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°C. Extracts were centrifuged at 10,000xg, filtered

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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 Ciocalteau procedure using gallic acid as standard. Aliquots of the extracts were mixed with Folin-Ciocalteau reagent for 5 min, 7% Na2CO3 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. To aliquots of the extracts was added 4 ml distilled water, followed by 0.3 ml of 5% NaNO2, 10% AlCl3 and 2 ml 1M NaOH. The solution was made up to 10 ml 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 was measured according to the method of Chung et al., 2002. 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 (IC50), 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. 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

### Table 1. Phenolics and antioxidant activity in methanol extracts of rhizomes

<table>
<thead>
<tr>
<th>Species</th>
<th>Total phenol content (mg GAE/100 g)</th>
<th>Antioxidant activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DPPH scavenging activity IC50 (mg)</td>
</tr>
<tr>
<td>C. aeruginosa</td>
<td>700 ± 2.3 d</td>
<td>0.64 ± 0.006 a</td>
</tr>
<tr>
<td>C. brog</td>
<td>480 ± 3.2 c</td>
<td>1.9 ± 0.10 b</td>
</tr>
<tr>
<td>C. malabarica</td>
<td>210 ± 3.5 a</td>
<td>4.2 ± 0.20 e</td>
</tr>
<tr>
<td>C. rakthakanta</td>
<td>340 ± 1.7 b</td>
<td>2.2 ± 0.06 c</td>
</tr>
<tr>
<td>C. sylvatica</td>
<td>210 ± 4.0 a</td>
<td>3.0 ± 0.06 d</td>
</tr>
</tbody>
</table>

*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≤ 0.05).

### Table 2. Phenolics, flavonoids and antioxidant activity in methanol extracts of leaves

<table>
<thead>
<tr>
<th>Species</th>
<th>Total phenol content (mg GAE/100 g)</th>
<th>Flavonoids (mg ECE/100 g)</th>
<th>Antioxidant Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>DPPH scavenging activity IC50 (mg)</td>
</tr>
<tr>
<td>C. aeruginosa</td>
<td>920 ± 5.5 c</td>
<td>300 ± 2.5 c</td>
<td>1.5 ± 0.06 b</td>
</tr>
<tr>
<td>C. brog</td>
<td>1480 ± 7.5 d</td>
<td>380 ± 1.5 e</td>
<td>1.1 ± 0.06 a</td>
</tr>
<tr>
<td>C. malabarica</td>
<td>840 ± 3.0 a</td>
<td>320 ± 1.7 d</td>
<td>1.1 ± 0.06 a</td>
</tr>
<tr>
<td>C. rakthakanta</td>
<td>880 ± 6.50 b</td>
<td>270 ± 2.1 a</td>
<td>1.5 ± 0.20 b</td>
</tr>
<tr>
<td>C. sylvatica</td>
<td>920 ± 9.0 c</td>
<td>280 ± 3.1 b</td>
<td>1.1 ± 0.06 a</td>
</tr>
</tbody>
</table>

*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≤ 0.05).
Antioxidant activity - The antioxidant potential of the methanol extracts of Curcuma species was determined by measuring the DPPH scavenging activity and ferric reducing power. Rhizomes had the highest scavenging activity (IC50 0.64) followed by C. rakthakanta. The variation in antioxidant activity and reducing power among the five species was not significant. 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.

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 (IC50 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 (EC50 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 mg (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 (R2 = 0.744).

Antioxidant activity: Methanol extracts of leaves had IC50 (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 EC50 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 (R2 = 0.137, 0.274) and ferric reducing activity (R2 = 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 Zingiberaceae 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 Curcuma 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. caesia 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 pharmaceutical 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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REFERENCES

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