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Evaluation of Antibacterial Activity of Twenty-Two Medicinal Plants Traditionally Used in Saudi Arabia against Pathogenic Bacteria

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Abstract

The high prevalence of infections due to multi-drug resistant bacteria represents a major challenge to public health worldwide. Additionally, the failure of many currently used antibiotics to kill multi-drug-resistant bacteria highlights the need to develop other antimicrobial agents such as antimicrobial peptides (AMPs). Antimicrobial peptides (AMPs), a growing class of natural and synthetic peptides that have a broad spectrum of targeted organisms, twenty two medicinal plants were selected for this study, sodium phosphate buffer (pH:7.2) used to isolate the protein and tested against 4 pathogenic strains such as Methicillin-resistant *S. aureus* (MRSA), *Acinetobacter baumannii, Escherichia coli*, and *Staphylococcus aureus*. Out of the 22 selected plants, we found 9 plants shown antimicrobial activity against tested pathogenic strains. In conclusion, screening plant peptides is a basic and far most important step in developing antimicrobial peptides from various medical plants. Peptidomics is a new tool that can be used to identify the functional impact of the natural peptides isolated which may have pharmacological and Industrial application.

Keywords: *Antimicrobial Activity; Agar Diffusion; Extraction; Pathogenic Strains*

Introduction

Protein is a vital nutrient that has an important role in how our bodies function. Although they have crucial role in building physical health, they have adverse effect due to excess protein which may have direct or indirect impact associated with many diseases [1]. Therefore, it has become important to have an eagle eye on the type of protein in diet to maintain the metabolism. Usually 65% per capita of protein source is from plants, whereas the rest may be from animal and other sources [2]. In this regard, many researches are focusing on bioactive properties of phytochemicals and plant based protein and peptides recently using as nutraceuticals to enhance health and reduce the disease risk.

Bacterial pathogens are associated with either causing a wide range of localized or systemic infections in human. Some pathogens like *Staphylococcus* usually form part of skin microbiota have potency to incite skin infections which can pose serious systemic inflammation sometimes may cause death [3]. Previous studies have also confirmed that people with cystic fibrosis leading to immunosuppression is mainly due to infection with some opportunistic pathogens such as *Mycobacterium, Pseudomonas* etc. [4,5]). AMPs are often the first line of defense against invading pathogens and play an important role in innate immunity [6]. AMPs are pervasive and act as host defenses against pathogens and present in different organisms [7]. AMPs occur in various molecular forms, most of them are linear peptides

produced from insects, plants and animals. Though, bacteria produces antibiotics which are polycyclic peptides and all major forms of life produces circular peptides including theta defensins from animals' bacteriocins from bacteria and cyclotides from plants [8,9]. As for current trends, the use of plant peptides has emerged as an alternative in the clinical treatment of bacterial infections. The excessive use of antibiotics has already contributed to the high level of antimicrobial resistance and therefore limits the therapeutic options prescribed usually by physicians. Additionally, it represents a great burden to healthcare costs due to long hospitalization period required for patients. To overcome these difficulties, the pharmaceutical industry is looking for new antibiotics, trying to modify existing treatment strategies or adding new alternative therapeutic approaches. At present scenario, AMPs derived from plants may offer limited application in the field of agriculture and medicine, more research in underway to dwell the benefits of AMPs which may have bactericidal affect against many pathogens [10]. In a recent development, researchers have come up with new approach peptidomics to identify biological active peptides which may have pharmacological active molecules that can be analyzed be means of mass spectroscopy further linked to advancement in bioinformatics [11]. Our present study is for screening and isolating antimicrobial proteins/peptides from some medicinal plants and to test the efficacy of the isolated proteins/peptides against some pathogenic strains.

Materials and Methods

Bacterial Strains

Four pathogenic bacteria namely Methicillin-resistant *S. aureus* (MRSA) (ATCC 43300), *S. aureus* (ATCC 29213), *A. baumannii* (ATCC BAA747), *E. coli* (ATCC 25922) were provided from the department of Clinical Laboratory Sciences, King Saud University, Riyadh, Saudi Arabia. All the strains were freshly sub cultured before the test using Nutrient agar.

Preparation of Extracts

The twenty-two medicinal plants were collected from in and around of Saudi Arabia. Plants were washed three times with sterile distilled water. After wash, the plant materials were air dried in shade at room temperature (25 to 30℃). Following the drying process, about 3,0 gm of sample was finely grinded with electric motor and incubated 50 ml of sodium phosphate buffer at pH 7.2. Next morning extracts were centrifuged at 5000 rpm for 5 minutes at 4^0C temperature and supernatant was filtered through Whatman filter paper 1 and resultant filtrate was stored at 4^0C till further use.

Inoculum preparation and Agar well diffusion assay

Overnight grown cultures of MRSA, *A. baumannii, E. coli* and *S. aureus* were inoculated in 5 ml of nutrient broth and concentration of the cells adjusted to 2×10^8 (CFU/ml) with Nutrient broth and 0.5 Mcfarland standard was maintained for all the cultures and used for streaking onto the agar plates for Antibacterial activity.

Antibacterial activity of the extract was conducted with a well diffusion agar-plate technique as described elsewhere. Briefly, 2.34g of High sensitivity testing agar (Hi-media, INDIA) was dissolved in 100 ml of sterile distilled water and then autoclaved for 15 minutes at 121°C. After Cooling, plates were inoculated with activated culture using sterile cotton swabs and the wells are created using sterile agar borer (7 mm). Following this, wells were filled with 3 mg/well of plant extract. A broad-spectrum standard antibiotics vancomycin (30 ug/ml) and gentamicin (15 ug/ml) were used as a positive control and sodium phosphate buffer as a negative control. The plates were then incubated for 24h at 37°C. Following incubation, the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm.

Results

Out of 22 selected plants, we found nine plants shown antimicrobial activity against tested pathogenic strains results shown in table 1. *Acacia nilotica* showed activity on MRSA with inhibition zone 11 mm in comparison to the positive control vancomycin 18 mm and *S. aureus* with inhibition zone 12 mm compared to positive control gentamycin 16 mm respectively. *Frangula alnus* showed activity on MRSA with inhibition zone of 11 mm compared to 18 mm vancomycin and *S. aureus* with inhibition zone of 9 mm compared to gentamycin with 16 mm respectively. *Rosmarinus officinalis* shown activity on MRSA with inhibition zone 15 mm compared to vancomycin with 16 mm

inhibition zone. *Thymus serpyllum* and *Hawthorn* also shown good activity on MRSA with inhibition zone 16 mm and 10 mm respectively compared to vancomycin with 16 mm inhibition zone. *Juniperus* shown good activity on MRSA 12 mm inhibition zone compared to vancomycin 16 mm inhibition and *S. aureus* with 8 mm inhibition compared to gentamycin with 16 mm zone. *Myrtus communis* showed good activity on MRSA of 18 mm, *A. baumannii* of 16 mm and *S. aureus* of 21 mm inhibition zone compared to vancomycin and gentamicin respectively. *Arctium lappa* and *Senna* shown activity on *S. aureus* of 21 mm inhibition compared to gentamycin with 16 mm inhibition zone. Of all tested plant protein extracts, no impact or activity was observed against Gram negative strain *E. coli* where positive control gentamicin has shown 18 mm inhibition zone.

Scientific Name	MRSA	A. baumannii	E. coli	S. aureus
Acacia nilotica	P 11mm	N	N	P 12mm
Cymbopogon	N	N	N	N
Alchemilla vulgaris	N	N	N	N
Salvia officinalis	N	N	N	N
Artemisia vulgaris	N	N	N	N
Trachyspermum ammi	N	N	N	N
Carum carvi	N	N	N	N
Frangula alnus	P 12mm	N	N	P 9mm
Foeniculum vulgare	N	N	N	N
Rosmarinus officinalis	P 15mm	N	N	N
Lavandula	N	N	N	N
Calendula officinalis	N	N	N	N
Thymus serpyllum	P 16mm	N	N	N
Crataegus	P 10mm	N	N	N
Juniperus	P 12mm	N	N	P 8mm
Equisetum	N	N	N	N
Myrtus communis	P 18mm	P 16mm	N	P 21mm
Cleome droserifolia	N	N	N	N
Arctium lappa	N	N	N	P 19mm
Costus indien	N	N	N	N
Senna	N	N	N	P 14mm
Hyssopus officinalis	N	N	N	N
Vancomycin (30ug/ml**)**	16mm	-	-	-
Gentamicin (15ug/ml)	-	18mm	18mm`	16mm

Table 1: *Antibacterial activity of protein extracts from selected medicinal plants against four pathogenic strains tested. N: Negative; P: Positive*

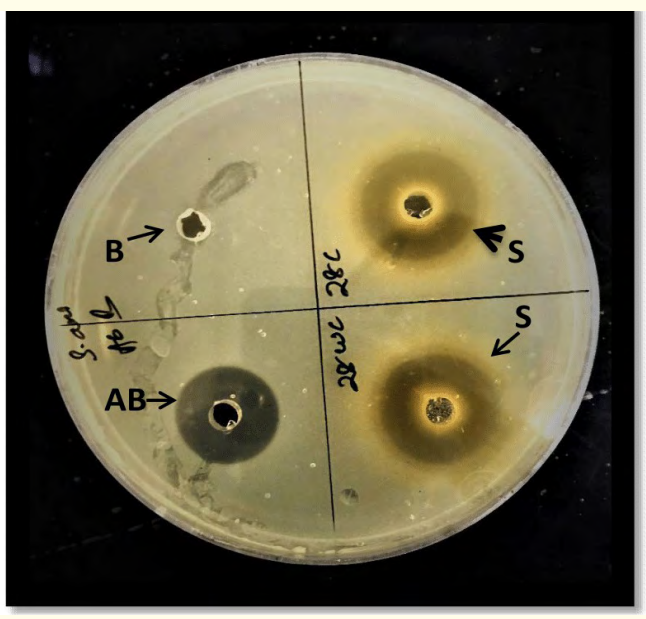

Figure 1: *Agar well diffusion test demonstrating inhibition zones by Myrtus communis leaf extract, Where: B: sodium phosphate buffer (negative control), AB: Gentamicin 15 ug/ml (positive control), S: Plant extract at concentrations of 3 mg/well, against S. aureus.*
pathogenic strains tested.

Discussion

In our present research work, screening of antimicrobial protein has been conducted using 22 medicinal plants mostly from native Saudi province (Table 1) and their antibacterial activity has been determined on four pathogenic bacterial strains. Our screening reports have shown good impact on different pathogenic strains that can be further used as a benchmark for isolating peptides from the plant source. Screening is the most essential step to identify the productivity and efficacy of the plants with respect to any sort of activity like antimicrobial anti-cancer and anti-fungal activity etc. The rapid increase in drug-resistant infections has delivered a serious question to antimicrobial therapies. The failing of the most efficient antibiotics to quell "superbugs" emphasizes the urgent need to promote other restraint agents. These peptides are prime candidates for development as modern therapeutic agents and supplement to orthodox antibiotic therapeutics since they comprehensively have a vast range of activity, are bactericidal as opposed to bacteriostatic and need a short contact period to trigger killing [12].

The protein extract of *Acacia nilotica* was found to be effective on both *S. aureus* and MRSA strains with zone of inhibition 12 and 11 mm, in the previous studies *Acacia nilotica* extracts showed substantial antimicrobial effects against antibiotic-resistant bacterial strains, by observing changes in bacterial cell morphology, cell membrane integrity and permeability [13]. *Frangula alnus* was also shown anti-staphylococcal effect on both *S. aureus* and MRSA strains, with zone of inhibition 9 and 12 mm. Previous research showed that *Frangula alnus* bark extracts have a potential anti-staphylococcal activity, where in our study we have used leaves protein extract [14].

We have found very good zone of inhibition with *Myrtus communis* protein extract on MRSA strain 18 mm, *Rosmarinus officinalis* and *Thymus serpyllum* also showed good zone of inhibition with 15 mm and 16 mm. whereas *S. aureus* shown highest sensitivity with zone of inhibition 21 mm towards the protein extracts of *Myrtus communis* and *A. baumannii* also showed good sensitivity with zone of inhibition 16 mm. *Arctium lappa* and *senna* found to be effective on *S. aureus* with zone of inhibition 19 mm and 14 mm. In the earlier studies, *Rosmarinus officinalis* oils and solvent extracts were shown antibacterial activity [15,16] our protein extract was found to be effective on MRSA strain.

High antibacterial activity of methanol, ethanol, and ethyl acetate leaf and berry of *Myrtus communis* extracts was observed against foodborne pathogens in the previous studies [17], and the present research showed good results on MRSA with protein extract. Our previous studies have shown promising results where we have done screening and characterization of some medicinal plants has reproduced activity on tested pathogens [18,19], high activity zone was seen in *Foeniculum Vulgare* where further studies were in progress to identify the peptide type and proactivity may have industrial and pharmacological benefits from these plant peptides.

Conclusion

In conclusion, screening plant proteins/peptides is a basic and far most important step in developing antimicrobial peptides from various medical plants and our study has come up with some good results where further proteomic analysis is in progress to ensure the activity of the isolated proteins/ peptides.

Competing Interests

The authors declare that they have no conflict of interests.

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