

Full Length Research Paper

## Antioxidant activity and total phenolic and flavonoid content of *Astragalus squarrosus* Bunge.

Jinous Asgarpanah<sup>1\*</sup>, Saeed Mohammadi Motamed<sup>2</sup>, Avishan Farzaneh<sup>1</sup>, Bahareh Ghanizadeh<sup>1</sup> and Simin Tomraee<sup>2</sup>

<sup>1</sup>Young Researchers Club (YRC), Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran, Iran.

<sup>2</sup>Department of Pharmacognosy, Pharmaceutical Sciences Branch, Islamic Azad University (IAU), Tehran, Iran.

Accepted 1 December, 2011

The antioxidant capacity of the flowering aerial parts of *Astragalus squarrosus* was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric thiocyanate methods. The phenolic and flavonoid content was also measured. *A. squarrosus* showed weak free radical scavenging activity with the DPPH method which might be due to its low phenolic constituents (23.3 mg/g) and flavonoid content (26.0 mg/g). Results obtained from ferric thiocyanate (FTC) assay revealed that the extract has antioxidative potential for chain-breaking inhibition of lipid peroxidation as it showed 69.0% inhibition when compared with butylated hydroxytoluene (BHT) (78.0% inhibition).

**Key words:** *Astragalus squarrosus*, antioxidant, phenolics, flavonoids.

### INTRODUCTION

There is increasing evidence indicating that reactive oxygen species (ROS) and free radical mediated reactions are involved in degenerative or pathological events such as aging, cancer, coronary heart ailments and Alzheimer's disease (Sun et al., 2004). Epidemiological studies have consistently shown that there is a clear significant positive association between the intake of fruits and vegetables and a reduced rate of heart disease, mortality, common cancers and other degenerative diseases as well as aging, and this is attributed to the fact that these foods may provide an optimal mix of phytochemicals such as natural antioxidants, fibres and other biotic compounds (Kaur and Kapoor, 2001).

Phenolic and flavonoid compounds are widely distributed plant constituents. They have traditionally been believed to play an important role in plant-herbivore interactions (Feeny, 1976; Swain, 1979). Plant phenolics have been implicated in food selection by many species

of animals as diverse like elephants (Jachmann, 1989) and ants (Seaman, 1984). Phenolics and flavonoids are important economically as agents for certain medicinal purposes. Medicinal values of these compounds are well known for a long time (Farnsworth, 1966).

*Astragalus* L. (Fabaceae) is generally considered as the largest genus of vascular plants with an estimated 2500 to 3000 species. *Astragalus* is widely distributed in temperate regions of the Northern Hemisphere. The greatest numbers of species are found in the arid, continental regions of western North America (400 species) and central Asia (2000 to 2500 species). Many species of *Astragalus* are useful as forage plants, to control erosion, as ornamentals or as medicinal plants (Hirovani et al., 1994; Baratta and Ruberto, 1997). The interest in chemical constituents of various species of the genus *Astragalus* has been increasing during the recent years. Many species of *Astragalus* have been investigated chemically for flavonoids, non-protein amino acid, saponins, alkaloids, nitro compounds, mucilages, sterols, etc. (Ebrahimzadeh et al., 2001).

The present report describes the total phenolics and flavonoids content of flowering aerial parts of *Astragalus squarrosus*, a plant native to the south, southeast and central parts of Iran. Since there is no evidence of the biological activity of this plant and also due to the

\*Corresponding author. E-mail: [asgarpanah@iaups.ac.ir](mailto:asgarpanah@iaups.ac.ir). Tel: 021 22640051. Fax: 021 22602059.

**Abbreviations:** DPPH, 1,1-Diphenyl-2-picrylhydrazyl; FTC, ferric thiocyanate; BHT, butylated hydroxytoluene.

interaction between phenolics and flavonoids content and the antioxidant property, we were prompted to determine the antioxidant activity of *A. squarrosus* by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric thiocyanate methods for the first time.

## MATERIALS AND METHODS

### Chemicals and general experimental procedure

Folin Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate and the other chemicals used in this experiment were of analytical grade purchased from Sigma-Aldrich.

*A. squarrosus* was collected from Golpayegan (Isfahan province, Iran) in May 2011 and identified by Dr. G. H. Amin at the Pharmacognosy Department, Islamic Azad University (IAU), Pharmaceutical Sciences Branch, Tehran, Iran. A voucher specimen (No. 343) has been deposited in the herbarium of the Department of Pharmacognosy, Islamic Azad University (IAU), Pharmaceutical Sciences Branch, Tehran, Iran.

### Extraction

300 g dried ground material was extracted by percolator apparatus using 3 L methanol (Merck). The extraction was repeated for 3 times. The extracts were concentrated by rotary evaporator apparatus and the solvent removed to produce a dark green gummy solid. The yield was 63 g total methanol extract. The resulting extract was kept in a clean vial in a dark and cool place for further test study.

### DPPH assay

The antioxidant activity of the extract and the standard was assessed on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity by modified method (Blois, 1958). The diluted working solutions of the test extract were prepared in methanol. BHT (butylated hydroxytoluene) was used as standard in 4 to 250 mg/L solution. 0.1 mM of DPPH was prepared in methanol and 3 ml of this solution was mixed with 1 ml of different concentrations of sample solution (600 to 3000 mg/L) and standard solution separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using Shimadzu Spectrophotometer. Methanol (1 ml) with DPPH solution (0.1 mM, 3 ml) was used as control. The optical density was recorded and % inhibition was calculated using the formula given below (Bors et al., 1992):

$$\text{Inhibition of DPPH activity (\%)} = (A - B / A) \times 100$$

Where, A is the optical density of the control and B is the optical density of the sample.

### Ferric thiocyanate (FTC) method

The antioxidant activity of the methanolic extract of *A. squarrosus* on inhibition of lipid peroxidation was determined according to the ferric thiocyanate method as reported by Kikuzaki and Nakatani (1993). A mixture containing the extract (4 ml) in absolute ethanol, final concentration: 200 µg/ml, 2.51% linoleic acid in absolute ethanol (4.1 ml), 0.05 M phosphate buffer pH 7 (8 ml) and distilled water (3.9 ml) was placed in a vial with a screw cap, and then

placed in an oven at 40 °C in the dark. To this solution (0.1 ml) was added 75% ethanol (9.7 ml) and 30% ammonium thiocyanate (0.1 ml). Three minutes after adding 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.1 ml) to the reaction mixture, the absorbance of red color was measured at 500 nm, each 24 h until one day after absorbance of the control (without sample) reached maximum. BHT was used as standard. % Inhibition of lipid peroxidation is calculated by equation:

$$\text{Inhibition (\%)} = (A_c - A_s / A_c) \times 100$$

Where,  $A_s$  is the absorbance of the sample on the day when the absorbance of the control is maximum and  $A_c$  is the absorbance of the control on the day when the absorbance of the control is maximum.

### Determination of flavonoids and phenolic compounds

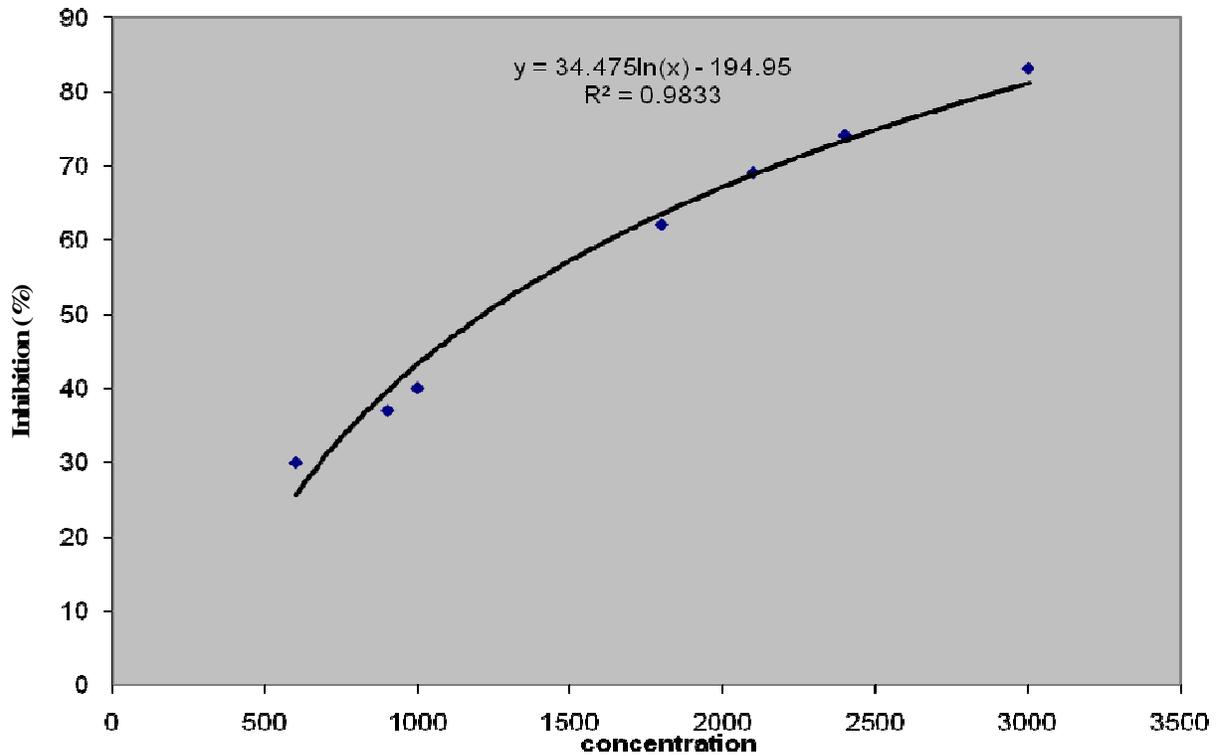
The content of total phenolic compounds in plant ethanolic extracts was determined by Folin–Ciocalteu method (1927). One millilitre of 0.01 g/ml methanolic plant extract was mixed with 5 ml Folin–Ciocalteu reagent (diluted tenfold with distilled water) and 4 ml (7.5 g/100 ml) sodium carbonate. After 1 h at room temperature, the absorption of clear solutions was read at 765 nm. For the preparation of calibration curve, different concentrations of ethanolic gallic acid solutions were mixed with the same reagents as described above, and after 30 min, the absorption of clear solutions was measured. The amount of total phenolic compounds was expressed as gallic acid equivalents (GAE) in milligrams per gram dry plant extract. The experiment was repeated thrice and the mean value was reported.

The content of flavonoids was determined using the aluminium chloride colorimetric method. One milliliter of 0.01 g/ml methanolic plant extract was mixed with 1 ml of 2%  $\text{AlCl}_3$  ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm. The results were expressed in mg rutin in g dry matter by comparison with standard rutin treated with the same conditions (Kumazawa et al., 2004).

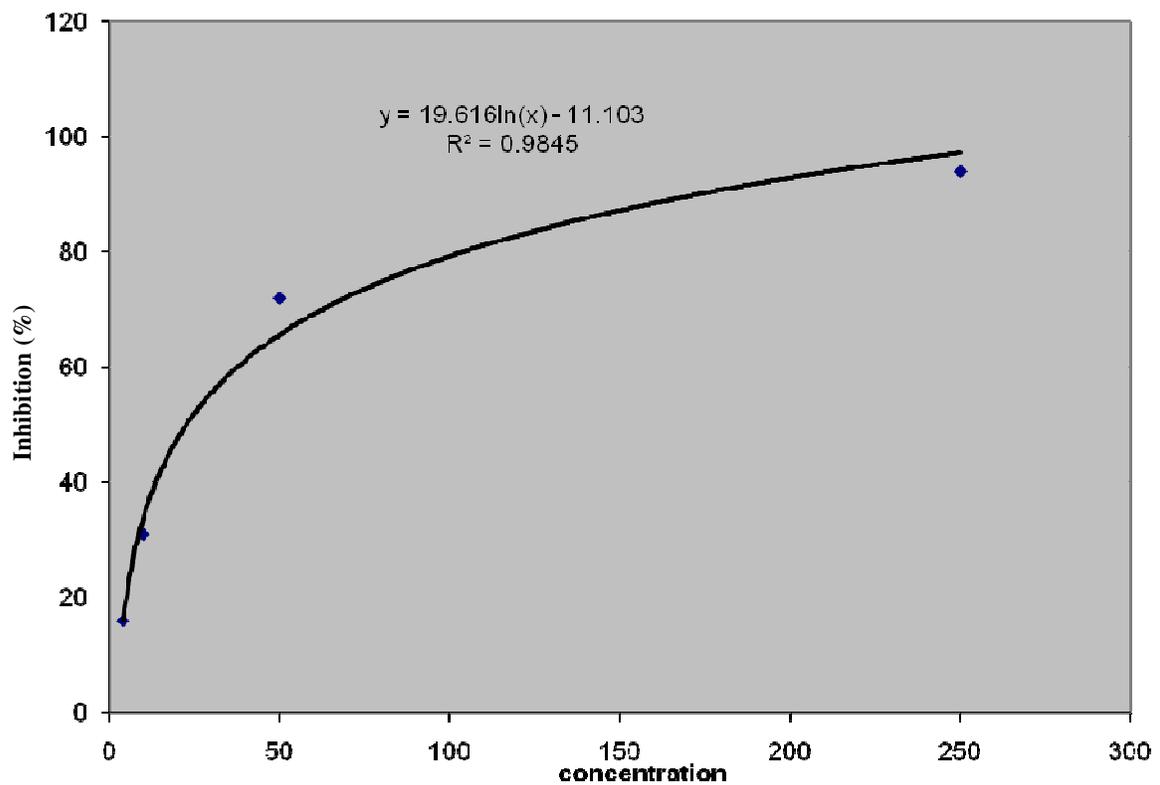
## RESULTS AND DISCUSSION

The DPPH radical scavenging activity of *A. squarrosus* is given in Figure 1. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The  $\text{IC}_{50}$  value of the extract was 1220 mg/L, as opposed to that of BHT ( $\text{IC}_{50} = 23$  mg/L), which is a well known antioxidant (Figure 2). From the above results, it can be concluded that the methanolic extract of *A. squarrosus* does not possess a potent antiradical activity when compared with BHT.

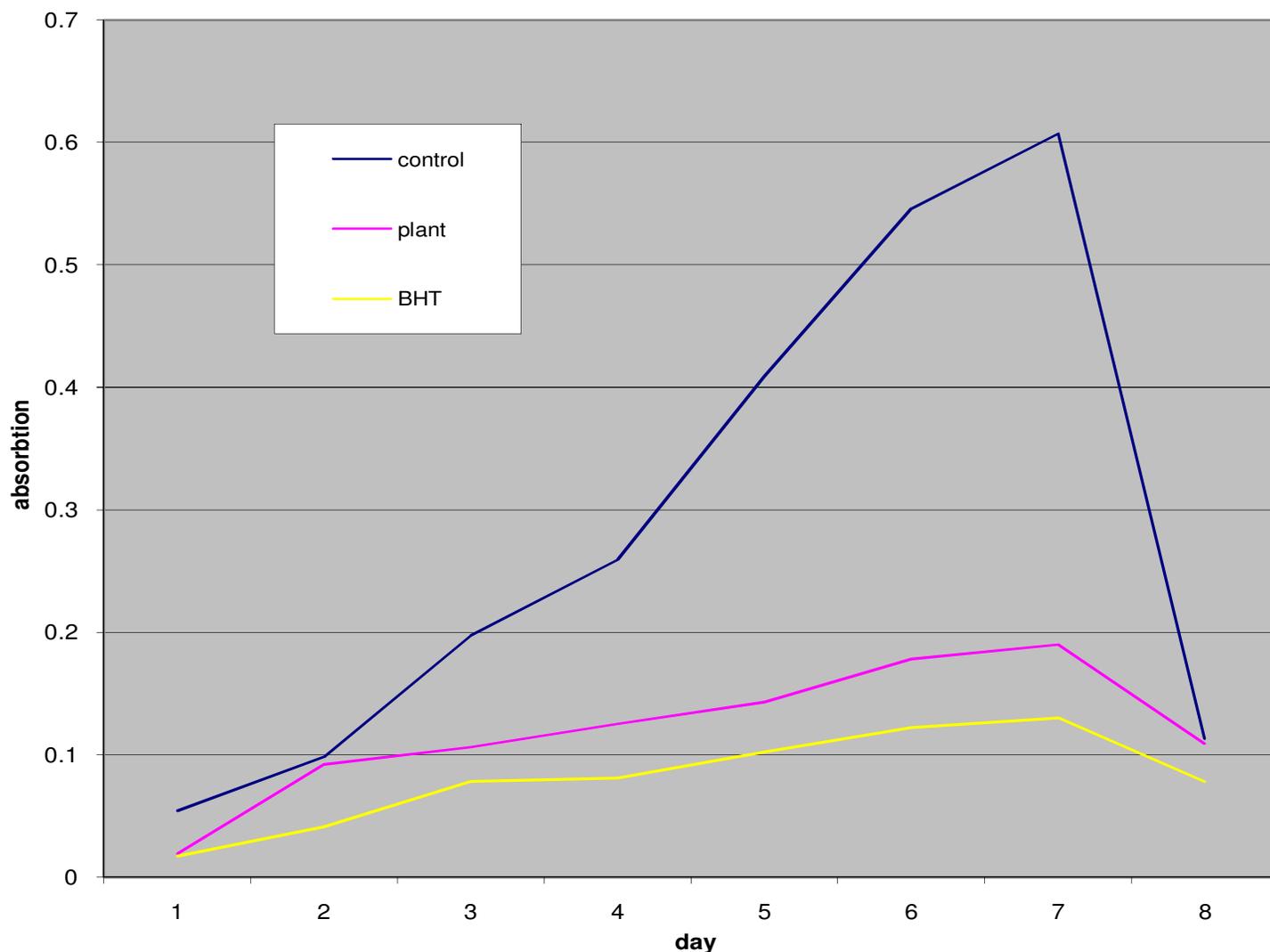
Ferric thiocyanate method was originally designed for measuring lipid peroxide content, whereby the endpoint measure is the amount of  $\text{Fe}^{2+}$  that is oxidized to  $\text{Fe}^{3+}$  by



**Figure 1.** DPPH radical scavenging activity of the methanolic extract of *A. squarrosus*. Values are the average of triplicate experiments.



**Figure 2.** DPPH radical scavenging activity of BHT. Values are the average of triplicate experiments.



**Figure 3.** Antioxidant activity of *A. squarrosus* extract by FTC method. A low absorbance value represents a high level of antioxidant activity.

lipid peroxides. The  $\text{Fe}^{3+}$ -thiocyanate complex produces a deep red color, which is detectable at 500 nm. The advantage of using ammonium thiocyanate over other coloring reagents is that binding of iron by thiocyanate ion is specific to  $\text{Fe}^{3+}$  only, and that the  $\text{Fe}^{3+}$ -thiocyanate complex produces a single absorbance peak at 500 nm. Results obtained from FTC assay (Figure 3) revealed that *A. squarrosus* extract carries the antioxidative potential for chain-breaking inhibition of lipid peroxidation as it has shown 69.0% inhibition when compared with BHT (78.0% inhibition).

Total phenolics concentration equivalents of gallic acid was estimated. Gallic acid being the most important polyphenol in natural products was used to determine the phenolics of *A. squarrosus* which is found to be 23.3 mg/g in plant extract. Total flavonoid content equivalents of rutin was estimated. Rutin being one of the most important flavonoid in natural products was used to

determine the flavonoid content of *A. squarrosus* which is found to be 26.0 mg/g in plant extract.

*A. squarrosus* showed low free radical scavenging activity which might be due to its low phenolic constituents and flavonoid content when compared with plants belonging to Lamiaceae family (a plant family with high flavonoid and phenolics content), especially that only some flavonoids with specific molecular structure show radical scavenging activity. On the other hand, results of studies on the antioxidant activity of the roots of *A. squarrosus* show significant inhibitory effect on DPPH radical, due to high polysaccharide content in the roots (Hao and Zhaobao, 2010), therefore weak free radical scavenging activity of *A. squarrosus* might be related to the lack of polysaccharides in the aerial parts of the plant as well.

Although, flavonoids can inhibit lipid peroxidation (Haraguchi, 2001), there are some reports that terpenoids

have more potent activity in lipid peroxidation inhibition (Olatunde et al., 2003; Pietri et al., 1997). For the lipid peroxidation inhibitory effects, besides the structural effects, other factors, such as the hydrophobicity of the compounds should be taken into account (Silva et al., 2005). There are various types of phytochemicals, including flavonoids, coumarins, xanthenes, phenylpropanoids and terpenoids, which are effective in preventing lipid peroxidation (Haraguchi, 2001). Synergistic effects of different compounds, existing in the extract of *A. squarrosus* might be responsible for their significant activities against lipid peroxidation.

Detailed determination of active metabolites in the plant extracts is therefore required for the comprehensive assessment of individual compounds showing DPPH scavenging and lipid peroxidation inhibition activity of *A. squarrosus*.

## ACKNOWLEDGEMENTS

Financial support from Young Researchers Club, Islamic Azad University (IAU), Pharmaceutical Sciences Branch, Tehran, Iran is gratefully acknowledged.

## REFERENCES

- Baratta FA, Ruberto G (1997). Cycloartane triterpene glycosides from *Astragalus siculus*. *Planta Med.* 63: 280-282.
- Blois MS (1958). Antioxidant determination by the use of a stable free radical. *Nature*, 181: 1198-1200.
- Bors W, Saran M, Elstner EF (1992). Screening for plant antioxidants. In: Springer, Berlin; *Modern Methods of Plant Analysis-Plant Toxin Anal.* 13: 277-295.
- Ebrahimzadeh H, Niknam N, Maassoumi AA (2001). The sterols of *Astragalus* species from Iran: GLC separation and quantification. *Biochem. Syst. Ecol.* 9: 393-404.
- Farnsworth NR (1966). Biological and phytochemical screening of plants. *J. Pharm. Sci.* 55: 25-76.
- Feeny P (1976). Plant apparency and chemical defense. *Recent Adv. Phytochem.* 10: 1-40.
- Folin O, Ciocalteu V (1927). On tyrosine and tryptophane determination in proteins. *J. Biol. Chem.* 27: 627-650.
- Hao S, Zhaobao W (2010). Effects on exercise endurance capacity and antioxidant properties of astragalus membranaceus polysaccharides (APS). *J. Med. Plants Res.* 4(10): 982-986.
- Haraguchi H (2001). Antioxidative plant constituents. In *Bioactive compounds from natural sources*, first published. New York: Taylor and Francis Inc. pp. 338-377.
- Hirotsani M, Zhou, Lui H, Furuya T (1994). Astragalosides from hairy root cultures of *Astragalus membranaceus*. *Phytochem.* 36: 665-670.
- Jachmann H (1989). Food selection by elephants in the "miombo" biome, in relation to leaf chemistry. *Biochem. Syst. Ecol.* 17: 5-24.
- Kaur C, Kapoor HC (2001). Antioxidants in fruits and vegetables – the millennium's health. *Int. J. Food, Sci. Technol.* 36: 703-725.
- Kikuzaki H, Nakatani N (1993). Antioxidant effects of some ginger constituents. *J. Food Sci.* 58, 1407-1410.
- Kumazawa S, Hamasaka T, Nakayama T (2004). Antioxidant activity of propolis of various geographic origins. *Food Chem.* 84: 329-339.
- Olatunde Farombi E, Ogundipe OO, Uhunwangho ES, Adeyanju MA, Olarenwaju Moody J (2003). Antioxidant properties of extracts from *Alchornea laxiflora* (Benth) Pax and Hoffman. *Phytother. Res.* 17: 713-716.
- Pietri S, Maurelli E, Drieu K, Culcasi M (1997). Cardioprotective and antioxidant effects of the terpenoid constituents of *Ginkgo biloba* extract. *J. Mol. Cell. Cardiol.* 29: 733-742.
- Seaman FC (1984). The effect of tannic acid and other phenolics on the growth of the fungus cultivated by the leaf cutting ant, *Myrmicorypta buenzlii*. *Biochem. Syst. Ecol.* 12: 155-158.
- Silva BA, Ferreres F, Malva JO, Dias AC (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.* 90: 157-167.
- Sun C, Wang J, Fang L, Gao X, Tan R (2004). Free radical scavenging and antioxidant activities of EPS2, an exopolysaccharide produced by a marine filamentous fungus *Keissleriella* sp. YS 4108. *Life Sci.* 75: 1063-1073.
- Swain T (1979). Tannins and lignins. In: *Herbivores: Their Interaction with Secondary Plant Metabolites.* (Eds.): G. Rosenthal and D.H. Janzen. Academic Press, New York, pp. 657-682.