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RESEARCH ARTICLE

Justification of Traditional Uses of *Asparagus Racemosus* (Shatavari) - A Miracle Herb

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Abstract:

Objective:

The ethanolic plant extracts of *Asparagus racemosus* and their partitionates were assessed for thrombolytic, membrane stabilizing, antimicrobial and antioxidant activity *in vitro*.

Methods:

The collected plant was dried and grinded. The coarse powder was shocked in 2000 mL of 90% methanol for several days then filtrated. At 40°C the volume of crude ethanolic extract (CEE) was reduced by a vacuum rotary evaporator followed by concentrated aqueous ethanol extractive was fractionated into petroleum ether (PSF), carbon tetrachloride (CTSF), chloroform soluble fraction (CSF) and aqueous (AQSF) soluble fractions.

Results:

In thrombolytic assay among all the extractives, carbon tetrachloride soluble fraction (CTSF) showed the highest clot lysis (41.48 ± 0.43%) activity as compared with standard streptokinase (65.16 ± 0.16%). With respect to the membrane stabilizing activity, chloroform soluble fraction (CSF) profoundly inhibited the lysis of erythrocytes (80.86 ± 0.36%) induced by osmosis, whereas reference standard acetyl salicylic acid (ASA) resulted in (88.99 ± 0.39%) inhibition of lysis. On the other hand, CTSF showed slightly higher level of protection against heat-induced hemolysis (85.68 ± 0.75%) as same as with ASA, (85.68 ± 0.75%)). In antimicrobial investigation, crude ethanol extract (CEE) and their partitioning fractions exhibited moderate inhibition of growth of some examined bacteria. The total phenolic content was found maximum (930.94 mg of GAE/gm) in CEE of *A. racemosus.* The antioxidant potentials in term of DPPH free radical scavenging assay showed EC50 values 2.62 µg/mL by aqueous soluble fraction (AQSF) in comparison to ascorbic acid 2.48 µg/mL as a reference standard.

Conclusions:

This study was conducted to validate the *A. racemosus* plant as thrombolytic, membrane stabilizing agent, antimicrobial and antioxidant.

Keywords: *Asparagus racemosus*, Thrombolytic, Membrane stabilizing, Antimicrobial, Antioxidant activity.

1. INTRODUCTION

Now a days almost all parts of medicinal plants are used for numerous diseases throughout the world. The experiments of medicinal plants for their folkloric use is the utmost real way to regulate the forthcoming lead compounds. Medicinal plants always provide new substances whereas the existing ones grow resistance in some cases *e.g.* antibiotics. Alkaloids, cardiac glycosides, quinines, phenols, flavonoids, saponin are the active constituents of

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medicinal plants which have many biological functions [1]. *Asparagus racemosus* is a very common medicinal plant grown in Bangladesh belongs to the family of *Asparagaceae* and commonly known as Asparagus, Wild Asparagus, Asparagus Root, and Satavar. It is a useful flowering medicinal plant of tropical and subtropical countries like Bangladesh [2]. *A. racemosus* is the valuable medicinal herb; its roots are used in ayurvedic medicine following a regimen of processing and drying. Traditionally plants of racemosus are used in nervous disorders, indigestion, tumors, burning of urine, upper respiratory tract infections, tuberculosis, cough bronchitis and general debility. Roots are also used externally to treat stiffness in the joints and few recent reports demonstrated some additional beneficial effects of this herb including antihepatotoxic, immunomodulatory, immunoadjuvant and antilithiatic effects [3]. The present study was designed to provide the scientific evidence for utilizing folkloric medicinal values as thrombolytic, membrane stabilizing, antimicrobial and free radical scavenging activity.

Fig. (1). Photography of the whole plant together with an image of the initial coarse powder (root).

2. MATERIALS AND METHODS

2.1. Collection and Identification of Plant Materials

The whole plant of *Asparagus racemosus* was collected from Pabna and identified by taxonomist of National Herbarium, Bangladesh situated in Mirpur in Dhaka. The sample is preserved in the Phytochemical Laboratory of the World University of Bangladesh for further reference, Fig. (**1**).

2.2. Chemicals and Reagents

Streptokinase vial was commercially purchased from (Trade name-S-Kinase from Popular pharmaceutical Ltd.) and Ciprofloxacin disk from Biotech Ltd. (India). Phosphate buffer, sodium hydroxide, hydrochloric acid, sodium carbonate, solvents (Ethanol, Pet-ether, Carbon tetrachloride, Chloroform) and also other necessary chemicals and reagents were of analytical grade.

2.3. Preparation of Plant Extract

The collected plants were shade dried for several days and dehydrated at 37°C overnight to aid crushing. The powders (200 gm) were soaked in 2000 ml of 90% ethyl alcohol. The filtrate was condensed by a vacuum rotary evaporator at 40°C. Concentrated aqueous ethanol extractive was fractionated by Kupchan protocol *et al.* protocol [4] and modified by Vanwagnen *et al.* method [5] to yield petroleum ether (PSF), carbon tetrachloride (CTSF), chloroform (CSF), and aqueous (AQSF) soluble fractions.

2.4. Phytochemical Screenings

The freshly prepared crude extract was qualitatively tested for the presence of various phytochemicals. These were identified by characteristic color changes using standard procedures, and the previously described standard method [6].

2.5. Streptokinase

Commercially available lyophilized Alteplase (Streptokinase) vial of 15, 00,000 I.U, was collected and 5 ml 0.9% NaCl was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis.

2.6. *In vitro* thrombolytic activity

The thrombolytic activity of the prepared extracts was evaluated by the method of Daginawala, where streptokinase (SK) was used as a standard drug [7, 8]. Venous blood (5 ml) was drawn from healthy volunteers, and transferred in different pre-weighed sterile tubes (1 ml/tube) to form clots. Then it was incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot formed. As a positive control, 100 µl of SK and 100 µl of isotonic (a negative non thrombolytic control) solution were separately added to two different clot containing tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, the released fluid was fully removed and vials were again weighed carefully to observe the difference in weight after clot disruption. Differences obtained in weight noted before and after clot lysis were expressed as percentage of clot lysis. The significance of the percentages of clot lysis of crude extract of weight difference was tested by the paired t-test analysis. Then statistical analysis was performed with the results of percentage of clot lysis by different fractionates, streptokinase and sterile water, where p value < 0.01 was considered statistically significant.

2.7. *In vitro* membrane stabilizing activity

The membrane stabilizing activity of the extract was evaluated by their ability to inhibit the lysis of human red blood cells, provoked osmotically by hypotonic solution or heat by the method developed by Omale [9].

2.8. Hypotonic Solution-Induced Hemolysis

Stock erythrocyte (RBC) suspension (0.5 ml) was prepared by mixing with 5 ml hypotonic solution (50 mM NaCl in 10 mM sodium phosphate buffered saline, pH 7.4), crude ethanolic extract (CEE) and their fractions (2 mg/ml each) and acetyl salicylic acid (0.1 mg/ml) was taken in different centrifuge tubes. The reference standard was used as the acetyl salicylic acid. In the Centrifuge Machine, the mixtures were centrifuged for 10 min at 3000 rpm, and incubated for 10 min at 25°C. The absorbance of supernatant part of the mixture was measured at 540 nm using UV/Visible spectrophotometer.

2.9. Heat-induced Haemolysis

Two duplicate sets of centrifuge tubes were taken containing 2 mg/ml of crude ethanolic extract (CEE) of *A. racemosus* and its fractions (PSF, CSF, CTSF, and AQSF) solution and 5 ml of buffered isotonic NaCl solution. ASA at a concentration of 0.1 mg/ml for positive control and negative control containing only 5 ml of the isotonic buffered solution was maintained. By inversion, erythrocyte suspension (30 µl) was added to all the tubes and mixed gently. First, one set of tubes was incubated in water bath at 54°C for 20 min and followed by the other set of tubes kept in an ice bath (0-5°C). The samples were centrifuged for 10 min at 3000 rpm at the end of the incubation period and the absorbance at 540 nm of the supernatant was measured [10, 11].

2.10. Antimicrobial Activity

The crude ethanolic extract and their fractions were screened for antimicrobial activity in comparison with the standard antibiotic Ciprofloxacin (5 µg/disc) *in vitro* by disc diffusion method using known bacterial strains [12]. About 10 µl of crude extract and its fractionates were added in the paper discs, then the dried paper disc with concentration of 400 µg/mL plant extract was placed aseptically on the organism containing agar surface. It was then kept at 4°C overnight followed by incubation at 37°C for 16 h to promote bacterial growth.

2.11. Total Phenolic Content Analysis

The total phenol contents were determined by using Folin-Ciocalteu reagent followed by the standard method [13, 14]. In a concise manner, 0.3 ml of each extractive was taken in individual volumetric flask (10 ml) containing 2.7 ml of Folin-Coicalteu (1: 10) phenol mixture. After 5 minutes, 2 ml of 7.5% sodium carbonate solution was effused to each test tube and shaken and then kept for 30 min in the dark place after warming at 45°C. A standard calibration curve was prepared by a series of solutions of gallic acid. Absorbance for samples and standard solutions were determined at 725

nm with an UV/Visible spectrophotometer against the blank. The total phenolic contents of extractives were determined from extrapolation of gallic acid standardized curve.

2.12. DPPH Free Radical Scavenging Activity

To conduct free radical scavenging activity for antioxidant assay, free radical 2, 2-diphenyl-1- picryl-hydrazyl-hydrate (DPPH) was used. In brief, the reaction mixture consisted of 2 mL of CEE and its extractives at a concentration of 5–500 µg/mL were added to 3 mL of 0.02 mg/mL (20 µg/mL) methanolic solution of DPPH followed by thorough vortexing of mixture which was incubated for 30 min in the dark place at room temperature. Some changes were observed in the color, suggesting that the higher the decolourization, the higher the reducing ability (from deep violet to light yellow). Following this, absorbance was measured against a blank (without sample) at 517 nm using UV/Visible spectrophotometer. Low absorbance of the reaction mixture indicated high free radical scavenging activity [15]. The results were compared with the percentage of inhibition at various concentration of positive ascorbic acid.

2.13. Statistical Analysis

Three replicates of each sample were used for each test to facilitate the statistical analysis and the data are presented as mean ± standard deviation (SD).

3. RESULTS AND DISCUSSION

The crude ethanol extracts of the plant of *A. racemosus* and their partitionates were used to determine the content of bioactive compounds and justify the traditional uses of thrombosis, inflammatory disorders, pathogenic bacteria and autoimmune diseases.

3.1. Phytochemical Screening Test

The qualitative phytochemical screenings revealed the presence of alkaloids, glycosides, steroids, flavonoids, tannins, proteins, resins, quinones, phenols and saponins.

3.2. Thrombolytic Activity

The extractives of plant *A. racemosus* were evaluated for thrombolytic activity, using a positive control Streptokinase (SK) that showed 65.16 ± 0.16% lysis of clot wherever a negative control sterile distilled water and exhibited a negligible percent of lysis of clot (8.20 ± 0.16%). The percentages of clot lysis of different extracts solution were observed in the following order, CTSF (41.48±0.43%), CSF (38.25±0.20%), CEE (36.24±0.10%), PSF (25.45±0.48%), and AQSF (17.74±0.25%). So, it was observed that significant (p value < 0.001) percentage of thrombolytic activity was exhibited by all extractives of *A. racemosus* Table (**1**). Thrombolytic agent like SK is used as a drug as it breaks the already formed thrombus (blood clot) in clinical settings where ischemia may be fatal [16]. This study showed that the thrombolytic activities of our test samples (CTSF, CSF, CEE, PSF, and AQSF) revealed positive results in comparison to the positive and negative controls. Thus all the results obtained in the study clearly indicated that *A. racemosus* extract is a remedy of possible clot lysis agent.

Table 1. Membrane stabilizing and thrombolytic activity of crude extracts and different fractions of plant *A. racemosus.*

Sample	Percent inhibition of hemolysis		Percent of clot lysis
	Hypotonic solution-induced	Heat-induced	
CEE	79.08±0.47	78.28±0.11	36.24±0.10
PSF	74.56±0.18	78.28±0.32	25.45±0.48
CTSF	73.90±0.70	85.68±0.75	41.48±0.43
CSF	80.86±0.36	72.05±0.39	38.25±0.20
AQSF	75.78±0.40	84.68±0.48	17.74±0.25
ASA	80.99 ±0.39	85.68±0.28	
SK			65.16±0.16
Negative control			8.20±0.16

Values are expressed as mean ± SD, (n=3).

3.3. Membrane Stabilizing Activity

The extractives of *A. racemosus* significantly protected the hemolysis of human erythrocytes membrane induced by

hypotonic-solution and heat as compared to the standard ASA. The membrane stabilizing activity against hypotonic solution induced stabilization obtained in the following order ASA (80.99±0.39%), CSF (80.86±0.36%), CEE (79.08±0.47%), AQSF (75.78±0.40%), PSF (74.56±0.18%) and CTSF (73.90±0.70%). In heat-induced method, the values of ASA, AQSF, PSF, CEE and CSF were (85.68±0.28%), (84.68±0.48%), (78.28±0.32%), (78.28±0.11%) and (72.05±0.39%), respectively. The presence of significant amount of phenolic compounds in the extract of *A. racemosus* revealed that the plant have potential to inhibit hemolysis of RBCs induced by hypotonic solution and heat induced (Table **1**).

3.4. Antimicrobial Activity

CEE and different fractions of *A. racemosus* were screened against the total 9 microorganisms in which 5 were gram positive and 4 were gram negative bacteria. In this study, crude extract and the fractions gave the zone of inhibition against these bacteria in the range of 6.5-14 mm (diameter) and the standard drug ciprofloxacin gave in the range of 14-22 mm (diameter) zone of inhibition. The crude extract of *A. racemosus* showed the maximum 14 mm zone of inhibition and the standard drug showed 19 mm of diameter zone of inhibition against *Vibrio parahimolyticus* in comparison to the examined Gram-negative bacteria and the results are given in Table (**2**). Extractives showed variable growth of inhibition against some examined bacteria and the growth inhibition was compared with the predictable standard drug ciprofloxacin. Ciprofloxacin is a broad-spectrum antibiotic; it shows its activity against both gram-positive and gram-negative bacteria. Considering the inhibited DNA gyrase, and a type II topoisomerase, topoisomerase IV, it is necessary to separate the bacterial DNA, thereby inhibiting mostly gram-negative pathogens fruitfully. According to the previous studies and literature review, a number of medicinal plants have been shown to possess anti-infective properties [17, 18]. The plant of *A. racemosus* may possess the similar properties, already found in our study. So we can expect the existence of antibacterial property in the plant of *A. racemosus*, which moderately inhibits the growth of both Gram (+) and Gram (–) bacteria.

Table 2. Antimicrobial activity of crude ethanol extract and various fractionates of *A. racemosus.*

Test organisms	Diameter of zone of inhibition(mm)					
	CEE	PSF	CSF	CTSF	AQSF	C-5µg/disc
Gram positive Bacteria						
Bacillus subtilis	10	10	8	11	9	15
Sarcinalutea	8		6.6	7		16
Staphylococcus aureus	7	12		7	9	18
Bacillus cereus	7	8	7	9		22
Gram negative Bacteria						
Escherichia coli	8		7	11	6.5	22
Shigella dysenteriae		6.5	7	9		14
Vibrio mimicus	6.5				-	17
vibrio parahimolyticus	14	7	11	14	8	19
salmonella typhi	7	9		11		20

Values are expressed as mean ± SD, (n=3).

3.5. Total Phenolic Contents Determination

The antioxidant potential was determined by total phenolic contents of CEE and different extractives of *A. racemosus* ranging from 221.16 mg of GAE/gm to 550.14mg of GAE/g of extractives (Table **3**).

Table 3. Total phenol compounds and DPPH free radical scavenging activity of crude ethanol extract and its different partitionates of plant of *A. racemosus.*

Sample/ Standard	Total phenol compounds (mg of GAE/g)	DPPH Free radical scavenging activity (EC_{50} µg/ml)
AQSF	550.14	2.62
PSF	434.22	3.79
CSF	345.2	3.88
CEE	320.11	5.70
CTSF	221.16	6.20
Vitamin c		2.48

3.6. Antioxidant Activity

The antioxidant potential was also determined in terms of DPPH free radical scavenging assay of *A. racemosus,* showing the EC_{50} value within the range of 2.62 µg/ml to 6.20µg/ml. Among the crude ethanolic extract and fractionates, the highest free radical scavenging activity showed by AQSF which was 2.62 µg/ml followed by PSF being 3.79 µg/ml, CSF being 3.88 µg/ml, CEE being 5.70µg/ml and CTSF being 6.20 µg/ml in comparison with ascorbic acid (2.48 µg/ml) as a standard Table (**3**). Outcome of our study revealed that changes in color (implying that the higher the decolourisation, the higher is the reducing ability, from deep violet to light yellow) followed by low absorbance of the reaction mixture indicated a high free radical scavenging activity. AQSF contains maximum total phenolic compound of 550.12 mg of GAE/g of extractives and EC_{50} value of AQSF was 2.62 µg/ml, which was the lowest among the extractives. So we can conclude that there exists a significant correlation between the total phenolic contents and DPPH free radical scavenging activity.

CONCLUSION

This study was conducted to validate the *A. racemosus* plant as thrombolytic, membrane stabilizing, antimicrobial and antioxidant. It can be concluded from the above convincing evidence that the plant *A. racemosus* possesses remarkable thrombolytic, membrane stabilizing, antimicrobial activity and antioxidant. However, further chemical and pharmacological studies are necessary to elucidate the detailed mechanism of action behind this and isolate the responsible active principles.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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