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

King Saud University

Saudi Journal of Biological Sciences

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ORIGINAL ARTICLE

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

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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-

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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

and incubated at 37 °C overnight and the activated culture was used for streaking onto the agar plates for antimicrobial sensitivity.

2.5. Agar well diffusion assay

The antibacterial activity of the crude protein extracts was determined by Agar well diffusion assay (Reeves, 1989). 2.34 g of high sensitivity testing agar was dissolved in 100 ml of distilled water and autoclaved at 121 °C for 15 min. Before transferring this medium in sterilized petri plates, it was allowed to cool and then was poured into the petri plates and allowed to solidify. After this, it is inoculated with activated culture using sterile cotton swabs. And the wells are created using sterile agar borer and the wells were filled by adding 25 µl of crude protein extracts and were incubated at 37 °C for 12–24 h. Three replicates were prepared from each sample. The extracts having antimicrobial activity, inhibit the microbial growth and the clear zones were formed. The zone of inhibition was measured in millimeters.

3. Results

Our results showed that various buffer pH have different protein extractability percentages for the seed extracts used (Table 1). The protein was extracted by sodium phosphate citrate buffer. Final concentration of the protein in various extracts was found to be 107.8 µg/ml in *F. vulgare* at pH (5.2) (109.79 µg/ml) in *C. sativus* at pH (7.8) (109.58 µg/ml) in *A. majus*), at pH (6.8), (109.37 µg/ml) in *A. ascolinicum* at pH (5.8), (110.8 µg/ml) in *C. intybus* at pH (7.4) and (109.56 µg/ml) in *R. vesicarius* at pH (7.6). In sodium acetate buffer, at pH (6.5) the concentrations of the protein were *F. vulgare* (179.4 µg/ml), *C. sativus* (149.7 µg/ml), *A. majus* (139.7 µg/ml), *A. ascolinicum* (143.26 µg/ml), *C. intybus* (146.6 µg/ml) and *R. vesicarius* (76.98 µg/ml). Overall comparison of different pH and buffer systems showed that sodium phosphate buffer at pH (7.4) in *C. intybus* and Sodium acetate buffer pH (6.5) in *F. vulgare* had maximum protein extractability and Sodium acetate buffer pH (6.5) the highest protein concentration was found in *F. vulgare* (179.4 µg/ml) whereas the lowest concentration in *R. vesicarius* (76.98 µg/ml) was observed.

Table 1 Concentration of protein extracts of different pH.

S. No.	Name of the plant	pH	Concentration (µg/ml)
1.	*Foeniculum vulgare*	5.2	107.8
		6.5	179.4
2.	*Cucumis sativus*	7.8	109.79
		6.5	149.7
3.	*Ammi majus*	6.8	109.58
		6.5	139.7
4.	*Allium ascolinicum*	5.8	109.37
		6.5	143.26
5.	*Cichorium intybus*	7.4	110.8
		6.5	146.6
6.	*Rumex vesicarius*	7.6	109.56
		6.5	76.98

3.1. Antimicrobial activity of protein extracts obtained from different plant species

Antibacterial activity of different plant extracts prepared in different pH and buffers was tested against different bacterial strains which were studied. Antibacterial activity of different plant extracts prepared in sodium acetate buffer pH (6.5) was tested against different bacterial strains. Among all the extracts *F. vulgare* showed inhibition in all the bacterial strains used. The extract prepared in sodium phosphate citrate buffer at different pH exhibited good results. The highly strong activity was observed in the seed extracts of *A. ascolinicum* at pH (5.8) against *E. coli* and *P. vulgaris* with zone of inhibition 17 mm, protein concentration (2.73 µg) which is found to be more than standard antibiotic Chloramphenicol (25 mcg) which showed zone of inhibition 8 mm and the least activity was found in *P. aeruginosa* with zone of inhibition 7.5 mm with the same protein concentration (2.73 µg) in *R. vesicarius* whereas the same was found to be very effective on *S. aureus* with zone of inhibition 16 mm with protein concentration (2.73 µg) at pH (7.6). The crude protein extracts of *A. majus* at pH (6.8) were found to be very effective against *S. aureus*, *E. coli* and *P. vulgaris* with zone of inhibition 14 mm, 12.5 mm and 12 mm with protein concentration (2.73 µg). *Nigella sativa* showed good inhibition against *S. aureus* and *P. aeruginosa* with zone of inhibition 10.5 mm and 8 mm and other two bacterial strains were found to be resistant. The highest activity was found against *E. coli*, *P. vulgaris* and *S. aureus* strains with zone of inhibition 12.5 mm, 12 mm and 12 mm, even *P. aeruginosa* was also found to be almost equally sensitive with a zone of inhibition 11 mm. The extracts of *C. sativus* was found to be effective on *P. aeruginosa* and *P. vulgaris* with zone of inhibition 10 mm and 6 mm. Likewise *C. intybus* was found to be effective on *P. aeruginosa*, *E. coli* and *P. vulgaris* with a zone of inhibition 10 mm, 11 mm and 10 mm. And the extracts of *C. sativus* and *R. vesicarius* had shown no activity on all the bacterial strains. Most of the strains found to have shown better sensitivity compared with the standard antibiotic Chloramphenicol (25 mcg) (Table 2).

4. Discussion

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 (Ashish et al., 2013). 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 (Mothana et al., 2009). Seed extract of different medicinal plants were screened for antibacterial activity. The method for the determination of antibacterial activity was agar well diffusion Assay and the zones of inhibition were measured. Zone of inhibition varied among the samples. For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60% and 90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare (WHO, 2002). Antibacterial activity

Table 2 Diameter of zone of inhibition of different plant seed extracts.

S. No.	Plant name	pH	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Proteus vulgaris*	Concentration (μg)
Zone of inhibition (mm)							
1.	*Foeniculum vulgare*	5.2	10	0	0	0	2.69
		6.5	12	11	12.5	12	4.48
2.	*Cucumis sativus*	7.8	10	10	0	0	2.74
		6.5	0	10	0	6	3.74
3.	*Ammi majus*	6.8	14	0	12.5	12	2.73
		6.5	7	0	0	8	3.49
4.	*Allium ascolinicum*	5.8	15	12.5	17	17	2.73
		6.5	0	0	0	0	3.58
5.	*Cichorium intybus*	7.4	12	0	12	15.5	2.77
		6.5	0	10	11	10	3.66
6.	*Rumex vesicarius*	7.6	16	7.5	0	15	2.73
		6.5	0	0	0	0	1.92
7.	Chloramphenicol (25 mcg)	–	21	08	08	08	25

of different plant extracts prepared in sodium acetate buffer pH (6.5) and sodium phosphate citrate buffer pH [5.2], [7.8], [6.8], [5.8], [7.4], [7.6] was tested against different bacterial strains. Among all the extracts *F. vulgare* showed inhibition in all the bacterial strains used. The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000). Intensive care physicians consider antibiotic resistant bacteria a significant or major problem in the treatment of patients (Lepape, 2009). Increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains (Alviano and Alviano 2009; Hemaiswarya et al., 2008).

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The results from our study have shown extremely strong activity in the seed extracts of *A. ascolinicum* at pH (5.8) against *E. coli* and *P. vulgaris* with zone of inhibition 17 mm, protein concentration(2.73 μg) which is found to be more than standard antibiotic Chloramphenicol (25 mcg) which showed zone of inhibition 8 mm. Our study is in agreement with other previous studies. Shallot or red onion (*Allium ascalonicum* L.) is a versatile vegetable used as an ingredient in many Asian dishes and also used as medicinal plant having antimicrobial effect (Dankert et al., 1979), which is having the properties like cancer risk reduction (Fukushima et al., 1997), also acts as a effective model in anti-hypertension (Sakai et al., 2003), anti-thrombosis (Ali et al., 1999) and anti-diabetes (El-Demerdash et al., 2005).

In herbal medicine, crude plant extracts are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes et al., 2007). *A. majus* at pH (6.8) was found to be very effective against *S. aureus*, *E. coli* and *P. vulgaris* with zone of inhibition 14 mm, 12.5 mm and 12 mm with protein concentration (2.73 μg). *A. ascolinicum* at pH (5.8) against *E. coli* and *P. vulgaris* with zone of inhibition 17 mm, protein concentration (2.73 μg) and the least activity was found in *P. aeruginosa* with zone of inhibition 7.5 mm with the same protein concentration (2.73 μg). The consonance of the work was done using CH_2Cl_2/MeOH (1:1) extract, (Zellagui et al., 2011) the extract exhibited varying degrees of antibacterial activity depending on the dose used. *A. majus* is used for the treatment of leukoderma and psoriasis (Kumar, 1998). The constituents (xanthotoxin, imperatorin, isopimpinellin, bergapten and isoimperatorin) isolated from the seeds are used to prepare cream and lotion to cure skin diseases. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005). Medicinal plant extracts offer considerable potential for the development of new agents effective against infections that are currently difficult to treat (Iwu et al., 1999).

5. Conclusion

These plants can be used to discover bioactive natural products in the form of antimicrobial proteins and peptides that may serve for the development of new pharmaceuticals. Such screening of various natural antimicrobial proteins and peptides supports the traditional knowledge of local users and it is a preliminary scientific validation for the use of these plants for the antibacterial activity. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings.

Acknowledgements

We are grateful to the Director General of Central Council for Research in Unani Medicine, Ministry of health and family welfare, New Delhi, and Department of Laboratory Sciences, College of Applied Medical sciences, King Saud University for financial assistant, encouragement and providing facilities for carrying out present study.

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