

Original Article

PHYTOCHEMICAL ANALYSIS, ANTIMICROBIAL ACTIVITY AND ASSESSMENT OF POTENTIAL COMPOUNDS BY THIN LAYER CHROMATOGRAPHY OF ETHANOL FRACTION OF *ASPARAGUS RACEMOSUS* ROOTS

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ABSTRACT

**Objectives:** *Asparagus racemosus* (AR) Willd, is commonly known as Shatavari, belongs to family Liliaceae, and has potent medicinal values. The present study deals with detail Pharmacological evaluation of ethanol fraction of AR.

**Methods:** Preliminary phytochemical screening, antimicrobial and antioxidant activity, qualitative analysis of antioxidant and flavonoid detection, total phenol and flavonoid content were measured.

**Results:** Phytochemical screening showed the presence of various phytoconstituents like phytosterols, phenols, flavonoids, saponins and carbohydrates. Ethanol fraction of *Asparagus racemosus* roots have maximum relative percentage inhibition against gram (-) bacteria. The root displayed significant DPPH free radical scavenging activity against standard ascorbic acid. Thin layer chromatography of the ethanol fraction was also performed to determine the active principles. Four major spots obtained with the Rf values 0.20, 0.22, 0.24 and 0.58. Total flavonoid and phenol content was 188.3 mg/g quercetin equivalent and 165.22mg/g gallic acid equivalent respectively.

**Conclusion:** The findings of the current study shows that *Asparagus racemosus* is a good source of various phytochemicals and can be useful to progress and surge further scientific investigation to prevent and management of different diseases by their medicinal properties.

**Keywords:** *Asparagus Racemosus*, Phytochemical, Antimicrobial, Antioxidant, Flavonoid.

INTRODUCTION

Plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management and treatment of many intractable diseases. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [1]. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world [2]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine [3]. *Asparagus racemosus* is commonly known as Shatavari or Shatamuli has been used in ayurvedic medicine from long past. The roots are cylindrical, fleshy and tuberous.

The roots are 30-100 cm in length, 1-2 cm in thickness and yellowish-cream in colour. The roots contain long needle shaped structure known as pith which is meant for the conduction of water [4]. It is one of the best rejuvenative drugs of classical Ayurveda. Its pharmacological applications, particularly from the root extracts, have recently been found to possess a phytoestrogenic effect, an effect on neurodegenerative disorders, as well as antidiarrhoeal [5], antidyspepsia, adaptogenic, antiulcer, cardioprotective, antibacterial [6], immunoadjuvant and antitussive effects.

The root extracts of AR have been employed in two major forms as methanolic and aqueous extracts, the products of which include root extract in tablet form, root powder in tablet form and root extract in syrup form [7]. A study was done on different extracts of AR to reduce acetaminophen induced uremia and hydromethanol extract shows maximum protective effect to reduce uremia [8]. Thus this study is aimed to demonstrate and determine the phytochemical analysis of ethanol fraction separated from hydromethanol extract

of AR roots including antimicrobial activity, preliminary phytochemical screening by TLC for flavonoids and antioxidants, determining total antioxidants, flavonoids, phenols etc.

METHODS AND MATERIALS

Collection of plant material

Roots of *Asparagus racemosus* (AR) was collected from Gopali, Kharagpur, Paschim Medinipur, district of West Bengal. The material was identified by the taxonomist of the Botany Department at the Raja N. L. Khan Women's College, Midnapore. The voucher specimens were deposited in the Department of Botany, Raja N. L. Khan Women's College.

Preparation of ethanol fraction of AR root

Roots of AR were washed and cut into small pieces. The plant parts were shade dried at 37 °C and then crushed in an electrical grinder. A total of 100 g root dust of said plant material was then washed in 400 mL of hexane for 24 h to remove the greasy pigmented non polar materials. Then the hexane was discarded and residue was dissolved in hydromethanol (4:6) for 2 h in a soxhlet apparatus. Then the extracts were filtered through Whatman No. 1 filter paper and the filtrate was evaporated in a rotary vacuum evaporator.

These extracts were used for separation and fractionation of compounds. The hydromethanol extract was then dissolved ethyl acetate for 2 h in a soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried in the air. The ethyl acetate extract was dissolved in 250 mL ethanol for 2 h in a soxhlet apparatus. Then it was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried under reduced pressure at 40 °C on a rotary evaporator. Then the ethanol fraction was stored in refrigerator for further study.

### Bacterial strain and culture conditions

Two Gram negative and two Gram positive indicator bacteria was used for antimicrobial assay respectively, *Escherichia coli* (*E. coli*) (MTCC 443), *Klebsiella pneumoniae* (*K. pneumoniae*) (MTCC 109), *Staphylococcus aureus* (*S. aureus*) (MTCC 3160) and *Streptococcus mutans* (*S. mutans*) (MTCC 890) were provided by microbiological laboratory and clinical detection center Midnapur (West Medinipur, India). They were cultured in tryptone soy broth or agar (TSB or TSA) in aerobic condition at 37 °C.

### Qualitative phytochemical analysis

Ethanol fraction of *AR* were subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids etc. [9-11].

### Antimicrobial analysis

The antimicrobial activity was determined in the ethanol fraction of *AR* using agar well diffusion method. The antibacterial activities of *AR* (concentration 50 µgml<sup>-1</sup>) were tested against two Gram-positive (*S. aureus*, *S. mutans*) and two Gram negative (*K. pneumoniae*, *E. coli*) human pathogenic bacteria. Zone of inhibition of ethanol fraction of *AR* root was compared with standards like chloramphenicol for antibacterial activity [12].

### Determination of relative percentage inhibition

The relative percentage inhibition of the test *AR* fraction with respect to positive control was calculated by using the following formula [13].

Where,

$$\text{Relative percentage inhibition of the test fraction} = \frac{100 \times x - y}{z - y}$$

x: total area of inhibition of the test fraction

y: total area of inhibition of the solvent

z: total area of inhibition of the standard drug

The total area of the inhibition was calculated by using area =  $\pi r^2$

where, r = radius of zone of inhibition.

### TLC analysis for antioxidant constituents

The TLC plates were developed in methanol: chloroform: hexane (7:2:1, v/v/v) and sprayed with 0.05% DPPH reagent. Purple colour of DPPH reagent was bleached by yellow spots which was the indication of positive antioxidant activity. Ethanol fraction of *AR* root was spotted on the plate against ascorbic acid [14].

### TLC for flavonoid

TLC was performed on the 20 × 20 cm plates precoated with microcrystalline cellulose (Camag, Muttnanez, Switzerland). A volume of 1 µL of 1% ethanolic solutions of *AR* fraction was spotted on the plate. One-dimensional TLC analysis was performed with chloroform: ethyl acetate: formic acid in volume ratio 10:8:2 as mobile phase. Spots were observed after spraying with the anisaldehyde sulphuric acid reagent.

Rf Value - It is a ratio of distance travelled by the sample and distance travelled by the solvent.

$$\text{Rf Value} = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

### Antioxidant activity determination by DPPH free radical scavenging assay

DPPH radical scavenging activity of the *AR* ethanol fraction was measured by DPPH free radical scavenging assay method [15]. For this study, different concentrations of sample and ascorbic acid (standard) were prepared with ethanol (Sigma- Aldrich) as the test solutions. About 1 mL of each prepared concentrations were placed into test tubes and 0.5 mL of 1 mmol/L DPPH solution in methanol was added. The test tubes were incubated for 15 min and the absorbance was read at 517 nm. A blank solution consisted of DPPH dissolved in same amount of methanol. The DPPH radical scavenging activity percentage was calculated by using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{AR fraction}}}{A_{\text{control}}} \times 100$$

### Determination of Total Phenolic Content

Total phenolic contents in the ethanol fraction of *AR* was determined by the Folin-Ciocalteu reagent method [16]. 1 ml of the ethanolic fraction (1mg/ml) /standard solution was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for color development. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solution contained the solvent used to dissolve the test fraction. The total content of phenolic compounds in plant fraction in gallic acid equivalent (GAE) was calculated using the following equation:  $C = (c \times V)/m$  where; C= total content of phenolic compounds, mg/gm plant fraction, in GAE, c= concentration of gallic acid established from the calibration curve (mg/ml), V = volume of fraction in ml, m = weight of crude plant extract in gm.

### Determination of Total Flavonoids Content

Aluminum chloride colorimetric method was used for flavonoids determination [17]. 1 ml of the ethanolic fraction (1mg/ml) /standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank.

The total content of flavonoid compounds of plant methanol extracts in quercetin equivalent was calculated by the following equation:  $C = (c \times V)/m$  where; C = total content of flavonoid compounds, mg/gm plant fraction, in quercetin equivalent, c = concentration of quercetin established from the calibration curve in mg/ml, V = volume of fraction in ml and m = weight of crude plant fraction in gm.

## RESULTS

### Preliminary phytochemical screening

From the preliminary phytochemical screening, we found that the ethanol fraction of *AR* roots possess Phytosterols, Carbohydrates, Flavonoids, Phenolic Compounds & Tannins (Table 1).

**Table 1: Phytochemical analysis of ethanol fraction of *Asparagus racemosus***

| S. No. | Phytochemicals               | Name of biochemical test   | Ethanol fraction |
|--------|------------------------------|----------------------------|------------------|
| 1      | Phytosterols                 | Salkowski reaction         | +                |
| 2      | Triterpenoids                | Liebermann-Burchard's test | -                |
| 3      | Saponins                     | Foam test                  | ++               |
| 4      | Alkaloids                    | Dragendroff's test         | -                |
| 5      | Carbohydrates                | Molisch's test             | +                |
| 6      | Flavonoids                   | Lead Acetate test          | ++               |
| 7      | Proteins & Amino acids       | Ninhydrin test             | -                |
| 8.     | Tannins & Phenolic compounds | Ferric chloride test       | ++               |
| 9.     | Glycosides                   | Keller-Killiani test       | +                |
| 10.    | Lactones                     | Legal's test               | -                |

Table 2 Antimicrobial activities of ethanol fraction of *Asparagus racemosus*

| Sample and standard                           | Diameter of Zone of inhibition (mm)* |                             |                              |                         |
|---|--------------------------------------|-----------------------------|------------------------------|-------------------------|
|   | <i>Staphylococcus aureus</i>         | <i>Streptococcus mutans</i> | <i>Klebsiella pneumoniae</i> | <i>Escherichia coli</i> |
| Ethanol fraction(50µgml <sup>-1</sup> )       | 19.6± 0.24                           | 23.6±0.83                   | 28.5±1.28                    | 34.1±0.54               |
| Chloramphenical (std) (50µgml <sup>-1</sup> ) | 25.0±1.57                            | 28.0±1.02                   | 31.0±0.84                    | 38.0±0.44               |

\*The zone of inhibition (mm) taken as average. Values are expressed as mean ± SD

Table 3: Relative percentage inhibition of ethanol fraction of *Asparagus racemosus* roots

| Test organisms               | Relative percentage Inhibition (%) |
|------------------------------|------------------------------------|
| <i>Staphylococcus aureus</i> | 78.4                               |
| <i>Streptococcus mutans</i>  | 84.28                              |
| <i>Klebsiella pneumoniae</i> | 91.93                              |
| <i>Escherichia coli</i>      | 89.73                              |

### Antimicrobial activity

The results of our investigations showed that the ethanol fraction of AR roots exhibited antimicrobial activities against some of the test bacterial isolates. Here antimicrobial activity was assessed at the conc. of 50µgml<sup>-1</sup> and compared with the activity of standard Chloramphenical. Gram negative bacteria shows more activity than gram positive bacteria also relative percentage inhibition was shown maximum by gram negative bacteria (Table 2 and 3).

### TLC analysis for antioxidant constituents

Qualitative antioxidant activity was measured by thin layer chromatography in mobile phase methanol: chloroform: hexane (7:2:1, v/v/v) and sprayed with 0.05% DPPH reagent and ascorbic acid was used as standard. Yellow color spot on the TLC plates indicate the presence of antioxidant compounds (Fig 1).



Fig. 1: TLC analysis for antioxidant property in ethanol fraction of AR root.

AR- Ethanol fraction of *Asparagus racemosus*

STD- Ascorbic acid

### TLC analysis for flavonoid constituents

Ethanol fraction of AR was chromatographed on TLC plates and best resolution was obtained in, chloroform: ethyl acetate: formic acid (10:8:2) and the plates were derivatized with anisaldehyde-sulphuric acid reagent. After using these spraying reagents four distinct spots were observed with Rf values 0.20, 0.22, 0.24 and 0.58 (Table 5, Fig 3).

### Total Antioxidant activity

The percentage inhibition of DPPH radicals by ethanol fraction of AR was shown in Figure 2. In our experiment the antioxidant capacity was compared with the standard ascorbic acid at different concentrations. It may be due to its high phenol and flavonoid contents (Fig 2).

### Total Phenolic compounds and Flavonoid Content

The quantitative determination of the total phenolic content, expressed as gallic acid equivalent per gram of AR ethanol fraction. Phenol contents of the ethanol fraction of AR was 165.22mg/g. Total flavonoid content of the AR fraction was expressed as Quercetin equivalent per gram of sample showed the content values of 188.3 mg/g shown in table no four.

Table 4: Total phenol and flavonoid contents of the ethanol fraction of *Asparagus racemosus*

| AR Fraction | Total Phenol Contents (mg/gm, Gallic Acid Equivalent) | Total Flavonoid Contents (mg/gm, Quercetin Equivalent) |
|-------------|---|--|
| Ethanol     | 165.22 ± 8.26   | 188.33 ± 7.33  |

Data are expressed as Mean ± SD

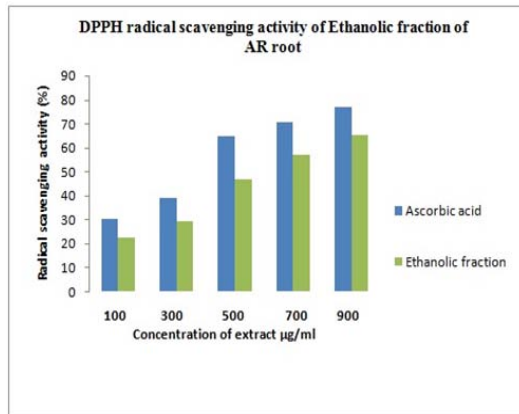
### DISCUSSION

After the preliminary phytochemical analysis it has been shown that AR root contains different type of compounds like phytosterols, saponins, phenols, flavonoids, antioxidants etc (Table 1). It has good antimicrobial activity against both gram (+) and gram (-) organisms whereas maximum inhibition was shown in case of gram (-) organisms (Table 2). The results of antimicrobial activity of ethanol fraction was compared with the positive control (Standard drugs: Chloramphenical) for evaluating their relative percentage inhibition (Table 3). It was showed that the fraction have maximum relative percentage inhibition against *Klebsiella pneumonia* (91.93), followed by *Escherichia coli* (89.73), *Streptococcus mutans* (84.28) and *Staphylococcus aureus* (78.4).

Antioxidants are major compounds which believed to have protection against certain diseases by preventing the deleterious effect of free radical mediated processes in cell membrane and by reducing the susceptibility of tissue to oxidative stress. For qualitative analysis of antioxidant activity on TLC plates with the standard ascorbic acid, there was a yellow band like standard indicate the presence of antioxidant compounds (Fig 1). In DPPH scavenging activity, reducing power of ethanol fraction of AR indicates that it may consist of polyphenolic compounds that usually show great reducing power. Antioxidant activity of was measured compared with ascorbic acid in different concentrations (Fig 2).

Flavonoids are good free radical scavengers, donate hydrogen atoms. Free radical scavenging activity of flavonoids are also distinguished [18]. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS. Phenols are well established to exhibit antioxidant activity, contribute to human health. This study denotes

the presence of flavonoids by four separate bands in the TLC plate with the Rf values 0.20, 0.22, 0.24 and 0.58 (Table 5, Fig 3) from the ethanol fraction of AR.



**Fig. 2: Comparative DPPH radical scavenging activity of Ethanol fraction of *Asparagus racemosus* root and ascorbic acid at different concentrations.**



**Fig. 3: TLC analysis for flavonoid compounds in ethanol fraction of AR root.**

Total phenol and flavonoid content of ethanol fraction of AR was 165.22 mg/g gallic acid equivalent and 188.3 mg/g quercetin equivalent respectively (Table 4). Results denote, the roots of AR possess phenols and flavonoids, chiefly responsible for antioxidant activity. It was reported that the presence of natural flavonoids could show the antioxidant and free radical scavenging properties [19] which was confirmed by our experiment. According to our investigation, the high contents of flavonoids in the ethanol fraction of *Asparagus racemosus* can explain its high radical scavenging activity.

**Table 5: Detection of flavonoids through TLC**

| Fraction | Solvent  | Spray reagent               | No of spots | Rf value                                    |
|----------|--|-----------------------------|-------------|---|
| Ethanol  | chloroform: ethyl acetate : formic acid (10:8:2) | anisaldehyde sulphuric acid | 4           | A -0.20<br>B - 0.22<br>C - 0.24<br>D - 0.58 |

## CONCLUSION

The results obtained from the above experiment showed that *Asparagus racemosus* roots contains different major antioxidative compounds like flavonoids, phenols in high amount which may be helpful for treatment of diseases as well to suppress the growth of many pathogenic organisms. Further investigations may be carried out to isolate new compounds by HPLC and NMR study to characterize these compounds from the plant roots and to evaluate its bioactivities as it is necessary to introduce new biologically safe phytochemicals.

## CONFLICT OF INTERESTS

Declared None

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