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Comparative study of Antioxidant activity of root of Asparagus Racemosus in different solvents

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Abstract

Asparagus racemosus is are of the most frequently used herbs in Indian Traditional medicine. *Asparagus racemosus* are medicinal plants and process a variety of biological properties such as being antioxidants, immunostimulants, antiinflammatory, antihipatotoxic, antibacteial, antioxytocic and reproductive agents. The objective of the present study was antioxidant activity of different solvent extracts of root of *A. racemosus* Antioxidant capacity measurements were carried out by DPPH, methods. Total phenolic contents, flavonoid contents and total antioxidant capacity were determined by the Folin-Ciocalteu method and the aluminium chloride colarimetric method.

Key Words : Asparagus racemosus, DPPH, Antioxidant, Phenolic, Flavonoid.

Introduction

Asparagus species (Family Liliaceae) are medicinal plants. *Asparagus racemosus* Willd. Root has been traditionally in Ayurveda. It is commonly known as Shatavari or Satamuli is found in all over India. Ayurvedic literature claimed several therapeutic attributes for the root of *A. racemosus* and has been specially recommended in case of galactogogue.[1] In Indian System of Medicine *A. racemosus* is an important medicinal plant and its root paste or root juice has been used in various ailments and as health tonic[1,3] *A. racemosus* used for prevent ageing, impart immunity, improve mental functions, nervous disorders, tumour, inflammation and neuropathy.[4] Literature review showed that root extract of *A. racemosus* has antiulcer activity antioxidant, anti-diarrhoeal, anti-diabetio and immunomodulatory activities[5,6]

Recent reports on AR indicate that the root extracts show antioxidant an antidiarrheal activities in animal models.[7] *Asparagus racemosus* root extract was found to contain flavonoids, Polyphenols and Vitamin C, which were found to exhibit the greatest antioxidant activity. The objective of the present investigation is to determine the antioxidant value of different solvent extracts of the roots of *Asparagus racemosus*.

Antioxidant study was carried out on the basis of scavenging activity of the stable DPPH (1, 1-Diphenyl-2-picrylhydrazyl) free radical. The antioxidant value observed was due to their redox property of the phenolic compounds present in the root extract.

Materials and Methods

The roots were collected from Hisar (Haryana). The roots were washed with tap water and dried at 35^0C in an incubator. The dried material was then powdered with a mechanical grinder.

The powdered root (50 gms) was successively extracted in a Soxhlet extractor at high temperature using 300ml of distilled petroleum ether $(40\text{-}60)^0$C which was followed by ethanol, n-hexane, and chloroform. All extracts were filtered individually. All extracts were concentrated to dryness under a reduced pressure and controlled temperature using an evaporator and kept in refrigerator at 4^0C for investigation.

DPPH radical Scavenging assay :

The free radical scavanging capacity of the different solvent extracts of *Asparagus racemosus* roots was determined using DPPH. (1, 1-Diphenyl-2-picrylhyehazyl)[8-9]. An ascorbic acid solution was used as reference standard. A dose of each extract (15-1500 µg/ml) was added to a volume of 850 µg DPPH in absolute ethanol, incubated at room temperature in the dark for 30 minutes. The absorbance was read at 517 nm using Spectrophotometer. The absorbance values of DPPH solutions without or with sample added were the control and samples respectively and was read at 517 nm using a spectrophotometer.

Determination of total flavonoid contents :

Total flavonoids were determined by the aluminium chloride method.[10] Quercetin was used to make the standard. 2 ml of the different solvent extracts of *Asparagus racemosus* was mixed with 4 ml of methanol. Then add 0.3 ml aluminium chloride and 0.3 ml of 1 M potassium acetate. In this mixture add 6 ml of distilled water. Keep it at room temperature for 40 minutes. Then the absorbance of reaction mixture was measured at 410 nm with Spectrophotometer against blank. Methanol was taken as blank.

Total flavonoid contents were determined on the basis of absorbance and calculate as mg quercetin equivalent per gram of extract (mg QE/g powder).

Determination of total phenolic content :

Total phenolic contents in the extract was determined by the Folin-Ciocalteu reagent method.[(11)] 2 ml of the different solvent extracts was mixed with 6 ml Folin-Ciocalteu reagent diluted with water 1:10 v/v. The mixture was incubated at room temperature for 10 minutes. After that the mixture was mixed with 5 ml of 7% Sodium Carbonate solution and the contents were mixed thoroughly. The colour was developed and absorbance measured at 750 nm in Spectrophotometer against blank. Gallic acid was used to as standard.

Total Antioxidant capacity :

The total antioxidant capacity was compared with the standard ascorbic acid at different concentrations and was evaluated by the phosphomolybdenum method. It was expressed as ascorbic acid equivalent per gram of plant extract. 0.2 ml of extract and sub-fraction in ethanol, ascorbic acid used as standard and blank (ethanol) were combined with 2 ml of reagent mixture separately. After cooling the absorbance of each sample was measured at 690 nm against the blank. The total antioxidant activity is expressed as the number of equivalents of ascorbic acid and calculated by a equation A= (C x V)/m where A = total content of antioxidant compounds mg/gm plant extract, in ascorbic acid equivalent C = concentration of ascorbic acid from the curve in mg/ml V = volume of extract in ml m = weight of crude plant extract in gm.

Result and Discussion

DPPH radical Scavenging Assay :

The antioxidant activity of ascorbic acid by DPPH method was found to be greater than those of other extracts. There was significance decrease in concentration of the DPPH radical due to the scavenging ability of different extracts. Four extracts exhibited considerable DPPH radical Scavenging activity as indicated by IC_{50} values in Table-1. IC_{50} showed the potency of scavenging activity.

Table-1 : IC_{50} Values of different extracts of *Asparagus racemosus* in DPPH Scavenging assay

Extracts/standard	IC_{50} µg/ml
Petroleum ether	268.21
Ethanol	75.25
n-hexane	980.20
Chloroform	715.51
Ascorbic acid	5.570

Table 2 : Total Flavonoid contents Total Phenolic contents and total Antioxidant capacity of the different extracts of roots of *Asparagus racemosus*.

Extracts	Total Flavonoid contents (mg/gm) Quercetin equivalent	Total Phenolic contents (mg/gm Gallic acid equivalent)	Total antioxidant capacity (mg/gm) Ascorbic acid equivalent
Ethanol	125.70 ± 5.0	106.78 ± 2.55	635.912 ± 65.75
Petroleum ether	100.18 ± 2.2	52.42 ± 1.68	480.15 ± 50.85
n-hexane	68.4 ± 2.46	20.45 ± 2.98	310.55 ± 68.95
Chloroform	55.32 ± 3.80	77.75 ± 4.55	365.155 ± 20.75

Values are the mean of duplicate experiments and represented as mean ± SD.

In comparison to standard ascorbic acid ethanol and petroleum ether extract of *Asparagus racemosus* root displayed IC_{50} of 75.25 and 268.21 respectively. Chloroform and n-hexane are seen to have the least free radical scavenging activity.

Total Flavonoid contents :

Total Flavonoid contents of different extracts of *Asparagus racemosus* roots was determined by Aluminium chloride colorimetric method. Flavonoid contents of the extracts were found to decrease in the following order : ethanol extract > Petroleum ether extract > chloroform extract > n-hexane extract.

Total Phenolic content :

Folin-Ciocalteu reagent was used to determine the total phenolic content of the different solvent extracts of roots of *Asparagus racemosus* and were expressed as gallic acid equivalent per gram of plant extract. Phenol contents of the different solvent extracts were found to decrease in the following order ethanol extract > Chloroform extract > Petroleum ether > n-hexane extract (Table-2).

Total Antioxidant activity :

Total antioxidant capacity of different solvent extracts of roots of *Asparagus racemosus* was found to decrease in the following order : ethanol extract > petroleum ether extract > chloroform extract > n-hexane extract.

The result of the present study indicated the comparative study of antioxidant activity of different solvent extract of roots of *Asparagus racemosus*. The total phenolic contents and total flavonoids content in the ethanolic extract were also higher than other extract. Flavonoids play an important role in antioxidant system in roots of *Asparagus racemosus*.

The antioxidant properties of flavonoids are due to scavenging of free radicals. Chelation of metal ions and inhibition of enzymes responsible for free radical generations.[12, 13, 14] The present study explain the high contents of flavonoids in *Asparagus*

racemosus and its high radical scavenging activity. In DPPH scavenging activity the ethanol extract scavenged maximum than remaining three extracts which is may be due to its high phenol and flavonoid content.

Increase in the absorbance indicates increase in the antioxidant activity. Increase in the absorbance of the reaction mixture indicates the reducing power of the sample. Reducing power is associated with antioxidant activity.

Conclusion

Asparagus racemosus is a excellent plant with tremendous potential. Literature is available regarding its biological activities. The results of present study have shown highly phenolic and flavonoid contents. Further analysis is required to authenticate and find out bioactive compound from *Asparagus racemosus*.

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