



Antioxidant Potential of Methanolic Root Extract of *Asparagus racemosus* Linn

Waseem Raja* Tanzeela Jan**

1-Bhoj Mahavidyalya Bhopal M.P.

2-Department of Zoology Career College Bhopal M.P

Abstract

Aim: The present study deals with the determination of total phenolic and flavonoid contents, and evaluation of antioxidant activity of roots of *Asparagus racemosus* Linn. **Material and Method:** Antioxidant properties were determined by 1, 1- diphenyl, 2-picrylhydrazyl (DPPH), total phenols and flavonoid contents. **Result:** The total phenolic and flavonoid contents of the extracts were 125.20 ± 1.12 mg/g and 89 ± 2.01 mg/g respectively. **Conclusion:** The presence of high contents of phenols and flavonoids in the isolated fraction of *Asparagus racemosus* extract can explain its antioxidant potential.

KEYWORDS: Phenolics (phenols and flavonoids), ascorbic acid, DPPH, antioxidant; *Asparagus racemosus*

Introduction

A great number of modern medicines have been derived from plants that are considered as important sources of medicinal agents to treat different diseases. ⁽¹⁾ Plants have been important sources of compounds with potential medicinal activity since time immemorial. Indian sub-content is rich in such medicinal plants and Indian traditional medicinal system (Ayurveda) which is primarily based on plant based medicinals, has survived since thousands of years of time upto the present. ⁽²⁾ The complex biochemical reactions of the body and increased exposure to environmental toxicants and dietary xenobiotics result in the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative stress under different pathophysiological conditions. ⁽³⁾

Free radicals are highly reactive molecules or chemical species capable of independent existence. Their production however, multiplies several folds during pathological conditions. The release of oxygen free radicals has also been reported during the recovery phases from many pathological noxious stimuli to the cerebral tissues. ⁽⁴⁾

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources.

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydro-peroxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Several studies revealed that phenols, mainly flavonoids, from some medicinal plants are safe and bioactive, and have antioxidant properties and exert anticarcinogenic, antimutagenic, antitumor, antibacterial, antiviral, and anti-inflammatory effects. ⁽⁵⁾

There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature. ⁽⁶⁾

The active compounds in most parts of the medicinal plants have indirect or direct therapeutic effects and are used as medicinal agents. ⁽⁷⁾ With keeping these strategies and understanding in mind, here we explore one of the prevalent herbs (*Asparagus racemosus*). 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. ⁽⁸⁾

Asparagus racemosus is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicine. A lot of chemical analysis has been carried out on *A. racemosus*. The major reported constituent includes steroidal sponins, Shatavarian I, Shatavarian II, and glycoside-AR-4, with the two major ones being named Shatavarian I-IV. ⁽⁹⁾

Objectives of the study-

** Evaluation of antioxidant activity of isolated bioactive compounds.

Materials and Methods

Collection and Authentication of plant material

The plant material of *A.racemosus* was collected from the local surroundings at Bhopal (M.P). The plant material was identified and authenticated by Dr. Jagrati Tripathi, Unique College Bhopal (MP).The voucher specimens (**Herbarium; V. No. 03EA, 23/2013**) were kept in the herbarium of Bhoj Mahavidyalaya Bhopal (M.P.) for future reference.

Ethno medicinal uses of *Asparagus racemosus*

Asparagus racemosus (Satavar, Shatavari, or Shatmul) is a species of *Asparagus* common throughout India, Sri Lanka and Himaliyas. It grows one to two meters tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1300-1400 meters elevation. *A.racemosus* is an important medicinal plant of tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in indigenous systems of medicine. The genus *Asparagus* includes about 300 species around the world.

The genus is considered to be medicinally important because of the presence of steroidal saponins and sapogenins in various parts of the plant. Recently, it has been shown that *A.racemosus* contains 10 steroidal-saponins. Out of the 22 species of *Asparagus* recorded in India; *A.racemosus* is the one most commonly used in traditional medicine. Use of *A.racemosus* was mentioned in the ancient literature of Ayurveda (Charaka samhita).

DPPH free radical scavenging activity

Preparation of standard solution

Required quantity of ascorbic acid was dissolved in methanol and ethyl-acetate to give the concentration of 25, 50, 75, 100, and 125µg/ml.

Preparation of test sample

Stock solutions of samples were prepared by dissolving 5 mg of methanol in 5 ml of methanol to give concentration of 1mg/ml. Required volume of stock solution was dissolved in methanol to give the concentrations of 25, 50, 75, 100and125, /ml and same procedure was followed for ethylacetate.

Preparation of Control (DPPH solution)

0.5ml of 1M DPPH was added to 2.5ml of methanol and ethylacetate and absorbance was taken immediately at 517 nm and same for ethylacetate.

Protocol for estimation of DPPH scavenging activity

The method of ⁽¹⁰⁾ was used for the determination of scavenging activity of 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) free radical of the extract solution. A solution of 1mM DPPH in methanol and ethylacetate was prepared as a control and 50µl of this solution was added to 2.95 ml of solutions prepared in different concentrations (25,50, 75,100 and125µg/ml.) The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517nm. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity.

The ability of column fractions to scavenge DPPH radical was calculated by the equation

$$\text{DPPH radical scavening activity} = \frac{AC - AS}{AC} \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample of methanolic extract or standards.

Each experiment was carried out in triplicates.

Total phenolic and flavonoid contents

Determination of Total phenolic content (TPC) of column fractions SQ-1, SQ-2 and SQ-3

The column fractions SQ-1, SQ-2 and SQ-3 were analyzed for total phenolic content (TPC) using spectrophotometer by Folin-Ciocalteu method. Gallic acid was used as the reference standard. All determinations were carried out in triplicate and the results were expressed as mg/g gallic acid equivalent (GAE). Different concentrations i.e, 0.01, 0.02, 0.03, 0.04, 0.05 mg/ml of gallic acid, was prepared in methanol.

Concentrations of 0.1 – 1mg/ml of column fractions was also prepared in methanol. 0.5ml of each fraction sample was taken, mixed with 2.5 ml of (a 10 fold) dilute folin-Ciocalteu reagent and 2ml of 7.5% sodium carbonate solution. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature. The absorbance at 765 nm was measured after 30 minutes at 20° C and the calibration curve was drawn. To the similar reagent, 1 ml column fraction (40 mg/ml) was mixed as described above and after 1 hr. the absorbance was measured.

Determination of Total flavonoids content of column fractions SQ-1, SQ-2 and SQ-3

Aluminum chloride spectrophotometric method was used for flavonoids determination. The methanolic extract (0.5 ml of 200mg/ml FW) was diluted with 4 ml double distilled water. Diluted extracts of rhizome were mixed with 5% (0.3 ml) NaNO₂. 10% aluminum chloride was then added with reaction mixture. After 6 minutes, 2ml (1.0 M) NaOH and 2.4 ml D.D. water was added and mixed well. Thereafter, absorbance was measured at 510 nm in spectrophotometer. Standard solutions Rutin (10-100 µg/ml) was used as calibration curve.

Statistical Analysis

The results are expressed as the mean $\bar{x} \pm$ standard error. The data from biochemical determinations were analyzed using the Dunnett's test.

Results and Discussion

Antioxidant assay (DPPH Assay) of crude methanolic and ethyl-acetate extract of *A.racemosus* Linn.

The *A.racemosus* extracts were subjected to DPPH Assay and % inhibitions were calculated by following Method:

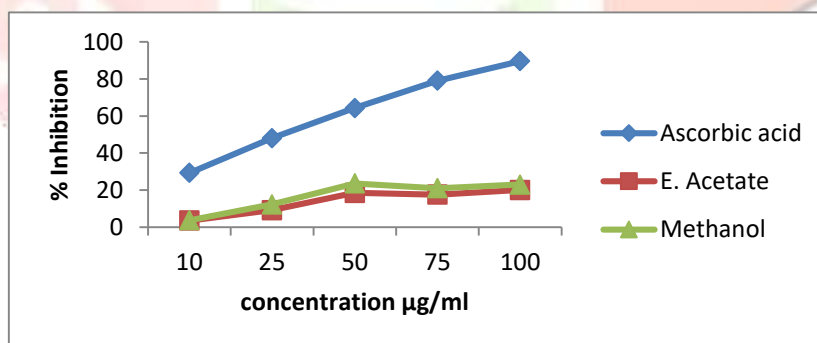
$$\% \text{ Anti-radical Activity} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100$$

Each experiment was carried out in triplicate.

Table 3: Evaluation of ethyl acetate and methanol extract of *A.racemosus* for antioxidant activity using DPPH Assay

| Conc. µg/ml | % Inhibition µg/ml | | |
|------------------------|--------------------|---------------|----------|
| | Ascorbic acid | Ethyl acetate | Methanol |
| 25 | 29.31 | 3.5 | 3.7 |
| 50 | 48.01 | 9.18 | 12.23 |
| 75 | 64.27 | 18.48 | 23.58 |
| 100 | 79.11 | 17.55 | 21.01 |
| 125 | 89.6 | 20.01 | 22.98 |
| IC ₅₀ Value | 55.19 | 259.30 | 220.42 |

Graph 1: Showing the effect of ethyl acetate and methanol extract of *A.racemosus* on accumulation of DPPH.



Ethyl acetate and methanol extracts of *A.racemosus* showed potent anti-oxidant activity in DPPH screening. The IC₅₀ value of ethyl acetate and methanol extract was 259.30 and 220.42 µg/ml respectively for DPPH assay. The known anti-oxidants ascorbic acid exhibited IC₅₀ value of 55.19 µg/ml for DPPH respectively. Thus antioxidant assay revealed that the methanolic extract of *A.racemosus* proved most potent antioxidant.

In-vitro antioxidant activity of column fractions SQ1, SQ2 and SQ3

The column fractions SQ1, SQ2 and SQ3 were subjected to DPPH Assay and % inhibitions were calculated as mentioned above.

Table 4: Evaluation of isolated bioactive fractions from methanolic extract of *A.racemosus* for antioxidant activity using DPPH Assay

| Conc. µg/ml | % Inhibition µg/ml | | | |
|------------------|--------------------|--------|-------|--------|
| | Ascorbic acid | SQ-I | SQ-II | SQ-III |
| 25 | 29.31 | 4.71 | 11.33 | 7.91 |
| 50 | 48.01 | 15.23 | 26.87 | 19.29 |
| 75 | 64.27 | 29.68 | 34.02 | 35.01 |
| 100 | 79.11 | 34.01 | 62.30 | 42.04 |
| 125 | 89.6 | 39.89 | 75.21 | 52.26 |
| IC ₅₀ | 55.19 | 146.17 | 84.68 | 117.13 |

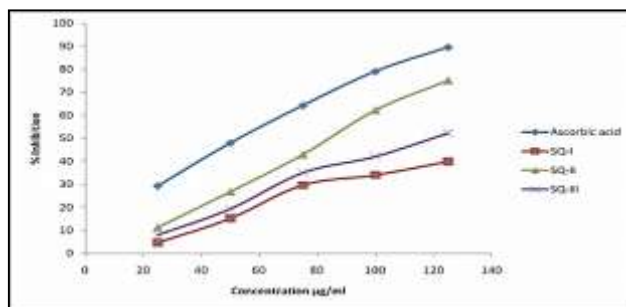
Graph 2: Showing the effect of isolated bioactive fractions from methanolic extract of *A. racemosus* on accumulation of DPPH.

Table 5: Total Phenolic content in Fractions expressed in mg/g equivalent to Gallic Acid.

| Fraction | Absorbance (Mean±SD) | Conc. of Fraction | Total Phenolic Content mg/g equivalent to Gallic Acid (Mean±SD) |
|----------|----------------------|-------------------|---|
| SQ-I | 0.689 ± 0.216 | 0.01mg/mL | 64.60 ± 2.01 |
| SQ-II | 0.992 ± 0.113 | 0.01mg/mL | 125.20 ± 1.12 |
| SQ-III | 0.793 ± 0.012 | 0.01mg/mL | 85.40 ± 1.36 |

Table 6: Total Flavonoid content in Fractions expressed in mg/g equivalent to Rutin.

| Fractions | Absorbance (Mean±SD) | Conc. of Fraction | Total Flavonoid Content mg/g equivalent to Rutin (Mean±SD) |
|-----------|----------------------|-------------------|--|
| SQ-I | 0.236 ± 0.005 | 0.01mg/mL | 10.5 ± 2.11 |
| SQ-II | 0.393 ± 0.003 | 0.01mg/mL | 89 ± 2.01 |
| SQ-III | 0.281 ± 0.002 | 0.01mg/mL | 33 ± 1.21 |

Discussion

Man from his first awakening, has sought to combat and control disease and pain, with assistance, inspiration and guidance from its natural environment. As far as life is concerned subject of priority is health. But despite effort to maintain good health, man and animals alike still confront disease conditions which are due to exposure to physio-pathological agents. This ability to activate the body defense mechanism or to protect the body system has been found to be present in some nature vegetation/herbal sources.

Natural products and their derivatives contribute more than half of all clinically administered drugs. ⁽¹¹⁾ They possess a significant position in drug discovery for treatment of infectious diseases. ⁽¹²⁾ During recent years herbal medicine has become an increasingly scientifically based system of healing. Of the several hundred thousand plant species around the world, only a small proportion has so far been investigated both phyto-chemically and pharmacologically. As a single plant may contain thousands of constituents, the possibilities of making new discoveries become evident. The selection of plant material is thus a crucial factor for the ultimate success of the identification of bioactive plant constituents. ⁽¹³⁾

Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals. ⁽¹⁴⁾ The active compounds in most parts of the medicinal plants have indirect or direct therapeutic effects and are used as medicinal agents. ⁽⁵⁾ Antioxidants act as a defense mechanism that protects against oxidative stress damage, and include compounds to remove or repair damaged molecules. It can prevent the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases. Plants are the source of medicines for preventive, curative, protective or promotive purposes. ⁽¹⁵⁾ However, the natural antioxidant compounds become important. ⁽¹⁶⁾

Flavonoids are mainly analysed in terms of their use for medicinal purposes. They exhibit anti-inflammatory properties: it was confirmed that they can also reduce the blood pressure, strengthen the cell walls of blood vessels, and improves the immune system. ⁽¹⁷⁾

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical analysis, saponins, phenols, and flavonoids were present in high amount in *A. racemosus* as compared to other phytoconstituents analyzed. The presence of alkaloids, phenolic compounds, tannins have been associated with various degrees of antioxidant activities. Therefore, antioxidant effects observed in this study may be due to the activity of one or a combination of some of the classes of compounds present in *A. racemosus*.

A. racemosus is a storehouse of a large number of anthraquinones. Anthraquinones are the largest class of natural quinones and occur more widely in the medicinal and dietary plants than other natural quinines. ⁽¹⁸⁾ *A. racemosus* is one of the well-known drugs in Ayurveda and its root extract have several pharmacological activities including antiulcer, antioxidant, antidiarrhoeal, antidiabetic and

immunomodulatory⁽¹⁹⁾ antifungal activity antibacterial activity, anti-abortifacient activity, Antioxytotoxic (shatavarin 4), spasmodic to uterus Hypoglycaemic, hypotensiv activity, anticoagulant activity.⁽²⁰⁾

Antioxidant potential of various crude extracts of *A.racemosus* extracts was determined on the basis of mostly used methods i.e, DPPH. The DPPH oxidative assay⁽²¹⁾ adopted is used worldwide in the quantification of radical-scavenging capacity. The antioxidant activity of the samples was expressed as IC₅₀ values. The IC₅₀ value was defined as the concentration (in µg/ml) of the standard or drug that inhibits the formation of DPPH radicals by 50%. The lowest IC₅₀ indicates the strongest ability of the extracts to act as DPPH radical scavengers. Methanol crude extract showed strongest ability to act as DPPH radical scavengers by showing lower IC₅₀ values of **220.42µg/ml** for methanol extract (**Table 3**) while as ethyl acetate showed IC₅₀ of **259.30 µg/ml**.

In present investigation the total phenolic contents of isolated fractions of methanol crude extracts of *A.racemosus* was determined by using the Folin-Ciocalteu reagent in terms of gallic acid equivalent. Galic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent to (GAE). Line of Regression ($y = 0.005x + 0.365$, $R^2 = 0.973$) from Gallic acid was used for estimation of unknown phenol content. The quantitative analysis of TPC of methanolic fraction of *A.racemosus* revealed that the SQ2 contains highest amounts of TPC (**125.20 ± 1.12 GAE/gm**), followed by SQ3 (**85.40 ± 1.36 mgGAE/gm**), whereas moderate amounts were recorded in SQ1 (**64.60 ± 2.01mgGAE/gm**).

The total flavonoid content of column fractions of methanolic crude extracts of *A.racemosus* was determined and was expressed as mg rutin/g dry weight (mg rutin/g DW). Rutin was used as standard compound for the quantification of total flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin/g DW). Line of regression ($y = 0.002x + 0.215$, $R^2 = 0.990$) from Rutin was used for estimation of unknown flavonoid content. The quantitative analysis of TFC in extracts revealed that methanolic fraction SQ2 contained highest amount of TFC (**89 ± 2.01 mgRE/gm**) followed by SQ3 (**33 ± 1.21 mgRE/gm**) whereas very less amount was found in SQ1 (**10.5 ± 2.11mgRE/gm**). The presence of high contents of phenols and flavonoids in the SQ2 fraction of *A.racemosus* extract can explain its antioxidant potential.

Conclusion

Antioxidant potential of isolated fractions was ascertained on the basis of DPPH Assay. IC₅₀ values obtained for these three fractions SQ1, SQ2 and SQ3 were **146.17µg/ml**, **84.68µg/ml** and **117.13 µg/ml** respectively. On the basis of above results it was concluded that SQ2 has lower IC₅₀ value and hence has highest antioxidant potential because the lowest IC₅₀ indicates the strongest ability to act as DPPH radical scavenger. The presence of high contents of phenols and flavonoids in the SQ2 fraction of *A.racemosus* extract can explain its antioxidant potential.

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