

Research Article

## Analysis of polyphenols and anti-oxidant activity of *Punarnava* (*Boerhavia diffusa* L.) from Himachal Pradesh-A North-Western State of India

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### Abstract

*Boerhavia diffusa* L. (family Nyctaginaceae) is also known as *Punarnava* in Indian medicine and has been used as *rasayana* herb in Ayurvedic medicine since ancient times. Plant has anti-aging, rejuvenating, cell-strengthening, mind-enhancing and preventative properties. The present study aims to investigate the different plant parts of the species *B. diffusa* for phytochemicals and antioxidants characterization for the first time from Bilaspur (31.33 °N 76.75 °E), Himachal Pradesh of North-Western India. The methanol: water extracts (leaf, stem and root) of *B. diffusa* were tested for *in vitro* antioxidant activity by various assays such as DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS [2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid)], FRAP (Ferric reducing antioxidant power assay) and RP-HPLC-PDA (Reverse Phase-High Performance Liquid Chromatography-Photo Diode Array Detector), which showed that *B. diffusa* root had the highest antioxidant activity i.e. DPPH (93.11±0.152<sup>g</sup> %), ABTS (89.70±0.207<sup>g</sup> %) and FRAP (88.67±0.251<sup>g</sup> %). This suggests that plant-derived polyphenols are good sources of natural antioxidants. Further, in the present study, three polyphenols, such as rutin, syringic acid, and epicatechin, were also determined through RP-HPLC-PDA method and found to be maximum in root extract. The results indicated that the methanolic extract of *B. diffusa* could be a potential herbal medicine against free radicals.

**Keywords:** Antioxidant activity, *Boerhavia diffusa* L., DPPH (1, 1-diphenyl-2-picrylhydrazyl), Polyphenols, RP-HPLC-PDA (Reverse Phase-High Performance Liquid Chromatography-Photo Diode Array Detector)

## INTRODUCTION

Herbal medicines have had a major impact on the health of many past and present cultures. Plant research has often shown that the active components of plants are essential for therapeutic efficacy. Medicinal plants can be a valuable source of new compounds for the development of effective treatments for various diseases. The pharmacological properties of natural plant products, including flavonoids, terpenoids, and steroid-derived compounds, are diverse and include antioxidant activity. An antioxidant is a compound that helps prevent oxidation reactions caused by free radicals, including singlet oxygen, superoxide, peroxy, hydroxyl, etc., to prevent or delay the formation of cells and cellular and tissue damage. In addition, antioxidant compounds can reduce the effects of free radicals and play an important role in maintaining health (Kataria, 1997). *Boerhavia diffusa* L. is a flowering plant belonging to the four o'clock-member family Nyctaginaceae and is native to the deserts of India. It is also known as *Punarnava* in Indian medicine. This perennial climber is identified as *Boerhavia*, a climber native to India. The name *Boerhavia* (L.) comes from the Dutch doctor Hermann Boerhaave of the 18th century. Plant has been found to have anti-aging, rejuvenating, cell-strengthening, mind-enhancing and preventative properties. In addition, it increases the body's resistance to foreign substances and improves the immune system (Government of India, 2007; Agrawal *et al.*, 2011; Oudhia, 2011; Pathak *et al.*, 2012; Mishra *et al.*, 2014). *B. diffusa* has been found to contain various chemical compounds such as flavonoids and isoflavones, as well as steroids and alkaloids. It has also been found to contain phenolic glycosides (Pandeya *et al.*, 2005; Sahu *et al.*, 2008; Pereira *et al.*, 2009 and Kaur, 2019). Some other chemical compounds found in this plant include punarnavoside (Jain and Khanna, 1989), boeravinone A-F (Kadota *et al.*, 1989; Lami *et al.*, 1990; Lami *et al.*, 1992), liriiodendrin (Lami *et al.*, 1990), hypoxanthine-9-larabinofuranoside (which is a type of hypoxanthine and used to treat hypoglycemia), eupalitin, repenone, repenol (Ahmed *et al.*, 1990), ursolic acid (Gupta and Ali, 1998),  $\beta$ -sitosterol (Gupta and Tandon, 2011), stigmasterol, campesterol, syringaresinol mono- $\beta$ -D-glucoside (Lami *et al.*, 1992), palmitic, oleic, heptad-stearic, arachidic and behenic acids (Misicic acid) (Gupta and Ali, 1998), gnetriacontan,  $\beta$ -ecdison, triacontanol (Gupta and Tandon, 2011), burhavisterol, burhadiffusene, diffusarotenoid, burhavianostenyl benzoate (Gupta and Ali, 1998). The plant is said to be diuretic, laxative and anti-inflammatory. At World level 40 species are distributed in tropical, subtropical and warm climates. India has 6 species - *B. diffusa*, *erecta*, *repanda*, *chinensis*, *hirsuta* and *rubicunda* - all of which have anti-inflammatory,

anti-nausea and anti-oxidant, anti-tumor, antibacterial and anti-hepatic properties. Many pharmacological studies have been reported such as anti-inflammatory, diuretic, laxative, antibacterial, anticonvulsant, hepatotoxic, antioxidant and ethnopharmacological activity (Patel *et al.*, 2014; Ekow Thomford *et al.*, 2018). The present study aimed to do phytochemical analysis of secondary metabolites from different parts (leaf, stem and root extracts) of *B. diffusa* by RP-HPLC-PDA method and to relate these findings to antioxidant activity, which are not previously known.

## MATERIALS AND METHODS

### Chemicals

Most of the chemicals used in the assay, including HPLC solvents acetonitrile, methanol and water, were of analytical grade and obtained from Merck, Life Science Pvt. Ltd. Mumbai, India. In addition, analytical standards for rutin, syringic acid, and epicatechin were obtained from Sigma-Aldrich, USA.

### Plant materials

Plant material (leaf, stem and root) of *B. diffusa* was collected from Bilaspur (31.33 °N 76.75 °E) Himachal Pradesh of Northwest India and identified by the Botanical Survey of India (B.S.I., Northern Circle), Dehradun (U.K.), further deposited in the Herbarium of Department of Botany, Eternal University, Baru Sahib, Himachal Pradesh (India) with accession numbers such as (BDL-101: *Boerhavia diffusa* Leaf, BDS-102: *Boerhavia diffusa* Stem, BDR-103: *Boerhavia diffusa* Root). The plant material was dried in the shade and ground for further extraction. The dried and ground plant parts were soaked for 12 hours at room temperature in a ratio of 80:20 (methanol: water). The mixture was filtered and the solvent was removed using a rotary evaporator, resulting in a crude extract.

### Antioxidant assay

#### 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

The leaf, stem and root extracts of *B. diffusa* were analyzed through standard DPPH assay with slight modifications (Blois, 1958). Methanol: water (80:20) crude plant extracts were prepared at a concentration of 1.0 mg/ml to perform the DPPH test, and L-ascorbic acid at a concentration of 1.0 mg/ml was used as a control. Ascorbic acid (vitamin C) acted as a rare anti-oxidant that provide reducing equivalent radical to form monodehydrosorbate, which specifically react with radicals, non-radicals and thus exhibit activity (Njus *et al.*, 2020). An alcoholic solution of 0.1 mM DPPH in methanol was prepared and 2 ml of this solution was added to 0.3 ml of extracts at different concentrations (50-1000 mg/ml) at room temperature and the reactivity was monitored after 30 minutes. Absorbance values at 517

nm were measured against a blank containing no extract. The per cent inhibition of radical scavenging activity was calculated using the following equation; Percentage Inhibition (%) =  $(A_{\text{Control}} - A_{\text{sample}} / A_{\text{Control}}) \times 100$  (1) Where  $A_{\text{control}}$  is the absorption of the control substance (L-ascorbic acid) and  $A_{\text{sample}}$  is the absorption of reaction mixture (in which the sample is present). All the tests were done in triplicate ( $n = 3$ ) and the mean values were calculated.

## 2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS) assay

To determine the radical scavenging activity of ABTS, the free radical scavenging activity of crude extracts (leaf, stem and root) of the species (*B. diffusa*) was used for the ABTS analysis. For the determination of ABTS (methanol: water: 80:20), the crude extracts of leaves, stems and roots of the species (80:20) were dissolved in the raw material concentration of 1.0 mg/ml. The cation radical (ABTS) was formed by the reaction between 7.0 mM ABTS in water and 2.45 mM potassium persulfate (1:1) stored in the dark at room temperature for 12-16 h before use. This experiment's total reaction time was 20 min (Re *et al.*, 1999; Sun *et al.*, 2014). Dissolving the ABTS solution in methanol gave an absorption range of 0.700-734 nm. After adding 5  $\mu\text{L}$  of the plant extract to a diluted ABTS solution (3.995 mL), the absorbance was determined 20 minutes after mixing. An appropriate solvent blank was used for each test. All measurements were repeated at least 3 times. The following equation was used to calculate the per cent inhibition at 734 nm;

$$\text{ABTS}^+ \text{ scavenging effect (\%)} = (\text{AB} - \text{AA}) / \text{AB} \times 100 \quad (2) \quad (2)$$

Where,

AB stands for absorbance of the ABTS radical + methanol and AA for the absorbance of the sample/standard (ABTS radical + Trolox)

Trolox was used as a standard.

## Ferric reducing antioxidant power (FRAP) assay

For the FRAP assay, methanol: water (80:20) extracts of different plant parts were dissolved in a concentration of 1 mg/ml and different dilutions were prepared for the FRAP assay. Various chemical solutions (0.2 M phosphate buffer (pH 6.6), 1% potassium ferricyanide solution, 10% trichloroacetic acid, 0.1 chloride solution, ascorbic acid (0.1%)) were prepared to evaluate the antioxidant activity in the FRAP test. The incubation period was 45 minutes for completion of the reaction (Vijayaakshmi *et al.*, 2016). Absorbance was measured with a Spectrophotometer at a wavelength of 700 nm. The principle of FRAP analysis is to increase the absorbance of reaction mixtures because the higher the absorbance, the higher the antioxidant activity. The antioxidant compounds in the samples form a colored complex with potassium ferricyanide, trichloroacetic

acid and ferric chloride.

## Reverse Phase-High Performance Liquid Chromatography-Photo Diode Array Detector (RP-HPLC-PDA) analysis of polyphenols

100 mg of each sample was air dried at 40°C. Grind and extract 3 times with 20 ml (80:20) methanol: water for 1 hour using a sonicator. Concentrated to dryness in vacuum at 45 °C, the dried extracts were re-dispersed in 2 ml of HPLC grade solvent, specifically HPLC grade methanol, filtered through a 0.22  $\mu\text{m}$  filter, and degassed for 1 minute. Samples with different concentrations were used for quantitative analysis. Stock solutions (rutin, syringic acid and epicatechin) (1 mg/2 ml) were prepared in 2 ml methanol (50:50 v/v) and in different amounts (1.0, 2.0, 3.0, 4.0 and 5.0  $\mu\text{g}/\text{ml}$ ) to construct a five-point calibration curve. A semi-finished HPLC-Waters system (binary pump (1525), column oscillator (50:50), temperature control module II, Waters Autosampler (2707), Waters Empower 3 software) was used for the separation of polyphenols. All samples and standards were filtered using a 0.22  $\mu\text{m}$  micropore filter. The sample material was Luna 5  $\mu\text{C}$ -18 (2), 100 Å (250 mm x 4.6 mm i.d., particle size 5  $\mu\text{m}$ ), manufactured by Phenomenex® in the USA. The mobile phase of methanol : water (0.3 acetic acid), pH=3.2, isocratic (70:30 v/v), flow rate 1.0 mL/min at 2200 Psi was used. The column temperature was kept constant at 40 °C. The injection volume of each sample was 5  $\mu\text{L}$ . Detection of the analytes (rutin, syringic acid and epicatechin) at a wavelength ( $\lambda=280$  nm) was performed using a PDA 2998. Peak area versus standard concentration data were processed by linear least squares regression. The regression equation derived from the standard curves was then used to estimate the analytes (rutin, syringic acid, and epicatechin) in the different samples.

## Statistical analysis

In the antioxidant assay, each experiment was performed in triplicate, and results were reported as mean  $\pm$  standard deviation of the mean. The statistical analysis was performed with IBM SPSS Software. The Duncan's test was used to compare the mean values and One-way analysis of variance (ANOVA). Variances were measured to be significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

### 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay

These results indicated that ascorbic acid acts as a standard and showed  $97.87 \pm 0.305^9$  % inhibition compared to the samples, i.e. stem, leaves and root extracts of *B. diffusa*. The results showed that the roots had the maximum antioxidant activity ( $93.11 \pm 0.152^9$  %) compared to the leaf and stem samples, which showed

(86.19±0.850<sup>g</sup> %) and (81.66±0.461<sup>g</sup> %) inhibition by DPPH assay at 1000 µg/ml (Table 1).

The DPPH test is a well-established, accurate and commonly used method to assess antioxidant activity. A stable free radical is a free radical whose electron has been transferred through the molecule. When H is added to the DPPH radicals, the color of the solution changes from purple to light yellow. The color change is inversely proportional to the antioxidant strength and concentration (Yang et al., 2008). In one of the previous studies, the ethanol extract of *B. diffusa* was tested *in vitro* DPPH antioxidant activity method showing inhibitory percentage such as 91.25±2.26 %, 90.39±1.23 % and 88.59±1.72 %, respectively (Ammar et al., 2014).

Another study reported that ethanol extract of *B. diffusa* scavenged DPPH radicals with a reduction potential of 0.65±0.02 mg/g ascorbic acid (Olaleye et al., 2010). In another study, the leaves were significantly more potent than the roots, with an IC<sub>25</sub> of 41 µg/ml and 1234 µg/ml, respectively. These general behaviours, i.e. leaves are more active than roots, were also confirmed in other research activities (Ignarro, 1999; Murad, 1999). The DPPH scavenging capacity, reductive potential and cytotoxicity were also studied by many workers (Teepica et al., 2009; Olaleye et al., 2010). In another study, the ethanolic extract demonstrated 81.94 % radical scavenging activity of DPPH, and chloroform extract demonstrated 42.58 % inhibitory activity at 1,000 µg/mL (compared to 88.02 % inhibitory activity of Quercetin). The maximum radical scavenging potential of DPPH at 1000 µg/ml was found to be 93.0 % and the percentage inhibition of cytotoxicity at 1000 µg/ml was 89.0% (Teepica et al., 2009). These previous results contradict the present report in which roots showed maximum inhibition, i.e. 93.11±0.152<sup>g</sup> %, compared to the leaf and stem samples at 1000 µg/ml.

One of the previous studies compared the bioavailability of ethanolic and ascorbic acid extracts. The free

radical inhibition of the ethanol extract was found to be a maximum 91.25±2.26 % at 1.50 mg/ml with an IC<sub>50</sub> value of 0.13±0.05 (Mohammad et al., 2011). *B. diffusa* aerial extracts exhibit concentration-dependent DPPH scavenging activity, with the ethanol extract having the strongest activity (EC<sub>50</sub>=9.92±0.5 µg/ml) (Olajumoke et al., 2018). These previous reports suggested that the roots of *B. diffusa* showed the highest antioxidant potential and corroborated many reports supporting the use of plant in traditional medicine (Gopal et al., 2010). The flavonoids are polyphenolic compounds acted as antioxidants, these reports also concur with the findings of other researchers (Middleton et al., 2000; Sharma et al., 2009). It was also reported that antioxidant activity in case of *B. diffusa* was associated with the presence of the rotenoids, Boeravinone G, H and D (Aviello et al., 2011; Poongothai et al., 2011; Piaru et al., 2012; Taher et al., 2012; Ahmed et al., 2012; Ravikumar et al., 2012). The DPPH method is fast, simple, sensitive, reproducible, and does not require special instruments (Clarke et al., 2013). The facts mentioned above indicated that the scavenging properties are usually due to polyphenols, which have a strong reducing power and act as the main antioxidant (Jomova et al., 2010; Mfengwana et al., 2019).

## 2, 2'-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) (ABTS) assay

The present results showed that the inhibition of standard Trolox was 95.97±1.154<sup>g</sup> %, while the inhibition of *B. diffusa* root was highest (89.70±0.208<sup>g</sup> %) at 1000 µg/ml compared to leaf (83.74±0.763<sup>g</sup> %) and stem (76.10±0.230<sup>g</sup> %) extracts (Table 2).

Since the ABTS method can be applied to both inorganic and organic solvent systems compared to other scavenging activity assays, it is more suitable for the determination of hydrophilic and lipophilic free radical scavenging activity, as mentioned in the previous articles (Rice-Evans et al., 1997; Yildirim et al., 2000). Ethanolic extracts of the plant can act as electron donors

**Table 1.** Percentage inhibition of leaf, stem, and root extracts of *Boerhavia diffusa* against DPPH assay (n=3)

Concentration (µg/mL)	Percentage Inhibition (%) of Ascorbic acid (Standard)	Percentage Inhibition (%) of Leaf (BDL-101)	Percentage Inhibition (%) of Stem (BDS-102)	Percentage Inhibition (%) of Root (BDR-103)
50	65.33±0.251 <sup>a</sup>	43.65±0.321 <sup>a</sup>	45.70±0.109 <sup>a</sup>	54.42±0.355 <sup>a</sup>
100	69.93±0.142 <sup>b</sup>	47.93±0.121 <sup>b</sup>	51.72±0.475 <sup>b</sup>	60.13±0.115 <sup>b</sup>
200	75.27±0.451 <sup>c</sup>	53.81±0.702 <sup>c</sup>	58.63±0.242 <sup>c</sup>	65.70±0.231 <sup>c</sup>
400	80.42±0.057 <sup>d</sup>	59.91±0.901 <sup>d</sup>	63.97±0.112 <sup>d</sup>	66.37±0.418 <sup>d</sup>
600	85.57±0.105 <sup>e</sup>	66.08±0.608 <sup>e</sup>	66.61±0.401 <sup>e</sup>	71.40±0.577 <sup>e</sup>
800	90.66±0.412 <sup>f</sup>	79.72±0.431 <sup>f</sup>	72.77±0.230 <sup>f</sup>	88.95±0.057 <sup>f</sup>
1000	97.87±0.305 <sup>g</sup>	86.19±0.850 <sup>g</sup>	81.66±0.461 <sup>g</sup>	93.11±0.152 <sup>g</sup>

BDL: *Boerhavia diffusa* Leaf, BDS: *Boerhavia diffusa* Stem, BDR: *Boerhavia diffusa* Root, Values are expressed in mean±SD (n=3), and were evaluated by One-way Anova and Duncan's test (p≤0.05). Statistical differences were indicated with different letters

**Table 2.** Percentage inhibition of leaf, stem, and root extracts of *Boerhavia diffusa* against ABTS assay ( $n=3$ )

Concentration ( $\mu\text{g/mL}$ )	Percentage Inhibition of Trolox (Standard) (%)	Percentage Inhibition (%) of Leaf (BDL-101)	Percentage Inhibition (%) of Stem (BDS-102)	Percentage Inhibition (%) of Root (BDR-103)
50	62.51 $\pm$ 0.131 <sup>a</sup>	43.30 $\pm$ 0.328 <sup>a</sup>	43.91 $\pm$ 0.118 <sup>a</sup>	43.49 $\pm$ 0.123 <sup>a</sup>
100	67.32 $\pm$ 0.341 <sup>b</sup>	46.15 $\pm$ 0.322 <sup>b</sup>	46.87 $\pm$ 0.102 <sup>b</sup>	46.22 $\pm$ 0.411 <sup>b</sup>
200	72.48 $\pm$ 0.114 <sup>c</sup>	52.67 $\pm$ 0.133 <sup>c</sup>	52.59 $\pm$ 0.318 <sup>c</sup>	53.57 $\pm$ 0.361 <sup>c</sup>
400	76.84 $\pm$ 0.105 <sup>d</sup>	59.10 $\pm$ 0.251 <sup>d</sup>	57.98 $\pm$ 0.113 <sup>d</sup>	59.23 $\pm$ 0.311 <sup>d</sup>
600	81.71 $\pm$ 0.228 <sup>e</sup>	65.54 $\pm$ 0.214 <sup>e</sup>	63.70 $\pm$ 0.446 <sup>e</sup>	65.14 $\pm$ 0.321 <sup>e</sup>
800	87.03 $\pm$ 0.143 <sup>f</sup>	71.90 $\pm$ 0.202 <sup>f</sup>	69.03 $\pm$ 0.057 <sup>f</sup>	77.93 $\pm$ 0.318 <sup>f</sup>
1000	95.97 $\pm$ 1.154 <sup>g</sup>	83.74 $\pm$ 0.763 <sup>g</sup>	76.10 $\pm$ 0.231 <sup>g</sup>	89.70 $\pm$ 0.208 <sup>g</sup>

BDL: *Boerhavia diffusa* Leaf, BDS: *Boerhavia diffusa* Stem, BDR: *Boerhavia diffusa* Root, Values are expressed in mean $\pm$ SD ( $n=3$ ), and were evaluated by One-way Anova and Duncan's test ( $p\leq 0.05$ ). Statistical differences were indicated with different letters

**Table 3.** Percentage inhibition of leaf, stem, and root extracts of *Boerhavia diffusa* against FRAP assay ( $n=3$ )

Concentration ( $\mu\text{g/mL}$ )	Percentage Inhibition (%) of Ascorbic acid (Standard)	Percentage Inhibition (%) of Leaf (BDL-101)	Percentage Inhibition (%) of Stem (BDS-102)	Percentage Inhibition (%) of Root (BDR-103)
50	51.19 $\pm$ 0.113 <sup>a</sup>	41.74 $\pm$ 0.107 <sup>a</sup>	41.62 $\pm$ 0.303 <sup>a</sup>	42.39 $\pm$ 0.363 <sup>a</sup>
100	53.89 $\pm$ 0.241 <sup>b</sup>	43.64 $\pm$ 0.416 <sup>b</sup>	50.60 $\pm$ 0.424 <sup>b</sup>	42.54 $\pm$ 0.206 <sup>b</sup>
200	55.11 $\pm$ 0.331 <sup>c</sup>	45.82 $\pm$ 0.262 <sup>c</sup>	53.03 $\pm$ 0.317 <sup>c</sup>	46.90 $\pm$ 0.311 <sup>c</sup>
400	61.96 $\pm$ 0.232 <sup>d</sup>	50.45 $\pm$ 0.116 <sup>d</sup>	55.55 $\pm$ 0.118 <sup>d</sup>	50.82 $\pm$ 0.621 <sup>d</sup>
600	69.63 $\pm$ 0.041 <sup>e</sup>	55.08 $\pm$ 0.312 <sup>e</sup>	58.16 $\pm$ 0.320 <sup>e</sup>	58.76 $\pm$ 0.401 <sup>e</sup>
800	77.55 $\pm$ 0.034 <sup>f</sup>	66.52 $\pm$ 0.321 <sup>f</sup>	65.91 $\pm$ 0.107 <sup>f</sup>	66.08 $\pm$ 0.148 <sup>f</sup>
1000	91.96 $\pm$ 0.202 <sup>g</sup>	81.50 $\pm$ 0.241 <sup>g</sup>	77.68 $\pm$ 0.272 <sup>g</sup>	88.67 $\pm$ 0.251 <sup>g</sup>

BDL: *Boerhavia diffusa* Leaf, BDS: *Boerhavia diffusa* Stem, BDR: *Boerhavia diffusa* Root, Values are expressed in mean $\pm$ SD ( $n=3$ ), and were evaluated by One-way Anova and Duncan's test ( $p\leq 0.05$ ). Statistical differences were indicated with different letters

and react with free radicals to form stable products, thus terminating the free radical chain reaction (Sharma *et al.*, 2007, Beegum Juna *et al.*, 2015). In previous work, different fractions of *B. diffusa* root extract were investigated and reported that ethanol fraction had better ABTS radical scavenging activity than chloroform, ethyl acetate and n-butanol fractions compared to the standard (98.27 $\pm$ 3.67 mg/ml) (Adeku *et al.*, 2022). The visible spectrophotometer absorbs the radical anion blue-green dye (ABTS) at 734 nm. The ability of plant components to act as antioxidants was demonstrated by the reduction of ABTS radical anion absorption (Venkatachalam *et al.*, 2012).

#### Ferric reducing antioxidant power (FRAP) assay

Ascorbic acid was taken as a standard and it showed 91.96 $\pm$ 0.202<sup>g</sup> % inhibition. Instead, the highest inhibition was reported in the roots of *B. diffusa* (88.67 $\pm$ 0.251<sup>g</sup> %) as compared to leaf (81.50 $\pm$ 0.241<sup>g</sup> %) and stem (77.68 $\pm$ 0.272<sup>g</sup> %) extracts at 1000  $\mu\text{g/ml}$ . The results are summarized here (Table 3). The FRAP analysis was expected to show a positive relationship between reducing power and phenolic power in *B. diffusa* extracts. Reducing properties are generally associated with compounds that donate hydrogen atoms via free radical chain termination (Duh *et al.*, 1999).

One of the earlier reports mentioned that ethanol extract of *B. diffusa* had a higher FRAP value than chloroform, petroleum ether and methanol extracts (Rachh *et al.*, 2009). At pH 6.6, potassium ferricyanide ( $\text{Fe}^{3+}$ ) was reduced to potassium ferrocyanide ( $\text{Fe}^{2+}$ ) in the presence of an antioxidant (Garba *et al.*, 2019). According to one report, the maximum absorbance of the ethanol extract of *B. diffusa* root was 0.35 nm obtained at 140.0 mg/ml extract concentration, while the maximum absorbance of gallic acid was 0.319 nm obtained at 100 mg/ml (Mohammad *et al.*, 2011). Previous results have shown stronger antioxidant activity of *B. diffusa* leaves than root extracts (Gopal *et al.*, 2010; Khalid *et al.*, 2011; Banjare *et al.*, 2012; Mahesh *et al.*, 2012). The results of many other studies are consistent with previous findings, which again characterized *B. diffusa* leaves as having a higher percentage of radical scavenging activity, as well as solvent extracts containing methanolic compounds, showing better inhibition compared to other solvent extracts (Devinder *et al.*, 2014; Kriti *et al.*, 2020).

#### Reverse Phase-High Performance Liquid Chromatography-Photo Diode Array Detector (RP-HPLC-PDA)

A high-performance liquid chromatography technique was used to quantify polyphenols in different extracts,

**Table 4.** Estimation of polyphenols in leaf, stem and root extracts of *Boerhavia diffusa* through HPLC (n=3)

Species	Sample ID	Rut. ( $\mu\text{g/mL}$ )	Epi. ( $\mu\text{g/mL}$ )	Syr. ( $\mu\text{g/mL}$ )
Boerhavia diffusa	BDL-101	1.270 $\pm$ 0.378	0.631 $\pm$ 0.017	0.281 $\pm$ 0.010
	BDS-102	1.021 $\pm$ 0.057	0.028 $\pm$ 0.027	0.235 $\pm$ 0.020
	BDR-103	2.132 $\pm$ 0.051	2.031 $\pm$ 0.039	1.203 $\pm$ 0.001

Rut.: Rutin, Syr.: Syringic acid, Epi.: Epicatechin, BDL: *Boerhavia diffusa* Leaf, BDS: *Boerhavia diffusa* Stem, BDR: *Boerhavia diffusa* Root, Values are expressed in mean $\pm$ SD (n=3)

namely, the leaves, stems, and roots of *B. diffusa*. RP-HPLC-PDA study of polyphenols showed that root extracts had higher concentrations of rutin and epicatechin than leaf and stem extracts. Rutin was found to have maximum value in *B. diffusa* root, i.e. 2.132 $\pm$ 0.051  $\mu\text{g/ml}$ . On the other hand, the content of rutin in leaves was found to be 1.270 $\pm$ 0.378  $\mu\text{g/ml}$  and the concentration of rutin in stem extract was quite low, i.e. 1.021 $\pm$ 0.057  $\mu\text{g/ml}$ . Epicatechin, a polyphenol, was estimated to be present in root, leaf and stem extracts. The epicatechin content in the root extract was the highest at 2.031 $\pm$ 0.039  $\mu\text{g/ml}$ , while in the leaf extract, it was 0.631 $\pm$ 0.017  $\mu\text{g/ml}$ , whereas in the stem extract it was low at 0.028 $\pm$ 0.027  $\mu\text{g/ml}$ . Further, the syringic acid was found to be higher at 1.203 $\pm$ 0.001  $\mu\text{g/ml}$  in root extract than 0.281 $\pm$ 0.010  $\mu\text{g/ml}$  leaf and 0.235 $\pm$ 0.020  $\mu\text{g/ml}$  in stem extracts (Table 4).

It has been reported that polyphenols often have phenolic hydroxyl groups that can donate an additional hydrogen or electron to a free radical, and the unpaired electrons move along the long conjugated aromatic structure (Lu et al., 2010). It has already been reported that plant roots contain less flavonoid than the leaves. From a statistical point of view, phenolics were much higher in the leaves than in the roots. These findings are consistent with the biological roles of phenolic compounds, especially flavonoids, in plants (Jordan, 2002).

## Conclusion

Antioxidant activity was evaluated using three antioxidant assays, DPPH, ABTS and FRAP, based on *B. diffusa* leaf, stem and root extracts. The results of the DPPH assay showed that the root extract had the highest antioxidant activity compared to the leaf and stem extracts. At the same time, ABTS analysis also showed that root extract had maximum inhibition at 1000  $\mu\text{l}$  concentration compared to leaf or stem samples. In addition, a test of reducing antioxidant capacity of iron (III) again showed the maximum inhibition percentage of the antioxidant activity in the root extract. It is suggested that the metabolically active components viz. (rutin, syringic acid, and epicatechin, etc.) of *B. diffusa* may be a potential herbal remedy against oxidative damage caused by free radicals. Polyphenols such as rutin, syringic acid and epicatechin, also checked by RP-HPLC-PDA were present in higher amounts, especially

in the root extract, followed by the leaf and stem extracts. Therefore, it is important to consider the various possible uses of *B. diffusa* roots and leaves in medicinal preparations.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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