CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIOXIDANT ACTIVITY OF AQUA ALCOHOLIC EXTRACT OF *SOLANUM NIGRUM* LEAVES

S Backialakshmi and J. Kalaimathi*
Department of Biochemistry
Sri Akilandeswari womens College
Wandiwash, TN, India

Abstract:
Solanum nigrum is a well know traditional medicinal plant used for the treatment of jaundice, asthma and ulcers and tuberculosis. It is an herbaceous annual weed. In the present study, we demonstrated the free radical scavenging activity of the aqua alcohol extracted leaf extract of Solanum nigrum. Crude aqua alcohol extract of Solanum nigrum leaves showed in vitro free radical scavenging activity in dose response manner. In vitro free radical scavenging activity of aqua alcohol extract of Solanum nigrum leaves was studied against DPPH, Hydroxyl, lipid peroxide, Super oxide anion, nitric oxide free radicals and reducing power. The results showed that Solanum nigrum plant possesses excellent free radical scavenging activity and significantly higher than the synthetic antioxidants. To conclude this investigation, aqua alcohol extract of Solanum nigrum leaves exhibit a good free radical scavenging activity and might be an alternate to synthetic antioxidants and they acts as potential therapeutic agents.

Keywords: *Solanum nigrum, Antioxidant, Medicinal plants*

*Corresponds to

Mrs. J. Kalaimathi
Assistant Professor
Department of Biochemistry
Sri Akilandeswari womens College
Wandiwash, TN, India
jkalaimathibio@gmail.com

QR code

Please cite this article in press as J. Kalaimathi et al , ***Preliminary Phytochemical Analysis And In Vitro Antioxidant Activity Of Aqua Alcoholic Extract Of Solanum Nigrum Leaves,*** *Indo Am. J. Pharm. Sci, 2015;2(7).*

INTRODUCTION

Free radicals are reactive oxygen species having unpaired electron in its outer orbital. Free radical is unstable compound that are quite reactive with other molecules due to the presence of unpaired electrons and converted into more stable compound [1]. These are very dangerous to living cells. Free radicals are generated in the human body during normal metabolic activities. Free radicals are formed during oxidation metabolism which takes place in mitochondria [2,3]. The most common free radicals are superoxide anion (O_2), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO) and reactive hydroxyl (OH) radicals, nitric oxide (NO) and peroxynitrite anion (ONOO), etc [4]. Excess production of these free radicals leads to cause cellular damage like diabetes mellitus, atherosclerosis, myocardial infarction, arthritis, anemia, asthma, inflammation, neurodegenerative diseases and carcinogenesis [5,6]. These diseases are caused by free radicals due to reacting with various bio molecules of body such as membrane lipids, nucleic acid, proteins and enzymes [7].

These harmful free radicals were scavenged by synthetic or plant derived compounds is called as antioxidant agent. Antioxidant agent inhibits the excess production of free radicals during metabolic activity. Synthetic antioxidant agents like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) [8]. These synthetic antioxidants have toxic effects and, consequently, restrictions have been imposed on their use [9,10]. These limitations increases demand for development of plant-derived natural antioxidants to scavenging the free radicals [11,12]. Natural antioxidant compounds from plants have been strong activity due to the presence of secondary metabolites like flavonoids, phenols, alkaloids, tannins, quinones, and proanthocyanidins [13,14,15]. These compounds inhibit and scavenge the free radicals and provide protection to human against diseases [16].

Solanum nigrum is an important medicinal valuable plant used in traditional medicine to cure dysentery, stomach complaints and fever. It is herbaceous plant belonging to the Solanaceae family. Whole plant has a medicinal properties[17]. The fruits are used as a tonic, laxative, and appetite stimulant; and also for treating asthma. The boiled extracts of leaves and berries are also used to alleviate liver-related ailments like jaundice. Solanum *nigrum* is considered to be antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, antipyretic. Aerial parts of *S.nigrum* has the ability to decrease the secretion of gastric acid, pepsin level and stimulate mucus secretion was reported by Akthar and Munir [18]. In this study, we demonstrated that free radical scavenging activity of aqua alcohol extract of *Solanum nigrum* leaves through performing DPPH assay, hydroxyl radical scavenging activity, super oxide radical scavenging activity, reducing power, nitric oxide assay etc.

MATERIALS AND METHODS

Plant Material

Leaves of *Solanum nigrum* was collected locally in and around Vandavasi.

Extract Preparation

Collected leaves were cleaned with water and shade dried at room temperature. Then dried leaves were powdered and extracted with 95% aqua alcohol in soxhlet apparatus for 24 hours. Then extracts were filtered and concentrated by dried in a vacuum desiccator. After evaporation the percentage yield for aqua alcohol extract was 7.9% and for antioxidant studies.

Non Enzymatic Antioxidant Assay

Free Radical Scavenging Assay (DPPH)

The DPPH free radical scavenging activity of aqua alcohol extract of *S. nigrum* leaves was determined by according to the method of Gyamfi (1999). Typically, different concentration (2-10 µg/ml) of plant extract was mixed with 1 ml of 0.1 mM DPPH in aqua alcohol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4) and incubated for 30 min. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. BHT was used as controls. The percent inhibition was calculated from the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] ×100

Nitric Oxide Radical Inhibition Assay

Nitric oxide radical inhibition activity of plant extract can be estimated by the use of Griess Illosvoy reaction (1999) with slight modifications i.e. using naphthyl ethylene diamine dihydrochloride (0.1% w/v). The reaction mixture prepared by mixing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *S. nigrum* leaf extract (10-100 µg/ml) or standard solution (rutin, 0.5 ml). This reaction mixture was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C.

The absorbance of these pink color solutions was measured at 540 nm against the corresponding blank solutions. Rutin used as a standard.

% Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] ×100

Superoxide Anion Scavenging Activity

Superoxide anion scavenging activity of *S. nigrum* extract was assayed described by Nishikimi method (1992) [25]. The reaction mixture containing 1ml of nitroblue tetrazolium (NBT) solution (156µM NBT in 100 mM phosphate buffer, pH 8), 1ml of NADH solution (468 µM in 100 mM phosphate buffer, pH 8) and different concentration (2-10 µg/ml) of plant extract sample. The reaction was initiated by adding 100µl of PMS solution (60µM PMS in 10mM, Phosphate buffer, pH 8) and incubated at incubated at 25ºC for 5minutes. The change in absorbance was recorded spectrophotometrically at 560 nm. Percentage inhibition was calculated using following equation:

% superoxide anion inhibition = [Abs of control – Abs of test sample/Abs of control] ×100

Estimation of Lipid Peroxidation Inhibition

The lipid peroxidation scavenging ability of the extracts and standards (BHA) was evaluated by ammonium thiocyanate method [19, 20]. Different concentrations (10-100 µg/ml) of plant extract were prepared in aqua alcohol. About 0.5 mL of plant extract was mixed with 2.5 mL of linoleic acid emulsion (0.284 g of linoleic acid, 0.284 g of Tween 40, 50 mL of Phosphate buffer 0.02 M, pH 7.0) and 2.0 mL of phosphate buffer (0.2 M, pH=7.0) and incubated at 37 °C. After incubation aliquots of 0.1 mL from the reaction mixture was taken and mixed with 4.7 mL of 75 % aqua alcohol, 0.1 mL of 30 % ammonium thiocyanate and 0.1 mL of ferrous chloride (0.02 M in 3.5 % HCl). The resulted reaction mixture were mixed well and allowed to stand for 5 min. The absorbance of the reaction mixture spectrophotometrically measured at 500 nm. Percentage of lipid peroxidation inhibition was calculated as,

% lipid peroxide inhibition = (Abs of the control- Abs of the extract sample0/Abs of the control × 100

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging assay using plant extract was performed by Halliwell method [21], with slight changes. To this assay, 1.0 ml of the reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), 500 µl solution of various concentrations of the *S. nigrum* (10 to 100 µg/ml), 200 µl of 200 µM $FeCl_3$ and 1.04 mM EDTA (1:1 v/v), 100 µl H_2O_2 (1.0 mM) and 100 µl ascorbic acid (1.0 mM) and incubated at 37°C for 1 hour. The amount of deoxyribose degradation was measured by the TBA reaction [22]. Measure the absorbance at about 532 nm against the blank solution. Vitamin E was used as a positive control.

% hydroxyl inhibition = (Abs of the control- Abs of the extract sample0/Abs of the control × 100

Reducing Power

The reducing power of aqua alcohol extract of *S. nigrum* leaves was determined according to the method of Yen and Chen [23]. Different concentrations of extracts (100-500 µg/ ml) were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%w/v). The reaction mixtures were incubated at 50°C for 20 min, and add 2.5 ml of trichloroacetic acid (10% w/v) to terminate the reaction. The resulted reaction mixture was centrifuged at 3000 rpm for 10 min and collected the upper layer of the solution. A 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1 %, w/v). The absorbance of the reaction mixture was measured at 700 nm. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract. Ascorbic acid was used as positive control.

Statistical Analysis

All the *in vitro* experimental results were mean ± S.D of five parallel measurements.

RESULTS AND DISCUSSION

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of aqua alcohol extract of *S. nigrum* leaves exhibited a significant dose dependent i.e. concentration of plant extract between 2 -10 µg/ml. IC50 values calculated as 50% of inhibition by plant extract concentration. The results of DPPH inhibition by aqua alcohol extract of plant are shown in Figure 1. The 50% radical scavenging activity was observed at 3.85µg/ml concentration of aqua alcohol extract. The IC 50 value of the extract was highly significant than the standard (5.65µg/ml).

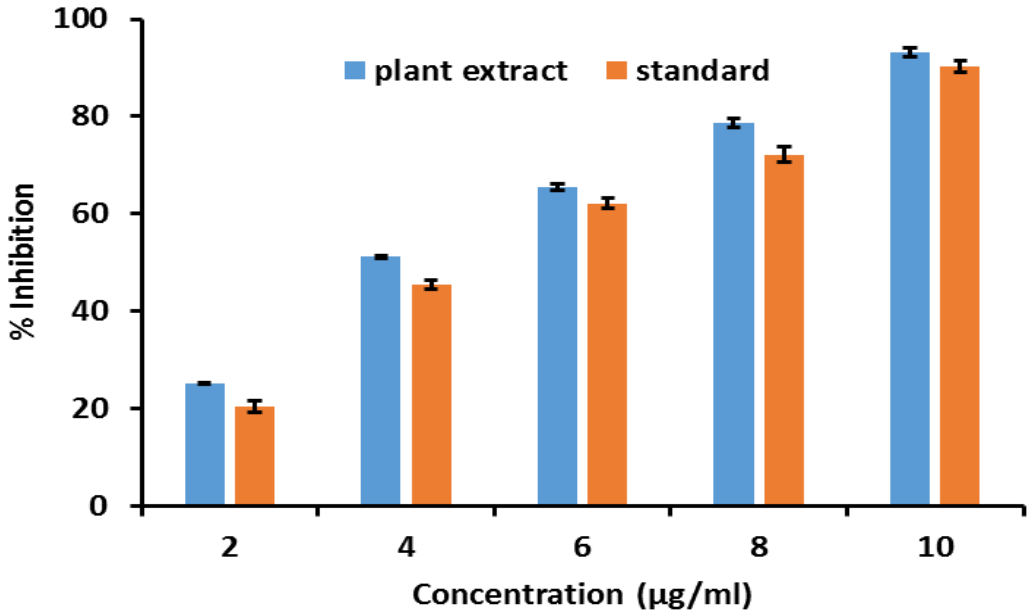

Fig 1: Effect of *S. nigrum* extract and Standard Vitamin C on Scavenging of DPPH radical Results are Mean ± S.D of five Parallel Measurements

Nitric Oxide Radical Inhibition Assay

The nitric oxide radical scavenging activity of plant extract was increased while increasing the plant extract concentration in a dose dependent manner (Figure 2). The IC50 value of the aqua alcohol extract was 22.45 µg/ml which was similar to that of standard (40.02 µg/ml). These results showed that aqua alcohol extract of *S. nigrum* leaves is known to be an excellent antioxidant agent.

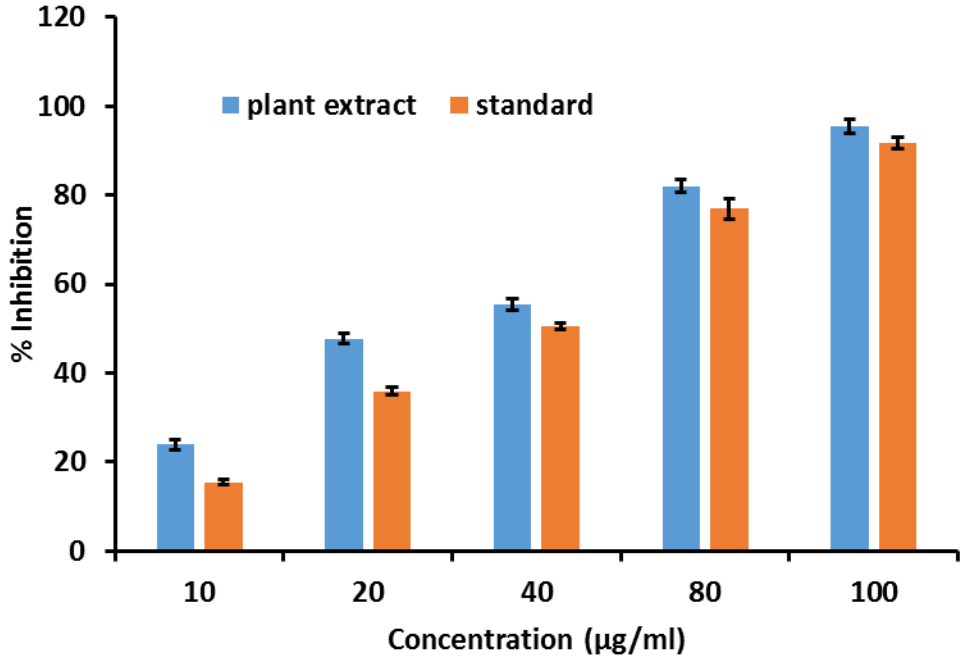

Fig : 2 Effect of *S. nigrum* Extract on Nitric Oxide Radical Inhibition Assay

Superoxide Anion Scavenging Activity

Superoxide anion free radical is a highly toxic radical that attacks a number of biological molecules. The superoxide anion derived from dissolved oxygen by Phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The results of superoxide anion scavenging activity of aqua alcohol extract of *S. nigrum* leaves presented in Figure 3. The decrease the absorbance at 560 nm with the plant extract thus indicates the consumption of superoxide anion in the reaction mixture. As mentioned in figure 3, the plant extract as well as curcumin showed the scavenging activity; IC50 values, 5.9 µg/ ml and 7.6 µg/ml, respectively. *S. nigrum* leaf extract efficiently scavenges the superoxide anion than the standards.

Lipid Peroxidation Assay

Lipid peroxidation inhibition assay was determined by using plant extract which compared with standard vitamin E. Figure 4 shows that the extracts have strong inhibiting activity in controlling lipid peroxidation. The scavenging activity was increased as increasing the concentration of plant extract from 10-100µg/ml. IC50 value was observed at 26.7 µg/ml had significantly 50 % inhibited lipid peroxidation than the standard.

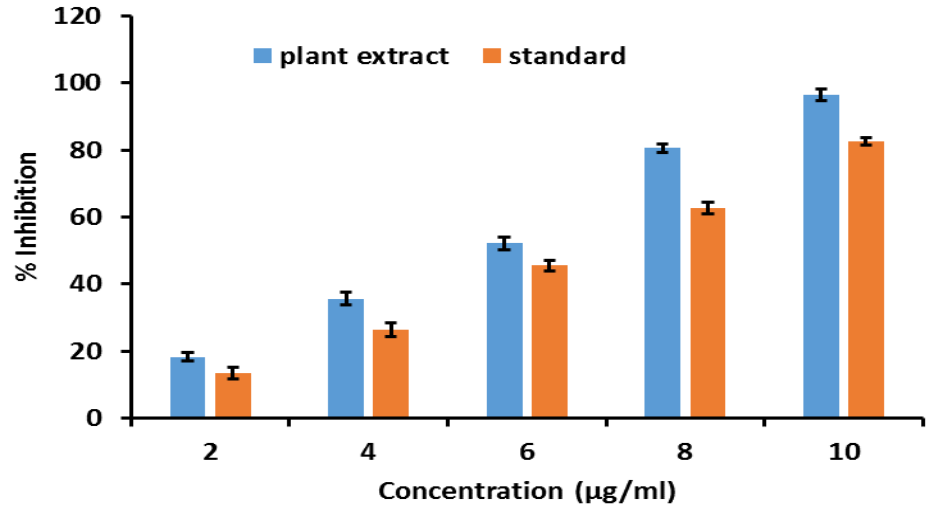

Fig 3: Superoxide Anion Scavenging Activity of *S. nigrum* Extract.

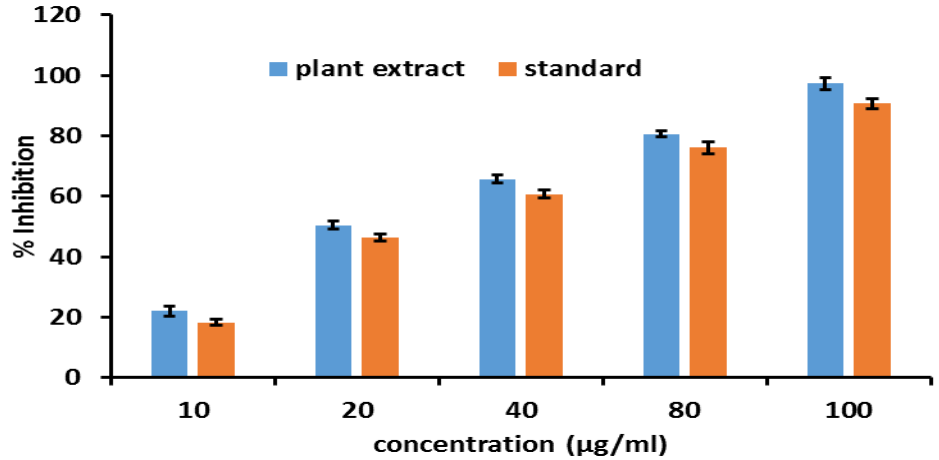

Fig 4: Lipid Peroxidation Assay of Aqua Alcoholic Extract of *S. nigrum*

Hydroxyl Radical Scavenging Assay

Figure 5 showed the aqua alcohol extract of *S. nigrum* plant leaves have high inhibition activity against hydroxyl radical. The scavenging activity of plant extract against hydroxyl radical was observed by deoxyribose assay in a concentration dependent manner. It showed hydroxyl radical scavenging activity with about 50 % at concentration of 30.0 µg/ml. The results are shown in Figure 5, the concentrations of 50% inhibition were found to be 30.0 µg/ml and 35.5 µg/ml for the extract and standard of vitamin E, respectively. The extract inhibition value was found to be lesser than the standard.

Reducing Power

The reducing power of plant extract was analyzed due to electron donating ability [24] (Blazovics et al 2003). Figure 6 shows the reducing capacity of the plant extract compared with butylated hydroxy toluene. The reducing power of extract of aqua alcoholic extract of *S. nigrum* was very effective and the activity was increased with increased concentration of sample. In the reaction mixture plant extract would reduce Fe^{3+} to Fe^{2+} by donating hydrogen. Thus aqua alcohol extract of *S. nigrum* exhibited excellent reducing activity.

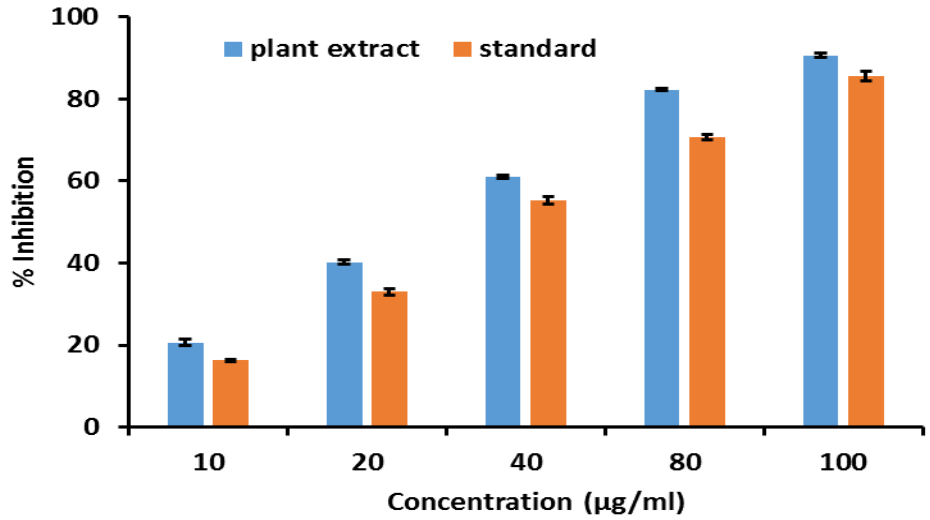

Fig 5: Hydroxyl Radical Scavenging Assay of Aqua Alcoholic Extract of *S. nigrum*

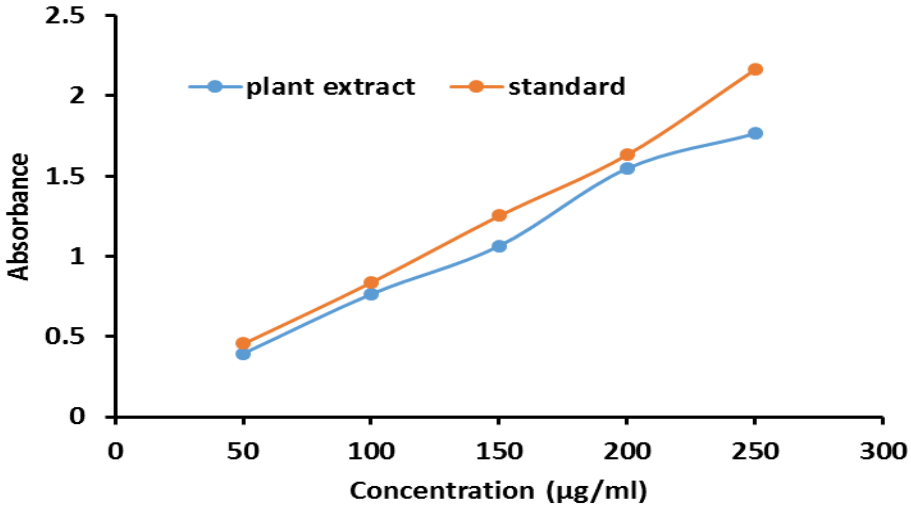

Fig 6: Reducing Power of Aqua Alcoholic Extract of *S. nigrum*

CONCLUSION

In this study, free radical scavenging activity of aqua alcohol extract of *Solanum nigrum* leaves was investigated. The results from this investigation indicates that *S. nigrum* exhibits excellent scavenging activity against free radicals such as DPPH, nitric oxide, superoxide anion, lipid peroxidase, hydroxyl and reducing power as compare to standard compounds. Extract of *S. nigrum* leaves scavenging free radicals with dose dependent manner. The present findings of the study suggested that *Solanum nigrum* could be a potential source of natural antioxidant that could replace the use of toxic synthetic antioxidants.

REFERENCES

1.Karlsson, J.,. Introduction to Nutraology and Radical Formation. *In: Antioxidants and Exercise*. Illinois: Human Kinetics Press, 1997;pp: 1-143.

2.Fahn, S. and G. Cohen,. The oxidative stress hypothesis in Parkinson's disease: Evidence supporting it. Ann. Neurol., 1992;32: 804-812.

3.Przedborski, S. and V. Jackson-Lewwis,. Experimental developents in movements disorders: update on proposed free radical mechanisms. Current Opinion Neurol., 1998;11: 335-339.

4. Valko M., et al. Free radicals and antioxidants in normal physiological functions and human disease, Int J Biochem Cell Biol. 2007; 39:pp. 44-84.

5. Jesberger, J.A. and J.S. Richardson,. Oxygen free radicals in brain dysfunction. Intl. J. Neurosci., 1991;57: 1-17.

6. Gülçin, M. Oktay, E. Kıreççi, I. Küfrevio lu, *Food. Chem.*, 2003, 83, 371-382.

7. Patel RM, Cibtech Journal of Bio-Protocols 2012;3:19-34.

8. Koksal, E., Gulcin, l.,. Antioxidant activity of cauliflower (Brassica oleracea L). Turk. J. Agric. For. 2008;32, 65–78.

9. Karthikkumar P, Kishore MP, Meenakshi M. Screening of antibacterial and antioxidant activities of leaves of *Eclipta prostrate* (L). *Scientific Research and Essay*, 2007, 2(4): 101-104.

10. Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. Antioxidant activity in some selected Indian medicinal plants. *African journal of Biotechnology*, 2008, 7(12): 1826-1828.

11. Kulisic, T., Radonic, A., Katalinic, V., Milosa, M., 2004. Use of different methods for testing antioxidative activity of oregano essential oil, analytical, nutritional and clinical methods. Food Chem. 85, 633–640.

12. Venkatesh R, Kalaivani K and Vidya R, Analysis of phytochemicals and free radical scavenging activity of *Solanum villosum* (Mill). - a traditional medicinal plant in southern India, World Journal of Pharmacy and Pharmaceutical Sciences, 2014;3(9): 741-755.

13. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Prindham JB, the relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad Res, 1995;22(4):375

14. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem, 2002; 76: 69-75.

15. Aqil F., I. Ahmed, Z. Mehmood, *Turk. J. Biol*., 2006, 30, 177-183

16. Polterat O. Antioxidants and free radical Scavengers of natural origin. Current Org. Chem. 1997;1: 415-440.

17. Jain SK. Medicinal Plants. *Thomson Press (India) Ltd*, 1968, 133-134.

18.Akthar, M.S. and Munir, M. Evaluation of antiulcerogenic effect of *S. nigrum*, *Brassica oleracea and Ocimim basilicum* in rats.J. Ethnopharm. 1989;27, 163-176.

19. Mitsuda H, Yasumoto K, Iwami K. Antioxidative action of indole compounds during the autoxidation of linoleic acid. Eiyo to Shokuryo 1966; 19: 210–214.

20. Yen GC, Hsieh CL. Antioxidant activity of extracts from Duzhong (*Eucommia ulmoides*) toward various lipid peroxidation models *in vitro*. J Sci Food Agri 1998; 46: 3952–3957.

21. Halliwell B, Gutteridge JM, Aruoma OI: The deoxyribose method:a simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. *Anal Biochem* 1987, 165:215-219.

22. Bouchet N, Barrier L, Fauconneau B: Radical Scavenging activity and antioxidant properties of tannins from *Guiera senegalensis* (Combretaceae). *Phytother Res* 1998, 12:159-162.

23.Yen GC and Chen HY. J Agri Food Chem 1995; 43(1): 27-32.

24. Blazovics, A., Lugasi, A., Szentmihalyi, K., Kery, A., *Acta Biological Szegediensis* 2003, 47, 99-102.

25.Nishikimi M., T. Kawai, K. Yagi, *J. Biol. Chem.*, 1992, 267, 21967-21972.