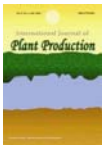

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A comparative study of phenol content and antioxidant activity between non-conventional *Curcuma caesia* Roxb. and *Curcuma amada* Roxb.

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Abstract

The present study aimed to investigate the phenol content and antioxidant activity of a non-conventional *Curcuma* sp. namely, *Curcuma caesia* in comparison with another species, *Curcuma amada*. The total phenol contents of the methanolic rhizome extracts of *C. amada* and *C. caesia* were 37.64 and 44.33 mg Tannic acid equivalents (TAE)/g dry material, respectively. The reducing power, and superoxide, ABTS and DPPH radical scavenging activities of *C. caesia* were higher than *C. amada*. These results supported that the non-conventional *C. caesia* could be an economically important plant species due to its antioxidant potential.

Keywords: *Curcuma amada*; *Curcuma caesia*; Rhizome; Total phenol; ABTS; DPPH; Antioxidant.

Introduction

The genus *Curcuma* under the family Zingiberaceae comprises of over 80 species of rhizomatous herbs. Among them, turmeric (*Curcuma longa* L.) is commonly used in the Indian traditional systems of medicine and also in several food stuff preparations for its medicinal properties. The biological effects of turmeric have been attributed to its chemical constituent, curcumin that has been widely studied for its anti-inflammatory, anti-angiogenic, antioxidant, wound healing and anti-cancer effects (Jayaprakasha et al., 2006). Rhizome of another species, *Curcuma amada* Roxb., popularly known as mango-ginger is having characteristic odour similar to raw mangoes (*Mangifera indica* L.) and used as major ingredient in the pickles, candies, salads, sauces and chutneys (Mridula and Jayachandran, 2001). However, the rhizome of yet another species, *Curcuma caesia* Roxb. is not under use besides, only limited work has been done on this species (Krishnaraj and Mathivanan, 2008; Krishnaraj et al., 2008). Therefore, the present work was aimed to analyze the total phenol content and antioxidant potential of the non-conventional *Curcuma* species, *C. caesia* in comparison with *C. amada*.

Materials and Methods

The rhizomes of *C. amada* and *C. caesia* were collected during May 2008 from the Dandakaranya forest, Malkanagiri District, Orissa, India, They were cut into small pieces (5 cm), shade dried and ground to fine powder. Known quantities of the ground rhizome materials were extracted with methanol using a Soxhlet apparatus for 16 h and the solvent was evaporated to dryness under reduced pressure. The residues were weighed and stored at 4 ºC until use.

The total phenol content of the methanolic rhizome extracts was determined by the method of Singleton and Rossi (1965). Extract of *C. amada* and *C. caesia*, 200 µL each was mixed with 1.0 mL of Folin-Ciocalteau reagent (1 X) and 1.0 mL of 7.5% sodium carbonate was added. After vortexing for 2 min, the tubes were incubated for 2 h at 28 ± 2 ºC and the absorbance was measured at 726 nm in a spectrophotometer. The total phenolic content was expressed as tannic acid equivalents (TAE) in mg/g dry material.

The dried rhizome extracts of *C. amada* and *C. caesia* were dissolved separately in methanol at 2.5 mg-15 mg/ mL, mixed with 5 mL phosphate buffer (0.2 M, pH 6.6) and 5 mL potassium ferricyanide (1%) and incubated at 50 °C for 20 min. Then, 5 mL of 10% TCA was added to the reaction mixture and centrifuged at 3000×g for 10 min. The upper layer of 5 mL solution was mixed with 5 mL of distilled water and 1 mL of 1% ferric chloride and the absorbance was read at 700 nm. The reducing power of the rhizome extracts was indicated by the increased absorbance (Siddhuraju et al., 2002).

Superoxide radical scavenging capacity was assayed by nitroblue tetrazolium (NBT) according to the method of Zhishen et al. (1999). The total volume of the reaction mixture was 5 mL and the concentration of riboflavin, methionine and NBT was 3×10^{-6}, 1×10^{-2} and 1×10^{-4} mol l^{-1}, respectively. The mixture was illuminated at 25 °C for 25 min and the un-illuminated reaction mixture was used as a blank and the absorbance (*A*) was measured at 560 nm. The rhizome extracts were added separately to the reaction mixtures in which O_2^- was being scavenged, thereby inhibiting the NBT reduction. The absorbance (A_1) was measured and the decrease in O_2^- was represented by A-A_1. The degree of the scavenging was calculated by the following equation:

Scavenging (%) = $(A\text{-}A_1/A) \times 100$

The free radical scavenging capacity of both the rhizome extracts was determined by ABTS (*2,2'-azinobis*-(*3-ethylbenzothiazoline-6-sulfonic acid*) radical cation decolorization assay (Re et al., 1999). The ABTS was dissolved in water to get 7 mM concentration. ABTS radical cation ($ABTS^{*+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark at 28 ± 2 ºC for 12-16 h before use. For the study of the test samples, the $ABTS^{*+}$ solution was diluted with absolute ethanol to an absorbance of 0.700 ± 0.02 at 734 nm and equilibrated at 30 °C. Reagent blank reading (A_0) was taken. After addition of 2.0 mL of diluted $ABTS^{*+}$ solution ($A734\ nm$ = 0.700 ± 0.02) to 20 µL of test sample, the absorbance was read at 30 °C exactly for 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. Methanolic solutions of pure compounds [(+)-catechin, ascorbic acid and quercetin] were also tested at 1 mg/ml concentration. All the determinations were done three times. The decrease in absorbance between A_0 and A_t was used to calculate the percentage of inhibition ABTS free radicals by the extracts according to the following formula:

$$PI = [(A_{C(0)}-A_{A(t)}) / A_{C(0)}] \times 100$$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidant at t = 6 min.

Free radical scavenging ability was also estimated according to the procedure described by Von Gadow et al. (1997) by the use of a stable DPPH radical (1,1-diphenyl-2-picrilhydrazyl).

The experimental data were analyzed by ANOVA using Agres (1994) Statistical Software Version 3.01. (Pascal International Software Solutions, USA).

Results and Discussion

The percentage rhizome extract yield of *C. amada* and *C. caesia* was 16% and 23% and the total phenolic content was 37.64 and 44.33 mg TAE/g dry materials, respectively. This variation is expected in plant extracts due to their constituents as well as the type of the phenolics and this may differ considerably between the genotypes of the same plant species (Erturk et al., 2009) and from species to species (Jang et al., 2007). These phenolics are responsible for antioxidant activity and hence, measurement of total phenolic content could be used to relate their antioxidant properties (Katalinic et al., 2006).

According to Yen & Duh (1993), the reducing power was associated with antioxidant activity and it was proven in the case of anthraquinones (Yen et al., 2000). In the present study, we observed a moderate reducing power with both the rhizomes. However, the reducing power of *C. caesia* is slightly higher than *C. amada* (Figure 1). Both the *C. amada* and *C. caesia* rhizomes extracts scavenged the superoxide molecules. However, this activity was higher in *C. caesia* than *C. amada* (Table 1). Similarly, The DPPH free radical scavenging ability of *C. caesia* rhizome extract was higher than the *C. amada* rhizome extract (data not shown). Further, the ABTS cation radical scavenging activity was statistically high in *C. caesia* than *C. amada* (Figure 2). These free radical scavenging effects might be due to the presence of phenolics as they are the important constituents of both the *Curcuma* rhizomes. Policegoudra et al. (2007) demonstrated the presence of flavanoids in the rhizome of *C. amada*, which are responsible for the antioxidant activity. Previously, Siddhuraju et al. (2002) reported that the plant extracts possess many free hydroxyl substitution, which might have great antisuperoxide properties. In summary, the total phenol content and antioxidant activity were significantly high in *C. caesia* rhizome extract than the *C. amada* rhizome extract. Although *C. amada* has already been introduced as a food additives, and no report has been available on the uses of *C. caesia* and hence, the present work assume significant.

Table 1. Superoxide radical scavenging activity of *C. amada* and *C. caesia* rhizome extracts.

Sample concentration (ppm)	Superoxide radical scavenging activity (%)	
	C. amada	*C. caesia*
5	4.615 ± 0.76	4.615 ± 0.76
10	7.948 ± 0.88	8.204 ± 1.17
15	11.794 ± 0.88	13.845 ± 1.53
20	18.717 ± 1.17	28.717 ± 1.93

Values are means of triplicates with standard deviation.

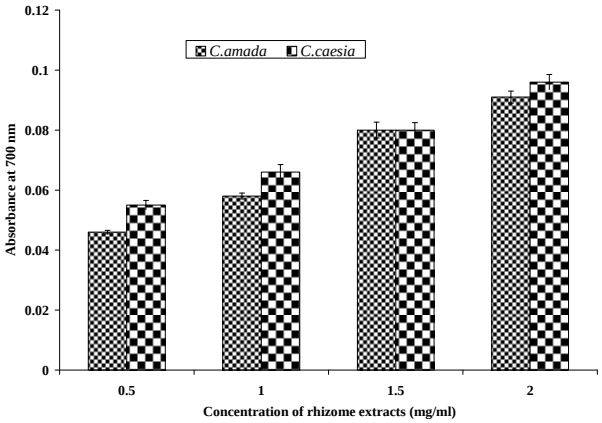

Figure 1. Reducing power of total phenolics of *C. amada* and *C. caesia.* Values are means of three replicates with standard deviation.

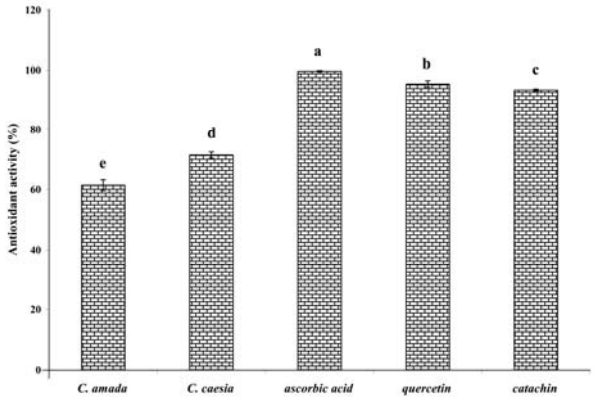

Figure 2. The percentage inhibition of free ABTS radical scavenging activity of *C. amada* and *C. caesia* extracts as compared to ascorbic acid, quercetin and catachin. Values are means of three replicates with standard deviation. Values in each column with different letter are significantly different at $P < 0.05$.

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