INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume3, Issue1)
Available online at: www.ijariit.com

In vitro Antioxidant Potential of Pure Fractions of *Eclipta alba*

Anjali Singh*
Department of Chemistry, M.M.H. College, Ghaziabad (UP), 201002
anjalisingh28@rediffmail.com

Ayodhya Singh
Department of Chemistry, M.M.H. College, Ghaziabad (UP), 20100.
dr.ayodhyasingh@gmail.com

Vandana Dwivedi
Department of Chemistry, M.M.H. College, Ghaziabad (UP), 201002
vandana.dwivedi23@yahoo.com

Abstract–Oxidative stress and Ultraviolet (UV) irradiation-induced skin damage, is involved in numerous diseases. Eclipta alba which belongs to Asteraceae family is used traditionally in Ayurvedic system of medicine in India for the treatment of liver diseases. Our study shows that water extract of E. alba has a potent effect in scavenging 1, 1-diphenyl-2-picrylhydrazyl (DPPH), chelating ferrous ion, and superoxide radicals, exhibiting IC_{50} values of 0.21 mg/mL, 1.20 mg/mL, and .49 mg/mL, respectively. Identification and quantification of the wedelolactone, one of the active constituents of the Eclipta alba plant extract, was carried out by HPLC analysis. The result of the present study indicates that the Eclipta alba extract shows a high amount of ascorbic acid, tannins, flavonoids, phenolics, contents. The hydroalcoholic extract of Eclipta alba effectively scavenged free radicals at all different concentrations and showed potent antioxidant potency. Eclipta alba extract shows antioxidative properties.

Keywords: Eclipta alba, DPPH, BHT, Hydroxyl radical, Superoxide radical.

I. INTRODUCTION

Medicinal plants derived natural products such as flavonoids, terpenoids, steroids, etc. have diverse pharmacological properties including antioxidant activity. Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals such as singlet oxygen, superoxide, peroxyl and hydroxyl radicals, etc., thereby preventing or delaying damage to the cells and tissues. The generation of free radicals is a feature of cellular function such as in the mitochondrial respiratory chain, in phagocytosis, and in arachidonic acid metabolism [1]. However, excessive production of free radicals impairs cell membrane integrity [2], causes defects in the susceptible proteins required for mRNA translation, and induces DNA damage and gene mutation [3]. Excess reactive oxidative stress (ROS) which is susceptible to redox dysregulation and oxidative stress is associated with many diseases including cancer [4], [5], diabetic retinopathy [6], and chronic inflammatory disease [7]. Excess reactive oxygen species is also associated with aging processes [8]. Biological systems evolved endogenous defense mechanisms including employing antioxidants and antioxidative enzymes, to help protect against free radical-induced cell damage [9], [10].

Reactive oxygen species [11] including hydrogen peroxide, superoxide anion, and singlet oxygen are significantly induced in the skin under UV irradiation [12].

Antioxidant compounds have potential to mitigate the effect of free radicals and play an important role as a health protecting factor [13]. *Eclipta alba* occurs throughout the whole of India. In India, the plant is known as bhangra, "bhringraj" or bhringraja. The branches are hairy, reddish brown and can grow up to 40 cm height. The roots are found growing at the thickened nodal points. The leaves are opposite, lance-like with a toothed edge and hairy. The flowers are white, small and arranged in small clusters. The plant has been reported to contain phytosterol, β-amyrin, triterpenes such as evaluation, echinocyte acid, flavones such as luteolin and coumarin such as wedelolactone [14].

The prime objective of the presently undertaken work was to find out antioxidative properties of the hydro-alcoholic extract of *Eclipta alba*.

II. MATERIALS AND METHODS

A. Chemicals

The chemicals used in the entire study were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India, and Sigma-Aldrich, was of analytical grade. HPLC grade methanol, acetonitrile, and acetic acid were obtained from Merk Specialties Pvt. Ltd., Mumbai, India.

B. Plant material

The whole plant of *Eclipta alba* was collected during September-October, 2010 from the Botanical Garden of the M.M.H. College, Ghaziabad Campus. Herbarium specimens were prepared and authenticated by Dr. R.M Johari, Associate Professor, a head of the Department of Botany, Gujarat University, and Ahmedabad.

C. Extract preparation

The whole plant of the plant was washed, air-dried, weighed, and ground to small pieces. The samples were then immersed in double distilled water (sample to water ratio 1:2 by weight) and boiled at 100°C for 20 min. After boiling, samples were filtered through Whatman No.1 paper with vacuum assistance. The water extracts were then freeze-dried and stored in dark bottle at 40°C. Before use, samples were dissolved in double distilled water at a concentration of 10 mg/mL to prepare a stock solution. The percent yield of the extract was calculated.

D. Qualitative and quantitative analysis

The hydro-alcoholic extracts of *Eclipta alba* were subjected to phytochemical analysis for the detection of the major chemical groups. Qualitative and quantitative estimation of phytochemical constituents were done as described below.

1) Qualitative analysis: Qualitative analysis of the plant extracts were carried out using standard methods for determining the presence of ascorbic acid, tannins, flavonoids, phenolics.

- Test for ascorbic acid: To the extract, one drop of 2, six dichlorophenolindophenol (DCPIP) solution was added. Formation of blue to red color indicates the presence of ascorbic acid.
  Test for tannins: 0.5 gm of the extract was dissolved in 20 ml distilled water in a test tube and then filtered. A few drops of 0.1% FeCl3 was added and observed for brownish green or blue-black color.
- Test for flavonoids: 5 ml of dilute ammonia solution was added to plant extract, followed by addition of concentrated H2SO4. A yellow colouration indicated the presence of flavonoids.
- Test for phenolics: The 50 gm of the extract was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic contents.

2) Quantitative analysis: Qualitative analysis of the plant extracts were carried out using standard methods.

- Ascorbic acid content: Ascorbic acid (vitamin C), is one of the most abundant antioxidants present in the plant was quantified by the method of Jagota and Dani (1982) [15]. The ascorbic acid content of plant extracts was expressed as µg/gm dry it. Of extract.
- Tannin content: Tannin content of the extracts was estimated by the method as described by (Price and Butler, 1977) [16]. Plant extract was allowed to react with $K_3Fe(CN)_6$-$FeCl_3$ reagent for five min, and the intensity of color developed was measured The tannin content of the extract was expressed as mg rutin equivalent/gm dry it. Of extract.
- Flavonoid content: The flavonoid content of the plant extract was estimated by the method of (Lamaison (Lamaison and Carnat, 1990) [17]. Briefly, 1.0 ml of plant extract was mixed with 1.0 ml of aluminum chloride reagent, and the resultant color was read at 430 nm. The flavonoid content of the extract was expressed as mg quercetin equivalent/gm dry wt. of extract.
- Total phenolic content: the Total phenolic content of the extract was estimated by the method as described by Singleton et al. (1999) [18]. Briefly, extract react with Folin-Ciocalteu reagent in the presence of sodium carbonate to form a blue colored complex which was read at 760 nm. Various concentrations of gallic acid were used to plot a standard curve. Total phenolic content of the extract was expressed as mg gallic acid equivalent/gm dry wt. of extract.

E. Antioxidative activity

The antioxidative potency of the plant extract was estimated by various chemical assay systems as described below:

1) DPPH Free Radical Scavenging Assay: The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen-donating antioxidant. The scavenging activity of WEP extracts on DPPH radicals was determined using a previously described method [19]. A total of 50 µL of various concentrations (0.01 mg/mL, 0.1 mg/mL, 0.3 mg/mL, 1 mg/mL, and 3 mg/mL) of WEP or chlorogenic acid were mixed with 150 µL of freshly prepared 1 mM DPPH in ethanol. Double distilled water was used as the vehicle control, and ascorbic acid (0.1 mg/mL, 0.3 mg/mL, 1 mg/mL, and 3 mg/mL) was used as positive control. The mixture was kept in darkness for 30 min. DPPH absorbance was then measured at 517 nm. Percent activity was calculated using the following equation:

The IC_{50} value, which is the sample concentration required for 50% inhibitory activity, was determined by interpolation. Each test was performed in triplicate.

2) Hydroxyl radical scavenging assay: The scavenging activity of the extract for Hydroxyl radical was estimated by the method of Halliwell et al. (1987), where radicals were generated from Fe^{+3}/ ascorbate/ EDTA/ H_2O_2 system by Fenton's reaction. [20] Briefly different concentrations of plant extracts were made to react with 2-deoxy-2-ribose, H_2O_2, $FeCl_3$ and EDTA. The reaction was initiated by the addition of ascorbic acid. After incubation for 90 min, the reaction was

terminated by addition of thiobarbituric acid (TBA) and resulting color was read at 590 nm. Percent inhibition by various concentrations of plant extract and IC50 of the extract was calculated.

3) *Superoxide radical scavenging assay***:** Scavenging activity of the extract for Superoxide radical was assessed by the method of Liu et al. (1997) [21]. In the PMS/NADH-NBT system, superoxide anion derived from dissolved O_2 by PMS/NADH coupling reaction reduces NBT. The addition of various concentrations of hydro-alcoholic extract resulted in decreased color intensity which was read at 560 nm against blank to determine the quantity of the formation generated. IC_{50} values of the extract were calculated.

4) *Fe^{+2} chelating capacity*: The Fe^{+2} chelating activity of the plant extract was estimated using the method of Dinis et al. 1994 [22]. Plant extract was allowed to react with ferrozine (5 mM) in the presence of $FeCl^3$ (2 mM). Blue colored Fe^{+2} – ferrozine complex formed was read at 562 nm. Chelating ability of the extract was compared with EDTA (0.01 mM). Percent inhibition and IC_{50} value for the extract were calculated by comparing test samples with the control.

5) *Reducing ability*: The reduction capacities of chlorogenic acid and BHT were significantly greater than those of hydro-alcoholic extract. However, at a concentration of 1 mg/mL, no significant differences in reducing power were observed between hydro-alcoholic extract, chlorogenic acid, and BHT. Thus, at 1 mg/mL concentration, the hydro-alcoholic extract has a similar efficacy to that of chlorogenic acid and BHT.

F. Statistical analysis

The findings were denoted as the means ± standard error of the mean (SEM). The IC_{50} values were computed by probit analysis. The data were statistically analyzed using specific software. The data were statistically determined by one-way Analysis of Variance at a significance level $p<0.05$. Also, linear regression analysis (R^2 value) was performed.

III. RESULTS

A. Phytochemical analyses of the plant extract

1) *Qualitative analysis*: Analysis of *Eclipta alba* qualitatively showed the presence of ascorbic acid, tannins, flavanoids, and phenolics contents.

Table: Qualitative and quantitative analysis of the extract of *Eclipta alba*

Phytochemical parameters ***Eclipta alba***	
Extract yield (%)	14.98 ± 1.34
Qualitative Analysis	
Ascorbic acid content	+
Tannin content	+
Flavanoid content	+
Phenolic content	+
Quantitative Analysis	
Ascorbic acid content	2.22 ± 0.26
Tannin content	40.50 ± 5.10
Flavonoid content	86.03 ± 1.88
Phenolic content	97.89 ± 2.29

Results are shown as mean ± SEM; n = 6.

Units:
Ascorbic acid - µg/gm dry wt. of extract.
Tannins – mg rutin equivalent/gm dry wt. of extract.
Flavonoids – mg quercetin equivalent/gm dry wt. of extract.
Phenolics – mg gallic acid equivalent/gm dry wt. of extract.

2) *Quantitative analysis*: Quantitative analysis showed the following results.

- Ascorbic acid content -- Quantification of ascorbic acid content showed that hydro- alcoholic extract of Eclipta alba contained 2.22 mg equivalent/gm dry weight of extract (Table)
- Tannin content -- Standard curve for tannin estimation was plotted using various concentrations of rutin. Tannin content of hydro-alcoholic extract of *Eclipta alba* was found to be 40.50 mg rutin equivalent/gm dry weight of tannin (Table).

Flavonoid content -- Flavanoid content of hydro-alcoholic extract of *Eclipta alba* was found to be 86.03 mg quercetin µg/gm dry weight of extract. (Table)

- Total phenolic content -- The concentration of TPC determined in hydro-alcoholic extract of *Eclipta alba* was 97.89 mg gallic acid equivalent/gm dry weight calculated using equation that was obtained from the standard gallic acid graph (Table).

B. Antioxidant activity

*1) DPPH radical scavenging assay***:** DPPH radical scavenging activity of various concentrations of *Eclipta alba* was found statistically significant ($p<0.05$). Decrease in absorbance due to the antioxidative effect of soluble solids of *Eclipta alba* was highest at 240 µg/ml concentration. *Eclipta alba* was found to be potent (80.25%). Scavenging effect of the *Eclipta alba* extract was concentration-dependent (R2 = 0.8016,). IC50 value for *Eclipta alba* extract was 50 µg/ml. (fig.)

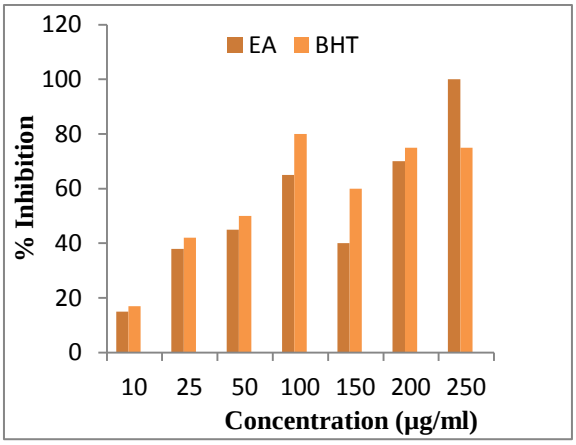

Fig. Showing DPPH activity

2) *Hydroxyl radical scavenging assay*: Scavenging capacity of extract for Hydroxyl radical is directly proportional to its antioxidative potency. The percent inhibition of hydroxyl radical increased significantly ($p<0.05$) with increasing concentrations of a hydro-alcoholic extract of *Eclipta alba* and. Maximum protection for *Eclipta alba* extract was 70.12%. The protective effect was concentration-dependent ($R^2 = 0.9650$) and was highest at 50 µg/ml concentration. IC_{50} values of Eclipta alba extract was 30 µg/ml.
3) *Superoxide radical scavenging assay***:** Different concentrations of *Eclipta alba* extract strongly scavenged superoxide radicals generated from PMS/NADH-NBT system The decrease in color intensity was observed with increasing concentration of extract indicating consumption of the radicals in the reactions mixture. The maximum effect was achieved at 300 µg/ml concentration of the extracts. The maximum scavenging effect found with *Eclipta alba* extract was 86.30%. The effect was concentration- dependent for the extracts (R^2 = 0.9762). Concentration required to scavenge 50% (IC_{50}) of the radicals was 160 µg/mL for *Eclipta alba* extract.
4) *Fe^{+2} chelating activity*: The addition of Eclipta alba extract reduced Ferrozine - Fe^{+2} complex which showed violet color in the presence of metal ions. Formation of colored chromophore is interrupted in the presence of chelating agents of Eclipta alba extract and resulted in decreased optical density. Maximum inhibition achieved with *Eclipta alba* extract was 79.15%. *Eclipta alba* extract chelated metal ions in a concentration dependent manner (R^2= 0.9750). IC_{50} values for *Eclipta alba* extract was 100 µg/ml.
5) *Reducing ability*: The presence of reductant in the tested extract of Eclipta alba showed the reduction in Fe^{+3}/ferricyanide complex to ferrous form (Fe^{+2}). Increasing concentrations of the extracts showed simultaneously increase of reducing power. The highest reducing ability was found with *Eclipta alba* extract (76.24%) at 250 µg/mL concentration. The increase in reducing the ability of the extracts was concentration- dependent (R^2 = 0.9872). IC_{50} values for reducing ability was 100 µg/mL for *Eclipta alba* extract.

IV. DISCUSSION

During our qualitative investigation assessment of phytochemical constituents of *Eclipta alba* plant extracts exnhibited presence of ascorbic acid, tannin, flavonoids and phenolics, contents. Our quantitative analysis showed the presence of phenolic content in *Eclipta alba* extract. A number of flavonoids and Phenolics was higher than other phytoconstituents. The phenolic compounds, flavonoids, tannins and alkaloids are known to possess antioxidant properties [24], [25]. In this quantitative study estimation of crude polyphenols from hydro-alcoholic extracts of *Eclipta alba* showed the presence of a significantly high amount of phytochemicals mainly responsible for its protective effect. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate the development of many diseases, like cancer, liver injury and cardiovascular diseases [26]. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are invovled [27]. Thus, this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been showed to have antioxidant activity [28]--[30].

Free radical scavenging properties are generally due to high reducing capacity of the polyphenols acting as primary antioxidants [31]. 1, 1-Diphenyl-2-pecryl-hydrazyl (DPPH) is a stable free radical which has an unpaired valence electron at one atom of Nitrogen Bridge [32]. 1, 1-Diphenyl-2-pecryl-hydrazyl (DPPH) assay has many properties, such as good stability, credible sensitivity, and feasibility [33]. Metal ions a major role in reactive oxygen species generation as they can change the state from reduced to oxidised causing the removal of an electron from various biomolecules [34]. Antioxidative effect of *Eclipta alba* extract is mainly denoted by the phytochemicals acting as reductant and free radical scavenger. An attempt was made in our study we tried to to isolate and characterize the major active component from *Eclipta alba*.

CONCLUSION

Our study showed the presence of antioxidant \activity in *Eclipta alba* plant extract.

ACKNOWLEDGEMENTS

Authors are thankful to Prof. H. S. Singh, Hon'ble Pro-Vice Chancellor, CCS University, Meerut for his constant encouragement. Special thanks are due to Prof. Gurmeet Singh, Head of the Deptt. of Chemistry, the University of Delhi for his blessings and suggestions to carry out such work. Thanks are also due to the Head of the Deptt. of Chemistry, MMH College, Ghaziabad for providing necessary facilities to carry out research work.

REFERENCES

[1] E. Róth, "Oxygen free radicals and their clinical implications," Acta chirurgica Hungarica, vol. 36, no. 1–4, pp. 302–305, 1997.

[2] E. Niki, "Lipid peroxidation: physiological levels and dual biological effects," Free Radical Biology and Medicine, vol. 47, no. 5, pp. 469–484, 2009.

[3] M. A. Birch-Machin and H. Swalwell, "How mitochondria record the effects of UV exposure and oxidative stress using human skin as a model tissue," Mutagenesis, vol. 25, no. 2, pp. 101–107, 2010.

[4] T. L. Gonçalves, F. Erthal, C. L. D. Corte et al., "Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women," Clinical Biochemistry, vol. 38, no. 12, pp. 1071–1075, 2005.

[5] F. Tas, H. Hansel, A. Belce et al., "Oxidative stress in breast cancer," Medical Oncology, vol. 22, no. 1, pp. 11–15, 2005.

[6] R. Mehta, N. Shangari, and P. J. O'Brien, "Preventing cell death induced by carbonyl stress, oxidative stress or mitochondrial toxins with vitamin B anti-AGE agents," Molecular Nutrition and Food Research, vol. 52, no. 3, pp. 379–385, 2008.

[7] Y. Maruyama, B. Lindholm, and P. Stenvinkel, "Inflammation and oxidative stress in ESRD—the role of myeloperoxidase," Journal of Nephrology, vol. 17, no. 8, pp. S72–S76, 2004.

[8] M. Carvour, C. Song, S. Kaul, V. Anantharam, A. Kanthasamy, and A. Kanthasamy, "Chronic low-dose oxidative stress induces caspase-3-dependent PKCδ proteolytic activation and apoptosis in a cell culture model of dopaminergic neurodegeneration," Annals of the New York Academy of Sciences, vol. 1139, pp. 197–205, 2008.

[9] A. T. Black, J. P. Gray, M. P. Shakarjian, D. L. Laskin, D. E. Heck, and J. D. Laskin, "Increased oxidative stress and antioxidant expression in mouse keratinocytes following exposure to paraquat," Toxicology and Applied Pharmacology, vol. 231, no. 3, pp. 384–392, 2008.

[10] A. S. Fernandes, J. Serejo, J. Gaspar et al., "Oxidative injury in V79 Chinese hamster cells: Protective role of the superoxide dismutase mimetic MnTM-4-PyP," Cell Biology and Toxicology, vol. 26, no. 2, pp. 91–101, 2010.

[11] W. B. Mors, M. C. Do Nascimento, J. P. Parente, M. H. Da Silva, P. A. Melo, and G. Suarez-Kurtz, "Neutralization of lethal and myotoxic activities of south american rattlesnake venom by extracts and constituents of the plant *Eclipta prostrata* (Asteraceae)," Toxicon, vol. 27, no. 9, pp. 1003–1009, 1989.

[12] K. Scharffetter-Kochanek, M. Wlaschek, P. Brenneisen, M. Schauen, R. Blaudschun, and J. Wenk, "UV-induced reactive oxygen species in photocarcinogenesis and photoaging," Biological Chemistry, vol. 378, no. 11, pp. 1247–1257, 1997.

[13] Kataria M, Singh L. Hepatoprotective effect of Liv. 52 and Kumaryasava on carbon tetrachloride induced hepatic damage in rats. Indian Journal of Experimental Biololgy. 1997; 35:255-57.

[14] Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. New Delhi: Council of Scientific and Industrial Res. 1966, 104.

[15] Jagota SK, Dani A. New colorimetric technique for the determination of vitamin C using folin-phenol reagent. Analytical biochemistry. 1982; 127:178-182.

[16] Price ML, Butler LG. Rapid visual estimation and spectrometric determination of tannin content of sporangium grain. Journal of Agricultural Food Chemistry. 1977; 25:1268-1273.

[17] Lamaison JL, Carnat A. Teneurs en acid rosmarinique en derives hydroxycinnamiquestotanx et activates antioxydents chez les Apiacees, les Borrabianacees et les Lamiaceesmedicinales. Pharmaceutica Acta Halvetiae 1900; 65:315-320.

[18] Singleton V, Orthofer R, Lumuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods in Enzymology 1999; 299:152-178.

[19] S. I. Abdelwahab, S. Mohan, M. Mohamed Elhassan et al., "Antiapoptotic and antioxidant properties of orthosiphon stamineus benth (Cat's Whiskers): intervention in the Bcl-2-mediated apoptotic pathway," Evidence-based Complementary and Alternative Medicine, vol. 2011, Article ID 156765, 10 pages, 2011.

[20] Halliwell B, Grootveld M. The measurement of free radical reactions in humans. FEBS Letters. 1987; 213:9-14.

[21] Lie F, Ooi VEC, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Science 1997; 60:763-771.

[22] Dinis TCP, Madeira VMC, Almeida MLM. Action of phenolic derivates (acetoamenophen, salyccilate and 5-aminosalycialate) as inhibitors of membrane lipid peroxidasetion and as peroxyl radical scavengers. Archives of Biochemistry and Biophysics 1994; 315:161-169.

[23] Gyamf MA, Yonamine M, Aniya Y. Free-radical scavenging action of medical herbs from Ghan: Thonningiasanguinea on experimentally-induced liver injuries. General Pharmacology. 1999; 32:661-667.

[24] Molina L, Constantinescu F, Michel L, Reimmann C, Duffy B, Defago G. Degradation of pathogen quorumsensing molecules by soil bacteria: a preventive and curative biological control mechanism. FEMS Microbiology Ecology 2003; 45:71-81.

[25] Gholivand K, Mahzouni HR, Pourayoubi M, Amiri S. Inorganic Chemistry 2010; 363:2318-2324.

[26] Liao KL, Yin MC. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: Importance of the partition coefficient. Journal of Agricultural and Food Chemistry. 2000; 48:2266-2270.

[27] Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant Activities of some Extracts of Thymus zygis. Free Radical Research 1997; 26:469-478.

[28] Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry. 1995; 43:2800-2802.

[29] Brown JE, Rice-Evans CA. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radical Research 1998; 29:247-255.

[30] Gil MI, Ferreres F, Tomás-Barberan FA. Effect of postharvest storage and processing on the antioxidant constituents (Flavonoids and Vitamin C) of fresh-cut spinach. Journal of Agricultural and Food Chemistry. 1999; 47:2213-2217.

[31] Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karagoz Y, Halici M et al. Comparison of antioxidant activity and phenolic content of three lichen species. Phytotherapy Research 2004; 18:938-941.

[32] Eklund PC, Langvik OK, Warna JP, Salmi TO, Willfor SM, Sioholm RE. Chemical studies on antioxidant mechanism and free radical scavenging properties of lignans. Organic and Biomolecular Chemistry 2005; 21:3336-3347.

[33] Ozcelik B, Lee JH, Min DB. Effects of light, oxygen, and pH on the absorbance of 2, 2-diphenyl-1-picrylhydrazyl. Journal of Food Science 2003; 68:487-490.

[34] Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Molecular and Cellular Biochemistry 2010; 345:91-104.

