

Phytochemical screening and antibacterial effect of root extract of *Boerhaavia diffusa* L. (Family Nyctaginaceae)

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Abstract: *Boerhaavia diffusa* belonging to the family of the Nyctaginaceae is mainly a diffused perennial herbaceous creeping weed of India. The present study deals with the investigation of phytochemical analysis and evaluation of the antimicrobial activity of the aqueous and methanolic extract of the roots of *Boerhaavia diffusa*. The result revealed the presence of alkaloid, glycoside, saponins, flavonoids, polysaccharides, steroid and tannin in both the root extracts. *B. diffusa* root extract possesses antimicrobial activity as the zone of inhibition was observed for both gram positive as well as gram negative bacterial strains.

Keywords: Alkaloid, Antimicrobial activity, *Boerhaavia diffusa*, Flavonoids, Phytochemical

INTRODUCTION

Boerhaavia diffusa L. (Nyctaginaceae) is a perennial herbaceous plant growing in tropical regions such as the Antilles, South America, India and Africa (Meena *et al.*, 2010). It is used in ayurvedic medicine system to treat various health problems. One of the most typical exemplary plants of the ayurvedic medicine is *Boerhaavia diffusa* Linn (Rastogi and Mehrotra, 1993; Akhilesh *et al.*, 1999). *Boerhaavia diffusa* (Spreading Hogweed in English), belongs to the family of the Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (Punarnava in Hindi) and of Brazil (known as *Erva tostão*). The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century (Mahesh *et al.*, 2012). *B. diffusa* is up to 1m long or more, having spreading branches. The stem is prostrate, woody or succulent, cylindrical, often purplish, hairy, and thickened at its nodes. The leaves are simple, thick, fleshy, and hairy, arranged in unequal pairs, green and glabrous above and usually white underneath. The shape of the leaves varies considerably ovate - oblong, round, or subcordate at the base and smooth above (Kirtikar and Basu, 1956). Six species are reported in India. This trailing herb is mainly collected after rainy season in India (Ayurvedic Pharmacopoeia of India, 2005). The whole plant analysis of *B. diffusa* is known to contain numerous phytochemicals constituents that include flavonoids, alkaloids, triterpenoids, steroids, lipids,

lignins, tannins, phlobaphenes and ursolic acid (Jones and Ennist, 1983; Jain and Singh, 1994; Lamela *et al.*, 1985). The root is mainly used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, edema, jaundice, menstrual disorders, anemia, liver, gall bladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers, diuretic documented in Indian Pharmacopoeia, digestive aid, laxative and a menstrual promoter (Ramachandra *et al.*, 2012). Traditionally, this plant has been extensively used in the treatment of dyspepsia, jaundice, enlargement of liver, abdominal pain and as antistress agent (Awasthi and Verma, 2006). The whole plant, specifically the roots have been screened to possess adoptogenic, antistress, antidiabetic and immunopotentiator activity and as antioxidant in different models (Sumanth and Mustafa, 2007). This plant has proved to be beneficial in the treatment of abdominal cancers and tumours. Studies have also shown that leaves have various nutritive components like Calcium, Sodium and Vitamin C due to which it can be included as an effective component of the diet (Rajpoot and Mishra, 2011). Despite the wide therapeutically use of *B. diffusa*, there are still no scientific data in the literature which clearly (i) demonstrate the existence of an antimicrobial activity and (ii) explain the mechanism of its action (Ramachandra *et al.*, 2012). Since *B. diffusa* is largely used in ayurveda medicine and the scientific data that explain such use are deficient, the purpose of our study was to investigate the

phytochemical constituents of *B. diffusa* and to evaluate the antimicrobial activity of the aqueous and methanolic extract of the roots of *B. diffusa*.

MATERIALS AND METHODS

Plant material: The roots of *Boerhaavia diffusa* were collected in September 2011 from local trader, Dehradun (Uttarakhand) and specimen was identified and authenticated at the Botanical Survey of India, Northern Zone, Dehradun with Accession No. 114549 and a sample deposited in the herbarium of BSI, Dehradun, U.K. All the chemicals used for phytochemical analysis were of analytical grade.

Preparation of the extract: The plant root materials were thoroughly washed, shade dried till a constant weight of sample obtained and then powdered mechanically using a blender. 100g of powdered material was soaked in 100ml of aqueous and methanol medium separately for 72 hours. It was filtered by using Whatman no.1 filter paper. The solvent was distilled out completely from the filtrate under the reduction pressure in Rota vapor (Kaur and Goel, 2009) and the weight of extract was measured.

Test microorganisms: Authentic pure cultures of bacterial strains- *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus* sps were obtained from Department of Microbiology, Shri Mahant Indresh Hospital, Dehradun, India.

Pharmacognostic study: Macroscopic and microscopic examination was done as per the guidelines of Ayurvedic Pharmacopoeia of India (2005).

Phytochemical screening

The preliminary phytochemical analysis of aqueous and methanol extracts was carried out using the standard methods described by Sazada *et al.*, 2009, Harborne, 2005, Trease and Evans, 1989.

Alkaloids-Tannic Acid Test: In the extract solution few drops of 10% tannic acid solution was added, buff precipitate was formed, and it conferred the presence of alkaloids.

Glycoside- Keller Killiani Test: Dissolve the extract in acetic acid containing traces of ferric chloride and transfer to a test tube containing sulphuric acid. At the junction, formation of a reddish brown colour, which gradually becomes blue, confirms the presence of glycosides.

Flavonoids - Alkaline Reagent Test: extract solution when treated with sodium hydroxide solution gives yellow or red color immediately.

Tannins- Vanillin Hydrochloride Test: extract solution when treated with few drops of Vanillin hydrochloride reagent gives purple color.

Saponins- Froth Test: place 1ml extract solution in a measuring cylinder. Make up the volume to 20ml using distill water. Shake for a minute. Presence of froth on top

confirms presence of saponins.

Proteins-Ninhydrin Test: Add two drops of freshly prepared 0.2% Ninhydrin reagent to the extract solution and heat. Development of blue colour reveals the presence of proteins, peptides or amino acids.

Carbohydrates-Molisch test: To 2 ml of the extract, add 1 ml of α -naphthol solution, and concentrated sulfuric acid through the sides of test tube. Purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.

Quinines-Sulfuric Acid test: To 2ml of test solution, 1 ml of conc. Sulphuric acid was added and was allowed to stand undisturbed for some time. Development of red color shows presence of quinines.

Phenols-Ferric Chloride test: extract solution gives blue-green color with few drops of ferric chloride.

Gum and Mucilage- 10ml extract added slowly to 25ml absolute alcohol with constant stirring. Presence of precipitate confirms gum and mucilage.

Steroids-Libermann-Burchard test: 1g extract dissolved in few drops of chloroform, 3ml acetic anhydride and 3ml glacial acetic acid. Drops of conc. H_2SO_4 added along the side of test tube. Appearance of Bluish-green color confirms steroids.

Lipid-Stain test: Press small amount of extract between two filter papers. Stain on filter paper one indicates presences of fats or lipids.

Determination of zones of inhibition by well diffusion method:

Methanol and aqueous root extract of *Boerhaavia diffusa* was prepared in sterile test tubes. Pure Kanamycin (50 μ l) as a standard antibiotic for comparison of the results was taken. The extracts and standard drugs were dissolved in Dimethyl Sulphoxide (DMSO). Sterile nutrient agar plates were prepared and incubated at 37 °C for 24 hours to check for any sort of contamination. The sterile nutrient agar medium in petridishes was uniformly smeared with test culture through pour plating technique. Well (5 mm) were made in each petridish to which 30 μ l of solvent extracts were added. For each treatment three replicates were maintained. Methanol and distilled water served as control for respective solvent extract. The petridishes were incubated at 37°C for 24 hours and the diameters of zone of inhibition measured in mm. Similar procedure was adopted for the pure kanamycin and the corresponding zone of diameters was compared accordingly (Satish *et al.*, 1999).

RESULTS AND DISCUSSION

The constant weight of dried plant root material out of 1000g was obtained as 960g (96%).

Macroscopic: White bitter tasting powder with a characteristic odour.

Microscopic: The powder showed characters like cork

Table 1. Successive extraction of *B. diffusa* root.

Solvent	Weight of initial root powder used	Weight of extract	Extractive value (%)	Color	Appearance
Aqueous	100 g	10.5 g	10.5 %	Dark brown	Sticky
Methanol	100 g	8.45 g	8.45 %	Light brown	Non-sticky

cells in surface view, acicular crystals and prismatic crystals of calcium oxalate, thin long narrow fibers with sharp pointed ends and narrow lumen, simple to compound oval to rounded starch grains were also observed, simple pitted vessels and few parenchyma with starch grains also seen.

Extractive value: Successive extraction of *B. diffusa* root gave extractive values of 10.5 and 8.45 for aqueous and methanol extract respectively as shown in Table 1.

Phytochemical results: Preliminary phytochemical results showed the presence or absence of certain phytochemicals in *B. diffusa*. The tests performed using water and methanolic extracts. Phytochemical test revealed the presence of alkaloid, glycoside, saponins, flavonoids, polysaccharides, steroid and tannin while absence of gum, mucilage, quinines and results are given in Table 2.

The *B. diffusa* plant contains a large number of such compounds as flavonoids (Pandey *et al.*, 2005), quercetin derivatives and alkaloids (Pereira *et al.*, 2009), lipids, steroids, carbohydrates, proteins, and glycoproteins (Khalid *et al.*, 2011; Khandelwal, 2002). Punarnavine boeravinones namely Boerhavinone A, Boerhavinone B, Boerhavinone C, Boerhavinone D, Boerhavinone E and Boerhavinone F (Chaudhary and Dantu, 2011; Lami *et al.*, 1991), hypoxanthine, punarnavoside (Jain and Khanna, 1989), have been isolated and studied in detail

Table 2. Phytochemical detection of components in *B. diffusa* root extracts.

Test	Root part	
	Aqueous extract	Methanol extract
Alkaloids	+	+
Glycoside	+	+
Flavinoids	+	+
Tannins	+	+
Saponins	+	+
Proteins	+	+
Carbohydrates	+	+
Quinines	-	-
Phenols	+	+
Gum	-	-
Mucilage	-	-
Steroids	+	+
Lipid	+	+

Table 3. Physico-chemical parameters of *B. diffusa*.

Parameters	Results
Description	Light brown
Foreign matter	<1.1%
pH	5.79
Loss on drying	3.00% w/w
Total ash	8.45% w/w
Acid-insoluble ash	1.11% w/w
Water-soluble ash	15.10% w/w
Alcohol-soluble ash	12.33% w/w

for their biological activity. Kokate *et al.*, 2005 has reported presence of 6% potassium nitrate and urosolic acid in roots of *B. diffusa*. The roots have been reported to contain alkaloids (punarnavine), rotenoids (boeravinones), flavonoids, amino acids, ligans (liriodendrons), β sitosterols, and tetracosanoic, esacosanoic, stearic and ursolic acids (Ujowundu *et al.*, 2008). These results support our result for presence of various phytoconstituents in *B. diffusa* roots.

Physico-chemical parameters: Physico-chemical parameters of *B. diffusa* are tabulated in Table.3. The results are in accordance to the Ayurvedic Pharmacopoeia of India.

Antibacterial activity: The antibacterial activity of root extracts of *B. diffusa* for few bacterial strains is presented in Table 4. The results clearly depicted that *B. diffusa* root extract has antimicrobial activity. The zone of inhibition was observed for both gram positive as well as gram negative bacterial strains that were *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus*. The methanolic extract of *B. diffusa* was observed to have comparatively more effect than aqueous extract. All the five bacterial strain had inhibited growth in methanolic extract while in aqueous extract four bacterial strains had inhibited growth. *In vitro* antimicrobial activity of methanol extract was significantly more on gram positive bacterial strains than gram negative ones. However aqueous extract of *B. diffusa* roots had almost equal inhibitory property on bacterial strains. Methanolic extract had maximum inhibition for *S. aureus* (17.5mm) and least for *E.coli* (8.5mm).The methanolic extract of *B. diffusa* showed significant antimicrobial activity with the zone of inhibition lying in the range of 8 mm to 18 mm. The

Table 4. Antibacterial activity of *B. diffusa* extracts.

Sl. No.	Bacterial strains		Zone of inhibition (mm)				
			Methanolic extract of <i>B.diffusa</i>	Control for methanolic extract- Methanol (30µl)	Aqueous extract of <i>B. diffusa</i>	Control for aqueous extract- distilled water (30µl)	Standard antibiotic- Kanamycin (30µg)
1	Gram positive	<i>Bacillus subtilis</i>	14.0	NIL	8.0	NIL	23.0
2	Bacteria	<i>Staphylococcus aureus</i>	17.5	NIL	9.0	NIL	24.0
3	Gram negative	<i>Escherichia coli</i>	8.5	NIL	8.0	NIL	22.0
4	Bacteria	<i>Klebsiella pneumonia</i>	15.0	NIL	10.0	NIL	20.0
5		<i>Pseudomonas aeruginosa</i>	15.6	NIL	NIL	NIL	20.0

aqueous extract of *B. diffusa* showed significant antimicrobial activity with the zone of inhibition lying in the range of 8mm to 10 mm. Kanamycin was used as positive control as an antibacterial antibiotic which produced the inhibition zones ranging between 20 and 24 mm. Negative control showed no formation of zone of inhibition, confirming that the inhibitory agent(s) was (were) actually from the plant extracts. The encouraging results indicate that the aqueous and methanolic root extracts of *B. diffusa* might be exploited as a natural drug for the treatment of several infectious diseases caused by these organisms and could be useful in understanding the relations between traditional cures and current medications. With this study we found that methanolic extract provides more consistent antibacterial activity. The methanolic extract of *P. harmala* showed strong antibacterial activity against gram positive than gram negative bacteria has been reported (Hayet *et al.*, 2010). Various researchers have already shown that gram positive bacteria are more susceptible towards medicinal plants extracts compared to gram negative bacteria. Difference might be due to the fact that the wall in gram positive bacteria is single layered while gram negative multilayered. Phytochemical constituents analysis of *B.diffusa* has been proved to contain alkaloids, tannins, saponins, flavonoids, phenols etc. These compounds presence might help explain the observed antibacterial activity of *B. diffusa* root extracts. The presence of bioactive compounds has been known to show medicinal as well as some physiological activity (Krishna *et al.*, 1997; Harbone, 2005). Abo and Ashidi (1999) have reported antimicrobial activity of pet ether, chloroform and methanol crude extracts of aerial parts, root of *B. diffusa* tested against both Gram positive and Gram negative bacteria by agar well method. The result revealed that the zone of inhibition of Petroleum ether extract ($16.83 \pm 0.12\text{mm}$), chloroform ($13.99 \pm 0.22\text{mm}$), methanol extract

($19.46 \pm 0.26\text{mm}$), the zone of inhibition is varying between $13.99 \pm 0.22\text{mm}$ to $19.46 \pm 0.26\text{mm}$. These data indicated that methanol crude extract of aerial part of plant exhibited strong antibacterial activity compared to petroleum ether extract. The chloroform extract of both aerial and root extract shown relatively less antibacterial activity. Ramachandra *et al.* (2012) has also reported *invitro* antibacterial potential of *B.diffusa*. The result revealed that methanol extract of root showed maximum activity against *S. aureus* ($17.49 \pm 0.42\text{mm}$), *P. aeruginosa* ($15.61 \pm 0.42\text{mm}$) and *K. pneumoniae* ($15.22 \pm 0.40\text{mm}$), compared to methanol extract, the Petroleum ether and chloroform extracts showed moderate activity against *B. subtilis* ($11.53 \pm 0.46\text{mm}$, $9.11 \pm 0.40\text{mm}$) and *A. tumefaciens* ($9.31 \pm 0.21\text{mm}$, $8.15 \pm 0.44\text{mm}$) respectively. All the three extracts showed less activity against *E. coli* ($7.89 \pm 0.2\text{mm}$, $5.95 \pm 0.0\text{mm}$, $9.17 \pm 0.17\text{mm}$) respectively. The above stated results are in accordance to the results obtained by this study.

Conclusion

Our research concludes presence of many phytoconstituents in *B. diffusa* root extracts which can provide various useful biological activities to this medicinal plant. The results clearly depicted that *B. diffusa* root extract has antimicrobial activity. Being naturally and widely present along with its cost-effectiveness can prove as an advantage of using *B. diffusa* as therapeutic drug.

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