IN VITRO ANTIOXIDANT POTENTIAL OF ETHANOLIC EXTRACT OF *MOMORDICA DIOICA* ROXB (CUCURBITACEAE)

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Summary

The free radical scavenging potential of the tuberous roots of *Momordica dioica* was studied by using different *in vitro* methods *viz.* DPPH radical scavenging, ABTS radical scavenging, iron chelating activity, total antioxidant capacity, and haemoglobin glycosylation assay. Its antioxidant activity was estimated by IC_{50} value and the values are 72.56 µg/ml (DPPH radical scavenging), 97.13 µg/ml (ABTS radical scavenging), and 56.59 µg/ml (iron chelating activity) for ethanolic extract. Also total antioxidant capacity of ethanolic extract was found to be 26.0 µg/ml equivalents to ascorbic acid. Ethanolic extract showed percentage inhibition of haemoglobin glycosylation at 66.63 and 74.14 at concentrations of 500 and 1000 µg/ml respectively, while that of standard DL α-tocopherol was 61.53 and 86.68 inhibition at same concentration by haemoglobin glycosylation assay method. In all the methods, the extract showed its ability to scavenge free radicals in a concentration dependent manner. The result indicates that *Momordica dioica* has moderate antioxidant activity.

Keyword: Scavenging, ABTS, DPPH, O-Phenanthroline, *Momordica dioica*

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Introduction

Free radicals have aroused significant interest among scientists in the past decade. Their broad ranges of effects in biological systems have drawn on the attention of many experimental works. Highly reactive free radicals, especially oxygen-derived radicals, which are formed by exogenous chemicals or endogenous metabolic processes in the human body, are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damage plays a significantly pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage[1].Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase or compounds such as ascorbic acid, tocopherol and glutathione[2]. When the mechanism of antioxidant protection becomes unbalanced by factors such as ageing, deterioration of physiological functions may occur resulting in diseases and accelerating ageing. However, antioxidant supplements may be used to help the human body to reduce oxidative damage.

Synthetic antioxidants are widely used because they are effective and cheaper than natural types. However, the safety and toxicity of synthetic antioxidants have been important concerns[3]. Much attention has been focused on the use of antioxidants especially natural antioxidants to inhibit lipid peroxidation or to protect the human body from the oxidative damage by free radicals.

Momordica diocia Roxb. is a perennial, dioicious climber with slender stem and yellow flowers[4] and is called giddahagala, karlekai, jangli karela, small bitter guard etc.[5]. The roots are large and reported to possesses abortifacient, spermicidal[6], anthelmintic, anti-inflammatory activities. Analgesic anticatatonic activities[7] and antifertility activities[8]

have also been reported. Phytochemical investigation of the tuberous roots ethanolic extract has been reported to contain carbohydrates, reducing sugars, traces of alkaloids, saponin glycosides of triterpenoid type and flavonoids. This paper describes the antioxidant activities of ethanolic extract of the root of *Momordica diocia* Roxb.

Materials and Methods

All chemicals and solvents used in the study were of analytical grade. Haemoglobin and DPPH (1, 1- Diphenyl-2-Picryl Hydrazyl) were obtained from Sigma Chemicals (St.louis, Mo, USA). ABTS was obtained from Himedia Laboratories Ltd. Mumbai, India. O-phenanthroline was obtained from National chemical, Baroda, India. Glucose, sulphuric acid, sodium phosphate and ammonium molybdate were obtained from Ranbaxy Fine Chemicals, Mumbai, India.

UV spectrophotometer (Shimadzu 1650 pc, Japan) and pH meter (Elico Ltd., India) were the instruments used for the study.

Plant material-The tuberous roots of the plant were collected from the farmhouse of Mr. Ganapaiah, Agriculturist, Konehousur, Choradi, Shimoga District in the month of Octomber, during fruiting season. Authentication of the plant was done by Dr. S.N. Yoganarasimhan, Head, Medicinal Plant Division, Regional Research Centre (Ayurvedic) Jayanagar, Bangalore.

Plant extract-The roots were made into small pieces, shade dried, coarsely powdered and extracted (500 gm) in a soxhelet extractor with ethanol (2 Ltr.). The crude extract was concentrated to dryness in rotary flash evaporator (Buchi type) under reduced pressure and controlled temperature (40-50^{o}C). The extract was preserved in a vacuum desiccators for subsequent use in study.

Antioxidant study

DPPH radical scavenging activity[11,12]- To 1 ml of various concentrations (2-1000 µg/ml) of ethanolic extract, 1 ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added to the test tube. An equal amount of methanol was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate (Fig1).

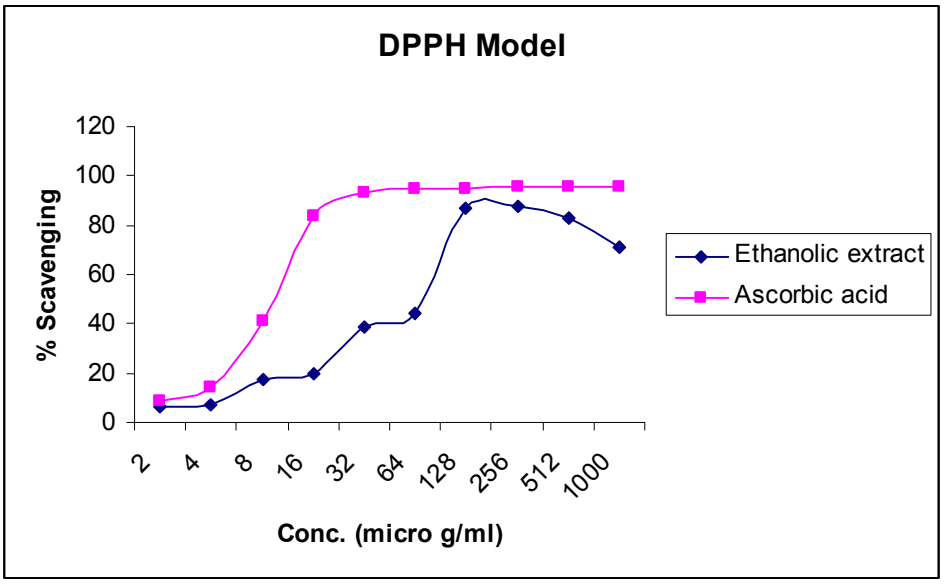

Fig 1. DPPH radical scavenging activity of different concentrations of *M. diocia* and ascorbic acid. Each value represents mean ±S.E.M.

ABTS radical scavenging activity[11, 12] -The ABTS radical cation preparation: ABTS 2 mM (0.0548g in 50 ml) was prepared in distilled water. Potassium per sulphate 70 mM (0.0189 g in 1 ml) was prepared in distilled water. 200 µl of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution is called as ABTS radical cation, which was used for the assay.

To the 0.5 ml of various concentration of extract, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4 was added. For control, instead of extract ethanol was

taken. The absorbance was measured at 734 nm. The experiment was performed in triplicate (Fig2).

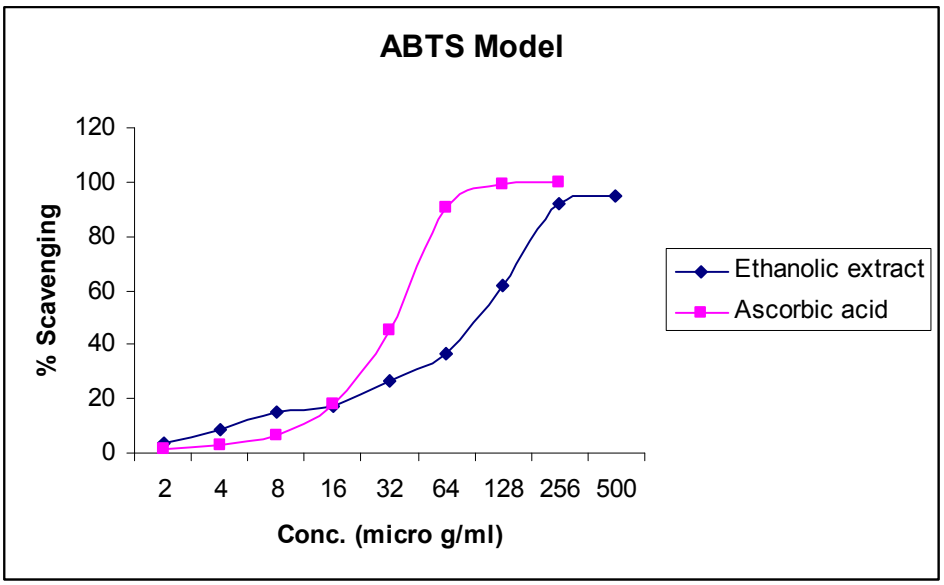

Fig 2. ABTS radical scavenging activity of different concentrations of *M. diocia* and ascorbic acid. Each value represents mean ±S.E.M.

Iron chelating activity[11,12] -The reaction mixture contain 1 ml 0.05% ortho-Phenanthroline in methanol, 2 ml ferric chloride 200 µM & 2 ml of various concentrations of the extract. The mixture was incubated at ambient temperature for 10 min, and then the absorbance of the same was measured at 510 nm. Ascorbic acid was added instead of test compound and Absorbance obtained taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without drug. The experiment was performed in triplicate (Fig 3)

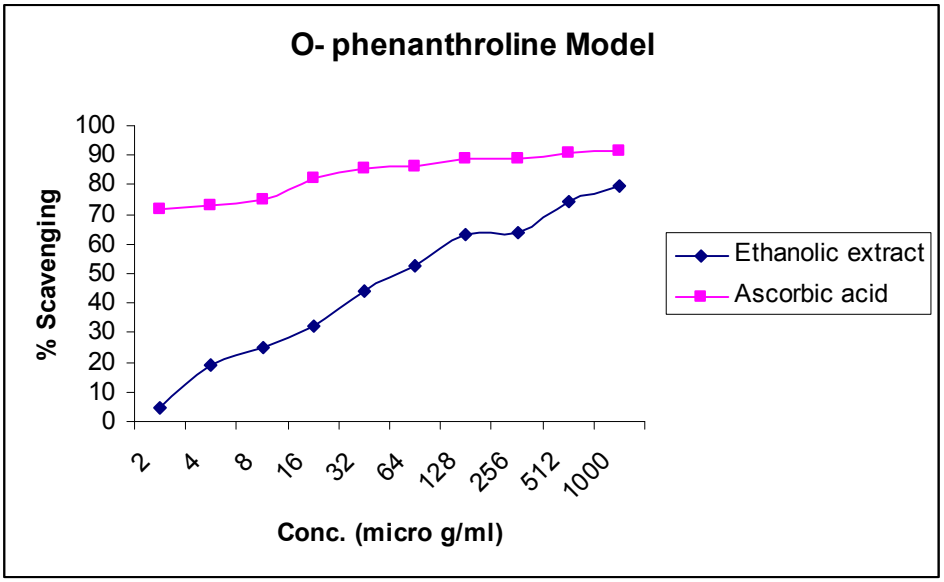

Fig 3. Iron chelating activity of different concentrations of *M. diocia* and ascorbic acid. Each value represents mean ±S.E.M.

Non-enzymatic haemoglobin glycosylation assay[13,14]-The antioxidant activity of extract was investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. The assay was performed by adding 1 ml of glucose solution, 1 ml of haemoglobin solution, and 1 ml of gentamycin (20 mg/ 100 ml), in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of haemoglobin in the presence of different concentration of extract and their absence were measured colorimetrically at 520 nm (Table 1)

Table 1: Antioxidant study by Non-enzymatic haemoglobin glycosylation assay

Sl. No.	Extract/ standard	Concentration (µg/ml)	% scavenging
1.	Ethanolic extract	0.5	66.63
		1.0	74.14
2.	Vitamin –E	0.5	61.53
		1.0	86.68

Total antioxidant capacity[15,16]**-** 0.1 ml of extract (10 mg/ml) dissolved in water was combined in eppendorf tube with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM Sodium Phosphate and 4 mM Ammonium Molybdate). The tubes were capped and incubated at 95^{o}C for 90 min. after cooling to room temperature; the absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid. (Table 2)

Table 2: Comparison of IC_{50} values of extract with standard

Sl .no.	Model	IC_{50} value of ethanolic extract (µg/ml)	IC_{50} value of ascorbic acid (µg/ml)
1.	DPPH radical scavenging activity	72.56	9.9982
2.	ABTS radical scavenging activity	97.13	35.21
3.	O-Phenanthroline Method	56.59	2.01
4.	Total antioxidant capacity	10 mg/ml Ethanolic extract of *M. diocia* is equivalent to 26.0 µg/ml of ascorbic acid	

Statistical Analysis-All results are expressed as mean ± S.E.M. Linear regression (Origin 6.0 version) analysis was used to calculate the IC_{50} values.

Results

Several concentrations, ranging from 2-1000 µg/ml of the ethanolic extract of *Momordica dioica* Roxb was tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extract in concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values (Table 1) were calculated for all the models. The maximum inhibitory concentration (IC_{50}) in all models was found to be 72.56 µg/ml (DPPH radical scavenging), 97.13 µg/ml (ABTS radical scavenging), and 56.59 µg/ml (iron chelating activity) for ethanolic extract. Also total antioxidant capacity of ethanolic extract was found to be 26.0 µgm/ml equivalents to ascorbic acid. Ethanolic extract showed percentage inhibition of haemoglobin glycosylation at 66.63 and 74.14 at concentrations

of 500 and 1000 µgm/ml respectively, while that of standard DL α-tocopherol was 61.53 and 86.68 inhibition at same concentration by haemoglobin glycosylation assay method. The result indicates that *Momordica dioica* has significant antioxidant activity.

Discussion

There is an extensive evidence to implicate free radicals in the development of degenerative diseases. Free radicals have been implicated in the causation of ailments such as diabetes, liver cirrhosis, nephrotoxicity, cancer etc. Together with other derivatives of oxygen, they are inevitable by-products of biological redox reaction. Reactive oxygen species such as superoxide anions, hydroxyl radical, and nitric oxide inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation [17,18].

DPPH is a stable free radical. When antioxidant reacts with this stable radical, the electron becomes paired off and bleaching of the colour stoichiometrically depends on the number of electrons taken up. From our findings, it may be postulated that *M. dioica* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles[19, 20].

ABTS is a decolorisation assay, which involves the direct generation of ABTS radical mono cation, which has a long wavelength absorption spectrum without the involvement of any intermediary radical. The antioxidant activity of the extract by this assay implies that action may be by either inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported in earlier studies[21].

Ortho substituted phenolic compounds may exert prooxidant effects by interacting with iron. O-phenanthroline quantitatively forms complexes with Fe^{2+}, which get disrupted in the presence of chelating agents. The ethanolic extract interfered with the formation of ferrous-o-phenanthroline complex, thereby suggesting that the extract has metal chelating activity. Iron stimulates lipid peroxidation by Fenton reaction and accelerate peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals which themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation. The metal chelating capacity is important, since it reduces concentration of the catalyzing transition metal in lipid peroxidation. It has been reported that the chelating agents, that form bonds with a metal are effective as secondary antioxidant because they reduce the redox potential, thereby stimulating the oxidized form of the metal ion. The observed results demonstrate a marked capacity of the extract for iron binding, suggesting that their action as a peroxidation protector may be related to its iron binding capacity[22, 26].

Haemoglobin glycosylation is an *in vitro* non-enzymatic method. Being an oxidation reaction, an antioxidant is expected to inhibit the reaction. The degree of haemoglycosylation *in vitro*, in the presence of different concentration of extract can be measured spectrophotometrically[13].

Total antioxidant capacity of the extract was calculated based on the formation of phosphomolybdenum complex which was measured spectrophotometrically at 695 nm[15.]

M. dioica extract exhibits its antioxidant action in several ways: removal of oxygen, scavenging of ROS, binding metal ions needed for catalysis of ROS and up regulation of endogenous antioxidant defenses.

Our finding shows that the ethanolic extract of *M. dioica* has moderate antioxidant activity.

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