Antimicrobial, hemagglutination and phytotoxic activity of crude ethanolic and aqueous extracts of Seriphidium kurramense

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Abstract

OBJECTIVE: To investigate the antimicrobial activity, hemagglutination and phytotoxic activity of crude ethanolic and aqueous extracts of Seriphidium kurramense.

METHODS: The extracts were analyzed by agar well diffusion assays against five bacterial species: Staphylococcus aureus (S. aureus), methicillin-resistant S. aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, and Salmonella typhi. The extracts were also screened against six fungal species — Aspergillus niger, Aspergillus flavus, Alternaria solani, Rhizoctonia solani, Fusarium solani and Pleurotus florida — using the agar tube diffusion method. Additionally, hemagglutination and phytotoxic activities of the crude ethanolic and aqueous extracts were assessed.

RESULTS: The crude ethanolic and aqueous extracts showed dose-dependent inhibition of the various tested fungal and bacterial strains. No hemagglutination activity was observed. Both the ethanolic and aqueous extracts showed dose-dependent phytotoxic activity toward Lemna minor.

CONCLUSION: The crude ethanolic and aqueous extracts of Seriphidium kurramense possess good antimicrobial and phytotoxic activities, but no hemagglutination activity.

Keywords: Anti-bacterial agents; Antifungal agents; Phytotoxic; Seriphidium kurramense

INTRODUCTION

Medicinal plants have been used for health purposes since ancient times. Plants are used widely as a source of powerful and effective drugs, for example, for treating different infectious diseases caused by microorganisms. Large numbers of medicinal plants are yet to be explored; among the approximately 250,000 to 500,000 plant species, only a small percentage have been investigated for their phytochemical and pharmacological activities. Interest in the use of medicinal plants as potential therapeutic agents is escalating in different parts of the world due to the increasing faith of people in plant-derived medicines and the side-effects and high costs of allopathic medicines. Many antimicrobial agents, such as phenols, tannins and alkaloids, are synthesized by plants and are helpful in sustaining human and animal health. Apart from these substances, medicinal plants also produce peptides and low-molecular-weight proteins that are potent against bacteria and fungi. These antimicrobial peptides perform their actions by degrading the cell wall, damaging the ribosomes, and inhibiting DNA synthesis and the cell cycle. Similarly, terpenoids, essential oils, coumarins and quinones isolated from various plants also have powerful antimicrobial activities.
da acuta, Spigeia anthelmia, Stachytarpheta cayennensis and Tridax procumbens are some of the important medicinal plants that possess good antimicrobial properties. Seriphidium kurramense (S. kurramense) is a potent medicinal plant that has not been explored previously. S. kurramense is a medicinal plant which is mostly found in the upper Kurram agency (FATA) Pakistan and part of Afghanistan border which touches Kurram agency. Local communities of the area used this plant for several purposes. The plants is used as an antielminic, anti-diabetic, and remedy for stomach problems. The plant is also used for isolation of serotonin that is a neurotransmitter. The plant is believed to have insecticide properties. The aim of this study was to evaluate the antimicrobial, hemagglutination and phytotoxic activities of aqueous and ethanolic extracts of S. kurramense in vitro.

MATERIALS AND METHODS

Plant material
The plant S. kurramense was collected from Nastikot, Parachinar, Kurram Agency, Federally Administered Tribal Area, Pakistan. The research was conducted in the Laboratory of Microbiology, Center of Biotechnology and Microbiology, University of Peshawar, Pakistan. Plant material was chopped into small pieces and then shade dried. Dried material was ground into fine powder using an electrical grinder. Powder was soaked in ethanol or distilled water for 2 weeks at room temperature and stirred occasionally. The ethanol-soluble and water-soluble materials were filtered using filter paper. The ethanolic extract was concentrated under vacuum at 40 °C by using a rotary evaporator. The aqueous extract was concentrated by keeping the filtrate in a water bath at 40 °C for 10 d. In both cases, a blackish-green crude extract was obtained; these extracts were used as test samples in the experiments described below.

Antibacterial assay
The effects of ethanolic and aqueous extracts of S. kurramense were tested against five different types of bacteria: Staphylococcus aureus, methicillin-resistant S. aureus (MRSA), Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli and Salmonella typhi. The assay was performed using standard procedures.10 Bacterial cultures were inoculated into test tubes containing nutrient broth (beef extract 1 g/L, yeast extract 2 g/L, peptone 5 g/L, sodium chloride 5 g/L). The samples were incubated overnight at 37 °C. On the next day, bacterial broth cultures were inoculated onto nutrient agar plates and evenly distributed. Wells were made in the agar medium. For the ethanolic extract, two concentrations (3 and 10 mg/mL) were used. For the aqueous extract, 5 and 10 mg/mL were used. A positive control (streptomycin) and negative control (dimethyl sulfoxide; DMSO) was used for comparison. All plates were incubated at 37 °C for 24 h. Tests were performed in triplicate. The plates were observed for inhibition zones. Zones diameters were measured using a ruler and data was recorded in millimeters to find out percent inhibition by comparing with inhibition zones of standard drug. The formula for percent inhibition is given below.

\[
\text{Percent Inhibition} = \left( \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \right) \times 100
\]

Antifungal assay
The antifungal activities of crude ethanolic and aqueous extracts of S. kurramense were checked against six different fungal strains: Alternaria solani, Aspergillus Niger, A. flavus, Rhizoctonia solani, Fusarium solani and Pleurortus flroida. Antifungal assay was performed using the agar tube diffusion method reported by Sheikh et al.11 The fungal species were grown on sterile potato dextrose agar medium. Ketoconazole (positive control) and DMSO (negative control) were used for comparison. Two concentrations of the extracts (5 and 10 mg/mL) were analyzed. Briefly, 4.8 mL of media along with 167 µL of test sample were poured into each test tube. Then, the tubes were placed in a tilted position, allowed to cool, and slants were prepared. Fungal cultures were inoculated into each test tube at the base of the slant. The cultures were allowed to grow for 3-7 d. Linear growth was recorded in the tubes. The experiment was conducted in triplicate. Percent growth inhibition was calculated as given below.

\[
\text{Inhibition of fungal growth} = \frac{\text{Linear growth th in test (mm)}}{\text{Linear growth th in cont (mm)}} \times 100
\]

Hemagglutination assay
The hemagglutination activity of test samples (crude ethanolic and aqueous extracts) was tested according to the procedure reported by Naqvi et al.12 Briefly, 30 mg/mL of stock solution was prepared by dissolving crude extracts in DMSO. On the same day, 5 mL of blood was taken from healthy individuals and was centrifuged at 600 x g for 5 min. Plasma was removed from each blood sample with a pipette. Erythrocytes were collected. Then, a 2% erythrocyte suspension was prepared in phosphate buffer. Test sample (1 mL) was placed in a test tube, and then 1 mL of erythrocyte suspension was added. The test tubes were then incubated at 37 °C for 30 min. Hemagglutination activity was recorded by examining button formation i.e. smooth button formation at the bottom of tube indicated negative result and rough button formation indicated positive result.

Phytotoxic activity
Assays were carried out to determine whether the ethanolic and aqueous S. kurramense extracts were toxic to Lemna minor. Phytotoxicity assays were performed according to McLaughlin et al.13 Three concentrations of the extracts were used — 10, 100, and 1000 µg/mL. Briefly, ethanolic and aqueous extracts were poured in-
to separate Petri dishes. E-medium (20 mL) was added to each Petri dish. Then, 20 healthy L. minor plants with a rosette of three fronds were added to each dish. The dishes were incubated at 28 °C for 7 d and observed for death of plants.

RESULTS

Antibacterial assay
Table 1 shows the average inhibition of bacterial strains by 3 mg/mL ethanolic and aqueous S. kurramense extracts. The ethanolic extract was ineffective against all the tested bacterial stains. The aqueous extract moderately inhibited (56% inhibition) the growth of MRSA and slightly inhibited (35.2% inhibition) the growth of non-methicillin resistant S. aureus. Table 2 shows the average inhibition of bacterial strains by 10 mg/mL ethanolic and aqueous S. kurramense extracts. The ethanolic extract showed its highest growth inhibition (74.4%) toward B. subtilis and its lowest inhibition (32.2%) toward K. pneumoniae. MRSA and E. coli were slightly inhibited, while non-methicillin resistant Staphylococcus aureus moderately inhibited by 10 mg/mL ethanolic extract. For 10 mg/mL aqueous extract, moderate inhibition was observed for MRSA, non-methicillin resistant S. aureus and B. subtilis. Slight inhibition was observed for E. coli, Salmonella typhi and K. pneumoniae.

Antifungal assay
Table 3 shows results of fungal inhibition assays using 5 mg/mL crude ethanolic and aqueous extracts of S. kurramense. For the ethanolic extract, good inhibition was observed for F. solani. Moderate inhibition was observed for Aspergillus niger and P. florida. Slight inhibition was observed for A. flavus and R. solani. The aqueous extract inhibited the growth of Alternaria solani by 51.5% and P. florida by 28.4% but did not inhibit the growth of the other tested fungi.

The average inhibition of fungal growth by 10 mg/mL S. kurramense ethanolic extract is given in Table 4. Good inhibition was observed against Aspergillus niger, F. solani and P. florida. Moderate activity was observed against A. flavus and R. solani. For 10 mg/mL aqueous extract, moderate activity was observed against A. solani and P. florida.

Hemagglutination assay
When the crude extracts of S. kurramense were reacted with human erythrocytes, smooth buttons formed at the bottom of the test tubes and agglutination was not seen. The crude extracts (at 15 mg/mL concentration) did not show hemagglutination activity against human erythrocytes, and thus we conclude that S. kurramense does not contain phytohemagglutinin.

Phytotoxic activity
The results of phytotoxicity assays are shown in Table 5. The phytotoxic activity of the ethanolic extract of S. kurramense toward L. minor was 25% at 10 µg/mL, 45% at 100 µg/mL, and 65% at 1000 µg/mL. The phytotoxic activity of the aqueous extracts was 20% at 10 µg/mL, 30% at 100 µg/mL, and 40% at 1000 µg/mL.

DISCUSSION

Many plant extracts are potent against many pathogen-
ic species of bacteria and fungi, for example, medicinal plants have antibacterial activities against Staphylococcus aureus, Pseudomonas aeruginosa, B. subtilis, and so on. Much information has been published on the usefulness of plants for the treatment of infectious diseases. For example, the leaf extract of Cassia occidentalis and C. auriculate showed substantial antibacterial activity.

The volatile oils of some plants including Piper nigrum, Syzygium aromaticum and others have been reported to have antimicrobial activity against animal pathogens, plant pathogens and food spoilage bacteria. Similarly, extracts from plants including basil, clove, guava, pomegranate, lemon, and jambolan have been found to possess antimicrobial activities. The growth of E. coli was inhibited by the methanol extract of Warburgia salutaris. The aqueous and organic extracts of different Palestinian medicinal plants showed antibacterial activity and Thymus vulgaris, T. origanum and Bunium bulbocastanum possessed antibacterial activity.

In the present study, crude ethanolic and aqueous extracts of S. kurramense showed promising antibacterial activities. Good activity was observed against human pathogenic non-methicillin resistant Staphylococcus aureus and MRSA (Tables 1 and 2). S. aureus causes human infections such as endocarditis, bacteremia, and osteoarticular, medical instrument-derived, skin, pleuropulmonary and soft tissue infections. Methicillin resistant S. aureus is a form of S. aureus that shows resistance to different penicillin-based antibiotics through the mecA gene and can cause serious infections of bone, joints, and the central nervous system, and pneumonia, bacteremia and endocarditis.

The S. kurramense extracts at 3 mg/mL showed no effect on the other tested species of bacteria in this study, but were inhibitory at 10 mg/mL.

Both the ethanolic and aqueous extracts of S. kurramense showed promising antifungal activities. Excellent activity was shown by the ethanolic extract against F. solani (Tables 3 and 4), a fungus that causes diseases in crops such as potato, pea and cucurbits. F. solani is also the causative agent of various human infections, including of skin and nail. Similar results were reported by Mohana and Raveesha who studies extracts of eight plants i.e. Argemone mexicana L., Caesalpinia coriaria (Jacq.) Willd, Decalepis hamiltonii Wight & Arn, Euphorbia tirucalli L, Leucas aspera Spr, Phyllanthus amarus Schum and Thonn, Tinospora cordifolia Miers and Tribulus terrestris L. The 10 mg/mL aqueous extract of S. kurramense was also active against F. solani. Murugan and Krishnan reported similar results for the extracts of Marchantia linearis. However, the aqueous S. kurramense extract showed no activity against Aspergillus niger. A previous study reported high antifungal

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<th>Table 5 Phytotoxic activity of crude extracts of Seriphidium kurramense toward Lemna minor (%)</th>
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<td>Concentration of extract (µg/mL)</td>
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activity of aqueous extract of Rosmarinus officinalis toward Candida albicans and Aspergillus niger. The ethanolic extracts of S. kurramense strongly inhibited the growth of A. niger. Ahmad et al. reported moderate antifungal activity of extracts of Caralluma tuberculata against Fusarium oxysporum. No inhibitory effects of aqueous extract of S. kurramense at 5 mg/mL concentration were observed against A. flavus, in contrast to the good activity reported by Lakshmeesha et al., who used Cinnamomum verum extracts against A. flavus. However, the ethanolic extract of S. kurramense was effective against A. flavus, as reported by Mahmoud et al. who used Neem leaf extracts against A. flavus. The aqueous S. kurramense extracts showed good activity against Alternaria solani. Similar good activities were reported by Jalander et al. who used aqueous extracts of Datura Sp against Alternaria solani and Fusarium oxysporum. However, no antifungal activity of the S. kurramense ethanolic extracts was observed against Alternaria solani, unlike the good activity reported by Ja-been et al. who studied Azadirachta indica extracts against Alternaria solani. The aqueous extract of S. kurramense had no effects on R. solani, in contrast to the report of Mangang and Chhetry who studies different plants (Artemisia Nilarigica, Artocarpous Integefolia, Citrus Maxima, Coix Lacryma Jobi, Hedychium Coronarium, Lantana Camara, Michelia Champaka, Paspflora Foetida, Punica Granatum and Strobilanthes Flaccidifliusi) extracts against R. solani. The inhibitory effect of the ethanolic extract was moderate, similar to the results reported by Aslam et al. who studied plant extracts of Adhatoda zeylanica, Azadirachta indica, Cap paris decidua, Dodonaea viscosa and Salvadora oleoides.

It was observed that the crude ethanolic extract of S. kurramense had higher phytotoxic activity (toward L. minor) than the aqueous extract. Medicinal plants with phytotoxic activity have been reported previously. Previous studies have shown that plant extracts contain flavonoids, which could be the active antimicrobial metabolites of S. kurramense.

In conclusion, many bacterial and fungal species cause infections in humans, animals and plants. The over use of antibacterial and antifungal drugs eventually leads to the development of antibiotic resistance among microbial strains. The use of medicinal plants against microbes provides a cheaper and non-harmful alternative to treat such infections. The crude ethanolic and aqueous extracts of S. kurramense were found to be potent against several bacterial and fungal species. These extracts can be purified to obtain the active ingredients that could be used to produce antimicrobial drugs. Both extracts had phytotoxic activity and so could be used in the preparation of herbicides.

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