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In Vitro Antioxidant and Cytotoxic Analysis of *Boerhaavia diffusa* Linn.

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ABSTRACT

The present study was carried out to evaluate the antioxidant and cytotoxic activity of unexploited plant, *Boerhaavia diffusa* indigenous to India. Different concentrations of the ethanolic whole-plant extracts (1000, 500, 250, 125, 50, 25, 12.5 µg/ml) were subjected to 1,1-diphenyl -2-picryl hydrazyl (DPPH) radical scavenging, reducing power activity and cytotoxic study against vero cell lines. The maximum DPPH radical scavenging potential was found to be 93% at 1000 µg/ml with IC_{50} (Inhibitory Concentration) value being 49.95±1.15 µg/ml. The maximum reducing power of the extract at 700nm was found to be 0.997±0.081 at 1000 µg/ml. The inhibition percentage with regard to cytotoxicity was found to be 89 % at 1000 µg/ml with IC_{50} value of 50±0.03 µg/ml.

KEY WORDS : *Boerhaavia diffusa*, Antioxidant activity, DPPH, Reducing Power, Cytotoxicity

INTRODUCTION

Boerhaavia diffusa (Linn.) (Syn. *B. repens* L.; *B. procumbens* Roxb: Sanskrit: "Punarnava") commonly called as Mukkurattai in Tamil language, belongs to the family Nyctaginaceae. This plant has been traditionally useful in all types of inflammations, strangury, leucorrhoea, ophthalmia, lumbago, scabies, cardiac disorders, jaundice, anemia, dyspepsia, cough, bronchitis and general debility (Nayar, 2000). The juice of fresh leaves of *B. diffusa* L. markedly reduces pain in mice (Hiruma-Lima *et* al., 2000). The hepatoprotective activity of *B. diffusa* L. roots has been reported (Rawat *et* al., 1997). In order to authenticate the traditional medicinal claims, the present investigation has been carried out to evaluate the antioxidant activity, reducing power and cytotoxic effects of *B. diffusa* whole plant extracts.

MATERIALS AND METHODS

Plant Material

The plant *B. diffusa* was collected from Pollachi in Tamil Nadu and authenticated in Botanical Survey of India,

Tamil Nadu Agricultural University, Coimbatore (accession No: JMS-82). The plant material was shade dried and powdered. About 50g of the air dried plant material was exhaustively extracted with 250ml of ethanol using soxhlet apparatus. The extract was concentrated to dryness. The crude extract was used for evaluation of antioxidant and cytotoxicity activities. Another 50g of the powdered plant material was extracted with hexane, chloroform, ethyl acetate and ethanol, the crude extracts were used for the preliminary photochemical screening.

Antioxidant assay

Different concentrations of ethanolic plant extract (1000, 500, 250, 125, 50, 25, 12.5 µg) were subjected to antioxidant assays using 1,1-diphenyl -2-picryl hydrazyl (DPPH) model (Blois, 1958) and reducing power model systems (Oyaizu,1986).

Test for DPPH Radical Scavenging Activity

Different concentrations of plant extracts sample was taken in test tubes and the volume were taken. Then 5ml of 0.1mM ethanolic solution of DPPH was added and the tubes were shaken vigorously. They were then allowed to stand at 35°C for 30 minutes. The control was prepared without any sample and ethanol was used for base line corrections in absorbance (OD) of samples measured at 517 nm. Radical scavenging activity was expressed as % (Percentage) scavenging activity and was calculated by the following formula

$$\% \text{ of radical scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

IC_{50} value was also calculated.

Test for Reducing Power

Different concentration (1000, 500, 250, 125, 50, 25µg) of plant extracts were taken in test tubes and the volume was adjusted to 1ml by the addition of DMSO. To that 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then kept in a 50°C water bath for 20 minutes. The resulting solution was then cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid and centrifuged at 3000 rpm for 10 minutes. The supernatant (5ml) was then mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride. The absorbance of 700nm was then detected after reaction for 10 minutes. The higher the absorbance represents the stronger the reducing power.

ASSAY OF CYTOTOXIC ACTIVITY

The vero cell lines (kidney carcinoma cells from African monkey) used for the assay were obtained from National Centre for Cell Science, Pune. The stock cells were cultured in RPMI-1640 with 10% Fetal Bovine Serum (FBS), Penicillin (100 IU/ml) Streptomycin (100 µg/ml) and amphotericin-B (5 µg/ml) in a humidified atmosphere of

5% CO_2 at 37°C unit confluent. The cells were dissociated with 0.2% trypsin in phosphate buffer saline solution. The stock cultures were grown in 25cm^2 tissue culture flasks and all cytotoxicity experiments were carried out in 6 well plates.

Viability Staining

Cytotoxic activity of ethanol extract of B. diffusa was analysed by Trypan Blue dye exclusion method adopted by (Ian Freshney, 1994) . Cell lines in exponential growth phase were washed with phosphate buffer saline (PBS) solution and trypsinized and re-suspended in complete culture media. Cells were plated at 30,000 cells/well in 6 well plates and incubated for 24 hours during which a partial monolayer forms. After incubation the cells were exposed to various concentration of the drugs, which is the plant extract (1000 µg/ml, 500 µg/ml, 250 µg/ml, 150µg/ml, 125 µg/ml, 50µg/ml and 25µg/ml). The control well received only maintenance of medium. The plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 24 hours. Morphological changes of drug treated cells were examined using an inverted microscope at different time intervals and compared with the cells serving as control. At the end of 24 hours incubation, cell viability was determined.

PHYTOCHEMICAL SCREENING

Phytochemical screening of plants was carried out to detect bioactive compounds using qualitative tests (Harborne, 1984)

RESULTS AND DISCUSSION

The *in vitro* antioxidant activity of *B. diffusa* using DPPH radical and reducing power was tested with different concentrations of ethanol extract and the percentage of scavenging and IC_{50} are given in table 1. The ethanol extract of *B. diffusa* showed potent antioxidant activity based on DPPH radical quenching and reducing power tests in a dose dependent manner. The ethanolic extract strongly scavenged DPPH radical with the IC_{50} being 49.95 ±1.15 µg/ml, which is comparable to that of Butyl Hydroxy Anisole (BHA). The extract also caused significant elevation of reducing power potential. The higher absorbancy at high concentration indicates the strong reducing power potential.

The presence of flavanoids might be responsible for the antioxidant activity of the plant. In very recent years, flavanoids, potent free radical scavengers have attracted a tremendous interest as possible therapeutics against free radical mediated diseases (Martin *et al* ,2006). Previous studies showed high DPPH antiradical activity of flavonoid compounds (Formica and Regelson, 1995) and many flavonoids posses DPPH radical quenching and reducing power capacity (Adreu *et al*., 2007) . The results of the present study demonstrated the extracts possessed strong antioxidant activity even though the activity is lesser than that of ascorbic acid. Thus, the medicinal claims of the plant being used in the treatment of jaundice may be in part due to the antioxidant activity.

Further, the *in vitro* screening of the ethanolic extracts of *B. diffusa* showed potential cytotoxic activity against the kidney cells from African monkey. The results obtained are shown in table 2. The results obtained from the present study show that the *B. diffusa* is moderately cytotoxic activity. The cytotoxic activity may be due to the presence of alkaloids in the plant.

CONCLUSION

The present study aimed to evaluate the possible antioxidant activity of the *B diffusa* used in the treatment of several diseases, but with no reports on its antioxidant potential. The results of the study revealed that the plant extracts have strong antioxidant activity, achieved by quenching capacity against DPPH radical, reducing power and remarkable cytotoxic activity. Further investigations are needed to provide some additional insight into the *invivo* antioxidant activity and cytotoxic activity of the plant.

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REFERENCES

Adreu, P.M., Braham, H ., Ben Jannet, H., Mighri, Z and Mathew, S. 2007. Antioxidant compounds from *Ebenus pinnata,* Fitoterapia**.** 78. 32-34.

Blois, M.S. 1958. Antioxidant determinations by the use of stable free radicals, Nature, 181.1199-1201.

Formica, J.V and Regelson, W.1995. Review of the biology of quercetin and related bioflavonoids, Food Chem. Toxicol. 33. 1061-1080

Harborne, J.B.1984.Phytochemical Methods Chapman and Hall, London, Sixth Edition, 27-34.

Hiruma-Lima, C.A., Gracioso, J.S., Bighetti, E.J.B., Germonsén Robineou, L and Souza Brito ARM. 2000. The juice of fresh leaves of Boerhaavia diffusa L (Nyctaginaceae)markedly reduces pain in mice, J. Ethnopharmacol. 71, 267-274.

Ian Freshney, R.1994. Culture of Animal Cells: a Manual of Basic Techniques**.** Fourth Edition. John Wiley & Sons, New York. 330-331.

Luis, J. C. F. Valdes, R. Martin, A. J. Carmana and Jesus Diaz, G. 2006, DPPH radical Scavenging activity two flavonoid glycosides from *Aconitum nepel* sp. *lustinacum*, Fitoterapia. 77. 469-471.

Nayar, I .C 2000. Glossary of Indian Medicinal Plants (CSIR Publication). 81,177.

Oyaizu, M . 1986. Studies on Products of browning reaction prepared from glycosamine. Japan J. Nutr. 44. 307-314.

Rawat, A.K.S. Mehrotra S., Tripathi, S. C. and Shome, U. 1997. Hepatoprotective activity of *Boerhaavia diffusa* L. roots –a popular Indian ethno medicine, J. Ethnopharmacol.56. 61-66.

Table 1: Free Radical scavenging activity by DPPH reduction and Reducing Power Activity of B. *diffusa*.

Concentration (µg/ml)	DPPH reduction inhibition (%)[a]	IC_{50}(µg/ml)	Concentration (µg/ml)	Reducing Power Absorbancy(700nm) [a]
Control	0	49.95±1.15	Control	0.076±0.004
1000	93±6.24		1000	0.997±0.081
500	91.36±5.85		500	0.644±0.022
250	82.13±6.24		250	0.365±0.015
125	71.5±6.24		125	0.168±0.012
50	60.87±4.00		50	0.115±0.003
25	54.97±5.42		25	0.080±0.003
12.5	48.54±5.45			
Ascorbic acid[b]		11.24±0.022		
BHA[c]		53.27±0.727	BHA[c]	0.683±0.008

[a] values are Mean ±SD (n =3) for the test groups and standards[b] Ascorbic acid (reference standard)

[c] BHA- Butylated hydroxyl Anisole (Reference Standard)

Table 2: Cytotoxicity Activity of ethanolic extract of *B. diffusa* against Vero Cell Lines.

Concentration (µg/ml)	Cytotoxic activity (%)	IC_{50} (µg/ml)
1000	89	50±0.23
500	71	
250	62	
150	58	
125	56	
50	51	
25	42	

Table 3: Results for Phytochemical screening.

Plant name	Part used	Extract name	AL	ST	GL	FL	SA	TA	TR	CG
Boerhaavia diffusa	Roots	HX	-	-	-	-	-	-	+	-
		CH	-	-	-	+	-	-	-	-
		EA	-	-	+	-	-	-	-	-
		ET	-	-	-	-	-	+	-	-

Abbreviations

HX- Hexane, CH- Chloroform, EA- Ethyl Acetate, ET- Ethanol, AL- Alkaloids, S- Steroids, GL- Glycosides, S- Steroids GL- Glycosides,
FL-Flavanoids, SA- Saponins, TA-Tannins, TR- Triterpenes, CG - Cardiac glycoside

'+' (Positive) indicates the presence of the bioactive compound.
'-' (Negative) indicates the absence test of the bioactive compound.