Saudi Journal of Biological Sciences (2014) **21**, 147–151

King Saud University

Saudi Journal of Biological Sciences

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains

Raid Al Akeel [a,*], Yazeed Al-Sheikh [a], Ayesha Mateen [b], Rabbani Syed [a], K. Janardhan [c], V.C. Gupta [b]

[a] *Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riaydh, Saudi Arabia*
[b] *Central Research Institute for Unani Medicine, Opp. ESI, Hyderabad, A.P., India*
[c] *P.G. Department of Biotechnology, A.V. College, Hyderabad, A.P., India*

Received 26 April 2013; revised 9 September 2013; accepted 10 September 2013
Available online 5 October 2013

KEYWORDS

Crude protein extracts;
Bacterial strains;
Antibacterial activity;
Agar well assay

Abstract A huge group of natural antimicrobial compounds are active against a large spectrum of bacterial strains causing infectious threat. The present study was conducted to investigate the crude extracts of antimicrobial protein and peptide efficacy from six medicinal plant seeds. Extraction was carried out in Sodium phosphate *citrate* buffer, and Sodium acetate buffer using different pH. Antimicrobial activities of these plants were determined by the microbiological technique using Agar well diffusion Assay. Extremely strong activity was observed in the seed extracts of *Allium ascolinicum* extracted in sodium phosphate citrate buffer at pH (5.8) against *Proteus vulgaris*, *Escherichia coli* and *Staphylococcus aureus* with zone of inhibition 17 mm, 17 mm and 15 mm and *Rumex vesicarius* at pH (7.6), *Ammi majus* at pH (6.8), *Cichorium intybus* at pH (7.4) and *Cucumis sativus* at pH (7.8) also showed better sensitivity against the bacterial strains with zone of inhibition ranges 16–10 mm and some of the strains were found to be resistant. Antibacterial activity pattern of different plant extracts prepared in sodium acetate buffer pH (6.5), among all the plant seed extracts used *Foeniculum vulgare* had shown good inhibition in all the bacterial strains used, with zone of inhibition ranges 11–12.5 mm, The extracts of *C. intybus* and *C. sativus* were found to be effective with zone of inhibition 11–6 mm and some of the strains were found to be resistant. Most of the strains found to have shown better sensitivity compared with the standard antibiotic Chloramphenicol (25 mcg). Our results showed that the plants used for our study are the richest source for anti-

* Corresponding author. Tel.: +966 541264918.
E-mail address: raalakeel@ksu.edu.sa (R. Al Akeel).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2013.09.003

microbial proteins and peptides and they may be used for industrial extraction and isolation of antimicrobial compounds which may find a place in medicine industry as constituents of antibiotics.

1. Introduction

Over the past 2 decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents (Bonjar et al., 2003; Tepe et al., 2004). According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). The indigenous system of medicine namely Ayurvedic, Siddha and Unani has been in existence for several centuries. This system of medicine supports the need of more than 70% of population residing in the rural areas. Besides the demands made by these systems as their raw materials, the demands of medicinal plants made by the modern pharmaceutical industries have also increased manifold (Bhattacharjee, 2001). Since a long period of time, plants have been a valuable source of natural products for maintaining human health and infections control because, microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents (Adenisa et al., 2000). Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Tapsell, 2006). Microbial infections pose a health problem throughout the world, and plants are a possible source of antimicrobial agents (Adenisa et al., 2000). Medicinal plants contain active principles which can be used as an alternative to cheap and effective herbal drugs against common bacterial infections (Kareru et al., 2008).

Even though pharmacological industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). In the present time, pathogenesis related plant proteins have been generally classified in accordance with their functional role in the formation of host plant immunity. On the other hand, considerable attention of researchers is attracted to a specific class of plant polypeptides capable of exerting an antimicrobial effect. The list of bactericidal and fungicidal plant proteins is being updated continuously. The search for new antibacterial compounds which have different mechanisms of action from those in current use is an alternative way for solving this problem. Seeds of plants have been reported to produce a number of peptides and proteins with antimicrobial activities (Wang et al., 2009). Many types of molecules with antibacterial activity have been isolated from plants (Boonnak et al., 2009; Mahabusarakam et al., 2008). Among them proteins and peptides with antimicrobial activity have recently been reported. They are recognized as important components of the innate defense system of bacteria, fungi, insects, animals and plants. Most of these defense proteins and peptides normally have multitasked activities. Some peptides can selectively inhibit gram positive or negative bacteria although antimicrobial peptides with gram positive and gram negative bacteria growth inhibiting ability have been reported (Reddy et al., 2004).

In addition, some peptides can inhibit other types of microorganisms including fungi and virus. Traditional antibiotics which have been previously used successfully for controlling bacterial pathogens are now less effective. This situation is due to the increasing antibiotic resistance which is currently shown by several bacteria (Costa et al., 2006). The aim of the present study is to study the antibacterial activity of six medicinal plants namely *Foeniculum vulgare*, *Cucumis sativus*, *Ammi majus*, *Allium ascolinicum*, *Cichorium intybus*, *Rumex vesicarius* against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus vulgaris* (ATCC 6380). The objectives of this research are to evaluate the potentiality of the crude protein extracts against the standard bacterial strain.

2. Materials and methods

2.1. Bacterial strain

Bacterial strains *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *P. vulgaris* (ATCC 6380) were purchased from Hi-Media laboratories.

2.2. Medicinal plants

The medicinal plant seeds were collected from Pharmacy of Central Research Institute of Unani Medicine, Hyderabad.

2.3. Extraction of antimicrobial proteins/peptides

Antimicrobial proteins and peptides were extracted using sodium phosphate citrate buffer (pH 5.2, 7.8, 6.8, 5.8, 7.4 and 7.6) and sodium acetate buffer (pH 6.5). The buffers were prepared and seeds of these medicinal plants were incubated at 28–30 °C and ground in these buffers and the extract was filtered using Whattmann filter paper No. 1. The crude extract isolated was saturated with 80% ammonium sulfate. The saturated extract was subjected for dialysis. After dialysis these samples were subjected to spectrophotometric analysis.

2.4. Culture medium and inoculum preparation

High sensitivity testing agar (Hi-Media) was used for checking antibacterial activity of crude protein extracts of different plant seeds against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *P. vulgaris* (ATCC 6380). The microbial strains were cultured on the slants in the sterilized Laminar Air Flow from the pure culture. These cultured slants were incubated at 37 °C for bacterial growth for 2–3 days. High sensitivity testing agar was mixed at a concentration of 23.4 g/1000 ml in distilled water and autoclaved at 121 °C for 15 min. A loop full from pure culture of a bacterial strain was mixed in the 10 ml of Nutrient broth medium