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Inhibition of antibiotics-resistant bacteria by natural modified proteins

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ABSTRACT **: Most antibiotics can not face multidrug-resistant bacteria (MDR), necessitating the search for new antibacterials based on natural resources. Native or modified natural legume proteins exhibited a wide range of potent antimicrobial properties. Sixteen bacterial isolates were mapped for antibiotic resistance according to CLSI, showing resistance range (42-92%) in case Gram-positive and (58-92%) in Gram-negative bacteria. White native Phaseolus vulgaris protein (NPP) was isolated from the seeds and methylated (MPP). The MIC range of MPP against 7 MDR bacteria was 10-25 times lower than NPP and could (1 MIC) considerably inhibit their 24hr liquid growth. MPP showed higher antibacterial effectiveness than Gentamycin, the most efficient antibiotic versus Gram-positive bacteria and the second most efficient against Gram-negative bacteria. However, MPP recorded MICs against the seven studied MDR bacteria in the 1-20 µg/mL range, which is the same for Gentamycin. The combination between Gentamycin and MPP produced synergistic effects against the seven studied bacteria, as confirmed by the Transmission Electron Microscopic images. The antimicrobial activity of MPP against the seven MDR bacteria remained stable after two years of cold storage at 8-10 C as contrasted to Gentamycin which lost 20-72% of its antimicrobial effectiveness.**

KEYWORDS: **AST., Methylated protein., Legume Protein., MIC**

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I. INTRODUCTION

Antimicrobial-resistant bacteria trigger a significant issue in public health **(Adams *et al.*, 2018)**. Hospitals frequently harbor antimicrobial and multidrug-resistant (MDR) bacterial strains, which likely hinders the global management of infectious diseases **(Terreni *et al.*, 2021)**. It is one of the most crucial issues associated with mortality and economic loss **(Safain *et al.*, 2020)**. Increased mortalities are currently attributed to nosocomial infections with antimicrobial resistance, presaging dire consequences in the future **(Zurabov & Zhilenkov, 2021)**. Approximately 0.7 million deaths occur yearly from MDR **(O'Neill., 2016)**. Because of their porins, cell wall composition, or efflux pumps, these bacteria were resistant to a number of antibiotics. An antimicrobial resistance pandemic is largely caused by drug resistance mechanisms, which involve either severe mutations in already-existing genes or the acquisition of emerging antibiotic-resistance genes through horizontal gene transfer **(Sun *et al.*, 2019)**. Nevertheless, antimicrobial resistance is believed to exist prior to the antibiotics' invention and its unlimited application in animal husbandry, hospitals, and low-income countries **(Ma *et al.*, 2020)**. *Acinetobacter baumannii*, *Pseudomonas aeruginosa, and Enterobacteriaceae* have been found to harbor colistin-resistance genes, whilst *Staphylococcus aureus* and *Enterococci* have been found to harbor methicillin- and vancomycin-resistant genes, respectively **(Snitser *et al.*, 2020).** Plasmid-mediated genes are accountable for the spread of carbapenemases in different bacteria **(Hishinuma *et al.*, 2020)**, Thus, the deteriorating effectiveness of antibiotics is becoming a severe challenge for modern medicine.

On the other hand, natural products offer a far superior route. Plant biodiversity offers a variety of chemical compounds with a range of medicinal uses, such as antiviral, antifungal, antibacterial, and anticancer properties **(Abdel-Hamid *et al.*, 2016)**. The antibacterial properties of basic proteins, herbs, spices, and herbal extracts have been known for a while **(Beuchat *et al.*, 1994; Osman *et al.*, 2014).** Amps or antimicrobial proteins and peptides are a well-known class of lead chemicals that combat microbial resistance. Despite the fact that certain antimicrobial peptides may not yet have the standard efficacy of available medications, their capacity to prevent the development of bacterial resistance mechanisms and their comparatively low toxicity

make them extremely intriguing. AMPPs often have high cationic amino acid concentrations **(Sitohy *et al.*, 2010 and 2013; Abdel-Shafi *et al.*, 2016)**, enabling their nonspecific binding to biological membranes **(Shai *et al.*, 2002, Mahgoub *et al.*, 2011).** The total leguminous seed protein was either void of or showing very low antibacterial activities. So, research efforts intended to enhance or create this activity either through releasing bioactive peptides through enzymatic hydrolysis **(Osman *et al.*, 2021; Saad *et al.*, 2021)** or through chemical modification to augment the protein-positive charges via esterification. Esterification is a well-known technique that can enhance the net positive charges on the surface of the modified proteins imparting them with antibacterial properties **(Sitohy *et al.*, 2000)**. This chemical modification proved effective in enhancing the antibacterial activities of native proteins **(Sitohy *et al.*, 2011; Abdel-Shafi *et al.*, 2016; Amer *et al.*, 2020).** In the current study, the potential antibacterial activity of methylated white kidney bean (*Phaseolus vulgaris)* protein was explained through its inhibitory effect on the most multidrug-resistant bacteria, after being mapped by the antibiotic sensitivity disc method.

Due to the increasing bacterial resistance to synthetic antimicrobial agents, there is a greater necessity than ever for natural antimicrobial substances that are efficient, non-toxic, and cause less risk to the environment. Antimicrobial agents cannot effectively treat these resistant microorganisms (such as bacteria and fungi), which causes diseases to persist and spread **(Jyoti *et al.*, 2014).**

2. Antibiotics resistant

The capability of an organism and future generations to live or reproduce under circumstances that would normally kill or inhibit other organisms of the same strain is known as resistance, according to **Cloete (2003)**. This capability might be transient or permanent. Particularly Gram-negative rods (e.g., *Escherichia coli, Klebsiella oxytoca, Klebsiella pneumonia, Salmonella* Typhimurium*, and Pseudomonas aeruginosa*) are becoming increasingly resistant to the majority of antibiotics currently in use.

3. Antibiotic resistant bacteria

The capacity of bacteria to protect themselves against an antibiotic's effects is known as antibiotic resistance i.e., antibiotic use will result in resistant bacteria surviving (or at least proliferating and multiplying more quickly than susceptible germs). In clinical terms, resistance denotes a bacterium's ability to flourish at the body's normal therapeutic antibiotic concentrations. As a result, using that medication to treat this illness will probably not be successful.

There are two pathways for bacteria to acquire all groups of resistance:

- Mutations in the bacterial DNA may result in resistance.
- They can obtain resistance genes from adjacent bacteria, "horizontal gene transfer" refers to this action.

Resistance may be maintained and will be passed on to subsequent generations as the bacterium divides or be transferred horizontally **(Hoffman *et al.*, 2001).** The resistant bacteria can spread via many ways including water, food, travel, and trade. The fundamentals behind the emergence and spread of antibiotic resistant bacteria are more thoroughly discussed in the sub-section that follows.

3.1. Antibiotic resistance mechanisms

3.1.1. Pump the antibiotic out of the bacterial cell:

Pumps are frequently found in the cell membrane or cell wall of bacteria. They are known as efflux pumps, they can transport various substances, such signal molecules, and nutrients, and they can also expel antibiotics from the bacterium, reducing the concentration of antibiotics inside the bacterial cell. Occasionally, modifications to the DNA of the bacterium may lead the bacterium to make more of a specific pump, elevating resistance **(Cox & Wright, 2013; Sun *et al.*, 2014).**

3.1.2. Permeability's reduction of the membrane that surrounds the bacterial cell.

Certain alterations to the bacterial membrane make it more challenging to pass via it. So, the organism receives fewer antibiotics **(Hoffman *et al.*, 2001).**

3.1.3. Destruction of the antibiotic:

By antibiotic-inactivating enzymes such β-lactamase that destroys the active component (the β-lactam ring) of penicillin, one of the most important drugs for treating infections in humans. Gram-negative bacteria, especially enteric and nonfermentive pathogens are the most significant β -lactamases because they collectively confer resistance to all β-lactam-containing antibiotics **(Bush *et al*.2020).**

3.1.4. Modify the antibiotic:

In some circumstances, bacteria can alter antibiotics by adding chemical groups using specific bacterial enzymes, which avoids the antibiotic from adhering to its receptor in the bacterial cell **(Hoffman *et al*., 2001)**

3.1.5. Camouflage the target:

Changes to the target's structure in the bacterium (caused by bacterial DNA mutations). might hinder the antibiotic from reaching the target. In addition, the bacteria can modify the target by adding new molecular groups, shielding it from the antibiotic **(Hoffman *et al*., 2001)**

3.1.6. Producing alternative proteins

Antibiotic-inhibited proteins can be substituted by those produced by a variety of bacteria. *Staphylococcus aureus*, for example, has the ability to utilize the resistance gene mecA and generate a distinct penicillin-binding protein. β-lactam antibiotics target these proteins because they are essential for the formation of the bacterial cell wall. Because of the changed penicillin-binding proteins (PBP) reduced affinity for β-lactam antibiotics. Based on this type of resistance, MRSA strains can generate (PBP) acquiring their resistance toward methicillin **(King *et al*., 2017) (Pandey *et al*., 2021).**

3.1.7. Target reprogram:

Bacteria can have the ability to create an alternative version of a required structure for instance , in contrast to susceptible bacteria, vancomycinresistant bacteria generate a distinct type of cell wall., with this kind of cell wall, the antibiotic does not interact as usual.

4. Common Gram-positive bacteria:

A) *Streptococcus pyogenes*

Streptococci are Gram-positive, catalase-negative, coagulase-negative, and they are typically found in chains or pairs. Major human-specific bacterial pathogen *Streptococcus pyogenes* causes a variety of symptoms, from mild localized infections to extremely deadly invasive infections **(Ibrahim *et al*; 2016)**. Because it exhibits antigen A on its cell wall, it has been categorized as group A according to the Lancefield serotyping system. As a result, this organism is frequently called beta-hemolytic group A *Streptococcus*. The most pathogenic bacterium in the entire genus is called *St. pyogenes*, also known as the flesh-eating bacteria The name pyogenes originates from the word pyogenic, which is a categorization for *Streptococci* linked to pus development. This microbe can cause a variety of infections, from minor ones like strep throat and impetigo to more dangerous ones like scarlet fever, glomerulonephritis, and necrotizing fasciitis **(Edwards and Baker, 2005).** However, strep throat can cause rheumatic fever if left untreated. Infections caused by *St. pyogenes* (Group A *Streptococcus*) usually are treatable with a variety of medications. The chance of dying from invasive group A streptococcal illness may be decreased with early treatment. However, in every case, even best medical care cannot stop death. Supportive care in an intensive care unit may be required for patients with extremely serious illnesses.

B) *Staphylococcus pasteuri*

Is a nonmotile, yellow-looking, coagulase-negative, Gram-positive bacteria. Louis Pasteur, a French microbiologist, was honored with the name. The Institute Pasteur, named for Pasteur, located in Paris, France, is where the species was first described **(Chesneau O., *et al* 1993)**. The first instance of machine injection-induced osteomyelitis caused by *Staphylococcus pasteuri*. Using mass spectrometry, *Staphylococcus pasteuri* was identified from blood cultures in a patient who had both endocarditis and osteomyelitis **(Petti CA, *et al* 2008)**. The bacterium is not a regular skin flora, but rather has been isolated indoor airborne bacteria **(Madsen AM., *et al* 2018)**, bird eye fluid **(Bezjian M, *et al* 2014)**, drinking water **(Faria C, *et al* 2009),** sea fish **(Regecova I, 2014)**. It can be overrepresented in the gastrointestinal tract in children with active celiac disease **(Sanchez E, *et al* 2013)** and can sometimes be found in platelet transfusions **(Savini V, *et al* 2009) (Savini V, *et al* 2008).**

C) *Bacillus cereus*

B. cereus is a facultatively anaerobic, Gram-positive species of the *Bacillus* genus that can produce endospores. *Bacillus cereus* and *Bacillus anthracis*, which are recognized as human pathogens; *Bacillus thuringiensis*, which is employed as a biopesticide; *Bacillus mycoides; Bacillus pseudomycoides*, which is characterized by rhizoidal formations; *Bacillus weihenstephanensis,* which includes psychrotolerant strains; and *Bacillus cytotoxicus*, the final species to be identified, make up the *B. cereus* group **(Guinebretière *et al*., 2013).**

***D) Staphylococcus aureus* (*S. aureus*)**

===*S. aureus* is most frequently responsible for skin infections. It is frequently found in the nasal and skin flora and is spherical in appearance. Approximately 20% of people have a chronic *S. aureus* infection. Additionally, *S. aureus* has been related to a wide range of illnesses, from life-threatening conditions like toxic shock syndrome, meningitis, pneumonia, osteomyelitis, bacteremia, endocarditis, and septicemia to relatively non-lethal skin infections like impetigo, carbuncles, pimples, cellulitis folliculitis, boils (furuncles), and blisters. Common causes include pathogens that affect the skin, soft tissues, respiratory system, joints, bones, and endovascular system.

It still ranks among the top five causes of nosocomial infections, frequently resulting in wound infections following surgery. **(Kluytmans *et al*., 1997; Enan *et al*., 2020).** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium that causes many complicated human diseases. MRSA is common in healthcare institutions, prisons, and nursing residences, where people with invasive devices, open wounds, and weakened immune functions are notable. MRSA has higher infection resistance than the normal population.

5. Common Gram-negative pathogenic bacteria:

A) *Salmonella* Typhimurium

Salmonella enterica serovar Typhimurium and Typhi are the causes of gastroenteritis and bacterial infection typhoid (enteric fever). The infection is systemic and is frequently contracted by ingesting contaminated food or beverage, usually from an oral or fecal source. The occurrence of typhoid fever may be a sign of poor personal and environmental hygiene. The disease can be mild or severe, but it can also be fatal **(WHO, 2008).** This bacterium responsible for many cases of illness and death in Nigeria and other tropical countries where the typhoid is common **(Ibekwe *et al*., 2008).** Even after the development of novel antimicrobial medicines and the widespread use of antibiotics, enteric fever has remained a serious public health concern.

B) *Proteus mirabilis*

P. mirabilis belongs to the family Enterobacteriaceae and is Gram-negative. It results in infections of the wounds, urinary system, respiratory diabetic foot ulcers, and tract, burns. *Proteus mirabilis* is highly resistant to several drugs, which may result in a failure of antibiotics therapy **(Fm *et al*.,2018).**

C) *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative, aerobic rod bacterium that plays a significant role in causing both community- and hospital-acquired illnesses. *P. aeruginosa* frequently causes nosocomial infections, such as pneumonia, UTIs, and bloodstream infections **(Driscoll *et al*., 2007).** Due to their high inherent resistance and ability to develop resistance to several antibiotics, *P. aeruginosa* are challenging to eliminate **(Breidenstein *et al*., 2011).**

D) *Klebsiella oxytoca*

K. oxytoca is a rod-shaped, Gram-negative bacterium that is closely correlated with *K. pneumoniae*. being indole-positive; and it also differs slightly from *K. pneumoniae* in growth characteristics in the ability to growth on melezitose, but not 3-hydroxybutyrate. *K. oxytoca* is a widespread environmental pathogen **(Gorkiewicz,2009).** *Klebsiella oxytoca* is currently being encountered in hospitalized patients after *Klebsiella pneumoniae* **(Vasaikar *et al.*, 2017).** For humans, 8–10% of the population's feces provide a fertile environment for *K. oxytoca* growth. **(Savino *et al.*, 2009).** According to **(Savino *et al.*,2011)**, *K. oxytoca* is currently acknowledged as a clinically significant opportunistic pathogen linked with nosocomial infections in hospitalized cases, particularly infants and neonates. Individuals with bacteremia, septicemia, soft tissue infectious diseases, septic arthritis, urinary infections, cholecystitis, and, more recently, colicky newborns have all had *K. oxytoca* isolated from their systems. Additionally, intestinal overgrowth of *K. oxytoca* was detected in children with celiac disease **(Sánchez *et al.*, 2013).**

6.Natural proteins and their use as antimicrobial agent alternatives

The antibacterial effects of spices, basic proteins, herbs, and their extracts have long been studied in this field **(Osman *et al.*, 2014 ;Enan *et al.*, 2020)**. Because of their biological richness, plants produce a lot of chemicals with antiviral, antifungal, antibacterial, and anticancer properties **(Penalver *et al.*, 2005; Abdel-Shafi *et al.*, 2019; Abdel-Shafi *et al.*, 2020)**. Legumes play a significant part in the traditional diets of many areas of the globe **(Yeheyis *et al.*, 2011)**.

6.1. Legume protein

Grain legumes are used widely in human and animal's food since they are excellent and affordable sources of essential elements like minerals, proteins, vitamins, lipids, and carbohydrates. Moreover, among researchers working on food product development in the past few decades, there has been an elevating trend to use the functional qualities of legume seed proteins in the creation of animal proteins for their manufacturing and physicochemical stability. Being the greatest well-known resource of vegetable proteins, soybean protein is also frequently employed as an essential food ingredient. However, alternatives to soy protein in food have been explored using protein sources extracted from other grain legumes like faba beans, peas, and lupin **(Gueguen & Cerletti, 1994).**

6.1.2. Mode of action of antimicrobial proteins

Cationic antimicrobial peptides are found in both plants and animals, they are critical components of intrinsic immunological processes that protect the body from broad pathogens, involving bacteria and viruses, through direct antimicrobial activity and immunoregulatory actions. Despite receiving a lot of hostility throughout the years, they occur naturally and have proven to be strong defensive tools **(Hancock, 2005).** However, very few resistant species strains have been discovered yet to antimicrobial agents. Under certain circumstances, the outer membranes of *Morganella*, *Burkholderia*, and *Serratia* bacteria have a decreased negative charge on the surface lipopolysaccharides (LPS). Other bacteria such as *Porphyromonas gingivalis* release proteases that break down peptides **(Devine & Hancock, 2002)**. The most of resistance mechanisms only significantly alter the MIC values by a factor of 2 to 4 **(Hancock, 2001).** These substances are therefore quite intriguing, and novel anti-infectives based on them are now being explored. Beta-sheets, loops, alpha-helixes, and extended structures, for example, are still present, as are various amino acid sequences **(Hancock, 2001).** The roughly 700 antimicrobial peptides that are known all have the same 3D structure according to **Hancock (2005)**, the molecules fold into amphipathic forms, which have both hydrophobic and charged sides.

Early in their biological activities, the majority of antimicrobial peptides interact with membranes; this fact could be described by the Shai-Matsuzaki-Huang model. Peptides are initially unstructured molecules in solution. When these molecules come into contact with the membrane, they take on a three-dimensional structure (such as an alpha-helix or beta-sheet) that makes them amphiphilic. This means that the lipid's head groups are directly engaged by the positive charge side of these molecules. The peptide then embeds itself in the outer leaflet of the membrane, weakening it further. New proof of this thinning has been produced using XRD and AFM **(Chen *et al.*, 2003).**

The channel can then be developed after that. This step of the procedure is more challenging, though. A number of ideas, including as the carpet model, the toroidal pore model, the barrel-stave model, and the micellar aggregate channel model, have been proposed to explain this behavior **(Wu *et al.*, 1999)**. The capability of each

model to be applied relies on the peptide **(Buffy *et al.*, 2004)** and the lipids' characteristics (elasticity, phase, hydration, and length of the hydrophobic chain) **(Dave *et al.*, 2005)**. In conclusion, the bacterial cells are eliminated in a variety of methods, including:

(i) Membrane depolarization **(Westerhoff *et al.*, 1989);**
(ii) Intracellular activities, such as macromolecular synthesis, are damaged **(Kragol *et al.*, 2001),**
(iii) Rupture of cell wall **(Bierbaum & Sahl, 1985)** and modification of membrane bilayer lipid content **(Matsuzaki,1999)**, or

In In extreme circumstances, micelles develop, leading to cell leakage **(Papo & Shai, 2005).**

While cationic peptides need rather high concentrations to be effective, their distinct mechanism of action makes them promising building blocks for developing new antimicrobial drugs **(Devine & Hancock, 2002)**. Due to the fact that these peptides' mode of action is dependent on charge-charge and hydrophobic interactions with the membrane bilayer, resistance is constrained by the fact that it would be too expensive or necessitate several mutational occurrences for a microbe to alter the structure or organization of its lipids in to reduce these interactions. Further, there is no certainty that a particular recognition site for protease cleavage exists due to the great sequence variation of these peptides. Another crucial aspect of avoiding resistance is the presence of secondary targets. Despite preferential binding to certain sites, cationic peptides may connect with some other substrates in the bacterial cell to influence processes, including macromolecular synthesis, cell wall formation or breakdown, and cell proliferation **(Hancock, 2001)**. Finally, it is important to remember that multicellular organisms often assault bacteria with several cationic peptides, hence reducing the likelihood of antibiotic resistance.

7.Chemical modifications

One of the earliest techniques for examining structure-function links was by chemically modifying endogenous proteins. Protein's primary structural changes can be made chemically to improve their biological functions and characteristics. Several structure-function relationships have been successfully investigated using this methodology. Systematic chemical modification of dietary proteins can modify their nutritional value, produce derivatives of amino acids that could be dangerous, and contaminate the food with poisonous substances.

The amino acid residues can be changed by heating at an acidic or alkaline pH. The four main types of chemical reactions (acylation, alkylation, phosphorylation, and esterification) are used to modify the side chains of amino acids.

Esterification involves three stages, the first stage is mixing the reactants (alcohol, protein, and acid). The second stage is the esterification process, which typically lasts from a few hours to several days at 4^{o}C, the final step is putting an end to the reaction and gathering the end result **(Chobert *et al.*, 1995).** By giving proteins and peptides an extra positive charge, antimicrobial and antibacterial activities are enhanced according to **(Berkhout *et al.*,2002),** negatively charged proteins have anti-HIV (human immunodeficiency virus) activity in vitro**.**

8. Enhancement of antimicrobial properties of a protein by esterification reaction

Cationic proteins or peptides with antibacterial activity may be produced using current biotechnology techniques. without the requirement for costly and time-consuming methods for separating the active protein component. The process of esterification is crucial for changing dietary proteins. As a result of blocking free carboxyl groups during esterification with various alcohols, the altered proteins become more acidic and have a higher net positive charge **(Sitohy & Osman, 2010).** The basicity of a changed protein is determined by the degree of esterification and, by extension, the initial concentration of amino acid residues that are basic.

9. Factors affecting protein esterification

Utilizing -lactoglobulin as a model, Chobert (2003) examined this factor impacting esterification and determined the following parameters:

a. Time-course of reaction influence:

The reaction time course was observed at 8, 16, 24, 48, 72, and 96 hours in an esterification technique employing β-lactoglobulin (3 percent protein content) dispersed in 95 percent ethanol in the existence of 0.7 N HCl. As the reaction time increased, the esterification percentage quickly increased to 11, 14, 23, 28, 32, and 36%, respectively. The esterification did not reach its optimum under the circumstances used even after 96 hours of reaction. Setting must be drastically changed to optimize the degree of alteration following lowered response times. For instance, the concentrations of acids and proteins might increase. The maximum esterification with methanol occurred after 24 hours with more dilute solutions, and this reaction duration is alcohol dependent.

b. Temperature influence:

After eight hours, the quantity of esterification increased with temperature. β-lactoglobulin esterified with 99.7% ethanol was used in comparative experiments at three different temperatures (4, 10, and 20°C) with a final concentration of 2% protein and 0.7N HCl. starting at 4, 10, and 20°C, or 2%, 4%, and 8%, respectively. After drying, the compounds produced at higher reaction temperatures developed a violet color, suggesting that high temperatures could result in adverse reactions **(Wilcox, 1967).** suggested that esterified compounds might become insoluble at higher temperatures. Greater temperatures should not be used, even though they might speed up reaction times, in order to avoid adverse reactions.

C. Water presence influence:

Water is needed during the esterification process in quantities sufficient to dissociate HCl and give the protons essential for the carboxyl groups activation (**Sitohy *et al.*, 2000).** By creating a layer of hydration around the protein molecules, the water in the medium can assist in organizing the hydrophobic moieties in the globulin core and improve access to the carboxyl groups for esterification **(Tanford,1980)**. When **Halpin & Richardson, (1985)** esterified β-lactoglobulin using 95 percent ethanol and anhydrous methanol, they assessed the need for water for the esterification process.

d. Alcohol presence influence:

Methanol was discovered to be the most reactive of the numerous alcohols studied for esterification, capable of esterifying a maximum of 97% of the carboxyl groups in β-lactoglobulin even in the existence of very small amounts of water (1%). Furthermore, the water content of the acid influences the final amount of water that is present in the reaction media **(Chobert, 2003).**

e. Protein concentration influence:

Different conditions were investigated to determine the effect of protein content. At 4 degrees Celsius, 24 hours of esterification in 95 percent ethanol and 0.7N HCl were carried out. At doses of 2, 3, 4, and 5%, -lactoglobulin was added. The degree of esterification was somewhat increased when the protein level was raised from 2 to 3 percent. However, the reaction was negatively impacted by increasing the concentration to 4 and 5 percent, leading to lower amounts of esterification. Protein carboxyl groups must first be activated by protons during esterification to interact with the alcohol. Therefore, the concentration of activated carboxyl groups, rather than the amount of proteins, is the most important factor for the process **(Sitohy *et al.*, 2000).**

10.Modified proteins mechanism of operation

CAMPs are widely distributed in the animal and plant and are essential for the innate immune system's defenses against a variety of pathogens, from bacteria to viruses, through both direct antibacterial activity and immunomodulatory effects (**Joey & Dirk, 2014).**

In comparison to traditional antibiotics, these peptides have a significant advantage since their capacity to target multiple systems makes it harder for bacteria to develop MDR against them **(Dennison *et al.*, 2013; Joey & Dirk, 2014).**

In addition to being "antimicrobial," AMPs have been shown to also stimulate the immune system. It has been proposed that these peptides should be referred to as "host-defense peptides" instead of "antimicrobial

peptides," with the latter term being assigned merely because of a feature that was first identified **(Hancock and Sahl, 2006).**

Only a few species of bacteria *Burkholderia*, *Morganella* or *Serratia* had resistance to AMPs. Via modifying their outer membranes lipopolysaccharides (LPS) reducing negative charge on their surface through two regulators PhoPQ and PmrAB under certain conditions. Other species, like *Porphyromonas gingivalis*, release proteases degrading peptides **(Devine & Hancock, 2002; Hancock,2005).**

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