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Original Article

Evaluation of the *in vitro* anti-oxidant activity of *Alternanthera brasiliana* leaves

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ABSTRACT

Background and aim: *Alternanthera brasiliana* belongs to the family, Amaranthaceae and is popularly known as Brazilian joyweed. It is a medicinal plant famous for its therapeutic effects in Brazil, South Africa and Nigeria amongst other countries. In the present study, the ethanol extract of the leaves of *A. brasiliana* was evaluated for its potential anti-oxidant activity.

Methods: This was carried out by determining the concentration of total phenols in the extract as well as using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, iron (II)-chelating, nitric oxide radical-scavenging, ferrous sulphate and carbon tetrachloride-induced lipid peroxidation assays.

Results: The results show that the concentration of total phenols in the extract was 0.031 ± 0.006 μg/ml of the extract. In addition, the percentage inhibition of DPPH radical exhibited by the increasing concentrations of the extract, iron (II)-chelating and nitric oxide radical-scavenging activities (in percent), percentage inhibitions of ferrous sulphate and carbon tetrachloride-induced lipid peroxidation by the extract ranged from 96.29% to 99.59%, 51.43% to 78.78%, 53.43% to 94.85%, 25.00% to 37.90% and 96.26% to 99.50% respectively. Results of the assays were comparable to those of the standard anti-oxidant (ascorbic acid).

Conclusion: The above data provide evidences that the ethanol extract of the leaves of *A. brasiliana* is rich in natural anti-oxidants and thus justify its use in folk medicine especially in the management of free radical-mediated disorders.

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1. Introduction

Medicinal plants are important sources of the therapeutic remedies of various diseases. World wide since ancient times, different parts of medicinal plants have been used to cure specific diseases. India is known for its rich diversity of medicinal plants and hence, is referred to as the Botanical Garden of the world.[1] Plants are significantly used medically in different countries and are a source of many potent and powerful drugs as: aspirin, codeine, vinblastine, morphine, vincristine, pilocarpine, cocaine, atropine and ephedrine amongst others. It is shown from a research that approximately one-fourth of the prescription dispensers from community pharmacies in the United States contains one or more

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ingredients of plant origin.[2] Plant-derived anti-oxidants are finding widespread recognition as preventive medicines. The damage caused by free radicals in the body and the role played by plants with antioxidants and/or free radical-mopping activity have been established.[3]

Alternanthera brasiliana (L.) Kuntz (Fig. 1) (Amaranthaceae) is a herbaceous plant commonly known in Brazil as penicillin or Brazilian joyweed. It is a neotropical native species which grows easily on poor and deforested soil. It is an ornamental as well as a medicinal plant found growing wild in bushes and along the road sides[4]; it is used therapeutically against inflammation, cough and diarrhoea in Brazilian popular medicine.[5] The extract of *A. brasiliana* leaves exhibited anti-nociceptive effect in mice, anti-microbial effect and anti-herpes simplex virus activity. Aqueous and ethanol extract of *A. brasiliana* leaves are able to block human mitogen-induced lymphocyte proliferation without any toxic effect.[6,7] Although the local traditional healers have ethnomedical knowledge on the medicinal values of *A. brasiliana*, not much has been done to scientifically validate/authenticate the medicinal values of this plant and the mechanisms of its diverse pharmacological actions. Hence, the present study was undertaken to investigate the anti-oxidant potential of the ethanol extract of the leaves of *A. brasiliana*.

2. Materials and methods

2.1. Plant

A. brasiliana leaves were locally harvested at Ogurugu road, Nsukka and were identified by Prof. (Mrs.) May Nwosu of the Department of Botany, University of Nigeria, Nsukka, Enugu State where the voucher specimens were deposited in the herbarium.

2.2. Preparation of the extract

A quantity (25 g) of powdered *A. brasiliana* leaves was weighed out and subjected to cold maceration in 125 ml of absolute ethanol for 24 h. The mixture was afterwards, filtered using Whatman No 1 filter paper. The filtrate was concentrated in an oven at 50 °C for 48 h and stored in a refrigerator at 4 °C until it was used.

Fig. 1 – *Alternanthera brasiliana*.

2.3. Animals

Six adult male Wistar rats of between 7 and 12 weeks old with average weight of 120 ± 20 g were obtained from the Animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were acclimatised for one week under a standard environmental condition with a 12 h light and dark cycle and maintained on a regular feed and water *ad libitum*. There was adherence to the Principles of Laboratory Animal Care.

2.4. Chemicals and reagents

The chemicals used for this study were of analytical grades and included: absolute ethanol (BDH Chemicals Ltd., Poole, England), ascorbic acid [standard anti-oxidant (Sigma–Aldrich, Inc., St. Louis, USA)], glacial acetic acid (BDH Chemicals Ltd., Poole, England), thiobarbituric acid [TBA (BDH Chemicals Ltd., Poole, England)], trichloro acetic acid [TCA (BDH Chemicals Ltd., Poole, England)], carbon tetrachloride (BDH Chemicals Ltd., Poole, England), potassium chloride (BDH Chemicals Ltd., Poole, England), dipotassium hydrogen phosphate (BDH Chemicals Ltd., Poole, England), phosphoric acid (BDH Chemicals Ltd., Poole, England), sulphanilamide (BDH Chemicals Ltd., Poole, England), sodium nitroprusside (BDH Chemicals Ltd., Poole, England), potassium ferricyanide (BDH Chemicals Ltd., Poole, England), phosphate buffer (pH 7.4), ferrous sulphate heptahydrate (BDH Chemicals Ltd., Poole, England), ferric chloride (BDH Chemicals Ltd., Poole, England), 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent, [N-(1-naphthyl)-ethylene diamine] Griess reagent, normal saline and distilled water.

2.5. Concentration of total phenols

The total phenolic content of the plant extract was determined by the method described by.[8]

2.6. DPPH radical – scavenging assay

The DPPH radical-scavenging activity of the extract was determined by the method reported by.[9]

2.7. Iron (II) – chelating activity

The ability of the ethanol extract of *A. brasiliana* to chelate Fe^{2+} was determined using a modified method of.[10]

2.8. Nitric oxide radical – scavenging activity

Nitric oxide radical-scavenging activity was performed as described by.[11]

2.9. Ferrous sulphate – induced lipid peroxidation assay

The method reported by[12] was used for this assay using 3 adult male Wistar rats.

2.10. Carbon tetrachloride – induced lipid peroxidation assay

Carbon tetrachloride-induced lipid peroxidation test was performed using 3 adult male Wistar rats according to the method described by.[13]

2.11. Statistical analysis

The results were expressed as means of three replicates ± standard errors of the means (SEM). Linear regression plots were generated using Microsoft Excel for Windows 7.

3. Results

3.1. Concentration of total phenols

The concentration of total phenols as evaluated using the equation generated from the standard curve of total phenols was 0.031 ± 0.006 μg/ml of the extract.

3.2. Effect of the extract on the scavenging of DPPH radical

The ability of the extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical varied significantly at different concentrations of the extract with the highest percentage inhibition of DPPH radical (99.50%) recorded at 0.1 μg/ml of the extract as shown in Fig. 2.

3.3. Effect of the extract on the chelation of iron (II)

Fig. 3 shows that the extract at different concentrations exhibited varying percentages of Fe^{2+} chelation and the ability of the extract to chelate Fe^{2+} dropped significantly with increase in the concentration of the extract as the highest and the lowest percentage chelation (78.38% and 51.43%) were recorded at 100 and 800 μg/ml of the extract respectively.

3.4. Effect of ascorbic acid on the chelation of iron (II)

Ascorbic acid at various concentrations exhibited different percentages of Fe^{2+} chelation in which case, its ability to chelate Fe^{2+} dropped significantly with increase in its concentration as the highest and the lowest percentage chelation (30.48% and 19.10%) were recorded at 100 and 400 μg/ml of ascorbic acid respectively (Fig. 4).

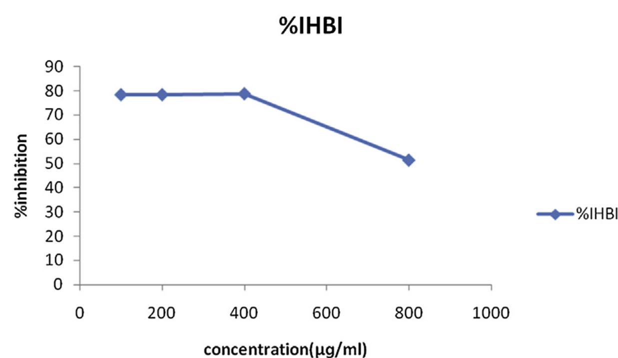

Fig. 3 – Effect of the extract on the chelation of iron (II).

3.5. Effect of the extract on the scavenging of nitric oxide radical

As shown in Fig. 5, different concentrations of the extract exhibited varying percentage scavenging activities. The ability of the extract to scavenge nitric oxide radical dropped significantly with increasing concentrations of the extract.

3.6. Effect of ascorbic acid on the scavenging of nitric oxide radical

The nitric oxide scavenging ability of ascorbic acid initially was rising with increasing concentration of ascorbic acid and later dropped as shown in Fig. 6.

3.7. Effect of the extract on ferrous sulphate – induced lipid peroxidation

Fig. 7 shows that the different concentrations of the extract exhibited different percentages of inhibition of ferrous sulphate-induced lipid peroxidation and the ability of the extract to cause the inhibition decreased with increase in the concentration of the extract as the highest inhibitory ability of the extract (37.90%) was recorded at 100 μg/ml of the extract

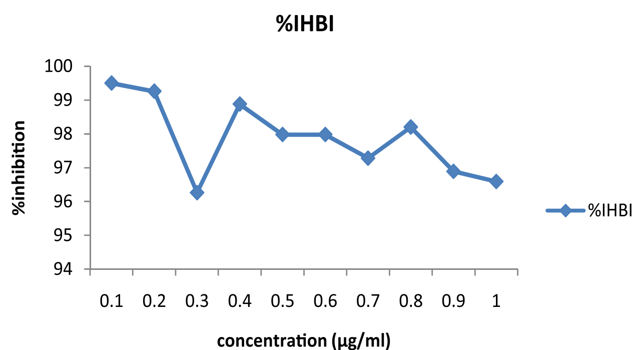

Fig. 2 – Effect of the extract on the scavenging of DPPH radical.

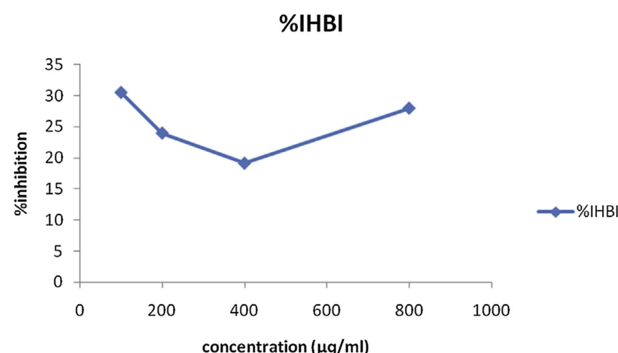

Fig. 4 – Effect of ascorbic acid on the chelation of iron (II).

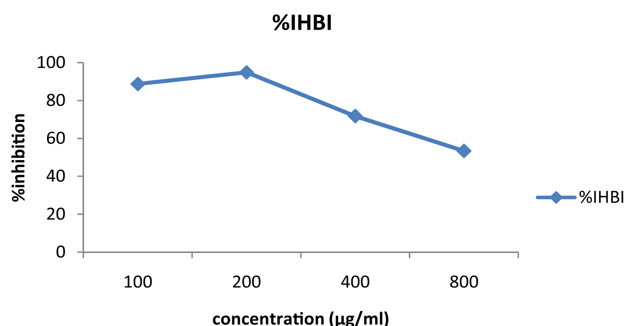

Fig. 5 – Effect of the extract on the scavenging of nitric oxide radical.

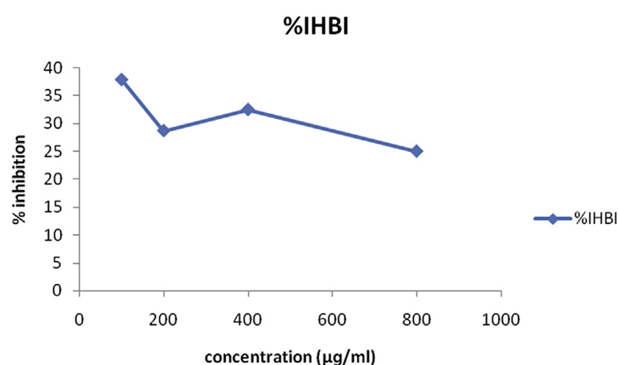

Fig. 7 – Effect of the extract on ferrous sulphate-induced lipid peroxidation.

and the lowest inhibitory ability (25.00%) was recorded at 800 μg/ml of the extract.

3.8. *Effect of ascorbic acid on ferrous sulphate – induced lipid peroxidation*

The ability of ascorbic acid to inhibit ferrous sulphate-induced lipid peroxidation decreased with increasing concentration of ascorbic acid (Fig. 8).

3.9. *Effect of the extract on carbon tetrachloride – induced lipid peroxidation*

As shown in Fig. 9, the percentage inhibitory ability of the extract on carbon tetrachloride-induced lipid peroxidation decreased as the concentration of the extract increased. The highest percentage inhibitory ability (99.54%) was recorded at 100 μg/ml of the extract while the lowest percentage inhibitory ability (99.45%) was recorded at 800 μg/ml of the extract.

3.10. *Effect of ascorbic acid on carbon tetrachloride – induced lipid peroxidation*

The percentage inhibitory ability of ascorbic acid on carbon tetrachloride-induced lipid peroxidation increased with increasing concentration of ascorbic acid as the highest percentage inhibitory ability was 99.96% at 800 μg/ml of ascorbic acid and the lowest percentage inhibitory ability was 99.93% at 100 μg/ml of ascorbic acid (Fig. 10).

4. Discussion

The ethanol extract of the leaves of *A. brasiliana* was evaluated for *in vitro* anti-oxidant activity in the present study. This was carried out by determining the concentration of total phenols in the extract as well as using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging, iron (II)-chelating, nitric oxide radical-scavenging, ferrous sulphate and carbon tetrachloride-induced lipid peroxidation assays.

The concentration of total phenols obtained in this study might be due to the polarity of ethanol. The total phenolic contents in plant extracts depend on the type of extract, i.e. the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.[14]

The extract demonstrated varied DPPH radical scavenging-effect. DPPH is a very stable free radical. Unlike the *in vivo*-generated free radicals such as the hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally fades when anti-oxidant molecules quench DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free radical attack on the DPPH molecule) and convert them into a colourless and or/bleached product (i.e. 1,1-diphenyl-2-

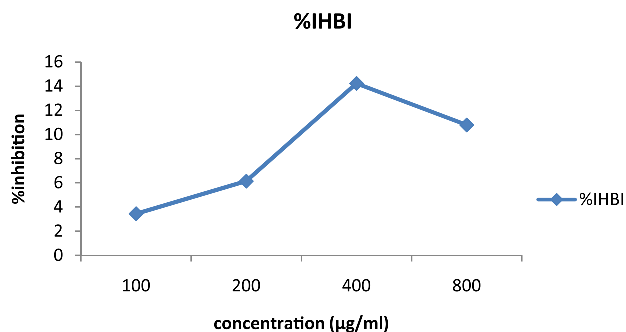

Fig. 6 – Effect of ascorbic acid on the scavenging of nitric oxide radical.

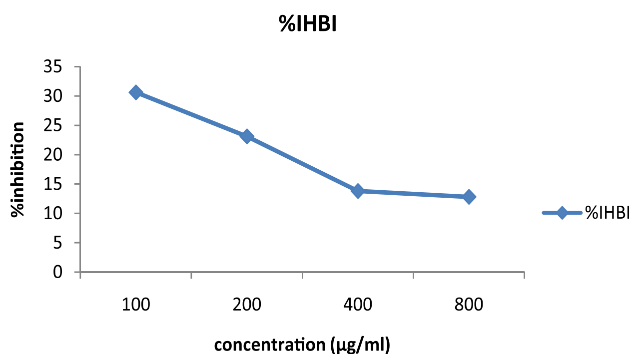

Fig. 8 – Effect of ascorbic acid on ferrous sulphate-induced lipid peroxidation.

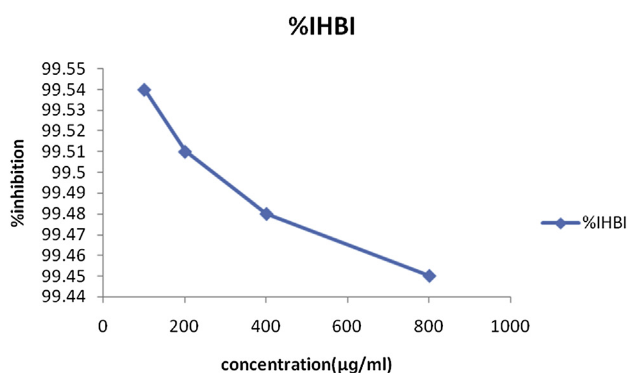

Fig. 9 – Effect of the extract on carbon tetrachloride-induced lipid peroxidation.

hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band.[9] The effect of anti-oxidants on DPPH radical is thought to be due to their hydrogen-donating ability. The result of this investigation demonstrates that the extract possesses strong scavenging effect on DPPH radical. This may be as a result of the concentration of total phenols in the extract. Phenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may contribute directly to their antioxidant action.[15]

The extract showed a strong capability of iron (II) chelation in a manner that is comparable to that of a standard anti-oxidant (ascorbic acid). This may be attributable to the anti-oxidant effect of total phenols. It is known that several mechanisms contribute to the anti-oxidant effect of phenolics in lipid system. These mechanisms are: suppression of the formation of reactive oxygen species (ROS) by inhibiting some enzymes, up-regulating or protecting anti-oxidant defence, scavenging free radicals especially ROS and capacity to chelate divalent metal ion involved in free radical production.[16]

That the extract exhibited a nitric oxide (NO)-scavenging activity implies an anti-oxidant activity. The contribution of NO to oxidative damage is increasingly becoming evident even though it has some beneficial effects. Excess production of NO has been associated with several ailments such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis.[17] This indicates that this medicinal plant might be potent and novel therapeutic agent for scavenging of nitric oxide and arresting of pathological conditions caused by excessive generation of nitric oxide and its oxidation product (peroxynitrite).

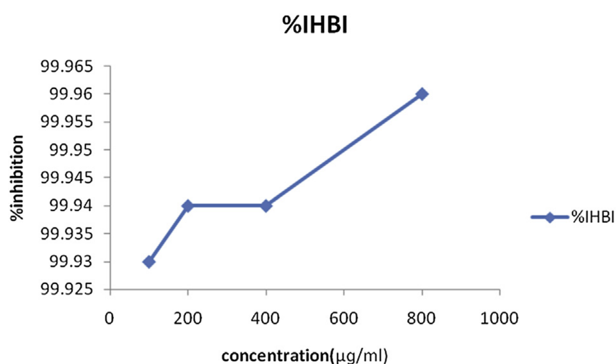

Fig. 10 – Effect of ascorbic acid on carbon tetrachloride-induced lipid peroxidation.

Anti-lipid peroxidative effect was exerted by the extract on ferrous sulphate-induced lipid peroxidation. Peroxidation of lipid is a natural phenomenon and occurs on its exposure to oxygen. Recently, free radical-induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygen toxicity, liver disorders, inflammation inter alia. Many natural and synthetic anti-oxidants are in use to prevent lipid peroxidation. Ferrous sulphate has been used as an inducer of lipid peroxidation. Production of thiobarbituric acid reactive substances [TBARS (an index of lipid peroxidation)] in normal conditions is very slow while in the presence of ferrous sulphate, it is relatively high. Initiation of lipid peroxidation by ferrous sulphate occurs through the ferryl–perferryl complex.[18] Anti-lipid peroxidative property of *A. brasiliana* might be either due to chelating or redox activity. The specific ratio of ferrous to ferric is important for induction of lipid peroxidation. It has been reported that at least 1:1 ratio of ferrous to ferric is critical for initiation of lipid peroxidation.[18] Anti-oxidant activity of *A. brasiliana* therefore, may result from multiple factors involving hydrogen or electron transfer, metal-chelating activity and synergistic activity and appears to be the result of many different activities.

The extract showed anti-lipid peroxidative effect on carbon tetrachloride-induced lipid peroxidation. Carbon tetrachloride (CCl_4) is metabolised by cytochrome P_{450} to reactive trichloromethyl radical ($\bullet CCl_3$). Trichloromethyl radical then combines with cellular lipids and proteins in the presence of oxygen to form a trichloromethyl peroxyl radical ($\bullet OOCCl_3$) which may attack lipids in the membrane of endoplasmic reticulum faster than trichloromethyl free radical. These radicals propagate a chain reaction leading to lipid peroxidation in cellular membranes, destruction of Ca^{2+} homeostasis that induces cell injury and finally results in cell death.[19] In line with the oxidative stress theory of CCl_4 toxicity, in the present study, the concentrations of TBARS remarkably increased and reduced in the CCl_4 and extract-treated rats respectively. It can be suggested from the result that the extract effectively protected the liver against the CCl_4-induced oxidative damage on the liver of the rats possibly through anti-oxidant and/or free radical-scavenging effects of phenolic compounds and other bioactive constituents that may be present in the extract.

In conclusion, the results of the present study generally imply that the leaves of *A. brasiliana* could be a potential source of natural anti-oxidant and may be greatly utilised as therapeutic agent in preventing or slowing oxidative stress-related diseases. The plant may also find relevance in cosmetic and food industries where anti-oxidants are used in fortifying products.

Conflicts of interest

All authors have none to declare.

REFERENCES

1. Saravanan P, Sathish KS, Vijay S. Evaluation of antioxidant and thrombolytic potential of *Alternanthera sessilis* Linn. *J Environ Sci Toxicol Food Technol*. 2013;2(5):1–4.
2. Nayak P, Nayak S, Kar DM. Pharmacological evaluation of ethanolic extract of the plant, *Alternanthera sessilis* against temperature regulation. *J Pharm Res*. 2010;3(6):1381–1383.
3. Aruoma OI. Methodological considerations of characterising potential anti-oxidant actions of bioactive components in plant foods. *Mutat Res*. 2003;523(524):9–12.
4. Ogedegbe AU, Ikhajiagbe B, Anoliefo GO. Growth response of *Alternanthera brasiliana* (L.) Kuntze in a waste oil-polluted soil. *J Emerg Trends Eng Appl Sci*. 2013;4(2):322–327.
5. Kumar S, Singh P, Mishra G, Srivastar S, Jha KK, Khosa RL. Phytopharmacological review of *Alternanthera brasiliana* (Amaranthaceae). *Asian J Plant Sci Res*. 2011;1(1):41–47.
6. Athukorala Y, Kim KN, Jeon YJ. Anti-proliferative and anti-oxidant properties of an enzymatic hydrolysate from algae. *Food Chemo Toxicol*. 2006;44(7):1065–1074.
7. Barua CC, Talakular A, Begum SA, Sarma DK, Pathak OC, Borah P. Anti-nociceptive activity of methanol extract of leaves of *Alternanthera brasiliana* Kuntze in animal model of nociception. *Pharmacol Online*. 2009;3:49–55.
8. Veliogu YS, Mazza G, Gao L, Oomah BD. Anti-oxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agric Food Chem*. 1998;46:4113–4117.
9. Amarowicz R, Pegg BR, Rahimi MP, Bar B, Weil JA. Free radical-scavenging capacity and anti-oxidant activity of selected plant species from the Canadian prairies. *Food Chem*. 2003;84:551–562.
10. Puntel RL, Nogueira CW, Rocha JTB. Kreb cycle intermediates modulate thiobarbituric acid reactive substances (TBARS) production in rats brain *in vitro*. *Neurochem Res*. 2005;30(2):225–235.
11. Marcocci I, Marguire JJ, Droy-lefaiz MT, Parker L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract. *Biochem Biophysical Res Commun*. 1994;201:745–755.
12. Okhawa H, Ohish N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*. 1979;9:357–358.
13. Comporti M. Three models of free radical-induced cell injury. *Chem Biol*. 1989;72:51–56.
14. Mohsen MS, Ammar SMA. Total phenolic contents and anti-oxidant activity of corn tassel extracts. *Food Chem*. 2008;112:595–598.
15. Tosun M, Ercisli S, Sengul M, Ozer H, Polat T. Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol Res*. 2009;41:175–181.
16. Malgorzata K, Jadwiga JL, Malgorzata E, Teresa A, Irena M, Wieslawa B. Protective effect of *Aquilegia vulgaris* (L.) on carbon tetrachloride-induced oxidative stress in rats. *Indian J Exp Biol*. 2007;45:702–711.
17. Etim OE, Egbuna CF, Odo CE, Udo MM, Awah FM. *In vitro* anti-oxidant and nitric oxide-scavenging activities of *Piper guineense* seeds. *Glob J Med Plants Indig Med*. 2013;2(7):475–484.
18. Vandana G, Mukta S. Protective effect of *Cinnamomum tejpata* on lipid peroxide formation in isolated rat liver homogenate. *Curr Res J Biol Sci*. 2010;2(4):246–249.
19. Rao GM, Rao CV, Pushpangadan P, Shirwaikar A. Hepatoprotective effects of rubiadin, a major constituent of *Rubia cordifolia* Linn. *J Ethnopharmacology*. 2006;103:484–488.