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Phytochemical Investigation and Evaluation of Antibacterial and Antioxidant Potentials of *Asparagus racemosus*

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Abstract: The ethanol extract of *Asparagus racemosus* Willd was examined for antbacterial and antioxidant properties. Phytochemical investigation was also done to identify the presence of phytochemical compounds. The ethanol extract at the concentration of 500 μg $disc^{-1}$ showed moderate antibacterial activity against *Staphylococcus saprophyticus, Enterococcus faecalies, Streptococcus agalactiae* and *Escherichia coli* with zone of inhibition of 7.77±0.37, 6.07±0.06, 10.10±0.11 and 6.00±0.04 mm, respectively while 250 μg $disc^{-1}$ of the extract did not reveal any zone of inhibition against the tested bacterial strains. Antioxidant activity of the ethanol extract was determined according to their scavenging activity of the stable DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical and 10% H_2SO_4. In the qualitative antioxidant assay, the extract showed free radical scavenging properties. Preliminary phytochemical analysis of the plant extract showed the presence of alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates which could be responsible for antibacterial and antioxidant properties justifying the ethnomedicinal applications of *Asparagus racemosus*. Thus, further advanced research is necessary to isolate and characterize the chemical compounds responsible for the therapeutic activities of the plant.

Key words: *Asparagus racemosus,* phytochemical, antioxidant, scavenging, antibacterial

INTRODUCTION

Asparagus racemosus Willd (Family: Liliaceae) is commonly known as Shatamuli (Ghani, 2003) which is widely used in traditional medicine in Bangladesh due to possessing high medicinal value (Hossain *et al*., 2006). The plant is widely used in diarrhoea and dysentery. It also possesses anthelmintic and antiseptic properties (Sinha and Biswas, 2011). The root extract of *Asparagus racemosus* has been used in ulcer, diabetes and immunomodulation. Several nervous disorders, dyspepsia, tumors, inflammation, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases can also be treated by this medicinal plant. Moreover, steroidal saponins (Shatavarins I-IV), isoflavones, asparagamine, racemosol, polysaccharides, mucilage are identified as major phytoconstituents in the plant (Chawla *et al*., 2011).

It is reported that *Asparagus racemosus* root extract inhibits the accumulation of oxidative damages and reduces the lipofuscin content in cardiac lysosomes. The enzyme activity is also restored due to the presence of enriched therapeutic phytoconstituents which improve the indices of oxidative stress related to aging (Velavan and Begum, 2007a). The modulatory activity of *Asparagus racemosus* has been observed on plasma glucose, insulin, insulin resistance index and metabolic liver enzymes in young and aged rats (Velavan and Begum, 2007b). In addition to this, methanol root extract of *Asparagus racemosus* shows cerebroprotective activity due to reduction of oxidative stress (Nandagopal *et al*., 2011). The root extract of the plant has significant effect in increasing milk secretion during lactation. It also shows the antihepatotoxic and antineoplastic activities (Chawla *et al*., 2011). In the present study, ethanol extract of the whole plant of *Asparagus racemosus* was undertaken to evaluate the antibacterial and antioxidant activities. The study was also carried out to identify different phytochemical compounds present in the plant extract.

MATERIALS AND METHODS

Collection of plant material: The whole plant of *Asparagus racemosus* Willd (Family: Liliaceae) was collected from Natore, Bangladesh in May, 2009 and

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the plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka. The voucher specimen of the plant was deposited in the Pharmacy Discipline, Khulna University, Bangladesh for future reference and the voucher specimen number is DACB 34216.

Preparation of extract of *Asparagus racemosus*: The collected plant was washed from external materials with tap water and dried under shade. After complete drying, the plant materials were cut into small pieces and then crushed in an electric grinder and then powdered. Finally, the powder was stored in a suitable container. About 500 g of powder was suspended in 1200 mL of 80% ethanol and kept at incubator at 37°C for 20 days. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then, it was filtered through filter paper and the filtrate thus obtained was evaporated by using a rotary evaporator to get a viscous mass which was then dried to get a dried ethanol extract (approx. yield value 16%). Finally, the extract was used for experimental purposes.

Microorganisms: The microorganisms used for the study were both gram positive bacterial strains such as *Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus agalactiae, Streptococcus pyogenes* and gram negative bacterial strains such as *Shigella boydii, Shigella sonnei, Shigella dysenteriae, Pseudomonas aeruginosa, Shigella flexneri* and *Escherichia coli* which were collected from the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

Standard drug: The standard drug, Kanamycin was collected from Beximco Pharmaceuticals Ltd. Dhaka, Bangladesh.

Preliminary phytochemical screening: The crude ethanol extract of *Asparagus racemosus* was subjected to preliminary phytochemical screening for the detection of major functional groups according to the standard procedures (Trease and Evans, 1989).

Antibacterial activity: Antibacterial activity of extract of *Asparagus racemosus* was tested by using the disc diffusion method (Bauer *et al*., 1966; Ahmed *et al*., 2003). To investigate the antibacterial activities, two different concentrations (250 and 500 μg $disc^{-1}$) of the extract were used while the drug Kanamycin at 30 μg $disc^{-1}$ was used as standard for antibacterial test. The experiments were carried out in triplicates. In this study, discs were impregnated with the extract sample and standard antibiotic (Kanamycin) while negative control discs were placed gently on the seeded agar plates with the help of sterile forceps to assure complete contact with medium surface. The plates were then inverted and kept in refrigeration for about 2 h at 4°C to allow the material to diffuse into a considerable area of the medium. Finally, the plates were incubated upside down at 37°C for 24 h. After proper incubation, the antibacterial activity of the test agent was determined by measuring the diameter of zone of inhibition in terms of millimeter with a slide calipers.

Determination of antioxidant activity: Antioxidant activity was determined on the basis of their scavenging activity of the stable DPPH free radical (Sadhu *et al*., 2003). The sample and ascorbic acid were spotted on commercially prepared TLC plat where ascorbic acid was used as standard. The chromatogram was developed by ascending technique using two types of solvent systems i.e., medium polar solvent system ($CHCl_3$:CH_3OH = 5:1) and polar solvent system ($CHCl_3$:CH_3OH:H_2O = 40:10:1). The solvent system was allowed to move up to a previously marked line. The plates were then dried naturally. The plates were viewed under UV detector both in short (254 nm) and long (360 nm) wavelength.

RESULTS

Preliminary phytochemical screening: The preliminary phytochemical screening of the plant extract identified the presence of alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates which are shown in Table 1.

Antibacterial activity: The antibacterial activity was assessed against a panel of 10 pathogenic bacterial strains (both gram positive and gram negative) at the dose of 250 and 500 μg $disc^{-1}$. Table 2 showed the results of antibacterial test. The obtained results were compared with the activity of the positive control, Kanamycin (30 μg $disc^{-1}$). At 250 μg $disc^{-1}$, the extract showed no activity whereas at 500 μg $disc^{-1}$, the extract showed activity only against *Staphylococcus saprophyticus, Enterococcus faecalies, streptococcus agalactiae* and *Escherichia coli* with zone of inhibition of 7.77±0.37, 6.07±0.06, 10.10±0.11 and 6.00±0.04 mm, respectively.

Antioxidant activity: Only qualitative antioxidant activity was assayed using DPPH and ascorbic acid. Results are represented in the Fig. 1 and 2 for the medium polar and

Table 1: Results of preliminary phytochemical analysis

Alkaloid	Glycoside	Steroid	Gum	Tannin	Saponin	Flavonoid	Carbohydrate
+	+	-	-	+	+	+	+

+: Present, -: Absent

Table 2: Antibacterial activities of ethanol extract of *Asparagus racemosus*

	Diameter of zone of inhibition (mm)			
Bacterial strains	Blank	Kanamycin (30 μg disc^{-1})	Extract (250 μg disc^{-1})	Extract (500 μg disc^{-1})
Gram (+) positive				
Staphylococcus saprophyticus	-	17.83±0.24	-	7.77±0.37
Enterococcus faecalis	-	15.25±0.54	-	6.07±0.06
Streptococcus agalactiae	-	20.07±0.06	-	10.10±0.11
Streptococcus pyogenes	-	26.23±0.59	-	-
Gram (-) negative				
Shigella boydii	-	19.65±0.18	-	-
Shigella sonnei	-	25.65±0.16	-	-
Shigella dysenteriae	-	19.75±0.12	-	-
Pseudomonas aeruginosa	-	16.05±0.43	-	-
Shigella flexneri	-	11.00±0.41	-	-
Escherichia coli	-	20.15±0.40	-	6.00±0.04

All values are expressed as Mean±SD of triplicate determination, SD: Standard deviation, (-): No inhibition

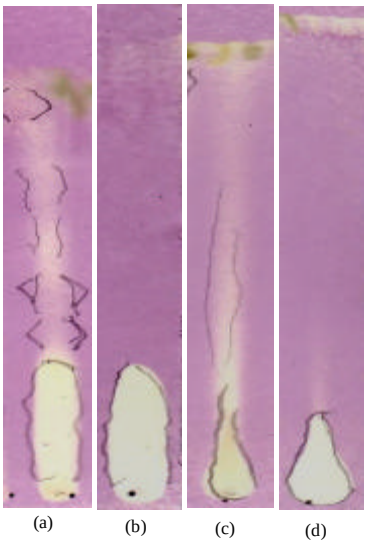

Fig. 1: Comparison of TLC plate for the extract of *Asparagus racemosus* with (a) standard ascorbic acid (b) after applying DPPH, (c) Chloroform:methanol = 5:1 as solvent and (d) Chloroform:methanol:water = 40:10:1 as solvent

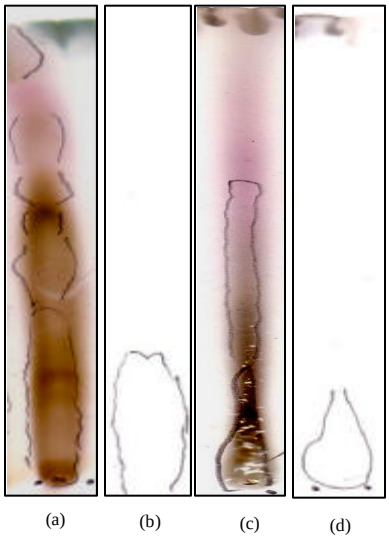

Fig. 2: Comparison of TLC plate for the extract of *Asparagus racemosus* with (a) standard ascorbic acid, (b) after applying 10% H_2SO_4, (c) Chloroform:methanol = 5:1 as solvent and (d) Chloroform:methanol:water = 40:10:1 as solvent

polar solvent system. DPPH (1,1-diphenyl-2-picryl hydrazyl) formed deep pink color when it was dissolved ethanol. When it was sprayed on the chromatogram of the extract, it formed pale yellow or yellow color which indicated the presence of antioxidants. Two spotted TLC plates were again subjected to universal spry reagent i.e., 10% H_2SO_4 and then heated on hot plate which indicated dark spot. Thus, the obtained results showed antioxidant activity.

DISCUSSION

Preliminary phytochemical analysis showed the presence of alkaloids, tannins, saponins, glycosides, flavonoids and carbohydrates as shown in Table 1. It was reported that the presence of natural flavonoids in the medicinal plants could show the antioxidant and free radical scavenging properties (Middleton and Kandaswami, 1992; Okwu and Orji, 2007) which was

confirmed by this study. Moreover, flavonoids (Zakaria *et al*., 2006) and tannins (Rahman *et al*., 2011) could possess antinociceptive properties. Tannins are also useful in the prevention of urinary tract infection and in the management of HIV (Agbafor *et al*., 2011). Thus, it could be suggested that the ethanol extract of the plant might possess antinociceptive and antiviral properties which should be investigated in future.

Antibacterial activity was tested by using disc diffusion method. The extract demonstrated the antibacterial activities against *Staphylococcus saprophyticus, Enterococcus faecalies, streptococcus agalactiae* and *Escherichia coli*. From this result, it could be concluded that the ethanol extract of *Asparagus racemosus* possessed mild antibacterial activity. It is reported that oxygen involves for the production of most of the free radicals in our body and thus the free radicals are referred to as reactive or reduced oxygen species. Free radicals cause cellular damage by reacting with the phospholipid bilayer of cellular membranes. This reaction results in the production of measurable end products, primarily malondialdehyde. The most effective way to eliminate free radicals is with the help of antioxidant nutrients such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin E) and beta-carotene (vitamin A) which can be found in vast amounts in fruits and vegetables. Literature review about the plant confirms the presence of polyhydric phenolic compounds, flavonoids, sesquiterpine etc. Any of these phytoconstituents can be responsible for the antioxidant activity of the crude extract. Phenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Phenolic compounds and flavonoids have also been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans *et al*., 1997; Jorgensen *et al*., 1999).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). In this study, antioxidant activity of the ethanol extract of the plant was determined on the basis of the scavenging activity of the stable DPPH free radical and 10% H_2SO_4. The result might partially support its traditional uses for different tumors. Further studies as lipid per-oxidation inhibition, xanthin oxidase inhibition, erythrocyte membrane stability and other studies are essential to characterize them as biological antioxidants.

CONCLUSION

The result obtained from the phytochemical screening showed the presence of several phytochemical compounds which might be responsible for antibacterial and antioxidant activities of the plant extract. Thus, these findings support the local uses of the plant extract in different infectious diseases in Bangladesh.

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