Phytopharmacology

Inforesights Publishing

# Antioxidant and antibacterial activities of oleoresins isolated from nine *Curcuma* species

Angel Gabriel Rajamma, Vimala Bai, Bala Nambisan*

Central Tuber Crops Research Institute, Sreekariyam, Trivandrum-695017, India

*Corresponding Author: balanambisan@yahoo.co.uk

**Received**: 7 February 2012, **Revised**: 13 February 2012 **Accepted**: 13 February 2012

**Abstract**

Oleoresins were extracted from rhizomes of nine starchy *Curcuma* species (*Curcuma aeruginosa, Curcuma. amada, Curcuma aromatica, Curcuma brog, Curcume caesia, Curcuma malabarica, Curcuma rakthakanta, Curcuma sylvatica* and *Curcuma zedoaria*) using dichloromethane and evaluated for antioxidant and antibacterial activity. The yield of oleoresin in the different species ranged from 4 to 15 % dry weight. Total phenols varied from 23 – 100 mg gallic acid equivalents (GAE) /g oleoresin. Oleoresins from all the species exhibited high DPPH radical scavenging activity and ferric reducing power, which had good correlation with phenolic content The oleoresins inhibited both g +ve (*S. aureus* and *B. subtilis*) and g-ve (*E. coli*) bacteria. Maximum sensitivity was observed in the case of *B. subtilis*. The results indicated that the oleoresins from these species (most of which are unutilized) would have good potential as additives for food and medicinal applications.

**Keywords**: *Curcuma* species; oleoresin; DPPH, Ferric reducing power; antibacterial

## Introduction

The genus *Curcuma* (family Zingiberaceae) comprises of more than 80 species of rhizomatous herbs which are widely used in traditional systems of medicines such as Ayurveda, Siddha, Unani, Homeopathy and Naturopathy. 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. (Naz et al, 2010, Mishra et al, 1997, Jayaprakasha et al, 2002, Sacchetti et al, 2005). 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. These species are active ingredients of traditional herbal medicines (jamu, ukon, yujin, gajutsu, ezhu) of Indonesia, Japan and China. The spec-

 

ies *C. aromatica* and *C. zedoaria* are active ingredients of yujin and ezhu in Chinese medicine (Sasikumar, 2005). *Curcuma aeruginosa* rhizome is traditionally used in the treatment of rheumatic conditions, cough, asthma and as an anthelmintic (Nasrullah, 2010). Rhizomes of *C. aromatica* possess carminative effect and are also used for bruises, sprains, bronchitis, cough and skin eruptions (Joy et al, 2001). *C.amada* is useful in treating bruises, sprains, wounds, skin diseases, bronchitis, asthma, diarrhoea etc (CSIR, 2001, Joy etal, 2001). Raw tuber paste of *C. aromatica*, *C. amada*, *C. zedoaria* is found to control of intestinal worms and *C. caesia* is applied for snake and scorpion bites (Joy et al, 2001,Tag et al, 2007). Rhizome paste of *C. caesia* is also applied externally to sprains and bruises (The Wealth of India, 2001). Starch extracted from *C. zedoaria* is used in the preparation of shoti starch, which is used as baby food. Rhizomes of *C. zedoaria* are used as appetiser, tonic, carminative, blood purifier and against worm infection, fever, diarrhoea and skin diseases (The Wealth of India, 2001, Joy et al, 2001).Rhizomes of *C.amada* and *C. sylvatica* are used in culinary preparations viz. pickles, salads and chutney (The Wealth of India, 2001, Sasikumar, 2005).

Their rhizomes and leaves of *Curcuma* species are aromatic, indicating the presence of volatiles/essential oils. Oleoresins from aromatic plants contain both the volatile essential oil and non-volatile resinous fraction. They have applications in food, cosmetic and pharmaceutical industry as flavouring agents and antimicrobials eg. Curcumin, essential oil and oleoresin from *Curcuma longa* rhizomes are commercially used in pharmaceutical, flavouring and perfumery industries. The essential oil composition and biological activity in species viz. *C.amada*, *C. aeruginosa, C. aromatica*, *C. xanthorrhiza*, *C. haritha* have been reported. (Mustafa et al 2005, Shafi Mohamed et al, 2003, Kojima et al, 1998) However, oleoresins from these species have not been extracted and utilized so far. In the present study oleoresins were isolated from nine rhizomatous *Curcuma* species, which included many unconventional species, and evaluated for phenolic content, antioxidant and antibacterial activity in order to explore their potential for medicinal/food applications.

## 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, India. The crop was raised in the farm of Central Tuber Crops Research Institute, Trivandrum. The rhizomes were harvested after 8 months, cut into small pieces and shade dried for 48 h. The dried samples were ground to a fine powder and used for extraction of oleoresin.

### *Chemicals*

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

### *Extraction of oleoresin*

 

Dry powdered samples of the different rhizomes were packed into glass columns and extracted continuously with dichloromethane at room temperature until extracts were colourless. Extracts were centrifuged at 10,000xg, filtered through Whatman 1 chromatography paper and concentrated using a rotary flash evaporator. Yield of oleoresin (OR) was recorded and samples were stored at $^{-}20\ ^{o}$ C for further analysis.

### *Determination of total phenolics*

Total phenols were determined by the Folin Ciocaltaeu procedure using gallic acid as standard (Lee et al, 2003). Aliquots of oleoresin diluted with dimethylsulphoxide were mixed with Folin-Ciocalteau reagent (1:1) 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)/g oleoresin.

### *Determination of antioxidant activity*

#### *DPPH free radical scavenging activity*

The DPPH radical scavenging activity was measured according to the method of Choi et al (2000) with some modifications. An aliquot was mixed with 100 µl Tween 20 (0.5 %) and 350 µl DPPH in methanol (0.5 mM) in the presence of 1500µl of Tris HCl Buffer (100 mM, PH 7.9). Tween 20 was used as an oil-in-water emulsifier. 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 oleoresin, and with oleoresin alone. The half-inhibition concentration values ($IC_{50}$) (the concentration of oleoresin (mg) at which the inhibition of DPPH radical is 50%), were calculated.

#### *Ferric reducing power*

The ferric reducing power was determined by the method of Duh et al (1999). Aliquots of oleoresin 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 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 (oleoresin concentration at which the A700 of the Prussian blue complex is 0.5) of the oleoresins were determined.

### *Antimicrobial activity*

The antibacterial activity of the oleoresins were evaluated against three standard bacterial strains which included the Gram positive *Staphylococcus aureus* (MTCC 902) and *Bacillus Subtilis* (MTCC No: 2756) and Gram negative *E. coli* (MTCC 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, 1990. The oleoresins were diluted with dimethyl sulphoxide (DMSO) to obtain a concentration of 10 mg/ml, filtered through 0.45 microfilter and aliquots of 100 µl were loaded on a 6 mm diameter disc, air dried and placed on sterile medium in a petri dish. Plates were incubated at 37$^{o}$C for 24 hr and antibacterial activity was evaluated by measuring the diameter of the inhibition zone. Standard antibiotic Ciprofloxacin (5 µg/disc) 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 antibacterial activity with phenol content.

## Results and Discussion

### *Yield of oleoresin*

Oleoresins obtained from all the *Curcuma* species were viscous in nature and brownish yellow in colour. Yield ranged from 4.0 to 15 % dw. *C. aeruginosa* had the highest yield followed by *C.amada* (8.8 %), *C. zedoaria* (8.4 %), *C. aromatica* (7.8%) *C. malabarica* (6.4 %) *C. rakthakanta* and *C. sylvatica* (6 %). *C. caesia*, *C. brog* had the lowest content (4, 5%).

### *Total Phenolic Content*

The oleoresin samples were assayed for total phenols after suitable dilution with DMSO. Total phenol content in the oleoresins ranged from 23 – 100 mg gallic acid equivalents (GAE)/g oleoresin. Higher phenol content was present in *C. zedoaria, C .aromatica*, *C. caesia, C. malabarica* and *C. rakthakanta,* followed by *C. brog* and *C. aeruginosa,* while lowest concentration was present in *C. amada* and *C. sylvatica.*

**Table 1. Total Phenol content and antioxidant activity in oleoresins from Curcuma species**

| Species | Total phenol content (mg GAE/g) | DPPH radical scavenging activity $IC_{50}$ (mg) | Ferric reducing power $EC_{50}$ (mg) |
|---|---|---|---|
| *C. aeruginosa* | 34.0 ± 0.58 b | 0.45 ± 0.01 e | 1.3 ± 0.06 f |
| *C. amada* | 23 ± 0.29 a | 0.78 ± 0.02 f | 2.6 ± 0.10 h |
| *C. aromatica* | 69 ± 2.1 f | 0.30 ± 0.02 b | 0.41 ± 0.02 a |
| *C. brog* | 40.0 ± 1.5 c | 0.45 ± 0.01 e | 0.82 ± 0.02 d |
| *C. caesia* | 63.0 ± 1.2 e | 0.32 ± 0.01 c | 0.50 ± 0.02 a,b |
| *C. malabarica* | 46 ± 1.3 d | 0.42 ± 0.02 d | 0.59 ± 0.01 b, c |
| *C. rakthakanta* | 46 ± 1.0 d | 0.44 ± 0.01 e | 1.1 ± 0.18 e |

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

 

Table 2. Antibacterial activity of Curcuma oleoresins

| Species | *B. subtilis* | *S. aureus* | *E. coli* |
|---|---|---|---|
| *C. aeruginosa* | 18 ± 0.5 a | 13.0 ± 0.6 b | 10.0 ± 0.1 b |
| *C. amada* | 26 ± 1.0 c | 15.0 ± 0.3 c | 13.0 ± 0.6 d |
| *C. aromatica* | 18 ± 0.6 a | 13.0 ± 0.5 b | 11.0 ± 0.3 c |
| *C. rakthakanta* | 20 ± 1.0 b | 13.0 ± 0.8 b | 10.0 ± 0.3 b |
| *C. sylvatica* | 18 ± 0.5 a | 13.0 ± 1.0 b | 10.0 ± 0.6 b |
| *C. zedoaria* | 18 ± 0.6 a | 11.0 ± 0.3 a | 9.0 ± 0.6 a |
| Ciprofloxacin | 27 ± 0.5 | 23 ± 0.4 | 23 ± 0.5 |

*Zone of inhibition (including 6 mm disc diameter) expressed in mm/ mg oleoresin are the mean of triplicate analysis ± standard deviation. Mean values followed by different letters in a column are significantly different ($p \leq 0.05$).

### *Antioxidant activity*

The antioxidant potential of the extracts was determined by measuring the DPPH scavenging activity and ferric reducing power. High variation in antioxidant activity was observed, with $IC_{50}$ values ranging from 0.15 – 0.81 mg and $EC_{50}$ values from 0.41 to 2.6 mg. Oleoresins from *C. zedoaria, C. aromatica* and *C. caesia*, showed high DPPH free radical scavenging activity ($IC_{50}$ values 0.15, 0.30 and 0.32), while lower activity was seen in C. *sylvatica* and *C.amada*. As in the case of DPPH scavenging activity, oleoresins from *C. zedoaria, C. aromatica* and *C. caesia* also showed the highest iron reducing power.

Analysis of variance indicated significant differences in phenol and antioxidant activity between the species. Among the nine species, *C. zedoaria, C. aromatica* and *C. caesia* oleoresins contained higher concentration of phenols and also exhibited the highest antioxidant activity. The correlation between total phenol content and antioxidant activity was analysed. Oleoresins with higher DPPH and ferric reducing power had higher phenol content. Correlation between phenol content and antioxidant activity in terms of DPPH radical scavenging activity and ferric reducing power was highly significant ($R^2 = 0.796$, $R^2 = 0.501$ respectively). Significant correlation was also noticed between DPPH radical scavenging activity and ferric reducing power ($R^2 = 0.825$).

### *Antimicrobial activity*

The antibacterial activity, measured in terms of the diameter of the zone of inhibition is shown in Table 2. Oleoresins from all species showed promising antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli* at a concentration of 1 mg except for *C. brog*, *C. caesia* and *C. malabarica* which produced inhibitory effects only at 5 fold higher concentration. The highest antibacterial activity was present in *C.amada* oleoresin. Among the three bacteria, *B. subtilis* was the most sensitive one. There was no correlation between antimicrobial activity and phenol content between all the species. However correlation was observed in *Staphylococcus aureus (*$R^2 = 0.520$ at 0.01 level), and *E. coli (*0.265 at 0.05 level). *Curcuma* oleoresins were highly active against *B. subtilis*. In spite of the lower phenol content, *C. amada* showed highest antibacterial activity against all three test micro organisms.

 

## Conclusion

The studies indicated that among the species used for the study, *C. amada* oleoresin had maximum antibacterial potential, while *C. zedoaria, C. aromatica* and *C. caesia* oleoresins had high antioxidant potential. The oleoresins from these *Curcuma* species could be utilized as natural antioxidants and antimicrobials in food and pharmaceutical industry in view of their antioxidant and antibacterial properties.

## Acknowledgements

The authors are grateful to Kerala State Council for Science Technology and Environment for financial support and to the Director CTCRI for providing facilities.

## Conflict of Interest statement

There is no conflict of interest associated with the authors of this paper.

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