



## *In vitro* Antioxidant Activity of *Coccinia Grandis* Root Extracts

Preeti Bhadauria<sup>\*a</sup>, Bharti Arora<sup>a</sup>, Birendra Vimal<sup>b</sup>, Akanksha Kulshrestha<sup>c</sup>

<sup>a</sup> Department of Pharmaceutical Chemistry, A.P.I.T.C.E, Agra, Uttar Pradesh, India

<sup>b</sup> Department of Pharmacology, Suresh Gyan Vihar School of Pharmacy, India

<sup>c</sup> Department of Pharmaceutical Sciences, Jaipur National University, India

Address for Correspondance: [preeti.ibs14@gmail.com](mailto:preeti.ibs14@gmail.com)

**ABSTRACT:** The present study was investigating the antioxidant activities of the various fractions of the hydromethanolic extract of the roots of *Coccinia grandis* L. Voigt. (Cucurbitaceae). The antioxidant activities of the fractions have been evaluated by using *in vitro* assays and were compared to standard antioxidants such as ascorbic acid,  $\alpha$ -tocopherol, curcumin and butylated hydroxyl toluene (BHT). All the fractions showed effective H donor activity, reducing power, free radical scavenging activity etc. The antioxidant property depends upon concentration and increased with increasing amount of the fractions. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the fractions. The results obtained in the present study indicate that the roots of *C. grandis* are a potential source of natural antioxidant. © 2011 IGJPS. All rights reserved.

**KEYWORDS:** *Coccinia Grandis*; Cucurbitaceae; Free Radicals; Antioxidant.

### INTRODUCTION

Antioxidants are agents which scavenge the free radicals and stop the damage caused by them. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA[1]. Antioxidants can be classified into two major classes i.e., enzymatic and non-enzymatic. The enzymatic antioxidants are produced endogenously and include superoxide dismutase, catalase, and glutathione peroxidase. The non-enzymatic antioxidants include tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are obtained from natural plant sources[2].

In India use of the different parts of several to cure specific ailments has been in vogue from ancient times. The indigenous system of medicine, namely, Ayurvedic, Siddha and Unani, has been in existence for several centuries. Some drug from Ayurveda approaching modern diseases, have already reached the market place[3].

The plant *coccinia grandis* belong to the family Cucurbitaceae commonly known as Ivy Gourd in English, Kovai in Tamil, Kovakka in Malayalam, kundru in Hindi. Description of plant are scandant shrubs, flowers white, Leaves alternate, fruit bright red in colour. It mainly present in asia and Africa[4].

*Coccinia grandis* voigt plays a major role in the medicinal properties. The plant part of *coccinia grandis* such as roots, leaves and fruits are used for numerous medicinal purposes like wound healing, ulcer, jaundice, diabetic and antipyretic. The roots also possesses antioxidant activity[5]. Natural antioxidants from plant extract provide a measure of production that slows the process of oxidative damage. Recent studies have shown that many flavanoids and related polyphenols contribute significantly to the total antioxidant activity of many plant extract[6]. Flavonoids are potent water-soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity[7-9]. The major group of phytochemicals that may contribute to antioxidant capacity of fruits includes polyphenols, carotenoids, and the traditional antioxidant vitamins such as vitamin C and E. The fruit polyphenols are the most important group of natural antioxidants because of their diversity and extensive distribution, and they possess the ability to scavenge both active oxygen species and electrophiles. The objective of the present study was to investigate the antioxidant activity of the different fractions of the hydromethanolic extract of the roots of *C. grandis* using *in vitro* models. Total phenolic and flavonoid content of the fractions were also determined in order to evaluate a relationship between the antioxidant activity and the phytochemical constituents.

## **MATERIALS & METHODS**

### *Collection of Plant Material*

Roots of *Coccinia indica* were collected from the locality of Bhopal (M.P.) India, during the month of September, 2011 and authenticated by Department of Pharmacognosy, RKDF College of Pharmacy, Bhopal. The voucher specimen is preserved for future reference.

### *Preparation of Extract*

Collected roots of *Coccinia indica* were air-dried under shade at room temperature and then crushed into coarse powder. This powder was extracted with methanol water (7:3) by soxhlet and the solvent was removed by evaporator. On removal of the solvent, a brownish colour residue was obtained. The yield was found to be 2.35 gm.

### *Chemicals*

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, gallic acid, rutin, ascorbic acid, BHA and Folin-Ciocalteu reagent were used. All other chemicals and reagents used were of analytical grade.

### *Phytochemical Screening*

Preliminary Phytochemical screening of the crude hydromethanolic extract of the roots was carried out.

### *In vitro Antioxidant Activity*

#### *a) DPPH Radical Scavenging Assay*

The free radical scavenging activity of the fractions was measured *in vitro* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (10). About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations (25-400 µg/ml). The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured 517 nm using a spectrophotometer. The % scavenging activity at different concentrations was determined and the IC<sub>50</sub> value of the fractions was compared with that of ascorbic acid, which was used as the standard. Decreasing

of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. DPPH radical-scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A<sub>0</sub> was the absorbance of the control (without extract) and A<sub>1</sub> was the absorbance in the presence of the extract.(11)

*b) Reducing Power Ability*

The reducing power was investigated by the Fe<sup>3+</sup>-Fe<sup>2+</sup> transformation in the presence of the fractions as described by (12). The Fe<sup>2+</sup> can be monitored by measuring the formation of Perl's Prussian blue at 700 nm . One ml of the fraction (50-800 µg/ml), 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide were incubated at 50°C for 30 min and 2.5 ml of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000g. About 2.5 ml of the supernatant was diluted with 2.5 ml of water and shaken with 0.5 ml of freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm. Butylated hydroxy toluene (50-800 µg/ml) was used as the standard. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

*c) Ferrous Chelating Ability*

The ferrous chelating ability of the fractions was monitor by measuring the formation of the ferrous ionferrozine complex. The reaction mixture containing 1.0 ml of different concentrations of the fractions (50-800 µg/ml) was mixed with 3.7 ml of methanol, 0.1 ml of 2 mM ferrous chloride and 0.2 ml of 5 mM ferrozine to initiate the reaction and the mixture was shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The percentage chelating effect on ferrozine-Fe<sup>2+</sup> complex was calculated. The IC<sub>50</sub> values were compared with ascorbic acid[13].

*d) Hydrogen Peroxide Scavenging Assay*

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the fractions (25-400 µg/ml) in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and the IC<sub>50</sub> values were compared with the standard, α-tocopherol[14].

*e) Nitric Oxide Radical Scavenging Assay*

This assay was performed according to the method described by[15]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by Griess reagent. The reaction mixture (3 ml) containing 10 mM sodium nitroprusside in phosphate buffered saline, and the fractions or the reference compound (curcumin) at different concentrations (50-800 µg/ml) were incubated at 25°C for 150 min. About 0.5 ml aliquot of the incubated sample was removed at 30 min intervals and 0.5 ml Griess reagent was added. The absorbance of the chromophore formed was measured at 546 nm. Inhibition of the nitric oxide generated was measured by comparing the absorbance values of control, fractions and curcumin (50-800 µg/ml).

*f) Estimation of Total Phenolic Content*

Total phenolic content was determined using Folin – Ciocalteu reagent as previously described[16]. Total phenol value was obtained from the regression

equation:

$$y = 0.0055x + 0.1139$$

and expressed as mg/g Gallic acid equivalent

using the formula:

$$C = c.V/M$$

Where C = total content of phenolic compounds in mg/g Gallic acid equivalent, c = the concentration of Gallic acid (mg/ml) established from the calibration curve, V = volume of extract, and m = the weight of pure plant Methanolic extract (g)[17].

*g) Estimation of Total Flavonoid Content*

The total flavonoid content of sample extracts was determined by the use of a slightly modified colorimetric method described previously (5). A 0.5 mL (1 mg/mL of methanol) extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% NaNO<sub>2</sub> solution. After 6 min of incubation, 0.15 mL of 10% AlCl<sub>3</sub> solution was added and allowed to stand for 6 min, and then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. All the values were expressed as g rutin equivalent (RE)/ 100 g of extract[18].

*h) Calculation of 50% Inhibitory Concentration (IC<sub>50</sub>)*

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions.

Percentage inhibition (I%) was calculated using the formula,

$$I \% = \frac{(A_c - A_s)}{A_c} \times 100$$

where A<sub>c</sub> is the absorbance of the control and A<sub>s</sub> is the absorbance of the sample.

*i) Statistical Analysis*

All experiments were performed in triplicate (n=3) and results were expressed as mean ± SEM. Statistical analysis was carried out with (SPSS package version 10.0) using ANOVA followed by Turkey's test (P<0.05).

## RESULTS & DISCUSSION

*Phytochemical screening*

Phytochemical screening of the crude hydromethanolic extract of the leaves of *Coccinia grandis* revealed the presence of flavonoids, saponins, phenols, tannins and terpenoids.

#### *DPPH assay*

All the fractions of *C. grandis* demonstrated H-donor activity. The highest DPPH radical scavenging activity was detected in chloroform fraction (IC<sub>50</sub> 0.135 mg/ml), followed by ethyl acetate, pet-ether and residual fractions (IC<sub>50</sub> 0.154, 0.39 and 0.8 mg/ml respectively) (**Table 1**). These activities are less than that of ascorbic acid. The scavenging ability increased towards the ethyl acetate fraction with increasing polarity of the solvent.

#### *Ferrous chelating ability*

Addition of the fractions of *C. grandis* interferes with the ferrous-ferrozine complex and the red colour of the complex decreased with the increasing concentrations of the fractions. All the fractions captured ferrous ions before ferrozine and thus have ferrous chelating ability. Among the fractions tested, the chloroform fraction showed the highest ferrous ion chelating ability (IC<sub>50</sub> 0.376 mg/ml). The abilities shown by pet-ether, ethyl acetate and residual fractions were almost similar (IC<sub>50</sub> 0.402, 0.433 and 0.505 mg/ml respectively). Ascorbic acid (IC<sub>50</sub> 0.017 mg/ml) showed the highest ferrous ion chelating ability (**Table 1**).

#### *Reducing power ability*

**Table 2** shows the reductive capabilities of different fractions of *C. grandis* when compared to the standard, BHT. Like the antioxidant activity, the reducing power increased with increasing amount of the fractions. The residual fraction of *C. grandis* showed the highest reducing ability (absorbance 0.620) than all the other fractions tested. However, the activity was less than the standard, BHT (absorbance 1.092). The pet-ether, chloroform and ethyl acetate fractions also showed significant activity indicating its reductive ability.

#### *Hydrogen peroxide scavenging assay*

All the fractions of *C. grandis* scavenged hydrogen peroxide in a concentration-dependent manner. The pet-ether fraction (PEF) of *C. grandis* showed strong H<sub>2</sub>O<sub>2</sub> scavenging activity (IC<sub>50</sub> 0.093 mg/ml) whereas that of the standard,  $\alpha$ -tocopherol was 0.075 mg/ml. The residual, chloroform and ethyl acetate fractions also showed significant scavenging activities (IC<sub>50</sub> of RF, CF and EAF were 0.135, 0.196 and 0.212 mg/ml respectively) when compared to the standard (**Table 1**).

#### *Nitric oxide radical scavenging assay*

The fractions of *C. grandis* effectively reduced the generation of nitric oxide from sodium nitroprusside. The chloroform fraction showed strong nitric oxide scavenging activity (IC<sub>50</sub> 0.173 mg/ml) and that of standard curcumin was 0.087 mg/ml. The residual fraction (0.470 mg/ml), pet-ether fraction (0.374 mg/ml) and ethyl acetate fraction (0.535 mg/ml) also showed good scavenging activities (**Table 1**).

**Table 1 Antioxidant Activity of Fraction of *Coccinia Grandis* Roots**

**IC50 (mg/ml)**

Fractions	DPPH	Fe <sup>2+</sup> chelating	H <sub>2</sub> O <sub>2</sub>	NO
Pet Ether	3.9 ± 0.93	0.402 ± 5.21	0.093 ± 0.02	0.374 ± 4.41
Chloroform	0.135 ± 0.02	0.376 ± 5.51	0.196 ± 4.0	0.173 ± 0.81
Ethyl acetate	0.154 ± 0.73	0.433 ± 22.00	0.212 ± 0.01	0.535 ± 4.31
Methanolic	0.8 ± 0.03	0.505 ± 12.12	0.135 ± 0.02	0.470 ± 7.20
Ascorbic	0.03 ± 0.01	0.017 ± 0.01		
Curcumin				0.087 ± 0.93
α – tocopherol		0.075 ± 0.73		

Results are expressed as mean ± SEM of three parallel measurements. Values within a column followed by different letters are significantly different (P<0.05)

**Table 2 Reducing power ability of different fraction coccinia grandis**

**Absorbance at 700 nm**

Fraction	50µg/ml	100µg/ml	200µg/ml	400µg/ml	800µg/ml
Pet ether	0.076±0.002	0.087±0.006	0.095±0.007	0.113±0.009	0.143±0.004*
Chloroform	0.067±0.003	0.112±0.003	0.183±0.003	0.231±0.021	0.429±0.003*
Ethyl acetate	0.231±0.001	0.283±0.006	0.355±0.006	0.436±0.007	0.476±0.005*
Methanolic	0.426±0.002	0.511±0.004	0.555±0.004	0.576±0.005	0.624±0.020*
BHT	0.095±0.002	0.218±0.005	0.318±0.003	0.645±0.002	1.089±0.002

Values are expressed as mean ± SEM of three parallel measurements. \* P<0.001 when compared with standard BHT.

**Table 3: Total antioxidant activity and total phenolic and flavonoid contents of different fractions of *Coccinia grandis***

Fractions	Total antioxidant activity ( $\mu\text{g vit E Eq} / 100\mu\text{g}$ )	Total phenolic content ( $\mu\text{g gallic Eq} / \text{mg}$ )	Total flavanoids content ( $\mu\text{g gallic Eq} / \text{mg}$ )
Pet ether	7.12 $\pm$ 0.03	73.22 $\pm$ 0.78	89.02 $\pm$ 1.0
Chloroform	29.33 $\pm$ 0.32	52.63 $\pm$ 0.86	12.01 $\pm$ 1.01
Ethyl acetate	8.32 $\pm$ 0.37	35.00 $\pm$ 0.12	15.34 $\pm$ 1.32
Methanolic	9.10 $\pm$ 0.03	28.01 $\pm$ 1.23	26.32 $\pm$ 1.42

Values are expressed as mean  $\pm$  SEM of 3 parallel measurement. Values with in a column followed by different letters are significantly different ( $P < 0.05$ ).

#### Total Phenolic and Flavonoid Content

Total phenolic content was estimated by using Folin-Ciocalteu reagent. Total phenolic content of the different fractions of *C. grandis* were solvent dependent and expressed as  $\mu\text{g}$  pyrocatechol equivalent.. The content of the total phenolics in the fractions decreased in the order of pet-ether > chloroform > ethyl acetate > residual fractions. The total flavonoid content in the fractions was expressed as  $\mu\text{g}$  quercetin equivalent. The pet-ether fraction of *C. grandis* showed highest amount of flavonoids among the fractions tested. The content of total flavonoids in the fractions decreased in the order of pet-ether fraction > residual fraction > ethyl acetate fraction > chloroform fraction (**Table 3**).

Free radical are known to play a vital role in a wide variety of pathological manifestations. Antioxidant fight with free radicals and protect us from various disease. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanism.

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts[19]. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol solution. It was reduced to a diphenylpicryl hydrazine, with the adding of the fractions in a concentration-dependent manner. The lessening in the number of DPPH molecules can be associated with the number of available hydroxyl groups. All the fractions showed significantly higher inhibition percentage (stronger hydrogen – donating ability) and positively correlated with total phenolic content.

The conversion of  $\text{Fe}^{3+}$  into  $\text{Fe}^{2+}$  in the presence of various fractions was calculated to determine the reducing power ability. The reducing ability of a compound generally depends on the existence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom[20]. The antioxidant principles present in the fractions of *C. grandis* cause reducing power ability.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell[21]. Thus, scavenging of  $\text{H}_2\text{O}_2$  is a measure of the antioxidant activity of

the fractions. All the fractions of *C. grandis* scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water.

In vitro inhibition of nitric oxide radical is a measure of antioxidant activity. Nitric oxide is a free radical which plays an important role in the pathogenesis of pain, inflammation, etc. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent[22]. The absorbance of the chromophore is measured at 546 nm in the presence of the fractions. All the fractions of *C. grandis* decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro. This may be due to the antioxidant principles in the fractions.

The metal chelating ability of the fractions of *C. grandis* was measured by the formation of ferrous ionferrozine complex. Ferrozine combines with ferrous ions forming a red coloured complex which absorbs at 562 nm[23]. It was reported that the chelating agents which form  $\sigma$  bond with a metal, are effective as secondary antioxidants, because they reduce the redox potential thereby stabilising the oxidised form of the metal ion[24]. The results of our study express that the fractions have an effective capacity for iron binding, suggesting its antioxidant potential. In addition, the metal chelating ability of the fractions demonstrated that they reduce the concentration of the catalysing transition metal involved in the peroxidation of lipids.

Phenolics are ever-present secondary metabolites in plants and possess a wide range of therapeutic uses. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. Total phenolic assay by using Folin-Ciocalteu reagent is a simple, convenient and reproducible method. It is employed usually in studying phenolic antioxidants[25]. Flavonoids are a group of polyphenolic compounds, which exhibit several biological effects such as antiinflammatory, antihepatotoxic, antiulcer, antiallergic, antiviral, anticancer activities. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are able of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidants[26]. The presence of high phenolic and flavonoid content in the fractions has contributed directly to the antioxidant activity by neutralizing the free radicals.

## **CONCLUSION**

Based on the results obtained, it may be concluded that all the fractions of the hydromethanolic extract of the roots of *C. grandis* showed strong antioxidant activity, reducing power ability, free radical scavenging activity and metal chelating ability when compared to standards such as ascorbic acid,  $\alpha$ -tocopherol, curcumin, and butylated hydroxytoluene. Further studies to evaluate the in vivo potential of the fractions in various animal models and the isolation and identification of the antioxidant principles in the roots of *Coccinia grandis* are being carried out.

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