In Vitro Bio Evaluation and Correlation of Antioxidant Activity of Different Extracts of Curcuma amada

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ABSTRACT

Mango ginger (Curcuma amada Roxb.) is a unique spice having morphological resemblance with ginger but imparts a raw mango flavour. They also used in the manufacture of pickles, chutney, salad and jam. 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 picryl 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 concentratons than Iron Reduction Test, FRAP and Phenols. This article brings to light the major active components present in C. amada that may be important from the pharmacological point of view.

Keyword: Curcuma Amada; Phenols; (FRAP); Iron Reduction Test; (DPPH).

INTRODUCTION

Curcuma amada is a unique spice having morphological resemblance with ginger (Zingiber officinale) but imparts a raw mango (Mangifera indica) flavour. The genus name Curcuma was coined by Linnaeus in 1753 in his Species Plantarum. The word probably derives from the Arabic word ‘karkum’, which means yellow colour [1, 2] Curcuma amada Roxb is commonly known as mango ginger. It is a perennial, rhizomatous, aromatic herb belonging to the family Zingiberaceae. This family is composed of 70–80 species of rhizomatous annual or perennial herbs [3, 4]. The genus originated in the Indo-Malayan region, and is widely distributed in the tropics of Asia to Africa and Australia [5].
The mango ginger rhizome was found to be a rich source of fibres and starch [6]. Additional health benefits of *C. amada* rhizome reported were biliousness, itching, skin diseases, asthma and inflammation due to injuries [7]. Mango ginger has a typical exotic flavour of raw unripe mango. Therefore, it is used as a basic ingredient in pickles, preserves, candies, sauces, curries, salads and so on [8, 9]. A rhizome paste has traditionally been used for healing of wounds, cuts and itching [10]. The external use of the rhizome paste for sprains and skin diseases is also an old practice. The rhizome has carminative properties, as well as being useful as a stomachic [11]. In present study we have evaluated the antioxidant potential of various solvent extracts of *Curcuma amada* 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 Hexane, Chloroform and 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 extracts were 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 [12]. 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 Picryl Hydrazyl radical scavenging assay (DPPH)**

The DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging assay was carried out as described earlier by [13]. 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.

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\text{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 [14]. 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 gram weight.

**Iron (III) to iron (II)-reducing activity**
The ability of the extracts to reduce iron (III) was assessed by the method of [15]. 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.

**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 [16]. DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds [17]. 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 [18]. Thus, the purple colour of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to α, α-diphenyl-β-picrylhydrazine (yellow coloured) [19].

![Graph](image)

*Y axis indicates the concentrations of the Plant extract (µg/ml)*

**Fig 1:** The correlation between solvent extracts of Phenols, FRAP, Iron reduction test and DPPH activity

According to [20] 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 [21][22]. 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 than Iron reduction test, FRAP and Phenols. In Phenols, FRAP and Iron reduction test hexane concentrations are completely absent. There was a good correlation between Iron reduction test, FRAP and antioxidant activity (DPPH) that support the idea of phenols as contributor of the antioxidant power of plants extracts.

CONCLUSION
The present study emphasizes the knowledge on the plant Curcuma amada Roxb.. The results showed that, Diphenyl picrial hydrazyl radical scavenging assay (DPPH) has more hexane, chloroform and methanolic concentrations than Iron reduction test, FRAP and Phenols. In Phenols, FRAP and Iron reduction test hexane concentrations are completely absent. There was a good correlation between Iron reduction test, FRAP and antioxidant activity (DPPH) that support the idea of phenols as contributor of the antioxidant power of plants extracts.

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CONFLICT OF INTEREST STATEMENT
The authors declare that they have no competing interests.

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