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Research Article

Phenolic content and antioxidant activity in five underutilized starchy *Curcuma* species

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ABSTRACT

Rhizomes and leaves of five underutilized *Curcuma* species viz. *C.aeruginosa, C.brog, C.malabarica, C.rakthakanta* and *C.sylvatica* were evaluated for total phenolic content, flavonoids and antioxidant activity. The total phenols in methanolic extracts of rhizomes ranged from 210 to 700 mg gallic acid equivalents/100g and in leaves from 840 to 1480 mg/100g. Flavonoid content in leaves ranged from 270 to 380 mg epicatechin equivalents/100 g dw. The leaves of all species had higher content of phenolics, DPPH radical scavenging activity and ferric reducing power as compared to rhizomes. There was good correlation between the phenol content and antioxidant activity in rhizomes, but not in leaves. The results of the study highlighted the potential of these unutilized *Curcuma* species (rhizomes and leaves) as a rich source of antioxidants for food and health.

KEY WORDS: *Curcuma* species; rhizomes; leaves; phenols; flavonoids; antioxidant activity

INTRODUCTION

The genus *Curcuma*, a member of the Zingiberaceae family, comprises of 80 species, some of which have been used in traditional systems of medicine (Ayurveda, Siddha, Unani) for a long time. Among them the most studied is *C.longa* which is known to possess tremendous therapeutic potency [1] .The medicinal properties of *C. longa* have been attributed to the presence of curcumin, essential oils and phenolics. *C.xanthorrhiza* and *C.zedoaria* also possess anti-inflammatory and antimicrobial properties and are used in traditional medicine. The rhizomes contain essential oils, phenolics, polysaccharides and small amounts of curcumin. Several lesser known *Curcuma* species include *C.aeruginosa, C.amada, C.aromatica, C.brog, C.caesia, C.malabarica, C.rakthakanta* and *C.sylvatica*. These species produce starchy rhizomes which are used as remedies for infections, inflammations, gastric and skin disorders but have not been evaluated scientifically for pharmacological activity. The rhizomes are aromatic but do not contain curcumin.

Curcuma plants (rhizomes and leaves) have a camphoraceous aroma and contain many functional compounds such as volatile oils, terpenes, phenolics and flavonoids, which are strong antioxidants. Phenolics and flavonoids possess pharmacological activity (anticarcinogenic, anti-inflammatory properties) due to their radical scavenging activity and lipid antiperoxidation effects [2,3]. Since free radicals are the cause for several major disorders, evaluation of antioxidant compounds/ activity in plants could result in the discovery of natural antioxidants with pharmacological and food value. The importance of phenolic compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known [4, 5]. Studies on the phenolic content and antioxidant properties of *Curcuma* rhizomes are limited to a few species such as *C. longa, C. zedoaria* and *C. xanthorrhiza* [6, 7]. Among the non curcumin species, *C. amada* and *C. caesia* were found to possess good antioxidant potential [8]. The present study evaluated the total phenols, flavonoids and antioxidant activity in rhizomes and leaves of five underutilized *Curcuma* species (*C.aeruginosa, C.brog, C.malabarica, C.rakthakanta* and *C.sylvatica*) in order to explore their pharmacological potential.

MATERIALS AND METHODS

The species (*C.aeruginosa, C.brog, C.malabarica, C.rakthakanta* and *C.sylvatica* were collected from the National Bureau of Plant Genetic Resources (Regional Station) Trichur, Kerala and maintained at Central Tuber Crops Research Institute, Trivandrum. The upper leaves were collected and air dried at room temperature while rhizomes were cut into small pieces and dried under similar conditions for 48h .The dried samples were ground to a fine powder.

Chemicals: Folin Ciocaltaeu reagent, gallic acid, epicatechin and 2, 2, Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Chemical Co. (St Louis MO) chemicals. All other chemicals used were of analytical grade.

Preparation of rhizome/leaf extracts: Methanolic extracts of rhizomes (500 mg) and leaves (250 mg) were prepared by extraction of dry samples thrice with methanol at 60^{o} C. Extracts were centrifuged at 10,000xg, filtered

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Table 1. Phenolics and antioxidant activity in methanol extracts of rhizomes [a]

Species	Total phenol content (mg GAE/100 g)	Antioxidant activity	
		DPPH scavenging activity IC_{50} (mg)	Ferric reducing power EC_{50} (mg)
C. aeruginosa	700 ± 2.3 d	0.64 ± 0.006 a	2.3 ± 0.10 a
C. brog	480 ± 3.2 c	1.9 ± 0.10 b	4.6 ± 0.10 b
C. malabarica	210 ± 3.5 a	4.2 ± 0.20 e	9.4 ± 0.15 d
C. rakthakanta	340 ± 1.7 b	2.2 ± 0.06 c	6.6 ± 0.10 c
C. sylvatica	210 ± 4.0 a	3.0 ± 0.06 d	10.6 ± 0.20 e

[a] Values (expressed on dry weight basis) are the mean of triplicate analysis ± standard deviation. Mean values followed by different letters in a column are significantly different ($p \leq 0.05$).

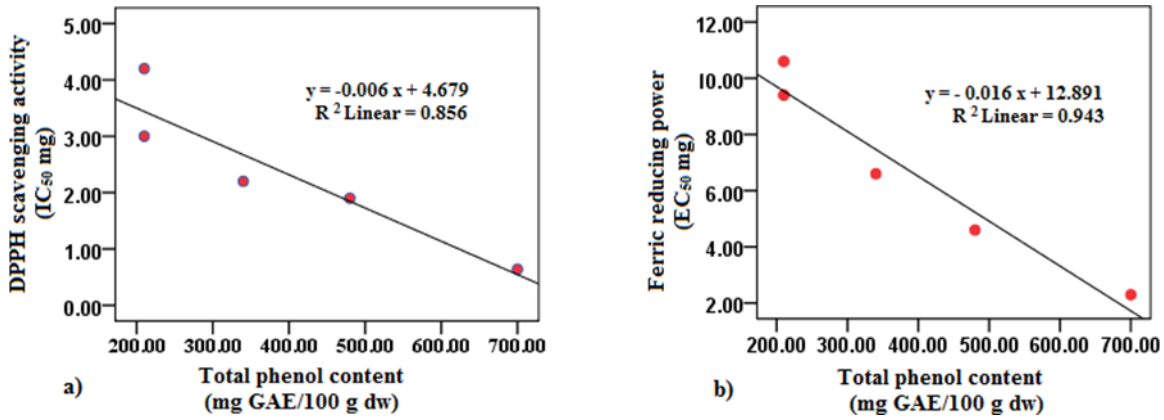

Fig.1. Correlation between total phenol content and a) DPPH (IC_{50}) and b) Ferric reducing power (EC_{50}) of methanolic extracts of rhizomes

Table 2. Phenolics, flavonoids and antioxidant activity in methanol extracts of leaves [b]

Species	Total phenol content (mg GAE/ 100 g)	Flavonoids (mg ECE/ 100 g)	Antioxidant Activity	
			DPPH scavenging power IC_{50} (mg)	Ferric Reducing Power EC_{50} (mg)
C. aeruginosa	920 ± 5.5 c	300 ± 2.5 c	1.5 ± 0.06 b	1.9 ± 0.10 c
C. brog	1480 ± 7.5 d	380 ± 1.5 e	1.1 ± 0.06 a	1.2 ± 0.10 a
C. malabarica	840 ± 3.0 a	320 ± 1.7 d	1.1 ± 0.06 a	1.4 ± 0.06 a, b
C. rakthakanta	880 ± 6.50 b	270 ± 2.1 a	1.5 ± 0.20 b	1.5 ± 0.10 b
C. sylvatica	920 ± 9.0 c	280 ± 3.1 b	1.1 ± 0.06 a	1.6 ± 0.02 b

[b] Values (expressed on dry weight basis) are the mean of triplicate analysis ± standard deviation. Mean values followed by different letters in a column are significantly different ($p \leq 0.05$).

through Whatman 1 chromatography paper and made up to a fixed volume with methanol.

Determination of total phenolics and flavonoids: Total phenols were determined by the Folin Ciocaltaeu procedure using gallic acid as standard [9] Aliquots of the extracts were mixed with Folin-Ciocalteau reagent for 5 min, 7% Na_2CO_3 was then added and final volume made up to 25 ml with distilled water. After 90 min the absorbance was measured at 750 nm. Phenolic content was expressed as mg gallic acid equivalents (GAE)/100 g tissue. Total flavonoids in leaf were determined by the method of Kim et al using epicatechin as standard [10.] To aliquots of the extracts was added 4 ml distilled water, followed by 0.3 ml of 5% $NaNO_2$, 10 % $AlCl_3$ and 2 ml 1M NaOH. The solution was made upto 10ml using deionized water and the absorbance was measured at 510 nm. Flavonoid content was expressed in terms of mg epicatechin equivalents (ECE) /100 g tissue

Determination of antioxidant activity: DPPH free radical scavenging activity- The DPPH radical scavenging activity was measured according to the method of Chung et al, 2002 [11]. Aliquots of tuber and leaf extracts were mixed with 1.2 ml of 80 µm DPPH in the presence of Tris buffer (1 M, pH 7.9). The reaction system was incubated in the dark for 20 min and decrease in absorbance at 517 nm was measured. Controls were run with DPPH and methanol, without addition of extract, and with extracts alone. The half-inhibition concentration values (IC_{50}), the tissue concentration at which the inhibition of DPPH radical is 50%, were calculated.

Reducing power: The ferric reducing power of the extracts was determined by the method of Duh et al [12]. Aliquots of extracts were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%) and incubated at 50°C for 20 min. Trichloroacetic acid solution (2.5 ml of 10 % TCA) was added to the

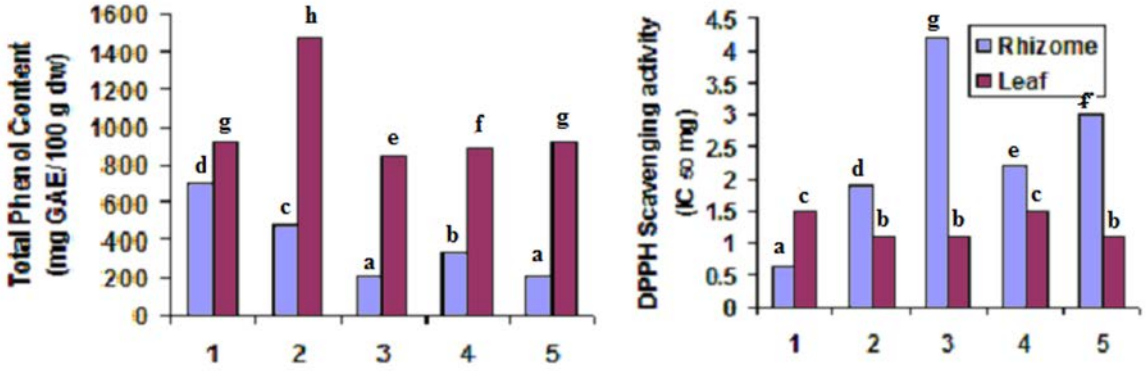

Fig.2. Phenol content and DPPH scavenging activity in rhizomes and leaves of *Curcuma* species (1-*C.aeruginosa*, 2 - *C.brog*, 3- *C.malabarica*, 4- *C.rakthakanta*, 5 - *C.sylvatica*). Values are means of three replicates. Values in each column with different letters are significantly different at $p<0.05$.

reaction mixture at room temperature. After centrifugation at 1000 g for 10 min, 2.5 ml of upper layer was mixed with equal volume of distilled water and 0.5 ml ferric chloride (0.1%).The absorbance at 700 nm was measured, increase in absorbance indicated increase in antioxidant activity and reducing power. The EC_{50} values (the effective tissue concentration at which the A700 of the Prussian blue complex is 0.5) of the extracts were determined.

STATISTICAL ANALYSIS

The data was expressed as the mean ± standard deviation (SD) of triplicates and then analysed using SPSS.17 (SPSS Inc. Chicago, Illinois, USA). One- way analysis of variance (ANOVA) and Duncans multiple range test ($p<0.05$) were used to determine the significance of the difference between means. Linear regression analysis was performed correlating antioxidant activity and phenol content.

RESULTS AND DISCUSSION

Rhizomes: Total phenol content (TPC)- The total phenol content in methanol extracts of the rhizomes of the five species was determined and found to range from 210 to 700 mg GAE/100 g (Table 1). *C. aeruginosa* had the highest phenolic content followed by *C. brog* and *C. rakthakanta*. The species *C. malabarica* and *C. sylvatica* had lower phenol content. Flavonoids were not detected in methanol extracts of *Curcuma* rhizomes.

Antioxidant activity- The antioxidant potential of the extracts was determined by measuring the DPPH scavenging activity and ferric reducing power. Rhizomes of *C.aeruginosa* showed highest DPPH free radical scavenging activity (IC_{50} 0.64)) followed by *C.brog* and *C. rakthakanta* (IC_{50} 1.9 and 2.2 respectively). Lower activity was observed in *C.sylvatica* and *C.malabarica* extracts. As in the case of DPPH scavenging activity, methanolic extracts of *C.aeruginosa* showed the highest iron reducing power (EC_{50} 2.3) followed by *C. brog and C. rakthakanta* (4.6 and 6.6) whereas *C.malabarica* and *C.sylvatica* had lowest value of reducing power.

Analysis of variance indicated significant differences in phenol and antioxidant activity between the species. Among the five species, *C. aeruginosa* rhizomes contained highest phenols and also exhibited the highest antioxidant activity. The correlation between total phenol content and antioxidant activity was analysed. Rhizome extracts with higher DPPH and Ferric reducing power had a higher phenol content and showed good correlation (Fig 1).

Leaves: Total phenol and flavonoid content- Total phenol content in methanol extracts of leaves ranged between 840 to 1480 mg GAE/100 g dw (Table 2). *C.brog* had highest phenol content 1480 mgGAE/100g) followed by *C.aeruginosa* and *C. sylvatica* (>900mg) and *C. malabarica* and *C. rakthakanta* the lowest content (< 900 mg /100g). Flavonoid content in leaves of the different species ranged from 270 to 380 mg ECE /100g (Table 2) and constituted approximately 25-38% of the total phenolics. There was good correlation between total phenols and flavonoids ($R^2 = 0.744$).

Antioxidant activity: Methanol extracts of leaves had IC_{50} (DPPH) values ranging from 1.1 to 1.5. *C.brog*, *C.malabarica* and *C.sylvatica* showed higher antioxidant activity compared to *C. aeruginosa* and *C. rakthakanta*. The DPPH scavenging activity was monitored at different concentration of extracts and found to increase linearly with increase in concentration of the extracts (data not shown). The ferric reducing power of leaf extracts of different *Curcuma* species had EC_{50} values ranging from 1.2 to 1.9 mg. *C.brog* had the highest iron reducing power and *C.aeruginosa* the lowest. The variation in reducing power among the other species was not significant.

Analysis of variance indicated significant differences in phenol but less significance in antioxidant activity between the different species. There was no correlation between the phenolic / flavonoid content in leaf extracts and the antioxidant activity as measured by both DPPH scavenging (R^2 = 0.137, 0.274) and ferric reducing activity (R^2 = 0.376, 0.413).

The content of total phenolics in leaves was two to five times higher than rhizomes in most of the species. The antioxidant activity in methanol extracts of leaves was also higher than rhizomes, with the exception of DPPH scavenging activity in *C.aeruginosa* rhizomes. Leaf

extracts also showed higher ferric reducing power as compared to rhizomes (Fig 2). A major difference between rhizomes and leaves was that there was good correlation between the phenol content and antioxidant activity in rhizomes, but not in leaves. This indicated that phenols were the main contributors of antioxidant activity in rhizomes, which may not be the case in leaves.

The rhizomes of the Zingiberacea family are known for their medicinal properties. Among *Curcuma* species, *C. longa* or turmeric is the most widely studied, both in terms of its pharmacological activity and phytochemical composition. Limited studies have been carried out in the other *Curcuma* species [7, 8, 13 - 16]. The essential oils from *C.aromatica* and *C. zedoaria* also possessed strong antifungal properties and methanol extracts of *C.zedoaria* inhibited COX-2 and NO production [17, 18]. The medicinal properties of species such as *C. longa, C. xanthorrhiza, C.zedoaria* etc is attributed to the presence of curcumin, which also contributes to the higher phenols and antioxidant activity [19, 20]. Rhizomes of unconventional species such as *C. amada* and *C. caesia*, have been shown to possess high antioxidant potential. A comparative study revealed that *C. ceasia* was superior to *C. amada* in terms of phenols and antioxidant activity [8]. The present studies focused on the evaluation of five underutilized *Curcuma* species for their phenolics and antioxidant activity species, in order to explore their potential for pharmacological applications. The studies revealed that the rhizomes of the five species were comparable or superior in terms of phenolic content, radical scavenging activity and reducing power to other non curcumin species (*C. aromatica, C. amada, C.caesia*). Our studies showed that in addition to rhizomes, the leaves of all five species contained high levels of phenols and antioxidant activity, which can therefore contribute to their pharmacological value. There was good agreement between the phenol content and antioxidant activity in rhizomes, as observed in the case of several other plants [21-23]. The beneficial effects of plant phenolics are mainly attributed to their antioxidant properties. They prevent the oxidation of LDL-lipoprotein, platelet aggregation, and damage of RBC and free radical scavenging activity [2, 24]. Since the chemical structures of phenolic compounds determine their properties, identification of the nature of the phenolic compounds in the different *Curcuma* species would be useful for further evaluation.

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