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Analysis of bioactive phytochemicals and evaluation of antioxidant activity of a medicinal plant, *Boerhaavia diffusa* L.

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Abstract: *Boerhaavia diffusa* L. (Family: Nyctaginaceae) commonly known as Punarnava is an herbaceous, spreading vine widely distributed in the tropical and subtropical regions in the world. The plants are a rich source of vitamins, minerals, protein and carbohydrate. The present study was carried out to determine the concentration of some bioactive phytochemicals (ascorbic acid, carotenoids, total phenolics, protein and carbohydrate) and their antioxidant activity in punarnava. Results showed the values for ascorbic acid (16.75±1.72 and 18.86±1.12 mg/100g of Fresh Weight), carotenoids (1.36±0.10 and 1.98±0.11 ìg/g of Fresh Weight), protein (122.975±6.27 and 134.45±6.23 mg/g of dry weight) and carbohydrate (56.67±5.77 and 60.11±5.23 mg/g of dry weight) for aqueous and methanolic of root extracts of *B.diffusa* respectively. Methanolic root extracts showed greater antioxidant activity than the aqueous extracts using DPPH method.

Keywords: Antioxidant property, *Boerhaavia diffusa*, DPPH, $EC_{50,}$ Flavonoid content

INTRODUCTION

It has been reported that the diets rich in vegetables, fruits and medicinal plants provide a wide range of antioxidant, phytochemicals such as polyphenolics, carotenoids, terpenoids, flavonoids, vitamins like E and C, glutathione and vegetable pigments (Vishwakarma *et al.*, 2012). These phytochemicals offer protection against cellular damage due to their ability to quench oxygen-derived free radicals by donating electrons, chelating redox active metals and by inhibiting lipo-oxygenases (Singh *et al.*, 2009a). It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of diseases (Singh *et al.*, 2009a). Phenolic compounds have the potential to function as antioxidants by scavenging the superoxide anion, hydroxyl radical, peroxy radical or quenching singlet oxygen and by inhibiting lipid peroxidation in biological systems (Izunya *et al.*, 2010). Oxidative stress, which results due to imbalance between the antioxidant defense system and the formation of Reactive Oxygen Species (ROS), may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases (Singh *et al.*, 2009b). *Boerhaavia diffusa* Linn. (Family: Nyctaginaceae) is an herbaceous spreading vine, cultivated (Parrotta, 2001, Indian Herbal Pharmacopoeia, 2002) in the tropical and subtropical regions of the world (Sahu *et al.*, 2008). The plant is a rich source of vitamins, minerals, protein and carbohydrate (Cho *et al.*, 2004) and contains a number of constituents mainly as alkaloids, others are flavonoids, saponins and steroids (Ujowundu *et al.*, 2008). It is also used in the treatment of diabetes (Pari and Amarnath, 2004). In the present study, the methanolic and aqueous extracts of the roots of *B. diffusa* have been screened for the antioxidant properties to assess the medicinal potential of the plant.

MATERIALS AND METHODS

The roots of *B. diffusa* were collected 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.

Extract preparation: Plants were air dried at room temperature for 3 weeks to get consistent weight. The dried plants were later ground to crude powder. Two hundred grams of crude powder plant material were shaken separately in methanol and aqueous medium respectively for 24 hrs on an orbital shaker at room temperature. Extracts were filtered using a Buckner funnel and Whatman No 1 filter paper. Each filtrate was

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concentrated to dryness under reduced pressure at 40°C through evaporator. The extract was resuspended in the aqueous and methanolic solvent for further estimation (Kaur and Goel, 2009).

Estimation of phytochemicals: Ascorbic acid content of plants was estimated by the method of Arlington (AOAC, 1984) and reported as mg $100g^{-1}$ fresh weight (FW) of tissues. Carotenoids were estimated by the method of Jensen (1978) and reported as µg g^{-1} FW. Protein content was estimated by the method of Lowry *et al.* (1951) and reported as mg g^{-1} of Distill Water (DW). Carbohydrate content was estimated by Anthrone method (Thomas and Hyman, 1956) and reported as mg g^{-1} of DW.

Analysis of total phenolic content: Total phenolic content in the *B. diffusa* extract was determined by the modified Folin- Ciocalteu method (Wolfe *et al.*, 2003). An aliquot of the extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The mixtures were allowed to stand for 30 min at 40°C for colour development. Reagent blank using distilled water was prepared. The total phenolic content was calculated with the help of calibration curve prepared by repeating the operation using 1ml of gallic acid solutions at concentrations (50,100, 150, 200, 250, 300 µg/ml) in DW.

Analysis of total flavonoid content: Total flavonoid content of *B. diffusa* was estimated by colorimetric method (Chang *et al.*, 2002). The extract was added in a volumetric flask (1 ml containing 10mg/ml) followed by DW. The extract was mixed with 5% solution of sodium nitrite. After 5 min 0.3 ml of 10% AlCl3 and after 6 minute 2 ml of 1M-NaOH was added. The volume was made to 10 ml with DW and the mixture of the volumetric flask were mixed thoroughly. The absorbance of mixture was measured at 510 nm against blank.

Antioxidant activity: Free radical scavenging activity (FRSA) was measured using DPPH solution in according to Amin *et al.* (2004) and expressed in the terms of efficiency concentration (EC_{50}). To different concentrations (10-320µg/ml) of the sample and standard solution (0.5-2.5 µg/ml gallic acid) methanol was added to make up the volume to 2925µl. To this 75µl of DPPH solution was added to make total volume of 3ml in a test tube. The reaction mixture was incubated for 15mins at room temperature. Decrease in absorbance values was calculated at 510nm.EC_{50} values was calculated from the percent inhibition values. Control was carried out without test sample and was maintained throughout the experiment.

Statistical analysis: Statistical analysis was done by employing two-tailed student t-test as described by Bennet and Franklin (1967). P value less than 0.02 were considered significant.

RESULTS AND DISCUSSION

Table 1 depicts the values of ascorbic acid, carotenoid, protein and carbohydrate content in aqueous and methanolic extract of *B. diffusa*. The carbohydrate contents were higher in the methanolic extracts compared to aqueous extract.

The fundamental role of ascorbic acid is to react non-enzymatically with superoxide, hydrogen peroxide, singlet oxygen etc. to minimize the damage caused by oxidative stress. Stephen *et al.* (2002) reported that the ascorbic acid has the ability to enhance the body's antioxidant defense and is important in the healing of ulcers and delays the onset of other diseases. Cioroi (2007) reported that ascorbic acid acts as antioxidant and the role of ascorbic acid is to neutralize free radicals.

Carotenoids are powerful antioxidants which help to scavenge free radicals and provide support to the body's immune system against infections. Epidemiological studies have shown that a high intake of carotenoid rich diet is associated with decreased incidence of cancers, cardiovascular diseases, age-related muscular degeneration and cataract formation. The protective role of carotenoids in the body is to diminish the degradation of antioxidant enzymes due to deactivation of singlet oxygen (Singh *et al.*, 2008a). The ability of carotenoids to quench singlet oxygen is related to its conjugated double bond system, and maximum protection is given by those having nine or more double bonds (Foote *et al.*, 1970).

It has been reported that proteins have excellent potential as antioxidant additives in foods because they can inhibit lipid peroxidation through multiple pathways including

Table 1. Ascorbic acid, Carotenoid, Protein and Carbohydrate content content in *B. diffusa*.

S. No.	Plant sample	Ascorbic acid (mg/100g of FW)	Carotenoid (µg/g of FW)	Protein (mg/g of DW)	Carbohydrate (mg/g of DW)
1	*B. diffusa* aqueous extract	16.75±1.72	1.36±0.10	122.97±6.27	56.67±5.77
2	*B. diffusa* methanolic extract	18.86±1.12	1.98±0.11	134.45±6.23	60.11±5.23
3	Quercetin (standard)	-	-	1.51±0.05	65.96±1.96

Values are mean ± SD of three replications; FW- Fresh weight; DW-Dry weight.

Table 2. Free radical scavenging activity of *B. diffusa* by DPPH method.

S.No.	Plant sample	Percentage inhibition at different concentrations (µg/ml)						EC_{50} (µg/ml)
		10	20	40	80	160	320	
1	*B. diffusa* aqueous extract	5.39	8.98	13.9	24.6	45.0	61.3	216.4
2	*B. diffusa* methanolic extract	2.1	4.9	11.7	27.4	49.0	79.0	149.3

Values are mean ± SD of three replicates

inactivation of reactive oxygen species, scavenging free radicals and chelation of prooxidative transition metals (Singh *et al.*, 2008b). Bhattacharjee and Sil (2006) demonstrated that the protein fraction of *P. niruri* protected liver damage caused by chemically induced oxidative stress in mice and hypothesized that it was probably due to increasing antioxidative defense. Annapoorani *et al.* (2006) also reported that the plant protein fraction of various plants has hepatoprotective activity against carbon tetrachloride induced free radical toxicity in Swiss albino male mice.Zhang *et al.* (2003) reported that the sulfated polysaccharide fraction from *Porphyra haltanesis*, an important alga in China, has antioxidant activity and a strong scavenging effect for superoxide radical and it inhibits lipid peroxidation.

The total phenolic content in *B. diffusa* methanolic extract was 252.83±1.31 ìg and water fraction was 254.42±1.82 ìg gallic acid was equivalent to /10 mg of extracts. Typical phenolics that possess antioxidant activity have been characterized as phenolic acid and flavonoids. Due to their useful antioxidant activity, phenolics have repeatedly been implicated as natural antioxidants in fruit, vegetable and other medicinal plants (Singh *et al.*, 2012).The flavonoid content of methanolic and water fraction in *B. diffusa* root was found to be 38.60±2.17, 38.59±2.10 ìg rutin was equivalent /10 mg extract respectively. Khalid *et al.* (2012) observed that *B. diffusa* root had minerals, organic acids, flavonoids and phenolic compounds which have been found to possess antioxidant, mast cells stabilizing effects.

The free radical scavenging actively of methanolic and aqueous extracts of *B. diffusa* are given in Table 2. EC_{50} calculated from values of % inhibition for standard gallic acid was 1.81 (µg/ml).

DPPH stable free radical method is an easy, rapid and sensitive phytometric assay method commonly employed for evaluating the antioxidant activity of a specific compound or plant extracts based on their capabilities to donate hydrogen (Kumazawa *et al.*, 2002). The fall in extinction and potential of antioxidant to scavenge free radicals have been correlated (Singh *et al.*, 2002). DPPH radical gives strong absorption at 510 nm with purple color. It is well known that free radicals are one of the causes of several diseases. Shisode and Kareppa (2011) reported that the root powder extracts of *Boerhaavia diffusa* had significant antioxidant activity. The presence of sufficient amount of vitamin C, alkaloids and flavonoids can be the cause for reduction of DPPH into hydrazine. Dhakarey *et al.* (2005) also correlated the phenolic content of *Rhododendron* with its antioxidant potential. Gopal *et al.*,2010 reported that *Boerhaavia diffusa* ethanolic root extract showed good antioxidant activity in all *in-vitro* free radical scavenging models.

The present study concluded that *B. diffusa* is an important medicinal plant that contains essential phytoconstituents that behave as antioxidants and thus can help in curing numerous ailments. The methanolic extract exhibited strong and effective *in vitro* antioxidant activity than aqueous extract of *B. diffusa* by chelation to metal ions as well as scavenging free radicals. The pesence of polyphenolics in the both extracts is mainly responsible for their overall antioxidant activity.

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