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*RESEARCH ARTICLE*

# EVALUATION OF ANTIOXIDANT ACTIVITY IN THE RHIZOME OF *ASPARAGUS RACEMOSUS* (ORNAMENTAL).

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## Abstract

*Asparagus racemosus* (ornamental) is a medicinal plant traditionally used for treatment of different diseases. Owing to the significance, the *in vitro* antioxidant activity was evaluated in the crude extract of rhizomes of *Asparagus racemosus*. The study included determination of enzymatic, non-enzymatic antioxidants and antioxidant ability of this plant. The results indicated the enzymatic antioxidants superoxide dismutase (12.65± 0.40 U/mg protein), catalase, (85.56 ± 0.22 U/mg protein), peroxidase (3.20 ± 0.12 U/mg protein) and ascorbic acid oxidase (8.90 ± 0.45 U/mg protein) showed good activity. The extract exhibited strong Diphenyl Picryl Hydrazyl **(**DPPH) radical scavenging, hydroxyl radical scavenging, $H_2O_2$ scavenging activity and metal chelation effect. The total antioxidant activity (TAA) 345.5± 0.66 Vit C Eq/gm extract, Reducing power activity 298.4 ± 0.34 Vit C Eq/gm extract and Ferric Reducing Antioxidant Power (FRAP) activity198.4±0.32 Vit C equivalents or FRAP U/gm extract showed good antioxidant ability of rhizomes of *Asparagus racemosus.* Hence this can be used as supplement of exogenous antioxidants to neutralize the reactive oxygen species and to relieve from oxidative stress.



## Introduction:-

An antioxidant is a molecule capable of decreasing or preventing the oxidation of other molecules that generate free radicals. Chemical compounds and reactions capable of generating potential toxic oxygen species / free radicals are referred to as 'pro-oxidants'. They attack macromolecules including protein, DNA and lipid causing cellular / tissue damage on the other hand, compounds and reactions disposing of these species, scavenging them suppressing their formation or opposing their actions are called antioxidants (Yamagishi and Matsui, 2011). In a normal cell there is an appropriate pro-oxidant: antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants are diminished. This state is called 'oxidative stress' and can result in serious cell damage if the stress is massive or prolonged (Cantafora *et al.*, 1993). The exogenous sources of ROS include electromagnetic radiation, cosmic radiation, UV light, ozone, cigarette smoke and low wavelength electromagnetic radiations and endogenous sources are mitochondrial electron transport chain and β-oxidation of fat (Yildrim *et al*.,2001; Davies,1995; Robinson *et al.*,1997; Buyukokuroglu *et al.*,2001).

Oxidative stress plays a significant pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, aging, adult respiratory distress syndromes and rheumatoid arthritis have all been correlated with

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oxidative damage (Abou-Karam and Shier, 1990; Afolayan and Meyer, 1997; Hernández *et al*., 2003; Chellaiah *et al*.,2006). Antioxidant therapy has gained an utmost importance in the treatment of free radical mediated diseases. Although oxidation reactions are crucial for life, they can also be damaging. Hence, living organisms have developed complex antioxidant systems to counteract reactive oxygen species.

Antioxidants are classified into enzymatic and nonenzymatic. The enzymatic antioxidants include superoxide dismutase, Catalase, peroxidase, ascorbate oxidase and glutathione peroxidase and nonenzymatic antioxidants are macromolecules such as albumin, ceruloplasmin and ferritin; and a variety of small molecules, including ascorbic acid, alpha-tocopherol, reduced glutathione, methionine, uric acid and bilirubin (Goraca and Skibska, 2005). The antioxidant defense system of living organism is not sufficient to cope up with the ROS due to high levels of free radicals. Hence the available endogenous antioxidant cannot fully neutralize the ROS. Supplementations of exogenous antioxidants fulfill this gap and completely neutralize the ROS. Medicinal plants may provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin, 2012).The properties of these plants have been studied in the recent scientific developments throughout the world, due to their potent antioxidant activities with no side effects and economic viability (Auudy *et al.,* 2003). Therefore such plants are being investigated for their antioxidant properties and due to the demand for natural antioxidants (Peschel *et al*., 2006). Various antioxidants, flavonoids and phenolic compounds widely distributed in plants have been reported with multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc (Miller, 1996).

*Asparagus racemosus* is a well known medicinal plant in ayurvedic rasayana which prevent ageing, increase longevity, impart immunity, improve mental function and add vigor and vitality to the body and also used in nervous disorders, dyspepsia, tumors, inflammation, hyperdipsia, neuropathy and hepatopathy (Sharma and Cikittsastana, 2001). It also has been reported to have potent adaptogenic activity (Rege, 2005) and antioxidant property (Kamat *et al.,* 2000).

Current research is to find out naturally occurring antioxidants of plant origin. The principle aim of this study was to evaluate the antioxidant activity in the buffer extract of rhizome of *Asparagus racemosus* (ornamental).

## Materials and methods:-

**Plant materials:-**

*Asparagus racemosus* was obtained from Padmapuram garden of Araku valley, Visakhapatnam district. *Asparagus racemosus* belongs to family *Asparagaceae*. The plant specimen was deposited in herbarium, Department of Botany, Andhra University. The voucher specimen number of *Asparagus racemosus* is 22237. The plant specimen was authenticated by Prof.S.B.Padal, Taxonomist, Department of Botany, Andhra University, Visakhapatnam. Rhizomes of *Asparagus racemosus* were used for evaluating antioxidant activity.

**Preparation of extract:-**

The plant rhizomes were washed, air dried, chopped in to fine pieces and ground using acid washed sand in a cold mortar and pestle and then homogenized with 0.1M Phosphate buffer, pH 7.6 at 4°C using tissue to buffer ratio of 1:10 (w/v). The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged at 6,500 rpm for 20 min at 4°C in a refrigerated centrifuge (Remi C-24 Plus). The supernatant solution was used for the evaluation of antioxidant analysis.

**Enzymatic antioxidants:-**

**Assay of superoxide dismutase:-**

The assay of superoxide dismutase was carried out based on the reduction of Nitroblue tetrazolium (NBT) (Beuchamp *et al.,* 1976). To 0.5 ml of rhizome ~~seed~~ extract, 1 ml 125 mM of Sodium Carbonate, 0.4 ml of 24 µM NBT and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1mM Hydroxylamine hydrochloride. Zero time and absorbance after 5 min at 25°C was taken at 560 nm using spectrophotometer (Systronics). Units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50 %. The specific activity was expressed in terms of Units per mg protein.

**Assay of Catalase:-**

Catalase activity was determined by the titrimetric method (Chance *et al.*, 1995). To 1 ml of rhizome ~~plant~~ extract, 5 ml of 300 µM phosphate buffer (pH 6.8) containing 100 µM hydrogen peroxide ($H_2O_2$) was added and left at 25°C for 1 min. The reaction was arrested by adding 10 ml of 2 % sulphuric acid and residual $H_2O_2$ was titrated with 0.01N potassium permanganate. Units of enzyme activity were expressed as ml of 0.01N potassium permanganate equivalents of $H_2O_2$ decomposed per mg protein per min.

**Assay of Peroxidase:-**
Assay of Peroxidase activity was carried out according to the procedure of Malik and Singh (1980). 3.5 ml of phosphate buffer (pH 6.5) was taken, 0.2 ml extract and 0.1 ml of freshly prepared O-dianisidine solution was added. The temperature of assay mixture was brought to 28°C - 30°C and then placed the cuvette in the spectrophotometer at 430 nm. Then, 0.2 ml of 0.2 M $H_2O_2$ was added and mixed. The initial absorbance and also at 30 sec intervals up to 3 min was recorded. A graph was plotted with the increase in absorbance against time. From the linear phase, the change in absorbance per min was read. The enzyme activity was expressed in units per mg protein per min.

**Assay of Ascorbate oxidase:-**
Assay of Ascorbate oxidase activity was carried out according to the procedure of Vines *et al.*, (1965).To 3.0 ml of ascorbate solution (0.003 %), 0.1 ml of extract was added and change in absorbance at 265 nm was measured at an interval of 30 sec for a period of 5 min. One unit of enzyme activity was expressed as 0.01 OD change per mg protein.

**Antioxidant ability assays**
**Total antioxidant activity**
The assay was based on the reduction of Mo (VI)-Mo (V) by the extracts and subsequent formation of a green phosphate Mo (V) complex at acidic pH (Prieto *et al*., 1999).

The extract (0.1 ml) was mixed with 3ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95ºC for 90 min and then the mixture was cooled to room temperature. The absorbance of the solution was measured at 695 nm against blank. The total antioxidant activity was expressed as Ascorbic acid equivalents in milligrams per gram tissue.

**Iron (III) to Iron (II) Reducing Activity (or) reducing power assay:-**
The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu (1986).

One ml of extract was mixed with 2.5 ml of 0.2 M phosphate buffer, pH 6.6, and 2.5 ml of 1% aqueous potassium hexacyano ferrate [$K_3$ Fe ($CN_6$)] solution. After 30 min of incubation at 50°C, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.5 ml of the supernatant ~~upper layer~~ was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm. The mean absorbance values were plotted against concentration and a linear regression analysis was checked. The results were expressed as Ascorbic acid equivalents (AscAE) in milligrams of ascorbic acid per gram of extract.

**Ferric Reducing Antioxidant Power Assay (FRAP) :-**
The total antioxidant power of the sample was assayed by the method of Benzie and Strain (1996).

At low PH, reduction of a ferric tripyridyl triazine [Fe (III)-TPTZ] complex to the ferrous form, which has an intense blue color, can be monitored by measuring the change in absorption at 593 nm. The reaction is nonspecific, in that any half-reaction that has a lower redox potential, under reaction conditions, than that of the ferric/ferrous half-reaction will drive the ferric (Fe III) to ferrous (Fe II) reaction. The change in absorbance therefore is directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture.

Three ml of FRAP (Ferric Reducing Antioxidant Power reagent) working reagent was taken in a test tube, then 100 µl of extract was added, and was vortex mixed, and the absorbance was read at 593nm against a reagent blank. The results were expressed as ascorbic acid equivalents (µ moles/ml) or FRAP units.

**Non enzymatic antioxidants:-**

**Diphenyl Picryl hydrazyl radical scavenging assay (DPPH Assay):-**
The DPPH assay was checked as described by Cuendet *et al*., (1997).
Five ml of DPPH (Diphenyl Picryl hydrazyl) solution in methanol was added to 50 µl of extract. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. Control was set up with buffer and reagent. Similarly positive controls were treated in the same way as test sample except sample replaced by positive standard. Butylated hydroxy toluene (BHT) was used as positive controls. Inhibition (I) of Diphenyl Picryl hydrazyl radical was calculated by
I= (Absorbance of control –Absorbance of test / Absorbance of control) x100.

**Hydroxyl Radical Scavenging activity:-**
Hydroxyl radical scavenging was checked by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the $Fe^{3+/}$Ascorbate/ EDTA/$H_2O_2$ system, a method checked by Gulhan Vardar-Unlu *et al*., (2003) and Ohkawa *et al*., (1979).

Rhizome~~Plant~~ extract (0.1ml) was added to the reaction mixture containing 0.1ml of 3.0 mM deoxyribose, 0.5ml of 0.1 mM $FeCl_3$, 0.5ml of 0.l mM EDTA, 0.5ml of 0.1mM ascorbic acid, 0.5ml of 1mM $H_2O_2$ and 0.8ml of 20 mM phosphate buffer, pH7.4, in a final volume of 3.0 ml. The reaction mixture was incubated at 37$^oC$ for 1 h. The formed thiobarbituric acid reactive substance (TBARS) was measured by adding 1.0 ml of thiobarbituric acid (TBA) and 1.0 ml of trichloroacetic acid (TCA) to the test tubes and incubated at 100$^oC$ for 20 min. After the mixture was cooled, absorbance was measured at 532 nm against a control containing deoxyribose and buffer. A blank was checked in similar way as the test except extract. Butylated hydroxy toluene (BHT) was used as positive control.
% Inhibition (I) of deoxyribose degradation was calculated by
%I= (Absorbance of control –Absorbance of test / Absorbance of control) x100.

**Metal chelating assay:-**
The metal chelating ability was determined according to the method of Eric Decker and Welch (1990).

The extract (5ml) was added to a solution of 0.1ml of 2 mM ferric chloride. The reaction was started by the addition of 0.2ml of 5 mM ferrozine solution and the reaction mixture was incubated for 10min at room temperature under shaking conditions. After incubation, the absorbance of the solution was measured at 562 nm. The percentage inhibition of Ferrozine - $Fe^{2+}$ complex formation was calculated using the formula given below. EDTA was used as positive controls.
% of inhibition = Control –Test /Control ×100.

**Hydrogen peroxide scavenging assay:-**
The ability of the extracts to scavenge hydrogen peroxide was determined according to the standard method by Ruch *et al*.,1989**.**

Rhizome~~Tuber~~ extract (0.1ml) was added to 4ml of phosphate buffer and mixed with 600µl of 40mM $H_2O_2$. Hydrogen peroxide concentration was determined spectrophotometrically at 230nm in the presence and absence of the extract. A control was set up without the extract. For each extract a separate blank solution containing the buffer solution without hydrogen peroxide was used. Butylated hydroxy toluene (BHT) was used as positive control.
Percentage of hydrogen peroxide scavenging was calculated as follows.
% of inhibition= (control-test/control) X 100

## Results and discussion:-

**Enzymatic Antioxidants:-**
The enzymatic antioxidants tested were superoxide dismutase, catalase, peroxidase, Ascorbic acid oxidase. These showed remarkable activity in the rhizome extract (Fig.1). Catalase showed maximum activity 85.56 ± 0.22U/ mg protein followed by SOD 12.65± 0.40 U/ mg protein, Ascorbic acid oxidase 8.90 ± 0.45 U/ mg protein and peroxidase 3.20 ± 0.12 U/mg protein.

**Antioxidant ability assays:-**
**Total antioxidant activity (TAA):-**
Total antioxidant activity (TAA) of the rhizome extract was found to be 345.5± 0.66 Ascorbic acid equivalents/gm extract.

**Reducing power assay:-**
Reducing power activity of the rhizome extract was found to be 298.4 ± 0.34 Ascorbic acid equivalents/gm extract.

**Ferric Reducing Antioxidant Power Assay (FRAP):-**
Ferric Reducing Antioxidant Power Assay (FRAP) activity of the rhizome extract was found to be 198.4±0.32 Ascorbic acid equivalents or FRAP U/gm extract.

**Non-enzymatic Assays:-**
DPPH, Hydroxyl radical scavenging, Metal chelation, $H_2O_2$ scavenging showed good percentage of inhibition as shown in Fig.2.

The nonenzymatic antioxidants were assayed with 5% and 10% rhizome extract of *A.racemosus* respectively**.** The DPPH radical scavenging (%) activity exerted was 52% and 80.25% of inhibition with 5% and 10% of extract respectively and that of standard Butylated hydroxy toluene BHT exhibited 92% at 1mg/ml. Hydroxyl radical scavenging (%) activity exerted by rhizome extract (5% and 10%) was 36% and 62% of inhibition respectively and that of standard Butylated hydroxy toluene BHT was 95% at 1mg/ml. Metal chelation (%) activity exerted by rhizome extract (5% and 10%) was 47% and 64% of inhibition respectively and that of EDTA was 90% at 1mg/ml. Hydrogen peroxide scavenging (%) activity exerted by extract (5% and 10%) was 47% and 64% of inhibition respectively and that of BHT was 90% at 1mg/ml.

## Discussion:-

Antioxidants are tremendously important substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. This investigation provides experimental support for the traditional medicinal plants. The rhizome of *Asparagus racemosus* exhibited good antioxidant activity. To assess this different radical scavenging assays has been performed to find antioxidant potential of this medicinal plant. The results confirm that the buffer extract of *Asparagus racemosus* was rich in enzymatic and nonenzymatic antioxidant levels.

Out of four enzymatic antioxidants studied, catalase (CAT) showed maximum activity followed by superoxide dismutase (SOD), ascorbate oxidase and peroxidase. The extract showed highest total antioxidant activity, good reducing power and ferric reducing power activity. The nonenzymatic antioxidants assay results revealed that this plant has maximum DPPH radical scavenging, metal chelation, Hydrogen peroxide scavenging and also Hydroxyl radical scavenging potential. The enzymatic antioxidants reported in *Tylophora pauciflora* were, 29.78±0.57U/mg protein and 39.87±0.51µmole of $H_2O_2$ consumed/min/mg protein for SOD and CAT respectively. Ascorbate oxidase activity was found to be 27.23±0.57 U/g sample (Thangarajan starlin, 2013).The leaf extract of *Alpinia purpurata* also reported with enzymatic antioxidants, peroxidases 173.12±9.40 µmol/g tissue, SOD 58.03±2.11 U/mg protein, CAT 46.70±2.35 µmol of $H_2O_2$ consumed/min/mg protein in high amount whereas ascorbate oxidase 17.41±2.46 U/g tissue (Chinthamony Arul Raj, 2016).The aqueous leaf extracts of *Juniperus oxycedrus* have the strongest scavenging activity against DPPH (17.91 ± 0.37 µg/ml), and reducing power activity (24.23 ± 0.07 µg/ml) (Meryem El Jemli,2016).This difference in antioxidant levels is due to source variation and assay method used.

In biochemical system, superoxide radical and $H_2O_2$ react together and form different forms of ROS viz. hydroxyl radical and singlet oxygen. They are responsible for various life threatening diseases like carcinogenesis, cytotoxicity and aging by altering or damaging DNA. From the experimental data it may be speculated that extract can prevent the damaging properties of ROS at cellular level as it may able to quench hydroxyl radical and scavenge ROS by the antioxidants.

DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva *et al.*, 2002; Suresh *et al*., 2008). DPPH becomes diamagnetic molecule after gets reduced into its hydrazine form by electron donation by the antioxidants. DPPH radicals are widely used to investigate the scavenging activity of several natural phytocompounds. The DPPH contains an odd electron, which is responsible for purple color and absorbance at 517 nm (Ferreira, 2007). The result of DPPH scavenging activity in this study indicates that the plant was potentially active. It is well known that ferrous ions ($Fe^{2+}$) are very effective peroxidation drivers in lipid systems, including edible oils (Halliwell, 1994). Ferric ions ($Fe^{3+}$) are less active. In general antioxidants possess

good reducing ability to convert $Fe^{3+}$ into $Fe^{2}$. The hydroxyl radical scavenging by rhizome extract of *A.racemosus* indicated its significant antioxidant potential.

## Conclusion:-

In conclusion, the free radical-scavenging activities evaluated from rhizome of *Asparagus racemosus* showed strong antioxidant activities. It was demonstrated that the use of several test methods for radical scavenging and antioxidant activity provides valuable data for better understanding of purposeful application of the plant extracts. Natural antioxidants have great importance as therapeutic agents in preventing or slowing the oxidative stress related degenerative diseases such as cancer and various other human ailments. Hence this plant has potential to be used even in food, cosmetics and medicinal preparations.

**Fig.1:-**Enzymatic Antioxidants.

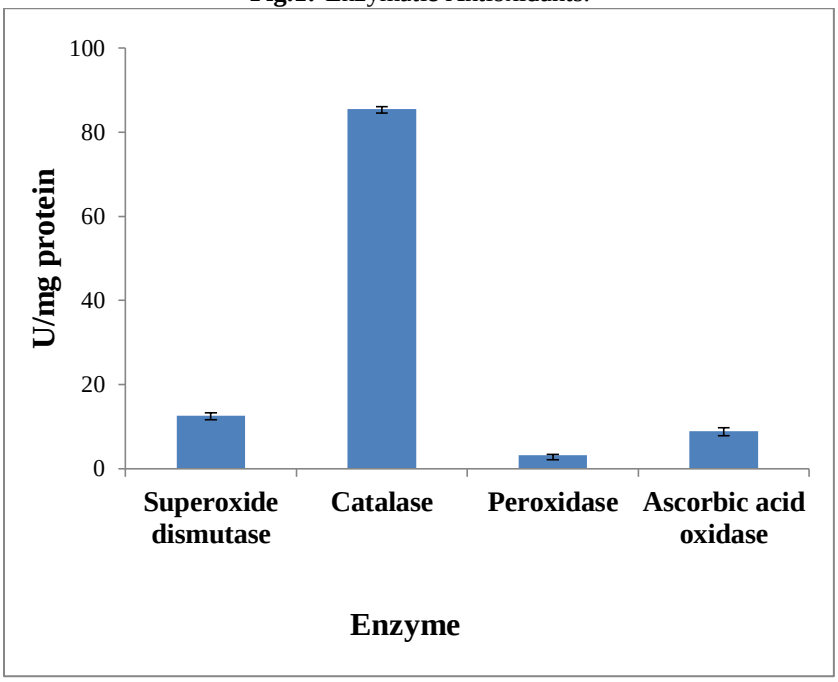
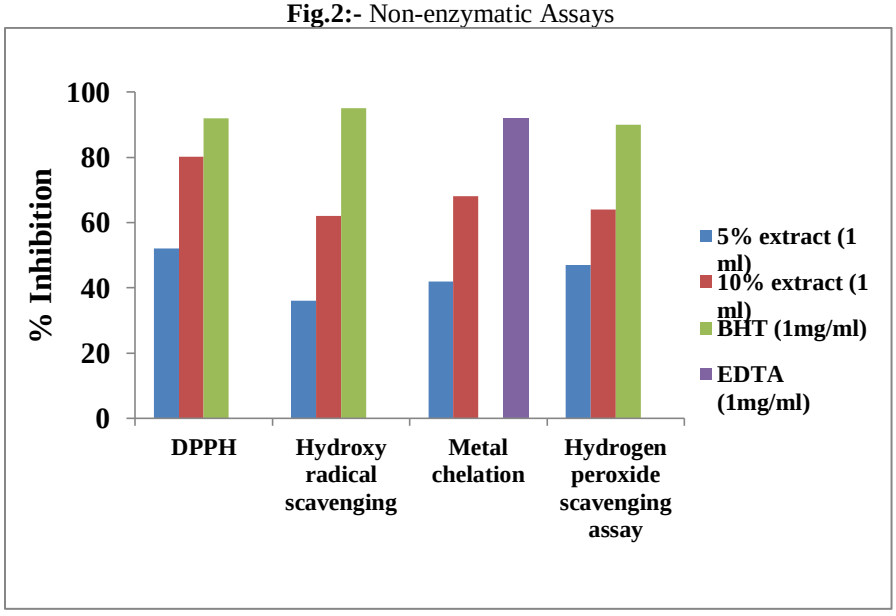



**Fig.2:-** Non-enzymatic Assays

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