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Comparative Antioxidant and Antiradical Potentials of Four Curcuma species

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Abstract: Various species belonging to the genus Curcuma are widely utilised in Asian cuisine and medicine. Among the various Curcuma species, *Curcuma longa* has well-established therapeutic and antioxidant effects. This study evaluated and compared the antioxidant and antiradical properties of four Curcuma species commonly used in India. The methanolic extracts were tested for DPPH radical scavenging activity, total phenolic content, total flavonoid content, total antioxidant activity (phosphomolybdenum method and beta-carotene linoleate model), hydrogen peroxide scavenging, reducing power, and metal chelating ability using various in vitro assays. According to the study, *Curcuma zedoaria* has the strongest antioxidant capacity, whereas *Curcuma amada* has a high total flavonoid concentration and metal chelating ability. When tested using the phosphomolybdenum technique, *Curcuma malabarica* had the highest value for antioxidant activity.

Introduction

Reactive Oxygen Species can damage proteins and DNA and are known to be implicated in the process of ageing, cancer, diabetes, and atherosclerosis (1-3). Antioxidants found in herbs, fruits, as well as vegetables are known to limit the start and spread of oxidising chain reactions by scavenging the free radicals (4, 5). Many medicinal plants have been useful in traditional medicine, and they are regarded as good sources of natural antioxidants (6). Due to the harmful effects, including potential carcinogenicity (7), of some synthetic antioxidants on human enzymes, researchers are interested in finding safer natural antioxidants from plants for medical use. Several *in vitro* methods are available for assessing the radical scavenging capacities, serving as useful tools for evaluating the antioxidant activity of various compounds. These methods can provide valuable insights into the effectiveness of antioxidants and contribute to advancements in this field.

Many of the plants belonging to the *Zingiberaceae* family have been utilized as spices in culinary traditions and traditional medicine around the world. This extensive family of rhizomatous plants originated in Asia and the Far East and has been cultivated for millennia. It represents a vital natural resource, offering a wide array of products, including food, spices, medicines, dyes, and perfumes.

Curcuma is a significant genus within the Zingiberaceae family, with some of the most extensively researched species including *Curcuma longa*, *Curcuma aromatica*, *Curcuma zedoaria*, *Curcuma aeruginosa*, *Curcuma xanthorrhiza*, and *Curcuma comosa*. These species are known to produce compounds that exhibit both antioxidant properties and various other biological effects. Additionally, *Curcuma amada*, *Curcuma ecalcarata*, and *Curcuma malabarica* are three more species utilized for medicinal purposes or as food in India and other Southeast Asian countries.

C. amada is a direct food component, although the starches of *C. ecalcarata* and *C. malabarica* are widely utilised. *C. amada* , which thrives extensively in South India, is commonly known as “mango ginger”. It features light to pale yellow rhizomes that bear a taste reminiscent of fresh mango (8). The primary compounds contributing to this mango-like flavor are cis-ocimene and delta-3-carene, both of which are present in the essential oil derived from its rhizomes (9). In traditional medicine, *C. amada* rhizomes are effectively utilized for treating sprains, contusions, and digestive issues. Research conducted on Albino rats clearly demonstrates that the ethanol extract of these rhizomes possesses significant anti-inflammatory properties (10). Furthermore, the phytochemistry of the rhizomes has been well-documented, confirming their medicinal potential (11-14).

C. ecalcarata, indigenous to Kerala, is utilized for its small yellow rhizomes, which are processed to produce starch (15, 16). The phytochemical composition of *C. ecalcarata* has been documented in existing literature (17). *C. malabarica* features blue-white flesh and emits a camphor-like fragrance (18). Its cooling and soothing properties make it a popular source of starch in meals across South India, especially for newborns and convalescents (19). Reports have highlighted the antimicrobial properties of its extracts (20) along with its phytoconstituents (21).

The yellowish rhizomes of *C. zedoaria* are characterised by their aromatic, bitter, and strong scent. The plant is used to treat conditions such as piles, bronchitis, asthma, and tumours, and also it serves as an antipyretic (8). Furthermore, *C. zedoaria* starch is believed to have therapeutic properties (22). While the phytochemical components of *C. zedoaria* rhizomes have been discovered (23), there have only been initial investigations on their antioxidant activities (24).

Although the antioxidant activity of *C. longa* is well-established and some early research on *C. zedoaria* is known, the antioxidant capacities of the other three, which are widely utilised in food items, have not been comparatively assessed. A comparison of the different species is essential for identifying their potential and enabling their utilisation as functional foods, in dietary supplements and pharmaceuticals. Therefore, utilising a variety of *in vitro* models, the current work had been set out to assess and compare the antioxidant activity of methanol extracts of *C. amada, C. ecalcarata, C. malabarica,* and *C. zedoaria*.

Experimental Section

Materials

Tween 20 (pharmaceutical grade, Merck, Germany); butylated hydroxyanisole (BHA; analytical grade, Sigma-Aldrich, USA); linoleic acid (analytical grade, Sigma-Aldrich, USA); ferrozine (≥97% purity, Sigma-Aldrich, USA); β-carotene (analytical grade, Sigma-Aldrich, USA); 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (analytical grade, Sigma-Aldrich, USA); Folin–Ciocalteu's reagent (Sigma-Aldrich, USA); hydrogen peroxide (30% w/v, analytical grade, Merck, Germany); potassium ferricyanide (analytical grade, Merck, Germany); ammonium molybdate (analytical grade, Merck, Germany); ferrous chloride ($FeCl_2$, analytical grade, Merck, Germany); and ferric chloride ($FeCl_3$, analytical grade, Merck, Germany) were used in this study.

Extraction

Rhizomes of *C. amada* (CA), *C. ecalcarata* (CE), *C. malabarica* (CM), and *C. zedoaria* (CZ) were sourced from medicinal plant producers in Kottayam District, Kerala. The rhizomes were cut, dried, and pulverized. Each 50 g of powdered rhizomes was extracted with methanol (2 x 200 mL) at room temperature for 48 h to obtain the crude extracts after solvent removal. These were then diluted with methanol to prepare different sample concentrations for antioxidant activity studies (25).

Free Radical Scavenging Activity

The free radical scavenging was evaluated as per the standard procedure in literature (26-28). To put it briefly, DPPH was combined with sample solution at various concentrations and absorbance was measured at 515 nm after the reaction mixture had been well agitated and kept in the dark. A reduction in the absorbance of the DPPH radical was used to assess the sample's ability to scavenge free radicals. BHA served as a benchmark.

Total Phenolic Content and Total Flavonoid Content

To assess the total phenolic content of the methanol extracts of the three rhizomes Folin Ciocalteau's phenol reagent was utilised (29, 30). The reagent was combined with suitably diluted samples, and the mixture was kept for 5 min. The absorbance at 760 nm was then measured against a reagent blank after adding sodium carbonate and again incubated at room temperature for two h. Gram gallic acid equivalents per 100 g extract was used to express the results.

The Woisky *et al.* technique was used to calculate the total flavonoid content (31). In short, a flavonoid-aluminum complex was formed by reacting aluminium chloride solution with 1 mL of suitably diluted sample, which was then kept for 5 min. Then sodium hydroxide solution was added at the sixth min, and then thoroughly mixed again and its absorbance was measured at 415 nm against a blank after 30 min at room temperature. For the blank, distilled water was used instead of $AlCl_3$. Gram quercetin equivalents per 100 g of extract were used to represent the overall flavonoid concentration.

Total Antioxidant Activity by Phosphomolybdenum Reagent

Using Prieto *et al.*'s approach, the formation of phosphomolybdenum complex was used to measure the overall antioxidant activity (32, 33). Briefly, the reagent solution consisting of H_2SO_4, ammonium molybdate and sodium phosphate were mixed with sample. After incubating at 95 °C in a water bath for around one and half h, the absorbance was measured at 695 nm against the blank. Gram ascorbic acid equivalents per 100 g extract were used to express the antioxidant activity.

Scavenging of Hydrogen Peroxide

Using the Ruch *et al.'s* approach, the capacity of methanol extracts to scavenge hydrogen peroxide was calculated (34). H_2O_2 (20 mM) was made into a solution in phosphate buffer of pH 7.4. Spectrophotometric measurement of H_2O_2 concentration at 230 nm was performed using a molar extinction value 81 $M^{-1}cm^{-1}$. As per the standard procedure, required amount of H_2O_2 in phosphate buffer was combined with extracts at different concentrations, and the mixture was left for 10 min. The absorbance of reaction mixtures at 230 nm was measured against a blank solution that comprised sample and phosphate buffer without H_2O_2 in order to determine the quantity of H_2O_2. The reference utilised was BHA. The percentage of H_2O_2 scavenging in the sample was calculated.

Reducing Power

The Oyaizu technique was used to determine the reducing power of methanol extracts (35). Potassium ferricyanide and phosphate buffer were combined with varying quantities of the sample in distilled water. For 20 min, the mixtures were incubated at 50 °C. After adding trichloroacetic acid to the sample solutions, they were centrifuged for ten min. Then, ferric chloride was added to the supernatant solution, and the absorbance at 700 nm was measured. Greater reducing power was shown by the reaction mixture's higher absorbance.

Beta-carotene – Linoleate Model

Using BHA as a standard, the antioxidant activity of methanol extracts was assessed using Hidalgo's (36) beta-carotene bleaching technique. Linoleic acid, tween 20, and beta-carotene were placed in a flask with a round bottom. Then chloroform was removed, the resultant emulsion was added to the sample. After measuring the absorbance, the

$$DR = \frac{Ln(\frac{a}{b})}{60}$$

Equation 1 | where *DR* = degradation rate, *a* is the initial absorbance at 470 nm, and *b* is the absorbance at 60 min.

$$AA = \left[\frac{DR_{control} - DR_{sample}}{DR_{control}}\right] \times 100\%$$

Equation 2 | *AA* = antioxidant activity (%).

mixture was placed in a water bath (50 °C). The absorbance was again read at the 60th minute. **Equation 1** was used to calculate the degradation rate. Antioxidant activity was then expressed as the percentage of inhibition relative to the control using **Equation 2**.

Metal Chelating Ability

Dinis *et al.*'s (37) approach was used to test the capacity of the methanol extracts to chelate ferrous ions. To summarise, sample of varied concentrations in methanol was treated with ferrous chloride followed by addition of ferrozine. After ten min, the absorbance was measured against a blank at 562 nm.

Statistical Analysis

All the experimental results are expressed as mean ± standard deviation (SD) of triplicate measurements. The results were processed using Microsoft Excel and Origin. A p value of <0.05 had been considered to be statistically significant.

Results and Discussion

Among the four different species of Curcuma rhizomes, the percentage yield of the extracts was obtained to be 7.24 % for *C. amada*, 6.98 % for *C. malabarica*, 6.13 % for *C. ecalcarata* and 8.32 % for *C. zedoaria*.

Assessing antioxidant activity of phytochemicals using a single technique in plant extracts is challenging because of the complex structure of the phytochemicals in them. As a result, several assays are employed for the assessment.

DPPH radical has been frequently utilised to assess the capacity of different natural products to scavenge free radicals (26). In order to provide a stable end product that prevents additional oxidation of the lipid, the antioxidants donate a H-atom from the phenolic OH-groups thereby stopping the free radical chain of oxidation (38). In comparison to other approaches, this approach requires a very short time. A persistent organic nitrogen radical, DPPH exhibits a UV lambda max of 515 nm in methanol. Since the change of colour is from purple to light yellow following reduction, a spectrophotometer may be used to track the progress of the reaction (39).

The reduction in absorbance corresponds to the concentration and antioxidant activity of the investigated samples. The EC_{50} value which is presented in **Table 1** was calculated from the graph which plotted percentage of scavenging activity against extract concentration. The efficiency of antioxidant capability is inversely proportional to their EC_{50} values. The results obtained (**Figure 1**) show that the radical scavenging activity is much lower than the synthetic antioxidant BHA; however, when the four *Curcuma* species were compared, CZ (EC_{50}: 2.38 ± 0.06 g/L) was determined to be a more effective radical scavenger than CM (EC_{50}: 5.08 ± 0.05 g/L), CA (EC_{50}: 5.57 ± 0.16 g/L), and CE (EC_{50}: 6.95 ± 0.17 g/L).

The Folin-Ciocalteau technique was used to determine the total phenolic content. A coloured product with a lambda max at 750 nm is produced when phenols are subjected to oxidation using a molybdotungstate reagent. According to the results, which were reported as g gallic acid equivalents per 100 g extract, CZ had a greater phenolic content than CA, CE, and CM. An estimated 7.33 ± 0.42 g, 7.79 ± 0.11 g, 8.77 ± 0.27 g, and 16.07 ± 0.30 g gallic acid equivalents were the total phenolic content of each 100 g of dried extracts of CA, CE, CM and CZ, respectively.

Aluminium chloride reagent quantified the total flavonoid content. It was calculated and presented as equivalents of quercetin. An estimated 5.96 ± 0.41 g, 3.49 ± 0.13 g, 5.28 ± 0.07 g, and 0.24 ± 0.01 g of quercetin equivalents were found in each 100 g of dried extracts of CA, CE, CM, and CZ. The data demonstrated that all four species had relatively low levels of flavonoids.

Table 1 displays the antioxidant activity of CA, CE, CM, and CZ methanol extracts at various doses. The green Mo (V) complex, which has a maximum absorbance at 695 nm, is formed when antioxidants reduce Mo (VI) to Mo (V) (32). This process is the basis of the phosphomolybdenum technique wherein the antioxidants contribute a hydrogen atom.

Gram ascorbic acid equivalents per 100 g of extract expressed the antioxidant activity. The results were suggestive that CM had higher antioxidant activity (47.94 ± 1.98 g AAE/100 g extract) than CZ (41.55 ± 1.19 g AAE/100 g extract), followed by CA (32.94 ± 0.54 g AAE/100 g extract) and CE (30.71 ± 2.10 g AAE/100 g extract).

Hydrogen peroxide which is an unstable metabolic product, is the source of singlet oxygen and hydroxyl radicals. These are produced by the Fenton reaction which

Table 1. Scavenging of DPPH radical, TPC, TFC and total AA of the rhizomes of *C. amada*, *C. ecalcarata*, *C. malabarica*, and *C. zedoaria*.

Extract	Scavenging of DPPH radical (EC_{50} (g/L))	Total phenolic content[a] (g/100 g)	Total flavonoid content[b] (g/100 g)	Total antioxidant activity[c] (g/100 g)
C. amada	5.57 ± 0.16	7.33 ± 0.42	5.96 ± 0.41	32.94 ± 0.54
C. ecalcarata	6.95 ± 0.17	7.79 ± 0.11	3.49 ± 0.13	30.71 ± 2.10
C. malabarica	5.08 ± 0.05	8.77 ± 0.27	5.28 ± 0.07	47.94 ± 1.98
C. zedoaria	2.38 ± 0.06	16.07 ± 0.30	0.24 ± 0.01	41.55 ± 1.19

Note: [a]Expressed as gram of gallic acid equivalents per 100 g extract, [b]Expressed as gram of quercetin equivalents per 100 g extract, and [c]Expressed as gram of ascorbic acid equivalents per 100 g extract.

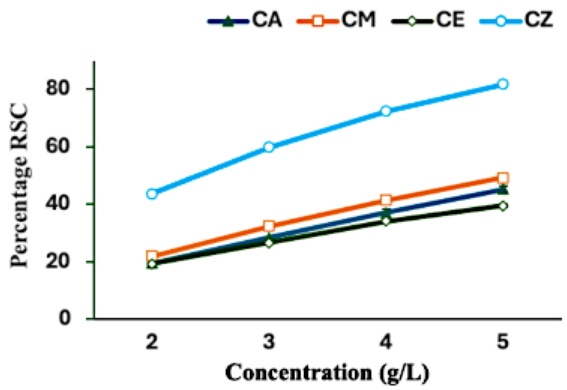

Figure 1. DPPH scavenging percentage of Curcuma extracts. CA = *Curcuma amada*, CE = *Curcuma ecalcarata*, CM = *Curcuma malabarica*, and CZ = *Curcuma zedoaria*.

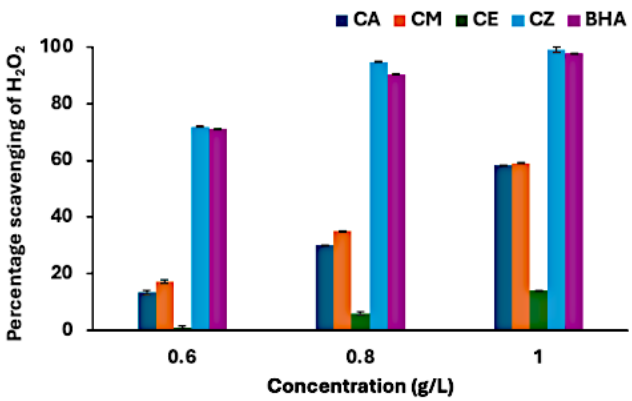

Figure 2. Percentage scavenging of hydrogen peroxide of extracts at various concentrations. CA = *Curcuma amada*, CE = *Curcuma ecalcarata*, CM = *Curcuma malabarica*, CZ = *Curcuma zedoaria*, and BHA = butylated hydroxyl anisole.

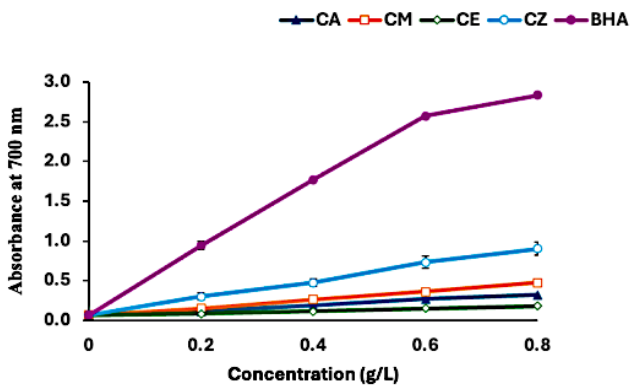

Figure 3. Reducing power of extracts at various concentrations. CA = *Curcuma amada*, CE = *Curcuma ecalcarata*, CM = *Curcuma malabarica*, CZ = *Curcuma zedoaria*, and BHA = butylated hydroxyl anisole.

thereby start lipid peroxidation, and are harmful to cells. Reactive oxygen species and hydrogen peroxide can harm a number of cellular constituents. Therefore, hydrogen peroxide elimination is crucial for the antioxidant defence of cells (40). All four extracts scavenged the hydrogen peroxide radical according to their dosages, as illustrated in **Figure 2**. The EC_{50} value of CZ (EC_{50}: 0.51 ± 0.01 g/L) was found to be superior than CM (EC_{50}: 0.92 ± 0.001 g/L) and CA (EC_{50}: 0.94 ± 0.002 g/L), and it was almost equivalent to that of BHA (EC_{50}: 0.47 ± 0.01 g/L). Since the EC_{50} value for CE could not be ascertained, it was identified to be a very poor hydrogen peroxide scavenger. BHA > CZ > CM > CA > CE was the order of the percentage of hydrogen peroxide scavenging.

Compounds which are associated with reducing properties show antioxidant activity by the donation of a H-atom to interrupt the chain of free radicals. Such species, reduces Fe^{3+}/Ferricyanide complex to the Fe^{2+} form in this assay. Perl's Prussian blue colour production at 700 nm can be used to track this process (41). The information obtained showed that CZ was a better reductant than CA, CE, and CM. The increased absorbance at 700 nm was found to vary with dosage. The findings are displayed in **Figure 3**. At concentrations between 0.2 and 0.8 g/L, the reducing power was in the following order: CZ > CM > CA > CE.

Using an aqueous emulsion system heat-induced oxidation can be used as a test for antioxidant activity. Linoleic acid together with beta-carotene can be used for the assay. When the antioxidant is absent, beta-carotene quickly discolours (42). The free radicals generated within the system are neutralised by a phenolic antioxidant, which minimises the amount of beta-carotene degradation. BHA was employed as a standard in this experiment. **Figure 4** displays the results of stronger antioxidant activity of CZ (99.2 ± 4.7 %) at a concentration of 0.2 mg of sample compared to BHA (91.8 ± 4.0 %), CA (44.3 ± 4.9 %), CE (50.4 ± 4.1 %), and CM (63.7 ± 4.6 %). Most remarkably, CZ was shown to neutralise the free radicals in the system and prevent α-carotene from bleaching, suggesting that the methanol extract of CZ contained more antioxidants.

Of the several types of metal ions, the Fe^{2+} ion is the most potent pro-oxidant. Transition metals are recognised to initiate and propagate lipid peroxidation. By stabilising transition metals, chelating compounds can prevent lipid oxidation (43). In this assay, ferrozine can combine with Fe^{2+} to form complexes. The red colour of the complex reduces when additional chelating agents are present because they interfere with the formation of the complex. There is a dose-dependent linear reduction in the absorbance of the ferrozine-Fe^{2+} complex. The extract can minimise the

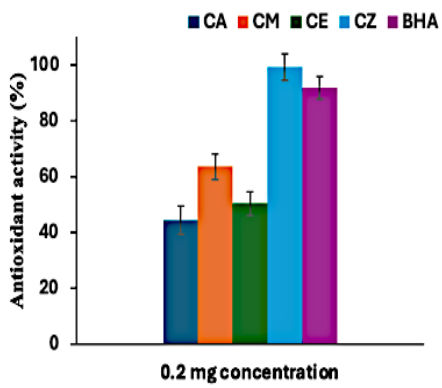

Figure 4. Percentage antioxidant activity of extracts at 0.2 mg concentration. CA = *Curcuma amada*, CE = *Curcuma ecalcarata*, CM = *Curcuma malabarica*, CZ = *Curcuma zedoaria*, and BHA = butylated hydroxyl anisole.

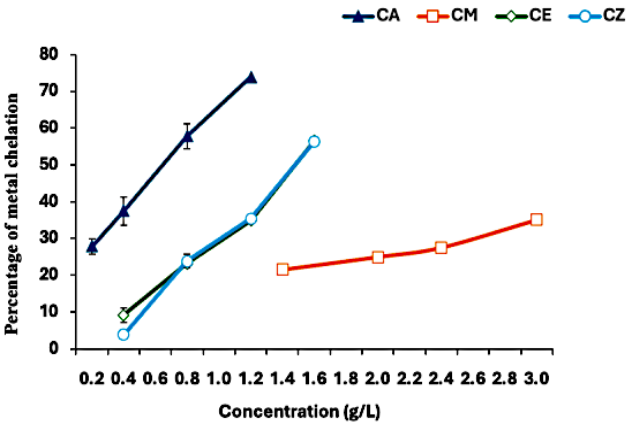

Figure 5. Percentage of metal chelation of extracts at various concentrations. CA = *Curcuma amada*, CE = *Curcuma ecalcarata*, CM = *Curcuma malabarica*, and CZ = *Curcuma zedoaria*.

superoxide-driven Fenton reaction which is the primary pathway to the production of active oxygen species, by lowering the concentration of the transition metal if it chelates with Fe^{2+}. **Figure 5** illustrates how the three extracts (CA, CE, and CZ) disrupted the formation of the ferrous as well as ferrozine complex in this test. This confirms that the extracts have chelating activity and can bind to ferrous ions, which means they may be employed as an efficient treatment to slow down Fe^{2+}-catalyzed lipid oxidation. It was discovered that CM was a poor metal chelator. Chelation occurs in the following order: CA > CE = CZ > CM since the EC_{50} value of CA (EC_{50}: 0.64 ± 0.017 g/L) was greater than CE (EC_{50}: 1.44 ± 0.012 g/L) and CZ (EC_{50}: 1.44 ± 0.020 g/L), and CM (EC_{50}: 4.88 ± 0.024 g/L).

Conclusion

The antioxidant capacity of the extracts of four *Curcuma* species was assessed and compared in this study using a variety of *in vitro* assay techniques. The current study provides information on the antioxidant capacity of methanol extracts of the four culinary and medicinal species of the genus *Curcuma*. According to the study, *C. zedoaria* has strong antioxidant activity, particularly because of its high phenolic content, reducing power, and ability to effectively scavenge DPPH radicals. Furthermore, *C. zedoaria* extract has a similar capacity to scavenge hydrogen peroxide as synthetic antioxidant BHA, and it prevents beta-carotene bleaching more efficiently. This study also demonstrates that *C. amada*, which is directly utilised in food preparation, has strong metal chelating activity and a high total flavonoid concentration. In some experiments, *C. malabarica* extract outperforms *C. zedoaria* extract and also shows strong antioxidant capabilities. Among the various species, it has the greatest antioxidant activity value when measured using the phosphomolybdenum technique. It was confirmed that *C. zedoaria* and *C. ecalcarata* extracts were equally efficient in metal chelation. This study concludes that all four *Curcuma* species have antioxidant properties useful for the preparation of pharmacological and nutraceutical products which helps to reduce oxidative cell damage and improve health. Ongoing *in vitro* studies will confirm their potential health benefits.

Declarations

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

Not applicable.

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Additional Information

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