IN-VITRO ANTIOXIDANT ACTIVITY OF *ASPARAGUS RACEMOSUS* ROOTS

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ABSTRACT

Objective: The objective of present study is to evaluate the antioxidant activity of methanolic extract of *Asparagus racemosus* roots (liliaceae). **Material and methods:** The antioxidant activity of the methanolic extract of *A. racemosus* was determined by using a method based on the reduction of methanolic solution of coloured-free radical 1, 1 diphenyl-1-2 picrylhydrazyl (DPPH). The radical scavenging activity of tested sample was expressed as an inhibition percentage. Butylated hydroxyl toluene was used as reference standard. The absorbances of all the dilutions were taken after 30 minutes at λ_{max} 517 nm using methanol as blank. **Results and Discussion:** The IC_{50} value of *A. racemosus* was 4158.8 whereas butylated hydroxyl toluene used as a standard showed an IC_{50} of 46.25µg. The absorbance of samples (Methanolic extract of *A. racemosus* and standard Butylated hydroxytoluene) were taken in triplicate. **Conclusion:** The present study showed that the methnolic extract of roots of *A. racemosus* have moderate free radical scavenging activity.

KEY WORDS: *Asparagus racemosus,* Satavari, liliaceae, DPPH, antioxidants, IC_{50}, Butylated hydroxyl toluene (BHT).

INTRODUCTION

Antioxidants or inhibitors of oxidation are the compounds which retard or prevent the oxidation in general and prolong the life of oxidizable matter [1]. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as reactive species scavenger. Polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity[2]. The antioxidant activities of the individual compounds may depend on structural factors, such as number of phenolic, hydroxyl or methoxyl groups and other structural features[3]. Among the antioxidative compounds vitamin A, C, E, selenium, carotenoids, ascorbic acid show very strong intensity of antioxidative activities[4].

A. racemosus is an indigenous medicinal plant of the family Liliaceae [5,6], commonly it is known as Satavari. It is found in all over India, especially in Northern India. It is important for its sapogenin content[7], the precursor of many pharmacologically active steroids. The roots of *A. racemosus* (Liliaceae) mentioned in Ayurveda, have been used to treat, Anticancer activity , antdysenteric activity, antifungal activity antibacterial activity, anti-inflammatory activities, antiulcer activity, antioxidant activity, anti-abortifacient activity (Shatvarin 1),

Antioxytoxic (shatavarin 4), spasmodic to uterus Hypoglycaemic, hypotensiv activity, anticoagulant activity [8]. A free radical is a compound with one or more unpaired electrons in its outer orbital [9]. Such unpaired electrons make these species very unstable and therefore quite reactive with other molecules due to the presence of unpaired electrons and try to pair their electrons and generate a more stable compound [10].

The molecule of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present). Representing the DPPH radical by Ẓand the donor molecule by AH, the primary reaction is

$Z^* + AH = ZH + A^*$ [1]

Where, ZH is the reduced form and Ạis free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant. The reaction [1] is therefore intended to provide the link with the reactions taking place in an oxidising system, such as the auto-oxidation of a lipid or other unsaturated substance; the DPPH molecule Ẓis thus intended to represent the free radicals formed in the system whose activity is suppressed by the substance AH[11].

MATERIAL AND METHODS

Collection and Identification of Plant Material

The plant material was collected from the Khari Baoli market, Old Delhi and was identified as *Asparagus racemosus* (Root). Famaly (liliaceae) by the Dr. Anjula Pandey (Taxonomist), National Bureau of Plant Genetic Resources (NBPGR), Pusa Campus, New Delhi. A voucher specimen (Specimen No: NHCP/NBPGR 2010-5/6650) is preserved in herbarium section of taxonomic deptt. of NBPGR, New Delhi and also in the Pharmacognosy laboratory, department of pharmacy, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh.

Preparation of plant material

The air dried roots were coarsely powdered and extraction was carried out in Soxhlet apparatus with help of methanol as a solvent. The methanolic extract was stored at 4°C.

Preparation of reagents

The 500 µM solution of DPPH was prepared by dissolving 23 mg of DPPH in 100 ml of methanol. TRIS [2-amino-2 (hydroxy methyl) propane 1-3di-ol] buffer (pH 7.4) was prepared by adding 0.605g of TRIS buffer in 30 ml of water and adding 0.33 ml of concentrated hydrochloric acid, diluted to 100 ml with distilled water. TRIS buffer prevents the sudden pH change during the preparation of test dilutions [12].

Preparation of reference standard solution

Various dilutions of butylated hydroxytoluene were made with concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45 & 50 µg per 0.5 ml of methanolic solution of butylated hydroxytoluene.

Preparation of sample solution and dilutions

Prepared the stock solution by dissolving .068 mg of *A. racemosus* extract into methanol and made up the volume to 25 ml with methanol. Prepared the initial dilutions from stock solution using volume 0.25 ml (125µg / 25ml), 0.50 ml (250 µg / 25 ml), 0.75 ml (375 µg / 25 ml), 1.0 ml (500 µg / 25 ml), 1.25 ml (625 µg / 25 ml), 1.5 ml (750 µg / 25 ml), 1.75 ml (875 µg / 25ml), 2.0 ml (1000 µg / 25 ml), 2.25 ml (1125 µg / 25 ml) and 2.5 ml (1250 µg / 25 ml). The volume make up was done in methanol. The final concentrations used for taking the absorbance are 25 µg, 50 µg, 75 µg, 100 µg, 1.25 µg, 1.5 µg, 1.75 µg, 200 µg, 225 µg, and 250 µg per ml.

Measurement of *in vitro* antioxidant activity

The antioxidant activity of the methanolic extract of *A. racemosus* was determined by using a method based on the reduction of methanolic solution of coloured-free radical 1, 1 diphenyl-1-2 picrylhydrazyl (DPPH). The radical scavenging activity of tested sample was expressed as an inhibition percentage. Butylated hydroxyl toluene was used as reference standard. In 5 ml volumetric flasks added 1 ml of DPPH solution, 0.5 ml of TRIS Buffer and 0.5 ml of final dilutions of different concentrations range prepared from *A. racemosus* (methanolic extract) stock solution and made up the volume to 5 ml with methanol. In same way prepared the control dilutions of DPPH, replacing 0.5 ml of prepared dilutions (the drug solution under investigation) with methanol. The absorbances of all the dilutions were taken after 30 minutes at λ_{max} 517 nm using methanol as blank.

Statistical analysis

The percentage inhibition was calculated using:

$$\text{Percentage Inhibition} = \frac{(Ac - As) \times 100}{Ac}$$

Where, Ac is absorbance of control; As is the absorbance of sample. IC_{50} value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC_{50} value was calculated by using the equation of line [13].

RESULTS AND DISCUSSION

The methnolic extract of *A. racemosus* tested for *in vitro* using DPPH showed moderate free radical scavenging activity, as evidenced by low IC_{50} values. Fig. 1 depicted that the IC_{50} value of *A. racemosus* was 4158.8 whereas butylated hydroxytoluene used as a standard showed an IC_{50} of 46.25µg. The absorbance of sample (Methanolic extract of *A. racemosus* and standard Butylated hydroxytoluene) were taken in triplicate. With the increase of concentration, the decrease of absorbance value and the calculated percentage inhibition has shown with the help of tables.

CONCLUSION

Polyphenolic compounds like flavonoids and phenolic acids, commonly found in plants, have been reported to have multiple biological effects, including antioxidant activity. The present study

demonstrates that *A. racemosus* shows *in vitro* antioxidant activity. *In vitro* study shows that *A. racemosus* has moderate free radical scavenging action. Antioxidant property of *A. racemosus* Methanolic extract can be attributed to the presence of flavonoids and polyphenols and which in turn may be responsible for its anti-stress effect.

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Table 1.1: List of materials

S. No.	Materials / chemicals	Manufacturer / Supplier
1.	1, 1-diphenyl-2-picryl-hydrazyl	Hi Media Laboratories Pvt. Ltd.
2.	Butylated hydroxytoluene	Rankem RFCL Limited
3.	TRIS [2-amino-2 (hydroxy methyl) propane 1-3di-ol] buffer (pH 7.4)	Qualigens Fine Chemicals
4.	Methanol	Rankem RFCL Limited

Table 1.2: List of instruments / equipments

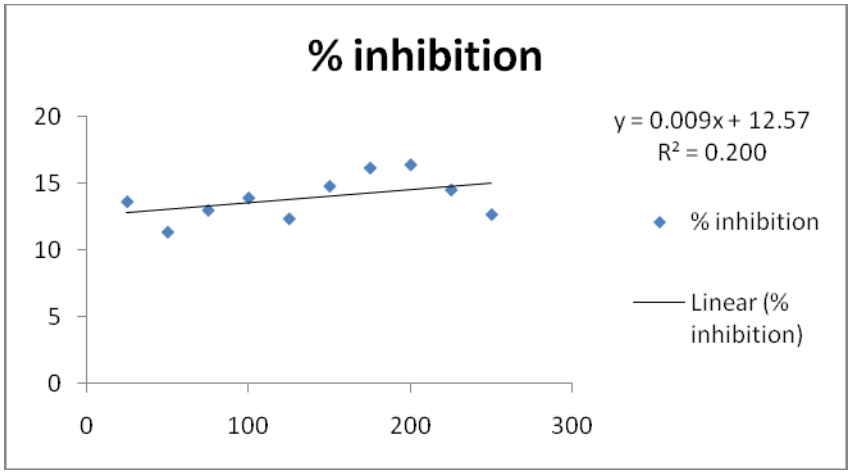
S. No.	Instrument / equipment	Make / model
1.	Weighing balance	WENSAR / ECB 300
2.	UV Visible Spectrophotometer	Shimadzu / UV 1700

Table 1.3: Values of absorbance and percentage inhibition with increase in concentration of methnolic extract of *A.racemosus*

Concentration(µg)	Absorbance	Percentage Inhibition
25	2.175 ± 0.03	13.65
50	2.214 ± 0.01	11.36
75	2.173 ± 0.05	13.01
100	2.150 ± 0.00	13.93
125	2.189 ±0.02	12.36
150	2.128 ±0.02	14.81
175	2.094 ±0.09	16.17
200	2.088 ±0.03	16.41
225	2.135 ±0.00	14.53
250	2.181 ±0.01	12.69

Table 1.4: Values of absorbance and percentage inhibition with increase in concentration of methanolic solution of Butylated hydroxytoluene (standard antioxidant)

Concentration(µg)	Absorbance	Percentage Inhibition
5	2.163 ± 0.01	7.78
10	1.996 ± 0.04	14.91
15	1.792 ± 0.06	23.58
20	1-601 ± 0.02	31.75
25	1.531 ± 0.09	34.72
30	1.375 ± 0.00	41.36
35	1.211 ± 0.01	48.36
40	1.111 ± 0.00	52.62
45	0.945 ± 0.01	59.69
50	0.892 ± 0.01	61.94



Concentration in µg/ml

Fig. 1.1: Free radical (DPPH) scavenging activity of *A. racemosus in vitro* systems.

Graphical representation of the concentration required inhibiting 50 percent of free radicals (Each point represents the mean percentage inhibition of triplicate experiments. Regression coefficient = 0.200, IC_{50} = 46.25.
X Axis = Concentration in µg; Y Axis = Percentage Inhibition; From the equation, When Y=50, X= **4158.8**

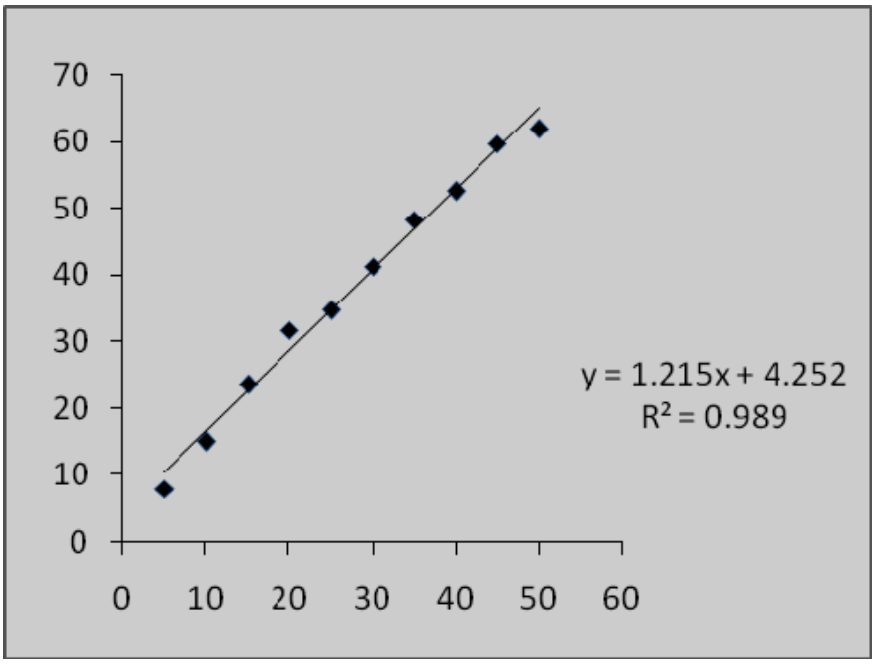


Fig. 1.2 Graphical representation of concentration (µg) vs percentage inhibition of methanolic solution of butylated hydroxytoluene (standard antioxidant)

X Axis = Concentration in µg; Y Axis = % Inhibition; from the equation, When Y=50, X= **46.25**.