



Phytopharmacological and Antioxidant Analysis of Hydroethanolic Extract of *Boerhavia diffusa*

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Article History:

Received on: 20.08.2019

Revised on: 15.11.2019

Accepted on: 21.11.2019

Keywords:

Boerhavia diffusa,
Phytochemicals,
Hydroethanolic extract,
Antioxidant property

ABSTRACT

Boerhavia diffusa, a well-known ethno-medicinal plant and were found in tropics and sub-tropics. *Boerhavia diffusa* is a traditional medicinal plant and they can cure many human ailments. It has most promising pharmacological activities include antifibrinolytic, immunosuppressive, diuretic, hepatoprotective, antidiabetic, anti-inflammatory, anticancer, immunomodulatory, antilympho proliferative, analgesic properties and also for the treatment of pulmonary tuberculosis. Due to their adverse side effects, the naturally occurring antioxidants has increased and replaced by the synthetic antioxidants. This study is focussed on the phytochemical evaluation and *in vitro* antioxidant activity of Hydroethanolic extract of *Boerhavia diffusa*. The qualitative chemical test exhibited the presence of phytochemicals like flavonoids, polyphenols, anthocyanins, coumarin, steroids, terpenoids, triterpenoids, glycosides, emodins and saponins. Quantitative analysis of total phenol, total flavonoid and total tannin was found to be 205.25mg/g, 121.65 mg/g and 61.41mg/g. DPPH, Total antioxidant activity, Superoxide anion radical, Nitric oxide and Hydroxy radical scavenging assay showed that *B.diffusa* was an excellent scavenger of these radicals. These results are an indication of the potent antioxidant property of the extract and may be responsible for some of the therapeutic uses of *Boerhavia diffusa*.

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ISSN: 0975-7538

DOI: <https://doi.org/10.26452/ijrps.v11i1.1904>

Production and Hosted by

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INTRODUCTION

Medicinal plants are of much more importance to the health of people and networks by and large (Singh *et al.*, 2011). The human body shows distinct physiological activity, which are produced

by the therapeutic value of plants that has some chemical substances. Alkaloids, Tannins, Flavonoids and Phenolic compounds are the bioactive compounds present in these plants. In Ayurveda (Indian system of the drug), different disorders and infections have been depicted in great details.

The World Health Organization (WHO) supports the utilization of the customary drug, and they are demonstrated to be adequate and safe (Jaffar *et al.*, 2011). Medicinal Plants are additionally added to nourishments implied for ladies who are for child-birth and nursing moms for medicinal purposes. They also add flavors for food. In addition, the utilization of herbal medicine for the treatment of infections and diseases is as old as humanity (Bhardwaj and Tanwar, 2011).

As referenced in old Indian books like Charak Samhita, Sushruta Samhita, Ras Tantra Sar,

Bhavprakash, Ras Tarang, Nayan Drastam and Astanghriday, Sushruta, the Father of Surgery in India before 500 BC inquired about that, and there are various plants which are utilized in different diseases.

As a juice, extract, kwath, paka, arka and so on, either single or in compound formulations with different plants (Dutt and Ambika, 2009). Therapeutic plants have no side reactions because of the presence of bioactive constituents. *Boerhavia diffusa* is a member from Nyctaginaceae family.

The stem is generally prostrate and woody. Stamens are typically a few in numbers and they have a peltate stigma (Kyselova et al., 2004). From ancient times Punarnava plays a significant role among medicinal herbs in India. The various disorders can be cured by different parts of Punarnava (*Boerhavia diffusa*).

The tribal people eat this plant as a vegetable in Purulia (West Bengal) and in Assam *Boerhavia*, leaves are cooked and eaten (Rajpoot and Mishra, 2011). *Boerhavia diffusa* growth will be more in the waste places during the rainy season. The roots can be used as a diuretic and laxative and are given for the treatment of jaundice. The herbal drug has developed and changed as the years progressed. Correspondingly Punarnava has been utilized in raw form as well as extract form in different disorders (Mahesh et al., 2012).

In Ayurveda, this medication is referred to be utilized as Mootrala (diuretic), Sothagha (Anti-inflammatory), Kasahara (Antitussive), jwarahara (antipyretic), Rasayana (rejuvenator). The root as therapeutic property, as well as the entire plant of this herb, is likewise being taken as diet by some tribal groups for which mainly the leaves are utilized.

This medication has been referred to as Tikta (bitter) and Kashaya (astringent) in Taste. The main helpful part is Panchanga (entire plant); however, the utilization of Moola (root) is generally given in Ayurveda classics. The current study clearly showed that the hydroethanolic extract of *Boerhavia diffusa* roots has many phytochemicals, which was assessed by qualitatively as well as quantitatively and also has potent antioxidant activities.

MATERIALS AND METHODS

Collection of the plant sample

The samples of plant *Boerhavia diffusa* Linn was collected from their characteristic natural surroundings and from the market. The plant *Boerhavia diffusa* was washed in tap water, air-dried and made

to a fine powder by a homogenizer. The dry powder acquired was maintained at room temperature in airtight containers.

Preparation of plant extract

The plant material is dried, powdered and 1 kg was extracted with 30:70 proportion of hydro ethanol for maceration periods (24 hr). At room temperature, the extraction was carried out with agitation at 150 rpm. After the maceration period, the soaked powder-solvent mixtures were separated through a Whatman filter paper No. 1 and utilized for phytochemical investigation and *in vitro* antioxidant studies.

Qualitative Analysis

Preliminary phytochemical analysis was carried out by utilizing a standard technique (Sofowora, 1993; Trease and Evans, 1989; Harborne, 1973).

Quantitative Analysis

The total phenolic content of *Boerhavia diffusa* was determined by the spectrophotometric method of (Kim et al., 2003) with slight modification. The total flavonoids assay was carried out, as stated by (Damodar et al., 2011). By using the colorimetric technique of Aluminum chloride, the total flavonoid content was determined. Total Tannin assay was led by (Bajaj and Devsharma, 1977) technique.

Antioxidant Assays

DPPH radical-scavenging activity was determined by the technique by (Shimada et al., 1992). By the phosphomolybdenum technique, the antioxidant activity of the extract was assessed, which was indicated by the methodology of (Prieto et al., 1999). The superoxide anion radicals scavenging activity was estimated by the strategy by (Liu et al., 1997). The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the strategy for (Yu et al., 2004). Nitric oxide radical scavenging activity was determined by the method by (Garrat, 1964).

RESULTS AND DISCUSSION

Qualitative Analysis

Boerhavia diffusa shows the presence of Phytoconstituents, which include flavonoids, polyphenols, anthocyanins, coumarin, steroids, terpenoids, triterpenoids, glycosides, emodins and saponins.

Table 1 shows the phytochemicals present in the hydroethanolic extract of *Boerhavia diffusa*. Flavonoids are the polyphenolic combinations and are dynamic against free radicals, free radical-mediated cell signaling, inflammation, allergies,

platelet aggregations, microorganisms, ulcers, infections, and tumors. In a hydroethanolic extract of the *Boerhavia diffusa*, flavonoids were observed to be available in high concentrations.

Quantitative Analysis

Total phenols, total flavonoids and Total tannin were assessed by the quantitative analysis. Total phenol shows the high measure in a hydroethanolic extract of *Boerhavia diffusa*. The total phenol content was observed to be 205.25mg/g, which was shown in Table 2 and Figure 1. Phenolic components have been considered for their pharmacological action against oxidative pressure, for example, inflammation, cardiovascular disease, antiulcer, anticancer, antioxidant, antispasmodic, cytotoxic and antidepressant activities (Mandal et al., 2010).

The total flavonoid content was observed to be 121.65 mg/g, which was predicted in Table 2 and Figure 2. Flavonoids are polyphenolic components and in historical periods, the flavonoids play a significant part in powerful therapeutic medications in various afflictions and their preparation has continued up to now. (Pridham, 1960; Cook, 1996) uncovered in his review that flavonoids can also acts as radical scavengers and as a result of the event of various beneficial therapeutic properties, flavonoids assumes an important part in the management of chronic ailments like cardiovascular diseases, cancer and some neurotic issue of duodenal and gastric ulcers, vascular fragilities, hypersensitivities, and viral and bacterial contaminations.

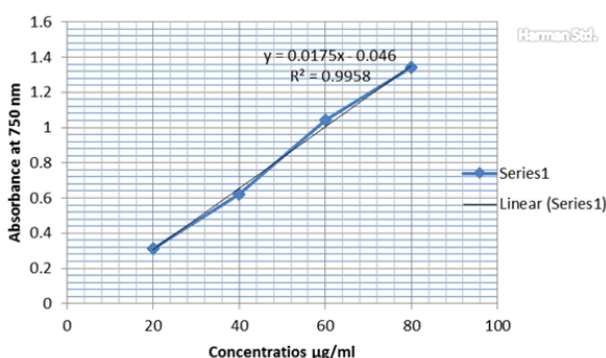


Figure 1: Linear regression for Total phenol content

The total tannin content was observed to be 61.41mg/g, which was shown in Table 2 and Figure 3. Tannins show antiviral, antibacterial, antiulcer and antioxidant activity. Tannin plays an important role in the treatment of piles, inflammation (Akiyama, 2001). The search for novel compounds for the improvement of prescriptions has ended turned out to be vital since the biological activity of the extract containing tannin is much

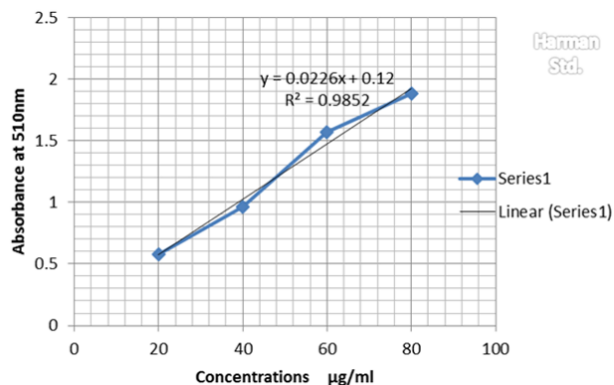


Figure 2: Linear regression for Total flavonoid content

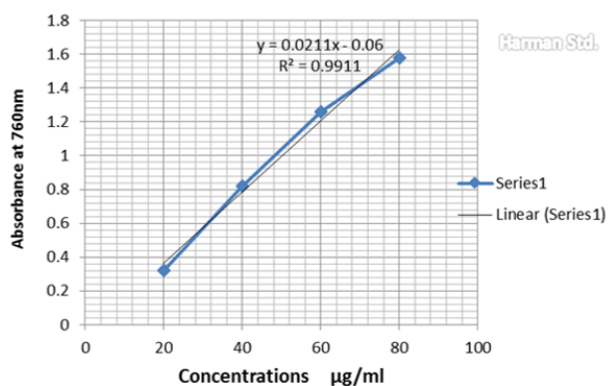


Figure 3: Linear regression for Total tannin content

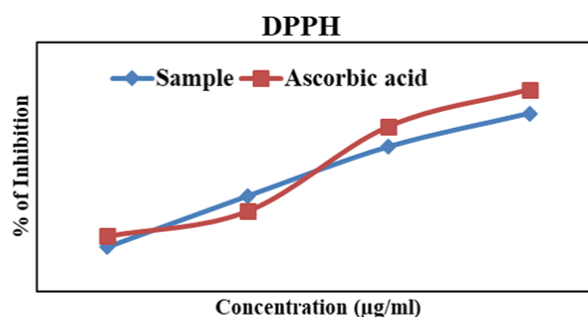


Figure 4: DPPH radical scavenging activity of *Boerhavia diffusa*

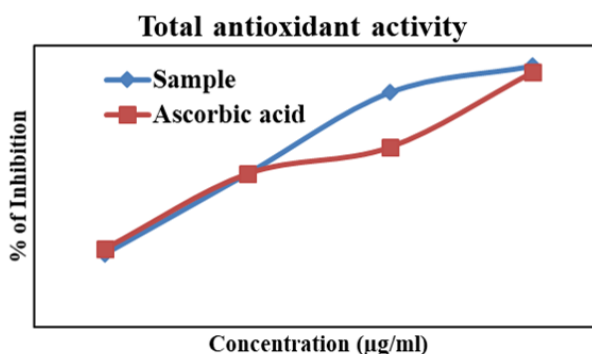


Figure 5: Total antioxidant activity of *Boerhavia diffusa*

Table 1: Qualitative analysis in Hydroethanolic Extract of *Boerhavia diffusa*

S.No	Test analysis	Hydro ethanolic extract
1	Tannin	-
2	Phlobatannin	-
3	Saponin	+
4	Flavonoids	++
5	Steroids	++
6	Terpenoids	++
7	Triterpenoids	++
8	Alkaloid	-
9	Anthroquinone	++
10	Polyphenol	+
11	Glycoside	++
12	Coumarins	++
13	Emodins	+
14	Anthocyanins	-

(-) Absent, (+) Present and(++) high concentration

Table 2: Quantitative analysis of Total phenol, Total flavonoids and Total tannin content of Hydroethanolic extract of *Boerhavia diffusa*

Sample	Total phenol (mg/g)	Flavonoids(mg/g)	Tannin (mg/g)
Hydroethanolic extract of <i>Boerhavia diffusa</i>	205.25	121.65	61.41

Table 3: DPPH radical scavenging activity of *Boerhavia diffusa*

Samples	Concentrations ($\mu\text{g/ml}$)				IC ₅₀ Value
	20	40	60	80	
Boerhavia diffusa (% of inhibition)	21.37 \pm 1.49	45.91 \pm 3.21	69.55 \pm 4.86	85.46 \pm 5.98	44.83
Ascorbic acid (Std.) (% of inhibition)	26.54 \pm 0.95	38.6 \pm 2.04	79.26 \pm 4.90	96.98 \pm 7.11	41.78

Values are expressed as Mean \pm SD for triplicates

Table 4: Total antioxidant activity of *Boerhavia diffusa*

Samples	Concentrations ($\mu\text{g/ml}$)				IC ₅₀ Value
	20	40	60	80	
Boerhavia diffusa (% of inhibition)	20.93 \pm 1.46	43.75 \pm 3.06	66.87 \pm 4.68	74.37 \pm 5.20	48.38
Ascorbic acid (Std.) (% of inhibition)	22.35 \pm 1.80	43.67 \pm 2.61	51.23 \pm 4.09	72.54 \pm 5.80	53.22

Values are expressed as Mean \pm SD for triplicates

Table 5: Superoxide anion radical scavenging activity of *Boerhavia diffusa*

Samples	Concentrations ($\mu\text{g/ml}$)				IC ₅₀ Value
	20	40	60	80	
Boerhavia diffusa (% of inhibition)	17.86 \pm 1.25	44.28 \pm 3.09	64.28 \pm 4.49	71.78 \pm 5.02	50.49
Ascorbic acid (Standard) (% of inhibition)	20.37 \pm 0.97	31.25 \pm 2.50	64.23 \pm 5.13	89.54 \pm 7.16	34.28

Values are expressed as Mean \pm SD for triplicates

Table 6: Hydroxyl radical scavenging activity of *Boerhavia diffusa*

Samples	Concentrations ($\mu\text{g/ml}$)				IC ₅₀ Value
	20	40	60	80	
Boerhavia diffusa (% of inhibition)	18.33 \pm 1.28	41.25 \pm 2.88	67.08 \pm 4.69	76.25 \pm 5.33	49.26
Ascorbic acid (Std.) (% of inhibition)	32.21 \pm 2.51	56.45 \pm 4.40	78.65 \pm 6.13	92.75 \pm 7.2	35.26

Values are expressed as Mean \pm SD for triplicates

Table 7: Nitric oxide scavenging activity of *Boerhavia diffusa*

Samples	Concentrations ($\mu\text{g/ml}$)				IC ₅₀ Value
	20	40	60	80	
Boerhavia diffusa (% of inhibition)	18.57 \pm 1.29	49.04 \pm 3.43	62.38 \pm 4.36	77.61 \pm 5.43	48.01
Ascorbic acid (Std.) (% of inhibition)	26.21 \pm 2.04	59.62 \pm 4.65	84.23 \pm 6.56	96.45 \pm 7.52	35.86

Values are expressed as Mean \pm SD for triplicates

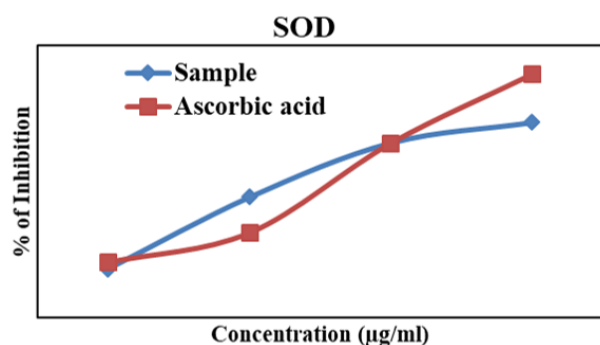


Figure 6: Superoxide anion radical scavenging activity of *Boerhavia diffusa*

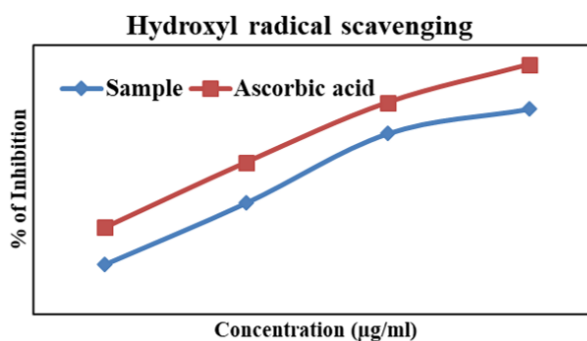


Figure 7: Hydroxyl radical scavenging activity of *Boerhavia diffusa*

reported (Mueller-Harvey, 1999). Table 2 predicts the quantitative analysis of phenol, flavonoids and tannin content of hydroethanolic extract of *B.diffusa*

Antioxidant assays

DPPH Assay

The DPPH assay was done with hydroethanolic

extract of *Boerhavia diffusa*, which shows the free radical scavenging activity of the extract increases with increasing concentration. The antioxidant capacity in the extract with DPPH solution can converts them into reduced form (Nuutila et al., 2003). The half inhibition concentration (IC₅₀) of the extract was 44.83, whereas standard ascorbic

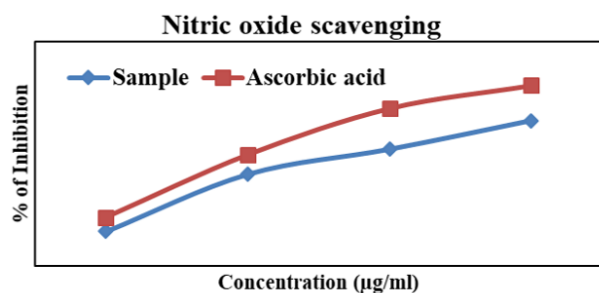


Figure 8: Nitric oxide scavenging activity of *Boerhavia diffusa*

acid was 41.78. Along these lines, *Boerhavia diffusa* predicts the effective DPPH radical scavenging activity. Table 3 and Figure 4 shows the IC_{50} value and the comparative graph among standard and the Hydroethanolic extract of *B.diffusa*.

Total Antioxidant Activity

The activity of Hydroethanolic extract of *B.diffusa* and its total antioxidant capacity was given in Table 4 and Figure 5. The result shows if the concentration increases, the antioxidant activity also increases. The half inhibition concentration (IC_{50}) of extract and ascorbic acid was $48.38\mu\text{g/mL}$ and $53.22\mu\text{g/mL}$, respectively. Thus, *Boerhavia diffusa* indicates effectively total antioxidant capacity.

Superoxide Anion Radical Scavenging Activity

The scavenging capacity of Hydroethanolic extract of *B.diffusa* displayed that the inhibition of formazan was recorded and predicted in Table 5 and Figure 6. Its IC_{50} value was 50.49, whereas standard ascorbic acid IC_{50} was 34.28. In this manner, *Boerhavia diffusa* demonstrates powerful SO radical scavenging activity. One of the strongest responsive oxygen species among the free radicals that are produced are Superoxide anion radical (Garrat, 1964). (Robak and Gryglewski, 1988) revealed that flavonoids are strong antioxidants mainly of their scavenging activity of superoxide anions.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging activity was carried out with hydroethanolic extract of *Boerhavia diffusa*. Its IC_{50} value was 49.26, whereas standard ascorbic acid IC_{50} was 35.26, which were shown in Table 6 and Figure 7. Thus *Boerhavia diffusa* comparatively proves its hydroxyl radical scavenging activity.

Nitric Oxide Scavenging Activity

Nitric oxide (NO) is a powerful pleiotropic mediator of a physiological process, for example, smooth muscle relaxant, neuronal signalling, inhibition of platelet aggregation and regulation of cell-mediated toxicity (Rice-Evans et al., 1997). The present

results demonstrated that the scavenging activity of nitric oxide was seen increasing in increasing concentration of the extract, which was predicted in Table 7 and Figure 8. The NO assay was examined for a hydroethanolic extract of *Boerhavia diffusa*. Its IC_{50} value was 42.34, whereas standard ascorbic acid was 35.36. In this way, *Boerhavia diffusa* indicates relatively viable NO radical scavenging activity.

CONCLUSIONS

The secondary metabolites of hydroethanolic extract of *Boerhavia diffusa* had various medical advantages, for example, high antioxidant activity gives some cardiovascular and weight reduction support. Moreover, *Boerhavia diffusa* can possibly be the best source of polyphenol compounds. The literature review of the *Boerhavia diffusa* gives us the information about different properties and details being utilized in different conditions like irritations, renal stones, anaemia, jaundice, eye diseases, joint pain, etc. the modern research has also proved the viability of roots has antidiabetic, antistress, adaptogenic, immunopotentiator and as antioxidant in different models. Studies have demonstrated that leaves have nutritive components like Vitamin C and Calcium because of which can be considered as an effective component of the diet. Consequently, it is very well be presumed that the hydroethanolic extract of *Boerhavia diffusa* has an effective role in scavenging free radicals and acts as the potential source of natural antioxidants. The presence of secondary metabolites such as phenols, flavonoids, tannin and alkaloids might be in charge of its antioxidant potential. Therefore, *Boerhavia diffusa* can serve as a potent natural drug for various diseases.

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