Research Article

Evaluation of antibacterial activity of *Asparagus racemosus* extract – An *in vitro* study

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ABSTRACT

Introduction: In Ayurveda, *Asparagus racemosus* is an amazing herb known as the "Queen of Herbs" because it promotes love and devotion. Shatavari is the main Ayurvedic rejuvenative tonic for the female, as is Withania for the male. This herb is highly effective in problems related with female reproductive system. *A. racemosus* is a well-known Ayurvedic rasayana which prevent aging, increase longevity, impart immunity, improve mental function, vigor, and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, and hepatopathy. The aim of the study was to evaluate the antimicrobial activity of *Allium cepa* against bacteria causing enteric infection. **Materials and Methods:** The screening of antibacterial activity of extracts was carried out using the disc diffusion method. The culture plates were allowed to stand on the working bench for 30 min for pre-diffusion and were then incubated in upright position at 37°C for 24 h. Standard antibiotic discs gentamycin (30 mcg/disc) were used as positive control. **Results:** The extracts at different concentrations exhibited antibacterial activity against the bacterial strains tested. The extract was more effective against *Lactobacillus acidophilus* with a zone of inhibition of 16 mm diameter (at conc. of 400 mg/ml) and with *Streptococcus mutans,* the zone of inhibition was found to be 15.8 mm (at conc. of 400 mg/ml). **Conclusion:** We conclude that the extracts of *A. racemosus* can be used as antimicrobial agent.

KEY WORDS: *Asparagus racemosus*, Disc diffusion technique, *Lactobacillus acidophilus*, *Streptococcus mutans*

INTRODUCTION

Asparagus racemosus belongs to the family Liliaceae and commonly known as Satawar, Satamuli, and Satavari found at low altitudes throughout India. *A. racemosus* is a woody climber growing to 1–2 m in height. The leaves are like pine needles, small and uniform and flowers are white and have small spikes. This plant belongs to the genus Asparagus which has recently moved from the subfamily Asparagae in the family Liliaceae to a newly created family Asparagaceae. In Ayurveda, this amazing herb is known as the "Queen of Herbs" because it promotes love and devotion.[1] Shatavari is the main Ayurvedic rejuvenative tonic for the female, as is Withania for the male. This herb is highly effective in problems related with female reproductive system. *A. racemosus* is a well-known Ayurvedic rasayana which prevent aging, increase longevity, impart immunity, improve mental function, vigor, and add vitality to the body and it is also used in nervous disorders, dyspepsia, tumors, inflammation, neuropathy, and hepatopathy.[2] A study of ancient classical Ayurvedic literature claimed several therapeutic attributes for the root of *A. Racemosus* and has been specially recommended in cases of threatened abortion and as a galactogogue. Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactogogue, and aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic, and as tonic.[3] Beneficial effects of the root of *A. racemosus* are suggested in nervous disorders, dyspepsia, diarrhea, dysentery, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity, and certain infectious diseases.

A. racemosus is phytoestrogens rich plant species. The majority of phytoestrogens belong to a large group of substituted phenolic compounds known

Access this article online

Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 06-11-2018; Revised on: 07-12-2018; Accepted on: 10-01-2019

as flavonoids.[4] Three classes of flavonoid, the isoflavones, coumestans, and prenylated flavonoids, are phytoestrogens that possess the most potent estrogenic activity. The phytoestrogen classes mentioned above have a similar structure to estradiol and are able to bind the estrogen receptor, preferably the ER although their binding affinity is lower than that of endogenous estradiol.[5] Earlier studies and literature survey revealed that the major bioactive constituents of Asparagus are a group of steroidal saponins. This plant also contains Vitamins A, B1, B2, C, and E, Mg, P, Ca, Fe, and folic acid. Other primary chemical constituents of Asparagus are essential oils, asparagine, arginine, tyrosine, flavonoids (kaempferol, quercetin, and rutin), resin, and tannin steroidal glycosides (asparagosides), bitter glycosides, asparagine, and flavonoids. Asparagine is a strong diuretic. In addition to these contain diosgenin and other saponins such as shatavarins I and IV reported by Ravi Kumar *et al*., in leaves and roots from *A. racemosus* also shatavarin V and shatavarin VI–X were reported in roots of *A. Racemosus*.[6-8] The major active constituents of *A. racemosus* are steroidal saponins (shatavarins I–IV) that are present in the roots. Shatavarin IV has been reported to display significant activity as an inhibitor of core Golgi enzymes transferase in cell-free assays and recently to exhibit immunomodulation activity against specific T-dependent antigens in immunocompromised animal.[9] Reports indicate that the pharmacological activities of *A. racemosus* root extract include antiulcer, antioxidant, and antidiarrheal, antidiabetic, and immune modulatory activities.

MATERIALS AND METHODS

Preparation Of Extracts (Aqueous Extract): (Cold Method Of Extraction)

The root samples of *A. racemosus* were shade dried at 37°C and were powdered using pestle and mortar. For cold maceration method, 10 g of the root powder was placed in 100 mL of natural distilled water in a conical flask, plugged with cotton, and then kept on a rotary shaker at 180–200 rpm for 72 h. After 72 h, the extract was filtered using muslin cloth. Then, the filtrate was lyophilized at −55°C. The lyophilized products were scraped and stored at 4°C in airtight vials. The final dried extract was dissolved in 10% of dimethyl sulfoxide, stored at 4°C, and subjected to antibacterial activity assay.

Bacterial Strains And Media

Streptococcus mutans and *Lactobacillus acidophilus* pure isolates used in this study were obtained from ATCC, USA. All samples were cultured and subcultured again for purity on blood agar plates. Colony morphology and Gram staining were carried out to confirm the identity of working strains.

Antimicrobial Susceptibility Testing

A 100 µl of bacterial suspension was spread on each nutrient agar plates. Different concentrations of extract (25, 50, 100, 200, and 400 mg/ml) have then used to impregnate in sterilized 6 mm blank discs. DMSO-loaded discs were used as negative controls for plant extract, respectively. All impregnated discs were ensured to be fully dried in 45°C incubator for 18–24 before the application on bacteria. The standard antibiotic disc used as positive controls was gentamycin (10 µg) for all strains. The discs which had been impregnated with extract using sterile forceps were applied on the inoculated Mueller-Hinton agar once it has completely dried. The discs were pressed gently to ensure uniform contact with agar surface. Then, the plates were inverted and incubated for 24 h at 37°C. The diameter of inhibition zone either around the treated discs or around the control discs was measured for the antibacterial activity assessment.[10-13] If present, their diameters were measured to the nearest whole millimeter with a ruler. All tests were carried out 3 times to ensure the reliability, and the average of the three replicates for each concentration of extract and antibiotic was calculated. Percentage inhibition of growth of bacterial microorganism was calculated after subtracting control from the values of inhibition diameter using control as standard.

RESULTS AND DISCUSSION

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in millimeter diameter. The results are given in Table 1.

The extract was more effective against *L. acidophilus* with a zone of inhibition of 16 mm diameter (at conc. of 400 mg/ml) and with *S. mutans,* the zone of inhibition was found to be 15.8 mm (at conc. of 400 mg/ml).

Oral diseases are major health problems with dental caries and periodontal diseases among the most important preventable global infectious diseases.[14] The present study was to evaluate the antibacterial activity of *A. racemosus* extracts against caries

Table 1: Antibacterial activity of ARE

Concentration of extract (ARE) (mg/ml)	Zone of inhibition in millimeter diameter	
	S. mutans	***L. acidophilus***
ARE (25)	7.1±0.61*	7.5±0.61*
ARE (50)	9.5±0.89**	10.4±1.0**
ARE (100)	12.1±1.0***	13.1±1.1***
ARE (200)	13.6±1.2***	14.5±1.3***
ARE (400)	15.8±1.3***	16.0±1.5***
Gentamycin	17.3±1.5***	17.5±1.0***
Negative control	No growth	No growth

NI means no inhibition zone. Each value is expressed as mean±SD (n=3). *P<0.05, **P<0.01, and ***P<0.001 statistically significant as compared with negative control. ARE: Asparagus racemosus extract

causing organism *S. mutans* and *L. acidophilus*. The results obtained from our study show that extracts have got a very good antibacterial activity against the selected oral pathogens.

CONCLUSION

This *in vitro* study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established.

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Source of support: Nil; Conflict of interest: None Declared