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Research Article

Isolation and Characterization of Peptides from *Momordica Charantia* L. and Their Antimicrobial Assay against Bacterial Isolates of Diabetic Foot Ulcer

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Abstract

Antimicrobial peptides (AMPs) are important elements of defense system in living organisms. They are tiny, cationic, amphipathic and highly efficient. AMPs have been divided into several groups based on their size, length and structure and play vital role in bacterial, viral and fungal resistance. In diabetic patients, wound healing is a complicated and major issue because once a wound has developed, it takes a long time to recover and therapies that are used in healing are expensive. Ethno medicinal plants possess small peptides (SP) that hinder biofilm production, check bacterial growth and support metalloproteinase activity for wound healing. Therefore, by eliminating biofilms these plants pave the drugs into wound wells thereby inhibiting bacterial moieties. The present study was carried out to isolate and characterize peptides having antibacterial activity from fruits of bitter melon (*Momordica charantia* L.). Crude seed extract of was prepared in Phosphate Buffer Saline (PBS) and antibacterial activity was checked on Luria Bertani (LB) broth agar plates against several bacteria such as *Escherichia coli, Staphylococcus aureus*, *Klebsiella pneumonia, Pseudomonas aeruginosa and Enterococcus facialis*. Seed extract was used for peptide(s) precipitation with different percentage of ammonium sulfate solution, homogeneity and molecular mass of protein fractions was determined by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS–PAGE). Purification of isolated protein/peptide was done by Gel Filtration Chromatography (GFC) using Sephadex G-50.

Keywords: *Antimicrobial peptide, Momordica charantia L, Escherichia coli, Staphylococcus aureus, Diabetic Foot Ulcer, Wound.*

1. Introduction

Patients with diabetes experience multiple pathological consequences in their foot such as ulceration, infection and/or destruction of deep tissues associated with neurologic abnormalities. They may also experience varying degrees of peripheral vascular disease and/or metabolic complications that could lead to in the lower limb gangrene and eventual which amputation.

Biofilm formation around the wound is the major problem in Diabetic Foot Ulcers (DFU). Reports reveal the presence of biofilms in at least 60 percent of chronic wounds (Malone *et al.*, 2017). 59 to 85% of diabetic foot ulcerations (DFUs) are polymicrobial due to the ability of various bacteria to form their microcolonies within the biofilm (Gompelman *et al.*, 2016). Some of these bacteria are *Staphylococcus*, *Streptococcus*, *Proteobacteria*, *Pseudomonas* etc. They play prominent a role in Diabetic foot ulcer (DFU). *S. aureus* has been reported in up to 43% of infected foot ulcers.

In the majority of living animals, antimicrobial peptides (AMPs) are evolutionarily conserved components of the innate immune response. They are found in many kinds of organisms, from prokaryotes to humans (Andrea *et al.*, 2007, Atousa *et al.*,

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2012). Cecropin, Dermaseptins, Magainin, Opistoporin, Cathelicidin, Proline rich, Glycine/arginine rich peptides, Brevinin peptides, Tachyplesin, Alpha defensins, Beta defensins, defensins, and Insect defensins are some of the most commonly reported AMPs (Wojciech, 2005). Few antimicrobial peptides have been identified from a wide variety of plant species' roots, seeds, flowers, stems, fruits and leaves and have proven their activity against pathogenic organisms such as viruses, bacteria, fungi, protozoa and parasites. Different types of AMPs have been found in plants, including Thionins, Defensins, Lipid transfer proteins, Puroindolines and Snakins. Immense necessity of treating drug-resistant bacteria with new generations of antibiotics, antimicrobial peptides are gaining greater attention (Hancock and Sahl, 2006).

Antimicrobial peptides (AMPs) are widely distributed in nature and play an essential role in the non-specific host defense system in addition to chemical substances. Initially, peptides from frog skin and insect lymph are shown to destroy *in vitro* bacterias. More than 800 AMPs have now been identified from insects, plants, amphibian, and mammals (Rydlo *et al.,* 2006).

According to peptide functions and structural similarity, they are divided into groups and families. Defensins, lipid transfer proteins (LPTs), lectins, pathogenesis-related proteins, chitin-binding proteins, ribosome-inactivating proteins, and digestive enzyme inhibitors are some of the most important proteinous molecules.

The major AMPs reported from plants ranged in size from 5 to 13 kDa. Even under harsh conditions, tiny AMPs have been found to have good antibacterial action.

Medicinal plants are known to have immuno-modulatory characteristics, and these plants are used to treat a variety of disorders involving the host defense mechanism (Arora *et al.,* 2021). Peptides are short amino acid (AA) sequences. They are usually <50 AA in length and are often stabilized by disulfide bonds (Hayashi *et al.,* 2012). They are designed to bind and modify a protein interaction of interest utilizing rational methods with high specificity. Therapeutic peptides have several key benefits over proteins and antibodies, including their tiny size, ease of synthesis, and ability to penetrate cell membranes. They are physiologically and chemically diverse with great efficiency, specificity affinity and have minimal drug-drug interaction. They don't accumulate in specific organs (such as the kidney or liver), which further help to reduce their toxic effects. They're also easier to synthesize and modify than recombinant antibodies or proteins (Boohaker, 2012), and they are less immunogenic (Cicero, 2017).

The present study aimed to extract, isolate and characterization of small peptides the seeds of *Momordica charantia* and evaluate their antimicrobial activity against some bacterial isolates of DFU like *Staphylococcus aureus*, *Escherichia coli, Klebsiella pneumoniae, Enterococcus facialis and Pseudomonas aeruginosa.*

2. Materials and Methods

2a. Collection of Plant Material – Plant specimens of *Momordica charantia* Linn. (Cucurbitaceae) were collected from the various localities' of the Udaipur (Rajasthan) region (24˚34'16.21"N and 73˚41'30.59"E). Herbarium sheets (Accession No BOT/201920/C/MC/347) of the specimens were prepared according to IRBN for the validation and authentication of plants; respective herbarium sheets were assigned taxonomical affiliations by the Department of Botany, B N University and were deposited for future reference.

2b. Preparation and Storage of Plant Samples - Test samples for antimicrobial activity of *M. charantia* small peptides were prepared by washing and sun drying the collected seeds. Subsequently, they were ground to fine powder, sieved and stored in airtight container in a refrigerator at 4^{o}C.

2c. Procurement of Bacterial Cultures - Bacterial cultures of *Staphylococcus aureus* (MCC 2043-T), *Pseudomonas aeruginosa* (MCC

2080) *Enterococcus faecalis* (MCC 2409), *Escherichia coli* (MCC 3671) and *Klebsiella pneumonia* (2451) were obtained from National Centre for Microbial Resource Pune. All cultures were sub-cultured at regular intervals on nutrient agar and stored at 4° C as well as at -20°C by making their suspension in 10% glycerol.

2d. Protein extraction from *M charantia* seed and their partial purification

Protein extraction from the seed was carried out by the method described by Rehman and Khanum (2011) with partial modifications. 10 g sample powder was blended with 100 ml phosphate buffer saline (pH 7.4) in an electric blender and homogenates were frozen and thawed thrice followed by centrifugation at 10,000 rpm/ 20 min/ 4˚C. This supernatant after treatment was used to evaluate antibacterial activity. Further supernatant was precipitated with different volumes of ammonium sulfate solution and the respective solution was centrifuged at 10,000 rpm/ 30 min/ 4˚C and pellets were dissolved in 1 ml of deionized water. The solubilized ammonium sulfate precipitate and supernatant were dialyzed at MWCO 3500 Da against distilled water and protein concentration was procured in crude extract, dissolved ammonium sulfate precipitate and supernatant.

2e. Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) SDS-PAGE

The dialyzed protein samples of both solubilized ammonium sulfate precipitates and supernatant were first used for SDS-PAGE analysis. Tricine {N-[2-Hydroxy-1, 1-bis (hydroxymethyl) ethyl] glycine} - SDS-PAGE was used for the separation of protein which are less than 30kDa. The protein pellets and supernatant were electrophoresed on 16% Tricine SDS-PAGE gel (Schagger, 2006; Jiang *et al*., 2016).

2f. Gel filtration chromatography

The protein pellets obtained from SDS-PAGE were purified through gel filtration chromatography. In gel filtration chromatography Sephadex G-50 column was used. The Sephadex G-50 spherical beads were allowed for swollen in buffer overnight. This chromatography buffer was prepared by mixing tris Cl 50mM and NaCl 50Mm in 1:1 ratio. The buffer was prepared in milliQ water, sterilized by autoclave and stored at 4°C till further use. The swollen Sephadex gel beads were used to pack the chromatography column (1.5×50cm), while packing the column Sephadex G-50 bead solution was added along the sides of the column to avoid the formation of air bubbles because air bubble interrupts the flow rate. The packed column was allowed to set and washed the column thrice with the same buffer. Now the protein sample added and allowed to move through the column. The flow rate was maintained under gravitation force during the separation process. The fractions of 1ml were collected and stored at 4°C. The optical density of fractions was calculated at 280nm.

2g. Reverse Phase High Performance Liquid Chromatography (RP-HPLC):

The Reverse Phase High Performance Liquid chromatographic (RP-HPLC) started with equilibrating the C18 silica column with solvent A which was prepared by mixing 999 ml Milli Q and 1 ml Triflouroacetic acid (TFA). It was sterilized using vacuum pump with 0.22µm pore size filter. The sample was applied to the chromatography column. The column was washed with the above prepared solvent A so that unbound molecules were removed. The gradual decrease in solvent A polarity was obtained by increasing the linear gradient from 100% solvent A to 70% solvent B (solvent B was prepared by mixing 999 ml Acetonitrile (ACN) which was of HPLC grade and 1ml Triflouroacetic acid) for 30 minutes at a flow rate 0.5ml/min The bound protein/peptide desorbed from the matrix according to their individual polarity. The absorbance of protein/ peptide was recorded at 214nm and 220nm. The single peptide/ protein were showed as separate peaks in graph. The separated peaks were collected manually from the HPLC unit. The collected peaks were concentrated using vaccum evapotator.

2h. Antibacterial assay:

The antimicrobial activity of the solubilized ammonium sulfate precipitates, supernatant of ammonium sulfate precipitates and fractions of gel chromatography was determined using the disc diffusion method (Bauer *et al.,* 1966). Sterile whatman filter paper discs of 6 mm in diameter were soaked in a 20 µg/ 30 µl test sample and placed on the surface of the agar plate. Chloramphenicol was used as a control. These plates were incubated overnight at 37°C. The results expressed as the mean ± SEM, indicate the standard deviation of the triplicate incubations in millimetre (mm). Excel statistical software was used to analyze the data (Borchardt *et al.,* 2008).

Macro dilution assay was performed to establish minimum inhibition concentration (MIC) values of isolated peptides against all bacterial cultures as described by Wang, 2022. The 108 CFU/ml test cultures were inoculated into LB broth containing 0-200 µg/ml antimicrobial protein preparation.

2i. Statistical analysis

All the experiments were carried out in triplicate thrice (n=3). The statistical analysis of the data was carried out by analysis of the variance (ANOVA). Results were considered significant when $p < 0.05$.

3. Results and Discussion

3a. SDS-PAGE Analysis

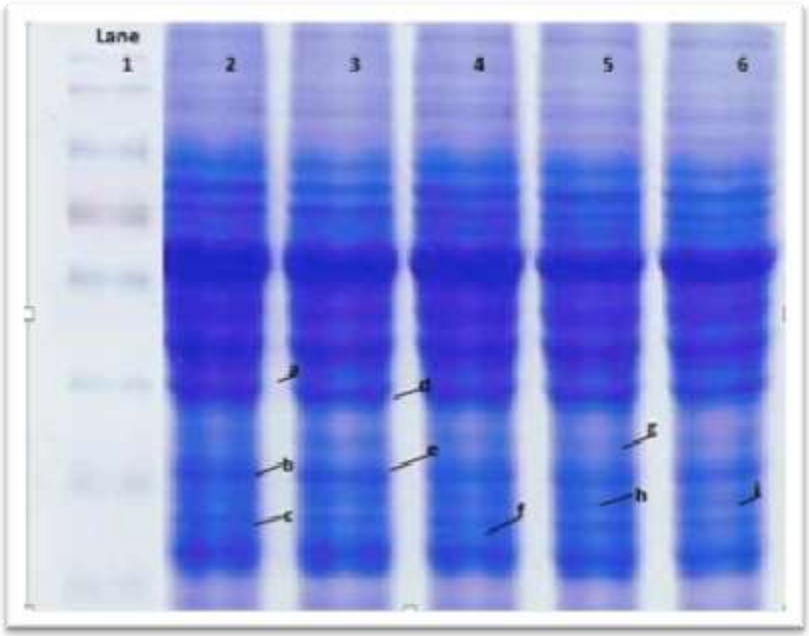


Figure: 3.1 SDS-PAGE analysis of different percentage of precipitated protein pellets of *Momordica charantia* seed extract. Lane 1: Supernatant; Lane 2: (a) 26.12 KDa, (b) 23.31 KDa, (C) 21.38 KDa in 90% protein pellet; Lane 3: (d) 25.07 KDa, (e) 22.32 KDa in 50% protein pellet; Lane 4: (f) 16.98 KDa in 25% protein pellet; Lane 5: (g) 23.21 KDa, (h) 17.28 KDa; Lane 6: (i) 16.63KDa in 75% protein pellet.

SDS-PAGE analysis was performed to ensure the protein integrity of *Momordica charantia* seed extract. Figure 3.1 displays a chromatogram depicting the elution profile of various proteins. It showed bands in a range of 16.63 KDa to 26.12 KDa. Protein extracts from *M.charantia* contained both major and minor proteins. In some previous studies the major proteins of *M.charantia* were identified to be of 10 KDa and 23 KDa (Poovitha and Parani 2020).

Momordica dioica seed extract SDS-PAGE analysis involving different percentages of precipitated protein pellets is displayed in Figure 3.1. The proteins bands in the

chromatogram range from 11.28 KDa to 25.12 KDa.

3b. Gel filteration chromatography

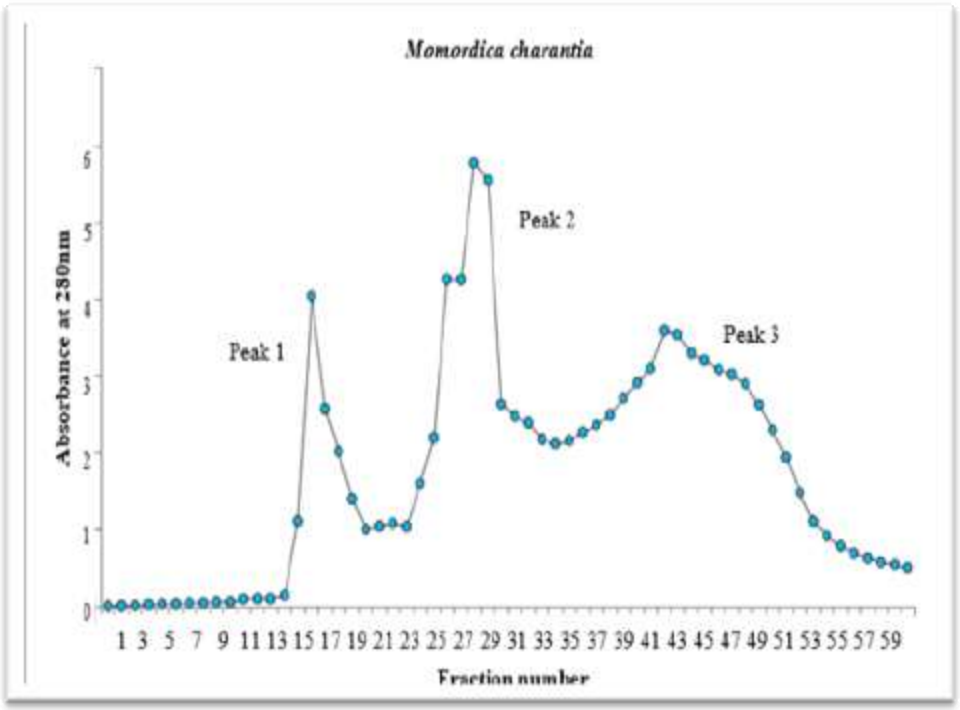


Figure 3.2: Elution profile of *Momordica charantia* protein pellet from gel filtration chromatography

Figure 3.2 exhibits the elution curve of a protein pellet from *Momordica charantia* using gel filtration chromatography. The peaks of each protein fraction were observed at 280 nm. The chromatogram shows three peaks. Peak 1 displays the proportion in the range of 15-21. Peak 2 shows the fraction number in the range of 25-29, whereas Peak 3 shows the fraction number in the range of 41-49. According to the yield, peak 2 of *Momordica charantia* has a greater peptide concentration than peak 1 and 3.

3c RP-HPLC (Reverse Phase- High Performance Liquid Chromatography) for characteristaion

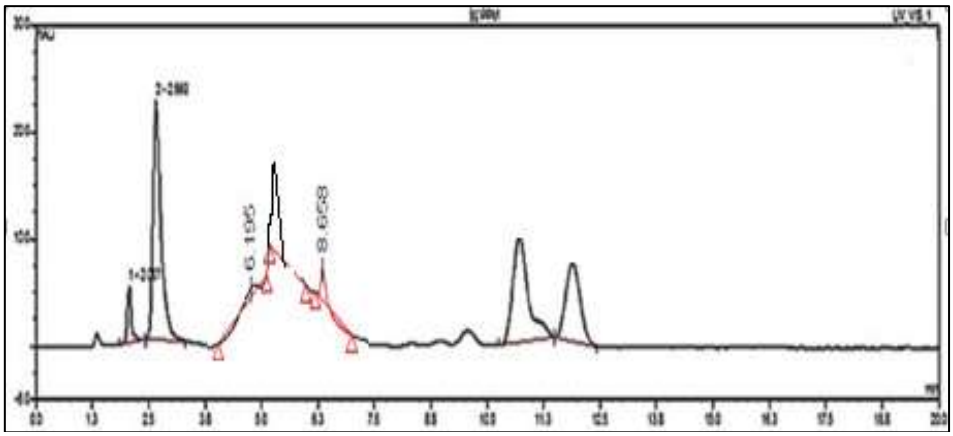


Figure 3.3: Reverse Phase Chromatogram of *Momordica charantia* protein pellet

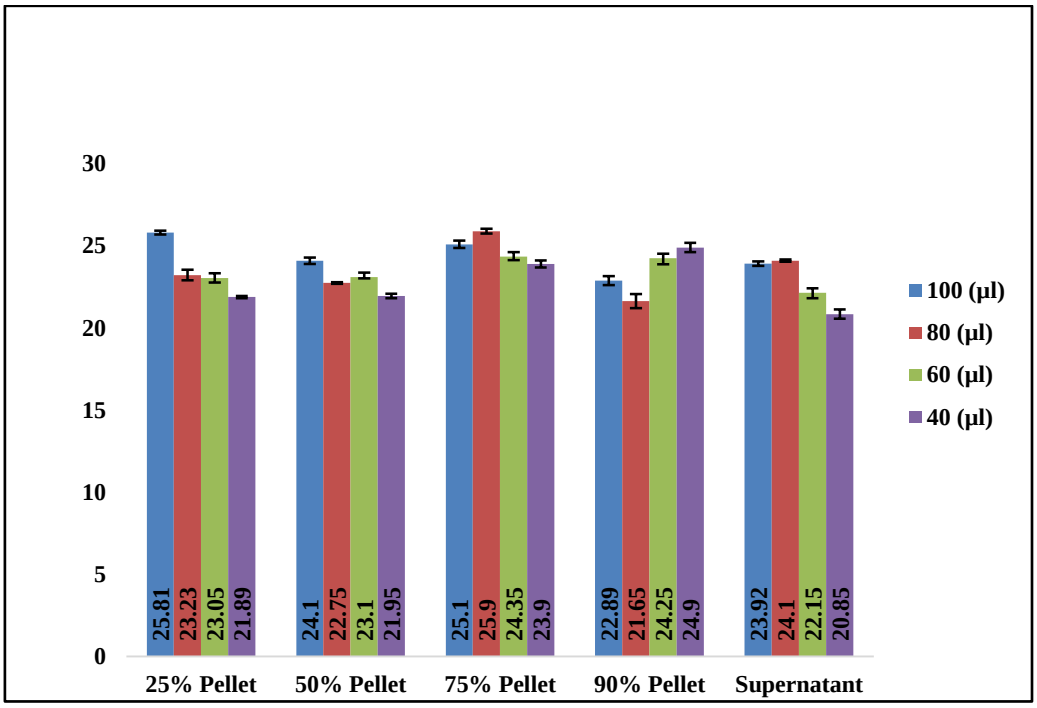
Figure 3.3 depicts the reverse phase chromatogram of *Momordica charantia* protein pellet. The chromatogram showed some significant height values that were clearly resolved. A total of 6 fractions were seen in the chromatogram. Elution time and peak height of the *Momordica charantia* samples are as follows: 19 min (1.207 AU), 27 min (2.286 AU), 50 min (6.196 AU), 63 min (8.658 AU). The findings obtained are in corroboration

with the previous investigations of Murugesan *et al.,* (2022).

3d. Antimicrobial activity in solubilized and supernatant of ammonium sulfate precipitates

Table: 3.1 Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Staphylococcus aureus*

S.No.	Peptide Sample	100 (µl)	80 (µl)	60 (µl)	40 (µl)
1.	25% Pellet	25.81 ± 0.12**	23.23 ± 0.33**	23.05 ± 0.28**	21.89 ± 0.07**
2.	50% Pellet	24.10 ± 0.19**	22.75 ± 0.05**	23.10 ±0.07**	21.95 ± 0.14*
3.	75% Pellet	25.10 ± 0.23**	25.90 ± 0.14**	24.35 ± 0.22**	23.90 ± 0.21**
4.	90% Pellet	22.89 ± 0.28**	21.65 ± 0.43**	24.25 ± 0.36**	24.90 ± 0.28**
5.	Supernatant	23.92 ± 0.14**	24.10 ± 0.07**	22.15 ± 0.33**	20.85 ± 0.28**
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

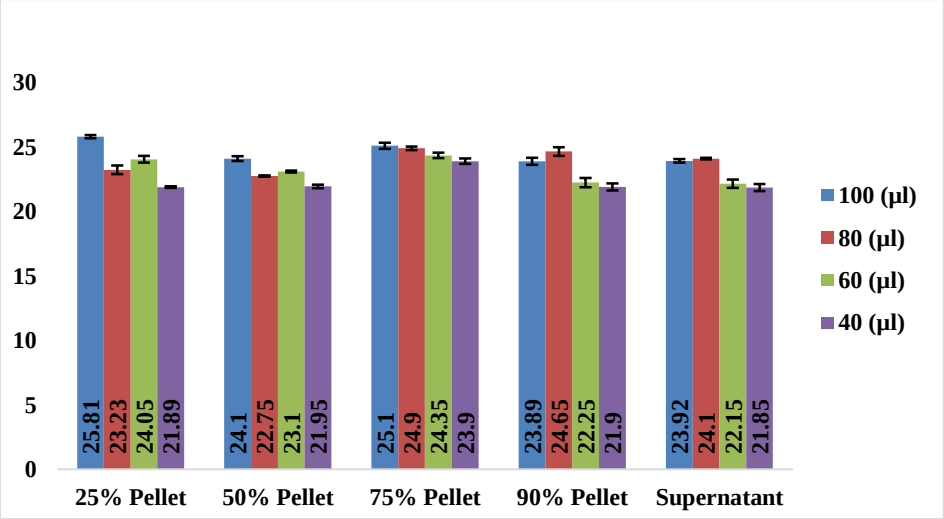


Graph 3.1: Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Staphylococcus aureus*

The protein extracted from *Momordica charantia* seeds was evaluated for antibacterial properties. *Staphylococcus aureus* was found to be susceptible to antimicrobial activity in the protein pellet obtained from a 25% ammonium sulphate precipitation seed extract, with a maximum inhibition zone measuring 25.81 ± 0.12mm and a minimum at 21.89 ± 0.07mm. The maximum inhibition zone measured in the 50% protein pellet derived from seed extract was 24.10 ± 0.19 mm at 100µl, whereas the minimum inhibition zone was 21.95 ± 0.14 mm at 40µl. The protein pellet obtained from 75% ammonium sulphate precipitation of seed extract showed maximum inhibitory zone of 25.90 ± 0.14mm at 80µl and minimum inhibitory zone at 23.90 ± 0.21mm at 40µl. 90% precipitated protein pellet derived exhibited maximum inhibition zone of 24.90 ± 0.28mm at 40µl and minimum inhibition zone of 21.65 ± 0.43mm at 80µl. The supernatant obtained after 95% ammonium precipitation showed inhibition zone of maximum 24.10 ± 0.07m at 80µl and minimum inhibition zone of 20.85 ± 0.28mm at 40 µl against *Staphylococcus aureus.*

Table: 3.2 Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Escherichia coli*

S.No.	Peptide Sample	100 (µl)	80 (µl)	60 (µl)	40 (µl)
1.	25% Pellet	25.81 ± 0.12**	23.23 ± 0.33*	24.05 ± 0.27**	21.89 ± 0.07**
2.	50% Pellet	24.10 ± 0.19**	22.75 ± 0.05**	23.10 ±0.07**	21.95 ± 0.14**
3.	75% Pellet	25.10 ± 0.23**	24.90 ± 0.14**	24.35 ± 0.22**	23.90 ± 0.21**
4.	90% Pellet	23.89 ± 0.27**	24.65 ± 0.33**	22.25 ± 0.36**	21.90 ± 0.27**
5.	Supernatant	23.92 ± 0.14**	24.10 ± 0.07*	22.15 ± 0.33**	21.85 ± 0.27**
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

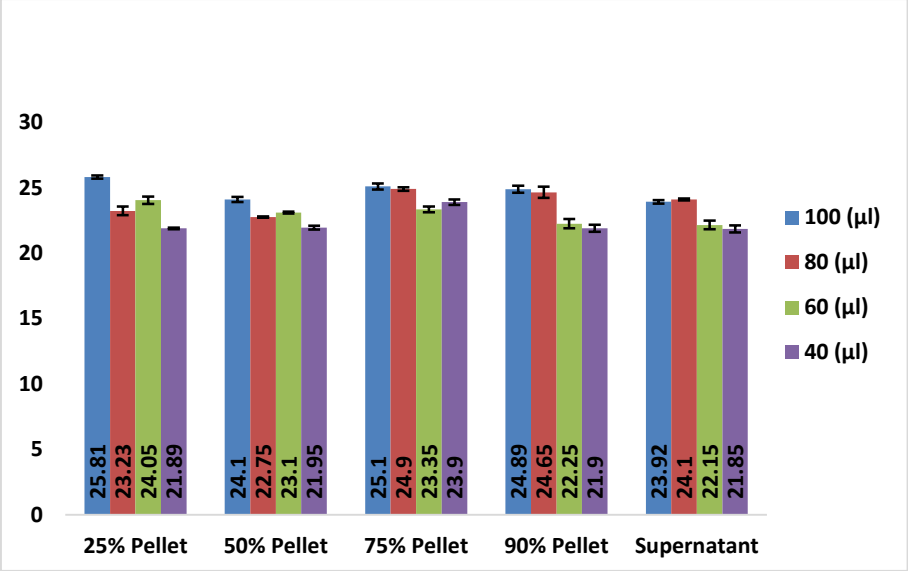


Graph 3.2: Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of Escherichia *coli*

The susceptibility of *Escherichia coli* to the antibacterial activity of *Momordica charantia* is illustrated in above table 3.2 and graph 3.2. The maximal inhibition zone measured 25.81 ± 0.12mm at 100 µl and the minimum inhibition zone was 21.89 ± 0.07mm at 40 µl in the protein pellet that was produced from a 25% ammonium sulphate precipitation seed extract. The 50% protein pellet derived from seed extract exhibited maximum inhibition zone measured 24.10 ± 0.19mm at 100µl and the minimum inhibition zone was 21.95 ± 0.14mm at 40µl. The maximal inhibitory zone measured in the protein pellet generated from the 75% ammonium sulphate precipitation of seed extract was 25.10 ± 0.23mm at 100µl, whereas the minimum inhibitory zone was 23.90 ± 0.21mm at 40µl. The 90% precipitated protein pellet produced a minimum inhibition zone of 21.90 ± 0.27 mm at 40µl and a maximum inhibition zone of 24.65 ± 0.33mm at 80µl. The supernatant following 95% ammonium precipitation revealed an inhibitory zone against *Escherichia coli* that was minimum 21.85 ± 0.27mm at 40 µl and maximum 24.10 ± 0.07 mm at 80 µl.

Table: 3.3 Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Klebsiella pneumoniae*

S.No.	Peptide Sample	100 (µl)	80 (µl)	60 (µl)	40 (µl)
1.	25% Pellet	25.81 ± 0.12**	23.23 ± 0.33**	24.05 ± 0.28**	21.89 ± 0.07**
2.	50% Pellet	24.10 ± 0.19**	22.75 ± 0.05**	23.10 ±0.07**	21.95 ± 0.14**
3.	75% Pellet	25.10 ± 0.23**	24.90 ± 0.14**	23.35 ± 0.22**	23.90 ± 0.21**
4.	90% Pellet	24.89 ± 0.27**	24.65 ± 0.43**	22.25 ± 0.36**	21.90 ± 0.27**
5.	Supernatant	23.92 ± 0.14**	24.10 ± 0.07**	22.15 ± 0.33**	21.85 ± 0.27*
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)**					

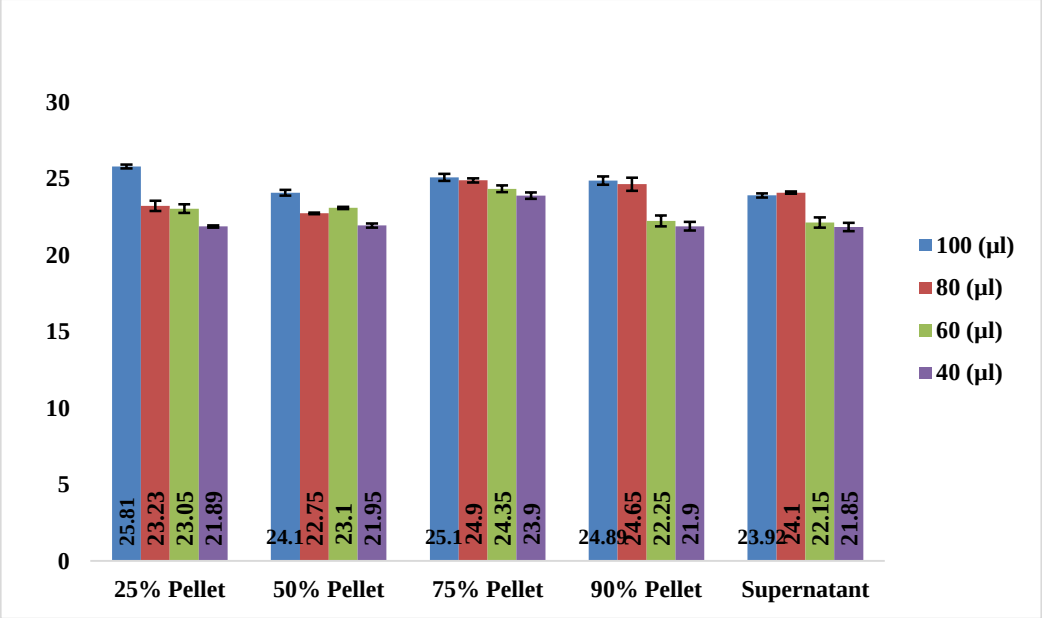


Graph 3.3: Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Klebsiella pneumoniae*

Klebsiella pneumoniae was found to be susceptible to antimicrobial activity in the protein pellet obtained from a 25% ammonium sulphate precipitation seed extract, with a maximum inhibition zone measuring 25.81 ± 0.12mm at 100 µl and a minimum at 21.89 ± 0.07mm at 40µl. The 50% protein pellet derived from seed extract exhibited maximum inhibition zone measured 24.10 ± 0.19mm at 100µl and the minimum inhibition zone was 21.95 ± 0.14mm at 40µl. The protein pellet obtained from 75% ammonium sulphate precipitation of seed extract showed maximum inhibitory zone of 25.10 ± 0.23mm at 100µl and minimum inhibitory zone at 23.35 ± 0.22mm at 60µl. The 90% precipitated protein pellet produced a minimum inhibition zone of 21.90 ± 0.27 mm at 40µl and a maximum inhibition zone of 24.89 ± 0.27mm at 100µl. The supernatant following 95% ammonium precipitation revealed an inhibitory zone against *Klebsiella pneumoniae* that was 21.85 ± 0.27 mm at 40 µl and maximum 24.10 ± 0.07 m at 80 µl.

Table: 3.4 Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Pseudomonas aeruginosa*

S.No.	Peptide Sample	100 (µl)	80 (µl)	60 (µl)	40 (µl)
1.	**25% Pellet**	25.81 ± 0.12**	23.23 ± 0.33**	23.05 ± 0.28**	21.89 ± 0.07**
2.	**50% Pellet**	24.10 ± 0.19**	22.75 ± 0.05**	23.10 ±0.07*	21.95 ± 0.14**
3.	**75% Pellet**	25.10 ± 0.23**	24.90 ± 0.14**	24.35 ± 0.22**	23.90 ± 0.21**
4.	**90% Pellet**	24.89 ± 0.28**	24.65 ± 0.43**	22.25 ± 0.36**	21.90 ± 0.28**
5.	**Supernatant**	23.92 ± 0.14**	24.10 ± 0.07**	22.15 ± 0.33*	21.85 ± 0.28**
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					

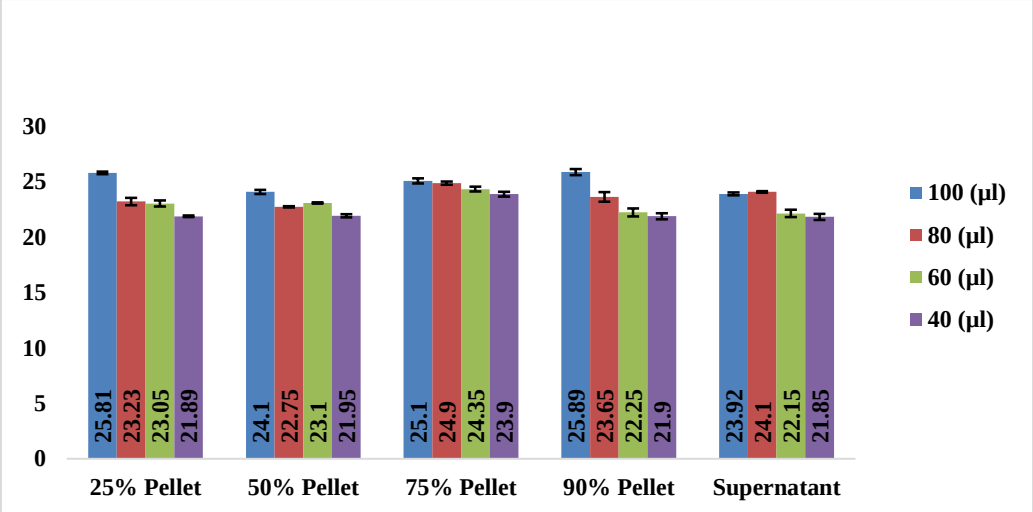


Graph 3.4: Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Pseudomonas aeruginosa*

Table 3.4 and Graph 3.4 show the sensitivity of *Pseudomonas aeroginosa* to the antimicrobial properties of *Momordica charantia*. With the protein pellet formed from a 25% ammonium sulphate precipitation seed extract, the maximal inhibition zone measured 25.81 ± 0.12mm at 100 µl, while the minimum inhibition zone measured 21.89 ± 0.07mm at 40 µl. The maximum inhibition zone measured 24.10 ± 0.19mm at 100µl and the minimum inhibition zone measured 21.95 ± 0.14mm at 40µl were observed in the 50% protein pellet that was generated from seed extract. In the protein pellet obtained from the 75% ammonium sulphate precipitation of seed extract, the highest inhibitory zone measured at 100µl was 25.10 ± 0.23mm, whereas the minimum inhibitory zone was 23.90 ± 0.21mm at 40µl. The 90% precipitated protein pellet showed a minimum inhibition zone of 21.90 ± 0.28mm at 40µl and a maximum inhibition zone of 24.89 ± 0.28mm at 100µl. The supernatant that was collected following 95% ammonium precipitation demonstrated an inhibitory zone against *Pseudomonas aeroginosa* that was at minimum range of 21.85 ± 0.28mm at 40 µl and maximum 24.10 ± 0.07 m at 80 µl.

Table: 3.5 Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Enterococcus faecalis*

S.No.	Peptide Sample	100 (µl)	80 (µl)	60 (µl)	40 (µl)
1.	25% Pellet	25.81 ± 0.12**	23.23 ± 0.33**	23.05 ± 0.28**	21.89 ± 0.07**
2.	50% Pellet	24.10 ± 0.19**	22.75 ± 0.05**	23.10 ±0.07**	21.95 ± 0.14**
3.	75% Pellet	25.10 ± 0.23*	24.90 ± 0.14**	24.35 ± 0.22**	23.90 ± 0.21**
4.	90% Pellet	25.89 ± 0.28**	23.65 ± 0.43**	22.25 ± 0.36*	21.90 ± 0.28**
5.	Supernatant	23.92 ± 0.14**	24.10 ± 0.07**	22.15 ± 0.33**	21.85 ± 0.28**
Mean values ± SD(n=3); P≥0.05 (NS), *P<0.1 (S), **P≤0.01 (HS)					



Graph 3.5: Zone of Inhibition (ZOI) of different volumes of peptide pellets isolated from *Momordica charantia* against different volumes of *Enterococcus faecalis*

The susceptibility of *Enterococcus faecalis* to the antibacterial activity of *Momordica charantia* is illustrated in above table and Graph. The maximal inhibition zone measured 25.81 ± 0.12mm at 100 µl and the minimum inhibition zone was 21.89 ± 0.07mm at 40 µl in the protein pellet that was produced from a 25% ammonium sulphate precipitation seed extract. The 50% protein pellet derived from seed extract exhibited maximum inhibition zone measured 24.10 ± 0.19mm at 100µl and the minimum inhibition zone was 21.95 ± 0.14mm at 40µl. The maximal inhibitory zone measured in the protein pellet generated from the 75% ammonium sulphate precipitation of seed extract was 25.10 ± 0.23mm at 100µl, whereas the minimum inhibitory zone was 23.90 ± 0.21mm at 40µl. The 90% precipitated protein pellet produced a minimum inhibition zone of 21.90 ± 0.28 mm at 40µl and a maximum inhibition zone of 25.89 ± 0.28mm at 100µl. The supernatant following 95% ammonium precipitation revealed an inhibitory zone against *Enterococcus faecalis* that was minimum 21.85 ± 0.27mm at 40 µl and maximum 24.10 ± 0.07 mm at 80 µl.

4. Conclusion

The present study is in agreement with the past studies, both in the present and past research work protein/peptide extracted was found to be potent against both Gram-positive and Gram-negative microorganisms though their efficacy was more prominent in Gram-positive bacteria as compared to Gram-negative bacteria (Golla *et al.,* 2016; Akeel *et al.,* 2018). AMPs are positively charged amphipathic molecules that use two major modes of action to specifically target and destroy bacteria (Li *et al.,* 2017). Not just at the primary sequence level, but also in terms of the 2D or 3D structure of the AMPs, it can be thought of as the balance between cationic and hydrophobic residues. AMPs cause biofilm membrane rupture in the first mechanism, resulting in cell death and lysis. In the second mechanism of action, AMPs penetrate cells without disrupting the membrane and block essential intracellular activities by binding to nucleic acids or intracellular proteins (Brogden, 2005;CF *et al.,* 2017).

All AMPs have hydrophobicity, which is defined as the percentage of hydrophobic residues in the peptide sequence, such as valine, leucine, isoleucine, alanine, methionine, phenylalanine, tyrosine, and tryptophan (typically 50 percent for AMPs). The degree to which water-soluble AMPs can partition into the membrane lipid bilayer is determined by their hydrophobicity. It is essential for membrane permeabilization; yet, high levels of hydrophobicity can cause toxicity in mammalian cells and antimicrobial selectivity loss. (Chen *et al., 2007*). The decrease in activity when the hydrophobicity is high could be owing to a higher possibility

of dimerization, which prevents the peptide from reaching the bacterial membrane. RBC lysis is also improved by increasing the hydrophobicity of the non-polar face of the amphipathic helix. This might be related to the membrane discrimination mechanism, in which higher hydrophobicity peptides permeate deeper into the RBC membrane's hydrophobic core (Chen *et al.,* 2005).

Based on the results of this study, it is concluded that seeds of *M. charantia* L. contains a low molecular mass peptide which is effective against *E.coli, S.aureus, E.facialis* but is least effective against *K. Pneumonia.* This peptide has more antimicrobial capacity towards Gram-positive as compared to Gram-negative bacteria.

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