Evaluation of Antibacterial Activities and Molecular Docking Studies of Di-Peptide Peptide

Article · December 2018

CITATIONS
0

READS
48

4 authors:

HITHESH KUMAR C K
Vellore Institute of Technology
6 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Shylesh Murthy
Bangalore University
16 PUBLICATIONS **0** CITATIONS
SEE PROFILE

H K Makari
I.D.S.G GOVERNMENT COLLEGE
45 PUBLICATIONS **274** CITATIONS
SEE PROFILE

Vivek Chandramohan
Siddaganga Institute of Technology
34 PUBLICATIONS **189** CITATIONS
SEE PROFILE

International Journal Of Public Mental Health And Neurosciences
ISSN No: 2394-4668
(An Official publication of Sarvasumana Association)

Evaluation of Antibacterial Activities and Molecular Docking Studies of Di-Peptide

Shylesh Murthy,
Makari Hanumanthappa
Department of Biotechnology,
IDSG Govt. College,
Chickmagalur - 577101, Karnataka, India.
shyleshias@gmail.com
makari.hk@gmail.com

Vivek Chandramohan,
Mousami Das
Department of Biotechnology,
Siddaganga Institute of Technology,
Tumkur- 572103, Karnataka, India.
vivekbioinf@gmail.com
dasmousumi9@gmail.com

Hithesh Kumar,
Genotypic Technologies Pvt. Ltd,
Bengaluru - 560094, Karnataka, India.
ckhithesh@gmail.com

***Abstract*:Antimicrobial peptides are potent agents with diverse structural and antimicrobial peptides, which represent one of the most promising future drugs for combating infections and microbial drug resistance. Understanding the versatile biological properties of antimicrobial peptides can be of extreme importance for clinical development of peptide-based therapeutics, this review is an attempt to illustrate the diversity of peptides reported for a potential. The search for potential antimicrobial peptide sequences was covering all the major peptide databases like Antimicrobial Peptide Database, Collection of Antimicrobial Peptide Databases (CAMP), Peptide Atlas, etc. Our Current study aimed at the discovery of potent Fmoc-Tryp-Ala-OMe was prepared and coupled with various amino acids and di-peptide these synthesized derivatives were characterized and screened for their antibacterial activity for various bacteria. Further, molecular docking studies revealed the potential of drug molecules. The result demonstrates the bioactive compound analysis and molecular docking studies, protein-ligand docking shows better binding interactions, they exhibit reasonable inhibitory activity and further employed to design derivatives with customized activities. These potential drug promises to overcome the bacterial drug resistance. Our research study helped in understanding the usefulness of synthetic peptides in treating diseases and further research can be continued, these have become a major challenge in *Insillico* and *Invitro* Studies.**

***Keywords*: Peptide, Antibacterial Activity, Drug target, Molecular Docking, Protein-Ligand Interactions, Drug Discovery.**

I. INTRODUCTION:

Biologically important peptides have only a few amino acid residues. Such peptides show large biological effects. Thus, the peptides are the biologically occurring short chains of amino acid monomers linked by peptide bonds. The covalent chemical bonds are formed when the carboxyl group of one amino acid reacts with the amino group of another. Peptides have recently been used in the study of protein structure and function. Varieties of peptides are involved in the mammalian oxygen-independent antimicrobial defense mechanism. Defensins are a family of small arginine and cysteine rich peptides that have been isolated from a variety of mammals, including rats, rabbits, and humans. Defensins are one of the largest and most studied families of antimicrobial peptides. Most defensins function by penetrating the microbial cell membrane by way of electrical attraction, and once embedded, forming a pore in the membrane. Which allows efflux. An imbalance of defensins in the skin may contribute to acne. Some inhibitory peptides are also

used in clinical research to examine the effects of peptides on the inhibition of cancer proteins and other diseases. The increasing prevalence of multi drug resistant strains of bacteria and recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection fighting strategies and new effective therapeutic agents. Therefore, the search for smaller chain peptide based drug analogues for the treatment of infectious diseases has become necessary. P.sudomonas aeruginosa is an opportunistic human and plant pathogen; infect immune individuals and people with cystic fibrosis. Many P.aeruginosa virulence factors, including Toxins, Proteases and Hemolysins, are released from the bacterial cells. These factors have been found to contribute to the virulence of *P.aeruginosain* animal models in vitro studies, and clinical studies. Whereas the E.coli Verocytotoxigenic produce a toxin that is lethal to cultured African green monkey kidney cells (Vero cells) but not to some other cultured cell types.

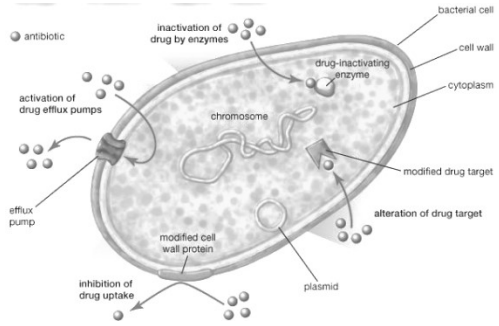


Fig. 1. Mechanism of antibiotic resistance

As per the ICU Book "The first rule of antibiotics is try not to use them, and the second rule is try not to use too many of them." Emergence of resistance often reflects evolutionary processes that take place during antibiotic therapy. The antibiotic treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibiotics. Under certain conditions, it may result in preferential growth of resistant bacteria, while the drug inhibits growth of susceptible bacteria. Antibiotics such as penicillin and erythromycin, which used to have a high efficiency against many bacterial species and strain, have become less effective, due to the increased resistance of many bacterial strains. Resistance may take the form of biodegradation of pharmaceuticals. Antibacterial resistance may impose a biological cost, thereby reducing fitness of resistant strains, which can limit the spread of antibacterial resistant bacteria. Thus, alternative for antibiotics is necessary. These molecules must be more effective than antibiotics and it must not prone to mutations quickly. Commonly used alternatives are resistance modifying agents, vaccines, antimicrobial peptides, bacteriophages etc. (Fig. 1) [10].

Amino acids are biologically important organic compounds composed of amine (-NH2) and carboxylic acid (-COOH) functional groups, along with a side-chain specific to each amino acid. The key elements of amino acids are carbon, hydrogen, oxygen and nitrogen though other elements are found in the side-chains of certain amino acids. The 20 amino acids that are encoded directly by the codons of the universal genetic code are called standard amino acids. The others are called non-standard amino acids. The standard amino acids are glycine, proline, alanine, valine, serine, leucine, isoleucine, lysine, tryptophan, arginine, aspartic acid, glutamic acid, methionine, tyrosine, threonine, phenylalanine, histidine, glutamine, arginine, and asparagine. Based on the side group of the amino acids, it can be classified as basic, acidic, polar and non-polar in nature. Peptides are short chains of amino acid monomers linked by peptide bonds. The covalent chemical bonds are formed when the carboxyl group of one amino acid reacts with the amino group of another amino acid, causing the release of a molecule of water (H20).Hence, the process is a dehydration synthesis reaction, also known as a condensation reaction. [2].

The pharmaceutical industry has continuously met this need by modifying existing antibiotics and developing newer antibiotics in a timely fashion. These successful efforts have produced the wide variety of currently available drug classes of Antibiotics, Glycopeptides, Macrolides, Ketolides, and Aminoglycosides etc. Similarly, there have been dramatic successes in developing effective antivirals to kill important clinical viral pathogens. However, the rapid emergence of resistance is even a greater

problem for life- threatening viral infections. Despite the success to date in antimicrobial development, the inexorable, ongoing emergence of resistance worldwide continues to spur the search for novel anti-infective to replace and supplement conventional antibiotics. Human Defensins, Cathelicidin, and a significant number of diverse AMPs from bacteria, viruses, plants, vertebrates, and invertebrates all appear to have a universal multidimensional signature that defines antimicrobial activity. Manipulation of this chemical structure to create designer synthetic peptides represents a promising strategy for the development of AMPs as a new class of drugs to prevent and treat systematic and topical infections. To date, the use of AMPs as single therapeutic antibiotic agents has received the most attention. Antimicrobial peptides (AMPs) have been considered as potential therapeutic sources of future antibiotics because of their broad-spectrum activities and different mechanisms of action compared to conventional antibiotics. Although AMPs possess considerable benefits as new generation antibiotics, their clinical and commercial development still have some limitations, such as potential toxicity, susceptibility to proteases, and high cost of peptide production. In order to overcome those obstacles, extensive efforts have been carried out. For instance, unusual Amino acids or Peptide-mimetics are introduced to avoid the proteolytic degradation and the design of short peptides retaining antimicrobial activities is proposed as a solution for the cost issue.

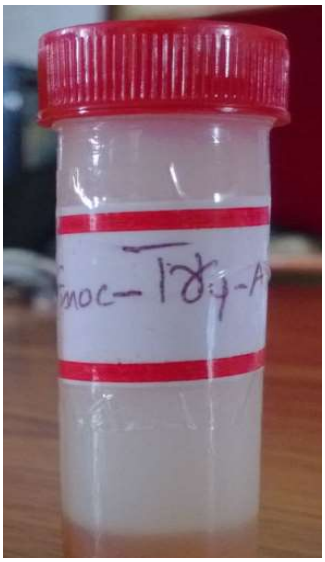
A pathogen is anything that can cause disease. Typically, the term is used to describe an infectious agent such as a virus, bacterium, prion, fungus, viroid, or parasite that causes disease in its host. The host may be an animal, a plant, a fungus or even another microorganism. Virulence evolves when that pathogen can spread from a diseased host, despite that host being much debilitated. Pseudomonas aeruginosa is a Gram negative, aerobic, bacillus with uni-polar motility. It is a member of the Gamma Proteobacteria class of bacteria. It is found in soil, water, skin flora and most man made environments throughout the world. It is an opportunistic pathogen. Its infections are generalized as inflammation and sepsis. If such colonization's occur in such as the lungs, the urinary tract, and kidneys, the results can be fatal. Elastae a major virulence factor of P.aeruginosa is used to inactivate EF2 in host cell. Without EF2, cells cannot synthesiseproteins, which in turn causes skin infections. Virulence of the enzyme will cause the same effect in plants and animals. [12] Escherichia coli is a Gram-negative rod (bacillus) in the family Enterobacteriaceae. Most E. coli are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such as exotoxins. The specific virulence factors can be used, together with the type of disease, to separate these organisms into pathogen types [38, 39].

In organic chemistry, peptides are the biopolymers formed from each individual amino acid, which are the building block of peptides and proteins. These composed of (NH2) amine group and carboxylic acid (COOH) groups along with a side chain specific to each amino acid. Peptides are synthesized by coupling the carboxyl group or C-terminal of one amino acid to the amino group or N-terminal of another. Chemical peptide synthesis starts at the C-terminal end of the peptide and ends at the N-terminal [1]. This is the opposite of protein biosynthesis, which starts at the N-terminal end. Energy minimization is done to ensure structural optimization of a protein complex. 3N Cartesian coordinates define a system of N atoms or 3N-6 internal coordinates. These define a multi-dimensional potential energy surface most minimization method can only go a downhill and so locate the closest minimum. No minimization method can guarantee the location of the global energy minimum. No method has proven the best for all problems. Stationary points as Minima (stable conformations), Maxima, and Saddle points (transition states) characterize it [2].

Molecular docking studies were performed to investigate the binding affinities and interaction modes between the inhibitors and the target using BioSolveIT FlexX. The docking score was noted down and docking poses were saved for reference. In addition, the docking was performed in Discovery Studio using Z- dock and based on the Z- scores

generated.The peptides were filtered and prioritized for synthesis. In the present study, we have tried to evaluate the anti-bacterial properties of Fmoc (9-Fluorenylmethoxycarbonyl). -Tryp-Ala-OMe against Escherichia coli (1HN9) and P.seusdomonous (4UMJ).

II. MATERIALS AND METHODS



1. Peptide synthesis: Fmoc-Tryp-Ala-OMe

Fig. 2. Peptide synthesis**.** Next step of the study was to synthesise the docked peptides. The solution phase peptide synthesis method was adopted which included the usage of less toxic chemicals and organic solvents.

The search for potential antimicrobial peptide sequences was done covering all the major peptide databases like Antimicrobial Peptide Database, Collection of Antimicrobial Peptide three Databases (CAMP), Peptide Atlas, etc. Through in depth literature survey and database information, **Fmoc-Tryp-Ala-OMe** peptide was screened for the synthesis process (Fig. 2) [33].

2. Target identification

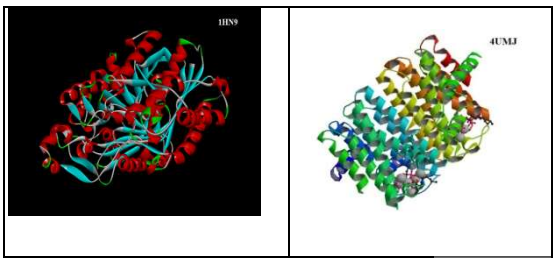


Fig. 3. Target identification. A,B. Structure of *E-coli*(1HN9) and*Pseudomonous aeruginosa* (4UMJ)**.** As per data base The PDB – ID (protein data bank) of the identified target is 1HN9 OF *E.coli* and 4UMJ of *Pseudomonoasaeruginosa.*

Ecthymagangrenosum is a characteristic dermatologic manifestation of severe and invasive caused due to *P.aeruginosa.* Elastae was chosen as drug target. It is an enzyme from the class of proteases that breakdown proteins. In bacteria, Elastae is considered a virulence factor like in *P.aeruginosa.* Elastae has been shown to disrupt tight junctions, cause proteolytic damage to tissue and contributes to the decrease in the ability of neutrophils to kill bacteria by phagocytosis. The PDB – ID of the identified target is 1HN9 OF *E. coli* and 4UMJ of *P.aeruginosa* (Fig. 3).

3. Energy Minimization

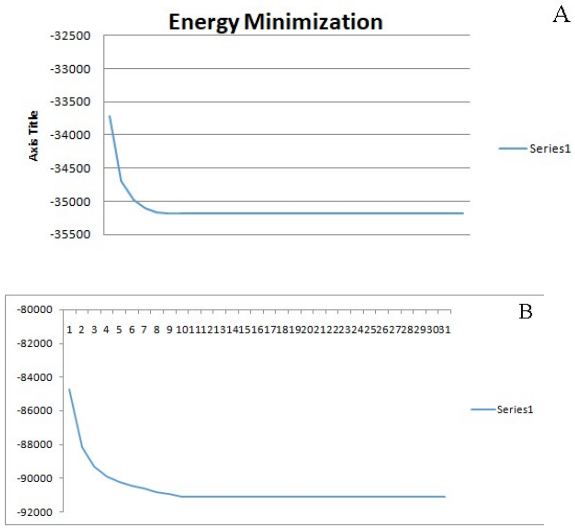


Fig. 4. A-B. Energy minimization.The energy minimization technique is useful for obtaining a static picture for comparison between systems. After protein minimization, the potential.

Before protein minimization, the potential energy of the protein was found to be -33713.99233 kcal/mol. Energy minimization (EM) is used to find positions of zero gradients for all atoms, in other words, a local energy minimum since lower energy states are more stable by relaxing the structure (Fig. 4). The energy minimization technique is useful for obtaining a static picture for comparison between systems. After protein minimization, the potential.

4. Binding site prediction

International Journal Of Public Mental Health And Neurosciences
ISSN No: 2394-4668
(An Official publication of Sarvasumana Association)

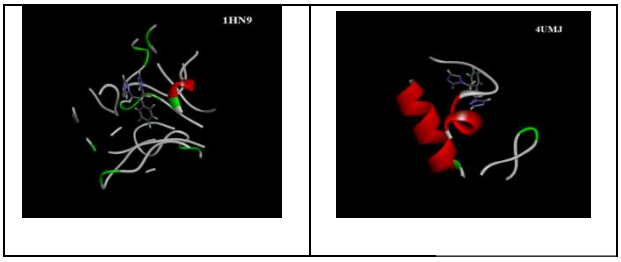


Fig. 5. Binding Site Prediction**.** A. Active site of *E coli* (1HN9). B. Active site of *Pseudomonas* (4UMJ). The active site of the protein was predicted using Discovery Studio 3.5. This study reveals the important residues in the target protein which are responsible for ligand binding, present in the active site or elsewhere.

Figure 5 shows the active site of the protein was predicted using Discovery Studio 3.5. It uses receptor activity method based on eraser algorithm. This study reveals the important residues in the target protein, which are responsible for ligand binding, present in the active site or elsewhere. [23]

5. Ligand preparation

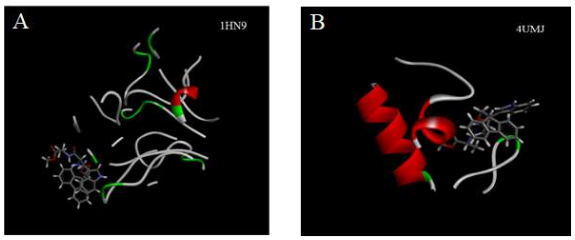


Fig. 6. Protein ligand docking. A. Complex docking of *E Coli* (1HN9) with Fmoc-Tryp-Ala-OMe. B. Complex docking of *Pseudomonous* (4UMJ) with Fmoc- Tryp-Ala-OMe. Protein ligand docking with standard antibiotic streptomycin**.** C. Complex Docking of (1HN9) with *Streptomycin*. D. Complex docking of (4UMJ) with Streptomycin. Molecular docking studies were performed to investigate the binding affinities and interaction modes between the inhibitors and the target using Bio SolveIT FlexX. The docking score was noted down and docking poses were saved for reference. Also, the docking was performed in Discovery Studio using Z- dock and based on the Z- scores generated, the peptides were filtered and prioritised for synthesis.

The drug likeliness of the screened peptides was investigated using Discovery Studio 3.5 and a library for those peptides was prepared. The energy of the ligand library was minimized using smart minimizer algorithm with the parameters of 200 steps and at RMS gradient 0.1. Each of the minimization methods were carried out with CHARMM force field. (Fig. 6) [7].

6. Protein ligand Docking

Molecular docking studies were performed to investigate the binding affinities and interaction modes between the inhibitors and the target using BioSolveIT FlexX. The docking score was noted down and docking poses were saved for reference. Also, the docking was performed in Discovery Studio using Z- dock and based on the Z- scores generated, the peptides were filtered and prioritised for synthesis. [7].

7. Antibacterial Activity

The antibacterial activity of the peptides was evaluated by well diffusion method. The nutrient broth was used for culturing the strains *P.aeruginosa* and *E coli* . The broth was incubated for 6 hours in a shaker. The absorbance was read at 600nm for each broth. The cultured strains were swabbed on Muller-Hinton agar plates. Uniform wells with a diameter of 5mm were punched in the media. water was used as a negative control and an antibiotic (streptomycin) was used for positive control. The concentration of samples was at 2000µg/ml throughout. The sample dissolved in DMSO was added at a volume of 100µl. The sample loaded plates were incubated for 24 hours at 37^0c. The incubation zones developed around the wells in each plate were measured and recorded [15].

8. Efficiency of peptides

The method employed for the checking the efficiency of peptides was broth dilution method. The 6 hour incubated culture of the *P. aeruginosa* was inoculated on Muller-Hinton Agar plates. Cultured plate with no inhibitor was taken as control. Peptides samples were diluted and spread on the plates in different volumes ranging from 50µl to300µl. same was followed in case of streptomycin an antibiotic. All the plates were incubated at 37^0C for 24 hours. Colonies grown on each plate were counted and compared with the control plate. The percent death of cells was calculated based on colony count.

III. RESULTS AND DISCUSSIONS

The study was aimed at *Insilico* and *Invitro* screening of potential antimicrobial peptides which could efficiently inhibit the action of pathogens like *Pseudomonas aeruginosa* and *E-coli*

1. Peptides Synthesis

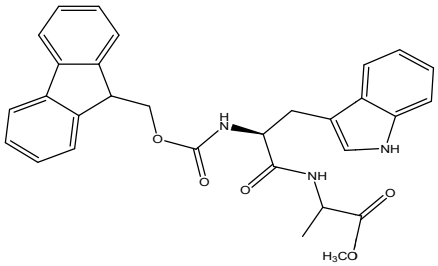


Fig. 7. Synthesis peptide: Fmoc-Tryp-Ala-OMe**.** The search for potential antimicrobial peptide sequences was done covering all the major peptide databases like Antimicrobial Peptide Database. Through in depth literature survey and database information, **Fmoc-Tryp-Ala-OMe** peptide was screened for the synthesis process.

The search for potential antimicrobial peptide sequences was done covering all the major peptide databases like Antimicrobial Peptide Database, Collection of Antimicrobial Peptide three Databases(CAMP), Peptide Atlas, etc. Through in depth literature survey and database information, **Fmoc-Tryp-Ala-OMe**peptide was screened. The synthesised peptide was taken for further investigation.

2. Target identification

The target structure retrived from PDB i.e. 1HN9 was acquired by X-ray diffraction and had resolution of 1.4.. 4UMJ has 2 domains - an Elstatase domain and neutral protease domain The structure available in the PDB database has 4 ligand molecules in it. They are calcium ions, N-(1-carboxy-3-phenylpropyl) phenylalanyl-alpha-alanine. Elastae is a polymeric protein isolated from the organisms *Psudomonas aeruginosa.* It has only one chain with 301 amino acid sequence. The gene responsible for this protein secretion is LasB.

3. Energy minimization

Before protein minimization, the potential energy of the protein was found to be -33713.99233kcal/mol. Energy minimization (EM) is used to find positions of zero gradients for ll atoms, in other words, a local energy minimum since lower energy states are more stable by relaxing the structure. The energy minimization technique is useful for obtaining a static picture for comparison between systems. After protein minimization, the potential energy of the protein was found to be -35186.13535 kcal/mol. Before protein minimization, the potential energy of the protein was found to be -84730.92751kcal/mol.

4. Binding Site Prediction

The A chain of 4UMJ contained 301 amino acid residues. Following were the residues found in A chain of the active site: ILE203, ARG1196, HIS204, SER202, ARG144, ASP1195, VAL1197, CYS146, ARG94, ARG95, GLU73, ARG47, SER429, ASN450, ARG428, SER449, SER448, SER392, TYR430, ASN450, GLN524, HIS447, ASP601, ASN450.

5. Protein ligand docking

The screened peptides were docked using DS 3.5 by using Z-Dock algorithm. The scores were generated from each peptide based on the hydrogen molecular interactions of best position of active site with the ligand. Peptides with the highest score were identified and synthesized (Table 1).

Table 1: Protein ligand docking scores with Fmoc-Tryp-Ala-OMe

Standard	**Bacterial target protein**	**Dock score**
Streptomycin	4UMJ	**-19.6385**
	1HN9	**-18.3609**
Peptide	**Bacteriial target protein**	**Dock score**
Fmoc-Try-Ala-OMe	4UMJ	**-22.35**
	1HN9	**-19.71**

The dock score of the peptides when docked with the active site of 4UMJ and 1HN9 were tabulated. Fmoc-Try-Ala-OMehad the best dock score of -19.71 and -22.35 respectively.

6. Antibacterial activity

Purified peptides were checked for their antibacterial activity. The method employed was well diffusion technique. The strains used for this method were *P.aeruginosa* and E.coli.

Figure shows the activity of peptides against gram negative *P.aeruginosa and Ecoli*

7. Efficiency check

Once the antibacterial activity was confirmed, the study was further carried out for checking the efficiency of each peptide. Number of colonies

grown on the control plate were compared with the number of colonies grown on the peptide containing plate.

Following Table shows the number of colonies grown on sample plates at different concentrations. Control plate contained around 200 colonies of *P.aeruginosa.* Tryptophan-Alanine was able to kill the maximum amount of cells compared to streptomycin. The % cell death of *P.aeruginosa* was highest when 200µl of Tryptophan-Alanine was added to the culture media and was found to be 83%. A graph of % cell death Vs. Volume of sample (Ala-Try) confirms the potency of peptides against bacteria.

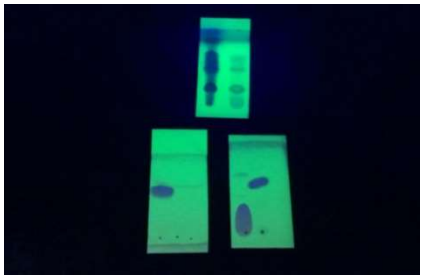


Fig. 8. TLC plates. The purity of peptides was analysed using Thin Layer Chromatography plates periodically until the purity of the sample was explains the of purification of peptides

Fig. 9. A. Antibacterial activity against *E. coli*. B. Antibacterial activity against *P.aeruginosa*. Purified peptides were checked for their antibacterial activity. The method employed was well diffusion technique. A-B. The strains used for this method were *P.aeruginosa* and *E. coli.* Figure shows the activity of peptides against gram negative *P.aeruginosa and E. coli*

Table 2: Antibacterial activity. The inhibition zones were measured and tabulated.

Organism	Sample(50 µg/ml)	Inhibition zones (mm)				Time(hours)
	(Peptide)	streptomycin	50 µl V	100 µl Vol	200 µl Vol	
					ol	
E,coli	Tryp-Ala	0	20	19	18	18
P.aeruginosa	Tryp-Ala	13	18	20	22	18
Streptomycin Susceptibility > 21 mm						
Streptomycin Resistivity < 14 mm						

Table 3: Efficiency check. Following Table shows the number of colonies grown on sample plates at different concentrations. Control plate contained around 200 colonies of *P.aeruginosa*

Sample	Volume of sample	Number of colonies	% cell death
Streptomycin	50	198	0.5
	100	182	9
	150	171	14
	200	185	7.5
	250	162	19
	300	175	12.5
Tryptophan-Alanine	50	82	59
	100	76	62
	150	74	63
	200	34	83
	250	45	77
	300	58	71

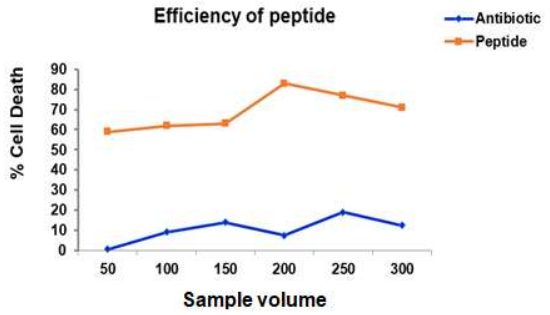


Fig. 10: Efficiency of the cell death rate of Antibiotic and peptide per sample volume.Tryptophan - Alanine was able to kill the maximum amount of cells compared to streptomycin. The % cell death of *P.aeruginosa* was highest when 200µl of Tryptophan - Alanine was added to the culture media and was found to be 83%. A graph of % cell death Vs. Volume of sample (Ala-Try) confirms the potency of peptides against bacteria.

IV. CONCLUSION

Our research study helped in understanding the usefulness of synthetic peptides in treating diseases that have become a major challenge globally. Small chain antimicrobial peptides especially Tryptophan-

Alanine residues were able to successfully inhibit the action of *E coli* and *Psudomonas aeruginosa* which had become antibiotic resistant. This two-gram negative and gram positive bacteria's was susceptible to the peptides and its action in a host organism can be controlled by stopping its growth. Bacterial cell death was accomplished by treating antimicrobial peptides against them. This study proved that small chain peptides can be efficient drugs with a considerable action against the bacteria which least react to antibiotics.

The study was based on the requirement of an efficient drug against antibiotic resistant pathogens. The results obtained were able to prove the efficacy of small chain peptide action in controlling the growth of these pathogens. Further research has to be done to study the anti-microbial aspects of the dipeptides.

REFERENCES

[1] Andrea Giuliani, Giovanna Pirri, Silvia FabioleNicoletto, 2007 Antimicrobial peptides: an overviewof a promising class of therapeutics. *Central European journel of pharmacy.2(1) 1–33*.

[2] Chakraborty, T. K., et al. (2004). "Sugar amino acids and related molecules: some recent developments." Journal of chemical sciences **116**(4): 187-207.

[3] Chen, H.-M., et al. (1996). "Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein." Journal of agricultural and food chemistry **44**(9): 2619-2623.

[4] Chen, R., et al. (2003). "ZDOCK: An initial-stage protein-docking algorithm." Proteins: Structure, Function, and Bioinformatics **52**(1): 80-87.

[5] Ciccotti, G., et al. (2014). Molecular Dynamics Simulation, MDPI AG Basel, Switzerland.

[6] David A. Phoenix, Sarah R. Dennison, and Frederick Harris, 2013. Antimicrobial Peptides: Their History, Evolution, andFunctional Promiscuity,*Wiley-VCH Verlag GmbH & Co. KGaA*.

[7] Dammalli, M., et al. (2014). "In silico analysis and identification of novel inhibitor for new H1N1 swine influenza virus." Asian Pacific Journal of Tropical Disease **4**: S635-S640.

[8] Ghahremanpour, M. M. and S. Sardari (2015). "The effect of structural parameters and positive charge distance on the interaction free energy of antimicrobial peptides with membrane surface." Journal of Biomolecular Structure and Dynamics **33**(3): 502-512.

[9] Giordano, A. "Bioactive Peptides in Cancer: Therapeutic Use and Delivery Strategies."

[10] Hancock, R. E. and D. P. Speert (2000). "Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment." Drug resistance updates **3**(4): 247-255.

[11] Høiby, N., et al. (2010). "Antibiotic resistance of bacterial biofilms." International journal of antimicrobial agents **35**(4): 322-332.

[12] Holden, M. T., et al. (2004). "Complete genomes of two clinical pseudomonas aureginosa: evidence for the rapid evolution of virulence and drug resistance." Proceedings of the National Academy of Sciences of the United States of America **101**(26): 9786-9791.

[13] Iscla, I., et al. (2015). "A new antibiotic with potent activity targets MscL." The Journal of antibiotics.

[14] K. C. Prakasha, G. M. Raghavendra, R. Harisha& D. Channe Gowda, 2011. Design, Synthesis and Antimicrobial screening of amino acids conjugated 2amino4arylthiazole derivatives, International Journal of Pharmacy and Pharmaceutical Sciences 3: 3.

[15] Kujumgiev, A., et al. (1999). "Antibacterial, antifungal and antiviral activity of propolis of different geographic origin." Journal of ethnopharmacology**64**(3): 235-240.

[16] Lindahl, E., et al. (2001). "GROMACS 3.0: a package for molecular simulation and trajectory analysis." Molecular modeling annual **7**(8): 306-317.

[17] Liu, F., et al. (2008). "The construction of a bioactive peptide database in Metazoa." Journal of proteome research **7**(9): 4119-4131.

[18] L Yin, J Jia, G.L Zhao, W.R Xu, L.D Tang, J.W Wang, 2011. Design, synthesis and antibacterial activity of novel N-formaylhydroxylamine derivatives as PDF inhibitors.*Indian J. Chem., Sec B*.

[19] Mah, T.-F., et al. (2003). "A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance." Nature **426**(6964): 306-310.

[20] MaciejJaskiewicz, MalgorzataOrlowska, Gabriela Olizarowicz, Dorian Migon, Daria Grzywacz, WojciechKamysz, 2015. Rapid Screening of Antimicrobial Synthetic Peptides. *Int J Pept Res Ther, Springer*.

[21] Michael R. Yeaman and Nannette Y. Yount, 2003. Mechanisms of Antimicrobial Peptide Action and Resistance, *American Society for Pharmacology and Experimental Therapeutics. 55:27–55*.

[22] Miranda, L. P., et al. (2000). "An activated O→ N acyl transfer auxiliary: efficient amide-backbone substitution of hindered "difficult" peptides." The Journal of organic chemistry **65**(18): 5460-5468.

[23] Munikumar, M., et al. (2012). "In silico identification of common putative drug targets among the pathogens of bacterial meningitis." Biochem Anal Biochem**1**(123): 2161-1009.1000123.

[24] N. B. Iannucci, R. González, O. Cascone and F. Albericio, 2011. Novel strategy for designing antimicrobial peptides: an answer to the development of drug resistance.*Communicating current research and technological advances,A Mendez-Vilas[Ed.].*

[25] Paungfoo-Lonhienne, C., et al. (2012). "Past, present and future of organic nutrients." Plant and Soil **359**(1-2): 1-18.

[26] Raganathan, S., et al. (2008). "Automated and accurate protein structure description: Distribution of ideal secondary structural units in natural proteins." arXiv preprint arXiv:0811.3587.

[27] Reller, L. B., et al. (2009). "Antimicrobial susceptibility testing: a review of general principles and contemporary practices." Clinical infectious diseases **49**(11): 1749-1755.

[28] Schmidberger JW, Schnell R, Schneider G ActaCrystallogr D BiolCrystallogr, 2015. Structural charecterization of substrate and inhibitor binding of farnesyl pyrophosphate synthase from Pseudomonas aeruginosa. Database of the U.S National Library of Medicine.

[29] Shang, S., et al. (2011). "Application of the logic of cysteine-free native chemical ligation to the synthesis of Human Parathyroid Hormone (hPTH)." Proceedings of the National Academy of Sciences **108**(15): 5986-5989.

[30] Stover, C., et al. (2000). "Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen." Nature **406**(6799): 959-964.

[31] Sundararajan, V. S., et al. (2012). "DAMPD: a manually curated antimicrobial peptide database." Nucleic acids research **40**(D1): D1108-D1112.

[32] Van Delden, C. and B. H. Iglewski (1998). "Cell-to-cell signaling and Pseudomonas aeruginosa infections." Emerging infectious diseases **4**(4): 551.

[33] Veronika Made, Sylvia Els-Heindl and Annette G. Beck-Sickinger, Beilstein J. Automated solid-phase peptide synthesisto obtain therapeutic peptides**,** 2014. *Org. Chem.10:1197–1212*.

[34] Victor Nizet, 2013. Antimicrobial Peptide Resistance Mechanisms ofHuman Bacterial Pathogens, *Mol. Biol*. 8: 223–238

[35] Wang, G., et al. (2009). "APD2: the updated antimicrobial peptide database and its application in peptide design." Nucleic acids research **37**(suppl 1): D933-D937.

[36] Wu, D. C., et al. (2011). "Pseudomonas Skin Infection." American journal of clinical dermatology **12**(3): 157-169.

[37] Xie, Z., et al. (2008). "Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate." Food Chemistry **111**(2): 370-376.

[38] Yeaman, M. R. and N. Y. Yount (2003). "Mechanisms of antimicrobial peptide action and resistance." Pharmacological reviews **55**(1): 27-55.

[39] Yoshida H, Kawai F, Obayashi E, Akashi S, Roper DI, Tame JR, Park SY J MolBiol, 2012. Crystal structures of penicillin-binding protein 3(PBP3) from methicillin-resistant pseudomonas aureginosa in the apo and cefotaxime-bond forms. Database of the U.S National Library of Medicine.

[40] Zhao, X., et al. (2013). "LAMP: a database linking antimicrobial peptides." PLoS One **8**(6): e66557.