Phytochemical Screening and Evaluation of Antioxidant Potential of *Boerhaavia diffusa* L. Roots

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Abstract- *Boerhaavia diffusa* L. belongs to the family Nyctaginaceae commonly known as "Punarnava" is one of the important plants of Ayurveda, Charaka Samhita and Sushruta Samhita. The plant has immense benefits as a Rasayana- in Rejuvenation benefits. The phytochemical screening was performed by Harborne Method (1998) and the antioxidant activity was assessed by DPPH free radical scavenging assay. The results showed the presence of Alkaloids, Carbohydrates, Glycosides, Amino Acids, Proteins, Tannins, Terpenes, steroids, flavonoids and Phenols. The DPPH free radical scavenging assay showed the IC₅₀values of *Boerhaavia diffusa* L. root extract as15.57 μ g/ml and that of ascorbic acid was 3.75 μ g/ml. The results conclude that *Boerhaavia diffusa* L. roots are a very good source of Antioxidants and a natural drug which has innumerable benefits.

Keywords: Boerhaavia, rejuvenation, phytochemicals, antioxidants.

1. INTRODUCTION



diffusa L. Boerhaavia known commonly as Raktapunarnava is aherbaceous plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds.

The plant is mentioned in the Atharveda with the name 'Punarnava'' because the top plant dries up during rainy season. Boerhaavia diffusa is one of the famous medicinal plants in India, South America and Africa. (Goyal et al., 2010) Boerhaavia diffusa L. has single, thick deep penetrating root bearing few rootlets occasionally brown. Root is stout and fusiform with a woody root stock. Stems are creeping, many arising from root stock and swollen at the nodes. Microscopically, the mature root of B. diffusa L. shows a complete ring of wood surrounding the ventral vascular region (Agarwal et al., 2011). Boerhaavia diffusa L. is widely used plant jaundice, hepatitis, oedema, anaemia, in inflammation, eye diseases, hepatoprotective, diuretic, anti-inflammatory, anti-stress, immunomodulation, anti-fertility, anti-microbial, insecticidal activities. anti-metastatic antiviral. activity, anti-diabetic, anti-proliferative, antiestrogenic activity, analgesic, antilymphoproliferative activity, antifibrinolytic activity, chemoprevention and bronchial asthma. (Agarwal *et al.*, 2011; Goyal *et al.*, 2010)

2. METHODOLOGY

The plant parts were collected from the Gujarat University campus identified by Prof. Hitesh Solanki at Department of Botany with the help of Flora of Gujarat (Shah, 1978) and the voucher specimen was submitted to Gujarat University Herbarium. The roots were collected, washed and dried in shade according to WHO Quality control standards. The roots when properly dried were grinded to fine powder and stored in airtight containers for further use. The dried powdered roots were extracted with methanol. 5 grams of powder was mixed 50 ml of methanol for 24 hours. Extracts were filtered, concentrated, dried and stored in refrigerator for further use.

PHYTOCHEMICAL ANALYSIS

Qualitative tests were performed for the following phytochemicals according to the Harborne method (1998)Alkaloids, Flavonoids, Carbohydrates, Glycosides, Steroids, Triterpenoids, Proteins, Amino Acids, Fixed oils and Fats, Tannins and Phenolics, Saponins, Gums, Mucilages. The presence of phytochemicals extracted in both the plant samples was confirmed by standard protocols. (Harborne, 1988)

Test for Alkaloids

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The alkaloids were detected using Dragendroff's test, Wagner's test, Mayer's test and Hager's test. Formation of Orange red, Reddish brown and Dull white precipitates indicated the presence of alkaloids.

Test for Carbohydrates

The carbohydrates were tested by using Benedict's test-Reddish brown precipitates, Fehling's test-Brick red colour, Molisch test-reddish violet ring indicated

the presence of carbohydrates.

Test for Glycosides

The glycosides were tested by Keller-Killiani testbrown ring to violet ring below brown ring indicated the presence of glycosides.

Test for Proteins and Amino Acids

The proteins were tested by Xanthoproteic testorange colour, Ninhydrin test-purple colour precipitates indicated the presence of proteins and amino acids.

Test for Tannins

The tannins and phenolics were tested by adding 2-3 drops of ferric chloride to 1ml of extract and the formation of a dark blue or greenish black colour shows the presence of tannins.

Test for Terpenes

Terpenes were tested by Salkowski's test. 2ml of extracts were mixed with 1ml of chloroform and conc. H_2SO_4 solution. A reddish brown colour at the interphase indicated the presence of terpenoids.

Test for Steroids

The steroids were tested by Libermann-Buchard Test-Brown ring formation and Libermann-sterol test- colours from red, violet, blue to green.

Test for Flavonoids

Flavonoids were detected by Shinoda test. Few ml extract with 50% methanol. Heat. Add Magnesium metal and few drops of conc. HCl-green to blue colouration. (Tiwari *et al.*, 2011)

Test for Phenols

Phenols were tested by Ferric Chloride test. Take few ml extract, add 5ml distilled water and few drops of 5% ferric chloride solution-Blue green colour indicated presence of phenols.

DPPH (2, 2-diphenyl-1-picrylhydrazyl Free Radical Scavenging Activity)

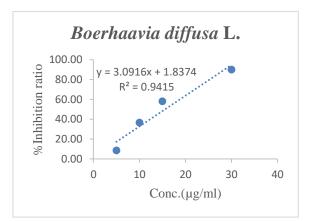
DPPH assay was performed according to Shekhar and Anju (2014) with slight modifications. Free radical Scavenging *A. aspera* leaves extract was measured by 2,2-diphenyl-1-picrylhydrazyl. In brief, 0.1mM solution of DPPH in methanol was prepared. This solution was added to 3ml of different extracts in methanol at different concentrations (5, 10, 15, 30 μ g/ml). The mixture was shaken vigorously and allowed to stand at room temperature for 30 mins. Then absorbance was measured at 517nm by using spectrophotometer (UV-VIS Shimadzu). Ascorbic Acid was used as reference standard compound and experiment was performed in triplicate.

The IC₅₀ value of the sample, which is the concentration of the sample required to inhibit 50% of the DPPH free radical was calculated using log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The present DPPH scavenging effect was calculated using the following equation: DPPH scavenging effect (%) or Percent inhibition= A_0 - A_1 / $A_0 \times 100$ where A_0 was the absorbance of test or standard sample.

| Group of Phytochemicals | Results |
|--------------------------|---------|
| Alkaloids | +++ |
| Carbohydrates | +++ |
| Glycosides | ++ |
| Proteins and Amino Acids | + |
| Tannins | +++ |
| Terpenes | ++ |
| Steroids | ++ |
| Flavonoids | +++ |
| Phenols | +++ |

3. RESULTS AND DISCUSSION:

Figure No. 1: Phytochemical Analysis of *Boerhaavia diffusa* L. roots methanolic extract.



Graph No. 1: DPPH Free Radical Scavenging Assay of *Boerhaavia diffusa* L. roots methanolic extract.

The whole plant has been of immense importance in Ayurveda. Hence, the roots were assessed for their phytochemical and antioxidant potential. Fig. No. 1

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represents the presence of various phytochemicals like Alkaloids, carbohydrates, glycosides, proteins, amino acids, tannins, terpenes, steroids, flavonoids and phenols. Graph 1. represents the DPPH free radical scavenging activity which showed an increase in the activity with the increase in the concentration of plant extracts. The IC₅₀ values of extract and standard were found to be 15.57µg/ml and 3.75µg/ml. The root extract possesses a very good activity in comparison to standard ascorbic acid. However, the use of synthetic antioxidants like BHA and BHT can be carcinogenic and liver damaging in the long run. So, *Boerhaavia diffusa* L. roots possess good potential to cure certain ailments and roots can be used for various formulation development.

4. CONCLUSION

Boerhaavia diffusa L. plant has been of immense importance for curing a number of diseases. Due to the presence of essential phytochemicals like alkaloids, carbohydrates, glycosides, proteins, amino acids, tannins, terpenes, steroids, flavonoids and phenols, the roots possess good antioxidant activity. The usage of roots for their excellent antioxidant potential is recommended from these studies. In comparison to the synthetic antioxidants, the use of roots is advisable. Also, the plant should be continued to be used in the formulations as described in our Ancient Literatures like Ayurveda, Sushruta Samhita and Charaka.

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