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 variables present were estimated by inductively coupled plasma-optical emission spectrometry (ICP-OES). Selected plant material contains 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 IC50 was found to be 73.132 µg/ml.

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

INTRODUCTION

Boerhavia 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 cancer1-4. The plant has gained lot of importance in the field of ethnopharmacology because of its various pharmacological and biological activities such as, immunosuppressive activity, antitumor activity, antioxidant activity, antiobiotic activity, antiproliferative and antiestrogenic activity, analgesic and anti-inflammatory activity, antibacterial activity, antistress and adaptogenic activity, antilymphoproliferative activity, nitric oxide scavenging activity, hepatoprotective activity, anti-viral activity, bronchial asthma, anti fibrinolytic activity, chemopreventive action, genetic diversity analysis, anti-convulsant activity5. Various phytochemical studies found that flavonoid glycosides, isoflavonoids (rotenoids), steroids (ecdysteroid), alkaloids, and phenolic and lignan glycosides are major active ingredients in this plant6. 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 profiles6. 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 needs7. 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 diseases8,9. 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
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.
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 (6x). 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 \textit{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°C 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 \textit{B. diffusa} L. root.\(^6\)

Determination of Moisture Content

10 g of \textit{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°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 weighings 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 \textit{B. diffusa} L. root powder was taken in a tarred silica dish and incinerated at the temperature not exceeding 450°C 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.

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\(IC_{50}\) (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 \textit{B. diffusa} L. root.

\[ y = 7.375x + 39.27 \quad R^2 = 0.928 \]

\[ y = 0.631x + 3.854 \quad R^2 = 0.961 \]
Table 3: Values of absorbance and % inhibition with increase in concentration of methanolic solution of ascorbic acid (standard antioxidant).

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>0.233±0.002</td>
<td>39.88%</td>
</tr>
<tr>
<td>1.60</td>
<td>0.192±0.003</td>
<td>50.47%</td>
</tr>
<tr>
<td>2.40</td>
<td>0.178±0.006</td>
<td>54.01%</td>
</tr>
<tr>
<td>3.20</td>
<td>0.142±0.004</td>
<td>63.39%</td>
</tr>
<tr>
<td>4.00</td>
<td>0.101±0.006</td>
<td>73.82%</td>
</tr>
<tr>
<td>4.80</td>
<td>0.073±0.008</td>
<td>81.05%</td>
</tr>
<tr>
<td>5.60</td>
<td>0.053±0.005</td>
<td>86.39%</td>
</tr>
<tr>
<td>6.40</td>
<td>0.043±0.007</td>
<td>88.98%</td>
</tr>
<tr>
<td>7.20</td>
<td>0.042±0.007</td>
<td>89.23%</td>
</tr>
<tr>
<td>8.00</td>
<td>0.039±0.008</td>
<td>90.01%</td>
</tr>
<tr>
<td>IC₅₀(µg/ml)</td>
<td></td>
<td>1.449</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.00</td>
<td>0.353</td>
<td>8.16</td>
</tr>
<tr>
<td>20.00</td>
<td>0.338</td>
<td>11.98</td>
</tr>
<tr>
<td>30.00</td>
<td>0.306</td>
<td>20.23</td>
</tr>
<tr>
<td>40.00</td>
<td>0.259</td>
<td>32.47</td>
</tr>
<tr>
<td>50.00</td>
<td>0.226</td>
<td>41.15</td>
</tr>
<tr>
<td>60.00</td>
<td>0.206</td>
<td>46.44</td>
</tr>
<tr>
<td>70.00</td>
<td>0.191</td>
<td>50.17</td>
</tr>
<tr>
<td>80.00</td>
<td>0.175</td>
<td>54.34</td>
</tr>
<tr>
<td>90.00</td>
<td>0.157</td>
<td>59.03</td>
</tr>
<tr>
<td>100.00</td>
<td>0.146</td>
<td>61.89</td>
</tr>
<tr>
<td>IC₅₀(µg/ml)</td>
<td>73.132</td>
<td></td>
</tr>
</tbody>
</table>

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 ashless filter paper (whatman filter paper#1). The filter paper was ignited in the silica crucible, cooled and acid soluble ash was calculated by weighing.

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.

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.

**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 IC₅₀ values of 1.449µg/ml, 73.132µg/ml, 73.132µg/ml, 73.132µg/ml, 73.132µg/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.

ACKNOWLEDGEMENT
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REFERENCES