IN VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF BOERHAVIA DIFFUSA LINN. (ROOTS) OBTAINED FROM FOUR DIFFERENT GEOGRAPHICAL REGIONS

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Abstract

Boerhavia diffusa Linn belonging to Nyctaginaceae family is highly reputed plant and has been widely employed in herbal medicine but no work has been carried out on comparison between the antioxidant activities obtained from four different geographical regions.

The antioxidant property of methanolic extract of Boerhavia diffusa Linn. was evaluated by estimation of Free radical scavenging activity (DPPH- radical scavenging activity), Total flavonoid contents and free reducing assay method The DPPH radical scavenging activity of **B. diffusa** was estimated by Inhibitory concentration (IC_{50}) at 517 nm and the free radical scavenging assay was analyzed by Folin - ciocalteu colorimetric method using ascorbic acid as standard, absorbance was reported at 765 nm.

Results were reported as DPPH radical scavenging activity (IC_{50} value) was 350 µg/ml and free scavenging assay was 76.85% (Bangalore) 75.22% (Jhansi),74.88% (pune),73.92% (Delhi) respectively at a concentration of 50 µg/ml.
Thus from ongoing study it is concluded that roots of B. diffusa Linn. possess significant Antioxidant activity and can be used for further research study. But the plant which is collected from Bangalore and Pune regions has maximum in vivo antioxidant activity. These shows the geographical conditions affect the phyto constituents of plant.

Key words: in vivo Anti-oxidant activity, Boerhavia diffusa (Linn) BD, Pune (P), Bangalore (B), Jhansi (J), Delhi (D).

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1. INTRODUCTION

Boerhaavia diffusa Linn. commonly known as Punarnava. The plant has drawn lot of attention due to its uses in Indian Traditional Medicine. The various parts of the plant are used in the treatment of cancer, jaundice, dyspepsia, inflammation, enlargement of spleen, abdominal pain and as an anti-stress agent. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases [1,6,18]. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as inflammation, diabetes, diabetes, Parkinson's and Alzheimer's diseases, atherosclerosis a as well as cancers. Reactive oxygen species are also said to be responsible for the human aging.

An antioxidant can be broadly defined as any substance that inhibits delays oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like Phenolics acids, polyphenols and Flavanoids scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Herbal plants considered as good antioxidant since ancient times[2,3,4].

B. diffusa Linn belonging to Nyctaginaceae family is highly reputed plant and has been widely employed in herbal medicine and aromatherapy . B.diffusa Linn. is well-known for its nutrition and health-promotion values. Different parts of B. diffusa Linn. have been used for the treatment of various human ailments such as itches, cuts, ulcers, swellings, bilious fever, catarrh, eczema, .antipsychotic etc. but no work has been carried out on the antioxidant activity of the roots extracts.

Hence, the current study was designed to evaluate the antioxidant activity of methanolic extracts of Boerhavia diffusa linn. (roots) obtained from four different geographical regions including by using DPPH scavenging assay, Total Flavonoids and Ferric Reducing Power method.

2. MATERIALS AND METHODS

Plant material

The roots of B. diffusa Linn. of all the four samples were procured from different botanical gardens and plants samples were identified and further confirmed by matching with the samples in the LWG herbarium .

Preparation of Sample:

Methanolic extract of roots of all four sample of B. diffusa Linn. were prepared through cold percolation by using 2 gm. of powdered material in 100 ml of methanol. The filtered solution were cooled and dried for the evaporation of the solvent. Then they were dissolved in methanol at concentration and used for further analysis [5].

Total Antioxidant Activity

1. **DPPH Radical Scavenging Assay:**

- A solution of 0.135 mm DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 1 ml of extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was vortexes thoroughly and left in the dark at room temperature for 30 minutes the absorbance of mixture was measured spectrophotometrically at 517 nm ascorbic acid, Quercetin and Ascorbic acid were used as references. The ability to scavenge DPPH radicals was calculated by the following equation:[19]. The result shown in **table 1and 2, Fig 1 and 2.**

DPPH radical scavenging activity (%)= [(Abs $_{control}$ - Abs $_{sample}$)] × 100
(Abs $_{control}$)

Where: Abs $_{control}$ is the absorbance of DPPH radical + methanol;
Abs $_{sample}$ is the absorbance of DPPH radical + sample extract / reference.

2. Total Flavonoid Content:

Prepare a stock solution (1mg/ml) of extract in methanol. From the stock solution take suitable quantity of the extract into 25ml volumetric flask and add 10ml of water and 1.5ml of folin ciocalteu reagent keep the mixture for 5 min and then add 4ml of 20% Na_2CO_3 and make up to 25ml with distilled water. Keep the mixture for 30 min and record absorbance at 765nm. Total flavonoid content was calculated as Querecetin (mg/ml) using the following equation based on the calibration curve: y = 73.56x + 0.073, R^2 = 0.997, where y was the absorbance and x was the Querecetin equivalent (mg/ml) [Ez Ordon L. A. A. et al., 2006]. The percentage of the total phenolic was calculated in triplicate with reference to the air dried drug.[16] The result is shown in **3 and 4.**

3. Ferric Reducing Power Assay:

Reducing power of extracts was determined as described by Oyaizu [1986].
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The solution of the extracts (0.2–1.0 mg) in 1 ml of distilled water was mixed with ml of 0.2 mol/L phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50oC for 20 min. Following this, 2.5 ml of 10% (w/v) Trichloroacetic acid was added and the mixture was then centrifuged at 1750 rpm for 10 min. A 2.5 ml aliquot of supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) FeCl3; the absorbance of the mixture was read at 700 nm [16,17].The result shown in **table 5 and 6.**

3. RESULT

- **DPPH Radical Scavenging Activity**: Methanolic extract of B.diffusa roots extract shows maximum inhibition of free radicals upto 76.85% (Bangalore) 75.22% (Jhansi),74.88% (pune),73.92% (Delhi) at a concentration of 50 µg/ml.

- Concentration of 5 ug/ml & 5 ug/ml. IC_{50} of sample was calculated as **385µg/ml**. while the standards Ascorbic acid shows maximum inhibition of 71.91% at concentration of 5 ug/ml .

Table 1: Absorbance at various concentrations (mg/ml) of in-vitro antioxidant activity:

Concentration	**0.02**	**0.04**	**0.06**	**0.08**	**0.1**	**control**
Ascorbic acid	0.5262	0.4048	0.3084	0.2333	0.4716	0.611
Quercetin	0.511	0.405	0.302	0.201	0.154	0.519
B. diffusa (B)	0.381	0.273	0.21	0.139	0.109	0.492
B. diffusa (P)	0.391	0.29	0.215	0.16	0.11	0.44
B. diffusa (J)	0.401	0.382	0.25	0.175	0.134	0.45
B. diffusa (D)	0.398	0.32	0.25	0.12	0.115	0.447

Table 2: Percentage of Standards and Sample at various concentrations (mg/ml)

Concentration	**0.02**	**0.04**	**0.06**	**0.08**	**0.1**
Ascorbic acid	13.879%	33.748%	49.525%	61.817%	71.915%
Quercetin	1.541%	21.965%	41.811%	61.272%	70.328%
B. diffusa (B)	22.561%	40.393%	54.148%	69.651%	76.201%
B. diffusa (P)	11.136%	34.091%	51.136%	63.636%	75.000%
B. diffusa (J)	10.889%	15.111%	44.444%	61.111%	70.222%
B. diffusa (D)	10.962%	28.412%	44.072%	73.154%	74.273%

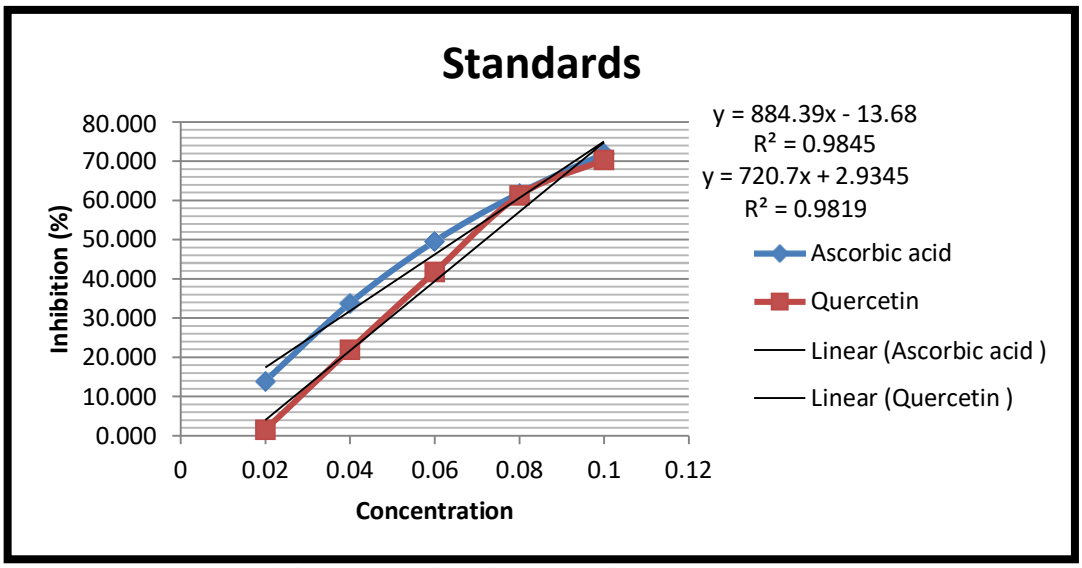

Figure 1. Graph between Absorbance and Concentration in DPPH assay

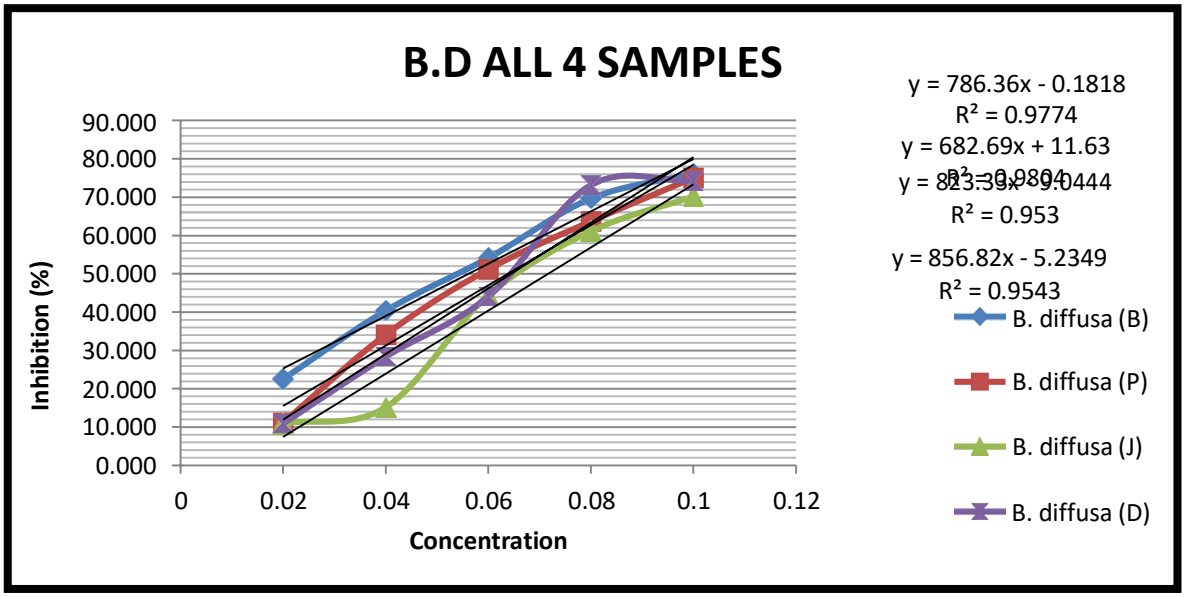

Fig 2. Graph between Absorbance and Concentration in DPPH assay

Total Flavonoid contents:

Table 3: Absorbance at various concentrations of standard and sample (mg/ml) of in-vitro antioxidant activity:

Concentration	0.004	0.008	0.012	0.016	0.020
Quericetin	0.3453	0.6692	0.9677	1.2711	1.5157
B.diffusa Linn. (B)	0.0015	0.0018	0.0018	0.0019	0.0018
B.diffusa Linn. (P)	0.0015	0.0018	0.0018	0.0019	0.0018
B.diffusa Linn. (J)	0.0013	0.0016	0.0016	0.0016	0.0017
B.diffusa Linn. (D)	0.0014	0.0017	0.0016	0.0016	0.0016

Table 4: Preparation of calibration curve for flavonoid content

S. no.	**Amount from stoke (ml)**	**2% aluminum chloride solution(ml)**	**Dist. Water (ml) up to**	**Conc. (mg/ml)**	**Abso. At 420 nm**			
					B.diffusa (B)	**B.diffusa (P)**	**B.diffusa (J)**	**B.diffusa (D)**
1	0.2	0.5	10	0.004	0.3453	0.3521	0.3421	0.3453
2	0.4	0.5	10	0.008	0.6692	0.6701	0.5682	0.5986
3	0.6	0.5	10	0.012	0.9677	0.9680	0.7456	0.8653
4	0.8	0.5	10	0.016	1.2711	1.2692	1.3652	1.3125
5	1	0.5	10	0.020	1.5157	1.5200	1.4510	1.4682
6	Blank	0.5	10	-	-	-	-	-

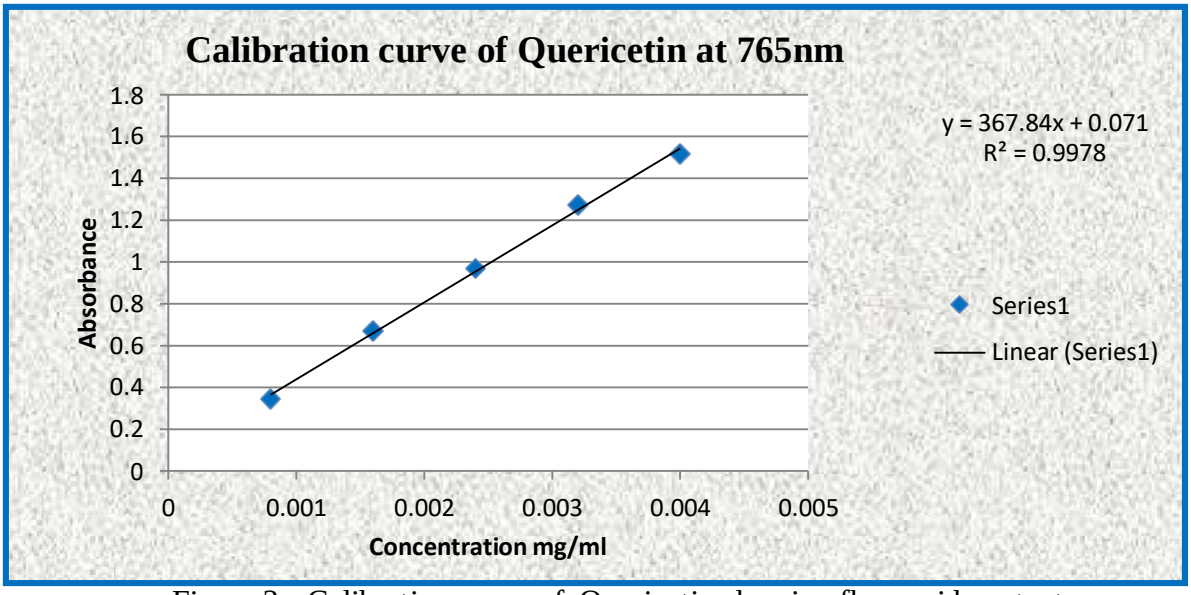

Figure 3:- Calibration curve of Quericetin showing flavonoid content

Ferric Reducing Power Assay:

Table 5: Preparation of dilutions for calibration curve (std. is Ascorbic acid).

S. No	Volume Taken From Stock (ml)	Distill Water upto 1ml	Phosphate buffer (pH 6.6) + Potassium ferricyanide (ml)	Final Volume Taken (ml)	Distill water (ml)	Ferric chloride (0.1% w/v)	Absorbance at 700 nm Ascorbic Acid
1	0.02	0.98	2.5 + 2.5	2.5	2.5	0.5	0.2363
2	0.04	0.96	2.5 + 2.5	2.5	2.5	0.5	0.2488
3	0.06	0.94	2.5 + 2.5	2.5	2.5	0.5	0.2899
4	0.08	0.92	2.5 + 2.5	2.5	2.5	0.5	0.3222
5	0.1	0.90	2.5 + 2.5	2.5	2.5	0.5	0.3539
6	Control	1.0	2.5 + 2.5	2.5	2.5	0.5	0.2363

Table 6: Absorbance at various concentrations (mg/ml) of in-vitro antioxidant activity:

Concentration	**Absorbance at 700 nm**				
	Ascorbic acid	B.Diffusa (B)	B.Diffusa (J)	B.Diffusa (P)	B.Diffusa (D)
1) 0 .020	0.2363	0.207	0.201	0.21	0.207
2) 0.040	0.2488	0.22	0.21	0.215	0.223
3) 0.060	0.2899	0.237	0.225	0.234	0.238
4) 0.080	0.3222	0.262	0.255	0.252	0.256
5) 0.10	0.3539	0.282	0.281	0.286	0.289

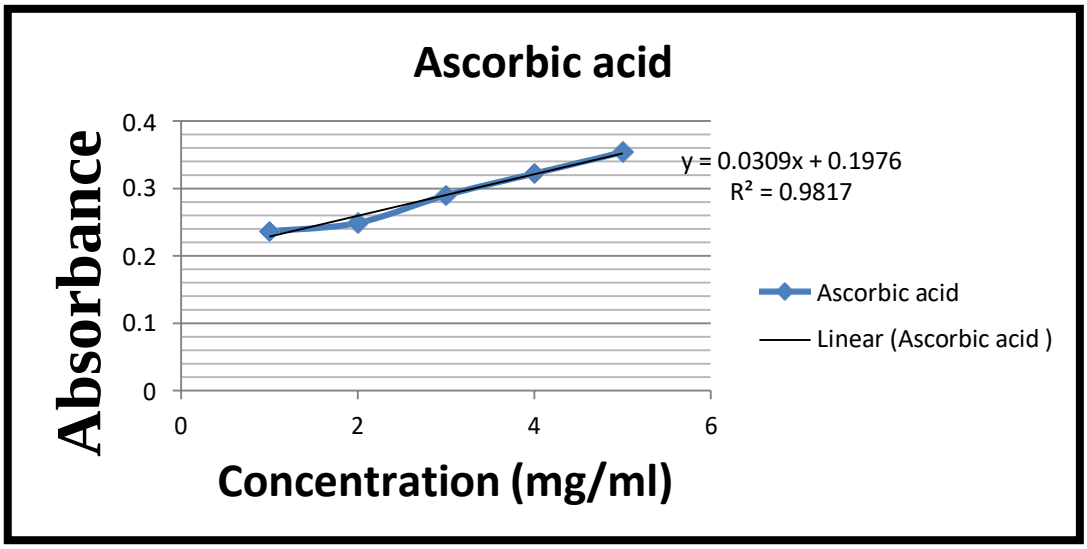

Figure4. Graph between Concentration & Absorbance in Ferric ion reducing assay of Ascorbic acid

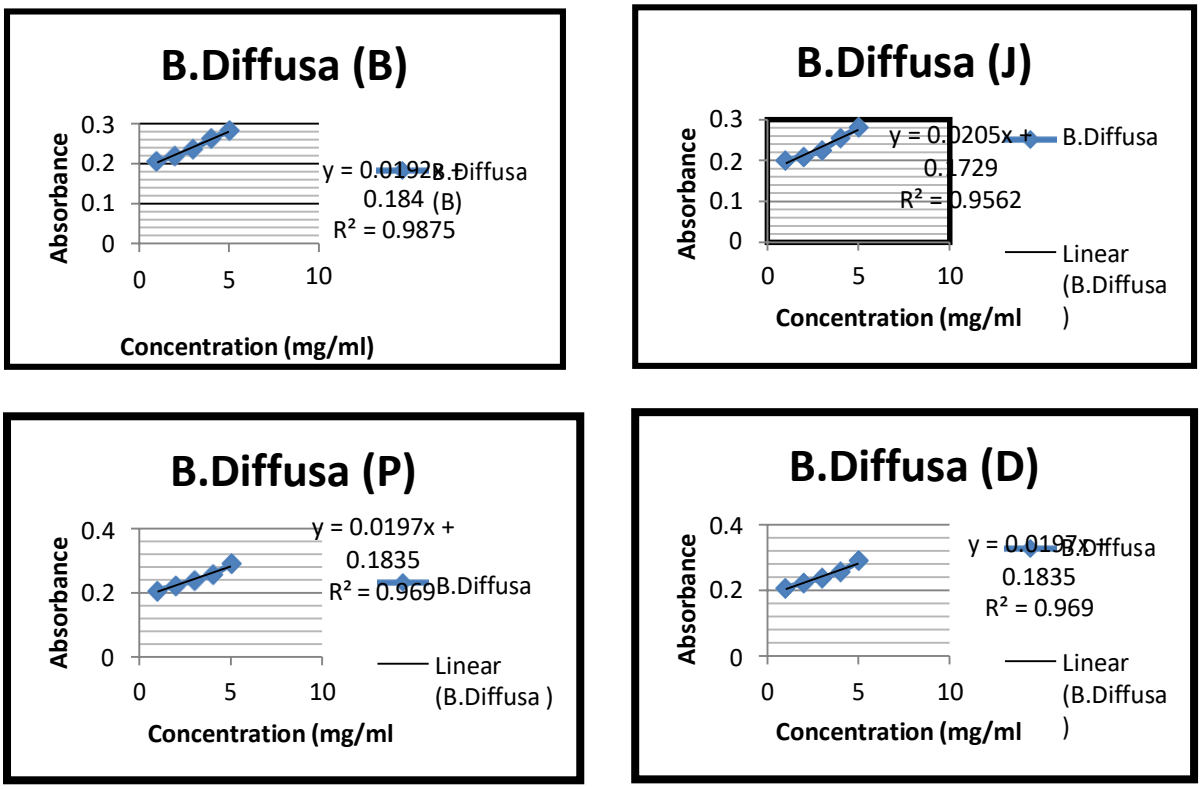

Figure 5 . Graph between Concentration & Absorbance in Ferric ion reducing assay of all B.diffusa samples

The reducing power of B. diffusa Linn extracts of four different samples using the potassium ferricyanide method is shown in **Figure 4.** The result indicates that the reducing ability of the extract increased with the concentration. as shown by the increasing optical density at **700 nm**.

4. DISCUSSION AND CONCLUTION

- **In-vitro DPPH free radical scavenging activity:** In-vitro DPPH free radical scavenging activity of the methanolic extract of four different samples of B.diffusa Linn. were compared with Ascorbic acid and Quericetin (standard used),. methanolic extract of Boerhavia diffusa Linn. roots extract shows maximum inhibition of free radicals up to 76.201% (Bangalore) 75.0% (Pune),70.2% (Jhansi),74.273% (Delhi) respectively , at a concentration of 50 µg/ml. The two standards Ascorbic acid and Quercetin shows maximum inhibition of 71.91% & 70.32% at concentration of 5 ug/ml for both the standards. As shown in table 1,2 and figure 1 and 2.

- . The DPPH anti-oxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants. Analysis by one way ANOVA shows that value of sample is comparable to that of Standards and hence it is stated that Antioxidant activity is maximum in Bangalore and Pune region samples.

- **Total flavonoid Content:** In – vitro TPC activity of the methanolic extract of roots of all the four samples of **B.**Ldiffusa Linn. collected from different geographical regions were compare with Quericetin (standard used).Results obtained in the present study revealed that the level flavonoind content in the methanolic extract of the roots of B.diffusa Linn. were 11.322±0.0640 mg/g which was compared to Quericetin. The result was tabulated in **table 3 and 4, Fig. 3.**

- **Ferric Reducing Power Assay:** The reducing power of four different extracts of B.diffusa Linn. using the potassium ferricyanide method is shown in **Fig. 4.** The result indicates that the reducing ability of the extracts increased with the concentration as shown by the increasing optical density at 700 nm. The result was tabulated in **table 5 and 6, Fig.4and 5.**

Reducing power is associated with its anti-oxidant activity and may serve as a significant reflection of the anti-oxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary anti-oxidants

Thus, the **plant have good in vivo antioxidant activity and could justify the ethnotherapeutic usage of this plant by the traditional healers. But the plant which is collected from Bangalore and Pune regions has maximum antioxidant activity. These shows the geographical conditions affect the Phyto constituents of plant.**

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Competing Interest:

All of the authors have nothing to declare as far as the conflict of interest is concerned.

5. REFERENCE:

1. Anonymous., (1989). The Ayurvedic Pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare, Part -1, Vol.1, Edition 1st, pp.17-18
2. Ashutoshkar., Pharmacognosy and Pharmacobiotechnology. New Age International. Edition 2nd pp.297-298.
3. Bray HC and Thorpe WV., (1954). Analysis of Phenolic compounds of interest in metabolism. Meth. Biochemical Analysis, Vol.1, pp. 27-52
4. Chiavaroli V, Giannini C, De Marco S, Chiarelli F, Mohn A. Unbalanced oxidant-antioxidant status and its effects in pediatric diseases. Redox Rep. 2011;16:101–107.[PubMed].
5. Evans W. C., (2006). Trease and Evans Pharmacognosy. Edition 15, Elsevier, New Delhi , pp. 138-140.
6. Evans W.C., (2008). Pharmacognosy. Edition15th, pp. 253.
7. EzOrdon LAA et al., (2006). Antioxidant activities of Sechiumedule (Jacq.) Swart extracts. Vol. 97, pp. 452-458.
8. Pooja, Lal V. K, and Verma A. "GC-MS and phyto pharmacological analysis of aqueous distillate of Boerhavia diffusa roots" , Int. J. Pharm. Pharm. Res., vol. 10, no. 2, 2017 , pp. 374-391.
9. Indian Herbal Pharmacopoeia. Mumbai: IDMR; 2002, pp. 88-97.
10. Kirtikar K, and Basu B. Indian Medicinal Plants. Delhi: Periodical Experts Book Agency; 1991.
11. Nayak P, and Thirunavoukkarasu M., "A review of the plant Boerhaavia diffusa : its chemistry , pharmacology and therapeutical potential", J Phyto pharm. , vol. 5, no. 2, 2016, pp. 83–92.
12. Mahesh A, Kumar H, Mk R, Devkar RA., " Detail Study on Boerhaavia Diffusa Plant for its Medicinal Importance-A Review", Res J Pharm Sci J Pharm Sci., vol. 1, no. 1, 2012, pp. 28–36.
13. WHO guidelines, Quality control methods for medicinal plant materials, A.I.T.B.S publishers and distributors, New Delhi, 2002, pp.6-84.
14. The Ayurvedic Pharmacopoeia of India, 1989, Government of India, Ministry of Health and Family Welfare, New Delhi, vol-2, pp. 65-, [10]Monali Patel, R.Verma," Antioxident activity of Boerhavia diffusa extract", 165.internation Joural of Pharmacognosy and Phytochemical Reserch6(3),2014: 598-605
15. Navan R, Bhalodia, Pankaj B. Nariya, R.N.Acharya In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn, 2013 Apr-Jun; 34(2): 209–214.
16. Mama Sirou, " Antioxidant and Free radical scavenging activity of various extracts of Boerhavia diffusa Linn. (Nictaginaceae), the Pharmaceutical and chemical journal, Vol-5, Issue (2), 2018: 62-7
17. Vijeta Gupta , Vijender Singh, " In vitro antioxidant activity of methanolic extract of Citrus limon Linn. (leaves), European Journal of Pharmaceutical and Medical Research. 2018; 6(1):636-641
18. Vijeta Gupta , Tarun Virmani , Vijender Singh, A Clinical evaluation of Boerhavia diffusa extract obtained from different geographical sources, ejpmr, vol 2020,7(11), 844-855
19. Vijeta Gupta , Tarun Virmani , Vijender Singh, AComparative Diagnostic account of the roots of Borehavia diffusa Linn. from four different geographical regions India, International Journal of Agriculture and Animal Production, 2022, Vol : 02 ,(03) :14-19
20. M.Grover, T Behle, T Virmani, Phytochemical Screening , antioxidant assay and cytotoxic profile of different extract of Chrysopogon zizanioides roots, Chemistry and Biodiversity, 2021, 18(8):e2100012.

21. C Singh, T Virmani, J Gupta, R Virmani, D Gahlawat, Antiobesity Potential of Boerhavia diffusa on Animal modal of obesity, January 2015 World Journal of Pharmaceutical Research 4990(4 11):1196-1206