISCUSSION

Plants are rich in metabolites and their production is regulated by various genetic and environmental factors and the primary role of these metabolites is plant defense and for humans, plants have long been used since ages as safe and effective sources of natural antioxidants [21,22].

There are many techniques to attain these metabolites from medicinal plants while extraction using various solvents is considered as the main process for isolating and recovering various phyto-constituents present in the plants. The effectiveness of extraction is subject to different solvents used with different polarities and at different pH, temperature and extraction time [17]. In the present study, *Curcuma zedoaria* extracts were prepared from the rhizomes collected from North-west and North-east locations using methanol and n-Hexane as solvents. Preliminary qualitative analysis revealed the presence of different metabolites in the extracts. However, from quantitative analysis of extracts phenolics were present in good amount in curcuma rhizomes. Similar results were observed by Rahayu [23] in rhizomes of *C. zedoaria* collected from Indonesia while studies on various *Curcuma* species collected from Japan, revealed highest phenolic content in the rhizomes of *C. longa* as compared to *C. aromatic* and *C. zedoaria* rhizomes [24]. Phenolics are considered as good antioxidants due to the presence of number of phenolic hydroxyls methoxy and carboxylic acid groups [25]. Several previous reports suggest presence of phenolics and flavonoids in the rhizomes of various *Curcuma* species collected from Malaysia, Thailand, Taiwan, India and hence it can be suggested that the content of phytochemicals in rhizomes is independent of geographical locations [26,27,28]. In the present study the content of flavonoids was also recorded high in the methanolic extracts from the rhizomes collected from North-west while tannin content was low. Since, tannins bind to dietary iron and prevent its absorption specifically of 'non-heme' iron present in plants [29]. So the presence of less tannins in zedoary rhizomes makes them better for human health consumption.

Also, biological properties of phenolics and flavonoids are due to their high antioxidant activity, as hydrogen donating free radicals. In normal cell metabolism, free radicals generated have one or more unpaired electrons as reactive oxygen species that reacts with free radicals [30]. Free radicals are mainly superoxide anion and hydroxyl radicals while H_2O_2 and the singled oxygen as non-free radicals. A significant amount of ferric reducing ability and free radical scavenging activity was observed in *C. zedoaria* rhizomes. Similarly, Rahman [31] also recorded and reported high antioxidant activity in the essential oils as compared to leaf extracts of *Curcuma zedoaria* while high antioxidant activity in *Curcuma leucorhiza* and *Hedychium rubrum* among various plants of *Zingiberaceae* family available in Manipur region were observed and reported [32]. Similar observation was made regarding high radical-scavenging activity and reducing power absorbance in more than eighty *Curcuma* species collected from Japan [33]. Hence, metabolites present in significant amount in the rhizomes of *C. zedoaria* are the main contributors for natural antioxidants in this plant species.

CONCLUSION

The present study reports the assessment and comparison of phytochemicals and antioxidant activity in the rhizomes of *C. zedoaria* collected from North-east and North-west location. High content of phenolics, flavonoids, and tannins were observed in methanolic extracts in comparison to n-Hexane extracts in rhizomes collected from North-west. The rhizomes showed good free radical scavenging activity and ferric reducing antioxidant potential. Presence of metabolites in significant amount in *C. zedoaria* rhizomes makes them a potential source of natural antioxidants that can play a pivotal role in preventing the diseases induced by oxidative stress.

ACKNOWLEDGMENTS

The authors are thankful to the NMPB, DST-FIST, RUSA, UGC-SAP, PURSE, Central Facility and DBT funded Bioinformatics Centre in School of Biotechnology for providing the basic facilities to carry out this research work.

Funding source

The work was supported by National Medicinal Plant Board (NMPB), (Project No. 18017/187/CSS/R&D/J&K-02/2017-18-NMPB-IV A), Government of India and Research and Seed Grant (RA/23/7204-11), University of Jammu, Jammu.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

Ritu Mahajan: Conceptualization, Project administration, Funding, Resources, Methodology, Supervision, Writing, review & editing; Shajaat Hussain: Experimentation, Data collection & analysis, Writing, review & editing; Nisha Kapoor: Writing, review & editing.

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