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Research Article

ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM NINE STARCHY *CURCUMA* SPECIES

ANGEL GR, VIMALA B AND BALA NAMBISAN

Central Tuber Crops Research Institute Sreekariyam, Thiruvananthapuram - 695017, India. Email: balanambisan@yahoo.co.uk

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ABSTRACT

The essential oils of nine starchy *Curcuma* species were isolated from the rhizomes by hydrodistillation and analysed for total phenols, DPPH scavenging activity and reducing power. Antibacterial activity was determined against *Bacillus subtilis, Staphylococcus aureus* and *E.coli*. The results showed that total phenolic content in the oils ranged from 4 – 83 µg gallic acid equivalents (GAE) /µl oil, the highest phenol content being present in *C. zedoaria*, *C. brog* and *C. caesia*, and lowest in *C. amada* and *C. sylvatica.* Most of the oils possessed high antioxidant activity, which was moderately correlated with phenolic content. All oils exhibited antibacterial activity and inhibited both g +ve (*S. aureus* and *B. subtilis*) and g-ve (*E. coli*) bacteria. *C.aeruginosa, C.amada* and *C.aromatica* oils produced maximum inhibitory activity against all the bacteria tested. Most of these *Curcuma* species are used in traditional medicine for their bactericidal and anti-inflammatory properties. Many are underutilized and there is tremendous scope for utilization of their essential oils as pharmaceutical and food additives.

Keywords: *Curcuma* species, Essential oil, Phenols, Antioxidant and Antibacterial activity.

INTRODUCTION

The genus *Curcuma* (family Zingiberaceae) comprises of more than 80 species of rhizomatous herbs. They occur in wild and cultivated forms and are widely distributed throughout the tropics of Asia, Africa and Australia. The most common species is *C. longa* or turmeric, which is used as a natural food colourant and as an ingredient in various medicinal formulations. The rhizomes of other *Curcuma* species (*C. aeruginosa, C. amada, C. aromatica, C. brog, C. caesia, C. malabarica, C. rakthakanta, C. sylvatica* and *C. zedoaria*) are also pharmacologically important but several of these species have not been exploited commercially. The rhizomes and leaves of most of the *Curcuma* species are aromatic, indicating the presence of volatiles/essential oils. Essential oils are commercially important plant volatiles employed extensively in pharmaceutical, flavouring and perfumery industries and possess a wide range of pharmacological properties. The essential oil of *C. longa* has been well studied and reported to contain ar-turmerone, turmerone, turmerol and zingiberene as the major constituents. Essential oils from *C. longa* and *C. zedoaria* possess antioxidant, antimicrobial, anti-inflammatory and cytotoxic properties [1-6]. Most of the other tuberising *Curcuma* species produce aromatic rhizomes which are rich in essential oils varying in chemical constituents but which remain unexplored for their pharmacological properties. Studies on their biological activity would be beneficial in medicinal applications. In the present study essential oils from nine rhizomatous *Curcuma* species, (which included several underutilized species), were isolated and evaluated for phenolic content and antioxidant power. The inhibitory activity of these oils against bacteria was determined, and the bioactivities of oils from different species were compared.

MATERIALS AND METHODS

Plant material

Nine tuberising *Curcuma* species namely *C. aeruginosa, C.amada, C. aromatica, C. brog, C. caesia, C. malabarica, C. rakthakanta, C. sylvatica* and *C. zedoaria* were collected from the National Bureau of Plant Genetic Resources (Regional Station) Trichur, Kerala. The rhizomes were planted and the crop was raised in the farm of Central Tuber Crops Research Institute, Trivandrum. The rhizomes were harvested after 8 months and fresh rhizomes used for the extraction of essential oil.

Chemicals

All chemicals used were of analytical grade. Gallic acid, DPPH (1, 1-diphenyl-2-picrylhydazyl, Butylated Hydroxy Toluene (BHT) and ascorbic acid were purchased from Sigma-Aldrich.

Isolation of the essential oils

Fresh rhizomes were washed, sliced, homogenised with distilled water and subjected to hydrodistillation for 4h using a Clevenger apparatus with a water cooled receiver. The essential oils were dried over anhydrous sodium sulphate, the volume measured and stored at $^{-}20^{o}$ C

Estimation of total phenol content

Total phenols were determined by the Folin Ciocaltaeu procedure using gallic acid as standard [7]. Aliquots of essential oil diluted with ethanol 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 µg gallic acid equivalents (GAE)/µl oil.

Antioxidant activity

(i) DPPH free radical scavenging activity

The DPPH radical scavenging activity was measured according to the method of Chung et al [8] with some modifications. Aliquots of diluted essential oil was mixed with 1530 µl of Tris-HCl buffer (100 mM, pH 7.9), 100 µl 0.5 % Tween 20 and 350 µl of DPPH solution (0.5 mM). 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 without addition of oil. The half-inhibition concentration values (IC_{50}), the volume of essential oil at which the inhibition of DPPH radical is 50%, were calculated.

(ii) Reducing power

The ferric reducing power of the oil was determined by the method of Duh et al [9]. Aliquots of oil 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^{o}C for 20 min. Trichloroacetic acid solution (2.5 ml of 10 % TCA) was added to the cooled reaction mixture. 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 concentration of oil at which the A700 of the Prussian blue complex is 0.5) of the oils were determined.

Antimicrobial activity

The antibacterial activity of the essential oils was evaluated against three standard bacterial strains which included the Gram positive

Staphylococcus aureus (MTCCNo: 902) and *Bacillus Subtilis* (MTCC No: 2756) and Gram negative *E.coli* (MTCC No: 2622). The bacterial strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology,Chandigarh, India.

Evaluation of *in vitro* antibacterial activity was carried out by the plate diffusion procedure as described by Perez et al [10]. The essential oils were diluted with dimethyl sulphoxide (DMSO) and aliquots were loaded on a 10 mm diameter disc, air dried and placed on sterile medium in a petri dish. Plates were incubated at 37°C for 24 hr and antibacterial activity was evaluated by measuring the diameter of the inhibition zone. Standard antibiotic Ciprofloxacin (5 µg/ml) and DMSO controls were included in the assay.

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

Yield of Essential oil

Yield of essential oil present in different species ranged from 0.25 to 1.4 % (ml/100 g fw). *C. zedoaria* had the highest yield, *C.brog, C.caesia, C.sylvatica* possessed ≥ 0.5 % yield, while *C.amada* had the lowest yield of 0.25% .

Total phenol content

The total phenol content of *Curcuma* essential oils showed large variation, ranging from 4 - 83 µg/µl oil.(Table1) *C.zedoaria* had the highest phenol content, followed by *C. brog, C.caesia* and *C. malabarica*. The lowest phenol content was observed in *C. sylvatica* oil and *C. amada* oils.

Antioxidant activity

The DPPH radical scavenging activity of the *Curcuma* oils showed a large variation, the IC_{50} values ranging from 1.6µg to 8µg. (Table 1). The highest activity was seen in *C. zedoaria,* (IC_{50}=1.6) followed by *C. aromatica* (IC_{50}= 3.9 µl). Mau et al 2003 also reported that the essential oil of *C zedoaria* was good in reducing power and excellent in DPPH scavenging activity Lowest activity was seen in *C.rakthakanta* and *C.malabarica* followed by other species including *C. sylvatica* oil and *C. amada*. Ferric reducing power also varied between the species, with *C.brog, C.caesia* and *C.malabarica* having the highest reducing power and lowest EC_{50} values of 1.5, 1.6 and 2.2. *C. sylvatica* oil and *C. amada* exhibited the lowest reducing activity. The IC_{50} values for the standards gallic acid and BHT were 9 and 20 µg respectively while EC_{50}values for gallic acid, BHT and ascorbic acid were 8.5 µg, 24 µg, and 34 µg respectively.

In most species there was significant correlation between the phenolic content and the DPPH scavenging activity (R^2 = 0.470) but lower correlation was observed with ferric reducing power (R^2 = 0.138). However, correlation between total phenol content and ferric reducing power was highly significant for *C.brog, C.caesia , C.malabarica* and *C. rakthakanta* oils (R^2 = 0.689).

Antimicrobial activity

The antibacterial activity, measured in terms of the diameter of the zone of inhibition, is shown in Table 2. Most of the essential oils exhibited antibacterial activity against all three bacteria used in the study. *C.aeruginosa, C.amada* and *C. aromatica* oils produced maximum inhibitory activity against all the bacteria tested. Among the three bacteria, *S. aureus* was the most sensitive one. Correlation of antimicrobial activity with phenol content was high for *B.subtilis* (R^2 = 0.371), while less correlation was noticed in the case of *E.coli* (R^2 = 0.048) and *S. aureus* (R^2 = 0.018). *C.sylvatica* oil, showed maximum activity against *B.Subtilis* and *C. caesia* towards *S. aureus*. Oils from *C. brog, C. caesia* and *C. malabarica* were inactive against *E. coli*. No difference in susceptibility was seen between g+ve and g-ve organisms. The antibacterial activity of the essential oil of *C. ceasia* and antifungal activity of essential oil of *C. aromatica* were earlier reported by Banerjee and Nigam [12] and Rao [13] respectively. Except for *C. zedoaria*, the essential oils of most of these *Curcuma* species have not been extensively investigated for antibacterial properties.

Table 1: Total phenol content and antioxidant activity in essential oils of Curcuma species

Species	Total phenol content (µg GAE /µl oil)	DPPHScavenging activity IC 50 (µl)	Ferric reducing power EC 50 (µl)
C. aromatica	7.5 ± 0.1 b	3.9 ± 0.06 b	2.5 ± 0.06 e
C. brog	36.3 ± 0.06 g	6.3 ± 0.13 d	1.5 ± 0.05 a
C. caesia	22.5 ± 0.12 f	6.3 ± 0.06 d	1.6 ± 0.1 b
C. malabarica	20 ± 0.58 e	7.0 ± 0.06 e	2.2 ± 0.06 c
C. rakthakanta	15 ± 0.58 d	7.9 ± 0.06 f	2.3 ± 0.06 d
C. sylvatica	3.8 ± 0.06 a	6.3 ± 0.06 d	24.2 ± 0.06 i
C. zedoaria	82.5 ± 0.06 h	1.6 ± 0.04	4.3 ± 0.06 g

Values are expressed as mean ± standard deviation. Means with different letters within a column are significantly different ($p<0.05$)

Table 2: Antibacterial activity of essential oils of *Curcuma species*

Species	*B. subtili*	*S. aureus*	*E.coli*
C.aeruginosa	28 ± 0.5 d	34 ± 0.5 e	28 ± 0.4 e
C.amada	28 ± 0.3 d	30 ± 0.5 d	28 ± 0.6 e
C.aromatica	28 ± 0.6 d	30 ± 1.0 d	28 ± 0.2 e
C.brog	15 ± 0.5 a	26 ± 1.0 c	7 ± 0.2 a
C.caesia	17 ± 0.3 c	40 ± 1.2 f	7 ± 0.2 a
C.malabarica	16 ± 0.4 b	28 ± 1.0 c	7 ± 0.1 a
C.rakthakanta	16 ± 0.5 b	18 ± 0.5 b	14 ± 0.2 c
C.sylvatica	32 ± 0.8 e	11 ± 0.3 a	12 ± 0.3 b
C.zedoaria	16 ± 0.5 b	20 ± 0.8 b	18 ± 0.3 d

10 µl oil samples were used for the assay.

Ciprofloxacin(5µg) was used as positive reference standard Values (diameter of zone of inhibition) are expressed as mean ± standard deviation. Means with different letters within a column are significantly different ($p<0.05$)

CONCLUSION

Essential oils are potential sources of antimicrobial compounds, comprising of mixtures of monoterpenes, sesquiterpenes, and various aliphatic hydrocarbons (Burt 2004). Their antibacterial properties have also been attributed to the presence of phenolic compounds. The present studies indicated that among the nine *Curcuma* species**,** *C. zedoaria*, *C. brog, C. caesia* and *C. malabarica* possessed higher content of phenolics. These species also had higher DPPH scavenging activity. *C. brog, C. caesia* and *C. malabarica* essential oils had higher reducing power. The species exhibiting highest antibacterial activity were *C.aeruginosa, C.amada* and *C. aromatica.* Most of these *Curcuma* species are used in traditional medicine for their bactericidal and anti-inflammatory properties. Many are underutilized and there is tremendous scope for utilization of their essential oils as pharmaceutical and food additives.

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