

**FREE RADICAL SCAVENGING ACTIVITY OF LEAVES OF  
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**ABSTRACT**

The oxidative degenerative disorders are caused due to the free radical accumulation in the body and there is a need for new antioxidants from plant sources to protect ourselves from the radicals. The present study was aimed at evaluating the free radical scavenging activity generated by the different extracts (petroleum ether, chloroform, ethanol and aqueous) of leaves of *Boerhavia diffusa*. The *in vitro* methods like DPPH, ABTS, nitric oxide, hydroxyl, hydrogen peroxide and superoxide radical scavenging activity were adopted for the study. The scavenging effect of the plant extracts and the standard ascorbic acid showed a dose dependent increase with increase in concentration in percent inhibition (20 – 100 µg/ml). The ethanolic extract of the leaves was found to possess a better scavenging activity at 100µg/ml concentration towards DPPH (60.8±0.21%), ABTS (71±0.31%), nitric oxide (80.4±0.1%), hydroxyl (76.4±0.21%), hydrogen peroxide (74.5±0.17%) and superoxide (79.6±0.26%) radicals. Hence the result of the present study confirms the antioxidant potential of the leaves of *Boerhavia diffusa*.

**KEYWORDS:** *Boerhavia diffusa*, antioxidants, free radicals, DPPH, ABTS

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## INTRODUCTION

In recent years there is an increasing awareness among people in prevention of free radical mediated disease and the role of antioxidants in health and diseases<sup>1</sup>. Free radicals are chemical species which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. The biological reactivity of free radicals and their roles in oxidative stress are subjects of considerable attention and controversy<sup>2</sup>. Medicinal plants typically contain several different pharmacologically active compounds that may act individually, additively or in synergy to improve health<sup>3</sup>. *Boerhavia diffusa* (*B. diffusa*) commonly known as punarnava in the Indian system of medicine and spreading hogweed in English, belonging to the family Nyctaginaceae. The plant possesses diuretic, cardiogenic, hypotensive and anticancerous activity<sup>4</sup>. All the parts of *Boerhavia diffusa* can be used as medicine: leaf, seed, stem and root. The present study is conducted to evaluate the free radical scavenging activity generated by the different extracts (petroleum ether, chloroform, ethanol and aqueous) of leaves of *Boerhavia diffusa*.

## MATERIALS AND METHODS

### (i) Collection and identification of plant samples

The experimental plant *Boerhavia diffusa* was collected from the areas in and around Coimbatore and duly authenticated from Botanical Survey of India, TNAU, Coimbatore. The authentication number is BSI/SRC/5/23/2013-14/Tech/1041. The fresh leaves of the plant were used for the assays.

### (ii) Chemicals

DPPH, ABTS, ammonium per sulphate, deoxy ribose, ferric chloride, EDTA, hydrogen peroxide, ascorbate, thiobarbituric acid, NBT, riboflavin, potassium dihydrogen phosphate were obtained from Himedia, Mumbai, India and Sigma, Chemico Co, USA. All other chemicals and solvents used were of analytical grade.

### (iii) Preparation of plant extract

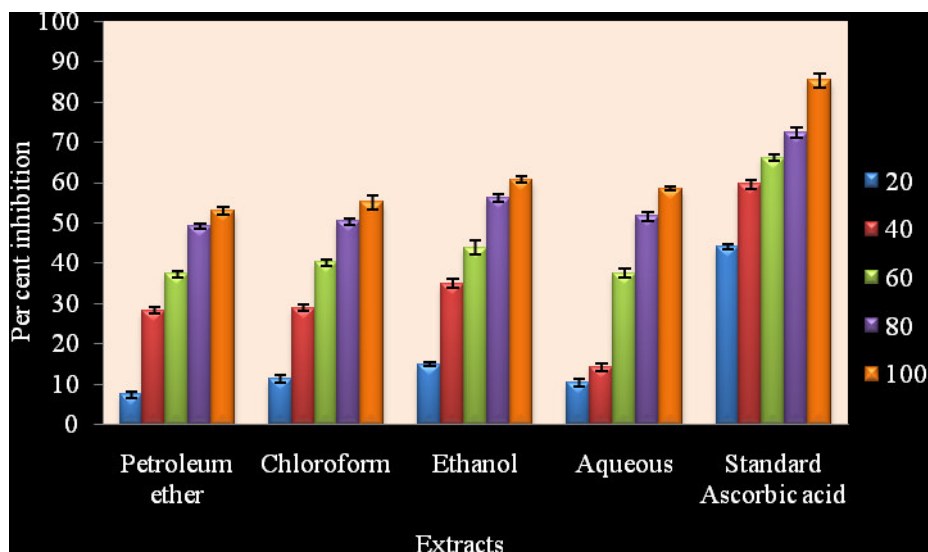
The collected leaves of the plant were washed thoroughly in running tap water and then with distilled water to remove sand and other dust particles adhered to it. They are then spread over a filter paper and air dried at room temperature to remove excess water. The fresh leaves of the plant was macerated finely using mortar and pestle and weighed 15 gram each into a thimble for sequential extraction with petroleum ether, chloroform, ethanol and aqueous using soxhlet apparatus. Then the extract was filtered through Whatman No.1 filter paper and concentrated in a rotary evaporator. The concentrated extracts were used for the further studies. Free radical scavenging assays of leaves of *Boerhavia diffusa* were assessed for scavenging the radicals of DPPH by Mensor *et al.* (2001)<sup>5</sup>, ABTS by Shirwaikar *et al.* (2006)<sup>6</sup>, inhibition of nitric oxide generation by Green and Hill, (1984)<sup>7</sup>, inhibition of hydroxyl radical by Elizabeth and Rao, (1990)<sup>8</sup>, inhibition of hydrogen peroxide radical by Ruch *et al.* (1989)<sup>9</sup> and inhibition of superoxide generation by Mc Cord and Fridovich, (1975)<sup>10</sup>.

## RESULTS AND DISCUSSION

### 1. DPPH radical scavenging activity of leaves of *Boerhavia diffusa*

DPPH scavenging activity of the extracts of *Boerhavia diffusa* is presented in Figure 1.

**Figure 1**  
**DPPH radical scavenging activity of leaves of *Boerhavia diffusa***



The scavenging activity of leaves of *Boerhavia diffusa* were found to be increased with increase in concentration from 20 – 100 µg/ml for the standard and the samples. The scavenging activity of all the solvent extracts was found to be less when compared to that of the standard ascorbic acid. Among the different extracts, the ethanol extract of leaves showed high DPPH scavenging activity at 100µg/ml (80.4±0.1%) with a low IC<sub>50</sub> value (71µg/ml) followed by aqueous, chloroform and petroleum ether. Ganie *et al.*<sup>11</sup> reported that the extracts of *Arnebia benthamii* exhibited the radical scavenging activity of 69.43±11.29% at the concentration of

700µg/ml. According to Thambiraj *et al.*<sup>12</sup> the methanolic extracts of leaves of *Acacia caesia* increased with increase in the concentration from 50 - 250 µg/ml 38.89% in 50 µg/ml of the extract to 72.22% in 250 µg/ml of extract. Hebbar *et al.*<sup>13</sup> showed that the aqueous leaf (IC<sub>50</sub> value = 12.61±0.00 µg/ml) of *Scheffera stellata* showed potent antioxidant activity than *Scheffera racemosa* which showed comparatively less antioxidant activity (IC<sub>50</sub> value = 24.03±0.03 µg/ml) when compared to the standard antioxidant ascorbic acid (IC<sub>50</sub> value = 11.5±0.00 µg/ml).

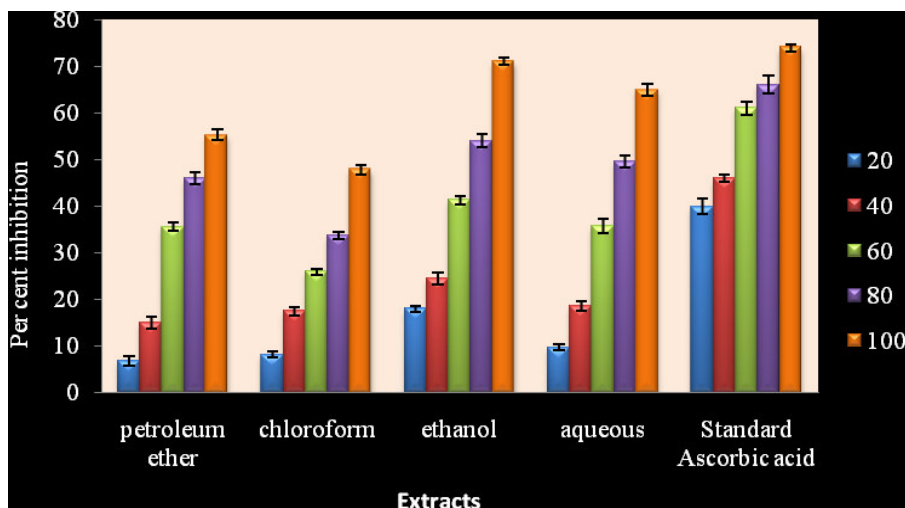
**Table 1**  
**IC<sub>50</sub> values of standard ascorbic acid and different solvent extracts of the leaves of *Boerhavia diffusa***

Radical scavenging activity	IC <sub>50</sub> value µg/ml				
	Petroleum ether	Chloroform	Ethanol	Aqueous	Ascorbic acid
DPPH radical	82 ± 3.0	90 ± 5.29	71.33 ± 2.08	78 ± 2.0	31.6 ± 1.53
ABTS radical	92 ± 4.0	106 ± 4.04	74.33 ± 3.22	80 ± 2.65	44 ± 2.65
Nitric oxide radical	96 ± 2.65	102 ± 2.0	61 ± 3.61	68 ± 3.0	60.33 ± 2.52
Hydroxyl radical	126 ± 3.60	88 ± 1.73	67 ± 2.65	73 ± 3.0	44 ± 4.58
Super oxide radical	96 ± 2.0	78 ± 3.61	62 ± 2.0	86 ± 1.73	40 ± 1.73
Hydrogen peroxide radical	110 ± 2.82	86 ± 2.65	52 ± 2.65	58 ± 3.0	58 ± 3.61

## 2. ABTS radical scavenging activity of leaves of *Boerhavia diffusa*

The ABTS radical scavenging activity of different solvent extracts of *Boerhavia diffusa* is shown in Figure 2.

**Figure 2**  
**ABTS radical scavenging activity of leaves of *Boerhavia diffusa***



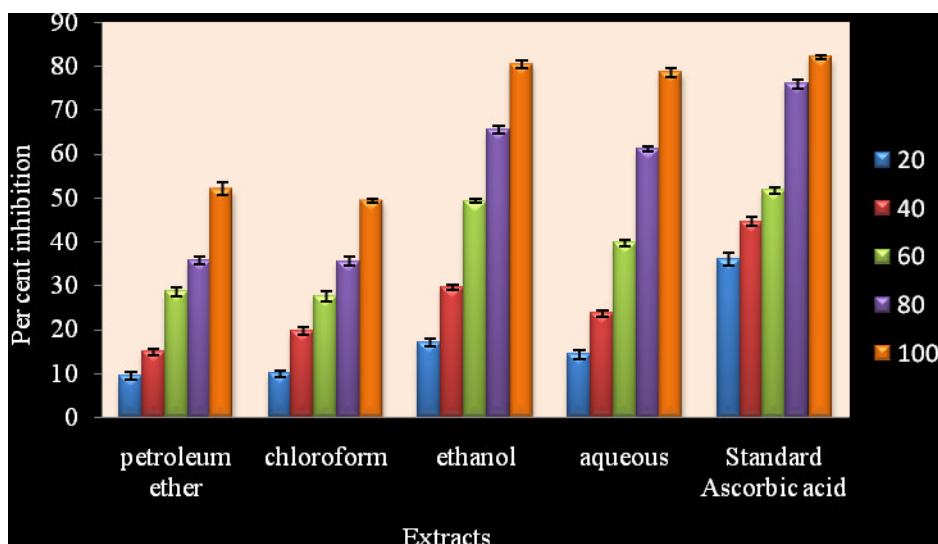
The potentiality of different solvent extracts (20 – 100  $\mu\text{g/ml}$ ) was assessed by its ability to quench ABTS. The ethanol extract of leaves of *Boerhavia diffusa* showed maximum free radical scavenging for ABTS assay at 100 $\mu\text{g/ml}$  concentration (71 $\pm$ 0.31%) with a low  $\text{IC}_{50}$  value of (74 $\mu\text{g/ml}$ ) followed by aqueous, petroleum ether and chloroform extracts. Ashafa *et al.*<sup>3</sup> reported that the leaves of *Felicia muricata* exhibited percentage

inhibition of 94.6%, 99.2%, 98.7%, 97.3% and 99.3% in aqueous, methanol, acetone, ethanol and butylated hydroxytoluene respectively at 0.05 mg/ml concentration. According to Iqbal *et al.*<sup>14</sup> the leaves from three varieties of mulberry namely *Morus alba* L., *Morus nigra* L. and *Morus rubra* L. showed significantly different ( $p < 0.05$ ) ability to scavenge the ABTS radical cation ranging from 6.12  $\pm$  0.53 to 9.89  $\pm$  0.87 mM.

## 3. Nitric oxide radical scavenging activity of leaves of *Boerhavia diffusa*

The Nitric oxide radical scavenging activity of leaves of *Boerhavia diffusa* is depicted in Figure 3.

**Figure 3**  
**Nitric oxide radical scavenging activity of leaves of *Boerhavia diffusa***



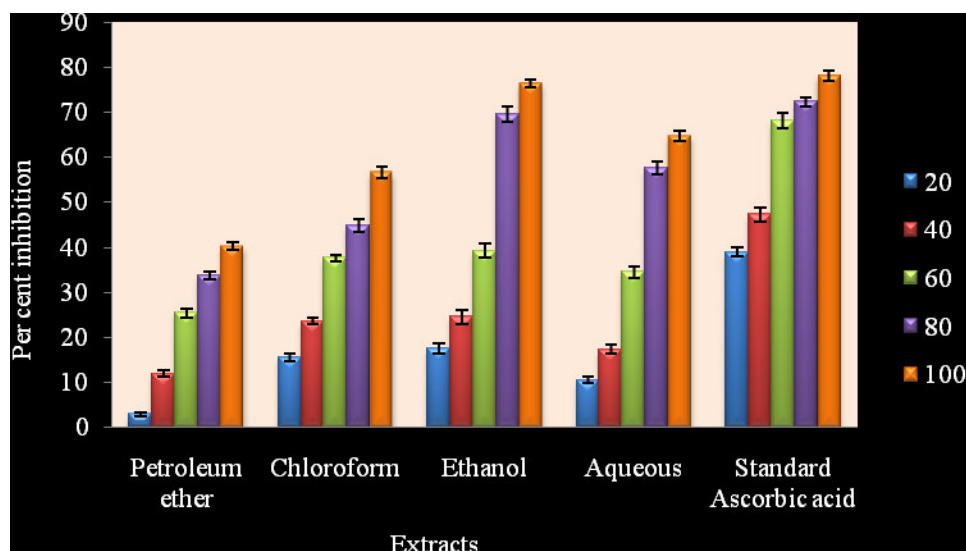
The results of the present study indicates that the ethanolic extract of leaves of *Boerhavia diffusa* showed highest radical scavenging activity at 100 $\mu$ g/ml concentration (80.4 $\pm$ 0.1%) followed by aqueous, petroleum ether and chloroform which was low when compared to the standard ascorbic acid. All the solvent extracts and standard has showed a dose dependent increase with increase in concentration from 20 – 100  $\mu$ g/ml. According to Ahirwal *et al.*<sup>15</sup> the methanolic extract of

*Gymnema sylvestre* showed a significant antioxidant activity of 11.4, 13.3, 15.2 and 17.7 % inhibition at 50, 100, 250, 500  $\mu$ g/ml respectively when compared with butylated hydroxyacetone. Hossain *et al.*<sup>16</sup> reported that the scavenging activity of nitric oxide by the ethanolic extract of *Ceriops decandra* showed a high activity of 67.4% at 100 $\mu$ g/ml, whereas ascorbic acid as a standard at the same concentration exhibited 81.1% inhibition.

#### 4. Hydroxyl radical scavenging activity of leaves of *Boerhavia diffusa*

Hydroxyl radical scavenging activity of leaves of *Boerhavia diffusa* is depicted in Figure 4.

**Figure 4**  
**Hydroxyl radical scavenging activity of leaves of *Boerhavia diffusa***



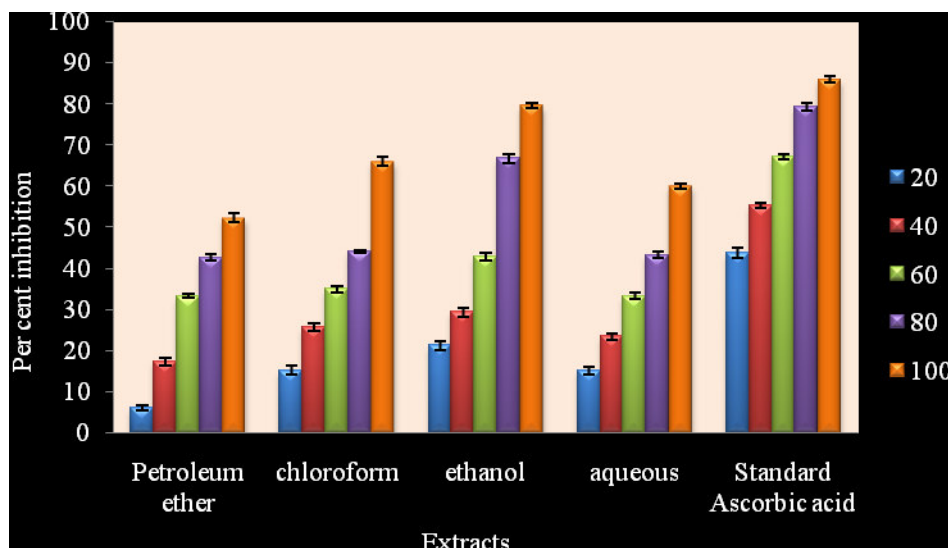
The concentrations ranging from 20 – 100  $\mu$ g/ml of different extracts were assessed for their hydroxyl radical scavenging activity. From figure 4, it is clear that the ethanol extract of leaves of *Boerhavia diffusa* showed greatest scavenging activity (76.4 $\pm$ 0.21%) at 100 $\mu$ g/ml concentration followed by aqueous, chloroform and petroleum ether with the IC<sub>50</sub> values of 67 $\mu$ g/ml, 73 $\mu$ g/ml, 88 $\mu$ g/ml and 126 $\mu$ g/ml respectively. The IC<sub>50</sub> value of

standard ascorbic acid was found to be 44 $\mu$ g/ml. Yang *et al.*<sup>17</sup> reported that the hydroxyl radical scavenging ability of ethanol extract of *Hibiscus sabdariffa* L. showed maximum scavenging activity of 95 per cent. Battu *et al.*<sup>18</sup> showed that the ethanolic extract and its fractions chloroform and hexane of leaves of *Aerva lanata* possess concentration dependent scavenging activity on hydroxyl radicals.

#### 5. Superoxide radical scavenging activity of leaves of *Boerhavia diffusa*

Superoxide radical scavenging activity of leaves of *Boerhavia diffusa* is given in Figure 5.

**Figure 5**  
**Superoxide radical scavenging activity of leaves of *Boerhavia diffusa***



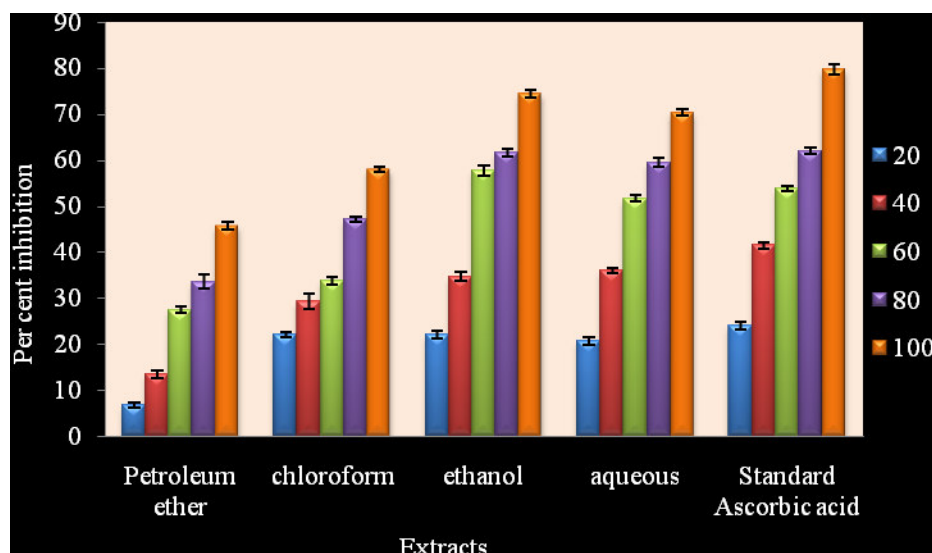
From figure 5, it is evident that all the extracts had shown a dose dependent increase with increase in concentration from 20 – 100 µg/ml. Among all the extracts, ethanol extract of leaves of *Boerhavia diffusa* showed greatest scavenging activity of (79.6±0.26%) at 100µg/ml concentration followed by chloroform, aqueous and petroleum ether. Rachh *et al.*<sup>19</sup> proved that *Gymnema sylvestre* extract showed a significant ( $p < 0.05$ ) dose response relationship for superoxide free

radical scavenging activity the maximum scavenging activity of 65.29% was observed at 100 µg/ml concentration. Govind *et al.*<sup>20</sup> stated that the superoxide scavenging activity of leaves of *Nerium indicum* was increased markedly with the decrease in absorbance. According to Khan *et al.*<sup>21</sup> the hexane extract of *Launaea procumbens* exhibited high radicals scavenging ( $IC_{50} 220.7 \pm 7.8 \mu\text{g/ml}$ ) which was comparatively high to ascorbic acid ( $IC_{50} 70.7 \pm 2.8 \mu\text{g/ml}$ ).

**6. Hydrogen peroxide radical scavenging activity of leaves of *Boerhavia diffusa***

Hydrogen peroxide radical scavenging activity of leaves of *Boerhavia diffusa* is shown in Figure 6.

**Figure 6**  
**Hydrogen peroxide radical scavenging activity of leaves of *Boerhavia diffusa***



A dose dependent increase with increase in concentration from 20 – 100 µg/ml was observed. Figure 6 clearly shows that the ethanol extract of leaves of *Boerhavia diffusa* showed maximum scavenging activity at 100µg/ml concentration (74.5±0.17%) followed by aqueous, chloroform and petroleum ether with a low IC<sub>50</sub> value of 52µg/ml. Meena *et al.*<sup>22</sup> showed that the leaves of *Baccopa monnieri* exhibited a significant higher ( $P<0.05$ ) antioxidant activity when compared to *Centella asiatica* at different concentrations. Vadnere *et al.*<sup>23</sup> proved that *Cicer arietinum L.*

(*Fabaceae*) showed an effective hydrogen peroxide scavenging activity.

## CONCLUSION

Based on the findings from the present study, it can be concluded that the ethanolic extract of the leaves of *Boerhavia diffusa* exhibited a promising free radical scavenging effect. Hence the ethanol extract of the leaves can be used as a therapeutic agent in treating free radical related disorders.

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