# *Peganum harmala* peptides (PhAMP) impede bacterial growth and biofilm formation in burn and surgical wound pathogens

**Rida Khalid[1], Qudsia Jaffar[2], Asima Tayyeb[1] and Uzma Qaisar[1*]**
[1]School of Biological Sciences, Quaide Azam Campus, University of the Punjab, Lahore Pakistan
[2]Biochemistry Department, University of Agriculture, Faisalabad, Pakistan

**Abstract**: Many clinical-pathogens have developed resistance against known antibiotics and there is an urgent need for the discovery of novel antibiotics. In this study, low molecular weight peptides were isolated from seeds/leaves of 20 medicinal plants and tested for their antibacterial activity against laboratory strains of *S.aureus* and *P.aeruginosa*. Peptides isolated from *Peganum harmala* (PhAMP) exhibited maximum activity against laboratory strains. As clinical-isolates are more virulent and resistant to antibiotics, we tested the potential of PhAMP on these bacterial strains isolated from infected wounds. Pathogens isolated from burn-wounds (*S. aureus, P. aeruginosa* and *K. pneumoniae*) and surgical-wounds (*P. aeruginosa* and *K. pneumoniae)* exhibited zones of inhibition against PhAMP when tested by disc diffusion method. Biofilm formation of wound pathogens in the presence/absence of PhAMP was analyzed to check its effect. Surgical-wound pathogens and *K. pneumoniae* from burn-wound showed significant reduction in biofilm formation and planktonic bacteria. While biofilms of *S. aureus* and *P. aeruginosa* from burn-wound showed resistance against PhAMP. An effective antibiotic treatment should not only inhibit but should also disrupt already developed biofilms. PhAMP was very effective in the disruption of developed biofilm of all pathogens after 36 hours. This data unravels the potential of PhAMP as a novel, natural antibiotic against clinical-pathogens.

**Keywords**: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, biofilm, planktonic cells

## INTRODUCTION

Our environment contains a huge number of microorganisms that can colonize epithelial and mucosal surfaces of multicellular organisms leading to severe damaging effects to host cells i.e. humans, animals or plants (Wilson, 2005). A variety of infectious diseases that can lead to unpredictable epidemics are the result of these diverse microorganisms (Morens *et al.*, 2004). Clinical nosocomial infections are the leading cause of morbidity and are much disastrous to human health (Nichols, 1991). Skin is the major barrier to pathogen entrance into the host cells and a wound disrupts this physical barrier. Thus, bacteria can easily breach into the host tissues from the damaged area (Giacometti *et al.*, 2000).

Microorganisms create a mesh of bacterial colonies named as biofilms and secrete extracellular polymeric substances. These bacterial cells are adherent to one another and also with a static surface (Hurlow *et al.*, 2015). Biofilms are extremely pathogenic and are notorious for their nosocomial infections as they are 1000 times more resistant than the normal strains. They are commonly produced in soft tissues, open wounds and infected immune compromised patients (Garrett *et al.*, 2008). Microbes attack and adhere to these open burns, surgical and diabetic wounds and develop biofilms there. These resistant biofilms are also resistant towards host immune system and many antibiotics that can lead to severe chronic effects (Jamal *et al.*, 2017).

There is an immense need for the pharmaceutical and scientific societies to discover new plant based antibiotics due to the emerging drug resistance (Savoia, 2012). A number of medicinal plants are being used traditionally due to the large scale implications in the treatment of pathogenic infections (Rios and Recio, 2005). Antimicrobial peptides isolated from the plant sources act as a part of host innate defense mechanism (Zasloff, 2002). These peptides have various modes of actions like disruption of bacterial cell membranes ultimately leading to the death of bacteria (Hancock and Rozek, 2002). Others include interaction with several metabolic components, proteins and virulence factors of these pathogens and inhibition of their formation by these peptides (Jenssen *et al.*, 2006). Moreover, these peptides can also target cytoplasmic components of bacteria (Iquebal and Rai, 2012). These peptides target the pathogens without causing much damage to the host cells.

In this study, low molecular weight antimicrobial peptides are isolated from the seeds and leaves of medicinal plants. These peptides are used to test the inhibitory effect on the bacterial growth of different pathogens isolated from burn and surgical wounds by various antimicrobial susceptibility assays. Further, these peptides are tested on the more resistant bacterial biofilms to have a clear understanding of their effect on the surgical and burn wound pathogens. The main aim of this study is to discover novel antimicrobial peptides from the plant sources that can act as a new class of antibiotic to treat nosocomial bacterial infections. It would be a major

**Corresponding author:* e-mail: uzma67@hotmail.com

breakthrough in the field of therapeutic development as limited work had been done so far on the plant peptides.

## MATERIALS AND METHODS

***Microbial strains and medicinal plants collection***

Laboratory strains of *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (MPAO1) were collected from Biochemistry Department, University of Agriculture, Faisalabad. Strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were isolated from burn wound samples collected from Burn Center, Jinnah Hospital, Lahore. Strains of *P. aeruginosa* and *K. pneumoniae* were collected from Microbiology Laboratory, Allama Iqbal Medical College, Lahore. All strains were tested for their purity using biochemical tests. All the experiments involving bacterial strains were performed in the biosafety cabinet according to the guidelines of bioethical committee of university of the Punjab, Lahore, Pakistan.

***Sample collection***

A total of 20 medicinal plants were screened for antimicrobial activity of the peptides isolated. The seeds of medicinal plants i.e. *Ricinus communis* (Castor bean), *Plantago ovata* (Ispaghul), *Cichorium intybus* (Cichory), *Foeniculum vulgare* (Fennel), *Silybum marianum* (Milk Thistle), *Brassica napus* (Canola), *Linum usitatissimum* (Flax), *Anethum graveolens* (Dill), *Carthamus tinctorius* (Safflower), *Lepidium sativum* (Water Cress), *Citrullus colocynthis* (Bitter Apple), *Momordica charantia* (Bitter Gourd), *Pisum sativum* (Pea) and *Peganum harmala* (Harmal) were obtained from Qarshi Herbarium Faisalabad. Fresh leaves i.e. *Mimosa pudica* (Touch-me-not), *Ocimum bacilicum* (Niaz Boo), *Psidium guajava* (Guava), *Croton tiglium* (Jamaal Gota), *Azadirachta indica* (Neem) and *Mentha spicata* (Mint) were obtained from Botanical Garden, University of the Punjab, Lahore.

***Extraction of antimicrobial peptides (AMPs)***

Extraction of peptides was done by using Sodium phosphate extraction buffer (10mM $Na_2HPO_4$, 15mM $NaH_2PO_4$,100mM KCl, 2mM EDTA, 1.5% PVPP, 1mM PMSF and 2mM thiourea) at pH 74 as mentioned previously (Jamil *et al.*, 2007, Cammue *et al.*, 1992). The seeds/leaves were ground to fine powder in liquid nitrogen using sterile mortar and pestle and mixed with chilled extraction buffer. Incubated the mixture at 4ºC for overnight with constant stirring. Centrifuged this extract twice at 7,000 rpm for 20 min at 4ºC. The supernatant was filtered using Whatman filter paper (pore size; 11 µm) at 4ºC and further passed through <10 kDa gel filtration columns (Amicon Ultra-15 Centrifugal Filter Unit, Merck Millipore). The eluted <10kDa sample was saturated with 80% ammonium sulphate and incubated overnight at 4ºC with constant stirring. Centrifuged this sample at 9,000 rpm for 15 min at 4ºC and collected the peptides pellet. The pellet was resuspended in autoclaved distilled water. Isolated samples were resolved on 12% SDS PAGE gel to check the quality and purity of peptides. The peptides were quantified using Bradford assay (Bradford, 1976).

***Disc diffusion assay***

To test the antibacterial activity, we used disc diffusion method (Bisignano *et al.*, 1999, Nostro *et al.*, 2000). Briefly, overnight LB cultures were added to molten LB agar at the ratio of 1:100 (v/v). Mixed well, poured in sterile petri plates and solidified. With the help of a sterile tweezers, filter paper discs (Whatman Grade AA DISCS 6mm, GE Healthcare Life Sciences) were carefully placed on prepared LB plates in Laminar Hood and 20µL of AMP's were loaded on the discs. Assay was performed in triplicates for each bacterial strain. These plates were incubated at 37ºC for 16 hrs. The AMPs having antimicrobial activity against any of the bacterial strain showed clear zone of inhibition around the discs. These zones were measured in millimeters (mm) using measuring scale. Distilled water was used as negative control while standard antibiotics (tetracycline, ampicillin and kanamycin) were used as positive control.

***Minimum inhibitory concentration (MIC)***

MIC is the lowest concentration of antimicrobial substance that completely inhibits visible bacterial growth. MIC of bacteria strains using peptides was calculated by broth dilution method (Wikler, 2009) in 24 well cell culture polystyrene plates after incubation at 37°C for 16 hours. The absorbance of these bacterial strains was taken at 600 nm by UV-Vis Spectrophotometer. Colony Forming Units (CFU's) of these bacterial strains were calculated by agar dilution method (Wikler, 2009).

***Biofilm formation and quantification***

Peptides from medicinal plants were tested for their effect on the biofilm formation of these bacterial strains. For this, biofilm assay was performed using 24 well cell culture polystyrene plates (Life Sciences). The overnight bacterial cultures were resuspended in LB broth to attain an approximate OD between 0.02-0.04 nm. The sterile plastic cover slips (Thermanox$^R$ Plastic Coverslips, 13 mm diameter) were aseptically transferred in 24 well cell culture plates with sterile tweezers. Peptides were added to inoculated media and poured in the wells. Covered with lid properly under sterile conditions and incubated for 16 hours at 37°C in shaking incubator. The growth of planktonic cells was observed at 600 nm after 16 hours of incubation.

Biofilms were gently washed with autoclaved distilled water to remove the planktonic cells and then stained with 1% crystal violet (1 mL per well) for 30 min. The stained biofilms were washed with autoclaved distilled water twice. 1 ml of absolute ethanol is poured in each well to

dissolve crystal violet absorbed by biofilms for 1-2 hours and OD is taken at 595 nm (Hammond *et al.*, 2010, Qaisar *et al.*, 2013). For qualitative analysis, crystal violet stained biofilms were visualized under light microscope (Nikon Eclipse TS100, Inverted Routine Microscope).

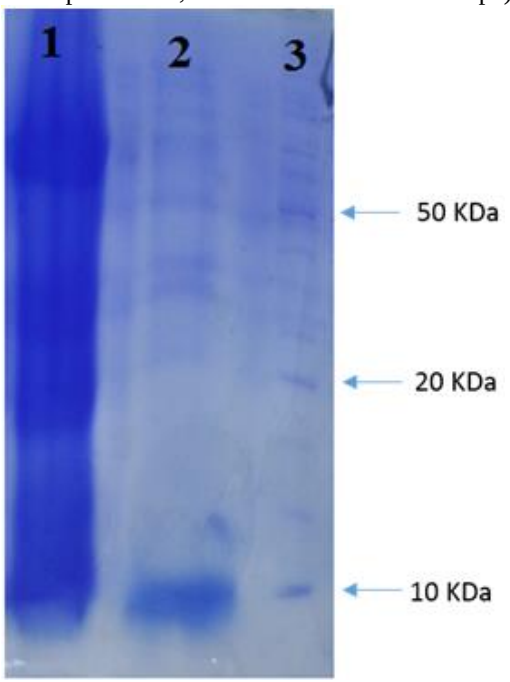


**Fig. 1**: SDS-PAGE gel showing proteins of *Pegnum harmala*, (1) partially purified total protein (2) peptides of less than 10KDa (3) protein ladder

***Biofilm disruption***

For biofilm disruption experiments, the biofilms were developed without addition of peptides. After 16 hours of development, biofilms were transferred in new sterile plate and fresh LB broth with or without peptides was added in each well. These plates were then incubated for 24, 30 and 45 hours at 37°C in shaking incubator. After reaching the required time point, biofilms were quantified by crystal violet assay (Friedman and Kolter, 2004). The growth of planktonic cells and the disruption of developed biofilms were observed at 600 nm and 595 nm respectively.

## RESULTS

***Antibacterial potential of medicinal plant peptides.***

Many plants produce small peptides as a part of defense mechanism against laboratory microorganisms. Medicinal plants are being used by humans since ancient times to treat various bacterial infections (Arora and Kaur, 1999). We isolated small peptides (fig. 1) from 20 medicinal plants (table 1) and tested their antimicrobial activity against laboratory bacterial strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. We used disc diffusion assay using 12.8 µg of peptides as described in the materials and methods section to measure the zones of inhibition exhibited by above bacterial strains and found that peptides from only few medicinal plants showed inhibitory effect against bacterial strains (table 1). *Pisum sativum*, *Psidium guajava* and *Pegnum harmala* exhibited antimicrobial effect on bacterial strains. *Peganum harmala* peptides (PhAMP) showed maximum zone of inhibition against laboratory *S. aureus* and *P. aeruginosa* strains (fig. 2 and table 1) and were selected for further studies.

***Antibacterial activity of Peganum harmala (PhAMP) against burn wound pathogens***

Burn wounds are prone to bacterial infections which is the major cause of morbidity and mortality in burn patients. We tested the anti-bacterial potential of PhAMP against burn wound pathogens. We isolated bacterial pathogen by taking swabs from burn wounds at Burn Center, Jinnah Hospital, Lahore. These bacterial isolates were identified by growing them on selective media and biochemical tests. Isolates were found to be *Klebsiella pneumoniae*, *P. aeruginosa* and *S. aureus*. We tested antibacterial activity of PhAMP against all burn wound pathogens by disc diffusion method. All three bacterial strains showed zones of inhibition (figs. 3A and 3B), *K. pneumoniae's* inhibitory zone had the diameter of 26.5 mm while this zone was 24.9 mm in *P. aeruginosa* and 31.6 mm in *S. aureus* (figs. 3A and 3B). PhAMP cleared burn wound pathogens significantly although these zones were smaller than laboratory strains (fig. 2) indicating that burn wound isolates are more resistant as compared to laboratory strains.

We checked the growth of burn wound pathogens in liquid LB medium containing different concentrations of PhAMP and found that *K. pneumoniae*, *P. aeruginosa* and *S. aureus* showed inhibition of bacterial growth with increasing concentrations of PhAMP. *P. aeruginosa* proved to be most sensitive and bacterial growth significantly reduced at only 3.7µg/mL of PhAMP. *S. aureus* which is gram positive bacterium, was more resistant than all other gram negative strains under investigation. *S. aureus* showed significant inhibition in growth at 18.5µg/mL of PhAMP (fig. 3C).

***Antibacterial activity of PhAMP against surgical wound pathogens***

Surgical wounds are prone to nosocomial infections which can be lethal if not treated with appropriate antibiotics. So it was necessary to test the effect of PhAMP on surgical wound pathogens. Bacterial pathogens harboring surgical wounds were collected from Microbiology Department at Allama Iqbal Medical College, Lahore. These pathogens were identified as *K. pneumoniae* and *P. aeruginosa*. Both bacterial strains showed sensitivity to PhAMP and significant clearing zones were observed (figs. 4A and 4B). PhAMP cleared 26.46 mm of zone of *K. pneumoniae* and 29.23mm of *P.*

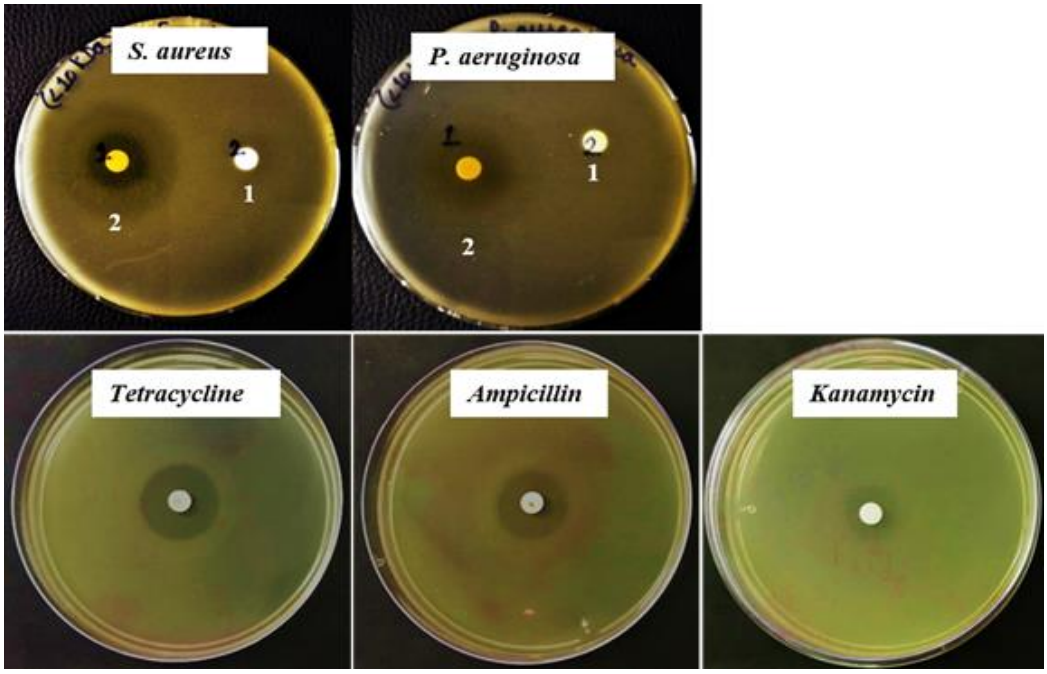


**Fig. 2**: Zone of inhibition of PhAMP (12.8µg) against laboratory strains of *S. aureus* and *P. aeruginosa* (Top row). 1 indicates water (negative control) while 2 indicated PhAMP. Standard antibiotics (12.8 µg each) were used as positive control (Bottom row).

*aeruginosa*. These zones were smaller than the ones observed in laboratory strains. (fig. 2).

In order to find minimum inhibitory concentrations of PhAMP, various concentrations (3.7µg, 7.4µg, 11.1µg, 14.8µg and 18.5µg) were used and their effect on pathogen growth was checked. *K. pneumoniae* and *P. aeruginosa* showed growth inhibition at 18.5 µg. With the increasing concentration of PhAMP, growth of pathogen reduced in both strains (fig. 4C). Growth inhibition was more pronounced in *K. pneumoniae* as compared to *P. aeruginosa* (fig. 4C).

***Inhibitory effect of PhAMP against pathogen biofilm formation***

We grew biofilms of different pathogens either in LB or LB supplemented with PhAMP and measured biofilm development by crystal violet assay while planktonic cell growth was also measured by taking $OD_{600}$ of each sample. Biofilms stained with crystal violet were visualized under light microscope and results are shown in fig. 6.

In case of burn wound pathogens, all pathogens exhibited significant decrease in the planktonic bacteria. This decrease was 3.3 fold in *P. aeruginosa*, 1.8 fold in *K. pneumoniae* and 1.2 fold in *S. aureus*. In case of biofilm formation, *P. aeruginosa* and *S. aureus* exhibited an increase in biofilm formation in the presence of PhAMP which indicates a shift from planktonic mode to more resistant biofilm mode. *K. pneumoniae* showed sensitivity to PhAMP in both planktonic and biofilm mode of growth and its growth significantly decreased (fig. 5A and fig. 6).

In surgical wound pathogens, both planktonic cell growth and biofilm development were significantly inhibited in both pathogens (fig. 5B). Similar results were obtained when biofilms were observed under light microscope (fig. 6). These results show that PhAMP can be applied as an antibacterial drug on surgical wounds to inhibit bacterial growth either in planktonic or biofilm mode of life.

***PhAMP induced disruption of biofilms produced by wound pathogens***

Biofilms develop on biological and non-biological surfaces which not only provide antibiotic-resistant shelter to bacteria but also serve as reservoir for spreading infection when situation is favorable. It is important to not only inhibit biofilm formation but also disrupt already developed biofilms. We allowed wound pathogens to develop biofilms on plastic discs for 16 hours and then treated with PhAMP + LB or LB to see whether developed biofilms are disrupted by PhAMP or not after 24, 30 and 45 hours of treatment.

Our results showed that In burn wound pathogens, biofilm showed resistance to PhAMP at 24 hours and showed enhancement in biofilm production while after 30 hours of treatment this resistance weakened and significant reduction in biofilm was observed. After 45 hours, there was disruption of biofilm in the control and treated samples due to decrease in the nutrients in the surrounding media (fig. 7A).

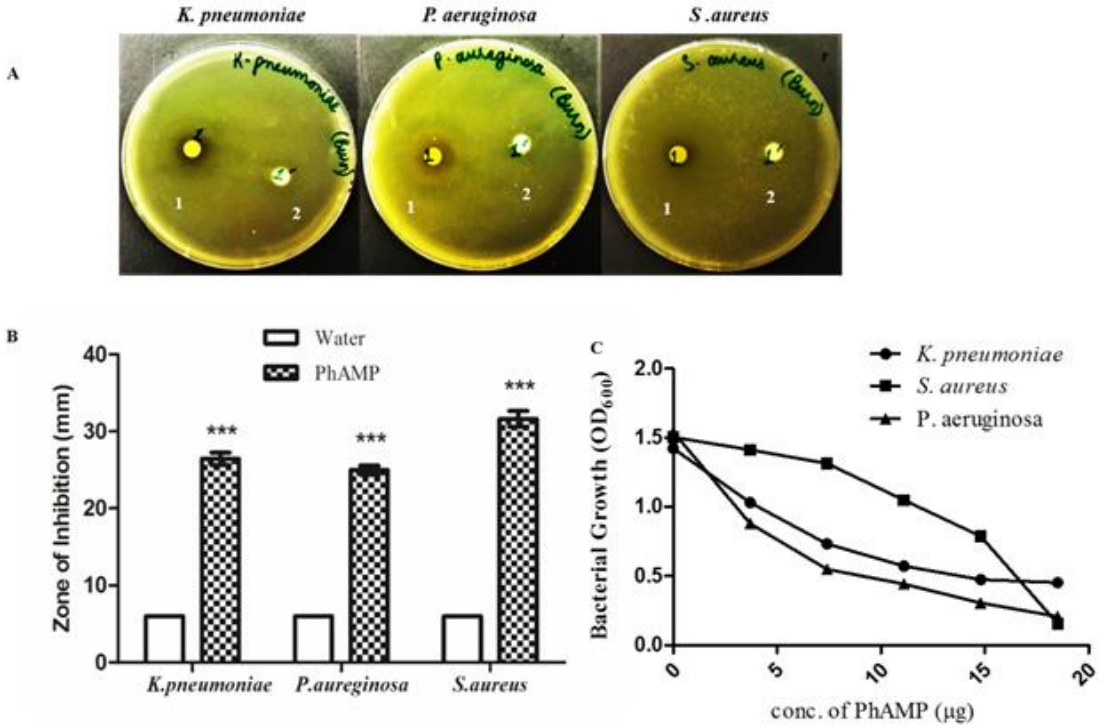


**Fig. 3**: Antibacterial activity of PhAMP against burn wound pathogens i.e *K. pneumoniae, P. aeruginosa* and *S. aureus* (A) Zones of inhibition of PhAMP. 1 indicates buffer while 2 indicates PhAMP. (B) Bar graphs of antibacterial activity of PhAMP and water. *** indicate p. value >0.001. Error bars represent standard deviation. (C) Growth of bacteria at different concentrations of PhAMP in LB broth.

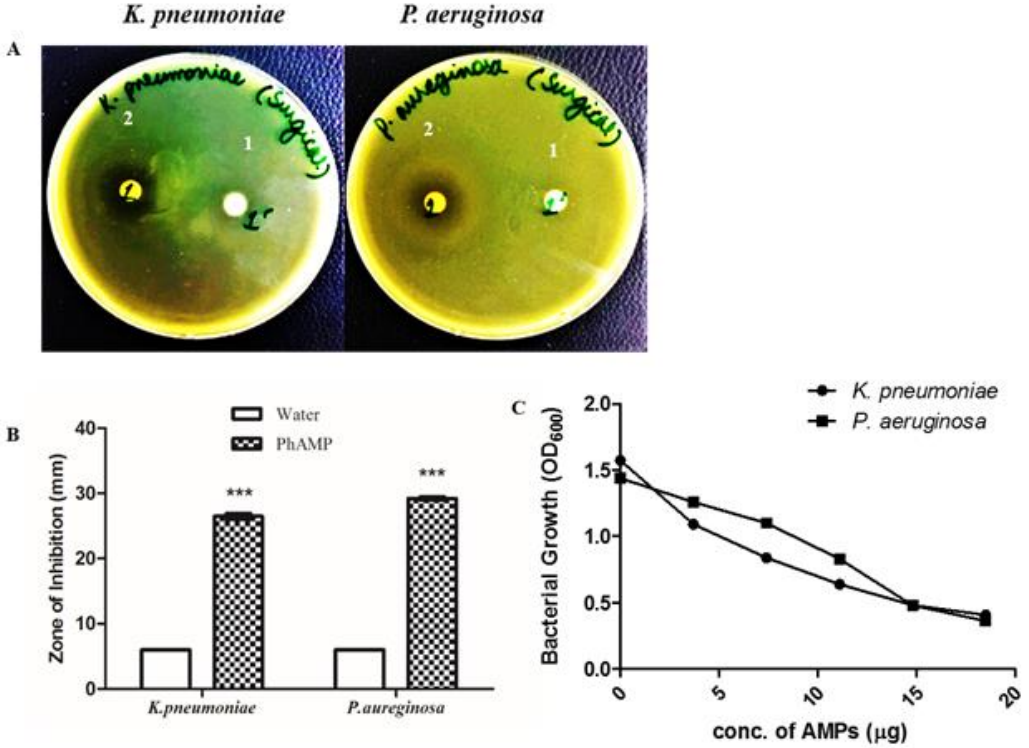


**Fig. 4**: Antibacterial activity of PhAMP against surgical wound pathogens i.e *K. pneumoniae* and *P. aeruginosa.* (A) Zone of inhibition of PhAMP. 1 indicates buffer while 2 indicates PhAMP (B) Bar graphs of antibacterial activity of PhAMP and water. *** indicate p. value >0.001. Error bars represent standard deviation. (C) Growth of bacteria at different concentrations of PhAMP in LB broth.

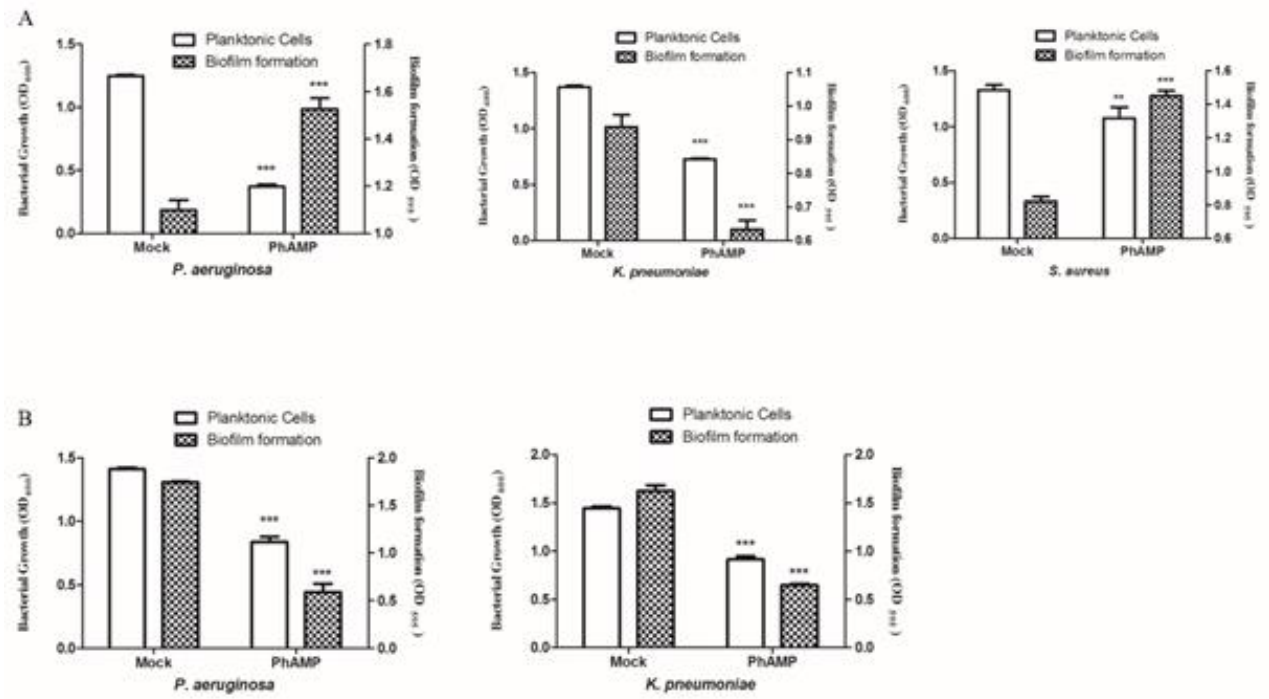


**Fig. 5**: Effect of PhAMP on biofilms formation and planktonic cell growth of burn wound pathogens i.e *P. aeruginosa*, *K. pneumoniae* and S. aureus (A). Surgical wound pathogens i.e *P. aeruginosa* and *K. pneumoniae*. Overnight cultures of each pathogen were sub-cultured either in LB or LB+PhAMP (18.5 µg) as described in the material and methods section. After 16 hrs of growth, planktonic cell growth was measured by taking $OD_{600}$ and biofilm formation was measured by crystal violet assay. Error bars represent the standard deviation (SD) between replicates. *** indicate p.value > 0.001 and ** indicate p.value > 0.01.

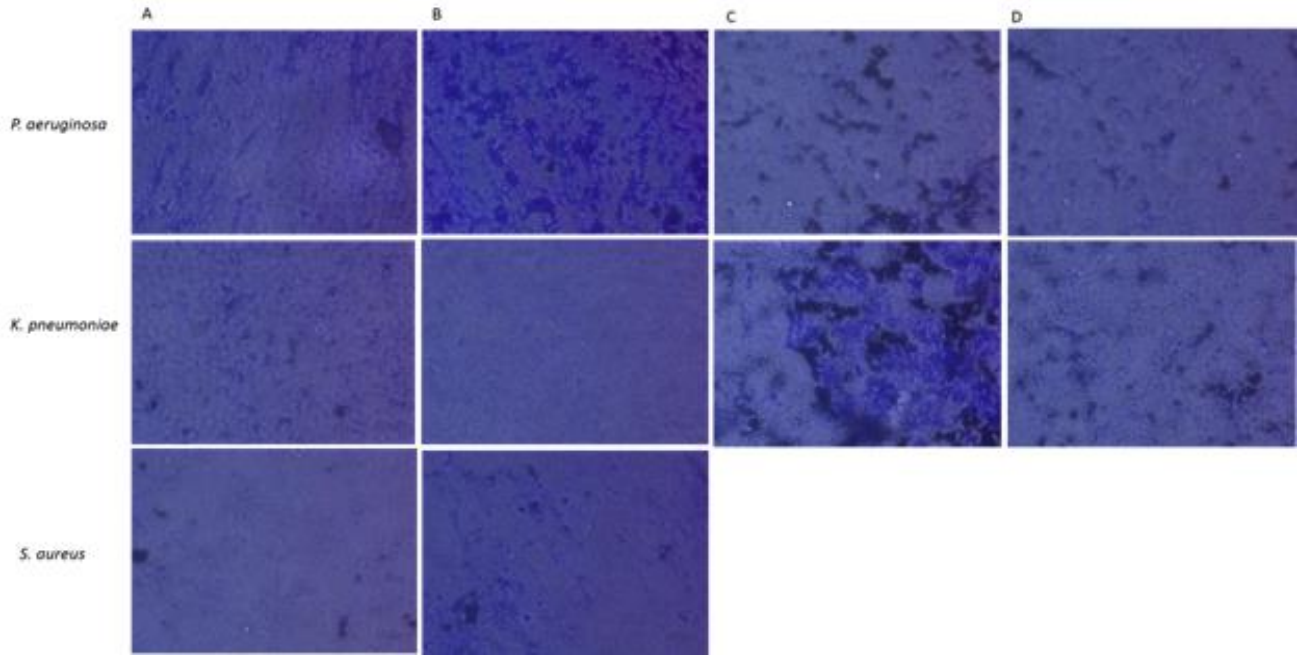


**Fig. 6**: Light microscopy analysis of biofilm formation of burn and surgical wound pathogens on treatment with PhAMP. Burn wound pathogens without treatment (A), Burn wound pathogens on treatment with PhAMP (B), Surgical wound pathogens without treatment (C) and Surgical wound pathogens on treatment with PhAMP (D)

**Table 1**: Antimicrobial activity of medicinal plant peptides against laboratory bacterial strains

| Sr. # | Botanical Names | Common Names | Parts used | *S. aureus*[1] | *S. aureus*[2] | *P. aeruginosa* |
|---|---|---|---|---|---|---|
| 1 | *Ricinus communis* | Castor Bean | Seeds | + | - | - |
| 2 | *Plantago ovate* | Ispaghul | Seeds | + | - | - |
| 3 | *Cichorium intybus* | Chicory | Seeds | - | - | - |
| 4 | *Foeniculum vulgare* | Fennel | Seeds | - | - | - |
| 5 | *Silybum marianum* | Milk Thistle | Seeds | - | - | - |
| 6 | *Brassica napus* | Canola | Seeds | - | + | - |
| 7 | *Linum usitatissimum* | Flax | Seeds | - | - | - |
| 8 | *Anethum graveolens* | Dill | Seeds | - | - | - |
| 9 | *Carthamus tinctorius* | Safflower | Seeds | - | - | - |
| 10 | *Lepidium sativum* | Water Cress | Seeds | - | - | - |
| 11 | *Citrullus colocynthis* | Bitter Apple | Seeds | + | - | - |
| 12 | *Momordica charantia* | Bitter Gourd | Seeds | + | - | - |
| 13 | *Pisum sativum* | Pea | Seeds | ++ | ++ | + |
| 14 | *Peganum harmala* | Harmal | Seeds | ++ | +++ | - |
| 15 | *Mimosa pudica* | Touch-me-not | Leaves | - | - | - |
| 16 | *Ocimum bacilicum* | Niaz Boo | Leaves | - | - | - |
| 17 | *Psidium guajava* | Guava | Leaves | + | + | + |
| 18 | *Croton tiglium* | Jamaal Gota | Leaves | - | - | - |
| 19 | *Azadirachta indica* | Neem | Leaves | - | - | - |
| 20 | *Mentha spicata* | Mint | Leaves | - | - | - |

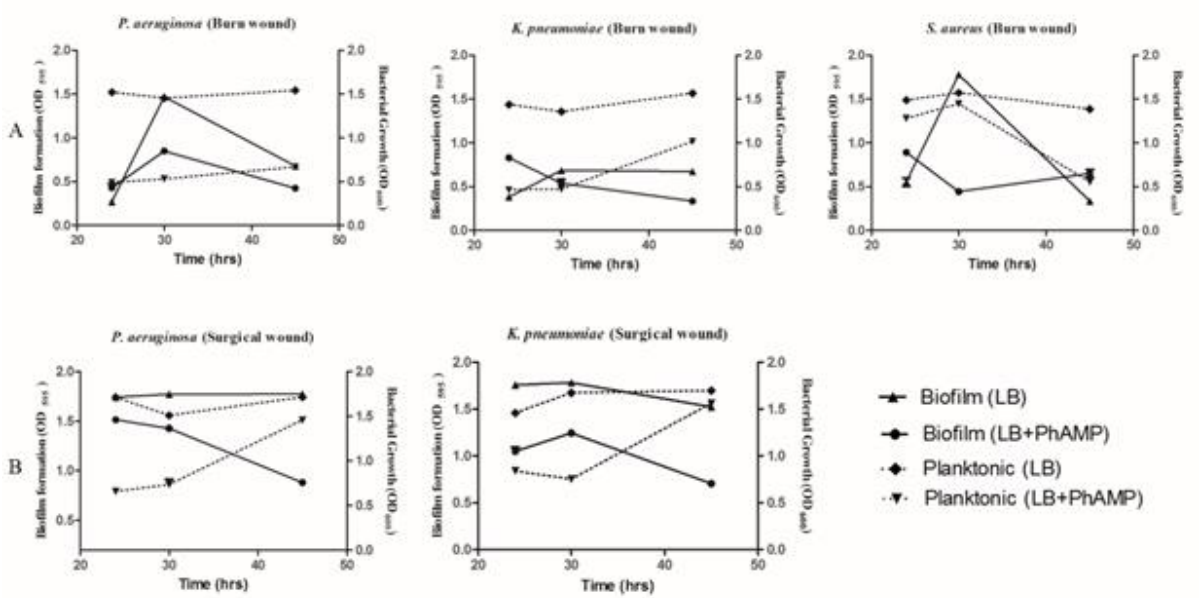


**Fig. 7**: PhAMP induced disruption of developed biofilms of wound pathogens. (A) Burn wound pathogens i.e. *P. aeruginosa, K. pneumoniae* and *S. aureus.* (B) Surgical wound pathogens i.e. *P. aeruginosa* and *K. pneumoniae*. Biofilms were developed for 16 hrs and treated with PhAMP. Disruption was observed after 24, 30 and 45 hrs of incubation. Planktonic cells were measured by taking $OD_{600}$ and disruption of biofilms was measured by crystal violet assay.

Biofilms of surgical wound pathogens were disrupted after 24 hours of treatment with PhAMP. This disruption was more pronounced at later time points of 30 and 45 hours (fig. 7B). After disruption of biofilms, bacteria were

exposed and killed by the activity of PhAMP as exhibited by the measurement of planktonic cell density. These results showed that PhAMP is very effective in clearing bacterial infections from surgical wounds by disrupting the developed biofilms.

## DISCUSSION

Antibiotic resistance is one of major challenges in biopharmaceutical research as microbial strains notorious for a number of infectious diseases have developed resistance against known antibiotics (Goossens *et al.*, 2005). Clinical strains are much more resistant than normal laboratory microorganisms. The infections caused by them are also difficult to eradicate (Lipsitch *et al.*, 2000). Now-a-days, research is going on to develop more economical ways to develop antibiotics with less side effects and more antimicrobial benefits (Coates and Hu, 2007). This study was carried out to test the potential of naturally producing small plant peptides to treat bacterial infections. A variety of medicinal plants were collected and antimicrobial proteins were extracted from the seeds and leaves by using sodium phosphate buffer (table 1). These peptides are then used to test antimicrobial potential on different laboratory, burn and surgical wound pathogens.

Out of twenty medicinal plants, antimicrobial peptides isolated from *Peganum harmala* (PhAMP) showed maximum antimicrobial potential (table 1). Modern phytotherapy have revealed the importance of *Peganum harmala* in the treatment of gastrointestinal, cardiovascular and neurologic diseases (Farzaei *et al.*, 2013). An alkaloid called 'Harmaline' isolated from *P. harmala* is known to be a pharmacologically active compound (Farouk *et al.*, 2008) which inhibits planktonic growth and biofilm formation of *S. aureus* (Xing *et al.*, 2012). Methanolic extract of roots and seeds of *Peganum harmala* were reported to have antibacterial potential. In this study, we focused mainly on small peptides (<10 KDa) isolated from this plant as low molecular weight peptides in other plants have shown their antimicrobial potential (Shao *et al.*, 1999). According to our knowledge no work was found for antimicrobial peptides isolated from *Peganum harmala* to have inhibitory effect on the biofilm formation and disruption.

Minimum inhibitory concentration (MIC) of the methanolic extracts from seeds and root of *Peganum harmala* against methicillin resistant *S. aureus* was 625 µg/mL (Darabpour *et al.*, 2011) while PhAMP have shown to have MIC value of 18.5µg/ml against all bacterial pathogens indicating that peptides isolated from the seeds of *Peganum harmala* are much more effective for resistant pathogens. Moreover, (Darabpour *et al.*, 2011) found that methanolic extracts were more effective against gram positive bacteria than gram negative. PhAMP in our study have shown antimicrobial activity against both gram negative and gram positive burn and surgical wound pathogens (figs. 3 and 4).

Antibiotic resistance was enhanced in all clinical isolates (figs. 3 and 4) as compared to corresponding laboratory strains of bacteria (fig. 2) confirming that clinical isolates develop antibiotic resistance (Savoia, 2012) and need for novel antibiotics especially consisting of bioactive molecules. In our results, surgical wound pathogens were less resistant than burn wound pathogens in both biofilm and planktonic mode of life (figs. 5 and 6). Biofilms of *P. aeruginosa* and *S. aureus* from burn wounds showed enhanced biofilm development on addition of PhAMP at MIC (figs. 5 and 6). However, at higher concentration of PhAMP, biofilm inhibition was observed.

Biofilm formation is one of the survival mechanisms of pathogenic bacteria against antimicrobial treatments and host immune response. Biofilms are much more resistant to antibiotics so it was important to test the effect of PhAMP on bacterial biofilm formation. In our study, biofilm disruption by PhAMP in burn wound pathogens and surgical wound pathogens was analyzed at 24 hours, 30 hours and 45 hours of treatment with PhAMP. In all pathogens maximum disruption of biofilms on application of PhAmP was observed at 30 hours as compared to control (fig. 7). At 45 hours, disruption of biofilm in control samples was also observed which could be due to depletion of nutrients in the growth medium with the passage of time.

## CONCLUSION

The research work presented here elaborates that antimicrobial peptides isolated from the seeds of *Peganum harmala* (PhAMP) exhibit excellent antimicrobial activity against the most resistant nosocomial burn and surgical wound pathogens. These peptides can impede bacterial growth as well as biofilm formation. Moreover, bacterial biofilms that are already developed can be disrupted by these antimicrobial peptides. This work showed the importance of development of novel and effective antimicrobial compounds from economical natural plant sources. Novel antimicrobial peptides can be a breakthrough in drug discovery as several resistant bacterial pathogens showed sensitivity against these peptides. Further research can be done to elaborate the mechanism lying behind the antimicrobial potential against these pathogens.

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