

Phytochemical, Nutritional and Antimicrobial Properties of *Boerhaavia Diffusa*

M. K. Thakur¹, Sulekha Pathak²

¹Department of Botany and Environmental Science, Govt. Model Science College (Auto.), Jabalpur. M. P., India

²Department of Botany, Govt. M.H. College of Home Science and Science for Women Jabalpur. M. P., India

Abstract: *Boerhaavia diffusa* Linn has attracted a lot of attention due to its prevalent uses in Ayurvedic system of medicine. It is widely used in jaundice, hepatitis, oedema, anemia, inflammation, eye diseases etc. The aim of present study was to evaluate the qualitative analysis of phytochemicals, because these phytochemicals are the basis of pharmacological properties of plant. Proximate analysis was done with the aim do determine the nutritional value, some macro-elements such as Ca, Na, K etc. play important function in many biochemical reactions. Antimicrobial activity of four solvent extracts (aqueous, methanol, ethyl acetate and petroleum ether) of *Boerhaavia diffusa* leaves was also tested against 10 human pathogenic microbes including both bacteria and fungi. Results of present study showed that plant has good nutritional value and rich in crude fiber and carbohydrates. Although antimicrobial activity was observed in only methanol and ethyl acetate extracts against *Staphylococcus aureus*, *Aspergillus flavus* and *Penicillium chrysogenum* aqueous and ethyl acetate extract did not show any inhibitory activity against any microbe. Thus it is concluded that plant play important role in strengthening, preventing and to some extent preventing the body from infections caused by infectious microbes.

Keywords: *Boerhaavia diffusa*, phytochemicals, Proximate analysis, Antimicrobial activity

1. Introduction

Plant based ingredients are commonly used as a food source and mostly the plant based secondary metabolites are taken with the food, which perform therapeutic as well as medicinal role in the body. *Boerhaavia diffusa* Linn. or spreading hog-weed, is belongs to the family Nyctaginaceae and is commonly known as, punarnava meaning that which rejuvenates or renews the body. It is a diffusely branched pubescent or glabrous, prostrate herb, abundantly occurring as a weed throughout India. *Boerhaavia diffusa* has single, thick deep penetrating tap root bearing few rootlets occasionally brown (Surange and Pendse, 1972). Root is stout and fusiform. Leaves are broadly ovate with slightly rounded or pointed apex and rounded base. The upper surface of the leaf is green but lower surface is white. The margin is entire. Flowers are bracteolate umbels and deep pink in color. The fruits are small 2.5 mm long, oval, oblong, pubescent, simple, achene bluntly five ribbed with single seed.

Boerhaavia diffusa It shows hepatoprotective activity (Das and Agarwal, 2011). The plant is used in epilepsy, pain in abdomen, dysentery and poison of scolopendrids (Jain and Tarafdar, 1970); in pneumonia, jaundice, anaemia, as blood purifier, in enlargement of spleen, as stomachic, emetic, laxative, expectorant, diuretic (Jha *et al.*, 1997); astringent, anti-asthmatic, as anti-inflammatory (Kapur, 1993), tonic in urinary troubles, uterine bleeding (Srivastava *et al.*, 1986), in dropsy, gonorrhoea, oedema. Root extract strengthen, tones and balances the liver (hepatotonic) (Rawat *et al.*, 1997). The fruits are used as a diuretic. The seeds are used as expectorant, carminative, tonic, anthelmintic in lumbago (Tripathi *et al.*, 1996). Thus the whole plant is believed to control different disorder. The present study was carried out to evaluate its nutritional and antimicrobial potential.

2. Material and Methods

Plant material- The plants were collected from South Civil lines area near Govt. Model Science College Jabalpur, M.P. The authentication of the plant was done by the available literature as well as by the State Forest Research Institute, Polipather, Jabalpur. The collected plant leaves were made thoroughly free from any foreign organic matter by washing, air dried under shade and grinded to powder by grinder.

Extraction of plant material- In order to perform a systematic phytochemical screening powdered leaves were extracted with an array of solvents. The usual technique involves extraction of phytochemicals by polar solvent directing towards non-polar solvents. For aqueous extraction cold percolation method was adopted (Harborne, 1998). The plant material was also extracted with methanol, ethyl acetate and petroleum ether by using Soxhlet apparatus by using successive extraction method.

Phytochemical Screening

Phytochemical screening was performed in the plant extracts obtained by extraction with different solvents using standard procedure as described by (Trease and Evans, 1983; Harborn, 1998 and Thimmaiah, 2004).

Proximate Analysis

The proximate analysis was done according to AOAC methods (A.O.A.C., 2007)

Moisture Content: One gram of the powdered sample was weighed in a clean crucible/ beaker of known weight. The sample was then dried in oven at 105°C for 8 h. The crucible/beaker was cooled and weighed to determine water loss in powdered sample.

$$\text{Moisture (\%)} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

Estimation of fat content: The apparatus used for estimation of fat is Soxhlet extractor. To determine the percentage of fat the dried sample of plant was extracted with petroleum ether. It was then distilled off completely and dried. The oil weight and percentage of oil was calculated.

Estimation of crude fiber: Briefly, 2 g of sample was subjected to acid and subsequent alkali treatment. Oxidative hydrolytic degradation of native cellulose and considerable degradation of lignin occurs. The residue obtain after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gives the crude fiber contents.

Estimation of ash Percentage: Weighed 2 g of each sample into the crucible and placed in to the Muffle furnace. Heating was started gradually until temperature of 600°C was reached. This temperature was maintained for 6 h. The crucible was then put inside desiccators and cooled. After cooling, the sample was reweighed and the percentage of ash was calculated.

$$\text{Ash \%} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} * 100$$

Estimation of Nitrogen (protein) percentage: (Jones, 1988; Subbarao, 1993) - Nitrogen was estimated using micro Kjeldahl method. In this method, 50 mg sample was digested by boiling with concentrated sulfuric acid in the presence of catalyst copper sulfate till the formation of the clear solution. The ammonia was released by the addition of excess sodium hydroxide in presence of compressed water vapors and was removed by steam distillation. The distillate was collected in 4% boric acid solution and titrated with standard hydrochloric acid using methylene blue as an indicator. Total protein was calculated by multiplying nitrogen percentage by 6.25.

Estimation of carbohydrates: (Krishnaweni *et al.*, 1984) - The phenol and sulfuric acid method is general method for carbohydrate estimation. It is a modification to the Anthrone's method of carbohydrate estimation. For this, 100 mg of sample was digested for 3 h with 2.5 N HCl and all the carbohydrate was converted into glucose which is further dehydrated to hydroxyl methyl furfural. The solution was neutralized with sodium carbonate. To each test tube, 1 ml of 5% aqueous phenol was added. One milliliter concentrated sulfuric acid was then carefully dispensed to each tube. The solution was allowed to stand for 20 min before taking the readings at 490 nm. The absorbance was converted to glucose concentration using a standard curve of D-glucose prepared in the same manner.

Nutritive Value: After estimation of protein, fat and carbohydrate, the nutritive value was calculated as per the following formula.

$$\text{Nutritive value (Kcal)} = 4x \text{ Protein\%} + 9x \text{ Fat\%} + 4x \text{ Carbohydrate\%}$$

Estimation of Mineral contents: Acid digestion method was used to digest the dried plant powder for mineral analysis. Organic matter of dry plant powder was wet oxidized with the sequential combination of perchloric acid, nitric acid (HNO₃) and sulphuric acid (H₂SO₄) (1:2.5:1) at

125°C temperature. After complete digestion the sample was cooled, diluted with distilled water up to final volume of 50 ml. The estimation of nutritionally important minerals i.e. P, Na, K, Ca, was done by spectrophotometer.

Antimicrobial Assay

Test organisms and procedure- The different extracts were screened for antimicrobial activity against ten clinically important microbes. These were three Gram negative bacteria i.e. *Escherichia coli*, *Salmonella abony* and *Pseudomonas aeruginosa*, two Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* and five fungi *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium solani* and *Penicillium chrysogenum*. The agar well diffusion method was used to determine the antimicrobial activity using Bauer-Kirby method (Bauer *et al.*, 1966). Erythromycin (10 µg) was used as a control antibiotic for Gram negative bacteria while Amikacin (30 µg) for Gram positive bacteria and Miconazole (30 µg) was used as control antibiotic for fungi. 50 µl of the plant extract (aqueous, methanol, ethyl acetate and petroleum ether) was placed into wells. The plates were incubated. After incubation period the results were expressed as diameter of the clearing zone (zone of inhibition).

3. Result

The aqueous extract of *Boerhaavia diffusa* showed the presence of alkaloids, saponins, resins, tannins, sterols, cardiac glycosides and triterpenes. The methanolic extract showed the presence of alkaloids, saponins and tannins. Both ethyl acetate and petroleum ether extract showed the presence of tannins only, while sterols were also present in petroleum ether extract (**Table 1**).

For the proximate analysis, the tests were done with three independent replicates and the data are presented as mean in **Table: 2 (Fig: 1)**.

Boerhaavia diffusa leaf aqueous as well as petroleum ether extract could not show any inhibition activity against any of the selected bacteria. But methanol extract showed some activity against Gram positive bacteria *Staphylococcus aureus* (10 mm) and ethyl acetate extract also showed inhibition of the same bacteria with zone of inhibition 7 mm (**Table: 3& Fig:2**).

Boerhaavia diffusa leaf aqueous and petroleum ether extract showed no activity against any of the test fungi. Methanol extract inhibits the growth of *Aspergillus flavus* and *Penicillium chrysogenum* with zone diameter 7 and 15 mm respectively. Ethyl acetate extract inhibits the growth of *Penicillium chrysogenum* only with zone diameter 13 mm (**Table: 4& Fig: 3**).

Table 1: Phytochemical analysis of different extracts of *Boerhaavia diffusa*

Constituents	Test	Extracts			
		Aqueous	Methanol	Ethyl acetate	Petroleum Ether
Alkaloids	Mayer's test	+	+	-	-
	Dragendroff's test	+	-	-	-
	Wagner's test	+	-	-	-
Saponins	Foam test	+	+	-	-
Flavonoids		-	-	-	-
Resins		+	-	-	-
Tannins	Gelatin test	+	+	-	-
	Lead acetate test	+	+	+	+
	Ferric chloride test	+	-	-	-
Sterols	Salkowski's test	+	-	-	+
Cardiac glycosides	Keller-killiani test	+	-	-	-
Triterpenes		+	-	-	-

Present +,
Absent -

Table 2: proximate and nutrient composition in *Boerhaavia diffusa*

Parameters	% concentration
Moisture	8.33
Total Ash	5.30
Crude Fibre	23.82
Protein	3.50
Carbohydrate	48.20
Fat	8.13

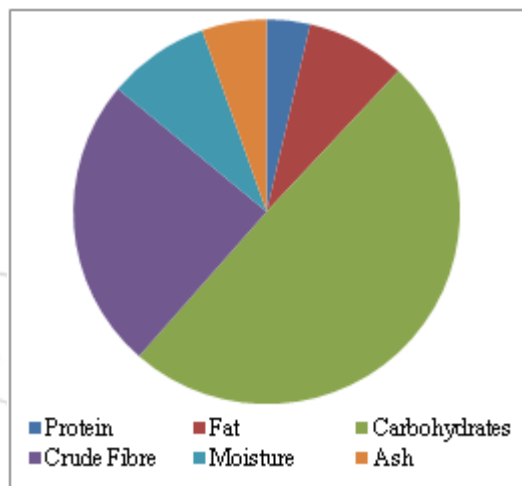


Figure 1: Proximate: Percentage of Major Nutrients In *Boerhaavia diffusa* Leaves

Table 3: Antibacterial activity of different extracts of *Boerhaavia diffusa*

Name of bacteria	Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control Erythromycin/Amikacin
<i>Escherichia coli</i>	0 mm	0 mm	0 mm	0 mm	23 mm
<i>Salmonella abony</i>	0 mm	0 mm	0 mm	0 mm	23 mm
<i>Pseudomonas aeruginosa</i>	0 mm	0 mm	0 mm	0 mm	24 mm
<i>Staphylococcus aureus</i>	0 mm	10 mm	7 mm	0 mm	26 mm
<i>Bacillus subtilis</i>	0 mm	0 mm	0 mm	0 mm	22 mm

Control-Erythromycin for Gram negative bacteria and Amikacin for Gram positive bacteria.

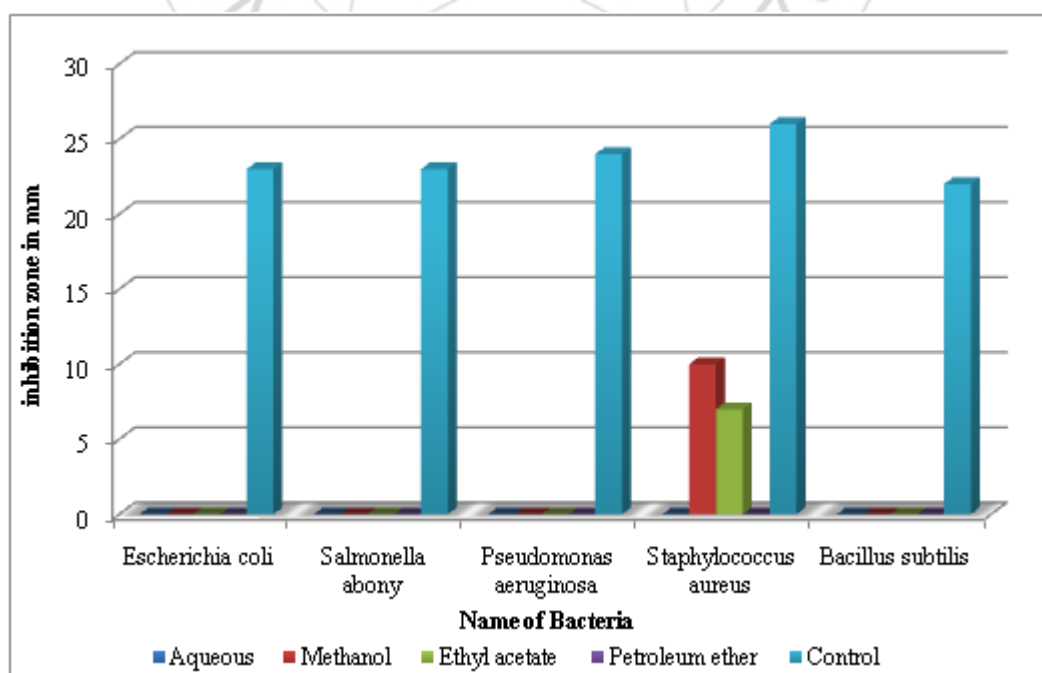


Figure 2: Antibacterial activity of different extracts of *Boerhaavia diffusa*

Table 4: Antifungal activity of different extracts of *Boerhaavia diffusa* -

Name of fungi	Aqueous	Methanol	Ethyl-acetate	Petroleum ether	Control (Miconazole)
<i>Candida albicans</i>	0 mm	0 mm	0 mm	0 mm	10 mm
<i>Aspergillus niger</i>	0 mm	0 mm	0 mm	0 mm	12 mm
<i>Aspergillus flavus</i>	0 mm	7 mm	0 mm	0 mm	12 mm
<i>Fusarium solani</i>	0 mm	0 mm	0 mm	0 mm	13 mm
<i>Penicillium chrysogenum</i>	0 mm	15 mm	13 mm	0 mm	19 mm

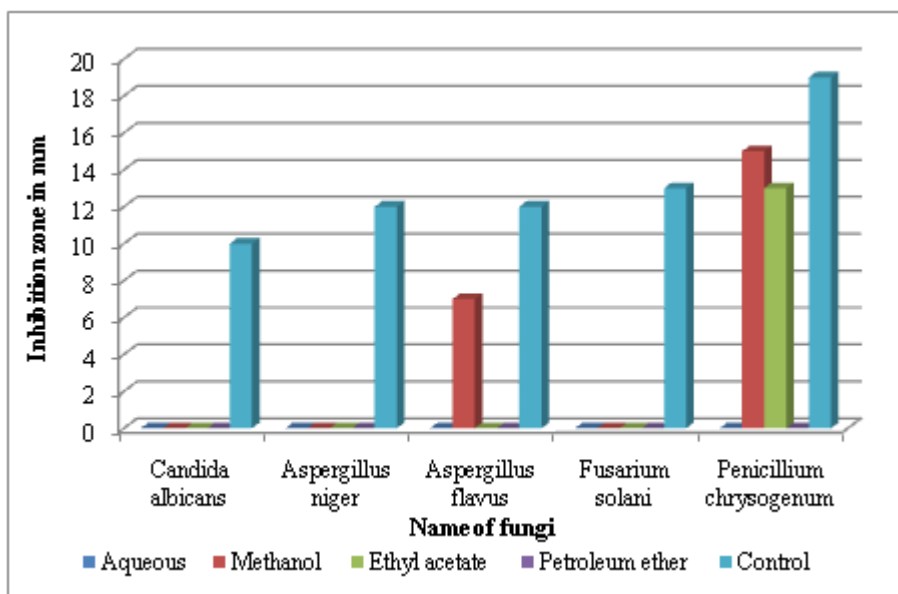


Figure 3: Antifungal activity of different extracts of *Boerhaavia diffusa*

4. Discussion

Plants may be classified as a biosynthetic laboratory not only for the chemicals that are utilized as food by human and animals but also for other compounds including alkaloids, terpenoids, flavonoids, glycosides etc. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by (Siddiqui *et al.*, 2009; Chitravadiu *et al.*, 2009; Ashok Kumar *et al.*, 2010; Savithamma *et al.*, 2011). As flavonoids having antioxidant property, it protects tissues against oxygen free radicals, they have a role in prevention of atherosclerosis, cancer, chronic inflammation and may inhibit aging. The polyphenols possess anti parasitic activity and monoterpenes have been reported to constitute anti-spasmodic, anti-neoplastic and anti-viral activities (Sharma, 2006). The presence of these phytochemical constituents revealed that the species may be used as a basic medicinal agent for analgesic, antispasmodic, antibacterial, anti-cancer, anti-inflammatory and anti-oxidant properties. The phytochemical profile of the species gives an insight into its value as a medicinal as well as highly nutritious one, safe for consumption both as a medicine and as a natural source for antioxidant and antioxidant promoting activities.

The percentage of fibre content of the plant was high (23.82) nutritionally it had been reported that food fibre aids absorption trace elements in the gut and reduce absorption of cholesterol (Le Veille and Sanberlich, 1966). Sodium and potassium are involved in maintaining water balance and acid-base balance and is the major extra cellular and intracellular mineral respectively. They are also involved in

the transport of some non-electrolytes. Calcium is found in the skeleton. Calcium is essential for the formation and maintenance of bone and for the blood clotting and muscle contraction processes. High quantity of the mineral calcium in the plant indicates its ability to regulate or control the osmotic balance of the body fluid and body pH (Subramanian *et al.*, 2012). Nutritive and antinutritive studies also performed by (Juna Beegum *et al.*, 2014) and shows that heavy metals such as lead, cadmium is low in this plant.

Das (2012) evaluated the antimicrobial activity of ethanolic extract of *Boerhaavia diffusa* by disc diffusion method and according to her growth of *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* was inhibited at 2000 µg/ml concentration and for *E. coli* minimum inhibitory concentration was 4000 µg/ml. In our findings methanol and ethyl acetate extract shows some activity against *Staphylococcus* but growth of other is not arrested. Possibly the region may be the use of 50 µg/ml conc. in this study which is not the minimum concentration for inhibition of growth of other bacteria. Results of antimicrobial study showed that methanol extract was more suitable when compared to other extracts.

5. Conclusion

The phytochemical and nutritional evaluation of *B. diffusa* indicates a high nutritive and pharmacological value of the plant. The plant screened for phytochemical constituent seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Based on the results of the present

investigation, it can be concluded that this plant can be considered as an ideal candidate for a holistic medical application.

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