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Full Length Research Article

Antioxidant and Thrombolytic Activities of Various Extracts of *Boerhavia diffusa* L. Leaves

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Abstract

Medicinal plants play a vital role in the primary health care system of India. Various parts of medicinal plants are used as raw drugs since they possess varied medicinal properties. In the present study investigated that the phytochemical and antioxidant activities of different extracts of *Boerhavia diffusa* leaves. Screening of phyto compounds and assay of antioxidant and thrombolytic activity were studied using the standard procedure. In the present study showed the various extracts of *B. diffusa leaves* showed the good source of phyto constituents. Among the extracts, ethanol extracts of *B. diffusa* (BDEE) showed the higher antioxidant, phytochemical and thrombolytic activities when compared with *BDPE (B. diffusa* petroleum ether extracts) and BDAE (*B. diffusa* Acetone extracts). BDEE had showed the highest amount of total phenol 292.38±3.48 mg GAE/g followed by total flavonoids 198.47±2.56 mg QE/g. The highest content of total phenolics and flavanoid in different extracts was found to be in the order of BDAE and BDPE respectively. In this study, BDEE showed highest percentage of clot lysis (20.38 ±2.74 %) and followed by BDAE 16.71±1.32%. The present study concluded that the plants *B. diffusa* have a potential source of medicinally important properties.

***Key words*:** *Boerhavia diffusa, Phytocomponents, Ethanol extracts,* Flavonoids, Antioxidant

Medicinal plants have been a part of modern lifestyle of a man and these plants are a source of important therapeutic aid for alienating human ailments. Majority of plants have medicinal properties, i.e., most pharmaceutical drugs are originally derived from plants. The scientific study of indigenous medicines is called Ethno pharmacology, which is an interdisciplinary science practiced all over the world. Medicinal plants have thus become a focal point to improve the present and future health needs against cancer. This is because secondary metabolites present in medicinal plants could maintain the health and cure various diseases including cancer with less harmful effects. The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities.

Free radicals are any chemical species capable of independent existence with one or more unpaired electrons in their outermost shell, which seek out and capture electrons from other substances to achieve neutrality. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, resulting in a chain reaction. The production of reactive oxygen species (ROS) is prevalent in the world's oceans and oxidative stress is an important component of the stress response in marine organisms exposed to a variety of environmental conditions, including thermal stress, exposure to ultraviolet radiation, pollution etc. As in the clinical setting, ROS are also important signal transduction molecules and mediators of damage in cellular processes, such as apoptosis and cell necrosis for marine organisms [1]. An important response to stress by aerobic cells is the production of ROS like the superoxide radical (O^{2-}), nitric oxide radical (NO^{-}), hydroxyl radical (OH^{-}), alkoxy radical (RO^{-}), singlet oxygen ($1O_2$) and toxic hydrogen peroxide (H_2O_2) molecules. ROS are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA, triggering a variety of patho -physiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity.

Boerhavia diffusa belongs to the family Nyctaginaceae commonly known as 'punarnava' and locally called 'thazuthama' which means rejuvenating the cells or renewing the body by eliminating excess water from it make it highly advantageous for the kidney and the liver. The plant acts on all important organ systems and rejuvenate them, giving a new life. In India it has a long history of use by indigenous and tribal people, and in Ayurvedic or natural herbal medicine. Ayurveda

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classified this plant as 'rasayana' herb which is said to possess properties like antiaging, reestablishing youth, strengthening youth, strengthening life and brain power, and disease prevention like jaundice [1], all of which imply that they increase the resistance of the body against onslaught [2]. Pharmacological studies have demonstrated that *B. diffusa* known to possess diuretic [3], antifertility [4], antifibrinolytic [5], immunomodulatory [6], antidiabetic [7], antiviral [8], antistress [9], antimicrobial [10], anti-inflammatory, hepatoprotective [11] and antimetastatic [12]. This prompted us to study the antioxidant activity of the species, *Boerhavia diffusa* L.

MATERIALS AND METHODS

Chemical required

Ferric chloride, lead acetate, conc.H_2SO_4, neutral ferric chloride, mercuric chloride, potassium iodide, Iodine, Dragendorff's reagent, acetic anhydride, chloroform, HCl, NH_3, ninhydrin solution, sodium nitrite, copper sulphate, potassium sodium tartrate, sodium hydroxide, ammonium hydroxide, amyl alcohol, methanol, acetic acid, ascorbic acid, DPPH, phosphate buffer, potassium ferricyanide, trichloroacetic acid, potassium persulphate, H_2O_2, sodium acetate buffer, $FeSO_4$, MTT, DMSO, distilled water were used in the experiment.

Collection of sample

B. diffusa leaf was collected from in and around region of Thanjavur, Tamil Nadu, India. Leaves were rinsed with distilled water left at room temperature for 7 days in the dark and then oven dried for 24 h at 50°C. Then the plant material made to coarse powder using mixer grinder. The powdered sample was stored in refrigerator for further use.

Preparation of extract

The Soxhlet apparatus was used for successive solvent extraction of the collected leaf powder of *Boerhavia diffusa*. 35g of plant powder was extracted with ethanol, petroleum ether and acetone for 12 hrs. After complete extraction, the contents of each extraction were concentrated by distillation. The concentrated extracts were evaporated to dryness and stored separately at 4 ºC in air tight containers for further experimental studies.

Phytochemical screening

Qualitative phytochemical analysis

Detection of tannins

a) *Ferric chloride test*: A small quantity of the extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of saponins

a) *Froth test*: about 0.2g of the various extracts was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of flavonoids

a) *Lead acetate test*: Each extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

b) *H_2SO_4 test*: Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

c) *$FeCl_3$ test*: To the alcoholic solution of the extract adds few drops of neutral ferric chloride solution. Appearance of green colour indicates presence of flavanoids.

Detection of alkaloids

a) *Mayer's test*: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Mayer's reagent: Mercuric chloride (1.358g) is dissolved in 60ml of water and potassium iodide (5g) is dissolved in 10ml of water. The two solutions are mixed and made up to 100ml with water.

b) *Wagner's test*: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Wagner's reagent: Iodine (1.2g) and potassium iodide (2g) is dissolved in 5ml of water and made up to 100ml with distilled water.

c) *Dragendorff's test*: To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added by the side of the test tube. A prominent red precipitate indicates test as positive.

Detection of steroids

a) *Liebermann- Burchard test*: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of terpenoids

a) *Salkowski's test*: 0.2g of each extract were mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) were carefully added to form a layer. A reddish-brown coloration of the inner face was indicating the presence of terpenoids.

Detection of anthroquinones

a) *Borntrager's test*: about 0.2g of each extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Detection of phenols

a) *Ferric chloride test*: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

b) *Lead acetate test*: The various extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Test for amino acids

Ninhydrin test to the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicates the presence of amino acids in sample.

Millon's test: Added 5 drops of million's reagent to 1 ml of test solution and heated on a water bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red color confirmed the test.

Detection of carbohydrates

a) *Fehling's test*: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Pottassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

b) *Benedict's test*: To a set of filtrates of various drugs' extracts, added equal volumes of Benedict's reagent and heated in boiling water bath for 5min. The appearance of green, yellow or red color indicated the presence of sugars.

Gum and mucilage

Alcohol precipitation: All the test solutions were mixed with absolute alcohol and dried in air and the residues were tested for swelling properties and didn't get positive results.

Detection of oils and resins

a) Spot test: Test extracts were applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Quantitative phytochemical analysis

Estimation of total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of each extract were pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The plant samples were made up to mark and left to react for 30 min for colour development. The estimation was done spectrophotometrically at 760 nm and the results were expressed as gallic acid equivalents (GAE) [13]

Estimation of total flavonoids

Ten grams of plant powder was repeatedly extracted with 100ml of ethanol, petroleum ether and acetone at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The results were expressed as quercetin equivalents (QE) [14].

Anti-oxidant activities

DPPH radical scavenging activity

DPPH radical scavenging activity was carried out by the method of Gyamfi *et al.* [15]. To 1.0 ml of 100.0 µM DPPH solution in ethanol, petroleum ether and acetone, equal volume of the different concentration of each extract of *B. diffusa* was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of ethanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated from the equation [(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100. IC_{50} value was calculated using Graph pad prism 5.0.

Reducing power assay

The sample together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50 °C water-bath for 20min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700 nm against each extract's concentration. Ascorbic acid was used as standard. Higher absorbance indicates higher reducing power [16].

ABTS assay

The 2, 2' –azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical cation scavenging activity was measured according to the method described by Re *et al*. [17]. ABTS was dissolved in water to a 7mM concentration. The ABTS radical action was produced by adding to the ABTs stock solution 2.45 mM potassium persulphate. The completion of radical generation was obtained in the dark at room temperature for 12-16h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70. to determine the scavenging activity, 1 mL of diluted ABTS solution was added to different concentration of plant extracts and the absorbance at 734 nm was measured 6 mins after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

Inhibition percentage (%IP) = [(Ac – As / Ac] × 100

Hydroxyl radical scavenging assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction [18]. The reaction mixture contained 60µl of 1mM FeCl3, 90µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2M phosphate buffer (pH 7.8), 150µl of 0.17 M H2O2, and 1.5mL of extract in various concentrations and it was incubated at room temperature for 5 min, absorbance was noted at 560nm. The % hydroxyl radical scavenging activity (HSRA) is calculated by the following formula:

% HSRA = [(Abscontrol – Abssample) / Abscontrol] × 100

Where;

Abscontrol is the absorbance of the control; Abssample is the absorbance of the extract / standard (ascorbic acid).

Nitric oxide scavenging assay

The assay is based on the principle that sodium nitropruside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interact with oxygen to produce nitrite ions, which can be measured by a Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO [19]. The samples of different extracts were prepared in various concentrations and mixed with 3 ml of 10mM sodium nitroprusside. The same reaction mixture without the extract served as the control. The reaction mixture was allowed to incubate at room temperature for 3hrs. Gallic acid was used as the standard for comparison. After incubation the samples were reacted with griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 2% phosphoric acid), the absorbance of the chromophores formed were read at 546nm. The reactions were done in triplicate and % scavenging activity was caculated using the following formula:

% Scavenging = [(Abscontrol – Abssample) / Abscontrol] × 100

Where;

Abscontrol is the absorbance of the control; Abssample is the absorbance of the extract/standard (gallic acid).

In vitro thrombolytic activity

3 mL venous blood drawn from healthy volunteer was transferred to 5 pre-weighed sterile eppendorf tubes (500 µL/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot formed. Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube - weight of tube alone). Each eppendorf tubes containing clot was properly labeled and 100 µL of BDHE, BDEA and BDME extracts (100 mg/mL) was added to the tubes separately [20]. As a positive control, 100 µL of streptokinase (CSL Behring GmbH, Germany) (3000 000 IU/mL) and as a negative control, 100 µL of normal saline (0.9% NaCl) were separately added to the control tubes. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was conducted on the blood samples of 10 volunteers (male=5; female=5) without a history of oral contraceptive or anticoagulant therapy for two weeks.

RESULTS AND DISCUSSION

Phytochemical analysis

Various solvents extracts were prepared and subjected to phytochemical analysis, which was found to have the secondary metabolites like alkaloids, flavonoids, terpenoids, steroids, glycosides, anthroquinones, tannins, phenols, amino acid, carbohydrate and saponins. Highest phytocompounds were presented in the ethanolic leave extracts of *B. diffusa* except gum, mucilage, oils and resins. The moderate amount was presented in the acetone. All the phytocompounds are presented only in the ethanolic extract to compare with other solvents (Table 1).

Table 1 Qualitative phytochemicals in various solvent extracts of *B. diffusa*

Phytochemical tests	BDPE	BDEE	BDAE
Tannin			
Ferric chloride test	+	++	+
Saponin			
Froth test	+	+	++
Flavonoids			
Lead acetate test	-	+++	+
H_2SO_4 test	+	-	-
FeCl3 Test	-	+	-
Alkaloid			
Mayer's test	+	++	+
Wagner's test	-	-	-
Dragendorff's test	-	-	+
Steroids			
Liebermann- Burchard test	++	+	+
Terpenoids			
Salkowski's test	-	++	+
Anthroquinones			
Borntrager's test	-	+++	-
Phenols			
Ferric chloride test	-	+++	+
Lead acetate test	+	-	-
Amino Acids			
Ninhydrin test	-	+	++
Millon's test	+	++	-
Carbohydrates			
Fehling's test	+	++	-
Benedict's test	-	-	+
Gum and Mucilage			
Alcohol Precipitation	-	-	-
Oils and Resins			
Spot test	-	-	-

BDEE-*B.diffusa* ethanol extracts, BDAE-*B.diffusa* Acetone extracts, BDPE-*B.diffusa* Petroleum ether extracts

+ Present, ++ moderately present, +++ Highest present, - Absent

Quantitative analysis

The phytochemicals such as flavonoids and phenols were quantitatively estimated in different extracts of *B. diffusa* leaves. BDEE had showed the highest amount of total phenol 292.38±3.48 mg GAE/g followed by total flavonoids 198.47±2.56 mg QE/g. The highest content of total phenolics and flavanoid in different extracts was found to be in the order of BDAE and BDPE respectively (Table 2). The present investigation showed significant variation in the phytocomponents like phenols and flavonoids.

Antioxidant activity

DPPH radical scavenging activity

The result showed the free radical (DPPH) scavenging activity of various extract of *Boerhavia diffusa*, expressing the activity in percentage inhibition. The result revealed that the ethanol extract exhibited the highest radical scavenging activity (77±2.0 µg/ml) at the concentration of 300 µg/ml (Fig 1). In the present results also indicate the more or equal to plant extracts and standard. Results of this assay suggest that the plant extract contain phytochemicals, capable of donating hydrogen to scavenge a free radical. DPPH (1, 1-diphenyl-2-picrylhydrazyl) analysis is one of the best known, accurate and frequently employed methods for evaluating antioxidant activity. It is a stable free radical because of its spare electron delocalization over the whole molecule. The degree of colour change is proportional to the potency and concentration of the antioxidants present in the extract [21].

Table 2 Quantitative phytochemical analysis of different extract of *B. diffusa*

Phytochemical	*B. diffusa* leaves extracts		
	BDEE	BDPE	BDAE
Total phenol (mg GAE/g)	292.38±3.48	275.23±3.15	262.17±3.48
Total flavonoid (mg QE /g)	198.47±2.56	182.35±2.70	167.25±2.92

Hydroxyl radical scavenging activity

The result showed that different extracts of *Boerhavia diffusa* have scavenging ability of OH- free radicals in a dose dependent manner at the concentration 10-300 µg/ml. The IC50 values for ethanol extracts were 167.3±2.7 µg/ml (Fig 1). Scavenging of hydroxyl radical is an important antioxidant activity because of its very high reactivity, which can cross the cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing the hydroxyl radical is very important for the protection of living systems [9]. BDEE showed strong hydroxyl radical scavenging ability.

Nitric oxide scavenging assay

The nitric oxide scavenging activity of different extracts of the plant can be ranked as BDEE> BDPE> BDAE indicating its strong nitric oxide scavenging capacity. The IC50 of NO activity was 127.6±1.8 µg/ml. Nitric oxide or reactive nitrogen species, formed during their action are very reactive with oxygen or with superoxides (Fig 1). These compounds are responsible for altering the structural and functional behaviour of many cellular components [22].

ABTS assay

Highest ABTS showed the BDAE (73±2.9%) followed by BDEE (58±2.9%) and BDPE (45±2.9%) at the concentration of 300 µg/ml. The standard ascorbic acid had highest activity (32±0.3% to 87±2.5%) at various concentrations level (50µg/ml to 300µg/ml). The ABTS (2, 2' –azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical assay is also one of the most commonly used methods to evaluate the antioxidant activity (Fig 1). ABTS is an excellent substrate for peroxidases and is frequently used to study the antioxidant properties of natural compounds [23]. The ethyl acetate fraction of *Evax pygmaea* showed strong ABTS radical scavenging and it nearly fully scavenged $ABTS^{+}$. Of the successively extracted *Aphanamixis polystachya* bark with hexane, ethyl acetate, methanol and water, the methanolic extract possessed potent ABTS scavenging activity [24].

Reducing power activity

Reducing capacity (RPA) is considered as a significant indicator of potential antioxidant activity of a sample. The presence of reluctant (i.e., antioxidants) causes reduction of $Fe^{3+/}$ferricyanide complex to the ferrous form. Higher absorbance indicated higher reducing power. Increase in OD determines the increase in reducing power. In the present study, ethanol leaves extract of *B. diffusa* possessed a good reducing power (81.7±4.2%) at 300 µg/mL followed by the minimum reducing power (41.4±2.5%) at 50 µg/mL concentrations. In the standard ascorbic acid showed good reducing power (84.1±2.6%) at 300 µg/mL concentrations (Fig 1). Hence, ethanolic extract of the plant may act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reaction [25].

Thrombolytic activity

Thrombolytic activity was observed in different extracts of *B. diffusa* and control streptokinase. In this study, BDEE showed highest percentage of clot lysis (20.38 ±2.74%) and followed by BDAE 16.71±1.32%. Positive control as Streptokinase showed the maximum percentage of clot lysis (43.10±4.75%). In this study, significantly influenced the thrombolytic activity was showed BDEE when compared with other extracts (Fig 2). In the previous study, methanol extracts of the plant showed significant thrombolytic activity compared with negative control [26]. BDEE contains various phytocompounds could involve for thrombolytic activity [27].

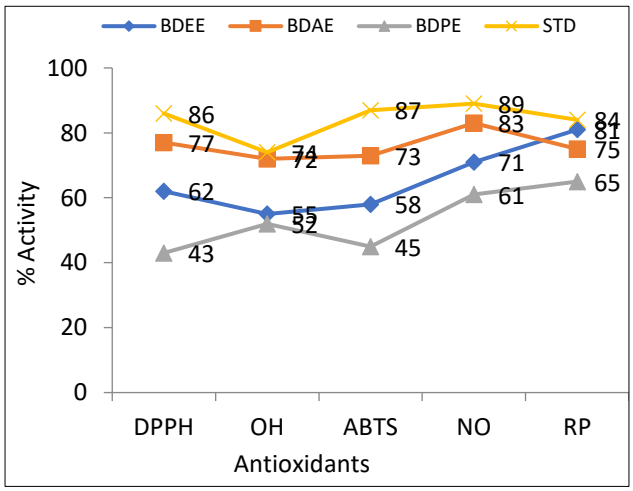

Fig 1 Antioxidant activity of different extract of *B. diffusa*

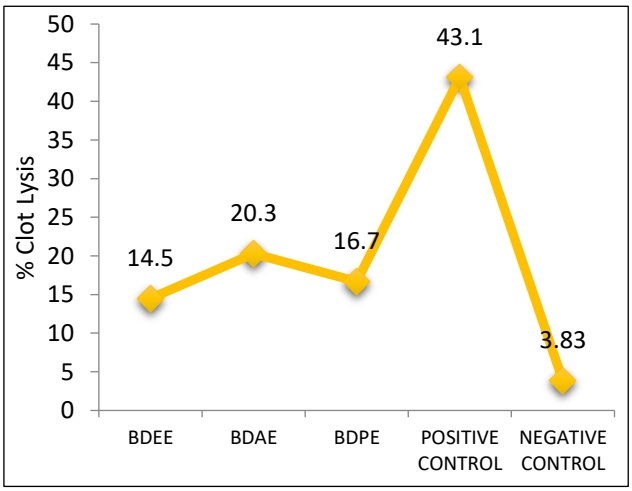

Fig 2 Thrombolytic activity of different extracts of *B. diffusa*

CONCLUSION

The present study indicates the presence of most of the phytocompounds in various extracts of *B. diffusa*. Total phenol and flavonoids contents were major contributors of antioxidant activity of *B. diffusa*. The present result concluded the *B. diffusa* extracts contained potential antioxidant and thrombolytic activity. The study found out that ethanol extract of the plant was the strongest radical scavenger among the three screened. *In vitro* assay studies indicated that the extract of *B. diffusa* is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Further investigations are needed to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

LITERATURE CITED

1. Rawat AKS, Mehrotra S, Tripathi SC, Shome U. 1997. Hepatoprotective activity of *Boerhaavia diffusa* L. roots- a popular Indian ethnomedicine. *Jr. Ethnopharmacology* 56(1): 61-66.
2. Govindarajan R, Vijayakumar M, Pushpangathan P. 2005. Antioxidant approach to disease management and the role of Rasayana herbs of Ayurveda. *Jr. Ethanopharmacology* 99(2): 165-178.
3. Desai SK, Gawali VS, Naik AB, D'souza LL. 2008. Potentiating effect of piperine on hepatoprotective activity of *Boerhaavia diffusa* to combat oxidative stress. *In. Jr. Pharmacology* 4: 393-397.
4. Barthwal M, Srivastava K. 1990. Histologic studies on endometrium of menstruating monkeys wearing IUDS: Comparative evaluation of drugs. *Adv. Contraception* 6: 113-124.
5. Sengul M, Yildiz H, Gungor N, Cetin B, Ecer Z, Ercisli S. 2009. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pak. Pharm.* 22: 102-106.

6. Mungantiwar AA, Nair AM, ShindeUA, Dikshit VJ, Saraf MN, Thankur VS, Sainis KB. 1999. Studies on the immunomodulatory effect of *Boerhaavia diffusa* alkoloidal fraction. *Jr. Ethnopharmacology* 65: 125-131.
7. Pari L, Satheesh MA. 2004. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *Jr. Ethnopharmacology* 91(1): 109-113.
8. Awasthi LP, Verma HN. 2006. *Boerhaavia diffusa*-a wild herb with potent biological and antimicrobial properties. *Asian Agri-Hist.* 10: 55-68.
9. Shirwaiar A, Shirwaiar A, Punitha ISR. 2007. Antioxidant studies on the methanol stem extract of *Coscinium femestratum*. *Nat. Prod. Science* 13: 40-45.
10. Gordon MH. 1990. The mechanism of antioxidant action *in vitro*. *In*: Food Antioxidants Hudson BJF, Elsevier Applied sciences, London, UK. pp 1-18.
11. Duh PD, Du PC, Yen GC. 1999. Action of methanolic extract of mung bean hulls as inhibitors of lipid peroxidation and non-lipid damage. *Food Chem. Toxicology* 37(11): 1055-1061.
12. Manu KA, Kuttan G. 2009. Anti-metastatic potential of Punarnavine, an alkaloid from *Boerhaavia diffusa* Linn. *Immunobiology* 214: 245-255.
13. Ainsworth EA, Gillespie KM. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat. Protoc.* 2(4): 875-877.
14. Dewanto V, Wu X, Adom KK, Liu RH. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* 50: 3010-3014.
15. Gyamfi MA, Yonamine M, Aniya Y. 1991. Free-radical scavenging action of medical herbs from Ghan: Thonningiasanguinea on experimentally-induced liver injuries. *General Pharmacology* 32: 661-667.
16. Oyaizu M. 1986. Studies on product on browning reaction prepared from glucose amine. *Japan Journal of Nutrition* 44: 307-315.
17. Re RN, Pellagrini A, Proteggenet A, Yang PM, Evans R. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med*. 26: 1231-1237.
18. Girish HV, Satish S. 2008. Antibacterial activity of important medicinal plants on human pathogenic bacteria-a comparative analysis. *World Applied Sci. Jr.* 5: 267-271.
19. Sumantha M, Mustafa SS. 2007. Antistress, adoptogenic and immunopotentiating activity roots of *Boerhaavia diffusa* in mice. *Int. Jr Pharmacology* 3: 416-420.
20. Khan IN, Habib MR, Rahman MM, Mannan A, Sarker MMI, Hawlader S. 2011. Thrombolytic potential of *Ocimum sanctum* L., *Curcuma longa* L., *Azadirachta indica* L. and *Anacardium occidentale* L. *Jr. Basic Clin. Pharm.* 2(3): 125-127.
21. Yang M, Rice-Evans C, Re R, Pellegrini N, Pannala A. 2008. Antioxidant activity applying an improved ABTS radical cat-ion decolorization assay. *Free Rad. Biol. Med.* 26: 1231-1237.
22. Seth RK, Khanna M, Chaudhary M, Singh S, Sarin JPS. 1986. Estimation of punarnavosides, a new antifibrinolytic compound from *Boerhaavia diffusa*. *Indian Drugs* 23: 583-584.
23. Reszka KJ, Britigan BE. 2007. Doxorubicin inhibits oxidation of 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by an actoperoxidase/H_2O_2 system by reacting with ABTS-derived radical. *Arch. Biochem. Biophysics* 466: 164-171.
24. Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. 2009. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Inter. Jr. Appl. Sci. Engineering* 3(2): 125-134.
25. Sharma A, Bhardwaj S, Mann AS, Jain A, Kharya M D. 2007. Screening methods of antioxidant activity an overview. *Pharmacognosy Review* 1(2): 232-238.
26. Apu AS, Liza MS, Jamaluddin AT, Howlader MA, Saha RK, Rizwan F, Nasrin N. 2012. Phytochemical screening and *in vitro* bioactivities of the extracts of aerial part of *Boerhavia diffusa* Linn. *Asian Pac. Jr. Trop. Biomed.* 2(9): 673-678.
27. Chaudhary G, Dantu PK. 2011. Morphological, phytochemical and pharmacological, studies on *Boerhaavia diffusa* L. *Jr. Med. Plants Research* 5(11): 2125-2130.