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Phytochemical screening and *in vitro* bioactivities of the extracts of aerial part of *Boerhavia diffusa* Linn.

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ABSTRACT

Objective: To investigate the bioactivities of crude *n*-hexane, ethyl acetate and methanol extracts of aerial part of *Boerhavia diffusa* Linn. (*B. diffusa*) and its phytochemical analysis. **Methods:** The identification of phytoconstituents and assay of antioxidant, thrombolytic, cytotoxic, antimicrobial activities were conducted using specific standard *in vitro* procedures. **Results:** The results showed that the plant extracts were a rich source of phytoconstituents. Methanol extract showed higher antioxidant, thrombolytic activity and less cytotoxic activity than those of *n*-hexane and ethyl acetate extracts of *B. diffusa*. Among the bioactivities, antioxidant activity was the most notable compared to the positive control and thus could be a potential rich source of natural antioxidant. In case of antimicrobial screening, crude extracts of the plant showed remarkable antibacterial activity against tested microorganisms. All the extracts showed significant inhibitory activity against *Candida albicuns*, at a concentration of 1000 μg/disc. **Conclusions:** The present findings suggest that, the plant widely available in Bangladesh, could be a prominent source of medicinally important natural compounds.

1. Introduction

Boerhavia is a genus of 40 species[1], almost all of which are widely distributed in tropical and sub-tropical areas of Asia, Africa, America and Australia[2]. Among those species, *Boerhavia diffusa* Linn. (Synonym: *Boerhavia* glabrata Blume; Family: Nyctaginaceae) (*B. diffusa*) is a most widely studied plant and has a long history of uses by the indigenous & tribal people and in Ayurvedic and Unani medicines. In Ayurvedic and Unani, the miracle medicinal plant finds to use as a cure for 22 ailments. In Brazilian pharmacopeia, 23 uses have been described for the plant, while in Africa and Middle East, the plant is prescribed for 14 ailments[1].

The vernacular names of *B. diffusa* include Gondhapurna, Punarnava (Bengali, Sanskrit); Pigweed, Spreading hog-weed (English), *etc.*[2]. The plant is an abundant perennial creeping or climbing herb that exhibits somewhat periodic efficacy, with its maximum activity being noticed in the month of May (Summer)[3].

The root and the whole plant of *B. diffusa* are used in traditional medicine for the treatment of diabetes, stress, dyspepsia, abdominal pain, inflammation, jaundice, enlargement of spleen, heart diseases, bacterial infections[4,5] and impotence[2]. It has also been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers, various hepatic disorders and as an antiviral agent[5,6]. In Nigerian folk medicine it has been widely used for the treatment of epilepsy[7], infertility and menstrual pain[8].

Pharmacological studies have demonstrated that *B. diffusa* known to possess anticonvulsant[4], diuretic, anti-inflammatory, antifibrinolytic[9], antibacterial[10], anti-hepatotoxic, anthelmintic, febrifuge, anti-leprosy, antiasthmatic, antiurethritis, antilymphoproliferative[11], antimetastatic[12], immunosuppressive[13], antidiabetic, antioxidant[14], immune-modulation[15], hepatoprotective[16], anti-nociceptive, nephroprotective[17], bacteria induced ulcer & diarrhea[18] and antiurolithiatic[19] activities.

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The main chemical ingredients of this plant include alkaloids (punarnavine), rotenoids (boeravinones A to J) and flavones[20].

A large number of research works on the phytochemistry, pharmacology and several other aspects have been conducted, but there have been no report on phytochemical screening and *in vitro* bioactivities of *B. diffusa*. collected from Bangladesh. So the present investigations were carried out to study the phytoconstituents and *in vitro* antioxidant, thrombolytic, cytotoxic and antimicrobial activities of *n*-hexane, ethyl acetate and methanol extracts of aerial part of *B. diffusa* available in Bangladesh.

2. Materials and methods

2.1. Plant collection and identification

The fresh aerial parts of the plant were collected from the surrounding of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during January, 2010 and identified by the taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka as *B. diffusa* Linn. A voucher specimen of the plant has been deposited (Accession No.: DACB 35440) in the herbarium for further reference.

2.2. Extraction of the plant material

Shade-dried and pulverized plant material (150 g × 3) was successively extracted with *n*-hexane (BDHE), ethyl acetate (BDEA) and methanol (BDME) by continuous hot extraction using Soxhlet apparatus at a temperature for 6 hours not exceeding the boiling points of the solvents. The extracts were concentrated with a rotary evaporator (IKA, Germany) at low temperature (40-50 ℃) and reduced pressure. The extracts (BDHE: 6.40 g, BDEA: 7.49 g, BDME: 8.83 g) were stored at 4 ℃ until used.

2.3. Phytochemical screening

The freshly prepared crude extracts of *B. diffusa* (BDHE, BDEA and BDME) were qualitatively tested for the presence of Alkaloids (Hager′s test), Flavonoids (Modified Ammonia test), Steroids (Salkowski test), Terpenoids (Modified Salkowski test), Reducing sugars (Fehling′s test), Saponins (Frothing test), Tannins ($FeCl_3$ test), Cardiac glycosides (Killer-Killani′s test) and Anthraquinones (Chloroform layer test)[21].

2.4. Determination of total phenolic content

The total phenolic content of the extracts were determined by using Folin-Ciocalteu reagent[22] using gallic acid as standard. The extracts were oxidized with 10% Folin-Ciocalteu reagent (Merck, Germany), and were neutralized with 700 mM sodium carbonate solution. The absorbance of the resulting blue color was measured at 765 nm after 60 minutes using UV-VIS spectrophotometer (Shimadzu, Japan). The total phenolic contents were determined from a standard curve prepared with gallic acid. The estimation of the phenolic compounds were carried out in triplicate and the results were expressed as mean±SD.

2.5. DPPH radical scavenging activity

The free-radical scavenging activity of *B. diffusa* extracts were measured by decrease in the absorbance of methanol solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl)[23]. A stock solution of DPPH (400 μg/mL) (Sigma-Aldrich, USA) was prepared in methanol, which gave initial absorbance of 0.197, and 100 μL of this stock solution was added to 5 mL of solutions of *B. diffusa* extracts of different concentrations (20-100 μg/mL). The solutions were then mixed properly and kept in dark for 20 minutes and the absorbances weremeasured at 517 nm. Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\%\ \text{free radical scvanging activity} = \frac{\text{Absorbance of contrl} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Then % inhibitions were plotted against respective concentrations used and from the graph IC_{50} was calculated. Ascorbic acid, a potential antioxidant was used as positive control.

2.6. Nitric oxide scavenging assay

Sodium nitroprusside (SNP) (5 mM) in phosphate buffer saline (PBS) was mixed with different concentration of extracts (5-200 μg/mL) of the plant dissolved in ethanol and incubated in dark at room temperature for 2 hours. 2 mL solution was withdrawn from the mixture and mixed with 1.2 mL of Griess reagent (1% sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride and 2% *o*-phosphoric acid) and the absorbances of the solutions were measured at 546 nm using UV-VIS spectrophotometer against blank. Ascorbic acid was used as a positive control and was treated in the same way with Griess reagent[24, 25].

$$\text{Nitric oxide scavenged}\ (\%) = \frac{\text{Absorbance of contrl} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

SNP in aqueous solution at physiological pH (7.2) spontaneously generates $NO^{\bullet}$[24, 25], which interacts with oxygen to produce nitrate and nitrite ions that can be estimated by the use of Griess reagent. Antioxidants (scavengers of $NO^{\bullet}$) compete with oxygen leading to reduced production of nitrate and nitrite ions and a pink color chromophore is formed[24, 25].