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Short Communication

Comparison of the antimicrobial activity of seed protein extracts from six medicinal plants against *Staphylococcus aureus* 6736152

Salahudin[1], Muhammad Afzal[1], Allah Bakhsh[2], M. Rauf Ahmad[2], Irfan Manzoor[1] and Sadia Liaquat[1*]

[1]Government College University Faisalabad, 38000, Pakistan; [2]National Centre of Excellence in Molecular Biology, 87-West Canal Bank Road, Thokar Niaz Baig, Lahore, 53700, Pakistan

Abstract: A large group of antimicrobial compounds termed as "natural antibiotics" are active against a large spectrum of microorganisms, including bacteria and filamentous fungi in addition to protozoan and metazoan parasites. The present study was conducted to investigate the antimicrobial peptides efficacy from six medicinal plants (*Azadirachata indica*, *Feniculum vulgare*, *Pegnanum hermala*, *Ricinus communis, Ocimum basilicum* and *Cichorium intybus*) against *Staphylococcus aureus* 6736152. The extraction was carried out in Potassium phosphate buffer, Sodium phosphate buffer and Sodium acetate buffer. The antimicrobial activities of these plants were determined by microbiological technique using disc diffusion method against *Staphylococcus aureus 6736152.* The minimum inhibitory concentrations (MICs) of these plants were also investigated. All the extracts exhibited antibacterial activity against *Staphylococcus aureus 6736152* but the stronger antibacterial activity was observed in *Peganum hermala* extract against *Staphylococcus aureus* with 40±1.5mm zone of inhibition. Our results showed that the *Pegnanum hermala* is a rich source for antimicrobial peptides and it may be used for industrial extraction and isolation of antimicrobial compounds which may found place in medicine industry as constituents of antibiotics.

Keywords: Antimicrobial activity, medicinal plants, *Staphylococcus aureus*.

مقارنة بين نشاط مضادات الميكروبات للبروتين البذور المستخلص من ستة نباتات طبية ضد *Staphylococcus aureus* 6736152

صلاح الدين [1] ، محمد ابفسل [1] ، الله بوكش [2]، م. رؤوف احمد [2] ، ارفان منزور [1] و ساديا لياكت[1]*

[1]جامعة الكلية الحكومية ـ فيصل اباد ، 38000 ، باكستان; [2] المركز الوطني للتميز في علم الاحياء الدقيقة ، 87- طريق وست قنال بنك ، طوكر نياس بيق ، لاهور ، 53700 ، باكستان

الملخص: مجموعة كبيرة من المركبات المضادة للميكروبات توصف باسم "المضادات الحيوية الطبيعية " التي تنشط ضد مجموعة واسعة من الكائنات الدقيقة، بما في ذلك البكتيريا والفطريات الخيطية بالإضافة إلى طفيليات protozoan and metazoan. وقد أجريت الدراسة الحالية للتحقق من فعالية الببتيدات المضادة للميكروبات من ستة نباتات طبية (*Azadirachata indica, Feniculum vulgare, Pegnanum hermala, Ricinus communis, Ocimum basilicum* and *Cichorium intybus*) ضد *Staphylococcus aureus* 6736152. وقد تم استخراج المستخلص في الفوسفات البوتاسيوم بطبقة عازلة والفوسفات الصوديوم بطبقة عازلة و الصوديوم خلات بطبقة عازلة. وتم تحديد الأنشطة المضادة للميكروبات من هذه النباتات باستخدام التقنية الميكروبيولوجية بطريقة الانتشار ضد *Staphylococcus aureus* 6736152. كما تم بحث تركيزات الحد الأدنى المثبطة لمجموعات هذه النباتات. المستخلصات اطهرت كل النشاط المضاد للبكتيريا مقارنة ب6736152 *Staphylococcus aureus* ولكن لوحظ نشاط أقوى مضاد للجراثيم في Peganum hermala المستخلص ضد *Staphylococcus aureus* مع 40 ± 1.5mm مجال تثبيط. وأظهرت النتائج التي توصلنا إليها أن *Pegnanum hermala* يعتبر مصدرا غنيا لالببتيدات المضادة للميكروبات ويمكن استخدامها للاستخراج الصناعي والعزل من المركبات المضادة للميكروبات التي قد وجد مكانا في الصناعات الدوائية كمكونات للمضادات الحيوية.

* Corresponding author, *Email*: abthebest@gmail.com

Introduction

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998).

The hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times have used (Capasso, 1998). Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins. Many are secondary metabolites, of which at least 12,000 have been isolated a number estimated to be less than 10% of the total. In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds (Tapsell, 2006). The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. This system of medicine caters to the needs of nearly seventy percent of population residing in the villages. Besides the demands made by these systems as their raw materials, the demands of medicinal plants made by the modern pharmaceutical industries has also increased manifold (Bhattacharjee, 2001).

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 in the last three decades, 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).

By 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 antimicrobial effect. The list of bactericidal and fungicidal plant proteins is being updated continuously. These are relatively small polypeptides (molecular weight, from 2 to 9 kDa) of similar structure (Molina et al., 1993; Koo et al., 1998; Tarchevskii et al., 2001).

According to one of the hypotheses discussed in the literature, molecules of antimicrobial peptides interact with the bacterial membrane giving rise to the formation of a transmembrane cluster (probably, an ion channel). This causes decrease in the membrane potential value and subsequent cytolysis (Taran et al., 2002).

The present study was conducted to compare the antimicrobial activity from six medicinal plants *Azadirachata indica*, *Feniculum vulgare*, *Pegnanum hermala*, *Ricinus communis, Ocimum basilicum* and *Cichorium intybus* against *Staphylococcus aureus* 6736152. The research work was conducted in Protein Molecular Biology Laboratory, Department of Chemistry and Biochemistry under a research grant funded by Higher Education Commission, Govt. of Pakistan. The objectives of this research were to (i) evaluate the potential of plant extracts and photochemical on standard microorganism strains, and (ii) investigate the synergistic effects of extracts with antimicrobial activity in

association with antibiotics against drugs resistant bacteria.

Materials and Methods

Bacterial strain

Bacterial strain, *Staphylococcus aureus 6736152* was provided by the Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad. The strain was biochemically tested for its purity.

Medicinal plants

The medicinal plants under study were selected on the basis of review and ethno pharmacologic effects, and seeds were collected from the local market of Faisalabad. The plants under study are easily accessible as they are cultivated on large scale in Pakistan.

Extraction of Antimicrobial Proteins

Antimicrobial proteins and peptides were extracted using 10mM of potassium phosphate buffer (pH 7.0), 10mM of sodium phosphate buffer (pH 7.0) and 3M of sodium acetate buffer (pH 5.2). The buffers were prepared and seeds of these medicinal plants were grinded in these buffers and extract was harvested in 10mL tube. This extract was further centrifuged at 10,000 rpm at 4^{o}C for 15-20 mins. The crude extract isolated was saturated with 80% ammonium sulphate. Centrifuged the crude extracts and collected with supernatant and residues were resuspended in buffer. Now the resuspended residues were eluted using gel filtration column and collected in 1.5mL tubes. These samples were subjected to Bradford assay (Bradford M., 1976).

Culture Medium and Inoculum Preparation

Nutrient agar (Oxoid) was used for checking antibacterial activity of seeds of different plants extract against *Staphylococcus aureus*. The microbial strains were cultured to 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. Nutrient broth (Oxoid) was mixed at a concentration of 1.3 g/100 mL in distilled water and autoclaved at 121°C for 15 minutes. A loop full from pure culture of a bacterial strain was mixed in the medium after cooling the flask and then placed in the shaker at 37°C for 24 hours. Inoculum for the strain were prepared in this manner and stored at 4°C.

Assay by Disc Diffusion Method

2.8 g Nutrient agar (Oxoid) was dissolved in 100 mL of distilled water and autoclaved at 121°C for 15 min. Before transferring this medium in sterilized Petri plates, allowed it to cool and then added inoculum to it, mixed it thoroughly and then poured it into the petri plates and allowed it to solidify. After this, small filter paper (whatman filter paper) discs were laid flat on growth medium and 100 µL of extract/protein was poured on each disc. Three replicates were prepared from each sample.

The Petri plates were then incubated at 37^{o}C for bacteria for 12 hours. The extracts having antimicrobial activity, inhibited the microbial growth, the inhibition and the clear zones were formed. The zone of inhibition was measured in millimeters using zone reader (Huynh et al. 2001).

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation (Andrews, 2001). Took 96 well plate (micro dilution plates), poured (100µL) of broth in each well and then poured 100µL of sample into first well and make two fold dilution of sample. Then added spores 10µL to each well, eleventh well was taken as negative and twelfth well was taken as positive control and incubated for 8 to 12 hours at 37°C for bacterial growth. Three replicates were prepared from each sample. Streaking was

done on the nutrient agar plates by taking a loop full from the first well and then from every third well taking eleventh as negative and twelfth as positive control. Then calculated the MIC values (Hancock, 1997).

Results and discussion

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.

Amount of protein and its antimicrobial activity after extraction with different types of buffers

The extracts of seeds of different medicinal plants were screened for antibacterial activity. The method for the determination of antibacterial activity was disc diffusion method and the zone of inhibition were also measured. Zone of inhibation varied among the samples. Some of the samples had moderate antibacterial activity (12-20 mm); strong antibacterial activity (21-30 mm); highly antibacterial strong activity (31-40 mm). Negative results of some plants indicate that either plants had no active compound or it has very low concentration or activity.

Our results showed that different buffers have different protein extractability percentages for different plants (Table-1). The highest percentage of protein (4.70µg/100mL) extracted by potassium phosphate was observed in *Azadirachata indica* and the lowest (4.09µg/100mL) was found in *Cicharium intybus.* In sodium phosphate buffer, the highest percentage was of *Ricinus communis* and *Azadirachata indica* (4.80µg/100mL) and the lowest was *Cicharium intybus* (4.0µg/100mL). In sodium acetate the highest percentage was of *Ricinus communis* and *Azadirachata indica* (5.52µg/100mL) whereas the lowest percentage was of *Ocimum basilicum* (4.10µg/100mL). Overall comparison of three buffers systems showed that sodium acetate buffer had maximum protein extractability and the highest protein concentration was found in *Azadirachata indica* whereas the lowest percentage was observed in by Sodium Phosphate buffer.

Table 1. Comparison of protein concentration (µg/100mL) in plant extract by different buffers.

Sl. No	Plants	Potassium phosphate buffer (µg/100mL)	Sodium phosphate buffer (µg/100mL)	Sodium acetate buffer (µg/100mL)
1	*Feniculum vulgare*	4.56	4.64	4.67
2	*Peganum hermala*	4.31	4.23	4.33
3	*Ricinus communis*	4.67	4.80	5.52
4	*Ocimum basilicum*	4.13	4.09	4.10
5	*Cicharium intybus*	4.09	4.00	4.14
6	*Azadirachata indica*	4.70	4.80	5.52

Antimicrobial activity of protein extracts obtained from different plant species

Antibacterial activity of different plants extracts prepared in potassium phosphate buffer against *Staphylococcus aureus* strain was studied. The extract prepared in potassium phosphate buffer exhibited good results. The highly strong activity was observed in the seed extracts of *Peganum hermala* against *Staphylococcus aureus*. The least activity was found in *Feniculum vulgare* but even that was much effective than the antibiotic which was used as positive control (Figure 1).

Antibacterial activity of different Plants extracts prepared in sodium phosphate buffer against *Staphylococcus aureus* strain was studied. There has been found a highly strong activity of *Peganum hermala* extracted in sodium phosphate buffer against *Staphylococcus aureus*. The least activity was found in *Feniculum vulgare* (Figure 1).

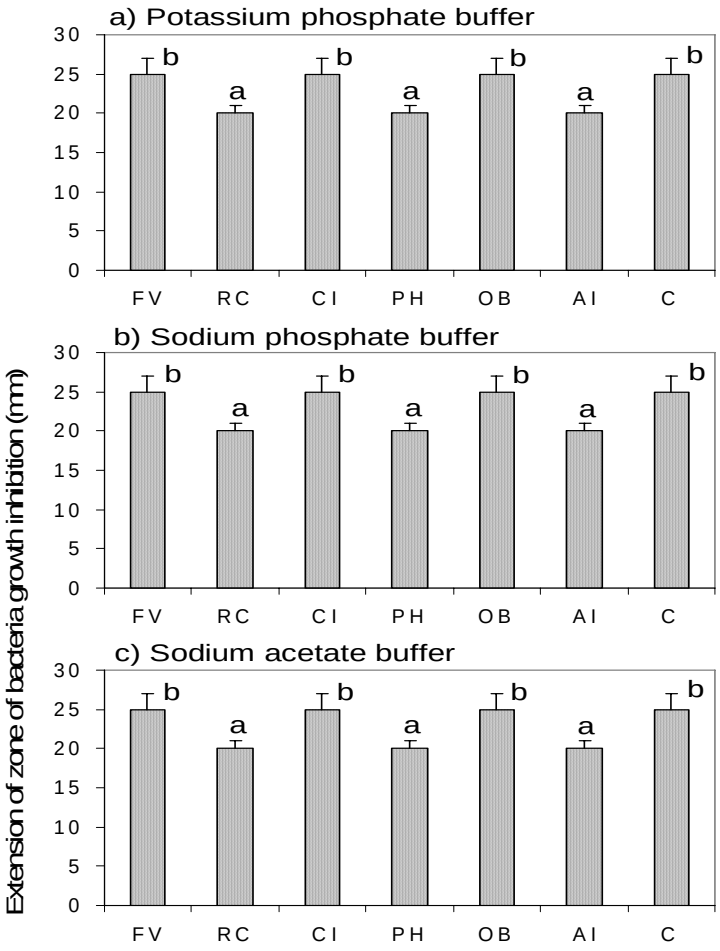

Figure-1: Extension of zone of bacteria growth inhibition around filter papers with proteins extracted by different buffers in mm.

Shown are the mean values of xx replicates ± standard deviation. Mean values followed by the same letter are not significantly different (Tukey's multiple comparison procedure; $P < 0.05$). FV: *Feniculum vulgare*; RC: *Ricinus communis*; C: Control.

As the seed extracts of *Peganum hermala* showed highly strong activity. So, from these results it is concluded that this plant is highly effective against the bacterial strains and we need to explore this plant for further use on a large scale. we conclude here from our study that *Pegnanum hermala* is very good candidate for the isolation of bioactive compound that play a vital role in the treatment of mastitis.

In this case, *Azadirachata indica* showed moderate activity against the strain. The least activity was found in *Feniculum vulgare* and *Ocimum basilicum* (Figure 1). Least MIC value was observed

in *Peganum hermala* being (3.82±0.23mg/mL±SD) and the highest value of the MIC was of *Cichorium intybus* (246.5±0.5mg/mL±SD) (Figure 2, Table 2).

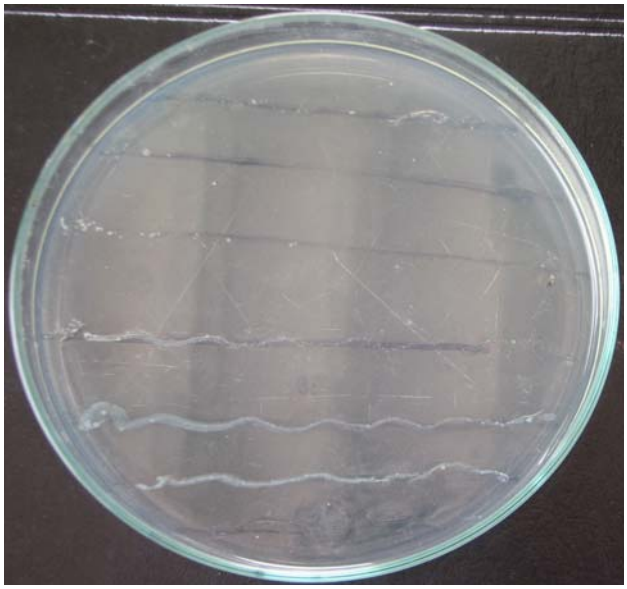

Figure 2. Minimum Inhibitory Concentration (MIC) of different medicinal plants extracted in potassium phosphate buffer against the selected bacterial strain *Staphylococcus aureus*.

Table 2. Minimum Inhibitory Concentration (MIC) of different medicinal plant extracts against the selected bacterial strain *Staphylococcus aureus 6736152.*

Sr. No	Medicinal Plants	MIC (mg/mL±SD)
1	*Feniculum vulgare*	124.64±0.17
2	*Peganum hermala*	16.45±0.21
3	*Ricinus communis*	15.29±0.04
4	*Ocimum basilicum*	58.75±0.25
5	*Cicharium intybus*	246.50±0.50
6	*Azadirachata indica*	3.83±0.22

From the data of MIC of seed extracts of the medicinal plants, it was concluded that *Peganum hermala* and *Azadirachata indica* were potent for detailed studies. These plants can be used to discover bioactive natural products that may serve for the development of new pharmaceuticals. Such screening of various natural organic compounds and identifying active agents is the need of the hour.

These findings support 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.

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