Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article

In vitro antioxidant activity of *Asparagus racemosus* root.

S.Velavan, K. Nagulendran, R. Mahesh, V. Hazeena Begum*

Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur 613 005, Tamilnadu, India.

**Author for Correspondence: E-mail address: drhazeena06@yahoo.co.in*

ABSTRACT - Free radicals are implicated for more than 80 diseases including Diabetes mellitus, arthritis, cancer, ageing. etc. In treatment of theses diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin. In Indian system of medicine *Asparagus racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic. To understand the mechanisms of pharmacological actions, the in vitro antioxidant activity of aqueous extract of *Asparagus racemosus* was investigated for activity of scavenging superoxide anion radicals, hydroxyl radical, nitric oxide radical, and hydrogen peroxide, metal chelation and reducing power. The extract was also studied for lipid peroxidation assay using young and aged rat brain mitochondria. In all the testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals, metal chelation, reducing power or inhibition of lipid peroxidation. The antioxidant property may be related to the antioxidant vitamins, phenolic acids and micronutrients present in the extract. These results clearly indicate that *Asparagus racemosus* is effective against free radical mediated diseases

KEYWORDS - Antioxidant activity, *Asparagus racemosus*, radical scavenging, lipid peroxidation.

INTRODUCTION

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals (1). Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (2). The most common reactive oxygen species (ROS) include superoxide ($0_2^{\cdot}$) anion, hydrogen peroxide (H_20_2), peroxyl ($ROO^{\cdot}$) radicals, and reactive hydroxyl ($OH^{\cdot}$) radicals. The nitrogen derived free radicals are nitric oxide ($NO^{\cdot}$) and peroxynitrite anion ($ONOO^{\cdot}$). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (3). In treatment of these diseases, antioxidant therapy has gained an immense importance. Current research is now directed towards finding naturally occurring antioxidants of plant origin.

Antioxidants have been reported to prevent oxidative damage by free radical and ROS, and may prevent the occurrence of disease, cancer and aging. It can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals, and also by acting as oxygen scavengers (4, 5). Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (6). Flavonoids and phenolic compounds widely distributed in plants which have been reported to exert multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic. etc. (7). They were also suggested to be a potential iron chelator (8, 9). Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidant and radical scavenging properties.

In Indian system of medicine *Asparagus racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic (10-12). *Asparagus racemosus* (Liliaceae) (Eng: Willd asparagus, Tamil: Thanner Vittan Kizhangu,) is a well known ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function, vigor and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, hyperdipsia, neuropathy, hepatopathy (13). Reports indicate that the pharmacological activities of *A. racemosus* root extract (ARRE) include antiulcer (14), antioxidant (15), anti-diarrhoeal (16, 17), antidiabetic (18) and immunomodulatory activities (19). ARRE contains saponins (20), alkaloids (21), polysaccharides (15), polyphenols, favonoids and

vitamin-C (16, 17). *Asparagus racemosus* has also been reported to posess adaptogenic activity (22).

Therefore, the objectives of the present study were to investigate the in vitro antioxidant activity of *Asparagus racemosus* root through the free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, nitric oxide scavenging, metal chelation, reducing power and inhibition of lipid peroxidation in young and aged rats.

MATERIALS AND METHODS

Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio- barbituric acid (TBA), potassium hexa cyano ferrate ($K_3Fe(CN)_6$), and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Preparation of aqueous extract of Asparagus racemosus

The roots of the *Asparagus racemosus* were collected from the kolli hills at Namakkal district, Tamil Nadu,. The collected roots were identified and authenticated by a botanist prof. Dr. S. Jegadeesan, Department of Environmental and Herbal science, Tamil University, Thanjavur, Tamil Nadu. A Voucher specimen (Specimen no: 29) has been deposited at the Herbarium of our department. The roots were cut into small pieces and shade dried at room temperature for 15 days, finely powdered and used for extraction. A required quantity of the powder (5g) was suspended in distilled water (600ml). The suspension was boiled until the quantity was reduced to 100ml. The resultant decoction was cooled and used in the present study. The concentration of resultant decoction was 50 mg/mL.

Total polyphenolic compounds

Total polyphenolic compounds were determined according to a protocol similar to that of Chandler and Dodds (23). *Asparagus racemosus* (1 ml) was mixed with 1 ml of 95% ethanol, 5 ml of distilled water and 0.5 ml of 50% Folin–Ciocalteu reagent. The mixture was allowed to react for 5 min and 1 ml of 5% Na_2CO_3 was added. Thereafter, it was thoroughly mixed and placed in the dark for 1 h and the absorbance was measured at 725 nm using UV–vis spectrophotometer. A gallic acid standard curve was obtained for the calculation of polyphenolic content. The concentration of polyphenols was expressed in terms of mg/100ml of sample.

Determination of ascorbic acid (vitamin C)

5 g of the sample was weighed into an extraction tube and 100 ml of EDTA/TCA (2:1) extracting solution were mixed and the mixture was shaken for 30 min, and transferred into a centrifuge tube and centrifuged at 800g for about 20 min. It was transferred into a 100 ml volumetric flask and made up to 100 ml mark with the extracting solution. 20 ml of the extract was pipetted into a volumetric flask and 1% starch indicator was added and titrated with 20% $CuSO_4$ solution to get a dark end point (24). The amount of vitamin C was calculated as mg/100 ml of plant sample.

Determination of Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination (25). 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The concentration of flavonoids was expressed in terms of mg/100ml of sample.

Superoxide anion scavenging activity assay

The scavenging activity of the *Asparagus racemosus* towards superoxide anion radicals was measured by the method of Liu, Ooi, and Chang (26). Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH, and oxygen. It was assayed by the reduction of nitroblue tetrazolium (NBT). In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 μM) solution, 0.75 ml of NADH (936 μM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 μM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer. The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Hydroxyl radical scavenging activity assay

The scavenging activity for hydroxyl radicals was measured with Fenton reaction (27). Reaction mixture contained 60 µl of 1.0mM $FeCl_3$, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2, and 1.5 ml of extract at various concentrations. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture at 560nm was measured with a spectrophotometer. The hydroxyl radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (28). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25°C for 150 min. From the incubated mixture 0.5 ml was taken out and added into 1.0 ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0\text{-}A_1) / A_0 \times 100)$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Hydrogen peroxide scavenging activity assay

Hydrogen peroxide scavenging activity of the extract was estimated by replacement titration (29). Aliquot of 1.0 ml of 0.1 mM H_2O_2 and 1.0 ml of various concentrations of extracts were mixed, followed by 2 drops of 3% ammonium molybdate, 10 ml of 2 M H_2SO_4 and 7.0 ml of 1.8 M KI. The mixed solution was titrated with 5.09 mM NaS_2O_3 until yellow color disappeared. Percentage of scavenging of hydrogen peroxide was calculated as:

$$\% \text{ Inhibition} = (V_0 - V_1) / V_0 \times 100$$

Where V_0 was volume of NaS_2O_3 solution used to titrate the control sample in the presence of hydrogen peroxide (without extract), V_1 was the volume of NaS_2O_3 solution used in the presence of the extract.

Fe^{2+} chelating activity assay

The chelating activity of the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis, Madeira, and Almeidam (30). To 0.5 ml of extract, 1.6 ml of deionized water and 0.05 ml of $FeCl_2$ (2 mM) was added. After 30 s, 0.1 ml ferrozine (5 mM) was added. Ferrozine reacted with the divalent iron to form stable magenta complex species that were very soluble in water. After 10 min at room temperature, the absorbance of the Fe^{2+}-Ferrozine complex was measured at 562 nm. The chelating activity of the extract for Fe^{2+} was calculated as:

$$\text{Chelating rate } (\%) = (A_0 - A_1) / A_0 \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract.

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu (31) with slight modifications. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power.

Lipid peroxidation inhibition assay using young and aged rat brain mitochondria

Young (3-4 months, 120-150 g) and aged (22-24months, 380-410g) Wistar albino rats were anaesthetized with Thiopentone sodium (50 mg /kg). Brain was excised and washed with 0.95 NaCl solution. Tissue homogenate was prepared in ice-cold 3 mM Tris buffer

containing 250 mM sucrose and 0.1 mM EDTA (pH 7.4). Differential ultra centrifugation and their protein content characterized the mitochondrial fraction. The inhibition of lipid peroxidation assay was determined according to the method of Okhawa, Ohishi, & Yagi (32) with minor modifications: 0.25 ml of mitochondria was mixed with 1.25 ml Tris-HCl buffer (pH 7.2), 1.0 ml 15 mM $FeSO_4$ solution and 0.5ml of extract at various concentrations. The mixture was incubated at 37^oC for 1 h, 0.1 ml of this reaction mixture was taken in a tube containing 1.5 ml 10% TCA. After 10 min tubes were centrifuged and supernatant was separated and mixed with 1.5 ml of 0.67% TBA in 50% acetic acid. The mixture was heated in a hot water bath at 85^oC for 30 min to complete the reaction. The intensity of pink colored complex formed was measured at 535nm. The values of MDA were expressed as nmol/mg of protein.

Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- windows based graphpad Instat (version 3) software. Results were expressed as graphically / mean± standard deviation.

RESULTS AND DISCUSSION

The phytochemical analysis of *Asparagus racemosus* root extract contain rich source of flavonoids 36.7±3.9 mg/100ml, polyphenol 88.2±9.3 mg/100ml and Vitamin-C 42.4 ± 5.1 mg/100ml. Polyphenol and flavonoids used for the prevention and cure of various diseases which is mainly associated with free radicals (9). Vitamin C acts as an antioxidant in biological systems and scavenge the free radicals thereby increase the antioxidant defence in the body. The effect of vitamin C and other putative antioxidants on biomarkers of oxidation have been studied in many pathological states that are thought to result from, or result in oxidative stress (32).

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (33). The superoxide anion radical scavenging activity of the extract from *Asparagus racemosus* assayed by the PMS-NADH system is shown in Table 1. The superoxide scavenging activity of *Asparagus racemosus* was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Asparagus racemosus* was 0.055 mg/ml. These results suggested that *Asparagus racemosus* had important superoxide radical scavenging effect.

Hydroxyl radical scavenging activity

Hydroxyl radical is very reactive and can be generated in biological cells through the Fenton reaction. Table 1 showed the *Asparagus racemosus* exhibited concentration dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system. The IC_{50} of *Asparagus racemosus* was 0.103 mg/ml. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution. Similarly, high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by specific functional groups (34).

Nitric oxide radical scavenging activity

Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities (35). *Asparagus racemosus* extract moderately inhibited nitric oxide in dose dependent manner (Table 1) with the IC_{50} was 0.898 mg/ml.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membranes rapidly, once inside the cell, H_2O_2 can probably react with Fe^{2+}, and possibly Cu^{2+} ions to form hydroxyl radical and this may be the origin of many of its toxic effects (36). It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. As shown in Fig. 1, *Asparagus racemosus* extract demonstrated hydrogen peroxide scavenging activity in a concentration dependent manner with an IC_{50} of 0.984 mg/ml.

The ferrous ion chelating activity

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of aqueous extract of *Asparagus racemosus*,

Table 1 - Radical scavenging activity of aqueous extract of Asparagus racemosus at different concentrations.

Concentration (µg/ml)	Superoxide radical scavenging %	Hydroxyl radical scavenging %	Nitric oxide radical scavenging %
10	30.25 ± 2.46	25.45 ± 3.15	11.25 ± 0.78
50	54.68 ± 2.78	45.56 ± 2.86	17.36 ± 2.12
100	86.96 ± 3.01	58.18 ± 2.69	21.53 ± 2.56
250	-	69.09 ± 3.15	29.12 ± 2.14
500	-	81.82 ± 2.87	35.68 ± 2.74
750	-	92.73 ± 3.22	44.36 ± 2.32
1000	-	94.55 ± 2.98	52.36 ± 2.41
IC_{50} (mg/ml)	0.055	0.103	0.898

Values are means ± SD (n=3).

Table 2 - Ferrous sulphate induced lipid peroxidation inhibition of aqueous extract of Asparagus racemosus at different concentrations in young and aged rat mitochondria.

Concentration (µg/ml)	MDA (nmol mg protein) Young	Aged
10	4.23 ± 0.25	7.76 ± 0.19
50	3.63 ± 0.34	6.55 ± 0.42
100	3.36 ± 0.19	5.72 ± 0.33
250	3.11 ± 0.22	5.23 ± 0.28
500	2.98 ± 0.31	4.82 ± 0.34
750	2.88 ± 0.42	4.69 ± 0.31
1000	2.79 ± 0.24	4.65 ± 0.27

Values are means ± SD (n=3).

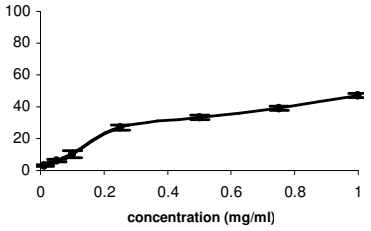

Fig. 1. H_2O_2 scavenging activity of Asparagus racemosus aqueous extract at different concentrations. Each value represents means ± SD (n=3).

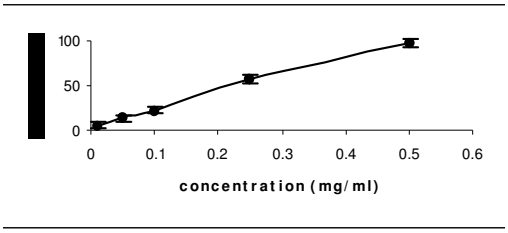

Fig. 2. Ferrous ion chelating activity of Asparagus racemosus aqueous extract at different concentrations. Each value represents means ± SD (n=3).

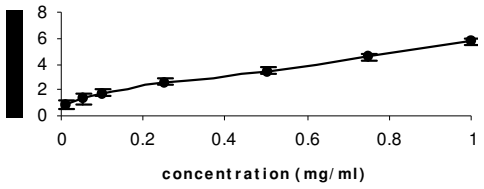

Fig. 3. Reducing power of Asparagus racemosus aqueous extract at different concentrations. Each value represents means ± SD (n=3). High absorbance at 700 nm indicates high reducing power.

indicating that have chelating activity with an IC_{50} of 0.241 mg/ml (Fig. 2). Ferrous iron can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydro-peroxides into peroxyl and alkoxyl radicals (37). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form s bonds with a metal are effective as secondary antioxidants because they reduce the redox potential, and thereby stabilize the oxidized form of the metal ion (38). Thus, *Asparagus racemosus* demonstrate a marked capacity for iron binding, suggesting their ability as a peroxidation protector that relates to the iron binding capacity.

Reducing power activity

For the measurements of the reducing ability, the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of *Asparagus racemosus*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (39- 41). Fig. 3 depicts the reductive effect of *Asparagus racemosus* Similar to the antioxidant activity, the reducing power of *Asparagus racemosus* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Asparagus racemosus* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Lipid peroxidation inhibition assay

Initiation of the lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex or through ·OH radical by Fenton's reaction. Table 2 shows that the *Asparagus racemosus* extract inhibited $FeSO_4$ induced lipid peroxidation in young and aged rat mitochondria as a dose dependent manner. The inhibition could be caused by absence of ferryl-perferryl complex or by scavenging the ·OH radical or the superoxide radicals or by changing the Fe^{3+}/Fe^{2+} or by reducing the rate of conversion of ferrous to ferric or by chelating the iron itself. Iron catalyses the generation of hydroxyl radicals from hydrogen peroxide and superoxide radicals. The hydroxyl radical is highly reactive and can damage biological molecules, when it reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids, lipid hydro-peroxides is produced (42). Lipid hydro-peroxide can be decomposed to produce alkoxy and peroxy radical which eventually yield numerous carbonyl products such as malondialdehyde (MDA). The carbonyl products are responsible for DNA damage, generation of cancer and aging related diseases (32). Thus the decrease in the MDA level in young and aged rat with the increase in the concentration of the extract indicates the role of the extract as an antioxidant.

In conclusion, the results of the present study show that the extract of *Asparagus racemosus* root which contains highest amount of flavonoids, polyohenols and vitamin-C exhibits the greatest antioxidant activity through the scavenging of free radicals such as superoxide, hydroxyl radical, hydrogen peroxide and nitric oxide, which participate in various pathophysiology of diseases including ageing. ARRE also exert iron chelating and reducing power activity. Overall, the plant extract is a source of natural antioxidants that can be important in disease prevention, health preservation and promotion of longevity promoter.

REFERENCES

1. J.M.C. Gutteridgde. Free radicals in disease processes: A complication of cause and consequence. *Free Radic. Res. Comm.* **19**: 141-158 (1995).
2. A. Tiwari. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. *Curr. sci.* **81:** p1179-1187 (2001).
3. D.A. Joyce. Oxygen radicals in disease. *Adv. Drug Reac. Bull.* **127:** 476-79 (1987).
4. M.E. Buyukokuroglu, I.Gulcin, M.Oktay, O.I. Kufrevioglu. In vitro antioxidant properties of dantrolene sodium. *Pharmacol. Res.* **44:** 491-95 (2001).

5. F. Shahidi, and P.D. Wanasundara. Phenolic antioxidants. *Cri. Rev. Food. Sci. Nutr.* **32:** 67-103 (1992).
6. B. Auudy, F. Ferreira, L. Blasina, F. Lafon, F. Arredondo, R. Dajas and P.C. Tripathi. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. *J. Ethnopharmacol.* **84:** 131-138 (2003).
7. A.L. Miller. Antioxidant flavonoids: structure, function and clinical usage. *Alt. Med. Rev.* **1:** p103-111 (1996).
8. R.F. Boyer, H.M. Clark and A.P. Laroche. Reduction and release of ferritin iron by plant phenolics. *J. Inorg. Biochem.* **32:** 171-181 (1988).
9. B. Havsteen. Flavonoids, a class of natural products of high pharmacological potency. *Biocchem. Pharmacol.* **30:** 1141-1148 (1983).
10. K.R. Krtikar and B.D. Basu. Indian material medica Vol. 3 Bishen Singh, Mahendra Pal singh Dehra Dun; 2499-2501 (1975).
11. A.K, Nadkarni. Nadkarnis *Indian material medica* Vol 1 popular prakasham Bombay; p 153 (1976).
12. R.K. Goyal, J. Singh, and H Lal. *Asparagus racemosus*-An update. *Ind. J. Med. Sci.* **57:** 408-14 (2003).
13. P.V. Sharma. Cikittsastana. *In: Ccaraka Samhita*, vol 2. Chaukhambha Orientalis, Varanasi, pp. 7-14 (2001).
14. K. Sairam, S. Priyambada, N.C. Aryya, R.K. Goel. Gastroduodenal ulcer protective activity of *Asparagus racemosus*: an experimental, biochemical and histological study. *J. Ethnopharmacol.* **86(1):** 1-10 (2003).
15. J.P. Kamat, K.K. Boloor, T.P. Devasagayam and S.R. Venkatachalam. Antioxdant properties of *Asparagus racemosus* against damaged induced by gamma radiation on rat liver mitochondria. *J. Ethanopharmacol.* **71:** 425-35 (2000).
16. N. Venkatesan, V. Thiyagarajan, S. Narayanan, A. Arul, S. Raja and S. Vijayakumar. Anti-diarrhoeal potential of *Asparagus racemosus* willd root extracts in laboratory animals. *J. Pharm. Pharmaceut. Sci.* **8 (1):** 39-45 (2005).
17. N.Visavadiya, and R.L. Narasimhacharya. Hypolipidemic and antioxidant activities of *Asparagus racemosus* in hypercholesteremic rats. *Ind. J. Pharmacol.* **37(6):** p376-80 (2005).
18. R. Govindarajan, M. Vijayakumar, Ch.V. Rao, V. Kumar, A.K.S. Rawat, and P. Pushpangadan. Action of *Asparagus racemosus* against streptozotcin-induced oxidative stress. *Nat. Prod. Sci.***10:** 177–181 (2004).
19. U. M. Thatte, S.A. Dahanukar. Comparative study of immunomodulating activity of Indian medicinal plants, lithium carbonate and glucan. *Meth. Find Exp. Clin. Pharmacol.* **10(10):** 639-44 (1988).
20. B. B. Gaitonde, and M.H. Jetmalani. Antioxytocic action of saponin isolated from *asparagus racemosus* Wild on uterine muscle. *Arch. Int. Pharmacodyn. Ther.* **1179:** 121-9 (1969).
21. TIFFNal. Sekine. Structure and relative stereochemistry of a new polycyclic alkaloid, sparagamine a, showing anit-oxytocin activity, isolated from *Asparagus racemosus. J. chem. soci.* Perkin Trans **1:** 391-3 (1995).
22. N.N. Rege, U.N. Thatte and S.A. Dahanuka. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytother. Res.* **13:** 275-291(1999).
23. S.F. Chandler and J.H. Dodds. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum. Plant Cell Reports.* **2:**1005-110 (1993).
24. M.Z. Barakat, S.K. Shehab, N. Darwish, E.I. Zahermy. Determination of ascorbic acid from plants. *Anal. Biochem.* **53:** 225-245 (1973).
25. C. Chang, M. Yang and H. Wen. Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anala.* **10:** 178-182(2002).
26. F. Liu, V. E. C.Ooi, and S. T.Chang. Free radical scavenging activity of mushroom polysaccharide extracts. *Life Sci.* **60:** 763-771 (1997).
27. W. Yu, Y. Zhao, and B. Shu. The radical scavenging activities of radix puerariae isoflavonoids: A chemiluminescence study. *Food Chem.* **86:** 525-529 (2004).
28. D. C. Garrat. *The quantitative analysis of drugs* Japan: Chapman and Hall. **vol. 3**: pp. 456-458 (1964).
29. X. Y. Zhang. *Principles of Chemical Analysis*. Beijing: China Science Press. pp. 275-276 (2000).
30. T. C. P. Dinis, V. M. C. Madeira, and L. M. Almeidam. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and peroxyl radicals scavengers. *Ach. Biochem. and Biophy.* **315:** 161-169 (1994).
31. M. Oyaizu. Studies on products of browning reactions: antioxidant activities of products of

browning reaction prepared from glucose amine. *Jap. J. Nutr*. **44:** 307-315 (1986).

32. R.A. Riemersma, K.F. Carruthers, R.A. Elton, K.A. Fox. Vitamin-C and the risk of acute myocardial infarction. *Am. J. Clin. Nutr*. **179(71):** 1181–1186 (2000).
33. H. Okhawa, N. Ohishi and K. Yagi. Assay for lipid peroxides in animal tissues by thio-barbituric acid reaction. *Anal. Biochem.* **95:** 351-358 (1979).
34. M. Korycka-Dahl and M. Richardson. Photogeneration of superoxide anion in serum of bovine milk and in model systems containing riboflavin and aminoacids. *J. Dairy Sci*. **61:** 400-407 (1978).
35. A. E. Hagerman, K. M. Riedl, Jones, G. A., sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., et al.. High molecular weight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. and Food Chem*. **46:** 1887-1892 (1998).
36. M. J. Miller, H.Sadowska-krowicka, S. Chotinaruemol, J. L Kakkis and D. A. Clark. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *The J. Pharmac. and Experi. Thera.* ***264:*** 11-16 (1993).
37. B. Halliwell and J. M. C. Gutteridge. *Free Radicals in Biology and Medicine* Oxford: Clarendon. pp. 419-422 (1993).
38. B. Halliwell. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The Am. J. Med.* **91:** S14-S22 (1991).
39. M. H. Gordon. The mechanism of the antioxidant action in vitro. In B. J. F. Hudson, *Food Antioxidants*. London: Elsevier; pp. 1-18 (1990).
40. A. T. Diplock. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? *Free Radic. Res.* ***27:*** 511-532 (1997).
41. A. Yildirim, A. Mavi, M. Oktay, A.A. Kara, O.F. Algur and V.Bilaloglu. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Salvia triloba* L.), and Black Tea (*Camellia sinensis*) extracts. *J. Agric. and Food Chem.* **48:** 5030-5034 (2000).
42. P. Valentao, E. Fernandes, F. Carvalho, P.B. Andrade, R. M Seabra, and M. L. Bastos. Studies on the antioxidant activity of *Lippia citriodoru* Infusion: scavenging effect on superoxide radical, hydroxyl radical and hypochlorous acid. *Biol. Pharm. Bull*. **25:** 1324-1327 (2002).