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In-vitro Anti-Oxidant Activity of Roots of *Boerhaavia diffusa* Linn

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ABSTRACT

The present study was undertaken to evaluate antioxidant activity of Chloroform, Ethanol, and Ethyl acetate fraction of *B. diffusa L* roots which might have improved its hepatoprotective action. Preliminary phytochemical testing showed the presence of high amount of tannins and phenolics in the various extracts of *B. diffusa L*. Herbal drugs containing free radical scavengers like phenolics, tannins and flavonoids are known for their therapeutic activity hence the present study was undertaken to evaluate antioxidant activity of different extracts of *B. diffusa L* roots which might have improved its reported pharmacological actions. *Invitro* nitric oxide scavenging activity, the percentage inhibition was 71.35%, 33.74%, 23.85% in ethanol, chloroform and ethyl acetate extracts at 250mcg/ml when compared with Curcumin at 62 mcg/ml showed only 84.7% inhibition respectively. The ethanol extract and ethyl acetate showed a biphasic response whereas the chloroform extract showed a dose dependent increase. In DPPH radial scavenging activity, the ethanol extract showed 81.94% inhibition and the chloroform extract showed 42.58% inhibition at 1000mcg/ml compared with 88.02 % inhibition by Quercetin. The above results suggest that roots of *B. diffusa L* were found to reveal antioxidant potential which supports the use of this plant in traditional medicine.

Keywords: Anti-Oxidant, *Boerhaavia diffusa*, Free Radicals, Phenolic compounds.

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INTRODUCTION

B. diffusa L is an herbaceous plant belongs to the family Nyctaginaceae. It is used successfully in the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain, tumors and cancers. The root powder mixed with mamira is used to treat eye disease. It is used to cure corneal ulcers and night blindness and helps restore virility in men. In the living system, free radicals of different forms are constantly generated for specific metabolic requirement. When the generation of these species exceeds the levels of antioxidant mechanism, they cause extensive damage to the cells leading to oxidative damage of tissues and biomolecules, eventually leading to disease condition, especially degenerative diseases and extensive lysis. The living system is protected from this by enzymes such as superoxide dismutase, glutathione peroxidase and catalase and certain endogenous antioxidants such as α-tocopherol, ascorbic acid, β-carotene and uric acid [1-2]. Since the endogenous antioxidants acting as intracellular defense systems protecting cells from free radical damage and extensive lysis, scavenging and diminishing the formation of oxygen-derived species are not 100% efficient, micro nutrients or antioxidants taken as supplements are particularly important in diminishing the cumulative oxidative damages [3-4].Various diseases Conditions are associated with free radical oxidative stress. Among currently available drugs, synthetic drugs do have potential adverse reactions and which can be minimized to greater extent through natural compounds. Still there are many natural drugs which are yet to be explored scientifically [5]. In the present study, preliminary phytochemical testing showed the presence of high amount of tannins and phenolics. The presence of high amount of tannins and phenolic prompted us to study the free radical scavenging activity of methanolic extract of *B. diffusa L root.*

MATERIAL AND METHODS

Collection and Processing

The roots of *B. diffusa L* was collected from medicinal garden inside the campus and cut into small fragments and dried until the fracture is uniform. The dried roots was granulated or powdered by using a blender and sieved to get uniform particles. The final uniform powder was used for the extraction of active constituents of the plant [6].

Preliminary Phytochemical Analysis

The chemical tests for various phytoconstituents in the ethanolic and chloroform extract were carried out. [7]

Preparation of Extracts

Freshly collected roots was died in shade, and then coarsely powdered in a blender. The coarse powder (100g) was extracted successively with Chloroform, Ethanol, Ethyl acetate, each 250ml in a Soxhlet apparatus for 24 hours. All the extracts were filtered through wattman No: 41 filter paper and evaporated on a water bath and finally dried in vacuum.

Experimental Method

Determination of total antioxidant activity

The formation of monaldehyde is the basis for the well-known TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100º) monaldehyde binds TBA to form a red complex that can be measured at 532 nm. TBA method was used to measure the carbonyl compound obtained by linoleic acid oxidation at later stage of lipid peroxidation. Absorbance of supernatant was measured at 532nm [8]. The results were tabulated in table: 2.

DPPH Radical Scavenging Activity

Radical scavenging activity was determined with a DPPH as a free radical by using various concentrations (1.5-1000 mcg/ml) of the sample prepared in ethanol and chloroform extracts. The decrease in absorbance was determined at 517nm after 30min at room temperature. Quercetin was used as standard. Antiradical activity is defined as the amount of inhibitor (phenolic compound) necessary to decrease the initial DPPH radical concentration by 50 % (EC-50) [9]. The results were tabulated in table: 3 & figure: 1.

Nitric Oxide (NO) Radical scavenging activity

Sample of various concentration were used to determine their effect on the NO radical scavenging activity using sodium nitroprusside generating NO system compared with their parent compound. The Griess reagent (1%sulfanilamide in 5% phosphoric acid and 0.1%N-(1-napthyl) ethylenediamine.2HCL in water) is added to sample which stiochiometrically reacts to form a chromophore whose absorbance was measured at 546nm Curcumin was used as standard [10-11]. The results were tabulated in table: 4& figure: 2.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis indicates tannins and flavonoids in root extract of *B. diffusa L*. Polyphenols particularly flavonoids and tannins are well known natural antioxidant. Thus, the antioxidant potential of root extract of *B. diffusa L* may be due to the presence of poly-phenolic compounds, which needs further analysis. The ethanolic extracts showed good antioxidant activity in all in-vitro free radical scavenging models when compared to chloroform and ethyl acetate extracts of *B. diffusa L* roots. *Invitro* nitric oxide scavenging activity, the percentage inhibition was 71.35%, 33.74%, 23.85% in ethanol, chloroform and ethyl acetate extracts at 250mcg/ml when compared with Curcumin at 62 mcg/ml showed only 84.7% inhibition respectively [12]. The ethanol extract and ethyl acetate showed a biphasic response where as the chloroform extract showed a dose dependent increase. In DPPH radial scavenging activity, the ethanol extract showed 81.94% inhibition and the chloroform extract showed 42.58% inhibition at 1000mcg/ml compared with 88.02 % inhibition by Quercetin. Both the extracts showed a dose dependent increase in activity. A

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dose dependent increase in total antioxidant activity was shown by ethanol extract of *B. diffusa L.* The values were expressed as grams equivalent to 1g vitamin E (mg/g) [13].

CONCLUSION

Invitro antioxidant and free radical scavenging activity studies on root extract of B. diffusa L revealed the presence of antioxidant activity. The antioxidant activity may be due to the presence of phenols, triterpenoids and flavonoids in the root. Further invivo studies will help us to reveal the mechanism of action and can be used as a potent molecule for radical scavenging.

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Table I: Preliminary phytochemical analysis of *B. diffusa L*

S. NO	PARTICULARS	ETHANOL EXTRACT	CHLOROFORM EXTRACT
1	Alkaloids	+	+
2	Terpenoids	+	+
3	Steroids	+	-
4	Coumarins	-	-
5	Tannins	+	+
6	Saponins	+	-
7	Flavanoids	+	+
8	Quioines	-	-
9	Phenols	+	+
10	Proteins	+	+
11	Carbohydrates	+	-
12	Glycosides	+	-
13	Gum	-	-
14	Starch	-	-
15	Volatile oil	-	-
16	Fixed oil	-	-

+PRESENT -ABSENT

Table II: *In vitro* total antioxidant activity of *B. diffusa L*

Concentration (mcg/ml)	Gram Equivalent To 1Gm of Vitamin E of Ethanol Extract
500	2.340
1000	2.863

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Table III: *In vitro* DPPH radial scavenging activity of *B. diffusa L*

Concentration (mcg/ml)	Percentage Inhibition			
	Ethanol Extract	Chloroform Extract	Ethyl Acetate Extract	Curcumin
1.5	0.25	-	0.12	76.8
3	11.1	3.52	0.87	80.4
7	13.75	4.26	3.12	81.7
15	14.66	4.83	4.39	82.4
30	20.26	6.32	5.62	83.9
62	31.25	10.45	7.45	84.7
125	60.45	21.96	14.63	85.6
250	71.35	25.55	23.85	86.9
500	64.36	30.64	21.52	87.8
1000	42.58	33.74	16.87	89.6

Table IV: Invitro Nitric Oxide Scavenging Activity of *B. diffusa L*

Concentration (mcg/ml)	Percentage Inhibition		
	Ethanol Extract	Chloroform Extract	Quercetin
1.5	0.68	O.19	-
3	1.36	0.39	-
7	7.24	2.33	-
15	10.58	3.56	-
30	21.45	9.26	80.99
62	45.23	14.25	86.63
125	72.69	25.68	86.43
250	78.25	36.24	87.18
500	80.56	39.65	87.74
1000	81.94	42.58	88.02

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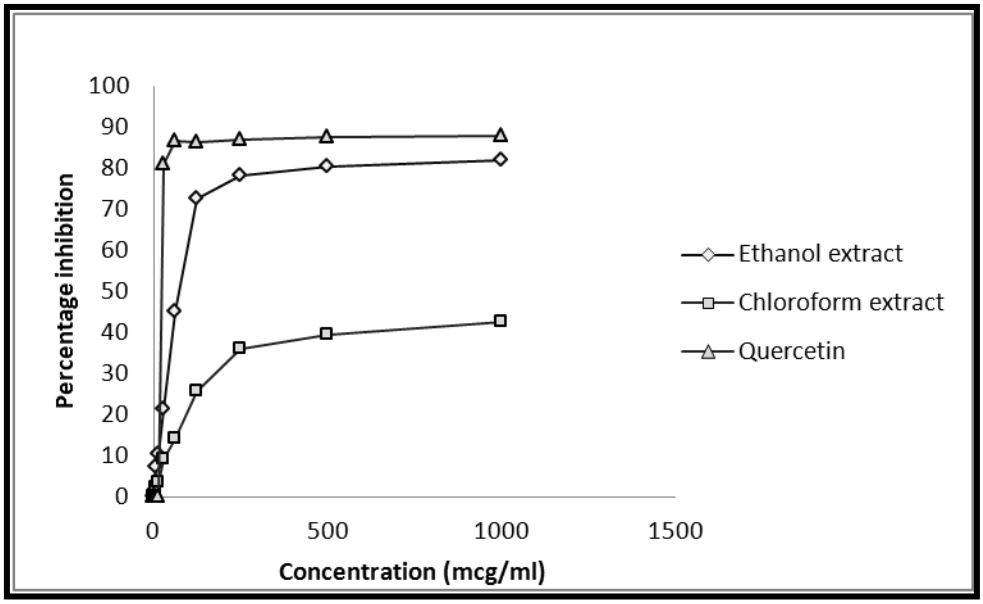

Fig I: ***In vitro*** **DPPH radial scavenging activity of** ***B. diffusa L***

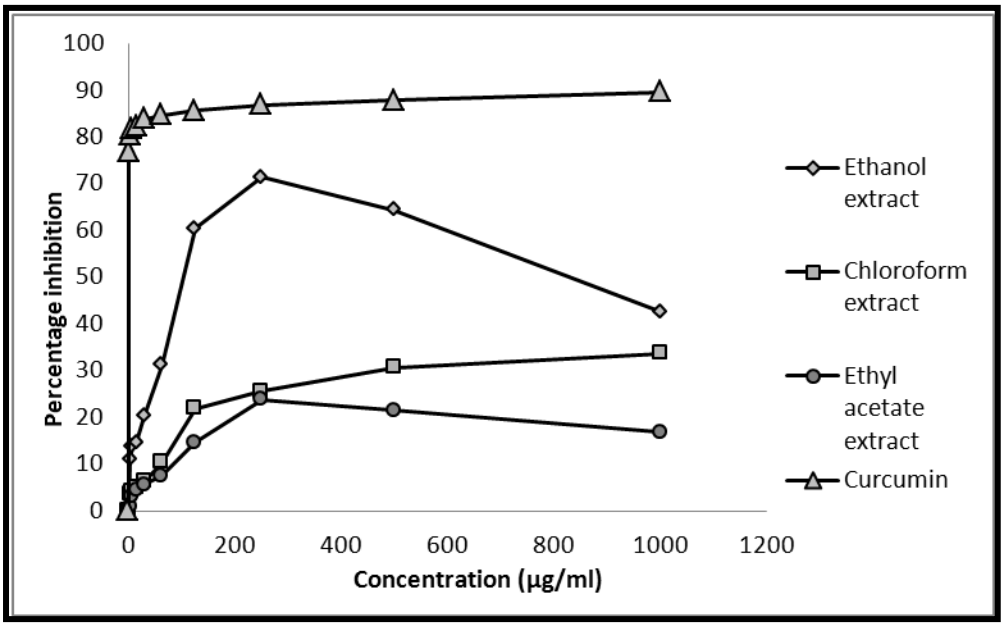

Fig II: ***In vitro*** **Nitric Oxide Scavenging Activity of** ***B. diffusa L***

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