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Antimicrobial Activity of Crude Protein Extracts of *Seriphidium kurramense*

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Current study was aimed at investigating antibacterial and antifungal activities of crude protein extracts of *Seriphidium kurramense*. Agar well diffusion assays were performed to screen crude protein extracts against methicillin resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtillus*, *Staphylococcus aureus* and *Salmonella typhi*. Antifungal assay was carried out against *Aspergillus niger, Aspergillus flavus*, *Alternaria solani*, *Rhizoctonia solani, Fusarium solani* and *Pleurotus florida*. Different dilutions of crude protein extracts were used. The protein extracts showed antimicrobial activity against bacterial and fungal strains. However, pure extracts were more potent than a 1:1 dilution of protein extracts.

Keywords: Antibacterial, Antifungal, Crude protein extract, *Seriphidium kurramense,* Medicinal plant.

INTRODUCTION

Microorganisms cause various types of diseases such as diarrhea, tooth decay, tuberculosis and anthrax. Traditionally various antibiotics have been used to control the activities of pathogenic microorganisms. But the use of antibiotics against pathogenic organisms is now less effective due to increase in antibiotic resistance shown by various pathogenic microbes [1]. According to the report of World Health Organization (WHO) medicinal plants are valuable sources to obtain variety of pharmaceutical natural products to maintain human health [2]. Thousands of various indigenous plants have been used for the treatment of different ailments [3-6]. Medicinal plants contain active pharmaceutical products which are low-cost and could be used as more effective herbal drugs against a variety of microbial diseases [7].

Different types of molecules with antimicrobial activity have been isolated from plants. However, little research has been done on the antimicrobial properties of plant proteins. There are some reports about peptides molecules with antimicrobial activities. For example, peptides with growth inhibiting ability of both Gram-positive and Gram-negative bacteria have been investigated [8]. Peptides can inhibit the growth of fungi and viruses also. The seeds of leguminous plants contain peptides and proteins with antimicrobial activities [9]. In an investigation, antimicrobial activities of seed protein extract from six different plants were checked against *Staphylococcus aureus* [10]. All extracts exhibited antibacterial activity against *staphylococcus aureus* but the extract from the *Peganum hermala* showed strong antibacterial activity [10]. The hyphal growth of *Trichoderma longibrachiatum* was inhibited by treating with osmotin protein [11]. The use of synthetic chemicals is risky for human, plants, animals and their environments. However, the antimicrobial proteins are natural, non-toxic and non-hazardous and could be used for the treatment of various diseases [12].

The seeds of plants contain many antimicrobial proteins including chitinases, β-1,3-glucanases, proteinase inhibitors and ribosome inactivating protein. Corn seeds contain a protein zeamatin which has potent activity against various types of fungi [13]. Crude protein extract isolated from the seed kernels of *Bauhinia acuminata* showed strong antibacterial activity against a variety of pathogenic bacteria including both Gram-positive and Gram-negative bacteria [14]. The heat stable protein extracts of *Morusindica* exhibited inhibitory activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aerueginosa* and *Bacillus subtilis* [15]. It is believed that antimicrobial peptides make interaction with bacterial membrane and form transmembrane cluster which reduces the membrane potential value and as a consequence cytolysis occurs [16]. Here we report antimicrobial properties of protein extracts of *Seriphidium kurramense*.

EXPERIMENTAL

The plant *Seriphidium kurramense* was collected from Nastikot, Parachinar, Kurram agency Federally Administrated Tribal Area (FATA), Pakistan.

TABLE-1
ANTIBACTERIAL ACTIVITY OF CRUDE PROTEIN EXTRACT OF *Seriphidium kurramense*

Bacterial strain	Zones of inhibition (mm)				
	0 µg	5 µg	10 µg	20 µg	Positive control
Methicillin resistant *Staphylococcus aureus*	0	5	12	21	25
Escherichia coli	0	6	10	18	28
Bacillus subtilis	0	4	7	14	23
Salmonella typhi	0	5	10	22	23
Staphylococcus aureus	0	6	11	22	23
Klebsiella pneumonia	0	3	7	16	25

Protein extraction: Crude protein was isolated using reported protocols [17]. Briefly, the plant material was ground in liquid nitrogen. After grinding, extraction buffer was added and slurry was made. The slurry was then transferred to micro-centrifuge tubes. The sample was then centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to a fresh tube. Protein extracts were then purified and stored at -20 °C.

Antibacterial assay: Six different bacterial strains *i.e.* methicillin resistant *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtillus*, *Staphylococcus aureus* and *Salmonella typhi* were used as test organisms. The assay was conducted according to the agar well diffusion method reported by Sheikh *et al*. [18]. Sterile nutrient broth medium was prepared and poured into sterile test tubes. Bacterial cultures were inoculated in test tubes with the help of flame sterilized inoculating loop. Samples were then incubated for 24 h at 37 °C. Sterile nutrient agar medium was poured into sterile Petri plates and allowed to solidify. Bacterial cultures were evenly distributed on nutrient agar plates using flame sterilized glass spreader. Wells were made into the medium using 6 mm cork borer. Various dilutions of crude protein extract (0, 5, 10 and 20 µg) contained in 25 µL were applied to these wells. Streptomycin (0.05 mg/mL) was used as positive control and DMSO as negative control. All plates were incubated at 37 °C for 24 h. Zones of inhibition were recorded the next day. All the tests were conducted in triplicate.

Antifungal assay: Antifungal activity was checked against *Aspergillus niger*, *Aspergillus flavus*, *Alternaria solani*, *Fusarium solani*, *Rhizoctonia solani* and *Pleurotus florida*. Antifungal activity was conducted using agar tube dilution method as described by Sheikh *et al*. [18]. Two dilutions (65 µg and 135 µg) of crude protein contained in 167 µL final volume were used. Ketoconazole (100 mg/mL) was used as standard antifungal drug while pure water was used as negative control. Slants were made containing 4.8 mL of medium mixed with 167 µL of test sample. Each test tube was inoculated with a loopful of fresh fungal culture. The inoculum was introduced at the base of slant by using flame sterilized inoculating loop. The tubes were incubated for 3-7 days and results were recorded. The percent growth inhibition was estimated by measuring linear fungal growth in test tube for each sample and both positive and negative controls. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Antibacterial assay: Crude protein extracts of *Seriphidium kurramense* were found effective against all the bacterial strains tested. The results are given in Table-1. The crude protein extracts showed antibacterial activity at all the concentrations checked. However, the extracts were most effective at 20 µg of protein extract and least effective at 5 µg while moderate activity was observed at 10 µg. Highest activity (22 mm zone diameter) was observed against *Salmonella typhi* and *Staphylococcus aureus* which is comparable to positive control (23 mm zone diameter). *Bacillus subtilis* seemed to be least affected (14 mm zone diameter) *Escherichia* and *Klebsiella pneumonia* were moderately inhibited.

Antifungal assay: The results of antifungal assay of crude protein extracts are shown in Table-2. The extracts showed good antifungal activity against four fungal species (*Rhizocotania solani*, *Alternaria solani*, *Aspergillus niger* and *Pleurotus florida*), moderate activity against *Aspergillus flavus* slight activity against *Fusarium solani* against 130 µg protein extract. The percent growth inhibition against 130 µg protein extract was 95.88 % for *Rhizocotania solani*, 91.68 % for *Alternaria solani*, 90.77 % for *Aspergillus niger*, 70.40 % for *Pleurotus florida*, 47.45 % for *Aspergillus flavus* and 8.57 % *Fusarium solani*. The percent growth inhibition against 65 µg of protein extract was 95.25 % for *Rhizocotania solani*, 85.7 % for *Alternaria solani*, 83.84 % for *Aspergillus niger*, 45.65 % for *Pleurotus florida*, 1.29 % for *Aspergillus flavus* and 0.00 % for *Fusarium solani*.

TABLE-2
ANTIFUNGAL ACTIVITY OF CRUDE PROTEIN EXTRACTS OF *Seriphidium kurramense*

Fungal species	Inhibition (%)		
	65 µg	130 µg	Positive control
Aspergillus niger	83.84	90.77	94.77
Aspergillus flavus	1.29	47.45	100.00
Alternaria solani	85.70	91.68	94.48
Fusarium solani	0	8.57	66.14
Rhizoctonia solani	95.25	95.88	66.00
Pleurotus florida	45.65	70.40	84.30

Antimicrobial proteins are produced by a number of plants as defense mechanism to combat infections [19]. These proteins are of small size generally less than 10 kDa, basic in nature, positively charged, rich in cystein content with disulphide bonds and posses amphiphilic shape [20]. These proteins are grouped into various classes like Defensins, Knottins, Hevein- and Vicilin-like peptides, Lipid transfer proteins, Snakins and Knottins *etc*. A general hypothesis regarding function of antibacterial proteins states that they interact with bacterial membrane which leads to the formation of *trans*-membrane channels that results in lowering membrane potential thus causing cytolysis [16].

A variety of plant species have been observed having antibacterial peptides. Most of these peptides have the ability to inhibit the activity of both Gram-positive and Gram-negative bacteria [21]. A protein from *Moringa oleifera* was screened against different bacterial species which showed maximum inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* [22]. The heat stable protein of three mulberry varieties was screened against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeuroginosa* and *Bacillus subtilis.* It was observed that the zone of inhibition increased with increase in concentration of heat stable protein [23]. Crude protein extracts of *Allium ascolinicum* showed strong antibacterial activity against *Proteus vulgaris, Escherichia coli and Staphylococcus aureus* [24].

A number of antimicrobial compounds are produced by plants to fight against the invading pathogens. Antifungal proteins produced by some plants play an important role against the attacking microorganisms [25]. In the present study, the effects of protein was found to be significant against fungal species. The protein was found to be potent against *A. niger* which was in accordance with the work reported by Jamil *et al*. [26] and Bhalodia and Shukla [27]. The protein extracts showed relatively less effectiveness against *A. flavus*. Similar results were reported by Moyne *et al*. [28]. The antifungal properties were found very prominent against *A. solani* similar to previous reports [12,29]. The growth of *F. solani* was inhibited to a maximum extent by the protein extract. Similar findings were reported by Mauch *et al*. [30] and Sela-Buurlage *et al*. [31]. Previous work also showed that various plant extracts possess potential antimicrobial compounds against microbes. Plant protein extracts could be used to treat various infectious diseases caused by microorganisms [32-34]. The results of antimicrobial studies of crude protein extracts of *Seriphidium kurramense* are promising and provide a basis for further exploitation of these proteins for biochemical characterization, isolation and structure elaboration of these proteins.

Conclusion

It is an established fact that many species of bacteria and fungi are pathogenic microorganisms and cause many health related issues. Consistent and excessive use of antibacterial and antifungal drugs has resulted in the evolution of resistant strains of bacteria and fungi. Hence, it is always desirable to find new antimicrobial drugs which are cheaper and without side effects. Medicinal plants are one of the possible solutions. The crude proteins of *Seriphidium kurramense* were potent against most of the bacterial and fungal species. These proteins could be isolated in pure form and utilized efficiently to serve as a valuable source for the production of cheaper antimicrobial drugs.

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