JTCM 中医杂志 Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2018 June 15; 38(3): 433-438
ISSN 0255-2922

RESEARCH ARTICLE

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

Kafeel Ahmad, Amjad Ali, Waqar Ahmed Afridi, Ramla Somayya, Muhammad Jawad Ullah

Kafeel Ahmad, Amjad Ali, Waqar Ahmed Afridi, Ramla Somayya, Muhammad Jawad Ullah, Centre of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa 25120, Pakistan

Correspondence to: Dr. Kafeel Ahmad, Assistant Prof., Centre of Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa 25120, Pakistan. kafeelpbg@gmail.com

Telephone: +92-3429143809

Accepted: March 30, 2017

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,[1] for example, for treating different infectious diseases caused by microorganisms.[2] Large numbers of medicinal plants are yet to be explored; among the approximately 250 000-500 000 plant species, only a small percentage have been investigated for their phytochemical and pharmacological activities.[3] Interest in the use of medicinal plants as potential therapeutic agents is escalating in different parts of the world[4] due to the increasing faith of people in plant-derived medicines and the side-effects and high costs of allopathic medicines.[5]

Many antimicrobial agents, such as phenols, tannins and alkaloids, are synthesized by plants and are helpful in sustaining human and animal health.[6] Apart from these substances, medicinal plants also produce peptides and low-molecular-weight proteins that are potent against bacteria and fungi.[7] These antimicrobial peptides perform their actions by degrading the cell wall, damaging the ribosomes, and inhibiting DNA synthesis and the cell cycle.[8] Similarly, terpenoids, essential oils, coumarins and quinones isolated from various plants also have powerful antimicrobial activities. Cleome rutidosperma, Etlingera coccinea, Euphorbia heterophylla, Richardia brasiliensis, Scoparia dulcis, Si-

da acuta, Spigelia anthelmia, Stachytarpheta cayennensis and Tridax procumbens are some of the important medicinal plants that possess good antimicrobial properties.[10] 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 anthelmintic, 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 insecticidal 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 Administrated 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 ℃ by using a rotary evaporator. The aqueous extract was concentrated by keeping the filtrate in a water bath at 40 ℃ 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.[11] 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 ℃. 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 ℃ 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.

Percent Inhibition = (Zone of Inhibition of Sample) / (Zone of Inhibiton of Standard) × 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 Pleurotus florida. 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.

$$\%Inhibition\ of\ fungal\ grow\,\text{th} = 100 - \frac{Linear\ grow\,\text{th}\ in\ test\ (mm)}{Linear\ grow\,\text{th}\ in\ con\,\text{tr}ol\ (mm)} \times 100$$

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 × *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 ℃ 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-