



## Evaluation of Antioxidant Potential in the Leaves and Seeds of *Boerhavia diffusa*

Vasundhara C.C.S, Gayathri Devi S.\*

Dept of Biochemistry, Biotechnology and Bioinformatics, Avinashilingam Inst for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India.

\*Corresponding author's E-mail: [gayathridevi.adu@gmail.com](mailto:gayathridevi.adu@gmail.com)

Accepted on: 22-01-2015; Finalized on: 28-02-2015.

### ABSTRACT

Plant and its products are rich source of phytochemicals and have found to possess a variety of biological activities including antioxidant potential. Natural antioxidants are in high demand for application as nutraceuticals, biopharmaceuticals as well as food additive because of consumer preference. The present study was carried out to investigate the status of enzymic and non-enzymic antioxidants in the leaves and seeds of *Boerhavia diffusa*. The enzymic antioxidants such as catalase, peroxidase, superoxide dismutase, glutathione reductase and glutathione-S-transferase and non-enzymic antioxidants like ascorbic acid,  $\alpha$ -tocopherol, total carotenoids, flavonoids, tannins and reduced glutathione were assessed as per the standard protocol. The activity of catalase in both the leaves and seeds of *Boerhavia diffusa* were found to be maximum ( $131.9 \pm 3.28$  and  $132.8 \pm 4.21$  U/g) when compared to other enzymes assessed. The non-enzymic antioxidants (total carotenoids, tannins and  $\alpha$ -tocopherol) were found to exhibit moderate activity in both the leaves and seeds of *Boerhavia diffusa*. From the results, it could be inferred that *Boerhavia diffusa* possesses antioxidant properties and could be used to treat disorders caused due to oxidative damage.

**Keywords:** antioxidants, superoxide dismutase, glutathione reductase,  $\alpha$ -tocopherol

### INTRODUCTION

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process.

Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer and cardiovascular diseases<sup>1</sup>.

Reactive oxygen species (ROS) are generated as a result of normal biochemical metabolism in the body which is due to high level of exposure to xenobiotics<sup>2</sup>.

A growing amount of evidence indicates a role of reactive oxygen species (ROS) such as peroxy radicals (ROO<sup>-</sup>), hydroxyl radical (HO<sup>-</sup>), superoxide anion (O<sub>2</sub><sup>-</sup>) and singlet oxygen (<sup>1</sup>O<sub>2</sub>) in the pathophysiology of aging and different degenerative diseases such as cancer, cardiovascular diseases, Alzheimer's disease and Parkinson's disease<sup>3</sup>.

The plant selected for the study is *Boerhavia diffusa* L. (Nyctaginaceae), commonly known as 'Punarnava' in Sanskrit and 'Hogweed' in English. It is an herbaceous plant species growing prostrate as a weed in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds.

In Ayurveda it is used for the treatment of edema, anaemia, dermatopathies, heart, renal, hepatic and inflammatory disorders<sup>4</sup>. Recently there has been an upsurge of interest in the therapeutic potentials of plants, also many other plant species have been investigated in the search for novel antioxidants.

Therefore in recent years, considerable attention has been directed towards the identification of plants with

antioxidant ability<sup>5</sup>. Hence the present study is focused to evaluate the phytochemical constituents and antioxidant potential in the leaves and seeds of *Boerhavia diffusa*.

### MATERIALS AND METHODS

#### Collection and Identification of Plant Material

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 and seeds of the plant were used for the assays.

#### Chemicals

Hydrogen peroxide, potassium hydrogen phosphate, potassium hydrogen buffer, ferrous chloride, ferric chloride, ferric cyanide, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra-acetic acid (EDTA), N-(1-naphthyl) ethylene diamine dihydrochloride, riboflavin, sodium nitroprusside, potassium persulfate were obtained from Himedia, loba and Merck. All other chemicals and solvents used were of analytical grade.

#### Enzymic Antioxidants

##### Estimation of Catalase Activity<sup>6</sup>

Homogenized the sample in a blender with M/15 phosphate buffer (pH 7.0) at 4°C and centrifuged. Stirred the sediment with cold M/15 phosphate buffer (pH 7.0), allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The combined supernatants were used for the assay. Read against a control cuvette containing the enzyme solution as in the experimental cuvette, but containing H<sub>2</sub>O<sub>2</sub> free M/15 phosphate buffer (pH 7.0). Pipetted into the



experimental cuvette 3.0 ml of H<sub>2</sub>O<sub>2</sub>-phosphate buffer (0.16 ml of H<sub>2</sub>O<sub>2</sub> made upto 100 ml with M/15 phosphate buffer, pH 7.0). Mixed in 0.01-0.04 ml sample and noted the time if required for a decrease in absorbance from 0.45 to 0.4. Calculated the concentration of H<sub>2</sub>O<sub>2</sub> using the extinction coefficient 0.036 per  $\mu$ mole per ml.

#### Estimation of Peroxidase Activity<sup>7</sup>

Macerated one gram of the sample with 5 ml (w/v) 0.1M phosphate buffer (pH 6.5) in a homogenizer. Centrifuged the homogenate at 300 g for 15 min. Used the supernatant as the enzyme source. All the procedures were carried out at 0-5°C. Pipetted out 3ml of 0.05 M pyrogallol solution and 0.5 to 1.0ml of enzyme extract in a test tube. Adjusted the spectrophotometer to read '0' at 400 nm. Added 0.5ml of 1% hydrogen peroxide in the test cuvette. Recorded the change in the absorbance every 30 seconds upto 3 minutes.

#### Determination of Superoxide Dismutase Activity<sup>8</sup>

The incubation medium contained in a final volume of 3.0 ml, 50 mM potassium phosphate buffer (pH 7.8), 45  $\mu$ M methionine, 5.3  $\mu$ M riboflavin, 84  $\mu$ M NBT and 20  $\mu$ M KCN. The tubes were placed in an aluminium foil-lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600 nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the enzyme. One unit of enzyme activity was defined as the amount of enzyme causing 50 per cent inhibition of the reduction of NBT.

#### Determination of Glutathione-S-transferase Activity<sup>9</sup>

GST conjugates with GSH and CDNB and the extent of conjugation causes a proportionate change in the absorption at 340nm. The sample was homogenized with Tris-HCl buffer (pH7.2). The homogenated sample was centrifuged at 4°C for 30 minutes at 8500rpm. The supernatant was used as the enzyme source. The estimation was done at 25°C under condition giving activities linear with respects to incubation time and protein concentration for at least 3 minutes.

The enzyme activity was determined by monitoring the change in absorbance at 340nm in a spectrophotometer. 0.1ml of both substrates GSH and CDNB was taken in 0.1M phosphate buffer (pH 6.5) at room temperature to make a volume of 2.9ml. The reaction was started by the addition of 0.1ml of sample to this mixture; the readings were recorded against distilled water blank for a minimum of three minutes.

The complete assay mixture without the sample served as the control to monitor nonspecific binding of the substrate. It was taken to ensure that final concentration of ethanol in the mixture was always less than 4%. GST activity was calculated using the extinction coefficient of the product formed and the values have been expressed as nmoles and CDNB conjugated/minutes/g sample.

#### Estimation of Polyphenol Oxidase<sup>10</sup>

The activity of polyphenol oxidase was estimated by the method of Esterbauer. Ground about 5g of the plant sample and made upto 20ml with the medium containing 50mM Tris-HCl (pH 7.2), 0.4M sorbitol and 10mM NaCl. Centrifuge the homogenate at 2000 rpm for 10 minutes and used the supernatant for the assay. Added 2.5ml of 0.2M phosphate buffer (pH 6.5), 0.3ml of catechol solution (0.01 M) into the cuvette and set the spectrophotometer at 495nm. Added the enzyme extract (0.2ml) and started recording the change in absorbance for every 30 seconds upto 5 minutes.

#### Determination of Glutathione Peroxidase Activity<sup>11</sup>

20 percent aqueous extract was prepared in 0.12 M phosphate buffer, pH 7.2, was used as the source of enzyme. The assay system contained 1 ml of 0.12 M potassium phosphate buffer, 0.1 ml of 15 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 6.3 mM oxidized glutathione and 0.1 ml of enzyme source and water in the final volume of 2 ml. Kept for 3 minutes. The 0.1 ml of NADPH was added. The absorbance at 340 nm was recorded at an interval of 15 seconds for 2-3 minutes. For each series of measurement controls were done that contained water instead of oxidized glutathione. The enzyme activity was expressed as mmoles of NADPH oxidized/minutes/g sample.

#### Non-enzymic Antioxidants

##### Estimation of Ascorbic acid<sup>12</sup>

One gram of the sample was homogenized in 10 ml of 4 per cent TCA. Centrifuged at 2000 rpm for 10 minutes. The supernatants obtained were treated with a pinch of activated charcoal. Shaken well and kept for 10 minutes. Centrifuged and 0.5 and 1.0 ml aliquots of this supernatant were taken for the assay. The assay volumes were made upto 2.0 ml with 4 per cent TCA. The working standard solution containing 20-100  $\mu$ g of ascorbate were pipetted out, the volumes of which were also made upto 2.0 ml with 4 per cent TCA. Added 0.5 ml of 2 per cent DNPH (2,4-dinitrophenyl hydrazine in 9 N H<sub>2</sub>SO<sub>4</sub>) reagent to all the tubes, followed by 2 drops of 10 per cent thiourea solution. Incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5 ml of 85 per cent sulphuric acid, in cold, drop by drop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540 nm. Calculated the content of ascorbic acid in the sample using the standard graph.

##### Estimation of $\alpha$ -tocopherol<sup>13</sup>

The samples were weighed (2.5 g) and added 50 ml of 0.1N sulphuric acid slowly without shaking. Stopped and allowed to stand overnight. The next day, the contents of the flask were shaken vigorously and filtered through Whatman No.1 filter paper, discarding the initial



10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation. Into three stoppered centrifuge tubes (test, standard and blank) pipetted out 1.5 ml of extract, 1.5 ml of the standard (D, L- $\alpha$ -tocopherol 10 mg/L in absolute alcohol, 91 mg of  $\alpha$ -tocopherol is equivalent to 100 mg of tocopherol acetate) and 1.5 ml of water respectively. To the test and blank added 1.5 ml of ethanol and to the standard, added 1.5 ml of water. Added 1.5 ml of xylene to all the tubes, stoppered, mixed well and centrifuged. Transferred 1.0 ml of xylene layer into another stoppered tube. Added 1.0 ml of 2, 2'-dipyridyl reagent (1.2 g/L n-propanol) to each tube, stoppered and mixed. Pipetted out 1.5 ml of the mixture into spectrophotometer cuvettes and read the extinction of test and standard against the blank at 460 nm.

Then, in turn, beginning with the blank, added 0.33 ml of ferric chloride solution. Mixed well and exactly after 15 minutes read the test and standard against the blank at 520 nm. The amount of vitamin E can be calculated using the formula:

$$\text{Amount of } \alpha \text{ tocopherol} = \frac{\text{Reading at 520nm} - \text{Reading at 460nm}}{\text{Reading of standard at 520nm} \times 0.29 \times 15}$$

#### Estimation of Total Carotenoids<sup>14</sup>

Weighed 5-10 g of the sample. Saponified for about 30 minutes in a shaking water bath at 37°C after extracting the sample in 12 percent alcoholic KOH. Transferred the saponified extract into a separating funnel (packed with glass wool and CaCO<sub>3</sub> containing 10 to 15 ml of petroleum ether) and mixed gently. Taken up the carotenoid pigments into the petroleum ether layer. Transferred the lower aqueous phase to another separating funnel, and the petroleum ether extract containing the carotenoid pigments to an amber coloured bottle.

Repeated the extraction of the aqueous phase similarly with petroleum ether until it is colourless. Discarded the aqueous phase. To the petroleum ether extract added a small quantity of anhydrous Na<sub>2</sub>SO<sub>4</sub> to remove turbidity. Noted the final volume of petroleum ether extract, and dilute if needed by a known dilution factor. The absorbance of the extract at 450 nm and 503 nm was noted in a spectrophotometer.

$$\text{Amount of total carotenoid present} = \frac{P \times 4 \times V \times 100}{W}$$

P = Optical density of the sample

V = Volume of the sample

W = Weight of the sample

#### Estimation of Reduced Glutathione<sup>15</sup>

One gram of the sample was homogenized in 5 per cent TCA to give a 20 per cent homogenate. The precipitated protein was centrifuged at 1000 rpm for 10 minutes. The homogenate was cooled on ice and 0.1 ml of the supernatant was taken for the estimation. The volume of the aliquot was made upto 1.0 ml with 0.2M sodium phosphate buffer (pH 8.0). 2 ml of freshly prepared DTNB

solution (0.6 mM in 0.2 M phosphate buffer, pH 8.0) was added to the tubes and the intensity of the yellow colour formed was read at 412 nm in a spectrophotometer after 10 minutes. The standards (2-10 nmoles GSH in 1.0 ml of 5 per cent TCA) were also treated in a similar manner.

#### Estimation of Flavonoids<sup>16</sup>

A portion of the sample was weighed out and the extraction was carried out in two steps, first, with methanol: H<sub>2</sub>O (9:1) and second with methanol: H<sub>2</sub>O (1:1).

At each step, sufficient solvent was added to make liquid slurry and the mixture was left for 6-12 hrs. Filtration to separate the extract from the sample was carried out rapidly by using a glass wool or cotton wool plug in the neck of a filter funnel. The two extracts were then combined and evaporated to about 1/3 the original volume or until most of the methanol had been removed.

The resultant aqueous extract was cleared off the contaminants such as fats, terpenes, chlorophylls and xanthophylls by extraction (in a separatory funnel) with hexane or chloroform. This was repeated several times and the extracts combined.

The solvent-extracted aqueous layer containing the bulk of the flavonoids was then concentrated. An aliquot of the extract was pipetted into a test tube and evaporated to dryness. Then added 4.0 ml of vanillin reagent (1 per cent vanillin in 70 per cent H<sub>2</sub>SO<sub>4</sub>) and heated for 15 minutes in a boiling water bath.

The standard (catechin 20-100  $\mu$ g) was also treated in the same manner. Then the absorbance was measured at 360 nm.

#### Estimation of Tannins<sup>17</sup>

Tannins are important polyphenolic compounds that act as antioxidants. Weighed 0.5 g of the sample in a 250 ml conical flask and added 75 ml water. Heated the flask gently and boiled for 30 min. Centrifuged at 2000 rpm for 20 min and collected the supernatant in 100 ml volumetric flask and made up the volume.

Transferred 1.0 ml of the sample extract to a 100 ml volumetric flask containing 75 ml alcohol. Added 5.0 ml of commercially available Folin-Denis reagent, 10 ml of 35 per cent sodium carbonate and diluted to 100 ml with water. Mixed well and read the absorbance at 700 nm after 30 minutes. The entire procedure was followed with a blank (water) and standards (20-100  $\mu$ g tannic acid).

## RESULTS AND DISCUSSION

### Activities of Enzymic antioxidants in the leaves and seeds of *Boerhavia diffusa*

The enzymic antioxidants such as catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione-S-transferase and glutathione peroxidase were assessed in the leaves and seeds of *Boerhavia diffusa* and their values are given in Table 1.



**Table 1:** Enzymic antioxidant in *Boerhavia diffusa*

S. No.	Enzymic antioxidants	Activity U/g		
		Leaves	Seeds	t – Test
1	Catalase <sup>1</sup>	131.9 ± 3.3	132.8 ± 4.2	0.238 <sup>ns</sup>
2	Peroxidase <sup>2</sup>	0.44 ± 0.06	0.68 ± 0.08	3.39**
3	Superoxide dismutase <sup>3</sup>	16.6 ± 0.12	10.0 ± 0.5	18.15**
4	Polyphenol oxidase	1.75 ± 0.18	0.17 ± 0.09	11.06**
5	Glutathione-S-transferase <sup>4</sup>	0.85 ± 0.16	0.60 ± 0.13	1.72*
6	Glutathione peroxidase (nmoles)	669 ± 1.23	624.1 ± 1.36	34.63**

Values are mean ± SD of six replicates

1. Amount of enzyme that brings about decrease in absorbance of 0.05 at 240nm.
  2. Change in absorbance/min/ g Sample
  3. Amount of SOD that cause 50% reduction in the extent of NBT oxidation
  4. Millimoles of CDNB-GSH conjugates/ min/ g sample
- \* - Significant at 5% level  
\*\* - Significant at 1% level  
ns – not significant

It is evident from Table 1 that both the leaves and seeds of *Boerhavia diffusa* possess considerable antioxidant activity. The activities of catalase, peroxidase, superoxide dismutase, polyphenol oxidase, glutathione-S-transferase and glutathione peroxidase (nmoles) were found to be 131.9 ± 3.28, 0.44 ± 0.06, 16.6 ± 0.12, 1.75 ± 0.18, 0.85 ± 0.16 and 669 ± 1.23 U/g of protein in the leaves and 132.8 ± 4.21, 0.68 ± 0.08, 10.0 ± 0.5, 0.17 ± 0.09, 0.60 ± 0.13 and 624 ± 1.36 U/g protein in the seeds of *Boerhavia diffusa*.

The enzyme catalase located in the peroxisomes converts H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub>. Devi<sup>18</sup> showed that the leaves of *Trichodesma indicum* was found to possess significant (p<0.05) catalase activity (117.5 ± 4.1 U/g) when compared to the leaves of *Cleome viscosa* (97 ± 0.7 U/g). Peroxidases refer to heme containing enzymes which are able to oxidise organic and inorganic compounds using hydrogen peroxide as co-substrate.

Das<sup>19</sup> have also reported that the fruits of *Terminalia bellirica* showed the highest peroxidase activity (0.2 ± 0.007 U/g) when compared to that of the leaves (0.1 ± 0.02 U/g).

Superoxide dismutase mainly act by quenching of superoxide (O<sub>2</sub><sup>-</sup>) to prevent inactivation of nitrous oxide by super oxide.

Rachh<sup>20</sup> proved that the leaf extract of *Andrographis paniculata* exerted highest superoxide activity (80.4) than the other antioxidants scavenged.

Polyphenol oxidase is a stress marker enzyme, produced as protective measure against diseases.

Similar results was also exhibited by Khatun<sup>21</sup> showed significantly high levels of polyphenol oxidase (P < 0.05) in the leaves of *Coleus forskohlii* Briq. (12.57 min/g dry tissue) than the stem.

### Activities of non-enzymic antioxidants in the leaves and seeds of *Boerhavia diffusa*

Apart from enzymic antioxidants, the non-enzymic antioxidants such as ascorbic acid, α-tocopherol, carotenoids, reduced glutathione, flavonoids and tannins are important in cellular system to curtail the reactive oxygen species. Table 2 depicts the levels of the non-enzymic antioxidants in the leaves and seeds of *Boerhavia diffusa*.

**Table 2:** Non-enzymic antioxidants in *Boerhavia diffusa*

S. No.	Non Enzymic antioxidants	Quantity per mg/g		
		Leaves	Seeds	t – Test
1	Ascorbic acid	0.28 ± 0.09	0.51 ± 0.11	2.29*
2	α-tocopherol	1.04 ± 0.12	0.51 ± 0.1	4.74**
3	Carotenoids	4.44 ± 0.13	4.08 ± 0.08	3.34**
4	Reduced glutathione (nmoles/g)	0.56 ± 0.13	0.90 ± 0.09	3.06**
5	Flavonoids	1.0 ± 0.1	1.2 ± 0.05	2.53*
6	Tannins	2.9 ± 0.08	3.42 ± 0.13	4.97**

\* - Significant at 5% level

\*\* - Significant at 1% level

The activities of non-enzymic antioxidants, ascorbic acid, α-tocopherol, carotenoids, reduced glutathione, flavonoids and tannins were found to be present in appreciable amounts in the leaves as well as the seeds of *Boerhavia diffusa*.

Ascorbic acid a water soluble non enzymic antioxidant is widely used as an alternative to synthetic antioxidants. Iqbal<sup>22</sup> showed a significant difference in the ascorbic acid content in the three different mulberry varieties. α-tocopherol a chain breaking antioxidant helps to minimize the consequences of lipid peroxidation in membranes. Ogunles<sup>23</sup> have reported that the α-tocopherol content in leaves of *Achyranthes aspera* was found to be moderate 0.40 ± 0.09 mg/g when compared to the other antioxidants. Abiramasundari<sup>24</sup> also showed that the leaves of *Aerva lanata* possessed moderate levels of reduced glutathione 0.10 ± 0.001mg/g. Flavonoids are phenolic compounds, present in several plants, inhibits lipid peroxidation and lipoxygenases. Patel<sup>5</sup> has reported that the leaves of *Hibiscus* (1.27mg/g) has exerted high levels of flavonoid content.

Our results were similar to that of Meena<sup>25</sup> (2012) who recorded a high content of tannins (2.52%) in the leaves of *Baccopa monnieri* when compared to that of *Centella asiatica* (1.91%).

### CONCLUSION

The findings of the present study clearly reveals the antioxidant properties of leaves and seeds of *Boerhavia diffusa*.

The levels of antioxidants were present in moderate amounts in both the leaves and seeds of *Boerhavia diffusa* and therefore could be used for the treatment of disorders caused due to oxidative damage.



## REFERENCES

1. Kaushik P, Kaushik D, Khokra SL. *In vivo* antioxidant activity of plant *Abutilon indicum*. J Pharm Educ Res, 2(1), 2011, 50-53.
2. Adebayo AH, Abolaji AO, Kela R, Ayepola OO, Olorunfemi TB, Taiwo OS. Antioxidant activities of the leaves of *Chrysophyllum albidum*. Pak J Pharm Sci, 24(4), 2011, 545-551.
3. Kratchanova M, Denev P, Ciz M, Lojek A, Mihailov A. Evaluation of antioxidant activity of medicinal plants containing. Biochimica Polonica, 57(2), 2010, 229–234.
4. Gomes AD, Vaidya VV, Bhagat RD, Gadgil JN. Separation and quantification of pharmacologically active markers Boeravinone B, Eupalitin-3-O- $\beta$ -D-galactopyranoside and  $\beta$ -sitosterol from *Boerhavia diffusa* Linn. and from marketed formulation. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3), 2013, 953-956.
5. Patel VR, Patel PR, Kajal SS. Antioxidant activity of some selected medicinal plants in western region of India. Advances in Biological Research, 4(1), 2010, 23-26.
6. Luck H. Methods of enzymatic analysis. Academic press, 1974, 885-894.
7. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyl adenine on dark-treated growing rice leaves, II changes in peroxidase activity. Plant cell Physiology, 24, 1995, 987-994.
8. Misra MP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem, 247, 1972, 31-70.
9. Habig WH, Pabst MJ, Jakoby W. The first enzymatic step in mercapturic acid IV formation. J Biol Chem, 249, 1974, 7130-7139.
10. Esterbauer H, Schwartz Z, Hayan M. A rapid assay for catechol oxidase and laccase using 2-nitro-5 thio benzoic acid. Anal Biochem, 489, 1977, 489-494.
11. David M, Richard JS. In methods of enzymatic analysis – 3 (Ed. Bergmeyer J. and Marianna Gra B). Verlag Chemie Wein Hein Dein Field, Beach Florida, Basel, 1983, 358.
12. Roe JH, Kuether A. The determination of ascorbic acid in whole blood and urine through 2, 4-dinitro phenyl hydrazine derivative of dehydroascorbic acid. J Biol Chem, 147, 1953, 399-404.
13. Rosenberg HR., 1992. Chemistry and Physiology of the vitamins. Inter Science Publishers, Inc. New York, 1992, 452-453.
14. Zakaria H, Simpson K, Brown PR, Krotulovic A. Use of reversed phase HPLC analysis for the determination of provitamin A, Carotenes in tomatoes. J Chromatography, 176, 1979, 109-117.
15. Moron MS, Bepierre JW, Mannerwick B. Levels of glutathione reductase and glutathione-S-transferase in rat lung and liver. Biochem Biophys Acta, 1979, 582.
16. Cameron GR, Milton RF, Allen JW. Estimation of flavonoids, Lancet, 1943, 179.
17. Schanderl SH. Methods in food analysis, Edition: Academic Press, New York, 1970, 709.
18. Devi GS, Sangeetha S, Das. MSC. Comparative evaluation of phytochemicals and antioxidant potential of *Cleome viscosa* and *Trichodesma indicum*. Int J Pharm Sci Rev Res, 23(1), 2013, 253-258.
19. Das MSC, Manila TN, Devi GS. Evaluation of antioxidant and *in vitro* free radical scavenging activity of *Terminalia bellirica*. Turning plants into medicines Novel Approaches, New India publishing agency, New Delhi, 2013, 59-78.
20. Rachh PR, Patel SR, Hirpar HV, Rupareliya MT, Rachh MR, Bhargava AS, Patel NM, Rom DCM. *In vitro* evaluation of antioxidant activity of *Gymnema sylvestre* R. Br. leaf extract. J Biol Plant Biol, 54(2), 2011, 141–148.
21. Khatun S, Chatterjee NC, Cakilcioglu U. Antioxidant activity of the medicinal plant *Coleus forskohlii* Briq. African Journal of Biotechnology, 10(13), 2011, 2530-2535.
22. Iqbal S, Younas U, Sirajuddin, Chan KW, Sarfraz RA, Uddin Md. K. Proximate composition and antioxidant potential of leaves from three varieties of mulberry (*morus* sp.): a comparative study. Int J Mol Sci, 13, 2012, 6651-6664.
23. Ogunles M, Okiei W, Azeez V, Obakachi V, Osunsanmi M, Nkenchor G. Vitamin C contents of tropical vegetables and food determined by volumetric and titrimetric methods and their relevance to the medicinal uses of the plants. Int J Electrochem Sci, 5, 2010, 105-115.
24. Abiramasundari P, Priya V, Devi GS. Antioxidant potential and *in vitro* free radical scavenging activity of *Aerva lanata*. Trop Med Plants, 12 (1), 2011, 1511-1525.
25. Meena H, pandey HK, Pandey P, Arya MC, Ahmed Z. Evaluation of antioxidant activity of two important memory enhancing medicinal plants *Baccopa monnieri* and *Centella asiatica*. Indian Journal of Pharmacology, 44(1), 2012, 114-117.

Source of Support: Nil, Conflict of Interest: None.

