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ANTIOXIDANT ACTIVITIES, PHENOLS AND FLAVONOID CONTENTS OF *CURCUMA LONGA* L. AND *CURCUMA CAESIA* ROXB. FOUND IN MANIPUR

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ABSTRACT
In the present study, a systematic record of the relative antioxidant activity in two selected medicinal plant species extracts is carried out. Findings indicated higher levels of total phenols (220 ± 0.060 mg/g, 60 ± 0.03 mg/g) and flavonoid (132 ± 0.03, 30 ± 0.06 mg/g dry weight of the root tissue) contents in the rhizome extracts of *C. longa* compared to *C. caesia*. The percentage of inhibition increases from 10μg/mL till 60 μg/mL and beyond which there is no increased in inhibition. The extend of inhibition by quercetin which is used as standard compound is 84%, *C. longa* 81.6% and *C. caesia* 76.24% respectively.

INTRODUCTION

India has rich history of using plants for medicinal purposes. *Curcuma* species are well-known indigenous medicine. They are used for the treatments of various ailments and metabolic disorders. *Curcuma longa* L. has been used for centuries as a spice to give flavour. It is equally important as household remedy for various illness including hepatic and billiary disorders, skin diseases, sinusitis and as a tropical application for wounds and cut (Chopra *et al.,* 1958). It has been reported to possess anti-inflammatory, hepatoprotective antitumour, antiviral activities (Ammon and Wahl, 1991) anticancer activity and exhibits free radical scavenging property-antioxidant property (Jayaprakasha *et al.,* 2006). The rhizomes of *Curcuma casia* Roxb. (black Zedoary) are used in treating leucoderma, asthma, tumours, tuberculosis glands of necks and piles, bronchitis and enlargement of spleen (Sinha, 2001). The paste is applied on bruises, contusions, and rheumatic pains. The rhizomes are also used in dysentery, diarrhea and cough (Kumar, 2002). The essential oils of *Curcuma caesia* Roxb. is also known for its antifungal activities (Banerjee and Nigam, 1976).

Phenolic compounds and flavonoid which are widely distributed in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging, abilities, anti-inflammatory, anti-carcinogenic. Antioxidant therapy has gained an immense importance in the treatment of chronic diseases such as coronary heart diseases, atherosclerosis, cancer AIDS and ageing (Finkel and Holbrook 2002). Antioxidants have been reported to prevent oxidative damage caused by free radicals and may prevent the occurrence of diseases (Velavan *et al.,* 2007). It can interfere with the production of free radicals and also with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting oxygen scavengers (Buyukokuroglu *et al.*, 2001). Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the scientific development throughout the world, due to their potent anti-oxidant activities, no side effects and economic viability (Auudy *et al.*, 2003). The paper deals with the antioxidant potentials of *C. longa* L. and *C. caesia* Roxb. and correlates with phenolics and flavonoid contents.

MATERIALS AND METHODS

Rhizomes of *C. longa* and *Caesia* were collected from different districts of Manipur namely Chandel, Senapati, Thoubal, Churachandpur and Bishnupur during the end of the growth season (November-February). The rhizomes were washed thoroughly under tap water to make free from contaminants. These cleaned rhizomes were cut into small pieces and sun dried. The samples were further dried in a hot-air oven at 60ºC for 24h and then ground into powdered form and passed through a sieve (mesh 20). This sieved powdered form of the rhizomes were kept ready for analysis.

Total phenolic content

Phenolic contents were estimated by the method of Mc Donald *et al.* (2001). A known weight of the powdered *Curcuma*

Table 1: Total phenols and flavonoid content in the rhizome of *Curcuma longa* L. and *Curcuma caesia* Roxb

Name of the curcuma species	Total phenols (mg/g)	Total flavonoids (mg/g)
Curcuma longa L.	220 ± 0.06	132 ± 0.03
Curcuma caesia Roxb.	60 ± 0.03	30 ± 0.06

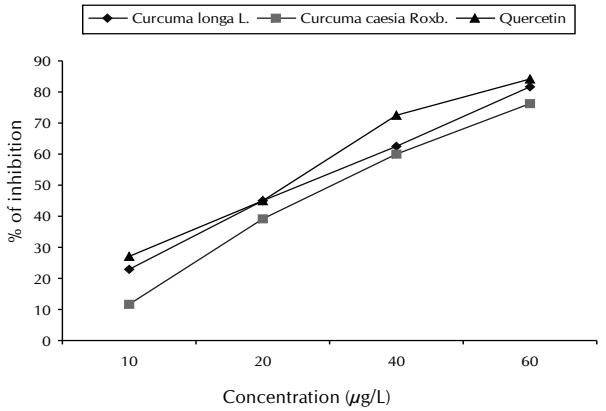

Figure 1: Showing percentage of inhibition by DPPH

samples were extracted in methanol by intermittent maceration upto 48h centrifuge and the supernatants were used for the estimations. Chlorogenic acid was used as the standard and absorbance was measured at 765nm.

Flavonoid content

Aluminum chloride spectrophotometeric method was used for flavonoid determinations (Chang *et al*., 2002). The reaction mixture comprised of 0.1mL of the extract, 0.1mL of aluminium chloride (10%), 0.1mL of potassium acetate (1 M) and 2.7mL of distilled water. It was kept at room temperature (27ºC) for 30 mins and the absorbance was measured at 415 nm using quercetin as the standard.

Anti-oxidant potentialities

Preparation of the extracts

10g of the powdered samples were extracted in a Soxhlet apparatus with 100 mL of ethanol (60ºC for 12 h). The samples were filtered through Whatman No.1 paper in Buchner funnel, the filtrate was freeze dried and weighed. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and quercetin were obtained from HiMedia. The method used by Fogliano *et al*. (1999) was adopted with suitable modifications to our experimental conditions. For qualitative assay, extracts and quercetin as standard (20mg) were dissolved in 1mL methanol, out of which 1mL was applied on TLC plates (Silica gel 60 F254; 20 × 20cm). These plates were sprayed with DPPH (0.002g 1.0mL) and exposed to daylight until discolouring of the background (6h). The resulting yellow colour on the plates was determined active antioxidant constituents. This method was also used for positive and negative control. For quantitative assay, each of the extracts (0.008g) was dissolved separately in 10 mL of methanol and various concentrations (60, 40, 20, 10 μg) were prepared. Each of the 2.5 mL test extract was mixed with DPPH (0.002g/10mL) and allowed 30 minutes for the reaction to occur. The absorbance of the colour developed was measured at 517 nm by a spectrophotometer. The negative control and positive control were also subjected to the same procedure. Three replicates were used and the average absorption was noted for each concentrations. Data were processed using excel and the concentrations that caused 50% reduction in absorbane (RC_{50}) were calculated. Percent inhibition of DPPH was calculated by following equation (Lee *et al*., 1998)

Table 2: Percent inhibition of DPPH by the rhizome extracts of *C. longa* L. and *C. caesia* Roxb

Name of the species	% Inhibition of DPPH			
	10μg/mL	20μg/mL	40μg/mL	60μg/mL
Curcuma longa L.	22.98	44.85	62.64	81.60
Curcuma caesia Roxb.	11.87	39.08	60	76.24
Quercetin	27.16	45.07	72.53	84

$$\% \text{ Inhibition} = 1-(A_1/A_2) \times 100$$

where A_1 is the absorbance of the test samples and A_2 the absorbance of control reaction.

RESULTS AND DISCUSSION

Phenolic compounds can act as antioxidants by radical scavenging in which they break the free radical chain reaction through hydrogen atom donation (Kosem and Moongkarndi, 2007). The resulting phenoxy radical can be reduced to its parent compound by enzymatic or non-enzymatic reactions (Sakihama *et al*., 2002). The level of total phenolic content was exhibited more in *C. longa* compared to rhizome of *C. casia*. Higher level of flavonoid content was observed in the rhizomes of *C. longa* (132 ± 0.03, and 30 ± 0.16 mg g^{-1} dry weight) as compared to the rhizomes of *C. caesia*. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler *et al*., 2003). In the present study the high contents of flavonoid in C. *longa* can probably explain its high anti-oxidant free radical scavenging activities and widely used in traditional medicines. The data obtained in the above study showed that the rhizomes of *C. longa* which contain maximum phenolic compound as well as flavonoid exhibited the great anti-oxidant activity. The results revealed that there is relation between phenol and flavonoid contents with antioxidant activites as previously reported by Pourmorad *et al*. (2006); Velavan *et al*. (2007).

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