



Evaluation of Bioactive Compounds and Free Radical Scavenging Potential of *Curcuma longa* L. Rhizomes

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ABSTRACT

The present investigation focuses on the phytochemical characterization and antioxidant potential of an ethanolic extract of *Curcuma longa* rhizomes. Standard qualitative phytochemical assays revealed the presence of carbohydrates, amino acids, saponins, alkaloids, tannins, phenols, flavonoids, glycosides, terpenoids, and phlobatannins. Quantitative estimations showed that the extract contains 75.6 ± 2.0 mg gallic acid equivalents (GAE)/g and 31.4 ± 3.2 mg rutin equivalents (RE)/g of phenols and flavonoids, respectively. Hydrogen peroxide scavenging activity increased proportionally up to $100 \mu\text{g/ml}$, after which a steady plateau was observed, similar to the activity of the gallic acid standard. The EC_{50} value of the extract was calculated as $41 \pm 2.3 \mu\text{g/ml}$, indicating a strong antioxidant capacity. The study reinforces the correlation between high phenolic and flavonoid content and the potent free radical scavenging properties of *Curcuma longa*.

Keywords: Phytochemicals, *Curcuma longa*, Polyphenols, Flavonoids, Antioxidant activity, H_2O_2 scavenging.

INTRODUCTION

Medicinal plants have long been valued for their therapeutic significance, primarily due to their rich reservoir of bioactive secondary metabolites [1]. In India, one such important medicinal plant is *Curcuma longa* L. (turmeric), a member of the Zingiberaceae family. It is a rhizomatous perennial herb cultivated widely in tropical and subtropical regions including India, Indonesia, Taiwan, China, and various African nations [2, 3]. Turmeric has been traditionally used as a dietary spice, natural colorant, and preservative, and it holds a prominent place in Ayurvedic, Unani, Siddha, and traditional Chinese medicine [4-6]. The rhizome contains several active phenolic compounds which are collectively known as curcuminoids (1-6%), which include curcumin (approx 77%), demethoxycurcumin (approx 18%) and bisdemethoxycurcumin (approx 5%), which are responsible for its characteristic color and pharmacological properties [7]. The concentrations of different curcuminoids may vary depending on the variety and environmental conditions of their place of growth. These compounds, along with volatile oils and nutritional components such as carbohydrates, proteins, fibers, and minerals, contribute to turmeric's antimicrobial, anti-inflammatory, antioxidant, anticlotting, and anticancer activities [8-10].

Despite extensive research on curcumin, comprehensive studies on crude extracts, particularly ethanol extracts that often contain a broader spectrum of phytochemicals remain essential. Such studies provide valuable insights into synergistic interactions among components that contribute to biological activity. Therefore, the

present study aims to evaluate the phytochemical constituents and antioxidant properties of the ethanolic extract of *C. longa* rhizomes, with special emphasis on phenolic and flavonoid content and hydrogen peroxide scavenging capacity.

MATERIALS AND METHODS

Collection and preparation of plant extract.

The rhizomes of most commonly grown species of *Curcuma longa* were collected from Madhepura district, Bihar. The collected rhizomes were then washed with distilled water followed by cutting and slicing into small fragments with the help of sterilized knife. This fragments were then spread onto a white sheet and left under shade for air drying for three weeks. When the moisture content of the fragments was completely lost, the fragments were grinded and processed into fine powder with the help of kitchen mill grinder. The fine powder was stored in an air tight zip-lock bag to prevent contamination. The stored fine powder was dissolved in 50% Ethanol. This mixture was then filtered with the help of Whatman No. 1 filter paper. The filtrate was then dried using a rotary evaporator under reduced pressure and $40-50^\circ\text{C}$ temperature and crude extract obtained was stored in a refrigerator (4°C) for future experiments.

Phytochemical Analysis

The qualitative phytochemical analysis was done for the detection of several phytochemicals found in *Curcuma longa* powder. Following tests were performed for this purpose [11-15].

Quantitative analysis of turmeric extract

The following tests were performed for determining the concentration and amount of the active compounds present in the prepared turmeric samples.

Evaluation of total phenols

Assessment of the total phenolic content of turmeric extract was done using Folin-Ciocalteu reagent with the help of Singleton and Rossi et al. (1965) method [16]. 200 μ l of the sample of turmeric extract (1mg/ml) was mixed with 1.5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and left for 5 minutes at 22^o C. 1.5ml of 6% sodium carbonate was mixed with this and kept at 22^o C for 90 minutes in dark. Further, this mixture was centrifuged for 5 minutes at 13000 rpm and left for incubation in dark for 45 minutes. Finally, absorbance was taken with the help of Biochrom spectrophotometer at 765 nm. The results were derived from a standard graph of Gallic acid. The values were demonstrated as mg of Gallic acid equivalents (GAE)/g of turmeric extract. This protocol was followed repeatedly for three consecutive days under the same laboratory setup and finally mean value was obtained [17].

Evaluation of total flavonoids

Evaluation of total flavonoid content of turmeric extract was done using aluminium chloride via calorimeter analysis. 1 ml (1mg/ml) of the turmeric extract was taken in a volumetric flask and 5ml of distilled water was added to it. To this 0.3ml of 5% sodium nitrite was mixed and left for incubation for 5 minutes at 37^o C. After this, 0.3ml of 10% AlCl₃ and 2 ml of 1M NaOH was added along with distilled water to make the final volume 10ml. After proper mixing of solution, absorbance was taken with the help of UV-visible spectrophotometer at 510 nm against blank [18]. At last the total flavonoid content was as mg of Rutin Equivalents (RE)

Anti-oxidative analysis of turmeric extract

Hydrogen peroxide scavenging activity

Determination of the hydrogen peroxide scavenging activity of turmeric extract was done using Bozin et al. (2008) method [19]. Turmeric extracts of different concentrations ranging from 25-100 μ g/ml was freshly prepared. 2.5 ml of phosphate buffer (0.1M, pH 7.4) and 0.5 ml of H₂O₂ (40mM) solution was mixed with 1ml of the extract and was left for incubation for 15 minutes at 25^o C. The measurement of absorbance of the solution was done at 230 nm against blank solution containing only turmeric extract. Following formula was used for calculating the percentage inhibition of turmeric extracts.

Percentage (%) Scavenged (H₂O₂) = Abs (control)-Abs (extract)/Abs (control) X100

Where, Abs (control) was the absorbance of the control (without extracts)

Abs (extract) was the absorbance in the presence of extracts.

RESULTS AND DISCUSSION

Both aqueous and ethanolic extracts showed the presence of tannins, flavonoids, glycosides, reducing sugars, phlobatannins, alkaloids, carbohydrates, and terpenoids (Table 1). Phenols, saponins, and steroids were absent in the aqueous extract but present in the ethanolic

extract. These results correlate with earlier findings by Swadhini et al. (2011) [20], while Sawant and Godghate (2013) reported variability in phenolic detection depending on extraction solvent [21].

Phenolic compounds are major contributors to antioxidant activity. The ethanolic extract exhibited 75.6 \pm 2.0 mg GAE/g of phenols and 31.4 \pm 3.2 mg RE/g of flavonoids (Table 2). These values are comparable to previously published reports of Maithilikarpagaselvi et al., (2020) for turmeric extracts [17]. The concentrations confirm that *C. longa* is a rich source of polyphenols, which are key molecules responsible for reducing oxidative stress. Figure 1 shows the total phenolic content of the ethanol extract of turmeric ranging from 10-100 μ g and Figure 2 represent the total flavonoid content of the ethanol extract of turmeric ranging from 10-100 μ g.

Hydrogen peroxide, though not a free radical itself, generates hydroxyl radicals upon reaction with metal ions, contributing to oxidative damage. The ethanolic extract of turmeric demonstrated strong scavenging activity, increasing progressively with extract concentration. The EC₅₀ value of 41 \pm 2.3 μ g/ml suggests efficient

Table 1: Phytochemical Profiling of Turmeric Extracts

Phytochemicals	Aqueous extract	Ethanolic extract
Phenols	-	+
Tannins	+	+
Flavonoids	+	+
Glycosides	+	+
Saponins	-	+
Steroids	-	-
Reducing sugars	+	+
Phlobatannins	+	+
Alkaloids	+	+
Carbohydrates	+	+
Proteins	+	-
Terpenoids	+	+

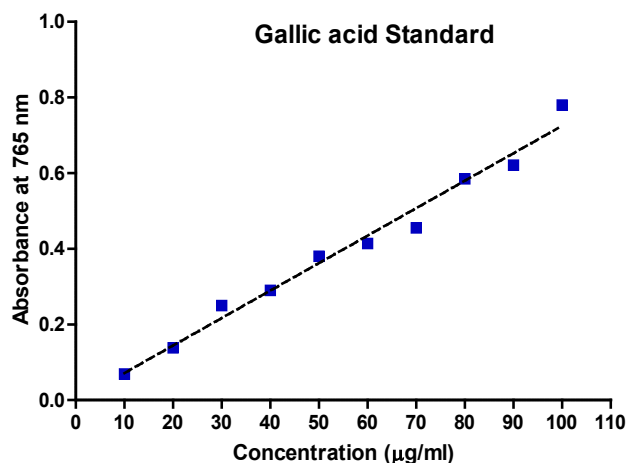


Figure 1: Total phenolic content in turmeric extract.

Table 2: Total phenolic and flavonoid contents as well as H₂O₂ scavenging activity of turmeric extract

Turmeric extract	Total phenol content (mg of Gallic acid equivalent (GAE)/g of extract)	Total flavonoid content (mg of Rutin equivalent (RE)/g of extract)	H ₂ O ₂ scavenging Assay (μg/mL)
Ethanol extract	75.6 ± 2.0	31.4 ± 3.2	41 ± 2.3

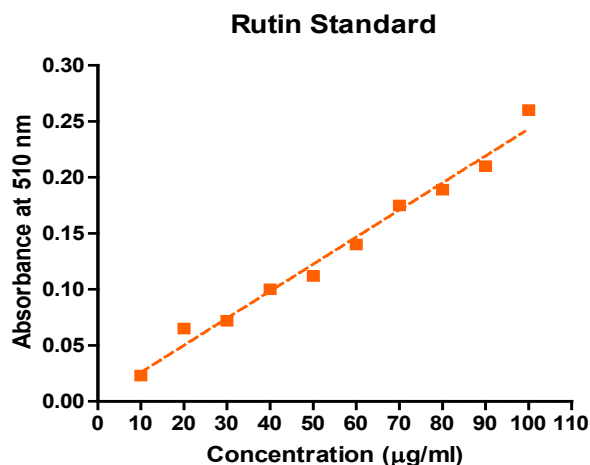
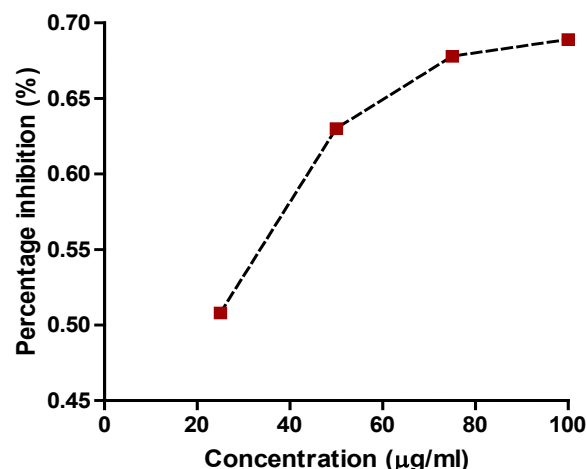


Fig. 2: Total flavonoid content in turmeric extract.

Fig. 3: H₂O₂ scavenging assay of turmeric extract

neutralization of H₂O₂, close to the standard antioxidant gallic acid (Figure 3). The plateau observed after 100 μg/ml indicates saturation of active sites, a typical phenomenon in antioxidant assays, reflecting maximal scavenging efficiency of the phytochemical constituents. This aligns with earlier research emphasizing turmeric's potent radical quenching ability [22,23].

CONCLUSION

The study provides a detailed analysis of the phytochemical composition and antioxidant properties of ethanolic turmeric extract. The presence of diverse bioactive compounds especially phenols and flavonoids, correlates strongly with its notable hydrogen peroxide scavenging activity. The EC₅₀ value of 41 ± 2.3 μg/ml demonstrates substantial antioxidant potential, comparable to standard gallic acid.

These findings suggest that *Curcuma longa* rhizome extract can serve as a promising natural antioxidant source for nutraceutical, pharmaceutical, and food industries. Further studies involving purification of active components and *in-vivo* antioxidant evaluation would help substantiate its therapeutic relevance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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