Synthesis of Peptide and their Conjugation with Silver Nanoparticles

Project report submitted in partial fulfillment of the requirement for the degree of Master of Technology

in

Biotechnology/Bioinformatics

By

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Under the supervision of

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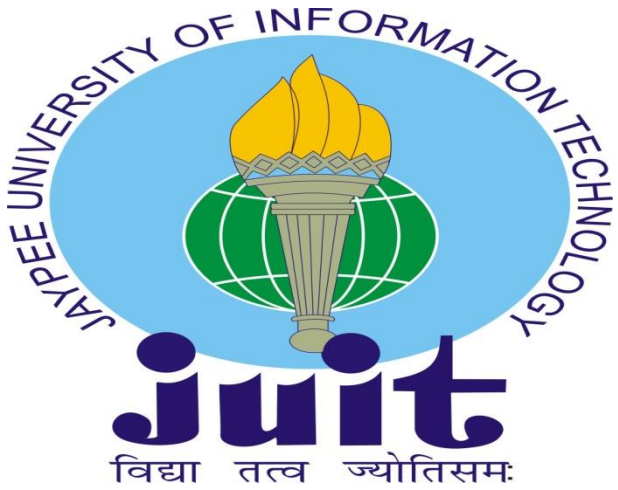
to



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DECLARATION

I hereby declare that the work reported in the M. Tech. thesis entitled **"Synthesis of Peptide and their conjugation with Silver Nanoparticles"** submitted at Jaypee University of Information Technology, Waknaghat, India, is an authentic record of my work carried out under the supervision of Dr. Gopal Singh Bisht , Dept. of Biotechnology and Bioinformatics, JUIT, Waknaghat, HP-173234, India. I have not submitted this work elsewhere for any other degree or diploma.

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Date:

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the M. Tech. thesis entitled **"Synthesis of Peptide and their conjugation with Silver Nanoparticles"**, submitted by Hita (212558) at Jaypee University of Information Technology, Waknaghat, India, is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

Signature of Supervisor

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ACKNOWLEDGEMENTS

We would like to thank God for guiding us throughout our academic journey and to acknowledge our project supervisor, Dr. Gopal Singh Bisht, for his undying support, priceless motivation and guidance throughout the project duration. Moreover, we extend our sincere gratitude towards the lecturers and the non-teaching workforce of the Department of Biotechnology and Bioinformatics towards their donation towards the success of this work.

The responsibility our associates played during the entire phase should not go unstated. Thank you all for your decent hold up and support. We deeply honored and obliged to you all.

As to our family, we appreciate the sustain help you have given us all over our academic expedition. This seek has not been easy but you have always gravely stood by our side.

Thank you.

Hita

ABSTRACT

Antibacterial peptides (AMPs) membrane-active peptides, and they typically only affect bacteria, fungi, and protozoans. Over the past few years, research has increasingly centered on AMPs as well as peptide conjugates containing AMPs. This recent research on AMP conjugates. Combining antimicrobial peptides (AMPs) with antibiotics or organometallic compounds is a strategy employed to enhance antibacterial effectiveness and target specificity, or the attachment to particles. Because antimicrobial conjugates have received so much attention in the literature thus far, many different scientific disciplines, including medicine, biology, biochemistry, and chemistry, appear to be particularly interested in this study issue. Antimicrobial peptides (AMPs) are a novel category of peptides, both natural and synthetic, that have been developed to target entities spanning viruses, bacteria, fungi, and parasites, among others, and are reviewed here along with recent advancements in the field. We list the main AMP subtypes, their methods of action, and the typical AMP resistance mechanisms. AMPs have emerged as promising candidates for future antibiotics. Their effectiveness exhibiting activity against a diverse array of microorganisms, AMPs employ distinct mechanisms of action. distinguishes them from current antibiotics, making them valuable therapeutic resources. Despite AMPs' many advantages as next-generation antibiotics, their clinical and commercial development is still constrained by issues such as possible toxicity, protease susceptibility, and expensive peptide manufacturing costs. Many efforts have been made to get around those challenges. For example, novel amino acids or peptido - mimetic compounds are added to prevent proteolytic degradation, and the construction of short peptides with antibacterial properties is suggested as a cost-effective option. Many vertebrates' innate immune systems contain proteolytically triggered peptides. These peptides have antibacterial action against bacteria, enveloped viruses, and fungi. Despite significant differences in sequence and taxonomy, most antibiotic peptides share a common mode of action, namely pathogen membrane permeabilization. In this research, we see that the peptide LP23 is conjugated with silver nanoparticles and they conjugated successfully. This is confirmed by the chemical method, UV spectrum and FTIR techniques. Purity is checked by the HPLC and mass is calculated by the Mass Spectroscopy. Conjugation helps in the stability of peptides and they’re in diagnostic and drug delivery.

CHAPTER 1- INTRODUCTION

AMPs present a fascinating class of antibiotics that exhibit distinctive antibacterial properties and a lower tendency for resistance development compared to conventional antibiotics. These membrane-active peptides primarily target bacteria, fungi, and protozoans in a selective manner.

In recent years, there has been a growing emphasis on the exploration of AMPs and peptide conjugates incorporating AMPs [1]. AMPs have emerged as promising and innovative antimicrobial agents, playing a crucial role in the defense mechanisms of the innate immune system. They demonstrate remarkable effectiveness against a diverse range of microorganisms, encompassing Gram-positive and Gram-negative bacteria, fungi, parasites, and viruses. The mechanism of action employed by AMPs is distinct and associated with their cationic amphipathic properties, which allow them to disrupt microbial membranes. Although the emergence of AMP-resistant strains is an anticipated consequence of the initial clinical use of AMPs, ongoing research endeavors aim to address this challenge and optimize their therapeutic potential and extensive research is being conducted in this area [2]. Lipopeptides, which consist of peptides attached to lipid chains, are versatile compounds with multiple functionalities. When lipopeptides incorporate hydrophilic peptide "headgroups," they can undergo aqueous self-assembly, forming amphiphilic molecules. The process of self-assembly gives rise to a densely populated surface of peptide groups in the lipopeptide nanostructure, often leading to heightened activity when compared to non-lipidated (and non-assembled) peptides. Proline, an essential amino acid, plays a pivotal role in peptides possessing catalytic properties. Numerous proline peptide gelatos, serving as effective catalysts for reactions such as the nitro-aldol reaction, have been investigated. These gelatos can assemble into various structures, including nanofibers, nano tapes, and micelles [3]. Contemporary medication is faced with the issue of a gradually expanding number of antimicrobial-safe microorganisms. This is largely a result of the maltreatment of anti-toxins, due to either their incorrect remedy or over-prescription, making microbes adjust furthermore, develop to oppose their executioner. Subsequently, new anti-infection agents as an alternative to a regular system of activity have been looked for trusting it would lessen the chance of microbial opposition. A promising source of these mixtures is antimicrobial peptides (AMPs) having a place with a different and transformative old gathering of atoms

that are significant effectors of inborn resistance. AMPs are given as the primary line of protection against microorganisms by most known creatures. A great deal of AMPs saturates and obliterate the cell film of microorganisms, causing harm that is challenging to modify. Therefore, microbial opposition happens with a low likelihood. The normal component of AMPs is a positive net charge and amphipathic structure, which decide their antimicrobial action and method of activity. Past examinations have shown that the formation of cationic antimicrobial peptides with greasy corrosive chains can work on their antimicrobial movement and balance of lipopolysaccharides. Besides, a connection of unsaturated fat to organically inert cationic peptides enriched the subsequent lipopeptides with lytic movement. Accordingly, short cationic lipopeptides, designed to safeguard the essential component of AMPs, appear to be encouraging specialists in a battle against attacking microorganisms [3]. Also, inferable from the surfactant-like design, they are able to go through self-gathering to a nanostructure in the arrangement. This element makes them potential candidates for the capability of the two emulsifiers and additives in food, beauty care products and the drug industry. They comprise electrostatic and hydrophobic co-operations after restricting to the anionic film has prompted no general restricting impacts in FTIR spectra [4]. Peptide amphiphiles (PAs) represent an awe-inspiring class of particles derived from peptides that possess the extraordinary capability of self-assembly. These remarkable entities consist of a bioactive head group intricately linked to a variable-length hydrophobic tail, which remarkably resembles an alkyl chain akin to a lipopeptide. The hydrophobic tail instills the molecule with unparalleled amphiphilic properties, thereby endowing it with the spontaneous ability to arrange itself in a liquid milieu, ultimately giving rise to exquisitely defined and meticulously controllable nanostructures embellished with peptides. The profound driving force underpinning this mesmerizing assembly phenomenon is the innate necessity to shield the hydrophobic tail from the encompassing solvent, while simultaneously allowing the hydrophilic peptide segment to gracefully grace the outermost layer of the assembled structures. In the fascinating realm of single-tail PAs, the self-assembly process predominantly begets resplendent fibrous architectures, where the hydrophobic tails luxuriate within the sanctity of the core, diligently secluded from the ambient solvent, while the hydrophilic heads proudly adorn the surface [5]. The captivating interplay between the hydrophobic alkyl tails and the exquisitely orchestrated β-sheet arrangement adopted by the peptide units orchestrates the emergence of the fibrous ensembles, although alternate modes of assembly, including micelles or vesicles, may manifest with their own intriguing allure. The awe-inspiring self-assembly orchestration remains a symphony that can be elegantly

choreographed by an ensemble of factors, including pH, temperature, concentration, and ionic strength, yielding an unprecedented level of control over the resulting nanostructures, as if conducting a symphony of molecular beauty.

Lipidated peptides possess an unparalleled allure as therapeutic agents, captivatingly offering the prospect of precisely tailoring their lipophilic characteristics to orchestrate a symphony of effects on absorption, distribution, metabolism, excretion, and bioavailability. The artful act of lipidation not only bestows upon these peptides exquisite stability but also enchants with the gift of prolonged half-life in the realm of the living, deftly facilitated by their affinity for carrier proteins, most notably the esteemed serum albumin. This divine union between lipidated peptides and carrier proteins gracefully delays renal clearance, as if time itself becomes an ethereal melody, and extends the symphony of their biological activity [6]. Behold the captivating realm of lipopeptides, those enchanting molecules where peptides become entwined with the allure of lipid chains, summoning forth the aqueous realm of self-assembly when the lipopeptides possess the celestial "headgroups" of hydrophilicity. Within this realm, myriad self-assembled structures emerge, adorned with the splendor of nanofibers, nanotapes, and micelles, each a testament to the symphony of ordered beauty that unfolds before our eyes. And in this grand orchestration of self-assembled lipopeptide nanostructures, the peptides cluster together in resplendent density upon the surface, a harmonious display that surpasses the unlipidated (unassembled) counterparts in the grandeur of their formation [3]. A spotlight is cast upon proline, that pivotal player within peptides bearing catalytic prowess, as it takes center stage in this enthralling performance. Lipopeptides, especially those graced with the presence of alkylated proline, bewitch us with their prowess as catalysts in the realm of direct aldol reactions, gracefully navigating both aqueous and organic solvents. Witness their virtuosity, delivering astounding yields and breathtaking enantioselectivities, particularly when immersed in oil-in-water emulsions, where they seem to conjure miracles from thin air [3].

Various manufactured peptides are critical business or drug items, running from the dipeptide sugar-substitute aspartame to clinically utilized chemicals, like oxytocin, adrenocorticotropic chemical, and calcitonin. This unit gives an outline of the field of manufactured peptides and proteins. It examines choosing strong help and normal coupling reagents. A peptide is an amino acid short chain (normally 2 to 50) connected by synthetic bonds which are also called peptide bonds. A more drawn-out chain of connected amino acids (at least 51) is a polypeptide. The proteins produced inside cells are produced using at least one or more

polypeptides. Peptides often act as hormones and are therefore natural couriers that move data starting with one tissue and then onto the next through the blood. Peptide and steroid chemicals are the two most famous sorts of chemicals [6]. For the irresistible illness therapy and deflecting flare-ups, the remedial utilization of peptides as the antigen part of subunit inoculations has recently made progress in different afflictions, like a disease. Subunit peptide-based immunizations inspire lower immunogenicity than the heft of inoculations against infectious including constricted or inactivated microorganisms, for the most part requiring rehashed dosages to accomplish viability [1].

1.1 Cationic Peptide as Antibacterial Agent

Cationic antimicrobial peptides are inherent components of various organisms, spanning from plants and insects to humans, naturally synthesized as an essential constituent of their defense mechanisms, vague safeguards against contaminations. Antimicrobial development can be described as a total term of each and every powerful norm (subject matter experts) that stifle the advancement of minute life forms, thwart the plan of microbial settlements and may wreck small-scale animals [7]. There is a dire need to find new classes of anti-toxins due to the rising improvement of antitoxin obstruction among major bacterial diseases. Cationic peptides are being created as anti-infective medicines as a result of clinical investigations. These peptides appear to have an effect or role in innate immunity in addition to their capacity to kill bacteria, and they have been able to upregulate the presence of numerous genes in eukaryotic cells [8]. The suppression of bacterial signaling by chemicals like lipopolysaccharide and lipoteichoic acid may be one of these functions. All species, from plants and insects to people, create cationic antimicrobial peptides as a significant component of their nonspecific, instantaneous disease prevention measures. Finding new classes of antibiotics is urgently needed since major bacterial infections are becoming increasingly resistant to existing medicines [9]. Cationic peptides are being created as anti-infective medicines as a result of clinical investigations. These peptides seem to function as innate immune effectors in addition to their capacity to kill bacteria, and they have the ability to upregulate the outflow of various qualities in eukaryotic cells. One of these functions could be to reduce the signaling produced by bacterial compounds like lipopolysaccharide and lipoteichoic acid[10].

1.2 Peptides as Therapeutic Agents

Therapeutic peptides are defined as naturally occurring short monomer chains of amino acids that are less than 100 amino acids in length. These molecules function by binding to particular cell surface receptors and activating intracellular pathways there. An extraordinary group of drug drugs known as remedial peptides is comprised of a progression of very much arranged amino acids, ordinarily with sub-atomic loads somewhere in the range of 500 and 5000 Da1. The pivotal studies focusing on natural human compounds like Cationic antimicrobial peptides are innate constituents of diverse organisms, including plants, insects, and humans, and are naturally produced as integral elements of their defense mechanisms. More than 80 peptide prescriptions have been endorsed universally because of exceptional headways starting from the making of the primary restorative peptide, insulin, in 1921. Accordingly, the production of peptide prescriptions has arisen as one of the most famous areas of concentrate in drug science [11]. Ion channel ligands, neurotransmitters, hormones, growth factors, and antibiotics are only a few examples of the common activities of therapeutic peptides. They connect to cell surface receptors in a way that is very similar to biologics, such as therapeutic proteins and antibodies, and they cause intracellular effects with high specificity and affinity [12]. However, therapeutic peptides have a lower cost of production and less immunogenicity than biologics. The long therapeutic history of small molecule drugs is well known, as is their inherent advantages, which include low manufacturing and sales costs, oral administration, and efficient membrane penetration [12]. The economic advantages of tiny molecules manufactured chemically or biologically are comparable to those of peptides and biologics (proteins or antibodies). On account of their little size and capacity to infiltrate cell layers, minuscule medications can be conveyed orally with more noteworthy patient consistence and security. They can also target intracellular molecules. Due to their small size, they find it difficult to properly suppress significant surface contacts, such as protein-protein interactions (PPIs). In contrast, peptide medicine's distinct physiochemical characteristics, such as their greater size and more adaptable spine, empower them to work as powerful analgesics [13]. The therapeutic utility of small molecules is constrained compared to peptide drugs due to their relatively narrower specificity. An example of this is seen with sorafenib and sunitinib, which are classified as tyrosine kinase inhibitors. These medications primarily target the tyrosine kinase domain of vascular endothelial growth factor (VEGF) receptors, leading to anti-angiogenic effects that are employed in cancer therapy. However, they also have interactions with other kinase

receptors, such as serine/threonine kinase receptors. Conversely, therapeutic peptides, which are derived from naturally occurring amino acids, encounter two inherent limitations: reduced in vivo stability and limited membrane permeability. These challenges significantly impede progress in the field of peptide-derived therapeutics [13]

1. The penetrability of films for peptides is low. Peptide length and amino corrosive arrangement are just two of the numerous boundaries that influence how porous the layer is to peptide drugs. Taking everything into account, can't go through cells. They reach intracellular targets across the membrane, restricting their applications in the creation of drugs [13].

2. The extracellular targets, including the glucagon-like peptide 1 (GLP-1) receptor, G-protein coupled receptors (GPCRs), and gonadotropin-releasing hormone (GnRH) receptor, constituted the focus of over 90% of peptides currently undergoing clinical research. Peptides don't hold up well in residing matters. Conventional peptides, consisting of amino acid chains connected by amide bonds do not possess the same structural robustness provided by secondary or tertiary arrangements. In the absence of protective measures, these amide linkages are susceptible to rapid hydrolysis or breakdown by chemicals present in living tissues upon exposure. As a result of these inherent chemical properties, peptides are inherently delicate, characterized by a short half-life and rapid clearance from the body [14].

Peptides used to treat diabetes mellitus therapeutically T2DM, which is frequent in middleaged and older persons, is brought on by an acquired insulin insufficiency. Peptide medications, such as insulin and GLP-1 receptor agonists (GLP-1RAs), have proved effective in treating T2DM. L-cells in the ileum release the endogenous growth hormone GLP-1. The receptors specific to Pancreatic β-cells, the peripheral and central nervous systems, the cardiovascular system (which includes the heart and blood vessels), the kidneys, lungs, and gastrointestinal mucosa are all found within their respective tissues. In a glucose-subordinate way, GLP-1 communicates with its receptor to advance satiety, limit the arrival of glucagon by islet cells, help the discharge of insulin, and delay stomach purging. GI-related unfavorable occasions (like sickness, retching, and loose bowels) and infusion site responses are the most continuous symptoms of GLP-1RA treatment, yet lengthy-acting GLP-1RAs make fewer side impacts and lower organization recurrence [13].

Cardiovascular infection treatment utilizing helpful peptides Cardiovascular sickness is right now the primary driver of mortality and dismalness overall among non-transmittable

diseases. Hypertension is widely acknowledged as a significant risk factor in the onset of cardiovascular disease and it is associated with factors such as salt retention, increased sympathetic nervous system activity, and the renin-angiotensin-aldosterone system (RAAS). The enzyme ACE2 plays a role in hydrolyzing angiotensin II into vasodilator angiotensin, indirectly contributing to the reduction of blood pressure. Expert (angiotensin-changing over catalyst) in the RAAS changes over angiotensin I into angiotensin II, which tightens veins and straightforwardly raises pulse. Consequently, the RAAS addresses the ideal objective for the administration of cardiovascular issues. Grown-ups with septicemia or other distributive shock were given the thumbs up by the FDA in 2017 to raise circulatory strain by intravenous imbuement of manufactured angiotensin II. Antibiotic overuse may further diminish the diversity of symbiotic bacteria, which is not helpful for therapy and may even make the condition worse; for instance, people with IBD (inflammatory bowel disease) are more likely to have used antibiotics during the two to five years before to diagnosis. Because of their selectivity, effectiveness, and low toxicity, peptide medicines have garnered a lot of interest in this sector. Because of their selectivity, effectiveness, and low toxicity, peptide medicines have garnered a lot of interest in this sector. The loss of the host-microbial interaction and significant alterations in the normal gut flora may be the causes of IBD. Constipation and intestinal obstruction are common in cystic fibrosis (CF) patients, and both conditions can progress to distal intestinal obstruction syndrome. In 2012, the FDA approved linaclotide, a guanylate cyclase C (GCC) receptor agonist, for the administration of steady stoppage. Albeit further exploration is expected to survey linaclotide's effect on the vehicle in CF model mice. Experimental investigations conducted in vivo have revealed that the early administration of β-casofensin, a peptide present in matured milk, effectively mitigated indomethacin-induced gastrointestinal damage and inflammation. This was achieved through the preservation of goblet cells and the acceleration of the wound-healing process [13]. Notably, the clinical, histological, and pathophysiological features observed in indomethacin-induced gastrointestinal injury bear similarities to those observed in Crohn's disease. This suggests the potential utility of β-casofensin as an adjunct therapeutic approach for managing Crohn's disease [14]. Gastric disease treatment utilizing helpful peptides. Stomach problems are a far-reaching worry since the gastric mucosa is quite possibly the most sensitive tissue in the two people and creatures. The essential drivers of stomach harm, which brings about gastritis and ulcers, incorporate Helicobacter pylori contamination, non-steroidal calming prescriptions, liquor, smoking, mind-set, and stress. Without brief therapy or with deficient treatment, stomach sickness can become ongoing, and supported long-haul harm emphatically raises the

possibility of creating gastric malignant growth. Right now, the gastric disease is the fourth most normal malignant growth to be analyzed around the world, and it is additionally the third and fifth most normal justification for malignant growth-related passing in all kinds of people, separately. The limited duration of action and poor oral absorption of peptide therapies have posed significant challenges. The short plasma half-life of peptides can be attributed to the presence of various peptidases and excretory systems that rapidly degrade and eliminate these molecules from the body [14]. Although this enzymatic susceptibility enables the body to efficiently regulate chemical levels for homeostasis, it presents obstacles in the development of effective peptide-based treatments tricky for some remedial advancement drives. the properties of peptides proposed for clinical testing in people. To make applicants more like medications, scientists began utilizing therapeutic science ways to deal with increments in their half-life, physiological strength, and receptor selectivity. Consequently, regular chemical peptide analogs with upgraded pharmacological qualities advanced into the facility. Oral bioavailability is one more obstruction to the improvement of peptidic drugs. Stomach-related catalysts made to separate amide obligations of the indistinguishable bonds in peptide chemicals might be really broken by ingested proteins, and the solid extremity and sub-atomic load of peptides seriously limit digestive penetrability [14]. Being that oral conveyance, as often as possible considered to be engaging for advancing patient consistence, the peptides were a less desirable alternative for indications requiring prolonged, outpatient treatment since they needed injection. Peptides form a distinctive subgroup of medicinal compounds that occupy a position between proteins and small molecules. They possess distinct therapeutic and physiological properties that differentiate them from both proteins and small molecules. It has the potential to function in therapies similarly to the natural route networks since it is an intrinsic signaling molecule. When a specific peptide hormone is deficient, some of them are even referred to as “replacement therapy” [13].

The best place to start when developing a novel medicine is with peptides. Due to their close bonds with targets, they have a desirable pharmacological profile, high specificity, and low toxicity. With regard to the effectiveness of human profiles, this specify has demonstrated that it is safe for commercial usage. By adding modules pertaining to active or passive transport, peptide medicines are often able to penetrate biological barriers [13]. The enhancement of peptide perforation is achieved by adding positively charged amino acids to the structure's terminal locations. Adrenocorticotropic hormone (ACTH) and insulin are two

examples of peptides from natural derivatives that were used as life-saving medications in the early 20th century. Peptides from many sources, including those from plants, animals, and the sea, offer a wide variety of possibilities and serve as treatments for upcoming drug development. Antimicrobial peptides are peptides that protect the human host from various microbial infections. They belong to a group of chemicals that mostly exhibit in host-environment interfaces such as the oral, gastrointestinal, and respiratory passageways. A manufactured restorative octapeptide called lanreotide was displayed to self-collect into nanotubes in water. Lanreotide is a cyclic octapeptide that is delivered as a development chemical inhibitor and can produce hydrogels, which are used as subcutaneous long-acting inserts to treat acromegaly. Likely purposes, like the nanofiltration of natural atoms, are featured by oneself gathering qualities of lanreotide in water that outcome in the making of nanotubes. It has been investigated if these vesicles may be used to transport photosensitizers. The peptide vesicles with phthalocyanine demonstrated an aggressive photodynamic reaction toward the cells, effectively killing them. At the measured ranges of concentrations, the vesicle forming peptides by themselves did not exhibit any cytotoxicity. As nanocarriers for the intracellular delivery of photosensitizers for photodynamic treatment, these peptide vesicles demonstrate potential [13]. There are more than 20 unmistakable human issues that are known to be connected with the development of stable protein totals, like amyloid fibrils. A few animal categories, be that as it may, have utilized working with conditions for their potential benefit. Novel inhibitors that have the potential to cure amyloid-associated disorders have been developed with the help of analysis of the amyloid production process. Animals, plants, bacteria, insects, and marine creatures are just a few of the sources that produce AMPs. Initially detected in invertebrates, animal-derived AMPs were eventually found in vertebrates as well. The sequences, structures, and mechanisms of these peptides are varied. Natural and abundant in biological molecules are the toxins produced by plants, animals, and marine sources. These AMPs can be created synthetically in addition to from natural sources. Synthetic AMP production techniques include the cultivation of industrial microbes, the use of genetically altered organisms, the breakdown of proteins by enzymes, and the extraction of AMPs from natural sources. The ability to modify species is continually improving because to advances in protein engineering [14].

1.3 Antimicrobial Peptides (AMPs): Classification

Prepare to be enraptured by the mesmerizing realm of antimicrobial peptides (AMPs), a kaleidoscope of boundless diversity and enchanting elegance. These extraordinary

compounds, hailing from the realms of α-helical, β-sheet, extended, and loop structures, weave a tapestry of awe-inspiring beauty. Behold the α-helical champions, magainin, LL-37, and cecropin, whose resplendent coils exude power and grace. Gaze in wonder at the β-sheet maestros, the defensins and protegrins, adorned with intricate disulfide bridges, their stability an ode to nature's ingenuity. Immerse yourself in the ethereal world of the extended AMPs, adorned with proline, tryptophan, arginine, and histidine, their irregular secondary structures a testament to their enigmatic allure. Traverse the mystical landscapes of indolicidin and bactenecins, veritable stars shining bright amidst the AMP constellation, their celestial beauty captivating all who dare to behold. And in the most intimate of AMP enclaves, discover the captivating serenity of the looped wonders, their harmonious disulfide bridges transforming them into mesmerizing works of art. Embark on this odyssey of discovery, where the relentless pursuit of synthetic AMPs unveils new horizons and pushes the boundaries of our imagination. Prepare to be spellbound by the symphony of antimicrobial peptides, a testament to the boundless creativity and infinite wonders of nature's grand design. The primary objectives include reducing the peptide size to enhance metabolic stability, bioavailability, and address concerns related to safety and immunogenicity. Shorter peptide sequences also offer the advantage of lower production costs. Furthermore, the integration of synthetic amino acids and alterations to the peptide bond within the sequence of AMPs offer promising opportunities to enhance their activity and effectiveness.

1.4 AMPs: Mechanism of Action

The mode of action of antimicrobial peptides (AMPs) revolves around their positive charge, allowing for specific interactions with the negatively charged outer membranes of microbial organisms. Bacterial surfaces, containing negatively charged components like lipoteichoic acid and lipopolysaccharides, facilitate this interaction [1]. Recent research has explored various physicochemical factors, including hydrophobicity, net charge, and helicity, as potential modulators of AMP activity. Prepare to embark on a thrilling journey into the intricate dance between antimicrobial peptides (AMPs) and pathogen membranes, where every move is filled with suspense and intrigue. Witness the mesmerizing transformation as AMPs undergo a metamorphosis, adopting well-defined secondary conformations upon contact with their targets. The grand performance begins with an electrifying attraction, as electrostatic forces draw the peptides towards the membrane, setting the stage for the epic battle to come [1]. Witness the grandeur of the toroidal-pore model, where the peptides assemble in a magnificent ensemble on the surface of the membrane causing the very fabric

of the membrane to bend and twist, forming a pore adorned with the intertwined beauty of the peptides and lipid head groups. A delicate balance is struck as the peptides maintain their connection with the phospholipid head groups, an essential harmony in the symphony of membrane disruption [2]. Enthrall yourself with the barrel-stave model, a mesmerizing spectacle of precision and coordination. The peptides gracefully gather at the outer membrane, ready to make their grand entrance into the heart of the cell membrane. Like skilled performers, the hydrophilic segments create a captivating core interior, while the hydrophobic regions intertwine with the lipids, creating a breathtaking display of interplay and tension [2]. Immerse yourself in the carpet-like model, a spectacle of captivating unity. Witness the captivating toroidal-pore model, where the peptides assemble in a magnificent formation on the surface of the membrane. With their unrivaled power, they induce permeabilization, by causing disruption to the membrane in a mesmerizing manner akin to a swirling detergent, the peptides create a spectacle, culminating in the formation of micelles, an awe-inspiring climax to the captivating show [2].In this enthralling saga, the stage is set for a battle of epic proportions. Witness the mesmerizing dance between AMPs and pathogen membranes, where structural changes, captivating models, and a relentless pursuit of victory converge in a symphony of destruction, as the forces of antimicrobial power triumph over their microbial foes. Brace yourself for an unforgettable experience, where science and imagination intertwine to create a spectacle that will leave you in awe of the extraordinary world of AMPs.While membrane permeabilization is a common pathway for most AMPs, they can also exert their antimicrobial effects through other mechanisms [2]. Some AMPs can penetrate pathogenic cells and target intracellular components, disrupting cell wall synthesis, inactivating essential enzymes, or affecting DNA, RNA, and protein synthesis [3].

1.5 Features of AMPs

They can operate as flexible effector molecules due to their interactions with different bacterial cell membranes. AMPs are valuable because of a range of their characteristics. Because tumor cells have an increased number of negatively charged phosphatidylserine components on their cell membranes, they have a greater tendency to activate AMPs. Making normal cells a preferred option for AMP as anticancer agents is so noteworthy [2]. According to a research, both in vivo and in vitro experiments revealed tumoricidal effectiveness against carcinoma and melanoma cells. In general, greater concentrations of these peptides are needed to achieve favorable tumoricidal effect, since studies reveal that cytotoxicity of tumor cells can only be identified at such concentrations due to the magnesium ion's inability to

attach to the membrane and enter deeper. Additionally, their ability to be altered by the proteases found in the extracellular matrix of tumor cells makes them significantly more susceptible to losing their tumoricidal function [2]. Furthermore, this problem was solved by AMP-encoded genes that were either directly inserted into the tumor cells or modified Through the substitution of peptides, the D-amino acids within tumor cells undergo a remarkable transformation, introducing a paradigm shift in their composition. terminal amidases. Host defense peptides (HDPs), which are tiny positively charged AMPs synthesized from the immune systems of those species that play a significant role in innate immunity, are another function of AMPs [1]. The majority of these HDPs aid in the infection of the host immune system and function as signal transduction cycle modulators by modifying the intracellular activity of signaling targets such protein kinases. These peptides function crucially in immune neuroendocrine interactions in addition to their antibacterial and immune regulatory functions. As administrative peptides, they contribute to the pathogenesis of corticostatic responses (stress action). AMPs have the ability to activate a variety of genes that participate in signal transduction networks at their sub inhibitory concentrations [3]. In this case, RNA polymerases' sigma factors are essential elements for figuring out promoter selectivity. Sigma factor substitution can cause RNA polymerases in the cell to malfunction, preventing it from producing gene transcription [2].

1.6 Advantages of AMPs

The excessive and prolonged use of traditional antibiotics as antibacterial agents has led to the emergence of bacterial mutations and the development of antibiotic resistance. To address this challenge, researchers in academia and the pharmaceutical industry have turned their attention to the advancement of alternative medications. Antimicrobial peptides (AMPs), a group of cationic peptides, have emerged as potential candidates to fulfill the role of antibiotics [7]. AMPs interact with the membranes of bacterial cells by neutralizing their charge, leading to membrane permeation and subsequent bacterial death. This unique mechanism of action reduces the likelihood of bacterial resistance. AMPs have exhibited a wide range of effectiveness against bacteria, fungi, and viruses. Importantly, they have shown minimal toxicity to host cells based on previous studies [7]. For example, Citrus-amp1, an AMP derived from citrus, exhibited low toxicity when tested on Galleria mellonella, while MTT assays revealed only modest cytotoxic effects on HT29 and Caco-2 cells [3].

The straightforward structure-activity relationship of AMPs makes them valuable for pharmaceutical development. They possess high water stability and solubility, further enhancing their potential [7]. Daptomycin, an AMP, has been utilized as an anionic antibacterial peptide to treat skin infections caused by Gram-positive bacteria. It has demonstrated inhibitory effects against S. aureus and Salmonella typhi, the causative agent of typhoid fever. AMPs have the potential to combat antibiotic-resistant bacteria and serve as effective antimicrobial agents [2]. The bactericidal action of AMPs is primarily attributed resulting in the creation of pores within the bacterial cytoplasmic membrane, disrupting ion flow and ultimately resulting in cell death. Therefore, AMPs offer a promising solution to tackle antibiotic resistance by serving as alternatives to conventional antibiotics [1]. Due to their powerful antibacterial properties, fast-acting kinetics, and wide range of effectiveness, AMPs are considered a viable class of antimicrobial drugs. However, one of the key challenges in utilizing AMPs for one limitation in treating microbial infections is the absence of specificity towards targeted microorganisms., potentially causing toxicity to normal cells. Some AMPs exhibit strong antibacterial effects but also display significant toxicity and hemolytic properties against normal cells [1].

1.7 Disadvantages of AMPs

AMPs have emerged as promising antimicrobial agents with the capability to address bacteria that have developed resistance to antibiotics, they exhibit the potential to combat antibiotic-resistant strains by primarily inducing the creation of openings in the cytoplasmic membrane of the bacteria is responsible for the formation of pores disrupting ion flow and ultimately leading to cell death [1]. This unique mechanism positions AMPs as viable alternatives to traditional antibiotics in the fight against antibiotic resistance. However, despite their distinct advantages, concerns have been raised regarding several limitations associated with their extensive use, which may contribute to the development of AMP resistance as bacteria adapt for survival [3].

One of the drawbacks of AMPs is their potential toxicity, as well as their immunogenicity and hemolytic activity in certain human cells. Additionally, the presence of high salt concentrations can potentially diminish the activity of AMPs, and the production costs associated with them can be substantial. These factors add complexity to the use of AMPs in medical applications. Differentiating between beneficial AMPs and those that may have negative effects presents a challenge. Some peptides have been found to induce adverse

effects, such as itching, burning, and discomfort, in mammalian cells in vitro. When administered systemically, they can disrupt cell membranes, leading to cell lysis. This motivates researchers to explore novel AMP molecules with lower toxicity profiles [1].

Furthermore, while AMPs generally have a low immunogenic response and do not interfere with host cell function, immunogenicity remains a concern in the development of peptide-based therapeutics. Structural features, such as modifications in peptide sequences (including modified amino acids) and the presence of aggregates, can influence the immunogenicity of AMPs. Additionally, certain AMPs exhibit hemolytic activity, which limits their therapeutic applicability. While some AMPs directly interact with host cells, resulting in their disintegration, most AMPs primarily bind to bacterial membranes through electrostatic interactions. It is noteworthy that amide peptides often display greater antibacterial activity, but also higher hemolytic potential compared to natural AMPs. The high amphiphilicity and hydrophobicity of AMPs contribute to their increased hemolytic effects [1].

Moreover, the hemolytic activity of AMPs can vary across different species. A study evaluating the hemolytic activity of 24 AMPs in cells from humans, dogs, rats, and cows revealed differences in response. Some AMPs exhibited minimal or no hemolytic activity in each species, while others showed varying degrees of hemolysis [2]. These factors underscore the challenges associated with the use of AMPs in medicine and highlight the need for further research to optimize their efficacy, minimize toxicity, and address concerns such as immunogenicity and hemolytic activity.

1.8 Approaches to tackle limitations:

1. Designing Peptidomimetics: Peptidomimetics based on endogenous peptides offer a promising approach in drug design, particularly as ligands for receptors, in comparison to antibodies or small molecules. The natural processing of endogenous precursor peptides, which undergo cleavage to produce biologically active metabolites, can be harnessed to develop treatments that predominantly the efficacy of these peptides is dependent on their active conformation. Peptides have traditionally been acknowledged for their ability to regulate cellular responses as receptor ligands, but emerging evidence suggests that they can also influence intracellular processes by targeting specific components. Peptides are continuously generated during cellular homeostasis through the breakdown and turnover of proteins. While most of these protein breakdown products are further hydrolyzed into individual amino acids for

protein synthesis or metabolic pathways, certain peptides serve as valuable templates for the development of novel therapeutic interventions. peptidomimetics.

2. Cyclization- The cyclization of a linear peptide can also be employed to improve the structural stiffness of the peptide. This can improve metabolic stability by locking the peptide into a less sensitive conformation to proteolytic enzymes (conformational limitations and/or selective molecular recognition).
3. Conjugation to polymer (e.g. PEGylation, PASylation)- peptide polymer conjugate (PPC) can be formed via coupling or polymerization of AMPs and functional polymers. Furthermore, PPC can self-assemble via electrostatic interaction, van der Waals forces, and other means to form a variety of different nanostructures, such as micelles, vesicles, and microspheres, to perform various specific functions.
4. Glycosylation- Glycosylation promotes protein global stability, and global stabilization is frequently accompanied by lower flexibility in the native form. It has previously been demonstrated that glycosylation improves LP23 stability against both heat and chemical denaturation. It helps in targeting particular organs and improving tissue biodistribution, improving penetration through biological membranes, boosting metabolic stability and decreasing clearance rate, receptor binding and amino acid side chain protection.

CHAPTER 2- LITERATURE SURVEY

Nanotechnology:

Conjugation of peptide to silver nanoparticle

Peptide conjugation with silver nanoparticles involves the joining together of peptides and nanoparticles to create a composite material with unique properties and applications. The term "conjugation" originates from Latin, meaning "to join together," and in organic chemistry, it refers to the connection of π systems, such as double bonds, resulting in their interconnection.

Nanotechnology utilizes distinctive characteristics the manipulation and utilization of materials at the nanoscale have become increasingly prominent across diverse industries, providing significant benefits and opportunities. It offers the potential for developing better designed and smarter products [15]. In the realm of medicine and healthcare, nanotechnology has paved the way for nanomedicine, which aims to combat prevalent diseases like cardiovascular disorders and cancer. Recent advancements in nanotechnology have revolutionized imaging techniques and drug delivery systems, as discussed in this review [15].

Nanoscience, the study of materials between 1 to 100 nanometers, serves as the foundation for nanotechnology. By manipulating structures at the atomic scale, nanotechnology enables the creation and modification of novel objects [15]. Nanomaterials, characterized by their at the nanoscale, they exhibit distinct properties in terms of optics, electricity, and magnetism., find applications in diverse fields, including electronics and medicine. Their remarkable functionality is attributed to their exceptional surface area-to-volume ratio. [16].

Nanomaterials operate on the principles of quantum mechanics, deviating from the conventional physics and chemistry rules. This allows for the design of functional systems and usable items at the molecular or atomic size. Nanotechnology has far-reaching implications across industries, offering enhanced products in areas such as healthcare, communications, and agriculture [16].

Nanomaterials are broadly classified into two categories. First, they can be blended or incorporated into existing products to enhance their overall performance by imparting unique

properties. Alternatively, nanomaterials like nanocrystals and nanoparticles can be directly employed to develop innovative and high-performance electronics, leveraging their distinct characteristics. These materials have the potential to revolutionize various industries due to their numerous advantages [16].

Nanomaterials are found in everyday products such as sunscreens, cosmetics, sporting goods, tires, and electronics. In the field of medicine, nanotechnologies have made significant strides in diagnostic procedures, imaging techniques, and medication administration [17]. Nanomaterials contribute to advancements in healthcare and the reduction of environmental impact in manufacturing by facilitating the mass production of cost-effective products with enhanced functionality and employing greener and cleaner manufacturing processes [18].

2.1 Nanotechnology in healthcare:

Nanomedicine, a field at the intersection of nanotechnology and medicine, focuses on utilizing nanoscale technologies and Nano-enabled approaches for medical applications. It encompasses a wide range of disciplines, including imaging, diagnostics, drug delivery, tissue engineering, implants, and therapeutics [19]. By harnessing the unique properties of nanomaterials and nanodevices, nanomedicine offers tremendous potential for advancing healthcare.

In the realm of diagnostics, nanotechnologies enable the development of highly sensitive imaging techniques and diagnostic tools that can detect diseases at early stages with improved accuracy [20]. Nanoparticles and nano sensors can be engineered to target specific biomarkers, allowing for precise disease identification and monitoring.

Drug delivery systems have been revolutionized by nanomedicine. Nanoparticles can encapsulate and deliver therapeutic agents to specific sites in the body, enhancing drug efficacy and reducing side effects. Nanocarriers can also overcome biological barriers and deliver drugs across cell membranes, enabling targeted and controlled release.

In tissue engineering, nanomaterials play a crucial role in scaffolds and implants. They can mimic the extracellular matrix and provide a favorable environment for cell growth, tissue regeneration, and organ transplantation. Nanotechnology enables precise control over the structure and properties of biomaterials, enhancing their biocompatibility and functionality.

Nanomedicine has also contributed to the development of innovative therapeutic approaches. Nanoparticles can be functionalized to selectively target diseased cells or tissues, delivering

therapeutic payloads with high precision. Additionally, nanoscale drug formulations can enhance drug solubility, stability, and bioavailability contribute to improved therapeutic outcomes.

Furthermore, nanotechnologies have shown promise in combating bacterial infections. Nanoparticles can be designed to possess antimicrobial properties, disrupting the integrity of bacterial cell membranes, or interfering with essential cellular processes. This opens new avenues for combating antibiotic-resistant bacteria and reducing the spread of infectious diseases.

Overall, nanomedicine holds great potential for revolutionizing medical practices and improving patient outcomes. Through the integration of nanotechnologies, we can envision more precise diagnostics, targeted therapies, enhanced drug delivery systems, and advanced tissue engineering approaches that will transform the landscape of healthcare.

2.2 Nanotechnology and cancer treatment:

Optimal efficacy of anticancer therapies relies on the precise delivery of therapeutic drugs to the target site without inducing any negative consequences. Surface modifications of nanoparticle carriers offer a strategy to enhance targeted drug delivery. One notable example of surface modification is the incorporation of PEG (polyethylene oxide) onto the nanoparticle surface. This modification not only improves the selectivity of drug absorption but also enhances the ability to target tumors. PEG incorporation prevents nanoparticles from being recognition by the immune system, enabling their circulation within the bloodstream until they reach the specific location of the tumor. An innovative application of this approach is the use of a vitamin E-based hydrogel in breast cancer treatment [21]. Herceptin, a monoclonal antibody used for breast cancer therapy, targets the human epidermal growth factor receptor 2 (HER2) on cancer cells [21]. The vitamin E-based hydrogel serves as a carrier for Herceptin, enabling sustained release over several weeks with a single dosage. The hydrogel-based drug delivery system demonstrates superior efficiency compared to traditional subcutaneous and intravenous routes, as Herceptin exhibits greater retention within the tumor. This approach enhances the anti-tumor efficacy of the drug. By harnessing the power of nanotechnologies, nanoparticles can undergo diverse modifications to extend their circulation time, enhance drug localization, optimize medication efficacy, and potentially mitigate the emergence of multidrug resistance [21].

2.3 Risk of nanotechnology:

While nanotechnology has garnered significant attention and interest from the public, it has also sparked debates regarding its safety and potential health hazards. The use of nanomaterials introduces new considerations in terms of understanding, predicting, and managing potential health risks [17]. Evidence has demonstrated that nanoparticles characterized by limited solubility can be more harmful, and toxic compared to larger particles on a mass-to-mass basis. Additionally, there are concerns about explosions and catalytic reactions associated with nanoparticles. However, it should be emphasized that only select nanomaterials, particularly those characterized by notable reactivity and mobility, are considered hazardous [18]. Until more comprehensive research provides evidence of the potential risks associated with nanomaterials, their mere presence in a laboratory setting does not pose a threat to human health or the environment [17].

Nevertheless, it is undeniable that nanotechnologies have contributed to enhancing the quality of life for patients by enabling advancements in the biotechnological, medical, and pharmaceutical industries [13]. These technologies have simplified healthcare procedures, ranging from diagnosis to therapeutic treatments and post-treatment monitoring. Ongoing efforts are focused on discovering and developing innovative nanomaterials that can revolutionize disease diagnostics and treatments, aiming for targeted, precise, potent, and long-lasting solutions. The ultimate goal is to make medical procedures more personalized, affordable, and safer. The future of nanotechnology lies in the responsible use of appropriate nanomaterials while minimizing any potential negative consequences [15]. It is of utmost importance to highlight that, akin to any other commodity, conducting risk assessments is vital prior to granting clinical and commercial approval for new nanotechnology-based products, in order to mitigate any plausible hazards to human health and the ecosystem [17]. Thorough life cycle assessments are imperative to ascertain the long-term sustainability and safety of their application [19].

2.4 Challenges and limitations of antimicrobial peptides and Nanotechnology:

The development of peptide-carrying drug delivery systems is still ongoing, and concerns regarding the safety of peptides in comparison to small molecule drugs are widespread. One drawback of peptide-based drugs is their susceptibility to in vivo proteolytic degradation. Examples such as Tyrothricin and Bacitracin, used as topical agents, have been associated with hemolysis and nephrotoxicity respectively [22]. Some antimicrobial peptides (AMPs)

tested in clinical trials have exhibited adverse effects and have been withdrawn from the market. This has prompted the pharmaceutical industry to focus on the topical application of AMPs rather than their parenteral administration, with the potential to address this limitation through the use of nanotechnology. However, the development of nano delivery systems comes with its own set of challenges.

Creating nano delivery systems involves various techniques that may expose peptides to organic solvents or subject them to shear stress through sonication or mechanical homogenization, which can affect their structure and activity [23]. The encapsulation of peptides within nano-carriers can lead to interactions between the peptide and carrier walls, influencing the release profile and potentially resulting in incomplete release. Stability and aggregation pose additional hurdles, as hydrophilic peptides may leak from hydrophobic systems in the presence of an aqueous environment. Some nano delivery methods require extreme temperatures that may denature the peptides [23]. Moreover, certain nano-carriers, like polymeric nanoparticles, rely on organic solvents during their fabrication, which can have toxic effects and impact the structure of encapsulated synthetic peptides, further complicating the use of AMPs in conjunction with nanotechnology [23].

Additional challenges encompass achieving optimal drug loading, selecting suitable polymers with acceptable safety profiles, and preserving the integrity of the peptides during processing and storage while maintaining their stability and bioactivity [22]. Overcoming these obstacles is crucial for successfully harnessing the potential of AMPs in combination with nanotechnology to enhance their safety, efficacy, and therapeutic applications.

2.5 AMPs-metal nanoparticles conjugates:

Researchers are seeking for a straightforward approach to make peptide nanoparticles due to the ease with which AMPs degrade. Metal nanoparticles, as we all know, have broad-spectrum antibacterial action and are widely employed in various medical and industrial goods, medical implants, and to battle multidrug-resistant bacteria. For a variety of uses, noble metal nanoparticles have been created [19]. They have been used for millennia to treat infections and are known for their exceptional antibacterial qualities on their own. These are desirable conjugation platforms to increase The effectiveness of antimicrobial peptides (AMPs) is attributed to their compact size and high surface area-to-volume ratio, among other unique characteristics. Among metal nanoparticles (NPs), silver (Ag) and gold (Au) NPs have been extensively studied due to their significant antibacterial properties. This article focuses

on the latest techniques for creating peptide-nanoparticle conjugates, with a specific emphasis on Ag and Au NPs. Advanced detection systems based on AgNPs and AuNPs offer enhanced sensitivity for detecting specific targets. In comparison to current Drug Delivery Systems (DDSs), metal NPs exhibit exceptional molecular-scale detection capabilities, providing significant advantages for therapeutic and diagnostic applications. Metal-based nanoparticles (MNPs), colloidal particles with distinct characteristics, possess unique optical behavior, electrical conductivity, and high thermal and chemical stability compared to their bulk counterparts. The bioconjugation potential of MNPs is a significant advantage, as they can selectively target cancer cells while minimizing damage to healthy cells, making them valuable for chemotherapy. The internalization and functionality of NPs are strongly influenced by their size and size distribution, which directly impact NP toxicity and in vivo dispersion. Smaller MNPs with larger surface area-to-volume ratios exhibit greater efficiency and reduced toxicity, resulting in enhanced microbicidal activity. Moreover, smaller nanoparticles exhibit prolonged circulation time without detection by immune cells, as phagocytes primarily remove particles larger than 200 nm. They are rapidly cleared from tissues through extravasation or renal clearance, thereby preventing accumulation in organs such as the liver and spleen.

MNPs come in various shapes, with spherical being the most common, although experiments have explored cubes, stars, rods, cones, and cages. NP shape is primarily determined by the surface-to-volume ratio, which influences the accessible surface area for biomolecule binding. Multivalence on the MNP surface can be achieved through functionalization with abundant functional ligands, ensuring conjugate binding to the target. However, polydispersity in MNPs can impact their biological activity. AMP-AgNP/AuNP conjugates exhibit cellular toxicity toward healthy cells and the negatively charged nucleus of eukaryotic cells. Despite the potential of AMPs, their widespread use is limited due to lower target binding affinity and selectivity compared to proteins, vulnerability to protease digestion in biological environments, short circulating half-lives necessitating frequent administrations, and limited ability to maintain innate folding structures when isolated from protein contexts [23].

Recent studies have demonstrated the effectiveness of nanoparticles in various biomedical applications, including drug delivery, diagnostics, and therapeutics, offering promising avenues for the development of novel treatments and diagnostic tools. However, further research and refinement are necessary to fully exploit the potential of nanoparticle-based

systems for enhancing medical interventions. technique to kill bacterial infections, including drug-resistant bacteria, and have a low likelihood of pathogen resistance development. Nanoparticles have emerged as promising candidates for targeted drug delivery, offering numerous advantages in terms of their ability to transport medications to specific sites in the body. When nanoparticles are combined with antibiotics, they exhibit synergistic effects that enhance antibacterial properties, allowing for reduced drug doses and minimizing toxicity to human cells. This approach of using nanoparticles as carriers for antibiotics represents a promising strategy for antibiotic therapy, delivering drugs directly to the site of infection. Additionally, silver nanoparticles (AgNPs) possess a unique property known as plasmon resonance, which can be harnessed for photo-activated drug release, further enhancing the precision and control of drug delivery [24].

2.6 Benefits of conjugation

In order to enhance its properties, the peptide was coupled with silver nanoparticles, leveraging the potential of nanotechnology in diagnostics and drug delivery. Nanomaterial-based drug delivery strategies have the capability to improve drug pharmacokinetics and pharmacodynamics. Various nanoparticles capable of binding drugs have been developed to combat drug-resistant bacterial infections, benefiting from their larger surface area for efficient drug administration. Silver, known for its antimicrobial properties against a wide range of pathogens, including bacteria, fungi, protozoa, and certain viruses, has been used by antibiotic-resistant bacteria to survive [25].

The combination of peptide and silver nanoparticles has shown significantly enhanced stability and activity compared to individual components. With its distinct properties, this molecule holds promise as a potential solution to antimicrobial resistance [26]. Encapsulating antimicrobial peptides (AMPs) within nanocarriers can improve therapeutic efficacy and selectivity by providing a protective shield and enabling controlled release directly into bacterial cells. Recent studies have explored the use of various nanostructures, such as nanofibers, nanovesicle-coated metallic nanoparticles, and self-assembled structures, for AMP delivery, with polymeric nanoparticles being the most promising approach [25].

The surface plasmon resonant characteristics of silver nanoparticles have been investigated in relation to aggregation, uptake, and interaction in living cells using advanced illumination techniques. Silver and its compounds have long been recognized for their antibacterial

properties, finding applications in water and food sanitation as well as infection control in medicine. Additionally, the optical characteristics of silver nanoparticles have attracted attention for advanced photonic and sensor applications [27].

Silver nanoparticles have garnered significant interest due to their remarkable antibacterial and catalytic capabilities. However, the use of hazardous compounds and the high energy consumption associated with their synthesis pose limitations for large-scale production. As an alternative, the biosynthesis of silver nanoparticles using microorganisms such as fungi, bacteria, and yeast has gained popularity as a simpler and more environmentally friendly method. Biologically synthesized silver nanoparticles exhibit stronger antibacterial activity and offer better control over size and shape compared to conventional chemical and physical processes [28].

By exploiting weak non-covalent interactions, natural biomolecules like peptides and proteins can form sophisticated supramolecular nanomaterials incorporating metals. The use of harmful chemical reagents in the synthesis of silver nanoparticles raises concerns for their biological applications. Supramolecular chemistry approaches have the potential to address this challenge in the design of peptide and protein-based nanomaterials [29]. The solubility of drugs plays a crucial role in their effectiveness, biocompatibility, biodegradability, and selectivity in therapy. Silver, due to its natural anti-inflammatory properties against various diseases, holds promise for enhancing therapy efficiency. In previous sections, we discussed assembly methodologies for silver-incorporating peptide and protein supramolecular nanomaterials.

The significance of silver-incorporated peptide and protein nanoparticles in drug delivery is further discussed, highlighting the role of biomolecules in improving biocompatibility as reducing and capping agents. Key considerations for designing drug delivery strategies involving silver-induced nanomaterials include stability, biocompatibility, biodegradability, absence of toxic chemicals in synthesis, capability for reactive oxygen species generation, and high drug payload capacity. Through ongoing research and development, the potential of these innovative nanomaterials for effective and targeted medication delivery continues to be explored.By leveraging coordination chemistry, silver metal can form complexes with a wide range of drugs that possess amine and carboxylic acid functional groups, leading to increased payload capacity in silver-based nanomaterials. What makes the use of silver-based supramolecular peptides and proteins as drug carriers particularly fascinating is the simplicity

of the synthesis approach, which does not necessitate harsh conditions. These nanoparticles can be synthesized in aqueous solutions at room temperature using standard laboratory equipment, making the process efficient and accessible.

The unique advantages offered by biological nanoparticles set them apart from conventional nanomaterials like carbon nanotubes and silicon nanostructures, which often require specialized equipment and high temperatures for production [29].

One promising approach for the green synthesis of silver nanoparticles involves the use of peptides and proteins as building blocks. These peptide and protein-based silver nanomaterials exhibit favorable physical characteristics. It is worth noting that silver has a well-established history of use in antimicrobial therapies. However, the biocompatibility of silver in its elemental form may raise concerns [29].

The utilization of silver nanoparticles to enhance the surface-to-volume ratio has proven to be an effective approach in increasing the bactericidal activity of silver ions.

By increasing the contact area with bacteria, the enhanced surface-to-volume ratio allows for more efficient interaction between silver nanoparticles and bacteria. In order for silver to exhibit its antibacterial effects, it must be in its ionic form. Therefore, maximizing the contact area of the nanoparticles enables a greater number of silver ions to interact with bacteria, leading to damage through multiple pathways. Silver nanoparticles have garnered significant interest in the field of wound care systems [30].

Similar mechanisms underlie the antimicrobial action of silver nanoparticles. The primary advantage of nanoparticles lies in their nanoscale size, which enhances their efficacy. The antibacterial activity and mode of action of silver nanoparticles have been studied by investigating membrane permeability, structural morphology, and the proliferation of E. coli. The data collected indicates an increase in the leakage of reducing saccharides and proteins, suggesting an augmentation in membrane permeability. The outer lipopolysaccharide (LPS) membrane plays a role in determining the permeability of the bacterial membrane. The shape and size of silver nanoparticles influence their antibacterial properties [30].

Nanomaterials can be found in various everyday products such as sunscreens, cosmetics, sporting goods, tires, and electronics. In the field of medical advancements, nanotechnologies have revolutionized diagnostic methods, imaging techniques, and drug delivery systems [15]. Nanoparticle drug delivery systems achieve specificity by combining drugs with nano-scaled

radioactive antibodies that are complementary to antigens on cancer cells. These approaches have demonstrated improved drug bioavailability, targeted drug delivery, and enhanced uptake of poorly soluble drugs [15].

Synthetic nanoparticles encompass a range of materials, including polymer conjugates, polymeric nanoparticles, lipid-based carriers like liposomes and micelles, dendrimers, carbon nanotubes, peptides, and gold nanoparticles, including nanocages. Due to their high surface area-to-volume ratio, they can accommodate a high density of ligands on their surface for targeted applications [31]

Nanoparticles consist of two main components: The physicochemical properties of the core material can be finely tuned by the presence of a surface modifier, allowing for precise control over nanoparticle characteristics. When proteins interact with nanoparticles, they can induce changes in protein conformation and promote local protein accumulation on the nanoparticle surface, which in turn may lead to aggregation. However, nanoparticles have the ability to capture and trap early aggregating intermediates, effectively inhibiting further aggregation processes [31]. With their high Gibbs free energy, nanoparticles exhibit an exceptional capacity for adsorption. By conjugating proteins to nanoparticles, not only is the system stabilized, but it also introduces biocompatible functionalities that enable additional biological interactions and connections. Furthermore, nanoparticles offer the advantage of carrying functional groups, such as amino and carboxylic groups, that can be leveraged for surface modifications. Additionally, nanoparticles demonstrate superior storage stability, in vivo stability following injection, and ease of customization during the manufacturing process [31].

The antibacterial activity of these nanoparticles is highly influenced by their size, shape, and surface modifications. When silver nanoparticles (AgNPs) are combined with other antimicrobial agents, their antibacterial effects are enhanced. Several research institutions have explored the potential of antibiotics to chelate with nanoparticles through the hydroxyl and amide groups present in antibiotic compounds. In human medicine, silver nanoparticle-based products have been utilized for wound dressings, impregnating catheters, and even during surgical procedures. Similarly, in veterinary medicine, silver nanoparticle-based treatments are often administered as drops, ointments, sprays, or gels to combat bacterial infections [32].The proper surface coating of nanoparticles plays a crucial role in improving their biological characteristics, such as reducing aggregation and enhancing solubility.

Biomimetic surface coatings can provide biocompatibility and facilitate interactions with biological systems [33].

Targeting angiogenesis in a tumor's microenvironment can allow tumour angiogenesis to be suppressed and anticancer medications to be delivered inside the tumour. Targeted anti-angiogenesis methods of administration that employ passively targeted approaches such as Improved Targeting tumors specifically can be accomplished by leveraging the Enhanced Permeability and Retention (EPR) effect and employing specific receptor-mediated interactions (active targeting). Peptides attached to silver nanoparticles have ability to combine as anticancer agents for drugs in one targeted anti-angiogenesis method[34]

Conjugation of antimicrobial peptides (AMPs) to tailored polymers offers an intriguing strategy to enhance AMP activity and stability. The design of the polymer must consider factors like availability of suitable functional groups, biocompatibility, biodegradability, and molecular weight within the renal excretion limit. Achieving a delicate balance between amphiphilicity and hydrophobicity is crucial in selecting appropriate AMP and polymer structures for conjugation. PEGylation, using PEG as the polymer, is a widely employed technique that enhances pharmacokinetics by prolonging half-life, increasing water solubility, reducing renal clearance, protecting against proteolytic degradation, and reducing immunogenicity by shielding antigenic epitopes. The coupling chemistry employed varies depending on the functional group, with N-hydroxysuccinimide (NHS) esters being commonly used for modifications involving amine groups (N-terminus or lysine). NHS esters exhibit high efficiency in linking to accessible amines.

2.7 Synthesis and Characterization of Short Peptide based molecules

The production of novel, elective nanostructures can deal with or potentially exemplify remedial/symptomatic synthetic substances while bringing down their harmfulness, upgrading their flow, and expanding in-vivo focusing on is an extreme yet fundamental undertaking. Peptides are among the clever materials produced using regular parts that stand out enough to be noticed attributable to their straightforward design, extremely high synthetic and actual solidness, assortment of arrangements and structures, effortlessness in (bio)molecular functionalization, and potential for mass amalgamation. Because of their innate biocompatibility, some of them can self-gather into nanotubes, - circles, - vesicles, or - shafts under innocuous circumstances. This opens up extra open doors in science and nanomedicine. through amino-and carboxyl get-togethers, their biodegradability,

manufactured reactivity, and surface strength. The design, size, plan, and surface study of these nanoplatforms can be acclimated to deliver nanostructures appropriate for natural purposes. The production of the direct peptide utilizing homogeneous coupling conditions, strong stage peptide combination, cluster handling, or stream science is the underlying move toward the blend of peptide nanoobjects. Second, the nanoobject will self-collect from the peptide. The sequence of the peptide will determine its auto-assembling characteristics, which may be influenced by outside variables including pH, temperature, chemical or light stimulation, as well as microfluidics. a procedure for producing peptides that involves gradually incorporating safeguarded amino acids into a peptide chain that is shaping and covalently connected to a strong sap molecule. This procedure simplifies it and speedy to eliminate the synthetic substances and waste from the pitch. Nano peptides gather diversely contingent upon the pH, temperature, ionic strength, salt substance, and dissolvable sort of the arrangement. As well as dealing with the outside natural elements influencing peptide auto-gathering, we can feature numerous manufactured strategies and systemic devices that can be utilized to drive on-line peptide self-gathering. It is important to change the sturdy nanostructures' construction, size, structure, surface science, and self-gathering for organic applications. Prior to involving them in biomedical applications, the gathering and physico-substance properties of short peptides should be assessed. The presentation of the amino acids should be overseen all through the short peptide combination. The two essential parts that manage the powers coordinating the self-gathering of peptides are the actual driving elements and the natural conditions. We will presently talk about their consequences for peptide self-congregations and its suggestions. At the point when peptide particles and their gatherings are in harmony in a watery arrangement, they take on the compliance that limits their all-out free energy. Hydrogen-bonding, π-πstacking, electrostatic, and hydrophobic interactions are the major factors engaged through bond interactions, the overall stability for assemblies. These non-covalent interchanges are genuinely unimportant for single peptides, yet when they get together, they can have energies that are commensurate to a weak covalent security. They decide the thermodynamically steady supramolecular structures and manage the state of peptides. The qualities of the peptide family or amino corrosive grouping will decide the sort of cooperation that controls the gathering. Truth be told, aliphatic (A, V, L, I, and M) and sweet-smelling (W, Y, and F) hydrophobic amino acids establish an overall hydrophobic climate and can partake in - stacking, which is significant for peptide collapsing, separately. The course of self-gathering is vigorously affected by ecological factors notwithstanding the intermolecular collaborations between peptide chains. We can list the pH, ionic strength,

temperature, peptide fixation, dissolvable extremity, and outer boosts here, in addition to other things. By changing the powers that these natural impacts can adjust the equilibria between various designs or even inside a similar construction, changing the last morphology. They can connect with peptides and in this way cause an adjustment of the primary compliance of those peptides (for instance, minimization). The Van der Waals and hydrophobic cooperations between self-gathered designs will be basically affected by changes to the peptide focus or dissolvable sort, though electrostatic associations between side chains as well as among peptides and dissolvable will be modified by changes to the solubilization medium's pH and ionic strength. To take advantage of supramolecular structures as medication conveyance frameworks, a few creators have concentrated on the underlying changes brought about by pH and tried different things with it to incline toward the ideal design or give the construction explicit highlights.

2.8 Characterization

Self-gathered peptide nanostructures might be portrayed with regards to their worldwide construction (peptide arrangement, size, measurement, charge thickness, shape, and CAC), engineering (auxiliary, tertiary, and quaternary design), and impact of a few outer improvements (pH, salts, temperature, and so on.). These portrayals are pivotal for planning the most biocompatible and bio distributable nanostructures with the ideal attributes under organic conditions for biomedical applications. Various characterization methods should be utilized couple because of their dynamic self-get together cycles, taking into consideration broad time and length scales. The standard strategies at present being used are either minute (atomic power microscopy [AFM], fluorescence microscopy]), dissipating [X-beam diffraction, little point Xbeam dispersing [SAXS], dynamic light dissipating [DLS]], or spectroscopic [nuclear attractive reverberation [NMR], infrared [IR], Raman, fluorescence, roundabout In specific examples, hypothetical and computational strategies are utilized to support the defense of exploratory information and the explanation of oneself collecting component. Imaging strategies (SEM, TEM, AFM, optical microscopy) that likewise put an accentuation on actual qualities, like warm, substance, and conformational strength [116], as opposed to primary association, can be utilized to decide the self-gathering morphology and mathematical boundaries (size, breadth) Figuring out the security attributes, vibrational modes, and covalent and noncovalent collaborations of the peptide structures engages the spectroscopic gadgets (Disc, NMR, Fourier-Change IR (FTIR)) to choose the discretionary plan (- helix, - sheet, erratic circled similarity, in a 5 nm range).Their designing could change

depending upon whether the assessment is finished in a response or a solid state. Peptide nanotubes have various constraints when used, as shown by X-bar crystallography and plan NMR, in light of their high nuclear weight, non-clear, and sometimes insoluble individual, as well likewise concerning enhancements responsive exceptional turns of events. Some assessment depicts the portrayal of dipeptides to make sense of their designing during self-get-together or to uncover cogathered nanostructures between two unquestionable dipeptides, as well as the effect of the solubilization media or the sound judgment overwhelming designs with different synthetic substances. After a couple of time of get together, AFM exhibited the production of circular micelle-type totals, which were then reworked into fibril helical designs with lengths of a few micrometers.CD with AFM photos for secondary structure. Peptide electrokinetic separations are effective biological compound characterisation techniques. They offer subtleties on peptide immaculateness, physico-synthetic and biochemical portrayals in their different configurations (old style or microprocessor), modes (forward looking and cross variety modes), modes (zone electrophoresis, isotachophoresis, gel electrophoresis, isoelectric focusing, affection electrophoresis, and methods (isoelectric focusing, gel electrophoresis, and electrophoresis). Different identifiers, including as UV, fluorescence, chemiluminescence, electrochemical, electrochemiluminescence, MS, NMR, and infrared spectroscopy, can be associated with electrokinetic partitions. Since electrokinetic techniques empower the assurance of actual properties like viable charge, dissemination coefficients, causticity (ionization) constants (pKa) of ionogenic gatherings, and restricting, they seem promising for an exhaustive physico-synthetic portrayal of peptides and their get together (affiliation, dependability, development, separation). For an intensive physico-synthetic portrayal of peptides and their get together, electrokinetic techniques seem promising on the grounds that they empower the assurance of physicochemical properties like powerful charge, pI, Mr, Stirs up radii, dispersion coefficients, causticity (ionization) constants (pKa) of ionogenic gatherings, and restricting affiliation, soundness, development.

CHAPTER 3- MATERIALS AND METHODS

Materials

The rink amide MBHA resin and Fmoc-Protected ornithine (Fmoc-Orn(Boc)-OH) were provided by Novabiochem (Mumbai, India). For acylation, myristic acid (Loba chemie), palmitic acid (Loba chemie), and stearic acid (Loba chemie) were used as fatty acids. N-hydroxybenzotrizole (HoBt), N'N- diisopropylcarbodimide (DIC), piperidine, N'N dimethylformamide (DMF), dimethylsulphoxide (DMSO), dichloromethane (DCM), and trifluoroacetic acid (TFA) were employed in the solid phase synthesis of lipidated peptidomimetic compounds. All purification solvents were of HPLC quality and obtained from Merck. Buffers were prepared using double-distilled water.

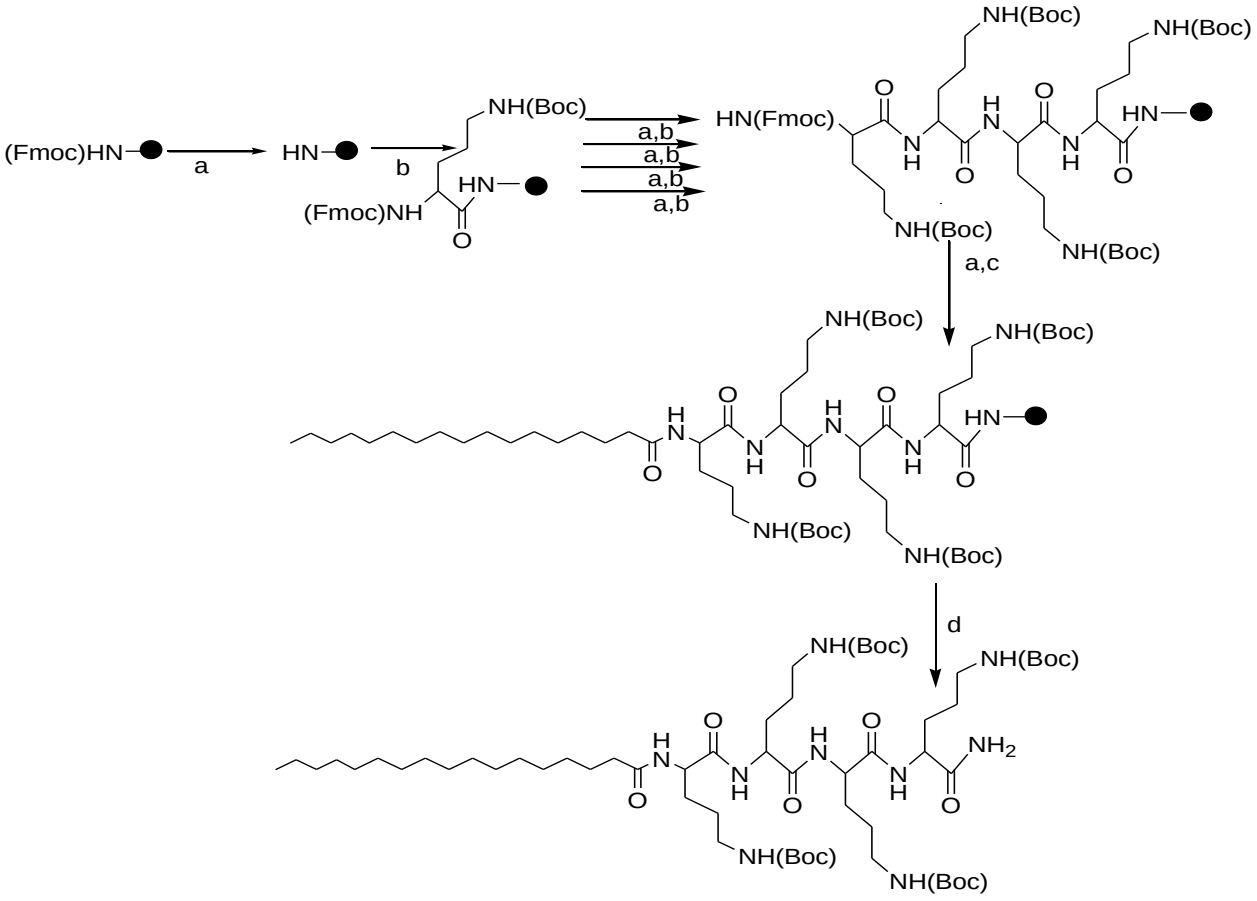
Methods

Peptide Synthesis- The SPPS fundamentals are given together an appraisal of the linkers and supports at present being used. Essential thoughts for the different SPPS processes, including mooring, deprotection, coupling response, and cleavage, as well as the possible issue of conglomeration and side responses, are totally covered. The presentation of key procedures, such as final cleavage, disulfide bridge, Fmoc-deprotection, resin handling, coupling, capping, and creation, is necessary for the synthesis of completely deprotected peptides.

LP 23 was made from rink amide resin using the conventional “Fmoc solid-phase synthesis”. Before being lyophilized for two days, the crude products were washed with cold ether at least eight times. The resulting compounds were exceptionally pure (>95%), according to analyses employing by HPLC method for purification. The peptide products were extremely soluble in water based solutions. After being dissolved in phosphate-buffered saline (PBS) (10 mM, pH 7.2) or water at a concentration of 2.0 mM, the peptide solutions had been sonicated in a wash bath for 30 minutes.

The SPPS fundamentals are given together an appraisal of the linkers and supports at present being used. Essential thoughts for the different SPPS processes, including deprotection, coupling response, and cleavage, as well as the possible issue of conglomeration and side responses, are totally covered. The presentation of key procedures, such as final cleavage,

disulfide bridge, Fmoc-deprotection, resin handling, coupling, capping, and creation, is necessary for the synthesis of completely deprotected peptides.



a: Deprotection with 20% piperidine in DMF
b: Fmoc-orn-boc/DIC/HoBt in DMF
c: Fatty acid/ DIC/HoBt in DMF
d: Cleavage (TFA:TIS:water, 95:2.5:2.5)

Fig 1: - Mechanism of LP-23 by Solid Phase Peptide Synthesis

Peptides were created using standard Fmoc SPS protocol using Rink MBHA resin as solid support. Deprotection of Fmoc protected resin was done using 20% piperidine in DMF. Once Fmoc group was removed, first Fmoc- protected amino acid (4 equivalent), HOBt (2 equivalent) and DIC (2 equivalent) in 2 ml DMF was coupled. Kaiser test verified coupling completion. The Fmoc group of the connected amino acid was deprotected after the initial coupling was completed. This was followed by the coupling of second fmoc protected amino acid. The deprotection and coupling processes were repeated several times until the desired peptide collection was built on resin. Finally, Fmoc group from last Fmoc-protected amino

acid coupled to resin was deprotected and the peptide's solid support was broken down using TFA cocktail. Cleaved peptide was precipitated in chilled diethyl ether. Diethyl ether was decanted and 6ml (ACN,water, 1:1) was added and kept at -80℃ for 4 hours before lyophilization. Samples were lyophilized and stored at -20℃.

Kaiser Test: - To determine free amino functional groups, utilise the Kaiser test kit. This test kit is frequently used in solid-phase peptide synthesis (SPPS) to ensure that incomplete peptide chains are not generated by checking the precision of amino acid coupling processes. The test includes 50 mL of ninhydrin solution and 50 mL of phenol + KCN solution as reagents. The Kaiser test is a primary amine assay that is extremely sensitive. It is extensively employed in solid phase peptide synthesis to check the completion of coupling processes. When ninhydrin reacts with the peptide-resin's deprotected N-terminal amine group, it generates a vivid blue colour. Ninhydrin has the ability to identify amine groups in proteins and amino acids. Because skin contains amino acids, ninhydrin becomes blue when it comes into touch with it. Amino acids have two functional groups, one carboxylic acid and the other an amine group [35].

Fig 2: - Blue colour shows Positive result of Kaiser Test

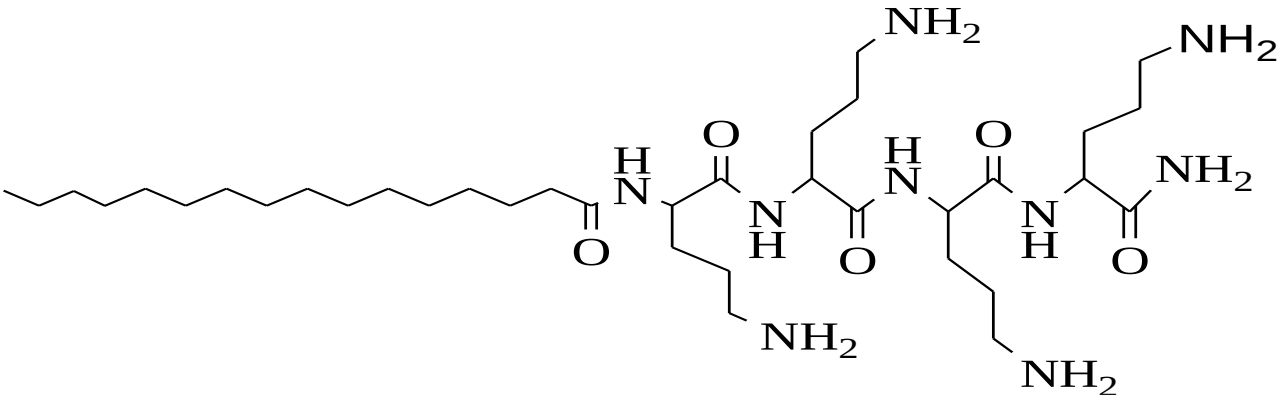


Fig 3: - Structure of LP 23

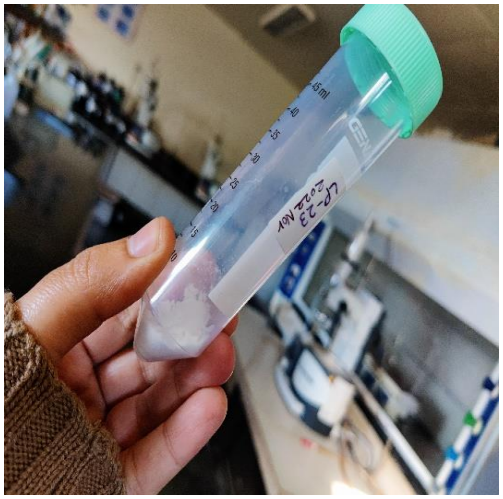


Fig 4:- Final Product of LP 23 after synthesis by solid phase method

Purification

Peptide LP-23 was examined using reverse-phase high performance liquid chromatography (RP-HPLC) with a C18 Waters column. The mass of LP-23 was determined through mass spectroscopy. For the HPLC analysis, a linear gradient method was employed, where Solution A (0.1% TFA in water) and Solution B (0.1% TFA in acetonitrile) were used as the mobile phase. The analysis was performed over a 30-minute period at a flow rate of 1 mL/min to separate and purify the peptide.

Nano-conjugate preparation- There are various phases involved in making nano-conjugates, and these procedures are covered in the following sections:

Silver nanoparticle preparation- silver nitrate (AgNO3) was converted into citrate capped silver nanoparticles (Ag NPs), with a diameter of around 10 nm, using sodium borohydride was used as the primary reducing agent, while trisodium citrate served as both the reducing and capping agent. The mixture of 2 mM sodium citrate and 2 mM sodium borohydride was combined using a magnetic stirrer at 60 °C for a duration of 30 minutes. The solution was then given 1.17 mM silver nitrate while the temperature was maintained at 90°C to produce the nanoparticle solution. Yellow to deep yellow coloration suggested the production of silver nanoparticles. A chemical approach was used to validate the Nano-silver creation, and the addition of sodium borohydride and trisodium citrate fully decolorized the yellow color [36].

Fig 5: - Change in color demonstrating development of silver nanoparticles

Nanoconjugate preparation- The conjugates were made by combining 500 µl of 2mM of AgNP at 298 K with 500 µl of 1 mM peptide (dissolved in water or buffer). AgNP is consequently diluted to 10 g/mL.

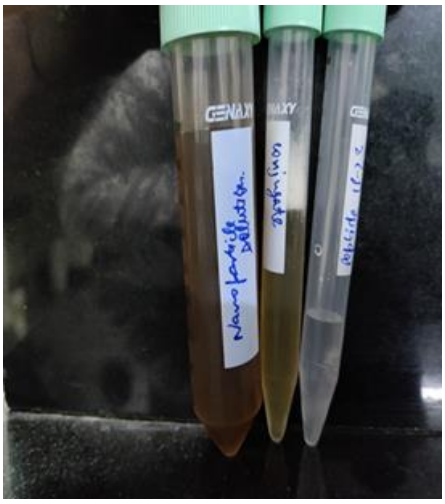


Fig 6: - Conjugate Solution

UV-VIS Spectroscopy- The UV-visible spectrum of Silver Nanoparticle and peptide LP-23 conjugates were obtained on a Thermo-Scientific UV-1800 UV-Vis spectrophotometer, equipped with a 1 nm slit width, utilizes a quartz cuvette with a 1 cm path length and operates within a wavelength range of 200-800nm. The silver nanoparticles-peptide conjugates were studied, and the results confirmed the presence of conjugation. Because of the existence of silver nanoparticle conjugation to the peptides, the UV spectroscopic peaks shifted from 421 to 410 nm as shown in figure.

Antibacterial Susceptibility Test-

Well-diffusion assay

1.The strains of bacteria were obtained from the Department of Biotechnology/Bioinformatics (JUIT).

2. A single colony were selected from culture plates and put into Luria broth, which was incubated at 37 degrees for 24 hours. The turbidity of the cultures was compared to the 0.5 Mc Farland standard after nightly growth.

3.McFarland standards are used as a guide, representing the volume in (1.5 x CFU/mL).

4. 0.05mL of 1% $BaCl_2$ and 9.95mL of 1% H_2SO_4 = 0.5 McFarland Solution.

5. For screening, a well-diffusion test was employed using samples at concentrations of 5mg/mL.

6.By serially diluting the bacterial inoculum, a bacterial suspension containing 1.5 x CFU/ml was generated.

7. 150uL of this bacterial solution was then equally dispersed over LB agar plates.

8. Wells were created and 50uL of sample dissolved in distilled water were poured into them.

9.Tetracyclin were employed as a positive control by the concentration of 1mg/ml.

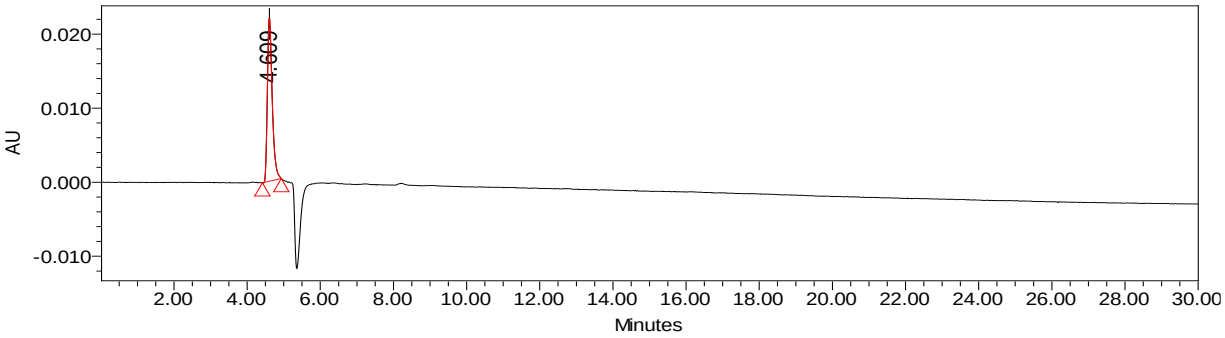
10. After incubating the plates at 37 degrees for 24 hours, the zones of inhibition were determined and compared to positive control that is tetracyclin.

CHAPTER 4- RESULTS

To resolve existing issues in the huge scope combination of manufactured peptides, enhancements and advances in the materials and reagents used for the amalgamation (tars, amino corrosive subsidiaries, coupling reagents, and so on) and refinement strategies have been made. With an accentuation on bringing down creation costs, raising yield and immaculateness, and getting confounded modifications, peptides are being made. Normally, peptide firms give peptides up to 15-20 amino acids at a sensible cost, both with and without non-regular or changed amino acids. The combination of longer peptides or peptides with post-translational adjustments is challenging for peptide researchers since they should manage conglomeration processes during the amalgamation, which prompts low yields and purities and habitually brings about inability to get the expected peptide. Current strategies for making longer peptides incorporate substance or enzymatic ligation. The use of Cys at the principal section's N-terminal is an unquestionable requirement, which is a disadvantage of this methodology. Longer peptides might be integrated utilizing Stake gums, pseudo prolines, and the expansion of chaotropic salts. Regardless of the way that SPPS is currently the best choice for peptide amalgamation, there are still issues that should be settled. Since huge scope Stake and unsaturated fat-based peptide changes can't be motorized, they are right now completed physically utilizing ordinary natural manufactured science. N-methylated amino acid incorporation is frequently challenging due to steric hindrance. Another unsolved problem is speeding up automated solid-phase peptide synthesis.

In this study we synthesize Lipopeptide LP 23 with palmitic fatty acid chain which carries 16 carbon in its chain. In the structure of LP 23 we join ornithine amino acid which increases the hydrophobicity of this compound.

HPLC Chromatogram of LP-23 at 220nm



Sr.no.	Retension time (min)	Area (µV*sec)	%area	Height (µV)
1	4.609	202761	100	22223

Table 1

Their molecular weight was confirmed using mass spectrometry (MS). Following that, purified HPLC fractions were lyophilized. 711.59 is the estimated mass, while 712 (M+1) is the calculated mass.

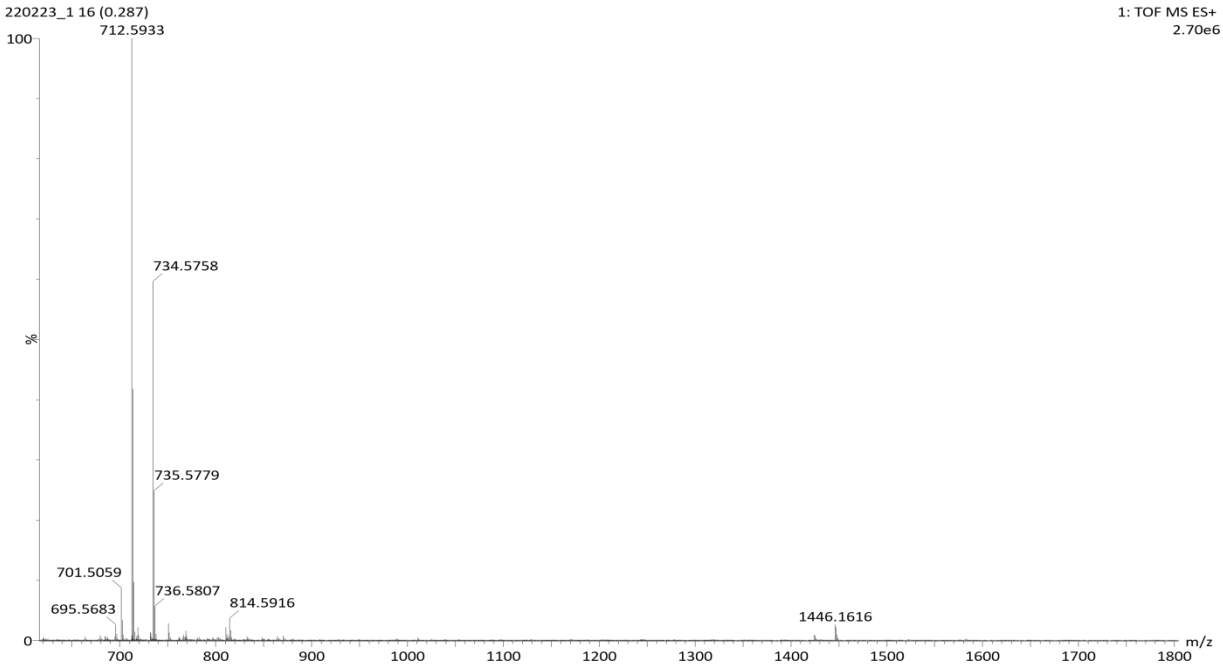


Fig 7: - Mass Spectrum of Peptide LP-23

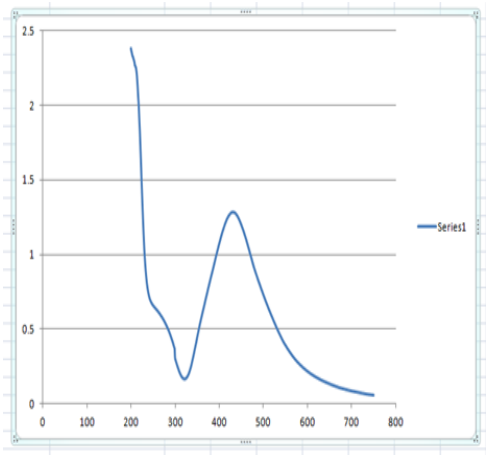


Fig 8: -UV Spectrum of Silver Nanoparticles

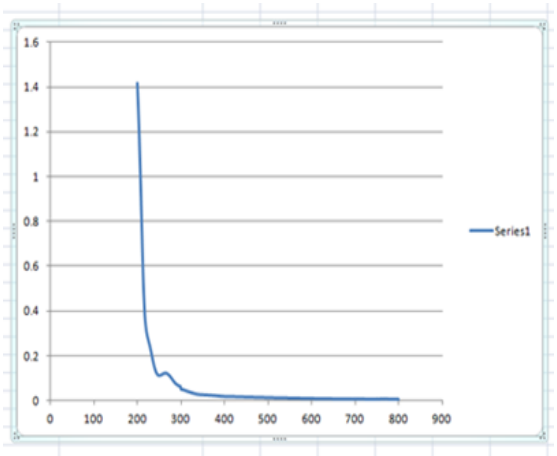


Fig 9: - UV Spectrum of Peptide LP-23

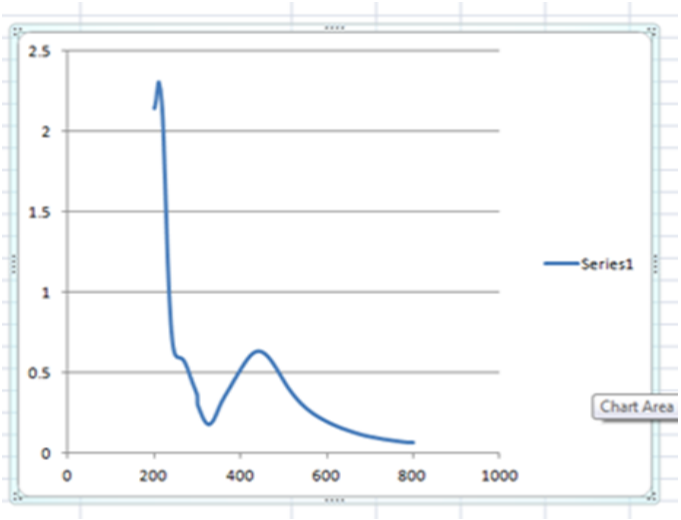


Fig 10 :- UV Spectrum of Peptide Conjugate with Silver Nanoparticles

Antibacterial activity:

Antibacterial activity of nanoparticles, peptide LP23 and conjugate (Nanoparticle + peptide) were tested against selected micro-organisms.

Our conjugate showed significant anti-microbial potential against the tested micro-organisms. *Staphylococcus aureus* and *E. coli* both shows susceptible organism as it showed greater zones of inhibition.

$$\text{ACTIVITY INDEX} = \frac{\text{ZONE OF INHIBITION OF SAMPLE}}{\text{ZONE OF INHIBITION OF REFERENCE}}$$

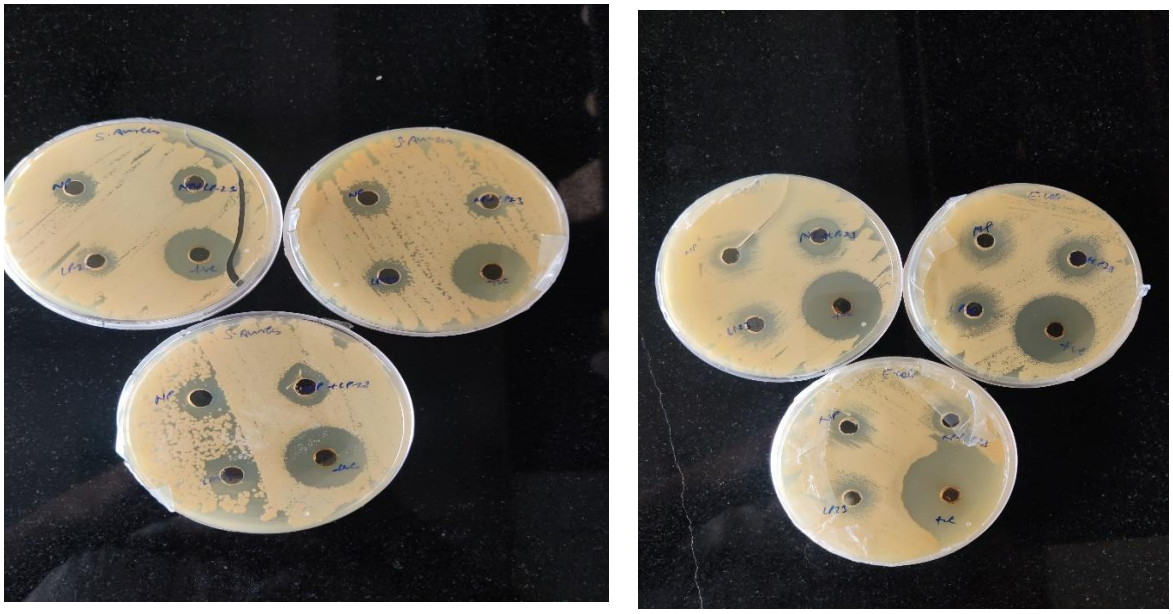


Fig 11: - Antibacterial evaluation results for standard reference strains: *E.coli* ATCC 25922 and *Staphylococcus aureus* MTCC 3160

	Zone of inhibition in cm for the strain *E.coli* ATCC 25922			
	Nanoparticle ($50\mu l/ml$)	LP 23 ($50\mu l/ml$)	Conjugate (Peptide LP 23) ($50\mu l/ml$)	Positive Control (Tetracyclin) (1mg/ml)
Plate 1	15mm	14mm	17mm	35mm
Plate 2	19mm	16mm	15mm	29mm
Plate 3	17mm	13mm	18mm	30mm

Table 2

	Zone of inhibition in cm for the strain *S. aureus* MTCC 3760			
	Nanoparticle ($50\mu l/ml$)	LP 23 ($50\mu l/ml$)	Conjugate (Peptide LP 23) ($50\mu l/ml$)	Positive Control (Tetracyclin) (1mg/ml)
Plate 1	15mm	12mm	15mm	25mm
Plate 2	14mm	11mm	15mm	26mm
Plate 3	14mm	14mm	14mm	27mm

Table 3

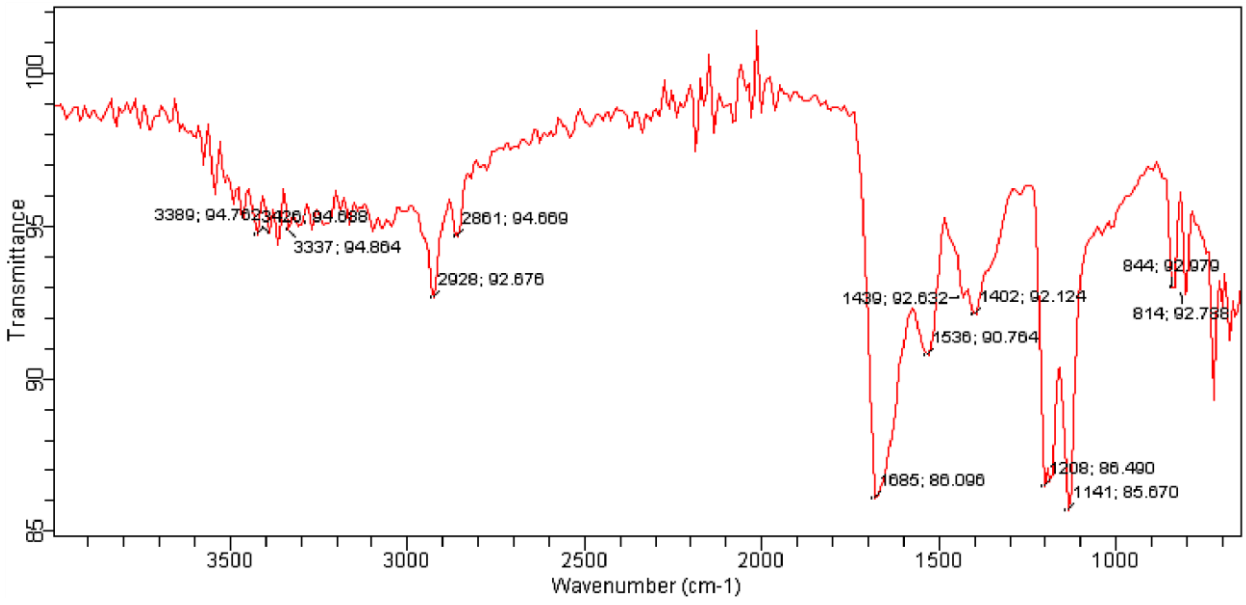


Fig 12 : - FTIR Spectrum of Conjugate (Peptide+Silver Nanoparticle)

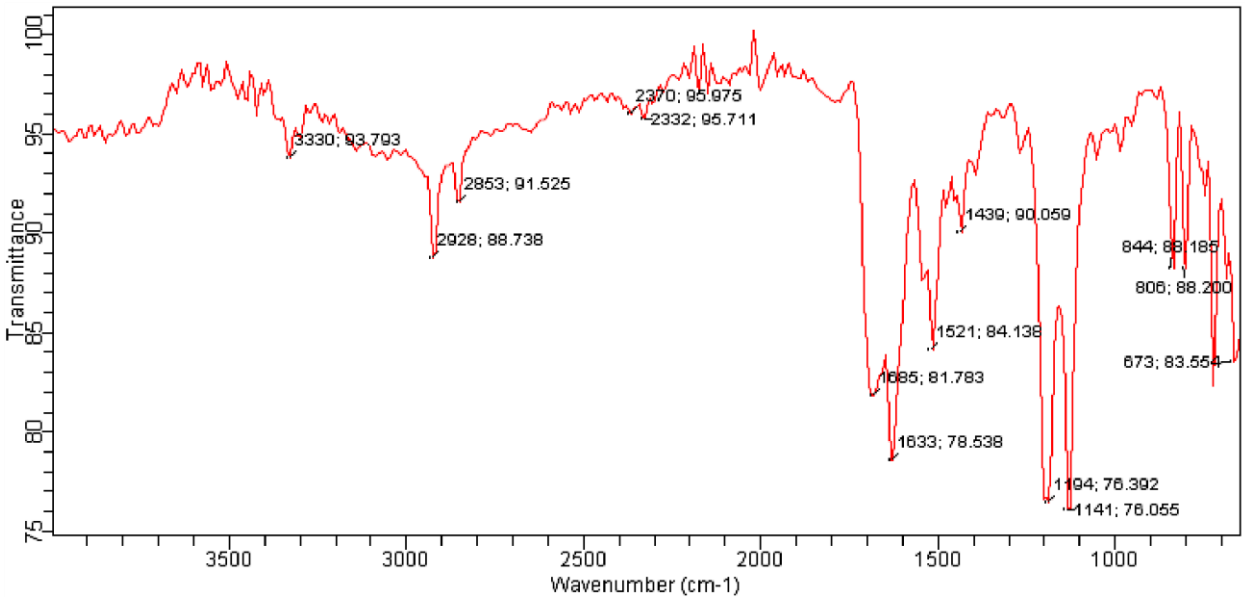


Fig 13: - FTIR Spectrum of Peptide LP-23

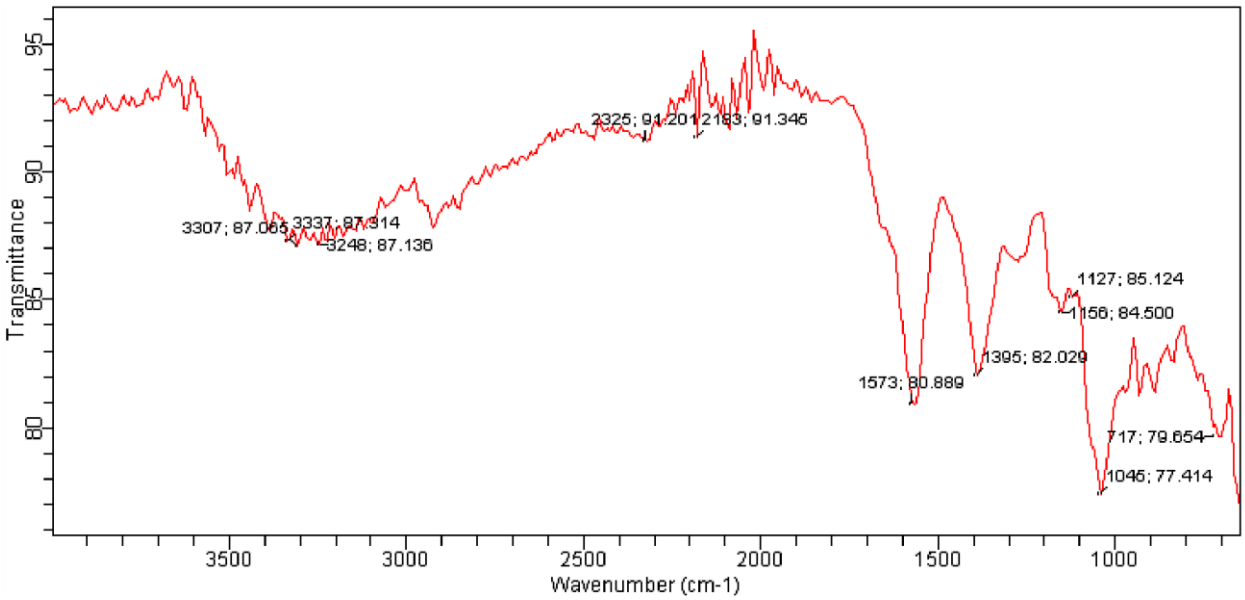


Fig 14 : - FTIR Spectrum of Silver Nanoparticle

As we can see that both the strains E. coli and S. Aureus both are susceptible to this test. We can also say that our synthesized compound LP-23 which is conjugated with silver nanoparticles shows potential against gram-positive and gram-negative bacteria. After this we can go for the MIC test which will tell about that how much concentration is required for the next process. Our product is feasible to work when we conjugate with the Silver nanoparticle and also it helps in the drug delivery systems.

CHAPTER 5 – CONCLUSION AND FUTURE ASPECTS

We synthesize our product by the solid phase peptide synthesis with the rink amide resin. Rink amide plays an important role in this because it works as a solid support in this synthesis. We have taken 100mg of the rink amide and after the synthesis we lyophilized our compound and we get 45mg after lyophilize the sample. After lyophilization we get 45mg final product. Next step is purification by HPLC method. We check our compound's purity by HPLC gradient method. We get the purity of our compound is 100%. Calculated mass of LP-23 is 711.57 and the observed mass is 712.59. We synthesize Silver nanoparticles by the chemical method and observe by the UV Spectroscopy and FTIR method. By the UV Spectrum we analyze the peak of silver nanoparticles, peptide LP-23 and conjugate. We get the peak at 421 nm of silver nanoparticle and for conjugate we get shifted peak at 410nm. After that we get the FTIR spectra of our compound. FTIR analysis of peptide conjugates shows that peptide LP -23 conjugate with silver nanoparticles in the range 1685 to the 1550 cm^{-1} which shows that it forms amide bond and conjugated with silver nanoparticle. This is successfully bond with each other and form anhydride bond and other bonds. So, this study shows that our compound LP-23 is successfully conjugated with the silver nanoparticles and it is potential to compound LP-23 exhibits activity against both gram-positive and gram-negative bacteria, demonstrating its broad-spectrum antimicrobial properties.

Future Aspects

This review, along these lines, may shape reason for future investigations like peptide portrayal, peptide forms with any metal particle, self - gathering by various strategies. This study may likewise be used in creating novel helpful medications from such antibacterial movement in mix with previous remedial to control, oversee and fix illnesses. Peptides have fostered an unmistakable restorative specialty since their beginning as synthetic substances extricated from dairy cattle organs, and they will keep on assuming a critical part in the drug business. By integrating new signs and atomic targets, using fresher synthetic procedures to increment sub-atomic variety, and planning worked on pharmacological qualities, peptide treatments have stayed aware of logical progression. The expected utilization of peptide-based prescriptions to novel targets is being investigated by research. Numerous peptide-addressable focuses for which no approved prescriptions have yet been created have shown helpful likely in early clinical preliminaries or in preclinical illness models.

CHAPTER 6 – REFERENCES

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