IN VITRO BIOEVALUATION OF ANTIOXIDANT ACTIVITY IN CURCUMA LONGA

1Prasanthi Donipati*, 2Dr. S. Hara Sreeramulu

1Research Scholar, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India - 530 013, INDIA
2Professor & Head of the Dept. of Biotechnology, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India-530 013, INDIA

Abstract

Turmeric is a spice derived from the rhizomes of Curcuma longa, which is a member of the ginger family (Zingiberaceae). Rhizomes are horizontal underground stems that send out shoots as well as root. The present study was undertaken to compare the antioxidant activity of hexane, chloroform and methanolic extract of rhizomes between PHENOLS, ferric-reducing antioxidant power assay (FRAP), IRON REDUCTION TEST, Diphenyl picrial hydrazyl radical scavenging assay (DPPH). A graph is plotted between enzymatic, non enzymatic antioxidant levels and concentration. The results showed that, Diphenyl picrial hydrazyl radical scavenging assay (DPPH) has more hexane, chloroform and methanolic concentrations. In IRON REDUCTION TEST, PHENOLS and FRAP, methanolic concentrations are present whereas hexane, chloroform concentrations were completely absent.

Keywords: Curcuma longa, PHENOLS, (FRAP), IRON REDUCTION TEST, (DPPH).

Corresponding Author:

Prasanthi Donipati
Research Scholar,
Dr.V.S.Krishna Govt. College,
Visakhapatnam, A.P, INDIA
E-mail: prashanthi.christopher@gmail.com
Phone: +91 9885653508

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INTRODUCTION

The genus *Curcuma* (family zingiberaceae) comprises of more than 80 species of rhizomatous herbs. They occur in wild and cultivated forms and are widely distributed throughout the tropics of Asia, Africa and Australia [1]. Most of the *Curcuma* species grow in mountainous areas of the world, but some common species are often cultivated in gardens and used as a spice, food preservative and coloring agent and as medicinal plants. The volatile oils of the rhizomes and leaves of these plants can be prepared by steam distillation or solvent extraction, and show a wide spectrum of medicinal applications [2]. In East Asia, the rhizome of *Curcuma longa* L. (turmeric) is widely used as a spice, coloring, flavoring, and traditional medicinal preparations. It has been also considered as an analgesic in the treatment of menstrual disorders, rheumatism, and traumatic diseases due to a number of components, such as monoterpenoids, sesquiterpenoids, and curcuminoids [3]. Furthermore, it has been noted that the materials of rhizome of *Curcuma longa* L. have antiplatelet [4], fungicidal [5], and repellent [6] properties. Curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin), the phenolic yellowish pigments of *Curcuma longa* L., have been suggested to have antioxidative, anticarcinogenic, antiinflammatory, and hypocholesterolemic activities. There are several studies that these beneficial properties of *Curcuma longa* L. have been associated to the antioxidant activity. In present study we have evaluated the antioxidant potential of various solvent extracts of *Curcuma longa* for antioxidant capacity assay.

MATERIAL AND METHODS

COLLECTION OF PLANT MATERIAL: The plant material used in present study was collected from (Gudala, Allavaram and Amalapuram) Andhra Pradesh. The plant materials were further identified in the Department of Botany, Dr.V.S.Krishna College, Visakhapatnam, India.

PREPARATION OF PLANT EXTRACTS

The rhizomes were cut into pieces and air dried at room temperature. The dried rhizomes were coarsely powdered and successfully extracted with methanol using Soxhlet extractor at a temperature of 55-60 °C for a period of 7-8 hrs and concentrated to dryness (crude extract). Extracts were filtered using Whatmann No.1 filter paper. The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.
ANTIOXIDANT CAPACITY ASSAY

FERRIC REDUCING OR ANTIOXIDANT POWER ASSAY (FRAP)
The total antioxidant power of the plant sample was assayed by the method as described earlier by [7]. The FRAP method for measuring the ferric reducing power (reduce the TPTZ-Fe (III) complex to TPTZ-Fe (II) complex ability) of plasma (FRAP) or plant extract. In the present FRAP assay, an aliquot of the samples (10-40 μl) was mixed with 3 ml of ferric-TPTZ-Fe (ii) reagent. The change in the absorbance was measured at 593 nm after initial mixing and up to 90 min. until it reached a plateau. Aqueous solution of known Fe (II) conc. (Feso4.7H2o) were used for calibration of the FRAP assay and Antioxidant. The results expressed as FRAP units.

DIPHENYL PICRIAL HYDRAZYL RADICAL SCAVENGING ASSAY (DPPH)
The DPPH (Diphenyl picrial hydrazyl) radical scavenging assay was carried out as described earlier by [8]. 5.0 ml of DPPH solution (0.004%) in methanol was added to 50 μl of plant extract. After 0.5 hrs of incubation period at room temperature, the absorbance was read against a blank containing a sample and methanol at 517 nm. Control containing the buffer and regent was carried out. Similarly positive controls are treated in the same way as test sample replaced by positive control. Butyl hydroxyl toline (BHT) used as positive control. Inhibition (I) Diphenyl picrial hydrazyl radical in present was calculated I the following way. Percentage of Inhibition (I) = Absorbance of control - Absorbance of test / Absorbance of control × 100.

TOTAL PHENOLIC COMPOUND ANALYSIS
The total phenolics were determined using the folin cio-caiteau reagent as reported by [9]. To 50 μl of each sample, 2.5 ml of folin cio-caiteau reagent and 2.5 ml of 7.5%(w/v) Na2CO3 was added and incubated at 45°C for 15 min. the absorbance values of all samples were measured in a spectrophotometer at 765 nm. The results were expressed as mg of Gallic acid equivalent per gm weight.

IRON (III) TO IRON (II)-REDUCING ACTIVITY
The ability of the extracts to reduce iron (III) was assessed by the method of [10]. A 1-ml aliquot of each extract, dissolved in water, was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of a 1% aqueous potassium hexacyanoferrate [K3Fe (CN) 6] solution. After a 30 min incubation at 50 C, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged for 10 min. A 2.5-ml aliquot of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous FeCl3, and the absorbance was recorded at 700 nm. Iron (III) reducing
activity was determined as ascorbic acid equivalents (mmol ascorbic acid/g extract). The values are presented as the means of triplicate analyses.

RESULT AND DISCUSSION

The importance of phenol compounds in plants as natural antioxidants and their use as substitutes to synthetic antioxidants in food additives is well known [11]. DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds [12]. The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals [13]. Thus, the purple colour of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to α, α-diphenyl-β-picrylhydrazine (yellow coloured) [14]. According to [15] scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants. In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plants extracts to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent [16,17]. A graph is plotted between enzymatic, non enzymatic antioxidant levels and concentrations of extracts shown in Fig 1. The results showed that, Diphenyl picrial hydrazyl radical scavenging assay (DPPH) has more hexane, chloroform and methanolic concentrations. In IRON REDUCTION TEST, PHENOLS and FRAP, methanolic concentrations are present whereas hexane, chloroform concentrations were completely absent. Free radicals are the cause for several major disorders. So, evaluation of antioxidant activity in plants could result in the discovery of natural antioxidants with pharmacological and food value.

![Graph showing correlation between solvent extracts of PHENOLS, FRAP, IRON REDUCTION TEST and DPPH activity](image-url)

**Fig. 1: The correlation between solvent extracts of PHENOLS, FRAP, IRON REDUCTION TEST and DPPH activity**

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CONCLUSION

The present study emphasizes the knowledge on the plant *Curcuma longa* Roxb. The rhizomes of the plant have enough bioactive properties as shown in the different animal model. The phytoconstituents are also proved to be identified. The results showed that, Diphenyl picrial hydrazyl radical scavenging assay (DPPH) has more hexane, chloroform and methanolic concentrations. In IRON REDUCTION TEST, PHENOLS and FRAP, methanolic concentrations are present whereas hexane, chloroform concenetrations were completely absent. This data may signify the investigations of different bio-active compounds from the plant *Curcuma longa* Roxb and the requisite level of activity (pharmacological & toxicological) would be considered for further scrutiny to develop the potential drug molecule.

REFERENCES


