

Original Article

ANTIOXIDANT EFFICACY AND CURCUMIN CONTENT OF TURMERIC (*CURCUMA-LONGA* L.) FLOWER

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ABSTRACT

Objective: The objective of the work is to evaluate the curcumin content, antioxidant activity and total phenol content of *Curcuma longa* flower.

Methods: Curcumin content were analyzed through HPLC-PDA analysis. The antioxidant activity of the flower was determined by free radical scavenging activity of DPPH, and the total phenol content was determined using the Folin-Ciocalteu reagent through standard protocols.

Results: The flowers had found about (3.87±0.5µg/g) of curcumin during HPLC-PDA analysis. The antioxidant activity (DPPH free radicals scavenging activity IC₅₀) of the turmeric flower (3.2 µg/ml) was found near to the standard ascorbic acid (2.6 µg/ml). In addition to these, a significant amount of phenolics (210.45±1.32 mg GAE/100 g) was present in the turmeric flowers.

Conclusion: The flowers of *curcuma longa* contains a significant amount of curcumin, antioxidant activity and phenol contents.

Keywords: Turmeric flower, Curcumin, Antioxidant activity, DPPH, HPLC

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INTRODUCTION

The turmeric (*Curcuma longa*), a rhizomatous herb of family Zingiberaceae, is used as a spice, coloring agent and traditional medicine since the ancient time in South Asian and Middle Eastern countries. Turmeric is everyday ingredients of food in Indian houses. The major and active curcuminoids of turmeric, curcumin (1, 7-bis (4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione) exhibits anticancer, antioxidant, anti arthritic, anti-malarial, anti-inflammatory activity [1-3]. The flower of the plant is rich in phenolics like curcuminoids and sesquiterpenoids. Ming *et al.* [4] first time studied the chemical composition of flowers, rhizomes and leaves of turmeric and concluded that monoterpenoids (85%) were major constituents in turmeric flower. The Sesquiterpenoids (Zingiberene, β-sesquiphellandrene, β-caryophyllene, ar-curcumene, β-bisabolene (E)-β-farnesene) were present in significant amount in flower oil.

Antioxidative nutraceuticals of food ingredients scavenge free radicals generated and protect the body by reducing impurity functioning of the immune system and improved vitality against infection. Free radicals are generated inside the body during different biochemical process occurring in the human body. The pharmacological importance of plant is determined by the amount of biochemicals and ingredients present in the plants. Different types of methodology have been used for the determination, separation and quantification of compounds in plants samples. There are a variety of methods available in the literature for the quantification of curcuminoids and sesquiterpenoids. The fresh turmeric extract has HPLC-UV diode-array and electrospray mass spectrometer method to analyze curcuminoids and sesquiterpenoids. Some improved HPLC method has been employed for separation and quantification of curcumin, desmethoxycurcumin and bisdemethoxycurcumin in turmeric [5, 6]. Several authors have reported the quantification and pharmacological importance of curcumin in the turmeric rhizome. In the present study, turmeric flower is used for the determination of curcumin content, antioxidant efficacy and total phenol content.

MATERIALS AND METHODS

Plant materials for curcumin quantification

For the quantification of curcumin and antioxidant activity of turmeric flower, young and mature flowers were collected from the

botanical garden of Banaras Hindu University, India (20° 18'N and 80 ° 36' E, elevation 80.71m). The flower of turmeric (1.0 g each) was treated with hexane (50 ml) by using a Soxhlet extractor (30 min) for extraction. The hexane was discarded through a rotary evaporator. Extracted samples were dissolved in 50 ml of methanol for 2 h and filtered through 0.2 µm Millipore filter.

Preparation of stock solution

The stock solutions of curcumin were prepared in them ethanol at a concentration of 0.5 mg/ml to 10 mg/ml.

Chemical and reference compound

All solvents and chemicals used were of either analytical or HPLC grade (E. Merck, Mumbai, India). A standard sample of curcumin obtained from Sigma-Aldrich, (Bangluru, India) and before use, all solvents were filtered through 0.2 µm Millipore membrane filter.

Equipment and chromatic condition

The HPLC analysis was performed on a system consisting of Hewlett-Packard quaternary HP1090 Series (Hewlett-Packard paloAtto CA, USA) with multi-wavelength Photodiode-Array detector between 200 nm to 500 nm and managed by computer system HP 9000 workstation. The quantification of compounds was performed by using Luna RP-C18 prepacked column (150 nm× 3m) with a particle size of 5 mm. Acetonitrile and 2% acetic acid 60:40(v/v) used as mobile phase with the flow rate of 0.7 ml/min and the injected volume was 20 µl.

Validation of HPLC methods

Identification and specificity of peaks: Identification of peak in the chromatogram was performed by HPLC-PDA detector by comparing the retention time of turmeric flower with the standard compounds. The UV spectra of a standard compound were matched with the UV-visible spectral sample track of turmeric. In both the standard and sample peak, Peak purity was greater than 0.9899. The standard curcumin samples solution ranging (0.5-10.0) mg/ml of curcumin were prepared and injected three times for linearity test. Linearity was found in the concentration range between 1-7µg with high reproducibility and accuracy. The correlation Coefficient (R²) of

curcumin was 0.9980 and the linear regression equation for the curve was $215e+004x-2.34e+004$ achieved.

Antioxidant activity of turmeric flower

DPPH radical scavenging assays on TLC

To determine the antioxidant activity of turmeric flower, 5 µl (1:10 dilution in methanol) was applied on TLC plate and developed in ethyl acetate and methanol (1:1). The solution of 0.25 DPPH (2,2-diphenyl-1-picrylhydrazil) in methanol solution was spread on the plate and kept at room temperature for 30 min. Development of yellow spot from the purple color on the plate, shown the confirmation of antioxidant activity of turmeric.

Free radical scavenging activity

The free radical scavenging activity of turmeric flower was measured by measuring the extent of bleaching of purple-colored DPPH solution to yellow. Different concentration (1 mg to 5 mg) of the samples were added to (5 ml) of 0.004% DPPH solution in methanol and kept for 30 min at the room temperature. The absorbance was taken against a blank at 517 nm using a spectrophotometer. Scavenging of DPPH free radical with a reduction in absorbance of the sample was taken as a measure of their antioxidant activity [7]. Ascorbic acid was used as positive control. IC₅₀, which represented the concentration of sample that caused 50% neutralization of DPPH radicals, was calculated from the graph plotting between percentage inhibition and concentration.

$$I \% = \left(\frac{A_{\text{Blank}} - A_{\text{sample}}}{A_{\text{Blank}}} \right) \times 100$$

Where, A_{Blank} is the absorbance of the control (Without test compound), and A_{sample} is the absorbance of the test compound.

Determination of total phenolic content of turmeric flower

Total phenolic contents of ethanolic extract of the flower were determined using the Folin-Ciocalteu reagent according to the method of [8]. A solution was made by mixing the 1000 µg sample mixed with 46 ml sterilized distilled water and 1 ml Folin-Ciocalteu reagent. The mixture was mixed by a shaker for 3 min to react. 3 ml aqueous solution of 2% Na₂CO₃ was then added to it was then added to it and left for 2 h incubation period at room temperature. The absorbance of each mixture was measured at 760 nm in the spectrophotometer. The same procedure was also applied to the standard solution of gallic acid, and an equation was obtained by the standard curve. Total phenolic contents of the turmeric flower were obtained by putting the absorbance value of the sample at 760 nm to standard curve and equation expressed as µg gallic acid equivalent/mg of the sample.

RESULTS AND DISCUSSION

Curcuminoid is an important component of turmeric. Among curcuminoids, curcumin is an important constituent. Extensive research in past half century has indicated the importance of curcumin in pharmacology. The HPLC chromatogram of turmeric flower at the wavelength of 425 nm observed two peaks at the retention time (RT) of 3.057 and 4.320. The peak at the retention time of 4.320 ± 0.056 was identified as curcumin. The purity of the compounds was checked through the standard of curcumin (RT 4.391) (fig. 1a and fig. 1b)

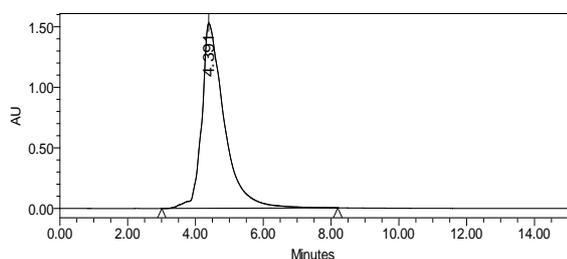


Fig. 1a: HPLC chromatogram of standard of curcumin at 425 nm

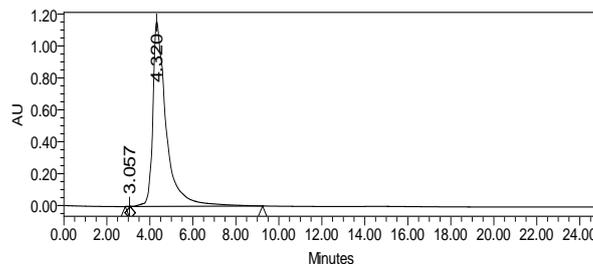


Fig. 1b: HPLC chromatogram of Curcuma longa flower at 425 nm

Analysis of curcumin content in the turmeric flower were performed using HPLC-PDA detector. The amount of curcumin was measured by comparing the peak area of turmeric flower chromatogram to the standard curved which were made by the running the different concentration of curcumin (0.5-6.0 mg/ml) (fig. 1c). Concentrations of curcumin were calculated on the basis of linear calibration function with regards to the dilution factor. In this study curcumin content in the turmeric, the flower was found in the range of $3.87 \pm 0.5 \mu\text{g/g}$ of the flower which proved as a good choice of curcumin.

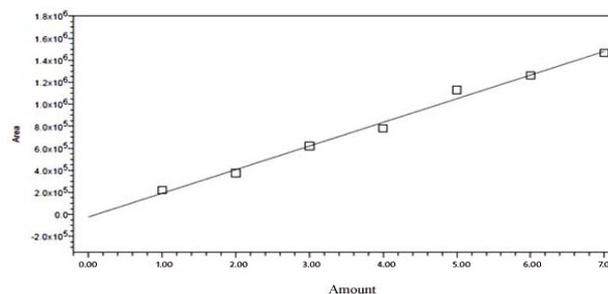


Fig. 1c: Linear curve of area vs. concentration of curcumin

Antioxidant activity of turmeric flower

The Antioxidant activity of the turmeric flower was evaluated by comparing the IC₅₀ of the ascorbic acid, BHT. During DPPH free radical scavenging activity the IC₅₀ of the turmeric flower is found to be $3.3 \pm 0.20 \mu\text{g/ml}$ which is closer to the ascorbic acid $2.6 \pm 0.25 \mu\text{g/ml}$ (fig. 2).

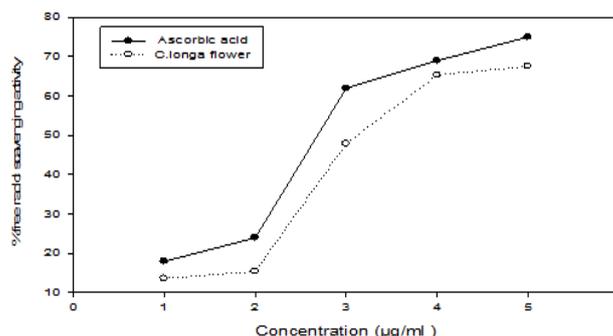


Fig. 2: Antioxidant activity of Curcuma longa flower

The low IC₅₀ values of turmeric flowers strengthen their application as a natural antioxidant in food preservatives and also in the human health to reduce the oxidation process. Antioxidants had various beneficial effects on human health, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene

(BHT) are often added to food. Nevertheless, this practice is being restrained due to their carcinogenicity, and synthetic antioxidants are known to prompt liver and kidney dysfunction [9, 10].

The antioxidant activity of the turmeric flower is not as effective as the synthetic one like ascorbic acid, but this may be used as a natural antioxidant. Antioxidant activity of the compound depends on upon the amount of phenolic compounds in the plants.

Total phenolic content (TPC) in the turmeric flower had been found in the range of 210.45 ± 1.32 (mg GAE/100 g). Amount quantified is based on the linear equation obtained from the gallic acid standard calibration curve (fig. 3).

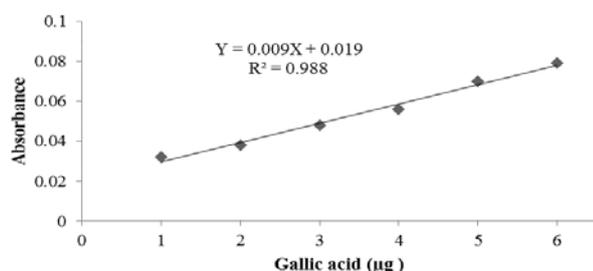


Fig. 3: Linear curve of Absorbance vs. concentration of standard graph of Gallic acid

Thus, TPC values were expressed as gallic acid equivalent (mg GAE/100 g samples). Phenolic compounds are hydroxylated derivatives of benzoic and cinnamic acids that are responsible for the antioxidant activity. The number of phenolic groups present in the structure of an antioxidant molecule is not always the only factor in determining its antioxidant activity. Additionally, polarity and hydrophobicity of antioxidants play an important factor in the antioxidant activity especially in the biomembrane systems [11].

In general, the antiradical and antioxidant activities of plant extracts are associated to the phenolic content [12]. The turmeric flower elicited the higher total phenolic content may be due to the fact that it contains phenol compounds like curcuminoids and sesquiterpenoids.

CONCLUSION

Turmeric flower contains a significant amount of curcumin ($3.87 \mu\text{g/g}$) determined by the HPLC-PDA detector analysis. The flowers also contain antioxidant activity (IC_{50}) $3.2 \mu\text{g/ml}$ closer to the standard ascorbic acid $2.6 \mu\text{g/ml}$, which showed importance in pharmacology and methanolic extracts of turmeric flower also contains 210.45 ± 1.32 (mg GAE/100 g) of phenolics.

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CONFLICT OF INTERESTS

The authors have no any conflict of interest

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