



# INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

## IN VITRO BIOEVALUATION OF ANTIOXIDANT ACTIVITY IN *CURCUMA CAESIA*

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Accepted Date: 17/09/2015; Published Date: 27/10/2015

**Abstract:** *Curcuma caesia* is commonly known as kali haldi and it belongs to the family Zingiberaceae. This herb is available throughout north-east, central India, Papi Hills of East Godavari, West Godavari, and Andhra Pradesh. In the traditional system of medicine, fresh and dried rhizomes of *Curcuma caesia* Roxb are used. 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, IRON REDUCTION TEST has more hexane, chloroform and methanolic concentrations than Diphenyl picrial hydrazyl radical scavenging assay (DPPH), PHENOLS, ferric-reducing antioxidant power assay (FRAP).

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



PAPER-QR CODE

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Access Online On:

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How to Cite This Article:

Prasanthi Donipati, IJPRBS, 2015; Volume 4(5): 179-184

## 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]. *Curcuma caesia* is commonly known as black turmeric which is an erect rhizomatous herb with large leaves found throughout the Himalayan region, north-east and central India. The paste of rhizome is used traditionally for the treatment of leucoderma, asthma, tumor, piles etc. Essential oil of *C. Caesia* has been known for its antifungal activity [2] It is Fresh rhizomes are aromatic with intense camphoraceous odor and are applied externally to sprain and bruises[3] It rhizomes is used for migraine, 2-4 drops of fresh juice is poured in nose. For longevity, impotence, infertility, irregular menstrual flow, a spoonful powder from dried rhizomes is mixed with a spoonful of honey or a cup of milk is taken twice a day. For gastric troubles, a fresh piece of rhizome is chewed [4]. The rhizomes of kali haldi have a high economical importance because of its putative medicinal properties. The rhizomes are used in the treatment of smooth muscle relaxant activity [5]. Haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmintic, aphrodisiac, inflammation, gonorrhoeal discharges, etc [6]. The inner part of the rhizome is bluish-black in colour and emits a characteristic sweet smell, due to presence of essential oil [7]. In present study we have evaluated the antioxidant potential of various solvent extracts of *Curcuma caesia* 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 [8]. 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  $\mu$ l) 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. ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) 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 [9]. 5.0 ml of DPPH solution (0.004%) in methanol was added to 50  $\mu$ l 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 reagent was carried out. Similarly positive controls are treated in the same way as test sample replaced by positive control. Butyl hydroxyl toluene (BHT) used as positive control. Inhibition (I) Diphenyl picrial hydrazyl radical in present was calculated in the following way. Percentage of Inhibition (I) =  $\frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$ .

### Total phenolic compound analysis

The total phenolics were determined using the folin cio-caiteau reagent as reported by [10]. To 50  $\mu$ l of each sample, 2.5 ml of folin cio-caiteau reagent and 2.5 ml of 7.5%(w/v)  $\text{Na}_2\text{CO}_3$  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.

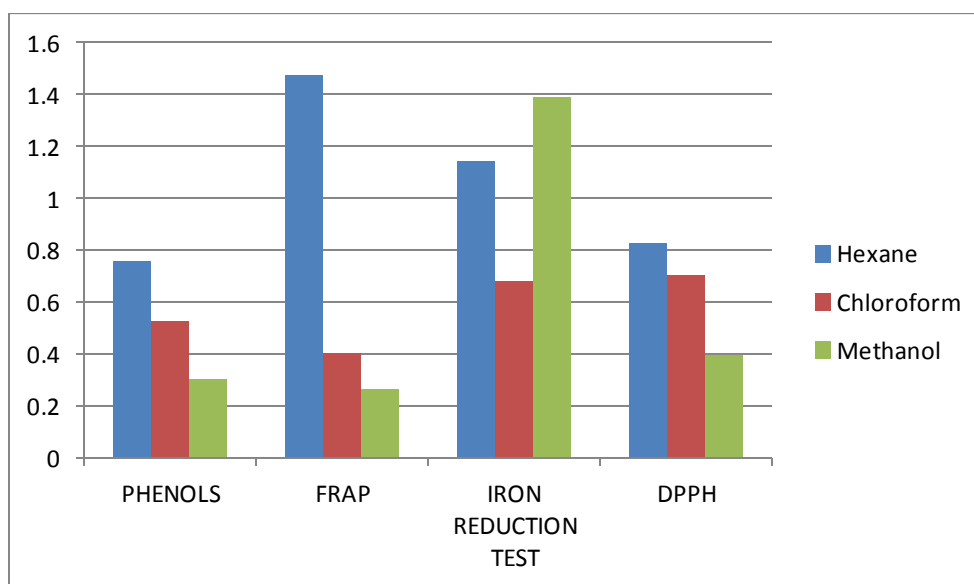
### Iron(III) to iron(II)-reducing activity

The ability of the extracts to reduce iron (III) was assessed by the method of [11]. 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 [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] 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  $\text{FeCl}_3$ , 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.

## RESULTS AND DISCUSSIONS

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. The importance of phenol compounds in plants as natural antioxidants and their use as

substitutes to synthetic antioxidants in food additives is well known [12]. DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds [13]. 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 [14]. Thus, the purple colour of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazine (yellow coloured) [15]. According to [16] 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 [17][18]. A graph is plotted between enzymatic, non enzymatic antioxidant levels and concentrations of extracts shown in Fig 1. The results showed that, IRON REDUCTION TEST has more hexane, chloroform and methanolic concentrations than Diphenyl picric hydrazyl radical scavenging assay (DPPH). In PHENOLS and FRAP, hexane, chloroform and methanolic concentrations are less when compared to IRON REDUCTION TEST and DPPH. There was a good correlation between IRON REDUCTION TEST and antioxidant activity (DPPH) that support the idea of phenols as contributor of the antioxidant power of plants extracts.



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

## CONCLUSION

The present study emphasizes the knowledge on the plant *Curcuma caesia* 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, IRON REDUCTION

TEST has more hexane, chloroform and methanolic concentrations than Diphenyl picrial hydrazyl radical scavenging assay (DPPH), PHENOLS, ferric-reducing antioxidant power assay (FRAP). This data may signify the investigations of different bio-active compounds from the plant *Curcuma caesia* Roxb and the requisite level of activity (pharmacological & toxicological) would be considered for further scrutiny to develop the potential drug molecule.

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