Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 1, 2014

Research Article

PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY IN *BOERHAVIA DIFFUSA*

RICHA BHARDWAJ*, ANKITA YADAV AND RA SHARMA

[1]Plant Physiology and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.
Email: niki86ster@gmail.com

Received: 05 Oct 2013, Revised and Accepted: 28 Oct 2013

ABSTRACT

Objective: The study aims to investigate the methanolic extract of different plant parts (leaves, stems, and roots) of *Boerhavia diffusa* L. (family Nyctaginaceae), for their antioxidant activity and were used for quantitative estimation of the total phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total flavonoid as Quericitin Equivalent (QE)) per gram dry weight, ascorbic acid content and Antioxidant potential of the plant was also evaluated.

Methods: The methanol extract of the leaves, stem, and root of *Boerhavia diffusa* were estimated for total antioxidant capacity using 1,1-diphenyl picrylhydrazyl (DPPH) free radical scavenging assay, total phenolic, flavonoid and ascorbic acid contents using spectrophotometric methods.

Results: Maximum Total Phenolic content was recorded in Leaves (24.5±1.703)mgGAE/gdw. Also Maximum Total Flavonoid content was found in Leaves (79.86±3.757)mQE/gdw). Maximum Ascorbic Acid content was found in Leaves (0.333±.166)mg/gdw while minimum in Roots (0.026±0.002)mg/gdw and Highest radical scavenging activity is observed in stems with IC_{50} value 90.8±2.275.

Conclusion: It signifies that the Plant-derived phenolics and flavonoids represents good sources of natural antioxidants, From the above results it seen that this plant exhibits significant antioxidant activity.

Keywords: DPPH *radical scavenging assay*, Ascorbic acid, TFC, TPC.

INTRODUCTION

Boerhaavia diffusa L. (Nyctaginaceae), a species of flowering plant in the four o'clock family commonly known as 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the waste land of India. The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice. Genus *Boerhaavia*, consisting of 40 species is distributed in tropical and subtropical regions and warm climate. It is found in Ceylon, Australia, Sudan and Malay Peninsula, extending to China, Africa, America and Islands of the Pacific. Among 40 species of Boerhaavia, 6 species are found in India, namely *B. diffusa, B.erecta, B. rependa, B. chinensis, B. hirsute* and *B. rubicunda*. *Boerhaavia diffusa* in India is found in warmer parts of the Scientific Name: *Boerhaavia diffusa* Linn. Syn. *B. repens; B. repens* Family: Nyctaginaceae, Family Name: Hog weed, Horse Purslane, Common Indian Names Gujarati: Dholia-saturdo, Moto-satoda. Hindi: Snathikari Kannada: Kommegida Marathi: Tambadivasu Sanskrit:Punarnava, Raktakanda, Shothaghni, Varshabhu Bengali: Punurnava Tamil: Mukaratee-Kirei Telugu: Punernava,Habitat: Grows as common weed., Useful Parts: Root, leaves and seeds**[1].**

Oxygen-free radicals, more generally known as reactive oxygen species (ROS) along with reactive nitrogen species (RNS) are well recognised for playing a dual role as both deleterious and beneficial species **[2].** ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. Oxidative stress contributes to many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthmal **[3].** Phenolic antioxidants inhibit lipid peroxidation by trapping the lipid alkoxyl radical. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules **[4].** During heavy metal stress phenolic compounds can act as metal chelators and on the other hand phenolics can directly scavenge molecular species of active oxygen **[5].** Zn is an essential component of numerous proteins involved in the defense against oxidative stress **[6].** Ascorbic acid is the principal biologically active form but *L*-dehydroascorbic acid, an oxidation product, It functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals. As an antioxidant, it reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer **[7].** Studies have shown the involvement of Ascorbic Acid in the regulation of transcription and/ or stabilisation of specific mRNAs **[8].** Pretreatment with aqueous and ethanolic extracts decreased the activities of alkaline phosphatase, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and the level of bilirubin in the serum elevated by acetaminophen. The extracts also protected against acetaminophen induced lipid peroxidation **[9].**

MATERIALS AND METHODS

Plant material

The different plant parts (leaves,stems and roots) of *Boerhavia diffusa* were collected in month of october-december from university of rajasthan campus. It was washed with tap water , dried at room temperature and ground to fine powder. The species specimen was submitted to herbarium, department of botany, university of rajasthan. Jaipur, rajasthan, india and got the voucher specimen no. **RUBL211299**

Chemicals

All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Total Phenolic and Flavanoidal Content

Plant Extraction

2gm each of the dry material (leaves, stems and roots) was extracted with 25ml of methanol at room temperature for 48 hours, filtered through Whatman paper no 1 filter paper, stored and used for quantification.

Total Phenolic Content

Total phenolic compound contents were determined by the Folin-Ciocalteau method**[10-13]**.The extract samples (0.5 ml; 1;10 diluted) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were

then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ ml solutions of Gallic acid in methanol. Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound. Total phenolic content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total Phenolic concentration

C= Concentration of gallic acid from caliberation curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

Total Flavanoidal Content

Total flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method **[14,15]**, with slight modifications using quercetin as standard. 1ml of test material was added to 10ml volumetric flask containing 4ml of water. To above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5mins 0.3ml of 10% AlCl3 was added. After 6min, 2ml of 1M NaOH was added and the total volume was made upto 10ml with distilled water. Then the solutions were mixed well and absorbance was measured against blank at 510nm. Total flavanoidal content of the extracts was expressed in milligram of quercetin equivalents/gdw. Total flavanoidal content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total flavanoidal concentration

C= Concentration of gallic acid from caliberation curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

Ascorbic acid Content

Extraction procedure

Each of the fresh experiment materials (400mg) was homogenize thoroughly with 10 ml of acetate buffer (pH 4.8) and centrifuged (1200 rpm, 20 min.). The supernatants were separately collected, out of which 1 ml was measured to other test tube, 4 ml of 4% trichloroacetic acid (TCA) was added, left overnight and later, centrifuged **[16].** To the supernatant of each sample, 1 ml of the colour reagent (prepared by mixing 90 ml of 2.2%, 2,4-dinitrophenylhydrazine in 10N H_2SO_4, 5 ml of 5% thiourea and 5 ml of 0.6% $CuSO_4$ solution), was added and incubated at 57% for 45 min. Later, on cooling 7 ml of 65% H_2SO_4 was added to each mixture and cooled again.

Quantification

From the stock solution of ascorbic acid (10 mg/100 ml in 4% TCA), varied concentrations (0.01 to 0.09 mg/ml) were prepared in different test tube. The volume of each was raised to 5 ml by adding 4% TCA solution and left overnight at the room temperature. To these, 1 ml of the color reagent added. Later, to each of these, 7 ml of 65% H_2SO_4 was added, brought to the room temperature and the ODs were measured at 540 nm in a spectrophotometer against a blank. A regression curve was computed between the main optical density and the concentration of standard ascorbic acid, which followed Beer's Law.

DPPH Radical Scavenging Activity

Plant extraction

10gm each of the plant material was soxhlet extracted with methanol for 24hours. The extract was filtered with Whatman filter paper no 1 and the crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40–50°C). The extract was preserved in vacuum desiccators for subsequent use in antioxidant assay.

Assay

The antioxidant activities were determined using 1, 1,diphenyl-2-picryhydrazyl (DPPH) as a free radical. Experiments were initiated by preparing a 0.25mM solution of DPPH and 1mg/ml solution of different plant parts extracts (stock) in methanol was prepared. To the methanolic solutions of DPPH an equal volume of the extract dissolved in methanol was added at various concentrations. An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as a control. Experiment was performed in triplicate **[17,18,19].** A control reaction was carried out without the test sample. Absorbance values were corrected for radicals decay using blank solution. The inhibitory effect of DPPH was calculated according to the following formula:

Linear graph of concentration Vs percentage inhibition was prepared IC_{50} values was calculated:

$$\% \text{ Inhibition} = [1 - (Abs_{SAMPLE} / Abs_{CONTROL})] \times 100$$

The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve [19-21].

Statistical analysis: Experimental results are expressed as means ± standard deviation (SD). All measurements were replicated three times. IC_{50} values were also calculated by linear regression analysis. Experiments results were further analyzed for Pearson correlation coefficient(r) between total phenolic, flavanoid and DPPH radical scavenging assay using the Microsoft Excel 2007 software and two way analysis of variance (ANOVA) was applied to investigate the differences among means by using Microsoft excel 2007 software. The values were considered to be significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Screening of methanolic extracts of different parts (leaves, stems, and roots) of *B.diffusa* were used for quantitative estimation of the total phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total flavonoid as Querctin Equivalent (QE)) per gram dry weight . maximum total phenolic content was recorded in leaves (24.5±1.703)mgGAE/gdw whereas lowest total phenolic content was recorded in roots (7 ±0.946) 125 mgGAE/gdw. Maximum total flavonoid content was recorded in leaves (79.86±3.757)mgQE/gdw, whereas lowest in roots(7.08±0.36) mgQE/gdw, also maximum ascorbic acid content is seen in leaves (0.333±0.166) and minimum is seen in roots (0.026±0.002) (Table 1).

Table 1: Total phenolic, flavonoidal and ascorbic acid content in different plant parts of *B. diffusa.*

Plant Part	Total Phenolic Content (mg GAE/gdw)	Total Flavonoidal Content (mg QE/gdw)	Ascorbic acid content
Leaves	24.5±1.703	79.86±3.757	0.333±0.166
Stem	7.77±0.780	39.375±1.653	0.10±0.025
Root	0.25±0.005	7.08±0.36	0.026±0.002

Antioxidant activity of methanolic extracts of different plant parts was measured by the using DPPH free radical scavenging assay. IC_{50} values of different plant parts are shown in Table 2. While graphs for %inhibition at different concentrations of different plant parts are shown in (Figure1-4). Antioxidant acitivity decreases in the order stem>leaves> roots. Stems show highest antioxidant acitivity (90.8±2.275) while roots show lowest antioxidant activity (398.03±4.351).

Table 2: The IC_{50} values of different plant parts of *B.diffusa* of DPPH radical scavenging assay

Plant part	IC_{50} values (µg/ml)
Leaves	195.25±4.487
Stem	90.8±2.275
Root	398.03±4.351

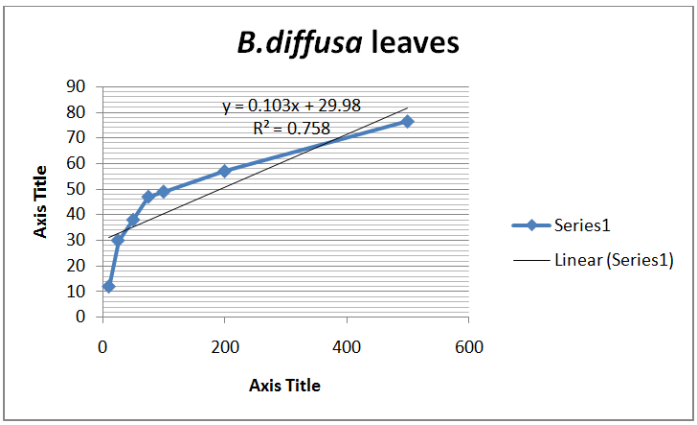

Fig. 1: DPPH Scavenger Assay of the methanol extract of leaves of *B.diffusa*

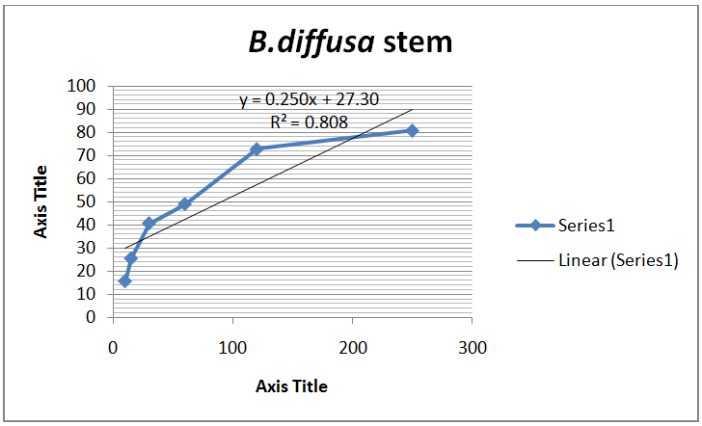

Fig. 2: DPPH Scavenger Assay of the methanol extract of stems of *B.diffusa.*

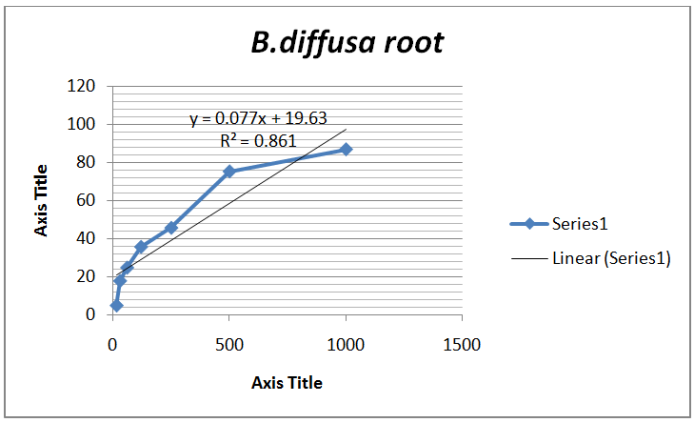

Fig. 3: DPPH Scavenger Assay of the methanol extract of roots of *B.diffusa*

Antioxidant activity is also correlated to the total phenolic, flavonoidal and ascorbic acid content of the plant for this we performed linear regression and correlation analysis of the values of the DPPH with TPC, TFC and ascorbic acid (Table 3).

Table 3: Correlation between IC_{50} values of DPPH assay, phenolic, flavonoid and ascorbic acid content of *B.diffusa*

Plant part	Phenols	Flavonoids	Ascorbic acid
Leaves	-0.7476	0.729	0.460
Stem	-0.999	-0.188	0.999
Root	0.721	0.960	0.9605

These analysis reveal that there is a positive correlation between IC_{50} and TPC of root while this is negative for leaves and stem. There is positive correlation between IC_{50} and TFC for leaves and root while negative correlation for stems. Also all the three plant parts leaves stem and roots show a positive correlation with the IC_{50} and ascorbic acid content. The high antioxidant activity in stems might be due to presence of high phenolic content. The medicinal actions of phenolics is mostly ascribed to their antioxidant capacity, free radical scavenging, chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways. The free radical scavenging and antioxidant activities of phenolics are dependent upon the arrangement of functional groups about the nuclear structure. Both the number and configuration of H-donating hydroxyl groups are the main structural features influencing the antioxidant capacity of phenolics **[20,21].**

1 way ANNOVA analysis for all the plant parts was also done (TABLE 4a, 4b, 4c) with taking into account only the total phenol, flavonoid and ascorbic acid content. With the above one way analysis it seen that for all plant parts leaves stem and roots. Fvalue > Fcirt , therefore with this ANNOVA analysis it is said that content of total phenols, flavonoids, and ascorbic acid is not similar that is their contents vary largely, in all different plant parts which is hence statistically proved.

Table 4a: ANOVA for leaves

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	9971.171	2	4985.585	877.1086	3.96E-08	5.143253
Within Groups	34.10468	6	5.684114			
Total	10005.28	8				

Table 4b: ANOVA for stem

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	2600.231	2	1300.115	1166.476	1.69E-08	5.1432528
Within Groups	6.6874	6	1.114567			
Total	2606.918	8				

Table 4c: ANOVA for roots

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	96.47592	2	48.23796	1108.972	1.96E-08	5.1432528
Within Groups	0.260988	6	0.043498			
Total	96.73691	8				

2 way ANNOVA analysis from Table 5 shows that statistically there is no significant difference between rows i.e. the content of phenols, flavonoids, ascorbic acid and DPPH free radical scavenging activity of plant parts is similar, as from table (F value < F crit), that is Fvalue of rows is acceptable. But when columns are taken into account there is a very significant difference between them i.e. content, the content of phenols and flavonoids and ascorbic acid DPPH free radical scavenging activity of different plant parts vary as F value > F crit for columns.

Table 5: ANNOVA analysis for DPPH, phenolic, flavonoidal and ascorbic acid content of *B.diffusa*.

Source of variation	Ss	Df	MS	F	P-value	Fcrit
Rows	9071.709	2	4535.854	0.637309	0.561078	5.143253
Coumns	102394.6	3	34131.53	4.795641	0.049194	4.757063
Error	42703.19	6	7117.198			
Total	154169.5	11				

Rows and columns for ANNOVA analysis

Plant part	TPC	TFC	Ascorbic acid	IC50
Leaves	24.5±1.703	79.86±3.757	0.333±0.166	195.25±4.487
Stem	7.77±0.780	39.375±1.653	0.10±0.025	90.8±2.275
Root	0.25±0.005	7.08±0.36	0.026±0.002	398.03±4.351

Also the positive correlation is seen between TFC and IC_{50} this reveals that antioxidant properties are also due to flavonoids as Flavonoids are an antioxidant group of compounds composed of flavonols, flavanols, anthocyanins, isoflavonoids, flavanones and flavones. All these sub-groups of compounds share the same diphenylpropane (C6C3C6) skeleton. The antioxidant properties are conferred on flavonoids by the phenolic hydroxyl groups attached to ring structures and they can act as reducing agents**[22]**

Due to presence of phenols and flavonoids and their correlation with TPC and TFC these studies suggest that phenols and flavonoids can be used to develop the novel phenolic synthetic antioxidants aimed

at retarding the effects of free radicals and oxidants**.** the antioxidant activity is also seen to be directly related to the content of ascorbic acid and total phenolics **[23,24].** The antioxidants are known to mediate their effect by plant extracts are quite safe and their toxicity is a not directly reacting with ROS, quenching them, they could be chelating the catalytic metal ions. Antioxidant activity of certain compounds have also been correlated to the amount of l-ascorbic acid in the medium **[25]** the phenolic antioxidants protect vitamin C against oxidative decomposition **[26]**, certain other compounds such as betacyanins have shown to have good antioxidant activity as ascorbic acid **[27],** also different varieties of mangoes also have shown to have a good antioxidant activity mangoe leaves had the highest DPPH scavenging capacities and ethanolic extract of arumanis mangoe had the highest ABTS scavenging activities. The positively high correlation between total phenolic content with DPPH and ABTS scavenging activities were given by four varieties mangoes **[28].**

CONCLUSION

The total phenolic, flavanoidal and ascorbic acid content and antioxidant activity of different plant parts was measured in the methanol extract of *B.diffusa.* All these vary significantly in different plant parts. Significant correlations was found between the antioxidant activities and total phenolic, flavanoid and ascorbic acid contents indicating that these phytocompounds are the major contributors of antioxidant capacities of this plant. The results of the present study suggests that *B.diffusa* contained potential antioxidant bioactive compounds, which if properly and extensively studied could provide source of biologically active drug candidates and it also shows its great importance as therapeutic agent in preventing or curing the diseases caused due to oxidative stress.

REFERENCES

1. Oudhia P. Traditional medicinal knowledge about useful herb Punarnava (*Boerhaavia diffusa*, family (Nyctaginaceae) in Chhattisgarh Plains, India, Available at: http://botanical.com/site/column_poudhia/138_punarnava.html.2011.
2. 2.Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160(1):1-40.
3. 3. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J 2012;5(1):9-19.
4. Milic B.L., Djilas S.M., Canadanovic-Brunet J.M. Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. Food Chem 1998;61: 443.
5. Michalak. Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal Stress. Polish J. of Environ. Stud 2006;15(4):523-530.
6. Jomova K, Valko M.(2011). Advances in metal-induced oxidative stress and human disease. Toxicology 2011;283(2-3):65-87.
7. Rekha C, Poornima G, Manasa M, Abhipsa V, Devi P, Kumar HTV and Kekuda TRP. Ascorbic Acid, Total Phenol Content and Antioxidant Activity of Fresh Juices of Four Ripe and Unripe Citrus Fruits. Chem Sci Trans 2012;1(2):303-310.
8. Arrigoni Oreste, De Tullio Mario C. Ascorbic acid: much more than just an antioxidant. Biochimica et Biophysica Acta 2002;1569:1-9.
9. Olaleye M T, Akinmoladun A C, Ogunboye A A, Akindahunsi A A. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* Linn against acetaminophen-induced liver damage in rats. Food and Chemical Toxicology 2010;48(8–9):2200–2205.
10. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem 2001;73:73-84.
11. Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR. Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol 2008a;32:43-49.
12. Ebrahimzadeh MA, Pourmorad F and Hafezi S. Antioxidant activities of Iranian corn silk. Turk. J.Biol 2008b;32:43-49.
13. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A and Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of Parrotia persica Mey. Pharmacologyonline 2008a;2:560-567.
14. Dewanto V, Wu X, Adom KK, and Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of agricultural and food chemistry 2002;50:3010-3014.
15. Sakanaka S, Tachibana Y, Okada Y. Prepation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food chemistry 2005; 89:569-575.
16. Roe, J.H. Kuenther, C.A. The determination of ascorbic acid in whole blood and urine through the 2,4-nitrophenylhydrazine derivative. J. Biol. Chem 1943;147:399-407.
17. John D. One hundred useful drugs of the Kani tribes of Trivandum forest divisions, Kerala, India. Inter. J. Crude Drug Res 1984;22:17-39.
18. Sreejayan N and Rao MNA. Free radical scavenging activity of curcuminoids. Drug Res 1996;46:169-178.
19. HSU CY. (2006). Antioxidant activity of extract from *Polygonum aviculare* L. Biol Res 2006; 39:281-288.
20. Fejes S, Blzovics A, Lugasi A, Lemberkovics E, Petri G, Kry A. In vitro antioxidant activity of *Anthriscus cerefolium* L.(Hoffm.) extracts. J Ethanopharmacol 2000;69:259-265.
21. Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI, Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radicals. Biochem pharmacol 1998;56:213-222.
22. Carocho M, Isabel C.F.R. Ferreira. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology 2013;51:15–25.
23. Fındık E, Ceylan M, Elmastas M (2011). Isoeugenol-based novel potent antioxidants: Synthesis and reactivity. European Journal of Medicinal Chemistry 2011;46:4618-4624.
24. Khatri D K, Juvekar P, Juvekar A R. Phytochemical investigation and *in vitro* antioxidant activities *Indigofera cordifolia* seed extracts. International Journal of Pharmacy and Pharmaceutical Sciences 2013;5(2):71-75.
25. Arnao M B, Cano A, Hernández-Ruiz J, García-Cánovas F, Acosta M. Inhibition byl-Ascorbic Acid and Other Antioxidants of the 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic Acid) Oxidation Catalyzed by Peroxidase: A New Approach for Determining Total Antioxidant Status of Foods. Analytical Biochemistry 1996;236(2):255–26.
26. Nicholas J. Miller, Catherine A. Rice-Evans. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chemistry 1997;60(3):331-337.
27. Napoleon A, Swetha S K, Angajala G. In-vitro anti oxidant and anti bacterial studies of betacyanin isolated from the bracts of *Bougainvillea glabra*. International Journal of Pharmacy and Pharmaceutical Sciences 2013;5(4):84-87.
28. Fidrianny I, Rahmiyani I, Wirasutisna K R. Antioxidant capacities from various leaves extracts of four varieties mangoes using DPPH, ABTS assays and correlation with Total Phenolic, Flavonoid, Carotenoid. International Journal of Pharmacy and Pharmaceutical Sciences 2013;5(4):189-194.