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Antibacterial activity of chemical constituents isolated from *Asparagus racemosus*

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Abstract

Asparagus racemosus is a medical extensively used in traditional medicine for various disorders including its use in infectious. So far, work has been done to identify its active constituents responsible for antiseptic folk use of this plant. In the current investigation, we have made an effort to identify its chemical constituents that might be partly responsible for antimicrobial properties. Extraction and isolation of plant extract lead to isolation of two nor-lignans and two steroidal triterpenes (compound **1** to **4**). All compound showed considerable antibacterial activities against *E. coli* and *S. aureus* while no significant activity was observed against *S. typhi*. This study highlighted the potential of *A. racemosus* to be further explored as a source of bioactive natural products.

Introduction

Asparagus racemosus is a climbing perennial herb. It has folk usage as a treatment for dyspepsia and gastric ulcers as well as being used as a galactogogue. Other folk medicinal properties generally attributed to this plant include emollient, cooling, nerve tonic, constipating, galactogogue, aphrodisiac, diuretic, rejuvenating, carminative, immunostimulant, gastroprotective and antiseptic effects (Patel and Patel, 2013; Ravishankar et al., 2012; Battuand Kumar, 2010). In literature, extensive work has been done on various pharmacological properties like phytoestrogen effects (Sharma et al., 1996), adaptogenic effect (Rege et al., 1999), hypolipidemic effect (Visavadiya and Narasimhacharya, 2005), immunomodulatory effects (Diwanay et al., 2004), antibacterial activity (Mandal et al., 2000; Patel and Patel, 2013; Ravishankar et al., 2012; Battu and Kumar, 2010), antidepressant (Singh et al., 2009; Meena et al., 2011), memory enhancing activity (Ojha et al., 2010), antidiabetic effects (Kanwar et al., 2010; Hannan et al., 2007, 2011), and antiulcer (Bhatnagar et al., 2005; Sairam et al., 2003).

We designed a preliminary study to explore its antibacterial activity of its active constituents. Previously, the plant extract of leaves (Patel and Patel, 2013; Battu and Kumar, 2010) and roots (Ravishankar et al., 2012) has showed considerable antibacterial activity against pathogenic bacteria (Mandal et al., 2000). In the current investigation, we have reported various isolated compounds from *A. racemosus* that might be at least partially responsible for its effect.

Materials and Methods

Plant

Plant material was collected from Malakand division of

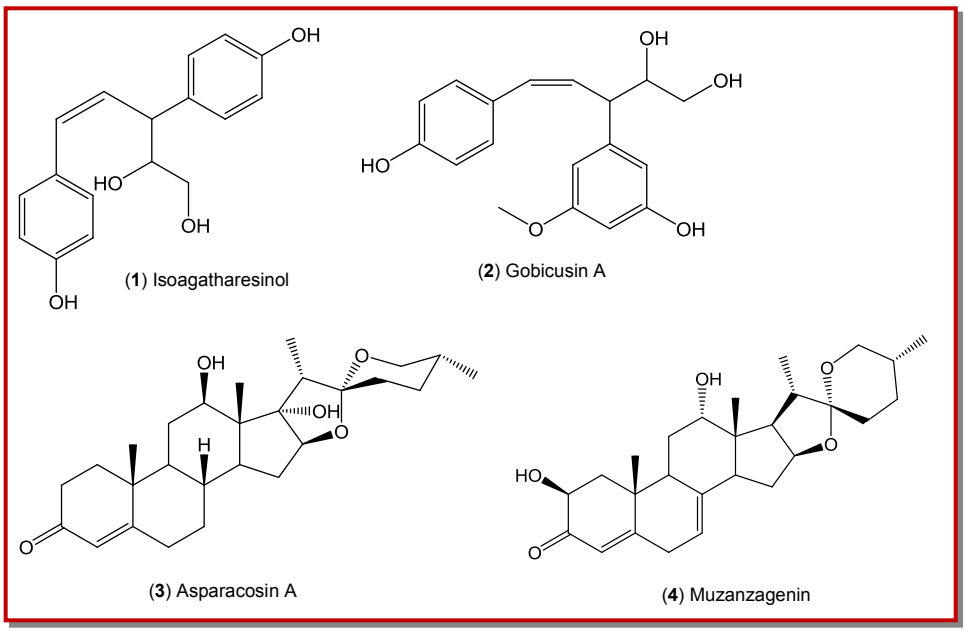

Figure 1: Chemical structures of the isolated compounds

the KPK Province, Pakistan, in April 2012. Plant material was dried under shade at room temperature for several weeks. After drying, plant material was subjected to grinding and pulverization, which was then stored at low temperature for further processing.

Extraction and isolation

Powdered plant material (16.2 kg) was macerated in distilled methanol for 72 hours, which was followed by filtration and evaporation at reduced pressure under vacuum using rotary evaporator at 40°C. This process was repeated four times that afforded crude methanolic extract (1.319 Kg). The crude methanolic extract was subjected to liquid-liquid extraction suing various organic solvents to get its successive fractions. After fractionation, *n*-hexane (112.3 g), dichloromethane (352.7 g), ethylacetate (482.9 g) and aqueous (372.4 g) fractions were obtained. Ethylacetate acetate fraction (350 g) was subjected to column chromatography (CC) using silica gel. Sample was eluted initially with increasing polarity of *n*-hexane in combination with dichloromethane (DCM) ranging from 10%DCM in *n*-hexane to 100%DCM. Afterwards, increasing polarity was used by combination of methanol with DCM, ranging from 1%methanol in DCM to 20%methanol in DCM. This process afforded 16 sub fractions (AE1 to AE16). Subfraction AE 7 (3.59 g) was subjected to further purification through CC using si gel. The sample was eluted with 4%methanol:DCM, as a gradient, which afforded compound **1** (142 mg and **2** (229 mg). Another subtraction AE9 was also purified via CC using si gel employing 6% methanol:DCM as a mobile phase, which provided compound **3** (87 mg) and **4** (176 mg). Chemical structures (Figure 1) of the isolated compounds **1** (isoagatharesinol; Yang et al., 2004), **2** (gobicusin A), **3** (asparacosin A; Zhang et al., 2004) and **4** (muzanzagenin; Oketch-Rabah et al., 1997) were identified by comparing their Mass and NMR data with reported in literature.

Antibacterial activity

In order to perform antibacterial activity various bacterial strains were used. Bacterial strains were *E. coli*, *S. aureus*, and *S. typhi*. Bacterial strains were grown and maintained on an agar slant at controlled temperature (4°C). The strains were activated for experiment at 37°C for 24 hours on nutrient agar (NA), before any experimental screening. Antimicrobial tests were carried out as reported earlier (Jan et al., 2009), according to disc-diffusion method. In medium, holes of 6 mm diameter were made on the MHA plate (8 mm thick) and filled with 150 µL of test samples (in different concentrations) or standard drug. The inoculated plates were incubated at 37°C for 24 hours. Anti-bacterial activity was evaluated by measuring the diameter of the zone of growth inhibition around the hole. The assay was repeated three times and the mean diameter was recorded. The minimum inhibitory concentration (MIC) was determined using the same method. Streptomycin, was used as standard antibiotic for comparison with test samples.

Results and Discussion

Bacterial infections are still posing a threat to humanity in successfully treating patients. New but effective antibacterial agents are very crucial for prompt therapeutic management of bacterial infections. Resistance to currently available drugs is an alarming and needs serious and timely efforts. In the current investigation, various compounds isolated from *A. racemosus* showed considerable antibacterial activity

against selected bacterial strains except *S. typhi* (Table I). *S. aureus* was the most susceptible bacterial strains, which is commonly associated with respiratory infections. Compound **2**, **3** and **4** showed significant antibacterial activity (Table I) against *S. aureus*, with MIC values 0.05 mg/mL, while compound **1** showed slightly lower activity against the same pathogen. In case of *E coli*, compound **2** and **4** showed significant activity with MIC values being 0.12 mg/mL. However, compound **3** showed lowest activity against *E. coli*. In case of compound **1**, moderate activity was observed. This preliminary data indicates promising potential of the plant to be further explored for discovery of new antibacterial agents to control ongoing crisis of merging resistance in clinical management of infectious diseases. Extensive work is required to identify molecular mechanisms involved behind the antibacterial effects of the isolated compound. Structural modifications and further experimental work is warranted to modify their potency and efficacy against bacterial pathogens.

Table I

Antibacterial activity of isolated compounds

Treatment	Minimum inhibitory concentration (mg/mL)		
	E. coli	*S. aureus*	*S. typhi*
Compound 1	0.25	0.12	n.d
Compound 2	0.12	0.05	n.d
Compound 3	0.5	0.05	n.d
Compound 4	0.12	0.05	n.d
Streptomycin	0.01	0.01	0.015

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