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Evaluation in comparative antioxidant activity of *Curcuma longa* & *Curcuma aromatica*

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ABSTRACT

The present study was aimed an investigation of phenol content, radical scavenging activity by DPPH method and to assess curcumin content of rhizomes in both the curcuma species to establish its potential as a therapeutic agent. The ethanolic fraction of *Curcuma longa* gave the highest yield of crude extract (3.35gm) and demonstrated highest free radical scavenging activity i.e. 74.61±0.02% due to presence of high amount of curcumin and phenolic content. In comparison to *C.longa*, *C.aromatica* showed moderate to poor antioxidant activity in compared to Ascorbic acid, DPPH free scavenging assay and IC_{50} value. The results indicate the use of *C.longa* as a source of an antioxidant while *C.aromatica* mat be used in food and cosmetic industries rather than pharmaceutical industries.

INTRODUCTION

The genus curcuma belongs to family Zingeberaceae and includes species *C.longa* and *C.aromatica* which are of high medicinal importance. *C.longa*, commonly known as turmeric, has been used as aromatic ingredient for cooking. Curcumin extracted from *C.longa* rhizome is an anti-inflammatory agent[1] and has anti-carcinogenic properties[2]. *C.aromatica*, a closely related species of *C.longa* also contain curcumin, rhizome powder of this species is of high caloric value and used as a substitute for baby food[3]. Cucuminoids exhibit free radical scavenging properties, antioxidant activity[1,4-7] and act as inhibitors of human immune deficiency virus type (HIV-1) intergrase[8].

Recent data suggest that curcumin and other antioxidant products from the rhizome of turmeric may be useful in the prevention or treatment of some age related degenerative processes[9]. The objective of this study was to evaluate the antioxidant activity in rhizomes of *C.longa* and *C.aromatica* in relation to curcumin and phenol content by using DPPH method.

MATERIALS AND METHODS

Plant materials

The rhizomes used in this study were collected from Koraput district of Orissa in the month of Dec-Jan 2010 and authenticated by Prof. R.N. Padhy, Department of Botany, B.J.B. Autonomous College, Bhubaneswar, Orissa. The voucher specimen (Number K046, K047) was deposited in the departmental herbarium for further references. Fresh rhizomes were rinsed with clean water to make it dust and debris free. The rhizomes were shade dried for a period of 4 weeks after which they were finely powdered.

Preparation of crude extracts

The dried and powered *C.longa* and *C.aromatica* rhizomes (100g) were extracted successively with water, methanol and ethanol for 2 days using a soxhlet apparatus. The supernatants were filtered through a

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nylon mesh and evaporated in vacuum evaporator to obtain the respective extracts and finally stored in vacuum desiccators until use.

Estimation of total phenol content

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described[10] Reading samples on a UV-vis spectrophotometer at 650 nm. Results were expressed as catechol equivalents (µg/mg).

Estimation of curcumin

Fresh rhizomes were cleaned and sliced into small pieces and air dried for two days. The samples were further dried in a hot-air oven at 50^{0}C for 24hr and then ground into powder. Total curcumin content was calculated using a standard curve. Analysis of each sample was carried out in triplicate. Standard solution was prepared according to the method of[11] Standard curcumin (2.00mg) was accurately weighed and transferred to a 5ml volumetric flask. Distilled ethanol was added and adjusted to a final concentration of 400µg/ml. from this solution, concentrations of 0.4, 0.8, 1.6, 2.0, 2.4, 3.2 and 4.0µg/ml were prepared and used for preparation of the calibration curve for preparing the sample solution from turmeric powder, the (100.00mg) of each sample was separately transferred to a 10ml volumetric flask, adjusted to 30µg/ml and measured at 420nm.

Assay for DPPH radical scavenging activity

Plant extraction

The modified extraction method of[12] was used with one gram of fresh rhizome ground using pestle and mortar with liquid nitrogen and then 10ml of 95% distillable ethanol was added before centrifuging at 6000rpm for 10min. finally, the clear sample was filtered using Whatman No.4 filter paper and kept in a capped bottle at -20^{0}C until used for antioxidant activity assay.

DPPH radical scavenging activity

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of[13] with modifications. A stock solution of the sample (100mg/ml) was diluted for five concentrations. The portion of sample solution (0.5ml) was mixed with 3.0ml of 0.1mM 1,1-diphenyl-2-picrylhydrazyl (DPPH, in 95% distillable ethanol) and allowed to stand at room temperature for 20min under light protection. The absorbance (A) was measured at 517nm. The scavenging activity of the samples corresponded to the intensity of quenching DPPH. The results were expressed as a percentage of inhibition. Ethanol was used as the control and the results were compared with the activity of a known antioxidant, ascorbic acid (Equation 1).

$$\% \text{ inhibition} = (1-(A_{sample}/A_{blank}))\times 100 \quad (1)$$

where A_{sample} = Absorbance of sample with DPPH; A_{blank} = Absorbance of control with DPPH

In the DPPH test, antioxidants were typically characterized by their EC_{50} value (effective concentration of sample required to scavenge 50% DPPH radicals). The results were obtained by linear regression analysis of the dose response curve plotted using % inhibition and concentration.

Statistical analysis

Experimental results are represented as mean±SE (Standard error of Mean). Student's t-test was used for the evaluation of data. The co-relation value was determined between phenol content and antioxidant activity (%) of *C.longa* and *C.aromatica*.

RESULTS AND DISCUSSION

Initially crude extracts were obtained by extractions with solvent of increasing polarity, viz. water, methanol and ethanol. The amounts of extracts are 1.90gm, 2.45gm and 3.35gm in *Curcuma longa* and 1.02gm, 1.45gm and 2.56gm in *Curcuma aromatic*a. The total phenolics in the extracts were determined spectrophotometrically by the Folin-ciocalteu method and the results were reported as Catechol equivalents mg/g. As revealed by the data (TABLE 1), the total phenol content of ethanol extract was higher than methanol and water extracts and they were as follow: 215mg/g, 158mg/g and 68mg/g in *C.longa* and 180mg/g, 123mg/g and 40mg/g in *C.aromatica* respectively. In the present study, the values of ethanolic and methanolic

TABLE 1 : Yield of crude extracts and total phenol contents in *C.longa* and *C.aromatica*

Solvent Used	Parts used	*Curcuma longa*		*Curcuma aromatica*	
		Crude Extracts (gm)	Phenol content (mg/g)	Crude Extracts (gm)	Phenol content (µg/ml)
Water	Rhizome	1.90	68	1.02	40
Methanol	Rhizome	2.45	158	1.45	123
Ethanol	Rhizome	3.35	215	2.56	180

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extracts were higher than those of aqueous ones. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic component. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity[14].

Curcumin content is reported to vary from one species to another. In the present study (Figure 1) we found that the curcumin content in *C.longa* showed a higher value i.e. 8.22(mg/100mg) in comparison to *C. aromatica* which is 6.07(mg/100mg). Several studies have shown that soil factors, including nutrients and level of acidity as well as the genus diversity, may affect the content of curcumin in plants.[15,16] Similar results are obtained by[17] who studied variation in the active constituents of *C.domestica* rhizomes collected from Nakhon Pathom, Central Thailand, where they found the highest curcumin content was 10.12% w/w. In addition,[18] reported that a sample from the South contained the highest total curcumin (8.99±0.83%w/w), while the lowest was found in the North (4.80±1.83%w/w) where the climate is cooler and the dry period is longer and more pronounced.

DPPH assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and sensitive enough to detect active ingredients at low concentrations. Antioxidant activity using DPPH radical scavenging assay reported with IC_{50} value is shown in the (TABLE 2) and (Figure 2). The lower the IC_{50} is the higher the antioxidant activity of the compound. IC_{50} value is lowest in ethanol extract of *C.longa* which is very close to ascorbic acid (Figure 3). Similar results are observed in *C.longa* by[19] and showed strong activity. Results of DPPH activity of a range of concentration (20µg/ml-200µg/ml) indicate that ethanol extract of *C.longa* was the strongest radical scavenger 74.61±0.02% in comparison to all the solvent extracts of *C.aromatica* (TABLE 2). Total curcumin content and antioxidant activity of 67 samples of *C.longa* from various locations of Thailand were analyzed. Highest antioxidant activity of *C.longa* was attributed to curcumin content of the test sample. An in vitro rhizome of *C.longa* was compared with *C.amada*, *C.aromatica* and

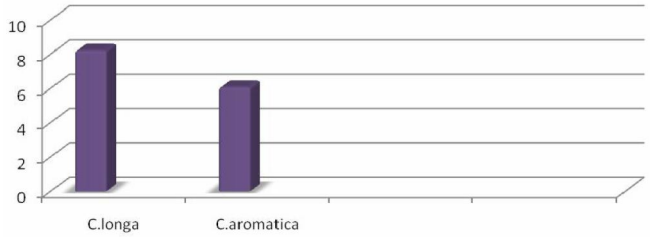

Figure 1 : Curcumin content in *C. longa* and *C.aromatica* (mg/100mg)

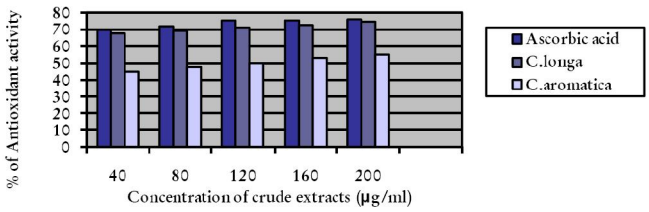

Figure 2 : Antioxidant activity (%) of *C.longa* and *C.aromatica* in comparison to ascorbic acid

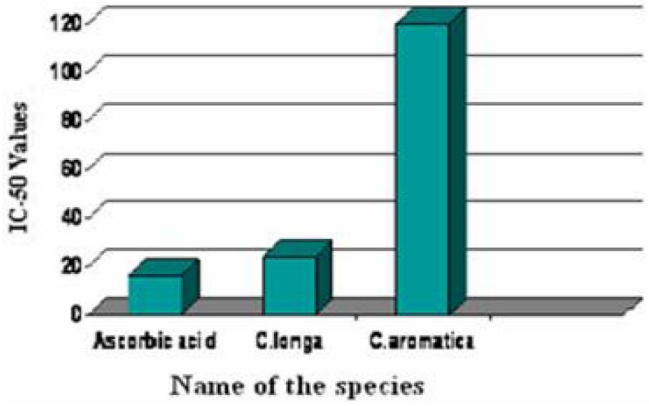

Figure 3 : IC_{50} values of *C.longa* and *C.aromatica* in comparison to ascorbic acid

TABLE 2 : Antioxidant activity (%) of *C.longa* and *C.aromatica*

Conc. of extracts (µg/ml)	Antioxidant activity (%)					
	Water		Methanol		Ethanol	
	C.longa	*C.aromatica*	*C.longa*	*C.aromatica*	*C.longa*	*C.aromatica*
40	26.15±0.01(NS)	25.38±0.02	53.07±0.04***	34.61±0.03	67.69±0.04***	44.61±0.06
80	30.00±0.03(NS)	27.69±0.08	56.92±0.06***	36.92±0.02	69.23±0.05***	47.61±0.03
120	32.30±0.04***	30.00±0.10	60.00±0.09***	40.76±0.03	70.76±0.04***	50.00±0.07
160	34.61±0.03***	32.30±0.07	61.53±0.07***	43.07±0.02	72.30±0.03***	53.07±0.02
200	36.92±0.02***	33.84±0.05	63.84±0.05***	46.92±0.04	74.61±0.02***	55.38±0.06

Note: Mean is expressed as, mean±S.E; Means with * mark are significant (p<0.001) and NS-Not significant**

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C.zedoaric. All accession of *C.longa* uniformly showed a high curcumin content[20]. It is well known that plant phenolics in general are highly effective free radical scavengers and antioxidants. We observed a correlation between the DPPH radical scavenging activity of the plant extracts and their phenol contents with the sample correlation coefficient r, 0.719 at $p<0.05$. The results showed that the antioxidant activity in turmeric was due to the presence of total phenol content.[21] Consequently the antioxidant activity of plant extracts are often explained with respect to their total curcumin content.[22]

CONCLUSION

Overall observations indicate the impressive antioxidant activity of *C.longa* (ethanolic extracts) in comparison to *C.aromatica*. The activity could be attributed to superior reducing power, higher phenol and curcumin content in *C.longa* turmeric powder is a proven antiseptic, carminative, somachic, appetizer and tonic[23] where as *C.aromatica* by its pleasant, camphoraceous aroma of rhizome is used to cure pimples, whitening of skin and also used as a blood purifier is not used as a condiment and due to poor antioxidant activity yet to find a place in pharmaceutical industries. However due to presence of some superior essential oil composition like α-pinene, β-pinene, 1,8-cineole, linalool in comparison to *C.longa* has a great potentialities in perfuming and pharmaceutical industries.[24]

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