



TOTAL PHENOLIC CONTENT & TOTAL FLAVANOID CONTENT OF *ASPARAGUS RACEMOSUS* LEAVES METHANOLIC EXTRACT

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ABSTRACT

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. *Asparagus racemosus* is a very important medicinal plant and used in various diseases. The methanolic leaf extract obtained through successive solvent extraction from *Asparagus racemosus* in order to prove the ethnopharmacological application of the plant in Indian folk medicine. The total phenolic content (TFC) and total flavonoid concentration were determined using spectrophotometric method. The TPC 126.4 ± 1.562 mg/g expressed as gallic acid equivalents and the total flavonoid concentration 47.6 ± 3.511 mg/g expressed as rutin equivalents. Methanolic extract of *Asparagus racemosus* showed the highest phenolic and flavonoid concentration. The quantitative estimation of total phenolic and total flavonoid contents showed the biological properties of the extracts might provide detailed evidence for the use of this plant in different medicines.

Keywords: *Asparagus racemosus*, Spectrophotometric method, TPC and TFC.

INTRODUCTION

Phytochemistry or plant chemistry has been developed in recent years as a distinct discipline between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances [1]. It is estimated that 70 to 80% of people worldwide depend on the traditional herbal medicines to meet their primary health care needs because it is safe. Plants contain varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids [2-3]. The majority of these bioactive compounds are imperative that the plants are important sources of treatment of various diseases in the world.

A. racemosus the common name 'Shatavari' means "She who possesses a hundred husbands" [4]. In Ayurveda *Asparagus racemosus* is known as the "Queen of Herbs". *Asparagus racemosus* leaves are reduced to needle-like sub erect, soft spines. *A. racemosus* is one of the most important medicinal plants used in indigenous system of medicine. Roots and leaves are the sources of drug. Roots are useful in nervous disorders, dyspepsia, diarrhea, dysentery, tumours, inflammations, burning

sensations, throat infections, tuberculosis, cough, bronchitis, gonorrhoea, leucorrhoea, leprosy, epilepsy, fatigue, hyperacidity, haemorrhoids, cardiac debility, and hypertension [5].

MATERIAL AND METHODS

Scientific Classification

Kingdom - Plantae
Family - Asparagaceae
Genus - *Asparagus*
Species - *Asparagus racemosus*

Collection and extraction of the plant

Plant sample of *Asparagus racemosus* leaves were collected from the Govt. Nursery of Bhopal & identified by Dr. Madhuri Modak, Professor, Department of Botany, Govt. M.V.M., College, Bhopal. The leaves of the plants were properly washed with tap water and then rinsed with distilled water. The washed leaves were shade dried and crushed to obtain powder. A quantity of 100g of the dried powder of *Asparagus racemosus* extracted with methanol using soxhlet apparatus. The extraction was done for 48 hours or till the cycles are completed. All the chlorophyll and lipids are deposited on the side of the flask and skill it was removed. To obtain the crude extract. Then

the extract was evaporated to dryness and then subjected to quantitative estimation of total phenolic content (TPC) and total flavonoid concentration (TFC).

Total Phenolic Content

The amount of total phenolic in extracts was determined with the Folin Ciocalteu reagent [6]. Gallic acid was used as a standard and the total phenolic were expressed as mg/g gallic acid equivalent (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1 mg/ml of plant extract were also prepared in methanol and 0.5 ml of each sample were introduced in to test and mixed with 2.5 ml of a 10 fold dilute folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 760 nm spectrometrically. All determination was performed in triplicate. The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically. Line of regression from Gallic acid was used for estimation of unknown phenol content. From standard curve of gallic acid line of regression was found to be

$$y = 0.005x + 0.065 \text{ and } R^2 = 0.976$$

Thus this was found to be good for the standard curve and the absorbance of test sample ($y = \text{absorbance}$) in line of regression of above mentioned GA.

Total Flavonoid Concentration

Total flavonoids were measured by a colorimetric assay according to Dewanto et al. An aliquot of diluted sample or standard solution of rutin was added to a 75 μl of NaNO_2 solution, and mixed for 6 min, before adding 0.15 mL AlCl_3 (100 g/L). After 5 min, 0.5 mL of NaOH was added. The final volume was adjusted to 2.5 ml with

distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin /g DW), through the calibration curve of Rutin. All samples were analysed in three replications. Line of regression from rutin was used for estimation of unknown flavonoid content [7-8]. From standard curve of rutin, line of regression was found to be

$$y = 0.001x - 0.118 \text{ and } R^2 = 0.985$$

The absorbance of test sample ($y = \text{absorbance}$) in line of regression of above mentioned rutin in a standard curve.

RESULT AND DISCUSSION

The methanolic extract was prepared to examine the total phenolic and total flavonoid concentration present in the leaves extract of *Asparagus racemosus*. The total phenolic contents examined in the plant extract with Folin Ciocalteu reagent; expressed in terms of gallic acid equivalent (the standard curve equation : $y = 0.005x + 0.065$ and $R^2 = 0.976$). The concentrations of total phenols are expressed as mg of GA/g of the extract and the concentration of flavonoid in methanolic extract of the *Asparagus racemosus* was determined using Colorimetric assay with NaNO_2 solution. The concentration of total flavonoids was expressed in terms of rutin equivalent (the standard curve equation: $y = 0.001x - 0.118$ and $R^2 = 0.985$), mg of Ru/g of the extract.

Flavonoids and phenolic acids are classes of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [9]. Methanolic extract of *Asparagus racemosus* have high concentration of total phenolic and flavonoid concentration.

Total Phenolic Content

Table 1. Standard Curve of Gallic Acid

S.No.	Concentration ($\mu\text{g/ml}$)	Absorbance
1	10	0.1098
2	20	0.1763
3	30	0.2468
4	40	0.2981
5	50	0.3258

Table 2. Total Phenolic Content in Methanolic Extract

S.No.	Absorbance	Concentration	Total Phenolic Content in mg/g equivalent of Gallic acid
1	0.702	1mg/ml	127.4
2	0.708	1mg/ml	124.6
3	0.701	1mg/ml	127.2
			126.4 ± 1.562

Total Flavonoid Contents**Table 3. Standard Curve of Rutin**

S.No.	Concentration ($\mu\text{g/ml}$)	Absorbance
1	10	0.135
2	20	0.151
3	30	0.165
4	40	0.177
5	50	0.201

Table 4. Total Flavonoid content in Methanolic Extract

S.No.	Absorbance	Concentration	Total Flavonoid Content in mg/g equivalent of Rutin
1	0.162	1mg/ml	44
2	0.166	1mg/ml	48
3	0.169	1mg/ml	51
			47.6 ± 3.511

CONCLUSION

The results of the study showed that the *Asparagus racemosus* possess the great medicinal value. It could be concluded that this plant is natural sources of antioxidant and other biological activities. Among the great variety of secondary compounds found in plants, presence of phenolics as one of the antimicrobial agents currently knows. Aromatic compounds such as phenol, phenolic acids, alkaloids, flavonoids, saponins and steroids have

been identified as antimicrobial agents. These compounds produced by plants are active against human pathogenic microorganisms. The present study carried out on the plant samples revealed the presence of medicinally active constituents. It is strongly believed that the phytochemical and various biological properties of the extracts might provide detailed evidence for the use of this plant in different medicines.

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