

Evaluation of Phytochemical and Antibacterial Potential of *Asparagus racemosus* Willd. Extracts against Respiratory Tract Pathogens

KISHLAY KUMAR¹, SANJAY KUMAR^{2*}, NAVNEET² AND SHIV SHANKER GAUTAM²

¹Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar- 246274 Uttarakhand, India

²Department of Botany and Microbiology, Gurukul Kangri University, Haridwar-249404, Uttarakhand, India

ABSTRACT

Now a days drug resistance is very common problem. In present study the antibacterial activity was studied against five bacterial pathogens i.e. *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655 and *Streptococcus pyogenes* MTCC 442. Shade dried root were crushed and extracted in petroleum ether (PET), acetone (ACE), methanol and water (H₂O) by using Soxhlet apparatus. The agar well diffusion method was adopted to examine antibacterial activity of extracts against the susceptible organisms. Erythromycin was used as positive control to determine the sensitivity of the strains. Phytochemical analysis was done for plant extract. The results showed that MeOH extract was most active as comparison to other extract. The maximum inhibition was found against *H. influenzae* (19 mm) followed by *S. pneumoniae* (18 mm), *S. pyogenes* (17 mm) and *S. aureus* (17 mm) respectively. The minimum inhibition was noted against *P. aruginosa* (15 mm). The phytochemical screening for MeOH extract has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpene, saponins and tannins.

Keywords: Antibacterial activity, *Asparagus racemosus*, agar well diffusion method, *Haemophilus influenzae*.

INTRODUCTION

The importance of herbs in the management of human ailments cannot be over underlined. The immense diversity met in flowering plants provide a great ecological security and contribute to raise economy of any country (Anonymous, 1975; Uniyal *et al.*, 2007). For thousands of years, traditional plant derived medicines have been used in most parts of the world and their use in fighting microbial disease is becoming the focus of intense study

Bhavnani and Ballow, 2000; Chariandy *et al.*, 1999).

Various natural or artificial particles of biological (microbial, plant or animal) origin suspended in the air. Such aerosols may consist of bacteria, fungi (spores and cell fragments), viruses, microbial toxins etc. Since, these particles are potentially related to various respiratory disease enter inside our body through air. Due to lower respiratory tract infections 2.64 million deaths was reported in 2001 in developing countries.

Asparagus racemosus belongs to the family Asparagaceae. It is commonly known as Satavari in Hindi. *A. racemosus* is recommended in traditional medicine for the prevention treatment of gastric ulcers, dyspepsia, diarrhea, nervous disorders (Sinha and Biswas, 2011). Besides use in the treatment of and dysentery, the plant also has antioxidant, immunostimulant, antidysepsia and antitussive effects (Goyal *et al.*, 2003). Therefore, aim of this study was to look into antibacterial and phytochemical screening of different extracts of *A. racemosus* against bacterial pathogens causing respiratory tract infections.

MATERIAL AND METHODS

Plant Material - Plant was collected from Natural habitat, Srinagar, Uttarakhand and authenticated at Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar. Collected roots were dried under shade at room temperature and crushed to small pieces by using pestle and mortar.

Preparation of Extract - Plant extracts were prepared by immersing 200 g of powdered plant material in 600 ml of four different solvents i.e. petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H₂O), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmed *et al.*, 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/ml for agar well diffusion method.

Test Microorganisms - The five bacterial strains causing respiratory infections used in this study were *Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442. Bacterial strains were procured from

Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of Inoculums - Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

Antibacterial testing - The antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmed *et al.*, 1998). 0.1 ml of 12-16 h incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and the mean values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimetre (mm) as observed from the clear zones surrounding the wells.

Phytochemical screening - Major phytochemicals, in the crude extracts of *A. racemosus* were subjected to phytochemical analysis to determine the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996).

Test for alkaloids - Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

Test for flavonoids - On addition of conc. HCl in methanolic extract of material, a red colour appeared which indicated the presence of flavonoids.

Test for glycosides - Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba(OH)₂. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H₂SO₄ and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

Test for Steroids - The extract mixed with 3 ml CHCl₃ and 2 ml conc. H₂SO₄ was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

Test for Saponins - Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Tannins - Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H₂SO₄ follow a yellow brown precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION

The results pertaining the antibacterial data against selected pathogens are represented in Table 1. MeOH extract was found most active against all test pathogens in comparison to other extracts. The maximum inhibition was found against *H. influenzae* (19 mm) followed by *S. pneumoniae* (18 mm), *S. pyogenes* (17 mm) and *S. aureus* (17 mm) respectively. The minimum inhibition was noted against *P. aeruginosa* (15 mm). *A. racemosus* crude extracts was found less active in comparison to positive control (erythromycin).

The root extract of *A. racemosus* has significant antibacterial activity against selected pathogens i.e. *H. influenzae*, *P.*

aeruginosa, *S. aureus*, *S. pneumoniae* and *S. pyogenes*. In a similar study, Uddin et al., 2012 reported the antibacterial susceptibility pattern of MeOH root extract of *A. racemosus* against bacterial strains including *K. pneumoniae*, *E. coli*, *P. alkaligenes*, *Proteus* sp., *S. typhi*, *V. cholerae* and *S. aureus*. Mandal et al., 2000 reported the MeOH extract of the roots of *A. racemosus* showed considerable *in vitro* antibacterial efficacy against *E. coli*, *S. dysenteriae*, *S. sonnei*, *S. flexneri*, *V. cholerae*, *S. typhi*, *S. typhimurium*, *P. putida*, *B. subtilis* and *S. aureus*.

Table:1 The inhibition zone diameters of various extracts of *A. racemosus* root

Microorganism	*Diameters of the inhibition zone (mm)				Positive Control (Erythromycin)
	PET	ACE	MeOH	H ₂ O	
<i>H. influenzae</i>	8	17	19	15	21
<i>P. aeruginosa</i>	-	11	15	13	17
<i>S. aureus</i>	9	15	17	15	31
<i>S. pneumoniae</i>	8	12	18	14	20
<i>S. pyogenes</i>	9	13	17	13	26

By study, it is concluded that *A. racemosus* can be used as herbal medicine to treat respiratory infections caused by tested pathogens as comparative to synthetic chemotherapeutic agents.

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