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Research Article

Physico-Chemical, Elemental and Antioxidant Investigation of *Boerhavia diffusa* L

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ABSTRACT
Present investigation was undertaken to determine the foreign matter, extractive values, moisture content, ash values, elemental content and anti-oxidant activity. Selected parameters were determined for the selected plant material. Physico-chemical parameters were used to standardize the plant material. Elemental study was undertaken to estimate the concentrations of elements present in *B. diffusa* L. root. Energy dispersive spectrometer (EDS) was used for elemental analysis in the field of quality control procedures and research concerned with plant samples and concentrations of various elements present were estimated by inductively coupled plasma-optical emission spectrometry (ICP-OES). Selected plant material contains he high levels of Ca, Fe and Mg; and the study indicates the presence of essential and potentially toxic elements are within the limit and *B. diffusa* L. root can be used on regular basis without any harmful effect. Antioxidant potential of the plant was studied using in vitro free radical model i.e. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) and IC_{50} was found to be 73.132 µg/ml.

Keywords: Boerhaavia diffusa L., Elemental analysis, ICP-OES, EDS, Antioxidant.

INTRODUCTION

Boerhaavia diffusa L. (Nyctaginaceae), commonly known as 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India. *Boerhavia diffusa* L. (Nyctaginaceae) is a green vegetable that is used as herbal medicine and functional food in many countries of the world such as Brazil, Republic of the Philippines, Vietnam and India for the treatment of kidney stones, urinary retention, urinary disorders, nephritis, hepatitis, diabetes, liver disorders and cancer[1-4]. The plant has gained lot of importance in the field of ethnopharmacology because of its various pharmacological and biological activities such as, immunosuppressive activity, antimetastatic activity, antioxidant activity, antidiabetic activity, antiproliferative and antiestrogenic activity, analgesic and anti-inflammatory activity, antibacterial activity, antistress and adoptogenic activity, antilymphoproliferative activity, nitric oxide scavenging activity, hepatoprotective activity, anti-viral activity, bronchial asthma, anti fibrinolytic activity, chemopreventive action, genetic diversity analysis, anticonvulsant activity[5]. Various phytochemical studies found that flavonoid glycosides, isoflavonoids (rotenoids), steroids (ecdysteroid), alkaloids, and phenolic and lignan glycosides are major active ingredients in this plant[2]. Traditional medicine incorporates health practices of plant, mineral and animal based medicines, applied alone or in combination to treat and prevent illnesses/maintain well being. Herbal medicines have been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the ayurvedic medicines is the lack of standard quality control profiles[6]. According to an estimate of WHO nearly 80% populations of developing countries relies on traditional medicines, mostly on plant drugs for their primary health care needs[7]. The plant is highly useful as immunomodulator. These immunomodulatory plants are comparatively a recent concept in phytomedicine. Immunomodulation using medicinal plants can provide an alternative approach to conventional chemotherapy for a variety of diseases[8,9]. Most of the traditional systems of medicines are effective but the need is just to validate them. For present study *Boerhavia diffusa* L. Root were taken for the standardization and investigated for elemental analysis and antioxidant activity.

MATERIALS AND METHODS

Materials

Plant Materials

Boerhavia diffusa L. root was purchased from local trade market of delhi and authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD), CSIR-NISCAIR. (Identification Ref. No.: NISCAIR/ RHMD/ Consult/ 2015/ 2933-126) *B. diffusa root was* crushed and powdered using grinder and

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Table 1: Standardization parameters with reference values.

S. No.	Parameter	Experimental Value (%age ±SEM)	Reference Value[12]
1.	Foreign Matter	1.187±0.001	Not more than 2%
2.	Extractive Value		
	Water	13.333±0.401	Not less than 9%
	Ethanol	4.533±0.111	Not less than 0.5%
	Chloroform	0.960±0.033	--
	Ethyl acetate	1.413±0.029	--
	Petroleum ether	2.213±0.029	--
3.	Moisture Content Value	7.667±0.437	Not more than 10%
4.	Ash values		
	Total Ash value	7.778±0.294	Not more than 10%
	Water soluble ash value	4.522±0.116	--
	Acid insoluble ash values	2.889±0.111	Not more than 3%

Table 2: Estimation of elements daily intake upon consumption of *b. Diffusa* l. Root (5g/day) by ICP-OES method.

S. No.	Element name	Amount of Elements present(mg/kg)	Daily intake range	Reference	
				Dosage (mg/day)	Parameter
1.	Ca	14677.365	73.386	1000.00	RLNI (SINU)
2.	Cd	Nd	0.000	0.06	PTDI (JECFA)
3.	Co	Nd	0.000	0.05-1.00	RLNI (ATSDR)
4.	Cr	2.372	0.012	0.05-0.20	RLNI (SINU)
5.	Cu	10.337	0.052	1.20	RLNI (SINU)
6.	Fe	327.348	1.637	10.00	RLNI (SINU)
7.	Mg	1863.265	9.316	150.00-500.00	RLNI (SINU)
8.	Mn	29.344	0.147	1.00-10.00	RLNI (SINU)
9.	Ni	3.498	0.018	3.00-7.00	PSL (ATSDR)
10.	Pb	8.725	0.044	0.21	PTDI (JECFA)
11.	Ti	Nd	0.000	0.0003	PSL (IPCS)
12.	V	0.639	0.003	0.01-0.02	PSL (EFSA)

PTDI: Provisional Tolerable Daily Intake, RLNI: Recommended Level of Nutrient Intake, PSL: Prescribed Safety Limit, EFSA: European Food Safety Authority[13,14], JEFCA: Joint FAO/WHO Expert Committee on Food Additive[13], SINU: Italian Society for Human Nutrition[13], EVM: Expert group on Vitamins and Minerals. ATSDR: Agency for Toxic Substances and Disease Registry[15,16], IPCS: International Programme on Chemical Safety[17].

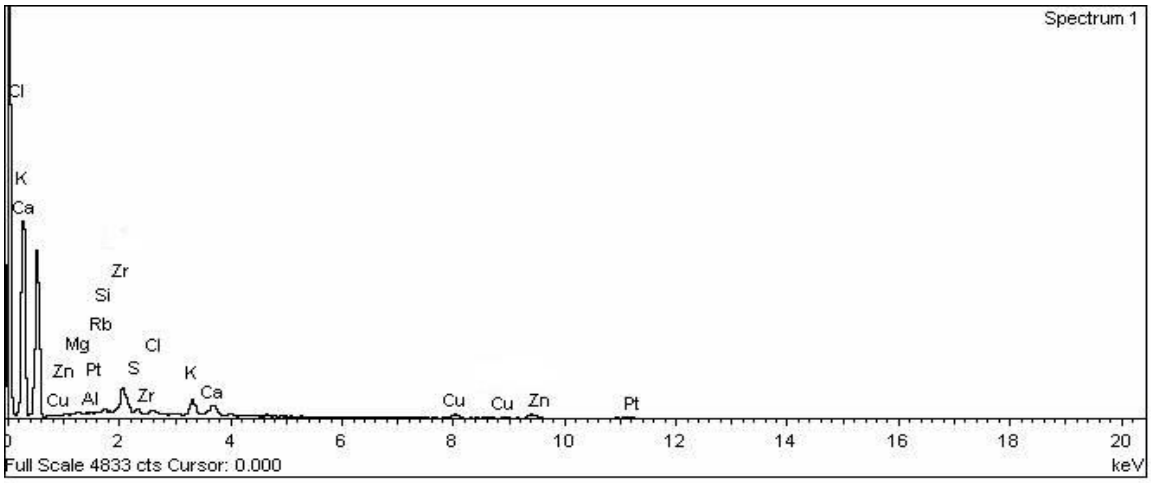
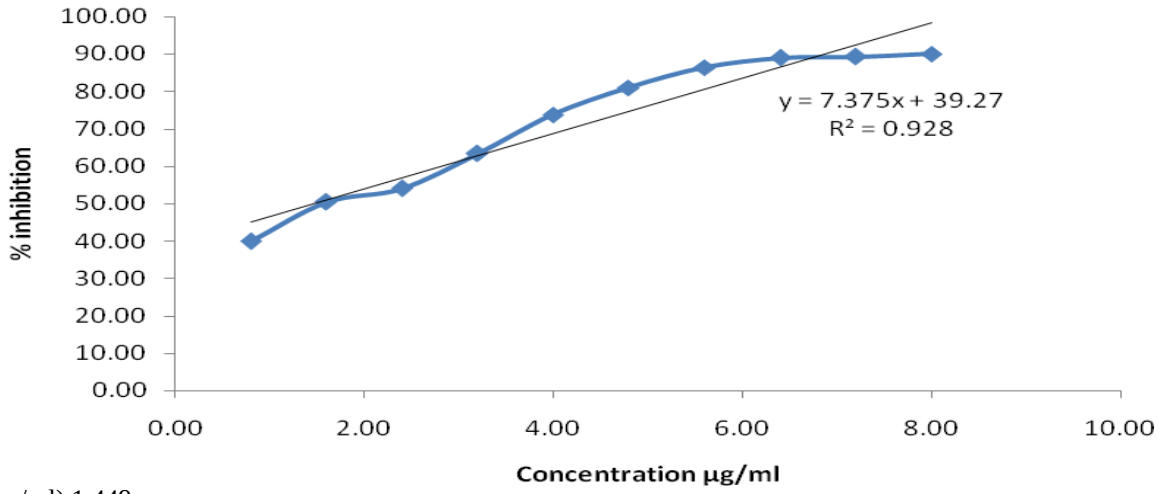


Figure 1: EDS spectrum of *B. diffusa* L. root.

passed through sieve number#85 and further used for investigation.

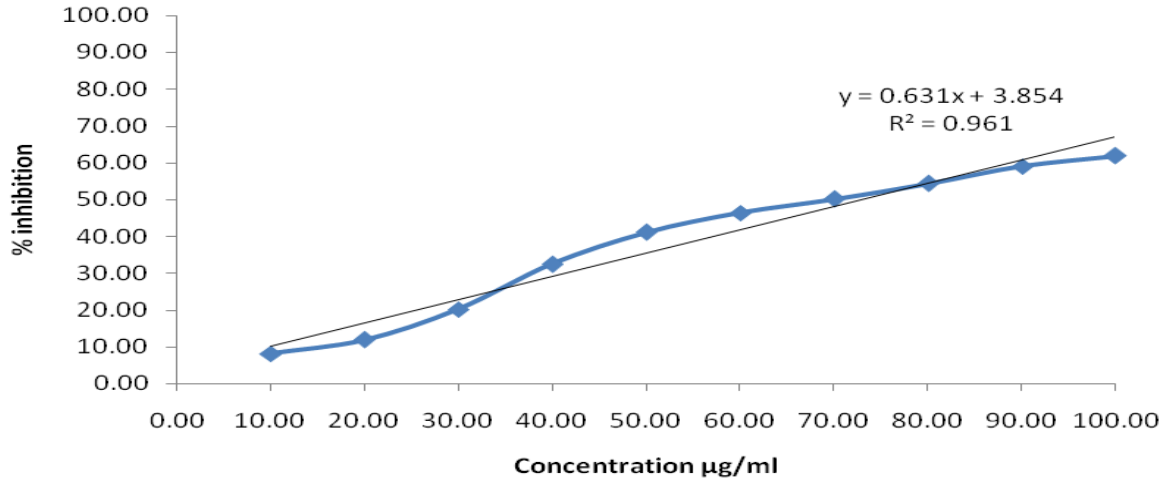
Chemicals and instrumentation

1, 1-diphenyl-2-picryl-hydrazyl and ascorbic acid were purchased from Sigma-Aldrich Pvt. Ltd.; methanol, concentrated nitric acid, concentrated hydrogen peroxide, concentrated hydrochloric acid were purchased from Rankem RFCL Limited. Weighing balance (Mettler Toledo AB265-S), UV-Visible Spectrophotometer (Shimadzu/UV-1700), Multiwave 3000 SOLV (Anton Paar) and Optical 2100DV inductively coupled plasma optical emission spectrometry (Perkin Elmer) were used



IC_{50} (µg/ml) 1.449

Figure 2: Graphical representation of antioxidant activity of ascorbic acid.



IC_{50} (µg/ml) 73.132

Figure 3: Graphical representation of antioxidant activity of *B. diffusa* L. root.

for weighing, spectrophotometric analysis, digestion and elemental analysis respectively.

Methods

Determination of Foreign matter

500 g of the drug sample was examined by spreading it on a thin layer. The foreign matter was detected by inspection with the unaided eye or by the use of a lens (6*x*). Separated and weighed the foreign matters and calculate the percentage of foreign matter present[6].

Determination of Extractive Values

5g of air dried Powder of *B. diffusa* L. root was taken and macerated with 100ml of solvent (i.e. methanol, water, chloroform, ethyl acetate and petroleum ether) in a closed flask for 24 hours, shaking frequently for the first 6 hrs and allowed to stand for 18 hrs; then filtered with precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and finally dried at 105℃ and weighed[12]. The percentage of the alcohol soluble, water soluble, chloroform soluble, ethyl acetate soluble and petroleum ether soluble extractive values were calculated with reference to air dried powder of *B. diffusa* L. root[6].

Determination of Moisture Content

10 g of *B. diffusa* L. root powder (without preliminary drying) was placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of the sample in the tarred evaporating dish dry at 105^0 C and continue the drying and weighing at 10 minutes interval until difference between two successive weights corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference. Finally moisture content was measured directly in percentage[6].

Determination of Ash values

Total Ash value: 3g of air dried *B. diffusa* L. root powder was taken in a tarred silica dish and incinerated at the temperature not exceeding 450℃ until free from carbon; cooled and weighed. The percentage of total ash value was calculated with reference to air dried powder.

Water Insoluble ash value

The total ash was boiled with 25 ml water for five minutes and filtered through an ashless filter paper (whatman filter paper#1). The filter paper was ignited in the silica crucible. Then cooled and the water insoluble ash was weighed. The water soluble ash was calculated

Table 3: Values of absorbance and % inhibition with increase in concentration of methanolic solution of ascorbic acid (standard antioxidant).

Conc. (µg/ml)	Absorbance	% Inhibition
0.80	0.233±0.002	39.88%
1.60	0.192±0.003	50.47%
2.40	0.178±0.006	54.01%
3.20	0.142±0.004	63.39%
4.00	0.101±0.006	73.82%
4.80	0.073±0.008	81.05%
5.60	0.053±0.005	86.39%
6.40	0.043±0.007	88.98%
7.20	0.042±0.007	89.23%
8.00	0.039±0.008	90.01%
IC_{50} (µg/ml)	1.449	

Table 4: Values of absorbance and % inhibition with increase in concentration of methanolic extract of *b. diffusa* l. root.

Conc. (µg/ml)	Absorbance	% Inhibition
10.00	0.353	8.16
20.00	0.338	11.98
30.00	0.306	20.23
40.00	0.259	32.47
50.00	0.226	41.15
60.00	0.206	46.44
70.00	0.191	50.17
80.00	0.175	54.34
90.00	0.157	59.03
100.00	0.146	61.89
IC_{50} (µg/ml)	73.132	

by subtracting the water insoluble ash from the total ash and percentage of water soluble ash was calculated with reference to the air dried drug.

Acid insoluble ash value

The total ash was boiled for five minutes with 25 ml of dilute hydrochloric acid and filtered through ash less filter paper (whatman filter paper#1). The filter paper was ignited in the silica crucible, cooled and acid soluble ash was calculated by weighing[6].

Elemental Analysis

The Energy Dispersive Spectrometer (EDS) was used to irradiate the samples and to collect its characteristic spectra. The system is fully controlled by an IBM PC using the software package XpertEase running under windows 3:1:1 and with the conditions like EDX Detector: Silicon, Window: SATW, Tilt (deg): 0.0, Elevation (deg): 33.0, Azimuth (deg): 0.0, Magnification: 750X, Accelerating voltage (kV): 20.00, working distance: 10mm. Before each run, the spectrometer is programmed by the user to operate under the appropriate fixed conditions for the sample using XpertEase. In the present work, the samples were irradiated under five different fixed conditions, namely Very light elements (VLE), Solids (S-V), Steels (ST), Medium elements (ME) and Very heavy elements (VHE). It is suitable for measuring the concentrations of the elements S through V using their K-Lines and the elements Ba through Sn using their L-lines[6]. Powdered sample (0.5 gm) was digested in Multiwave 3000 SOLV at 1400 watt for three hours in the solvent system of concentrated Nitric acid, concentrated hydrogen peroxide and concentrated hydrochloric acid in the ratio of 4:2:1; diluted to 100 ml and filtered. The heavy metals present in the sample were estimated quantitatively with the help of instrument ICP-OES. Calculation of the elements was done in mg/kg[10,11].

In vitro Antioxidant activity by DPPH (1,1-diphenyl-2-picryl hydrazyl) radicals scavenging[10]

Preparation of extract

Powdered sample was macerated in methanol for 72 hours, with occasional shaking. Macerate was decanted and filtered through whatman filter paper 1. The methanol extract was concentrated in vacuum and kept in a vacuum desiccator for complete removal of solvent. DPPH scavenging activity was measured by spectrophotometric method. *Preparation of reference standard solution:* 1ml of different concentrations of stock solution of ascorbic acid (50 µg/ml dissolved in methanol) i.e. 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2 & 8.0 µg/ml; 2ml of DPPH (100 µM) solution were taken and finally make up the volume up to 5.0 ml with methanol.

Preparation of sample solution and dilutions

10mg of methanolic extract of *B. diffusa L. Root* was dissolved in 10 ml of methanol to make stock solution; and the series of dilutions were done i.e. 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 & 100.0 µg/ml.

RESULTS AND DISCUSSION

Foreign matter values, Extractive values i.e. water soluble, ethanol soluble, chloroform soluble, ethyl acetate soluble, petroleum ether soluble extractive values, moisture content and ash values i.e. total ash, water soluble ash, acid insoluble ash values were determined. All the parameters were compared with reference value and found to be up to the standard as per Indian Pharmacopoeial standard. (Table 1)

Elemental Analysis

Elements modify the action of drug on the body. So the elemental analysis was carried out. Presence of different elements in the *B. diffusa* L. root is represented in EDS spectrum (figure 1.)

Element concentrations were determined by ICP-OES in the investigated formulations are reported in Table 2. Daily intake and reference values: The daily intake of each investigated element upon consumption of powdered drug is calculated taking into account the posology reported for dose, when present, or indications from the literature. Minimum and maximum amounts ingested daily are reported in the table.

Antioxidant activity

Methanolic extract of *B. diffusa* L. root was evaluated for antioxidant properties by using DPPH method. A result of antioxidant activity was compared with ascorbic acid, a standard antioxidant. As observed in Figures 2, DPPH screening has shown the IC_{50} values of 1.449µg/ml, 73.132µg/ml, for ascorbic acid and methanolic extract of *B. diffusa* L. root respectively. Methanolic extract shows

potent antioxidant activity. (Table 3, Table 4, Figure 2 & Figure 3)

CONCLUSION

The present study revealed that the set parameters for investigation can be used for correct identification of the *B. diffusa* L. root; selected plant part was found to be genuine and passed the standardization parameter as per the Indian pharmacopoeial standards. The analysis of *B. diffusa* L. root was passed the maximum tolerable limit of the elements. The comparison between the calculated daily intake of each element upon use of the investigated plant and reference values showed that the all the elements were present within the limit. The present study shows good antioxidant potential of methanolic extract of *B. diffusa* L.

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