Research Article

Total antioxidant capacity and iron chelating activities of methanolic leaf extract of *Boerhavia diffusa* (Linn.)

M. Muthulingam[1]*, K. Krishna Chaithanya[2]

ABSTRACT

Introduction: An oxidative stress results from the imbalance between the free radicals such as reactive oxygen species (ROS) and ROS reactive nitrogen species produced in the body, and the antioxidant system mechanisms have been responsible for oxidative diseases. *Boerhavia diffusa* (Linn.) is an important medicinal plant used in Indian Traditional Medicinal System for curing diseases. **Objective:** The objective of the present study was to evaluate the total antioxidant and metal chelating activity of the methanolic leaf extract of *B. diffusa*. **Materials and Methods:** The total antioxidant activity and iron chelating activity of the methanolic leaf extract of *B. diffusa* were performed using standard procedures. **Results:** The results have indicated that the total antioxidant activity of the methanolic leaf extract of *B. diffusa* was 89.03/500 µg/ml and showed maximum Fe^{2+} chelating of 72.76% with an IC_{50} value of 180 µg/ml. **Conclusion:** The present study concluded that the methanolic leaf extract of *B. diffusa* can be used as a potential source of natural antioxidants.

KEY WORDS: Antioxidant assay, *Boerhavia diffusa*, Free radicals, Oxidative stress iron chelating

INTRODUCTION

Oxidative stress is defining as an imbalance between antioxidant and prooxidant species. Oxidative stress occurs when the generation of free radicals and reactive intermediates in a system exceeds the system's ability to neutralize and eliminate them.[1,2] Oxidative stress is associated with cardiovascular diseases, atherosclerosis, hypertension, and diabetes mellitus.[3] Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), hydroxyl radical (OH), superoxide anion (O_2^-), reactive nitrogen species (RNS), and peroxy nitrate ($ONOO^-$) contribute prooxidant/antioxidant imbalance. In inflammatory conditions, both ROS and RNS are considered to be toxic in their upregulated state due to lower activity of antioxidant system. Both enzymatic and non-enzymatic systems contribute free radical scavenging within the cell, thus protecting from radical-induced inflammation and cancer. Plant-derived antioxidants such as ascorbic acid rutin and quercetin play a protective role by scavenging the generated free radicals, and hence, they are highly beneficial to cure the diseases caused by oxidative stress.

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Medicinal plants have been used to treat various health ailments for a long period of time in different countries. In Asia and African continents, the most of the population uses traditional medicine for primary health care according to the World Health Organization report in 2003, and there are >20,000 plant species used in traditional medicine.[4] With the development of modern technology, more and more plant extracts have been found to be useful to medical practice.[5] Many researchers have established that plants produce effective numerous antioxidants that eliminate disease-causing ROS.[6] Based on growing attention on free radical biology, the plant-derived antioxidants are essential to compact against diseases.

Boerhavia diffusa Linn. which belongs to the Family *Nyctaginaceae* is usually known as "Punarnava" in the Indian System of Medicine. It is found in Asia, Africa, Australia, Sudan, and China. The entire plant of *B. diffusa* is used in traditional medicine for the treatment of diabetes, dyspepsia, abdominal pain, inflammation, cardiac diseases, and bacterial infections.[7] The objective of the present study was to evaluate the total antioxidant and metal chelating activity of the methanolic leaf extract of *B. diffusa* using standard *in vitro* antioxidant methods.

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MATERIALS AND METHODS

Plant Material Collection and Methanolic Extraction

The fresh leaves of *B. diffusa* were collected from Annamalai Nagar, Tamil Nadu, India, during the month of March 2015. The plant was authenticated by Dr. Subramanian, Professor, Department of Botany, Annamalai University, Tamil Nadu, India.

The fresh leaves of *B. diffusa* were properly washed with distilled water, shade-dried, and coarse powdered. Powder weighing 100 g was packed in Soxhlet apparatus. The extraction was continued until the color of the solvent in the siphon tube became colorless. Extract of methanol was subjected to evaporation at room temperature until a semisolid mass was obtained.

Total Antioxidant Activity (Phosphomolybdenum Reduction Assay)

The whole antioxidant activity of the methanolic leaf extract of *B. diffusa* was assessed by phosphomolybdenum assay method[8] which is based on the reduction of Mo VI–Mo V by the methanolic leaf extract of *B. diffusa* and formation of green phosphate - Mo V complex in acidic condition. Briefly, an aliquot of 0.1 ml of methanolic leaf extract (1 mg/ml) and standard ascorbic acid with different concentrations ranging from 25, 50, 100, 200, 500 µg/ml were incubated with 1 ml of reagent solution containing (0.6 ml sulfuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min, and then, the absorbance was measured at 695 nm using ultraviolet visible spectrophotometer against blank (0.3 ml of methanol is used as blank in the place of extract), after cooling to room temperature. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid.

$$\%\,Total\ antioxidant\ activity = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Metal Chelating Assay[9]

The reaction mixture containing 1 ml of o-phenanthroline, 2 ml of ferric chloride, and 2 ml of various concentrations of the methanolic leaf extract of *B. diffusa* of 25, 50, 100, 200, and 500 µg/ml in a final volume of 5 ml was incubated for 10 min at ambient temperature. The absorbance at 510 nm was recorded. Ascorbic acid was added instead of extract and absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without extract. All tests were performed in triplicates ($n = 3$).

$$\%\,\text{Chelating activty} = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Statistical Analysis

The experimental results were expressed as mean ± SEM of three replicates, where applicable, the data were subjected to one-way analysis of variance (ANOVA) and 2-ANOVA. All these analyses were done by GraphPad Prism software program (Version 6.0) and MS Office 2010 version, and $P < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The total antioxidant capacity was increased by increasing the concentration of the methanolic leaf extract of *B. diffusa*. The total antioxidant capacity of methanolic leaf extract of *B. diffusa* was expressed as a number of equivalents of ascorbic acid. As shown in Table 1, the total antioxidant activity of methanolic leaf extract of *B. diffusa* was 89.03 µg Ascorbic acid equivalent /500 µg of extract. The standard calibration curve is shown in Figure 1. Purena and Bhatt[10] reported that the total antioxidant activity of ethanolic extract of *Kadam* was expressed as 302.13 ± 36.8 µg/mg as equivalent as ascorbic acid.

As shown in Figure 2, at a concentration of 500 µg/ml, the methanolic leaf extract of *B. diffusa* and ascorbic acid was 72.76% and 80.27% with IC_{50} value of 180 µg/ml and 176 µg/ml, respectively. Muthulingam and Chaithanya[11] reported that the methanolic leaf extract of *B. diffusa* interrupted the formation of the ferrozine–Fe^{2+} complex by showing 76.21% of chelating activity with an IC_{50} of 205 µg/ml.

The phenolic constituents exert their action either by scavenging the ROS or protecting the antioxidant defense mechanisms.[12] Muthulingam and

Table 1: Determination of total antioxidant activity of the methanolic extract of *B. diffusa* by phosphomolybdenum assay

Description	Concentration (µg/ml)	Absorbance	Ascorbic acid equivalent (µg/g) of extract
Control	-		
B. diffusa	25	0.096	15.05
	50	0.260	40.75
	100	0.336	52.66
	200	0.472	73.98
	500	0.568	89.03

B. diffusa: Boerhavia diffusa

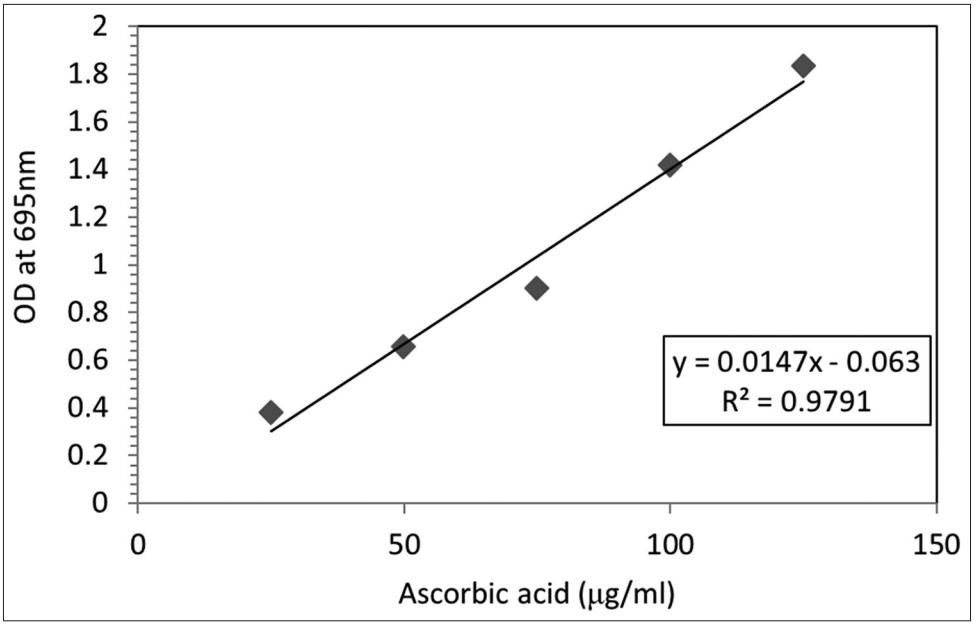

Figure 1: Calibration curve for total antioxidant capacity using ascorbic acid as standard

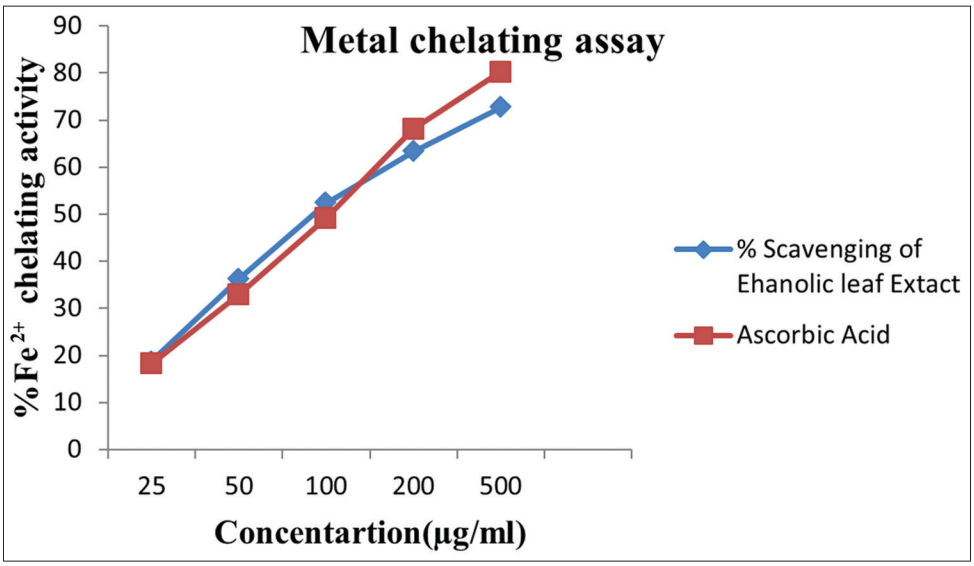

Figure 2: Fe^{2+}chelating activity of the methanolic leaf extract of *Boerhavia diffusa* compared to that of standard ascorbic acid. Each value is expressed as mean ± standard deviation (n = 3)

Chaithanya[13] reported that the methanolic leaf extract of *B. diffusa* contained considerable amounts of tannins (98.25 µg/ml tannic acid equivalents/g), flavonoids (173.75 µg/ml quercetin equivalents/g), and phenolic compounds (82.37 µg/ml, gallic acid equivalents/g), and from this study, it was revealed that the methanolic leaf extract of *B. diffusa* extract has significant total activity and metal chelating activity due to the presence of bioactive compounds such as tannins, flavonoids, and phenolic compounds.

CONCLUSION

The methanolic leaf extract of *B. diffusa* shown the total antioxidant activity with significant chelating capacity of Fe^{2+} activity due to the presence of promising bioactive compounds that would be responsible for *in vitro* antioxidant activity. Hence, the methanolic leaf extract of *B. diffusa* as an excellent source of bioactive compounds that can be further used for the developed into natural antioxidant medicines for combating oxidative stress-related diseases.

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