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EVALUATION OF ENZYMATIC AND NON ENZYMATIC ANTIOXIDANT ACTIVITY OF *ARISTOLOCHIA BRACTEATA* RETZ

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ABSTRACT

This study significantly focused on the enzymatic and non enzymatic antioxidant activity of *Aristolochia bracteata* Retz. belongs to the family *Aristolochia* ceae which is exploited for the treatment of various free radical mediated ailments. Plant is mainly used for antidote and skin disease. Catalase activity, Superoxide dismutase, reduced Glutathione, Ascorbic acid, α-Tocopherol and total Carotenoids were estimated by following methods. The enzymatic assay shows best activity in Catalase activity (28.3±0.14 µ/mg), reduced Glutathione (49.7±0.08 µ/mg) followed by Superoxide dismutase (17.8±0.25 µ/mg) respectively and in non enzymatic assay Carotenoids (283.8±6.14mg/g) shows significant results. Thus this plant can be suggested as an alternative source for antioxidants.

KEY WORDS: Enzymatic, Non-Enzymatic, *Aristolochia bracteata*.

INTRODUCTION

Oxygen is a highly reactive atom which is capable of damaging molecules commonly called "Free radicals". Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, and immune system decline and brain dysfunction[2]. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Plants are sources of natural antioxidants, and some of the compounds have significant anti-oxidative properties and health benefits[4]. The *Aristolochia*ceae family contains about 400 species in 7 genera of cosmopolitan distribution,

many of them are of economic importance due to aristolochic acids and terpenoids. The genus *Aristolochia* finds a prominent place in different Indian Systems of Medicine. The different ethnic communities in India have used many species of *Aristolochia* in the treatment of various human ailments. *Aristolochia* bractea commonly called as "worm killer" in English and "Aadutheendaapaalai" in Tamil. Plant parts (Whole plant, Leaves, seed and root) Traditional uses as dermatitis, allergic disorder, leprosy, jaundice, worms, fever, mosquito repellent, anodyne, purgative, emmenagogu, anti-inflammatory, dermatitis, rashes, skin disease, for scorpion sting, antipyretic, snake bite, antiulcer, amenorrhoea, antihelmintic , antiplasmodial, antibacterial, anti inflammatory analgesics, Syphilis, gonorrhoea and eczema[14]. According to Ayurveda the leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders[1]. So the anti oxidant activity was studied in enzymic and non enzymic properties of *Aristolochia bracteate*s.

MATERIALS AND METHODS

Collection of plant materials

*Aristolochia bracteate*s leaves were collected from Nammakal district during November 2013. Plant materials (leaves) were washed with distilled water and shade dried. The dried samples were manually ground to a fine powder. The coarsely powdered parts were exhaustively extracted with methanol for 8 h using Soxhlet apparatus. The filtrate was then evaporated to dryness under reduced pressure using rotary vaccum evaporator. The extracts were lyophilized until further use.

Estimation of catalase activity

Catalase activity was carried out by the method of Luck[7].The sample is homogenized in a pre chilled mortar and pestle with M/150 phosphate buffer (assay buffer diluted 10 times) at 1-4^{o}C and centrifuged. Sediment is stirred with cold phosphate buffer, allowed to stand in the cold with occasional shaking and then repeated the extraction once or twice. The extraction should not take more than 24 hr. The combined supernatants were used for the assay. Fresh extract is used for assay. OD is measured against a control cuvette 3 ml of H_2O_2 containing the enzyme solution as in the phosphate buffer (M/15) at 240nm. 0.01-0.04 ml sample is mixed H_2O_2 phosphate buffer and time is noted for a decrease in absorbance from 0.45 to 0.4. Calculated the concentration of H_2 O_2 using the extinction coefficient 0.036K Mole/ml.

Estimation of superoxide dismutase (SOD) Activity

Activity of superoxide dismutase (SOD) was determined by the method of Misra and Fridovich[8]. The incubation medium contained a final volume of 3ml, 50ml potassium phosphate buffer (pH 7.8), 45KM methionine, 84KM Nitro blue tetrozolium (NBT) and 20mM potassium cyanide. The tubes were placed in aluminium foil- lined box maintained at 25°C and equipped with 15W fluorescent lamps. Reduced NBT was measured spectrophotometrically at 600nm after exposure to light for 10 minutes. The maximum reduction was evaluated in the absence of the amount of enzyme giving 50% inhibition of the reduction of NBT.

Estimation of reduced glutathione

The assay of glutathione reductase was done according to the procedure of David and Richard[3]. 20% aqueous extract was prepared in 0.12M phosphate buffer (pH7.2) was used as the source of enzyme. The assay system contained 1ml of 0.12M Potassium buffer, 0.1ml of 15mM EDTA, 0.1ml of 10mM Sodium azide and 0.1 ml of 6.3mM oxidized glutathione were added and the volume was made up to 2 ml with water. The mixture was kept at room temperature for three minutes and 0.1ml of NADPH was added. The absorbance at 340nm was recorded at intervals of 15seconds for 2 to 3 minutes. One unit of GR is expressed as µM of NADPH oxidized/ minute/gram sample

Estimation of ascorbic acid

The estimation of ascorbic acid in the sample was done using the method of Roe and Keuther[10]. About 1g of the sample and homogenized in 4% TCA up to 10 ml. Sample and homogenized in 4% TCA up to 10 ml. The supernatants obtained were treated with a pinch of activated charcoal. Shaken well and kept for 10 minutes. Centrifuged once again to remove the charcoal residue. Noted the volume of the clear supernatants obtained and this supernatant were used for assay. The assay volumes were made up 2.0ml with 4%TLC. The working standard solution 0.2 to 1.0 ml containing 20-100µg of ascorbate respectively were taken into clean dry test tube, the volume of which were also made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent is added to all the test tubes, followed by 2 drops of 10% thiourea solution. Incubated at 37ºC for 3 hours. The osazones formed were dissolved in 2.5 ml of 85% sulphuric acid, in cold, drop by drop, with no appreciable rise in temperature. To the blank alone, DNPH reagent and thio-urea were added after the addition of H_2SO_4. The tubes were incubated for 30 minutes at room temperature, and the absorbance was read

spectrophotometrically at 540nm.calculated the content of ascorbic acid in the sample using the standard graph.

Estimation of α-Tocopherol

The estimation of α-Tocopherol in the sample was analyzed using the Emmerie-Engel method as described by Rosenberg[11]. The sample was homogenized in a blender. Weighed accurately 2.5 g of the homogenized tissue into a conical flask. Added 50ml of 0.1N sulphuric acid slowly without shaking. Stoppard and allowed to stand overnight. The next day the content of the flask where shaken vigorously and filtered through Whatman No.1 filter paper, discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate the where used for the estimation. Into 3 Stoppard centrifuge tubes (test, standard and blank), pipette out 1.5ml of extract, 1.5ml of standard, 1.5ml of water respectively. To the test and blank, add 1.5ml of ethanol and to the standard, add 1.5ml of water. 1.5ml xylene is added to all the test tubes, Stoppard, mixed well and centrifuged. Transferred 1.0ml of xylene in to another Stoppard tube, take care of not to include any other ethanol or protein. Added 1ml of 2, 2-dipyridyl reagent to each tube, Stoppard and mixed. Pipette out 1.5 ml of the mixture into colorimeter cuvettes and read the extinction of the test and standard against the blank at 460nm. Then in turn beginning with the blank, added 0.33ml of ferric chloride solution. The amount of vitamin E can be calculated using the formula.

$$\text{Amount of α-tocopherol} = \frac{\text{Reading at 520nm- Reading at 460nm}}{\text{Reading of standard at 520nmX0.29X15}}$$

Estimation of total Carotenoids

The estimation of total carotenoids was done by the method described by Zakaria et al., [15]. Weighed 5-10g of the sample saponified for about 30 minutes in a shaking water bath at 37ºC after extracting the sample in 12% alcoholic KOH. Transferred the saponified extract in to a separating funnel packed with glass wool and $CaCO_3$ containing 10 to15ml of petroleum ether layer. Transferred the lower aqueous phase to another separating funnel, and the petroleum ether extract containing the carotenoid pigments to amber coloured bottle. Repeated the extraction of the aqueous phase. To the petroleum ether extract, add a small quantity of anhydrous Na_2SO_4 to remove the turbidity. Finally volume of the petroleum ether extract is noted and diluted if needed by a known dilution factor. The absorbance of the extract at 450nm and 503nm was noted in a spectrophotometer.

$$\text{Amount of total carotenoids present} = \frac{\text{P X 4 X V X 100 X 45}}{\text{W}}$$

P= Optical Density of the Sample, V=Volume of the Sample, W=Weight of the Sample

RESULT AND DISCUSSION

The chemical reactions that occur naturally are essential for controlling the metabolic process occurring in the living organisms. Free radicals or reactive oxygen species are introduced as a product of normal metabolic function in to the living system. phenolic compounds are mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[9]. The results obtained for some enzymic and non enzymic antioxidants of the *Aristolochia bracteata* are tabulated (Table-1-2).

The catalase is one of the most potent catalysis known. The catalase catalyses the conversion of H_2O_2, a powerful and potentially harmful oxidizing agent, Which oxidise toxins including phenols, formic acid, formaldehyde and alcohol. Catalase was found to be important in the inactivation of many environmental mutagens and has much industrial importance. The activity of catalase in the methanolic extract of the leaves of *Aristolochia bracteata* was found to be 28.3µ/mg protein (Table-1).

The activity of SOD in the methanolic extract of the leaves of *Aristolochia bracteate* was found to be 17.8µ/mg (Table-1). SOD has scavenging effect on the ROS, superoxide and hence the leaves of the plant can be used to prevent the oxidative stress and the diseases caused by ROS. Similarly Karan et al.,[6] the superoxide scavenging effect of chloroform extract of *Aristolochia indica* was found to be 8.498 µg/ml. Curcumin used as reference standard, which showed 40.11% inhibition at a concentration of 10 µg/ml in A. *indica*. Glutathione has antioxidant properties since the thiol group in its cysteine mosty is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is reduced by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidises and glutaredoxins, as well as directly it will react with oxidants. Due to its high concentration and its central role in maintaining the cells rebut state, glutathione is one of the most important cellular antioxidants. *Aristolochia bracteata* leaf extract has the highest activity (49.7µ/mg) (Table-1) Vitamins C is another non enzymatic antioxidant found in significant levels. As an antioxidant, the primary role ascorbate is donating electrons to neutralize reactive O_2 species including superoxide and hydroxyl free radicals. The activity of ascorbic acid in the methanolic extract of leaves of the plant is 0.28mg/g(Table-2). A-tocopherols interact with

the poly unsaturated acyl groups lipids, stabilize membranes, scavenge and quench various reactive oxygen species (ROS) and lipid soluble by productive stress. However ROS conditions, the excessive presence of reactive species can cause DNA, protein and lipid oxidation, which can cause cellular failure and neuronal death[5]. The activity of α- tocopherols in the plant methanolic leaf extract was 7.3mg/g. (Table-2)

Total carotenoids are organic pigments which occur in plant. Their molecular structure makes them very efficient free radical scavengers, resulting in a powerful and oxidant affect. The activity of total carotenoids in the leaf methanolic extract of *A. bracteates* is 283.8 (Table-2) respectively. *A.bracteolata* is proved to have antioxidant property and insecticidal properties. The plant contain Aristolochic acid, has many medicinal properties to prevent various disease conditions[14].

Table-1 Estimation of enzymatic antioxidants in leaf extract of *Aristolochia bracteate*

Enzymatic Antioxidants (µ/MG)	Amount Of Enzymatic Antioxidants
Catalase[1]	28.3 ± 0.14
Superoxide dismutase[2]	17.8 ±0.25
Glutathione reductase[3]	49.7 ±0.08

Values are mean ±SD of triplicates

[1]amount of enzyme that brings about decrease in absorbance of 0.05 at 240nm

[2]amount of SOD that causes 50% reduction in the extent of NBT oxidation

[3]millimoles of NADPH oxidized/min/g sample

Table-2 Estimation of Non enzymatic antioxidants in leaf extract of *Aristolochia bracteate.*

Non Enzymatic Antioxidants (Mg/G)	Amount Of Non Enzymatic Antioxidants
Ascorbic acid	0.28 ± 0.002
α-tocopherol	7.3 ± 0.36
Total carotenoids	283.8 ± 0.14

Values are mean ±SD of triplicates

CONCLUSION

This study could be an answer to the people seeking for better therapeutic agents from natural source which is believed to be more efficient with little or no side effects when compared to the commonly used synthetic chemotherapeutic agents. The preset study verified the traditional use of A. *bracteata* for human ailments and partly explained its use in herbal medicine and as a source of antioxidants.

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