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Antioxidant activity of free and bound phenolics in *Curcuma longa*

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Abstract

The antioxidant activity of free and bound phenolics of turmeric (*Curcuma longa*) extract prepared by treating with enzyme and without enzyme was analyzed. The enzyme assisted extraction of *Curcuma longa* showed higher levels of antioxidant activity as evaluated by both free radical scavenging compared to that of non enzyme extracts. Higher level of antioxidant activity in *C. longa* has been attributed to the phenolic content in them. The free and bound phenolics of enzyme treated extract of *C. longa* showed high content of phenolic compounds (31.0 and 1.3 mg/g) compared to that of non enzymatic extract (28.8 and 3.5 mg/g). Our studies clearly suggest the presence of potent antioxidants in C. longa.

Keywords: Antioxidant; Phenolics; Curcuma longa

Introduction

Epidemiological studies have suggested an inverse relation between the consumption of polyphenol-rich foods and beverages and the risk of degenerative diseases, particularly cancers and cardiovascular diseases [1-2]. In this regard, there has been a great deal of interest in the screening and characterization of novel potentially therapeutic of compounds polyphenol-rich extracts from foods and medicinal plants [3-5]. The antioxidant properties of polyphenols and their ability to modulate the activity of various enzymes have been demonstrated in in vitro studies and are believed to be a primary mechanism for their biological effects [6]. However, the question remains of whether these properties demonstrated in in vitro studies are relevant to protect against oxidative damage in vivo, where polyphenols are at a very low concentrations depending on bioavailability and metabolism. In recent decades, various species of plants have been used and consumed due to the presence of high antioxidant activities. The extracts of medicinal plants and natural products become a great source of antioxidant and anti-ageing properties. Antioxidant is a protection agent which can terminate the initiation of oxidizing chain reactions and it can inhibit or delay oxidation by other molecules. Oxidation processes are important because it can control the production of free radicals and the unbalanced mechanism of antioxidant protection that can cause diseases and accelerated ageing.

Curcuma longa, a perennial herb and member of the Zingiberaceae family, grows to a height of three to five feet and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. Antitumour [7-8], hepatoprotective [9] and hypolipidemic [10], activities are reported for *Curcuma longa*. The main curcuminoid curcumin possesses anti-infammatory, antiarthritic, antioxidant [11-13], hepatoprotective activities [14-15]. The purposes of this study were to determine the content of total phenolics (Free and bound) and to evaluate antioxidant activity of *Curcuma longa*.

Materials and methods

Materials

Curcuma longa rhizome was purchased from a local market. 1,1-Diphenyl-2-picryl hydrazyl (DPPH), Folin-Ciocalteau reagent gallic acid were purchased from S.D. Fine-chem. Ltd; Mumbai. The other chemicals such as ferric chloride, sodium carbonate and solvents used were of the analytical grade purchased from a local chemical company.

Isolation of free phenolic acids

Free phenolics were isolated according to the method followed by [16] with some modification. Ten gram each of *C. longa* powder (in triplicate) was extracted (1:50, w/v) in 70% methanol (3x500mL, 2 h each), and the supernatants were obtained by centrifugation (Sigma 3-16K, USA) at 3000g for 15 min and concentrated by flash evaporation; the pH was adjusted to 1.5 with 4 N hydrochloric acid. Phenolic acids were separated by ethyl acetate phase separation (4x500 mL) and the pooled fractions were treated with anhydrous sodium sulphate, filtered and evaporated to dryness. Total phenolic acid was estimated spectrophotometrically by Folin-Ciocalteu method with gallic acid as the reference standard and expressed as gallic acid equivalent (GAE) in milligrams per gram dry weight (dw) of sample.

Isolation of bound phenolic acids

C. longa sample (10 g, each) were defatted with petroleum ether and chloroform (1:1, v/v) and extracted with 70% methanol (4x250 mL) to extract free phenolic acids. The dried samples were extracted with 1M sodium hydroxide (2x100 mL) containing 0.5% sodium borohydride under nitrogen atmosphere, and the clear supernatants were collected by centrifugation [17]. The combined supernatants (bound phenolics) were acidified with 4 N hydrochloric acid to pH 1.5 and the phenolic acids were extracted and quantified colorimetrically in the same way as free phenolic acids.

Enzyme assisted isolation of free and bound phenolic acid

Cellulase was quantified accurately and dispersed in 70% methanol to obtain enzyme solutions of concentrations (2.5 mg/mL) and pH was adjusted to 5.5 with 0.1 M HCl solution and shaken on a flat-bed orbital shaker for a period of 8 hr at a temperature of 50ºC. After the enzymatic treatment the same procedure is followed as mentioned above for the isolation of free and bound phenolics. All the experiments were performed in triplicate.

DPPH scavenging activity of bound and free phenolic fractions

The radical–scavenging activity of the extract was determined using Schmeda- Hirschmann et al. method [18]. Accurately 6 ml of DPPH (20μg/ml) methanolic solution was added to 20 μl of DMSO solution of each extract at room temperature. The mixture was shaken vigorously and absorbance was measured at about 517 nm using spectrophotometer. All tests were run in triplicate and mean values were taken for calculation.

Results and Discussion

Different proportions of free and bound phenolics were observed in enzyme treated extract and non enzyme treated extract of *C. longa*. The free phenolic content of enzyme treated extract of *C. longa* was 31.0 mg GAE/g, 12.2 % higher than that of (28.8 mg GAE/g) as measured by the Folin-Ciocalteu method. In addition, lower bound phenolics were observed in enzyme treated extract of *C. longa* (1.3 mg GAE/g) in comparison with that of bound phenolics (3.5 mg GAE/g). This indicates that enzyme has played a role in releasing the bound phenolics from the *C. longa* (Fig 1).

Antioxidant activities in various phenolics

Several studies reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties [19-21]. To evaluate the antioxidant activity of different phenolic

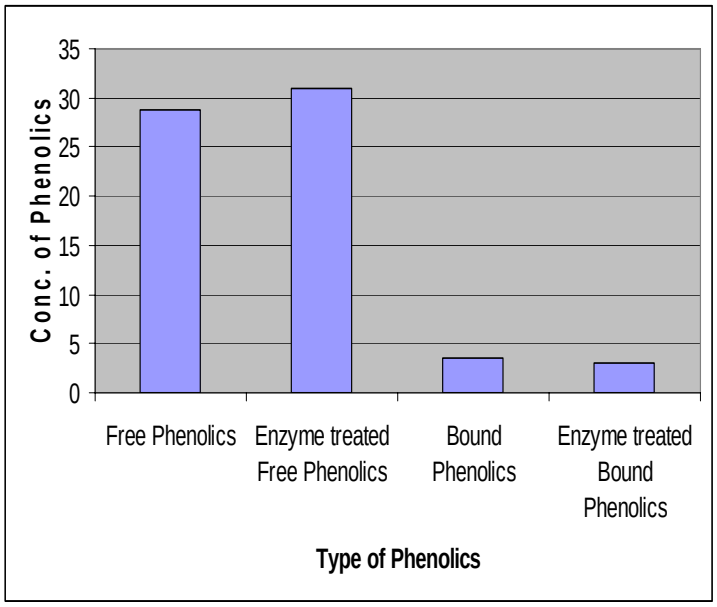

Figure. 1: Comparison of Enzyme treated and non enzyme treated release of Phenolics

fractions, the free radical scavenging activity by DPPH method were analyzed. Fig. 2 indicates a increase in the activity of enzyme treated free phenolic extract compare to bound phenolic fractions of *C. longa*.

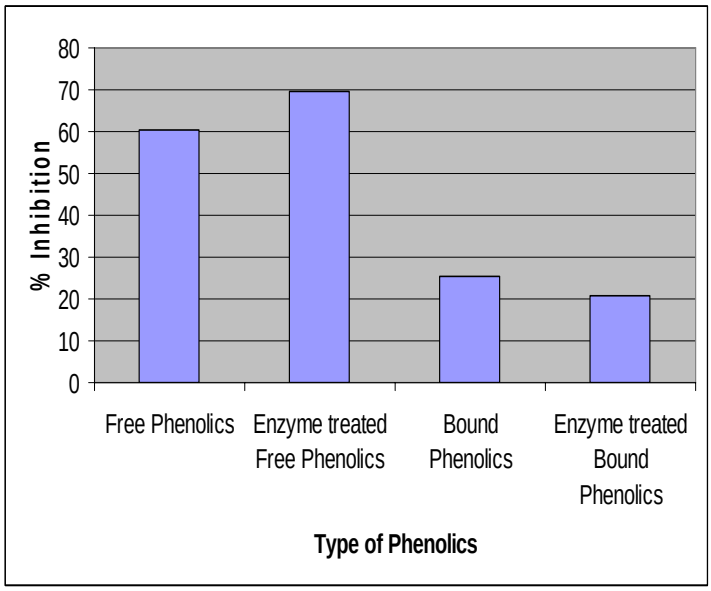

Figure. 2: Antioxidant activity of Phenolics of *Curcuma longa*.

Figure 3 shows the correlation between total phenolic content and DPPH assay of C.*longa*. Results showed a positive correlation coefficient between the total phenolic content and DPPH assay of plants extracts. A significant and linear relationship existed

between the antioxidant activity and phenolic content of turmeric, thus indicating that phenolic compounds are major contributors to antioxidant activity.

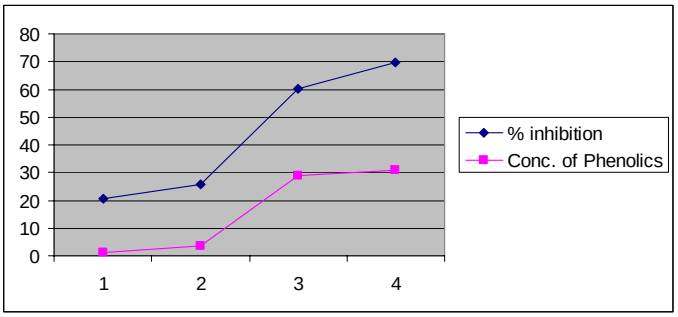

Figure 3: Correlation between total phenolics and antioxidant activity

Conclusion

The results obtained demonstrated that enzyme treated turmeric extract had the highest total phenolic content and antioxidant activity compared to non enzyme treated turmeric. There was a good correlation between total phenol content and antioxidant activity that support the idea of phenols as contributor of the antioxidant power of plants extracts.

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