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The Antimicrobial Activity of Camel Lactoferrin Peptide

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ABSTRACT

This study examines the effects of lactoferrin and black seed oil on microorganisms, as well as the extent to which they inhibit the growth of bacteria. Resistance to antibiotics has been outlined in previous research; therefore, a new strategy is required for sourcing natural biological materials for the extraction of antimicrobial peptides (AMPs). This new strategy should enable microbe efficacy, thus providing a new and innovative method for combatting microbes. Lactoferrin from humans and cows has undergone rigorous analysis though camel lactoferrin (CLF), but this analysis has also gone largely underreported. The current research study addresses CLF by evaluating its mechanisms of antimicrobial activity. This activity was observed in the contexts of Staphylococcus aureus (S. aureus), Streptococcus agalactiae, Streptococcus pneumonia, Streptococcus pyogenes and Candida albicans. The primary research aim of this study is to determine the mechanisms and properties of lactoferrin and black seed oil in the contexts of the five bacteria groups mentioned above. The materials used in the analysis included ethanol, sabouraud dextrose, agar, nutrient agar and filter paper discs (which were used for the AMP agent in sterile conditions—the disc diffusion method). AMPs, native and mutant (camel) lactoferrin peptides, nigella sative (NS) oil and Nigella sativa oil (black seeds) were also used. The microorganisms were synthesized from the lab. It has been found that lactoferrin is effective in bacterial applications. Gram-positive bacteria has also been found to be more efficient when the synergistic action of camel lactoferrin (SAoCL) mutant peptide is used with NS oil. This study shows that lactoferrin has promising results in the binding of iron in microbe growth prevention. Additionally, observations of SAoCL, the mutant peptide, show that SAoCL, alongside NS oil, hinders the further development of bacteria.

Keywords: Lactoferrin, Antimicrobial peptides

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INTRODUCTION

Human therapeutics came to provenance during the 1900s, and the field attained great accomplishments related to the discovery and advancement of antibiotics and antibacterial agents connected with the treatment of bacterial infections (Wood). A wealth of antibacterial agents to successfully treat contagious diseases on a global scale has been developed through antibiotics.

Bacterial agents' constant mutations to resist antibiotics, together with the occasional side effects of administering drugs, have increasingly restricted the use of antibiotics and antibacterial chemotherapeutics. As a result, there is a great need to research new families of drugs that can limit bacterial resistance but that have lower toxicity.

In 2013, Boucher et al. suggested that the increasing levels of antibiotic resistance highlighted limitations in antibiotic drug research. Lewis (2013) reported that the shift away from biological methods for isolating candidates and towards new chemical methodologies would have a severe impact on future antibiotic research. The lack of new drugs that are effective against pathogens resistant to antibiotics has led the scientific community to investigate antimicrobial peptides (AMPs) as alternative compounds for use with resistant organisms (Hancock & Sahl, 2006; Fox, 2013). As of September 2013, the Antimicrobial Peptide Database lists 2,398 AMPs, with the majority (80%) being cationic (CAMPs). Typically, AMPs have relatively low molecular weights (5 to 50 amino acids) and are generated by practically all organisms with distinctive immune systems. (Schmidtchen, Malmsten 2013) reported that CAMPs interact with bacterial membranes that possess anionic properties, resulting in efficient breakdowns in membrane function, followed by cell death (Wimley, 2010). This action has attracted significant attention from the scientific field, since high doses of CAMPs are thought to hinder bacterial resistance.

Infectious diseases that develop pathogenic bacterial resistance to current antibiotics remain a global problem.

Mangoni and Shai (2009) described two major threats to today's global population. The first of these is pathogens that are resistant to multiple available drugs, and the second is the beginning of septic shock, which occurs when antibiotics are as used as treatments.

The other one is connected to the influx of lipopolysaccharides (LPSs) from the cell dividers of gram-negative microorganisms.

Extracellular degradation and modifications to the cell walls of phospholipid membranes are two examples of the many mechanisms that result in bacterial resistance to AMPs, as reported by Yeaman and Yount (2003).

Feder et al. (2000) suggested that AMPs' mechanisms of action, compared to current antibiotics, are their principal advantage. These mechanisms involve the destruction of the bacterial membrane aimed at specific cytoplasmic matter, which allows AMPs to achieve improved antimicrobial activity (Lee et al., 2004).

The mechanism by which AMPs act is two-fold. The first step is membrane binding, which is controlled by electrostatic interactions. The second step is membrane permeation, which is dependent on the hydrophobicity of the peptide and its likelihood to partition from the plasma membrane (Lannucci et al., 2011).

AMPs are considered excellent candidates for anti-infective compounds because of their exclusive mode of action. Multiple AMPs have been known to destroy microorganisms and to neutralize certain toxicities of lipopolysaccharides (Mangoni & Shai, 2009). LPS affects septic shock; hence, the capability of AMPs to neutralize toxic effects is unique when compared with the capabilities of current antibiotics.

MATERIALS AND METHOD

Disk diffusion method

A variety of systems can be used to target the antimicrobial frailty of a microorganism pathogen. These typically include circle scattering, agar weakening or small-scale juices weakening and antimicrobial angle-strip dispersion. The circle dissemination strategy exhibited in this section represents a change to the Kirby-Bauer method that has been precisely institutionalized by CLSI and others. If performed unequivocally, as indicated by the accompanying convention, this system will yield information that can dependably anticipate the in vivo adequacy of the referenced medication. However, despite circle dispersion's ability to provide data allowing most antimicrobial specialists to translate a strain as defenseless, halfway or safe, this approach does not give exact data on negligible inhibitory fixation (MIC) (P faller et al., 2007).

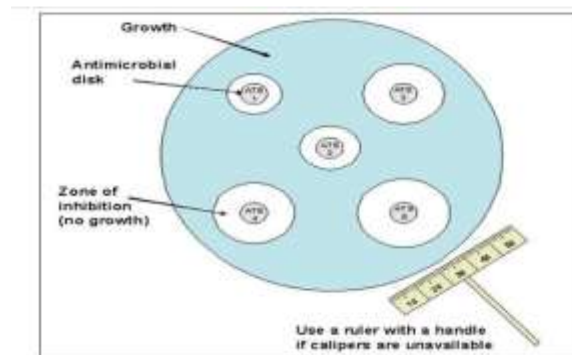

Figure 1: The antimicrobial vulnerability circle dispersion test: Rough plate position and estimation of inhibition zone

Materials

1. Ethanol, brain heart infusion, sabouraud dextrose, agar, nutrient agar
2. Disccs cut out from filter paper and put in a sterilized area for the AMP agent (disc diffusion method).
3. AMP, camel lactoferrin (native and mutant)
4. Nigella sativa oil (black seeds)

Microorganisms used

The peptides and oil are tested against five different microbes. Of these, four are associated with respiratory infections; these are called gram-positive bacteria: Streptococcus pneumonia, Streptococcus agalactiae, Streptococcus pyogenes, and Staphylococcus aureus. The last one, Candida albicans, is used for fungi.

In the present study, a mixed method approach is adopted in order to examine the effects of AMPs on the microorganisms. The procedure of this method involves preparing agar for each microbe. The first three types of bacteria can be prepared with each other via brain-heart infusion, while Staphylococcus aureus can be prepared using the material nutrient agar. The last type of bacteria, Candida albicans, is prepared using sabouraud agar. Through this method, 45 petri dishes are prepared from the brain-heart infusion and 15 from the nutrient agar. Another 15 are prepared with the sabouraud agar. After the materials are prepared, they are inoculated and spread. Each agar plate should include a specific microbe under a sterile condition. Then, the serialized discs (which should be cut out the previous day) are applied.

Native and mutant peptides

First, 10 µl of the mutant peptide is applied to three petri dishes. The same amount of native peptide is applied to another three petri dishes. This approach is applied to the five microbes mentioned above. Then, a negative control is created for both the native peptide and the mutant peptide. The negative control is made from PBS = Ethanol (430 µl from PBS and 100 µl from Ethanol). All these steps are conducted under sterilized conditions and near flame. All of the petri dishes are then placed in an incubator for 24 hours at 37˚C.

Examination of the vitro synergistic action of camel lactoferrin (mutant peptide) and Nigella sativa oil

A standard well agar disc diffusion approach was carried out to detect the antibacterial activity of the camel lactoferrin and the synergistic actions of the lactoferrin and the Nigella sativa oil against the microorganisms (S. aureus, streptococcus pneumonia, streptococcus agalactiae, streptococcus pyogenes and candida albicans). According to the protocol proposed by Cheesbrough, 5 µl each of

the oil and the peptide mutant are added to three petri dishes for all five microbes. Then, two controls are set for the two materials, as follows:

- Control A consists of 5 µl peptide and 5 µl of PBS
- Control B consists of 5 µl of PBS and 5 µl of oil Finally, all these are placed in an incubator room for 24 hours at 37˚C under sterilized conditions and near flame.

Different brands of Nigella sativa oil

Figure 2 illustrates different brands of Nigella sativa. Ten different brands of the black seed oil have been tested, such that 10 µl of each brand are placed into two petri dishes to serve as controls without any additions. Finally, all of these are placed in the incubator room for 24 hours at 37˚C under sterilized conditions and near flame.

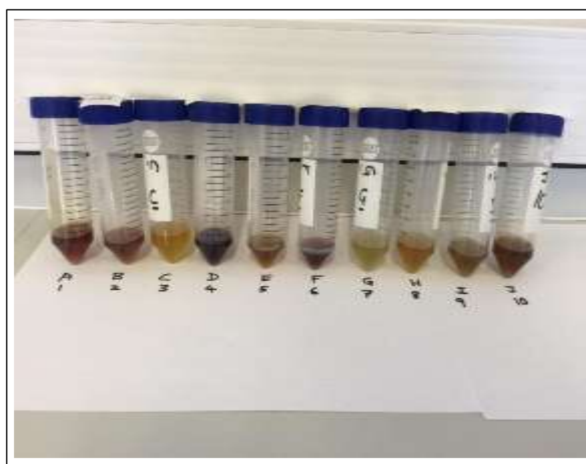

Figure 2: Different samples of BSO

Bioinformatics tools

Bioinformatics tools have been used to explore both lactoferrin peptides: namely, native and mutant peptides. Specifically, two proteomic bioinformatic tools were explored.

ProtParam tool

Multiple physical and chemical parameters can be calculated from a given protein sequence using the ProtParam tool (http://www.expasy.org/tools/protparam.html). When submitted as a Swiss-Prot or TrEMBL identifier or as a user-entered sequence (disregarding spaces and numerical characters), no other information about the protein of interest is required to enable the calculation. The section of the peptide sequence for which the analysis is required appears on an intermediary page when the identifier or sequence is entered.

Antimicrobial peptide database (APD) prediction

The APD database contains peptide data derived from other platforms (PubMed, PDB and Swiss-Prot) and provides the naming convention, classification, design, predicted structure and statistical

analysis of AMPs from all life kingdoms, including bacteria, fungi, plants and animals. In selecting specific, various-sized regions of peptide sequences for APD processing, a logical workflow is implemented to evaluate the amino acid composition (i.e., the charge and hydrophobicity of each specific region).

Helical wheel projection

(http://heliquest.ipmc.cnrs.fr)

As proposed by Armstrong and Zidovetzki (2009), hydrophilic (potentially negative) and hydrophobic (potentially positive) residues are represented by red and blue colours, respectively, according to the results generated by this analysis. The green colour, which decreases relative to hydrophobicity, depicts the most hydrophobic residues. The most hydrophilic (uncharged) residues are colored red, which, again, decreases in intensity relative to hydrophilicity. Potentially charged residues are represented by light blue, and yellow depicts a null degree of hydrophobicity.

GOR IV prediction method

(https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl? page=npsa_gor4.html). The specific secondary structures of mutant and native peptides were predicted through the application of the GOR algorithm (GOR IV). This algorithm calculates general location and percentage from potential parameters derived from 3-D protein structures drawn from experimental x-ray crystallography research. The quality of the data supplied by the databases was verified.

Furthermore, the capacity of amino acids to generate given structures was also evaluated by the GOR IV based on calculated based on whether adjacent amino acids had previously formed the particular structures.

RESULTS AND DISCUSSION

This study has determined various results concerning the peptides and their effects on microorganisms in the context of positive bacteria and gram-negative bacteria, as well as for nigella sativa oil (BSO). The study has also found results for the mixture of the peptide and the Nigella sativa oil in the context of the microorganisms.

Mutant peptide and native peptide

This experiment was conducted on two peptides. It sought to determine the differences between them, particularly in relation to the change in amino acid sequence.

Mutant peptide

It can be seen in Table 1 that the mutant had a significant effect on the Streptococcus pyogenes with a mean ZOI of 13.60mm. This is shown in the image below, which represents the total effect of the mutant on the bacteria Streptococcus pyogenes (Figure 3).

Table 1: Results for inoculated agar plates after treatment with 10 µl mutant peptides

Organism	Value
staphylococcus Aureus	0
	0
	0
	0 (Mean)
	0 (Standard Deviation)
Streptococcus Pyogens	11
	12
	18
	13.60 (Mean)
	3.79 (Standard Deviation)
Streptococcus Agalactia	14
	13
	13
	13.33 (Mean)
	0.58 (Standard Deviation)
Candida Albicans	0
	0
	0
	0 (Mean)
	0 (Standard Deviation)
Streptococcus Pneumonia	10
	11
	13
	11.33 (Mean)
	1.53 (Standard Deviation)

Legend: Mean; Original; Standard Deviation

Figure 3: Mutant peptide effect on Streptococcus pyogenes

Native peptide

Table 2 reveals that the effect of the native peptide on the micro-organism is especially strong for Streptococcus pneumonia, with a mean ZOI of 14mm. This effect is also displayed in the image below (Figure 4).

Table 2: Results for inoculated agar plates after treatment with 10 µl native peptides

Microorganism	Zone of inhibition (mm)
staphylococcus Aureus	14
	7
	9
	10
	3.61
Streptococcus Pyogens	14
	6
	6
	8.70
	4.62
Streptococcus Agalactia	13
	13
	13
	9.38
	0
Candida Albicans	7
	7
	7
	7.00
	0
Streptococcus Pneumonia	12
	13
	17
	14.00
	2.65

Legend
Mean
Original
Standard Deviation

Figure 4: Native peptide effect on Streptococcus pneumonia

Examination of the in vitro synergistic action of camel lactoferrin (mutant peptide) and Nigella sativa oil

Synergism can be defined as a process of combining two diverse material or compounds in order to develop and strengthen the sole activity of those material or compounds. If negative effects results emerge due to the combination, it would be called antagonism. While, when the effect is less than synergistic it is categorized as an additive or minor (Kumar, et. al., 2009).

The results indicated that camel lactoferrin exhibited synergistic actions with another antimicrobial activity (that of nigella sativa oil), as shown in Table 3 and Figure 5.

A standard well agar disc diffusion method was carried out to detect the antibacterial action of camel lactoferrin and the synergistic action of lactoferrin and Nigella sativa oil against the various microorganisms (S. aureus, Streptococcus pneumonia, Streptococcus agalactiae, Streptococcus pyogenes and Candida albicans). This table shows the extent to which the mutant peptide mixed

with the Nigella sativa oil affected the Streptococcus agalactiae. The results are shown in the picture below, as compared with controls A and B (in Figure 5 Figure 6, respectively).

Microorganism	Zone of Inhibition (mm)
staphylococcus Aureus	9
	13
	10
	10.67
	2.08
	11
	25
Streptococcus Pyogens	7
	9
	7
	7.67
	1.15
	10
	12
Streptococcus Agalactia	11
	30
	40
	27.00
	14.73
	15
	25
Candida Albicans	10
	7
	7
	8.00
	1.73
	10
	0
Streptococcus Pneumonia	5
	5
	6
	5.33
	0.58
	12
	7

Legend
Mean
Original
Control A
Control B
Standard Deviation

Table 3: Results for the inoculated agar plates following treatment with 5 µl mutant peptides with Nigella sativa oil

Figure 5: Mutant peptide and NSO effect on Streptococcus agalactiae

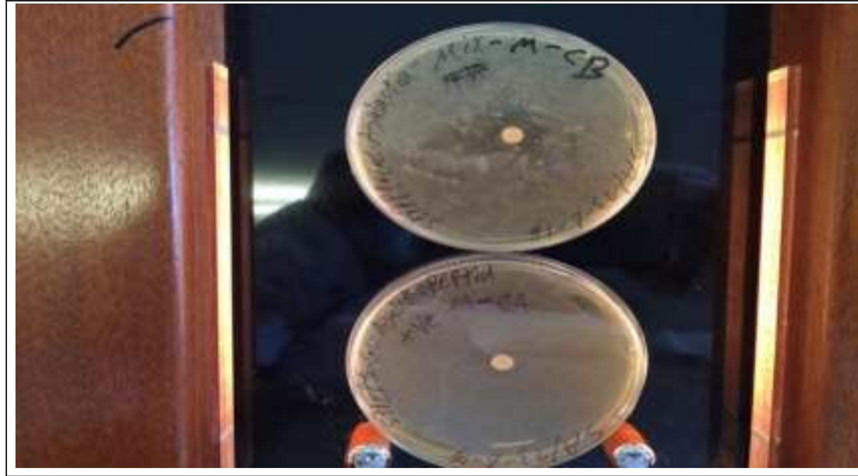

Figure 6: Controls A and B

Different brands of Nigella sativa oil

It is clear from the Table 4 that oil (10) is the most effective, while oil (9) is the least effective. It is worth noting that the selected brands have different colours. The greater the boldness of the oil colour (Figure 2), the greater the concentration of the black seed is (and vice versa). This may be attributed to the addition of various materials or to light dilution. It is also supported by the proportions mentioned in the table above.

Further, oil (10) has a great effect on the bacteria Streptococcus agalactiae, as shown in the picture below (Figure 7 and Figure 11). The ZOI is 39.00 mm, compared with the control (Figure 8).

Table 4: Results showing the ZOI following the treatment of different brands of black seed oil on inoculated agar plates

Microorganism	Zone of inhibition (mm)									
	1	2	3	4	5	6	7	8	9	10
Staphylococcus Aureus	11	9	5	9	5	15	5	5	0	22
	10	10	5	10	5	20	5	5	0	26
	7.33	9.50	5.00	9.50	5.00	17.50	5.00	5.00	0.00	24.00
	0.71	0.71	0	0.71	0	3.54	0	0	0	2.83
Streptococcus Pyogens	36	20	10	9	18	0	0	40	5	17
	41	9	11	12	18	9	0	10	5	18
	38.50	14.50	10.50	10.50	18.00	4.50	0.00	25.00	5.00	17.50
	17.68	7.78	0.71	2.12	0	6.36	0	21.21	0	0.71
Streptococcus Agalactia	17	0	55	48	40	30	5	14	10	40
	12	10	50	30	45	25	5	10	10	38
	14.75	5.00	52.50	39.00	42.50	27.50	5.00	12.00	10.00	39.00
	3.54	5.00	3.54	12.73	3.54	3.54	0	2.83	0	1.41
Candida Albicans	40	9	9	8	13	9	5	5	5	8
	45	8	7	7	10	10	5	5	5	12
	17.70	8.50	8.00	7.50	11.50	9.50	5.00	5.00	5.00	10.00
	3.54	0.71	1.41	0.71	2.12	0.71	0	0	0	2.83
Streptococcus Pneumonia	20	63	30	50	50	35	5	20	5	10
	20	59	50	40	55	35	5	20	5	9
	20.00	61.00	40.00	45.00	52.50	35.00	5.00	20.00	5.00	9.50
	0	2.83	14.14	7.07	3.54	0	0	0	0	0.71

Mean
original
Standard Deviation

Figure 7 Black seed oil (10) effect on Streptococcus agalactiae

Figure 8 Control for all brands of black seeds oil (No ZOI).

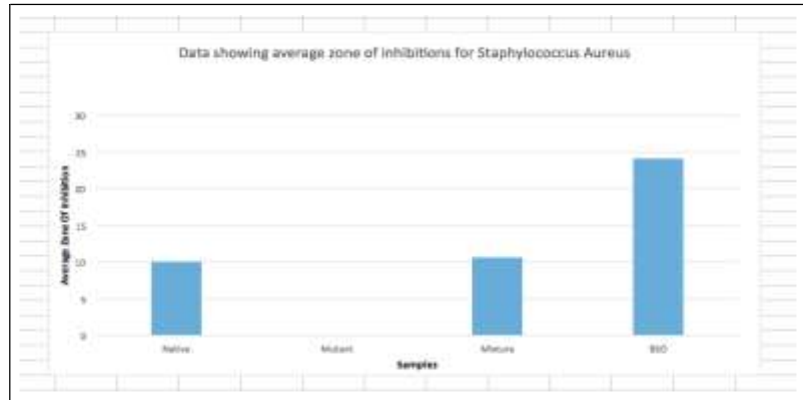

Figure 9 Antimicrobial activity of BSO and Lf against Staphylococcus aureus

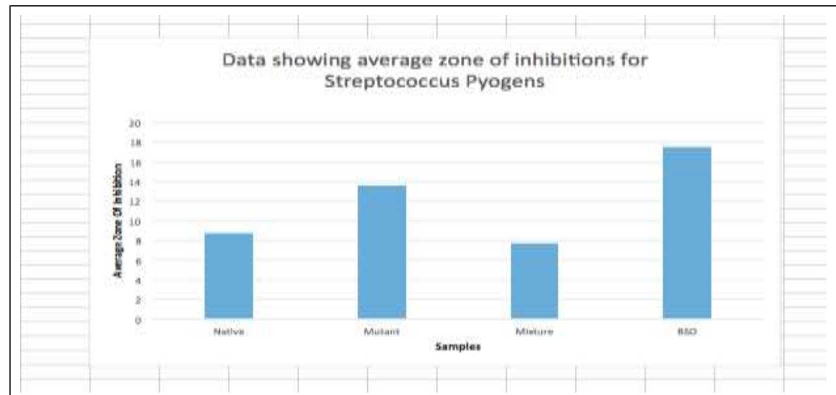

Figure 10 Antimicrobial activity of BSO and Lf against Streptococcus pyogenes

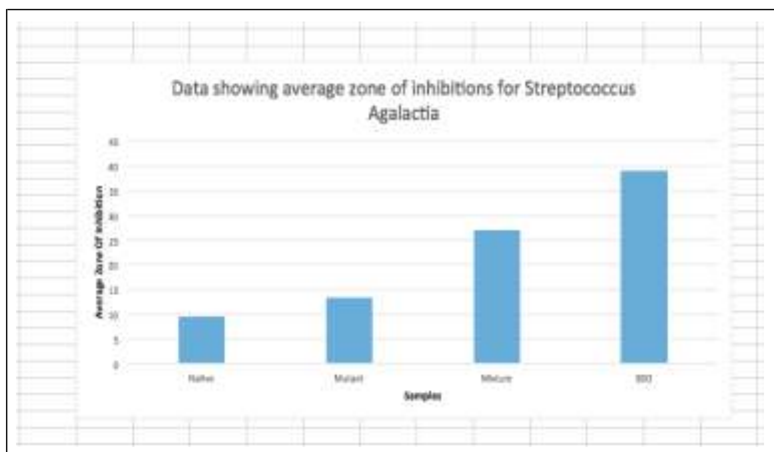

Figure 11 Antimicrobial activity of BSO and Lf against Streptococcus agalactiae

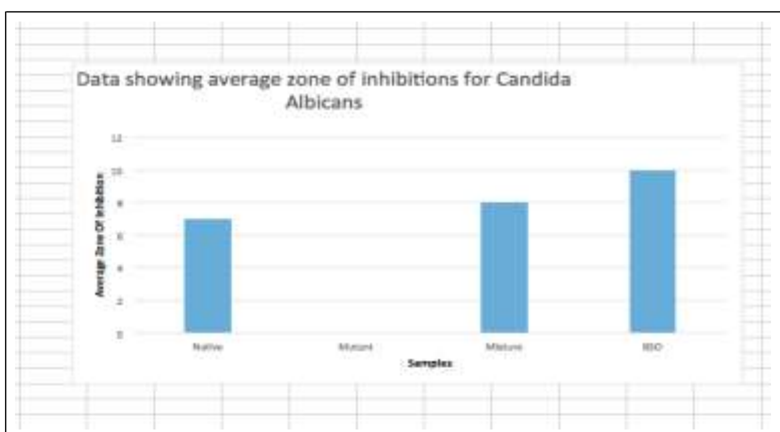

Figure 12 Antimicrobial activity of BSO and Lf against Candida albicans

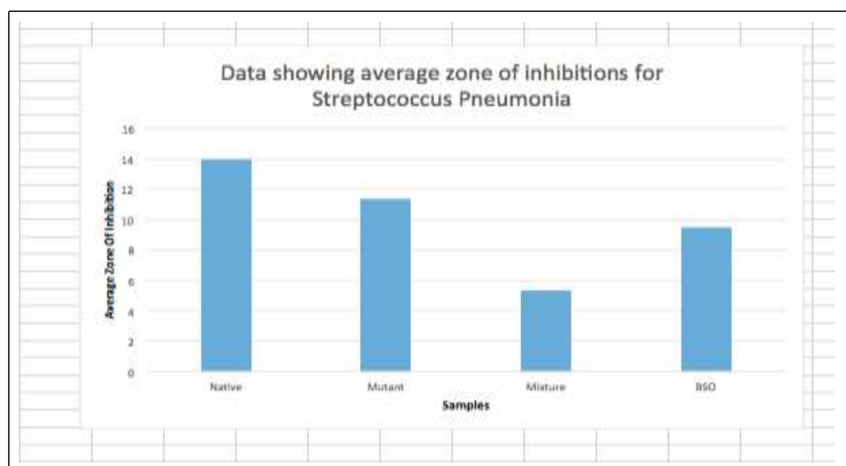

Figure 13 Antimicrobial activity of BSO and Lf against Streptococcus pneumonia

Mutant peptide

This section presents the results for the native peptide analyzed using ProtParam. The sequence was provided by Dr. Haris: AIRGLREKAKEVELRRAQKKW http://web.expasy.org/protparam/

Number of amino acids: 21

Molecular weight: 2566.0

Theoretical pI: 11.00

Native peptide

The input has 21 residues:

A- I- R- G- L- R- E- T- A- A- E- V- E- L- R- R- A- Q- V- V- W

Hydrophobic residues are shown in red, and P and G are shown in blue. The amino acid composition of the sequence:

Hydrophobic amino acid --------: 1, V: 3, L: 2, F: 0, C: 0, M: 0, A: 4, W: 1

Numbers of G and P -------G: 1, P: 0

Negatively charged amino acids -------E: 3, D: 0

Positively charged amino acids--------K: 0, R: 4, H: 0

Other amino acids -------T: 1, S: 0, Y: 0, Q: 1, N: 0

Percentage of each amino acid: Ile ratio = 4%

Val ratio = 14% Leu ratio = 9% Phe ratio = 0% Cys ratio = 0%

Met ratio = 0% Ala ratio = 19% Trp ratio = 4% Gly ratio = 4% Pro ratio = 0% Thr ratio = 4% Ser ratio = 0% Tyr ratio = 0% Gln ratio = 4% Asn ratio = 0% Glu ratio = 14% Asp ratio = 0% His ratio = 0% Lys ratio = 0% Arg ratio = 19%

The APD-defined total hydrophobic ratio = 52% The total net charge = + 1

GRAVY = -0.1

Wimley-White whole-residue hydrophobicity of the peptide = 7.64 kcal/mol The molecular weight of the input peptide = 2423.811

The molecular formula of the peptide = C106H180N35O30S0

Assuming cysteines are paired, the molar extinction coefficient of the peptide = 5550

If the cysteines are not paired (e.g., reduced HBD-1), the molar extinction coefficient of the peptide = 5550

Protein-binding Potential (Boman index) = 2.42 kcal/mol

The input sequence is: AIRGLRETAAEVELRRAQVVW A-I-R-G-L-R-E-T-A-A-E-V-E-L-R-R-A-Q-V-V-W-

A I R G L R E T A A E V E L R R A Q V V W A I R G L R E T A A E V E L R R A Q V V W

Hydrophobic residues are shown in red, and hydrophobic residues on the same surface are underlined.

Total hydrophobic residues on the same surface = 9.

Table 5: Comparison of the native peptide and the mutant peptide

Parameter	Native peptide	Mutant peptide
Number of amino acids	21	21
Molecular weight	2423.8	2566.0
Theoretical pI	9.49	11.00
Negatively charged residues	3	3
Positively charged residues	4	8
Net charge (overall charged residue)	+1	+5
Aliphatic index	116.19	83.81
Grand average of hydropathicity (GRAVY)	-0.100	1.295
Hydrophobic amino acids	11	8
Total hydrophobic ratio	52%	38%
Protein-binding Potential (Boman index)	2.42 kcal/mol	3.82 kcal/mol
Total hydrophobic residues on the same surface	9	7
Arginine (%)	19	19
Lysine (%)	0	19
Tryptophan (%)	4.8	4.8
Glycine (%)	4	4

Helical wheel projection

Mutant peptide

High hydrophobic quantities remain representative of the helical movement of mutant peptides. These include the following elements: alanine, leucine, valine, arginine, tryptophan, lysine, leucine, isoleucine, glutamic acid, glutamine and glycine. The hydrophilic residues include one glycine (Figure 14).

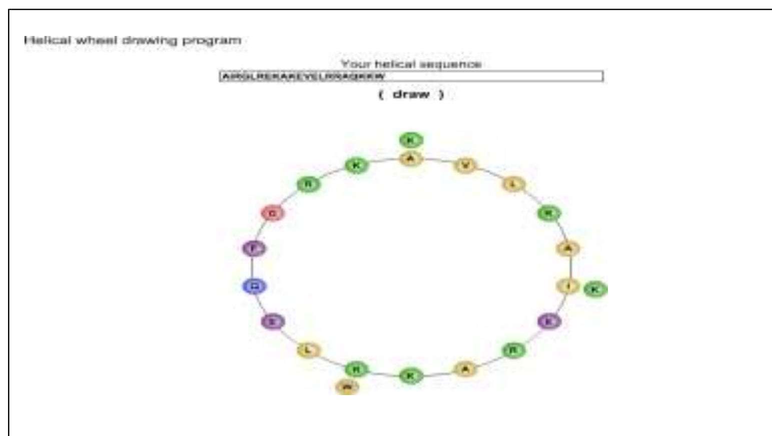

Figure 14: Helical wheel projection obtained for the mutant peptide

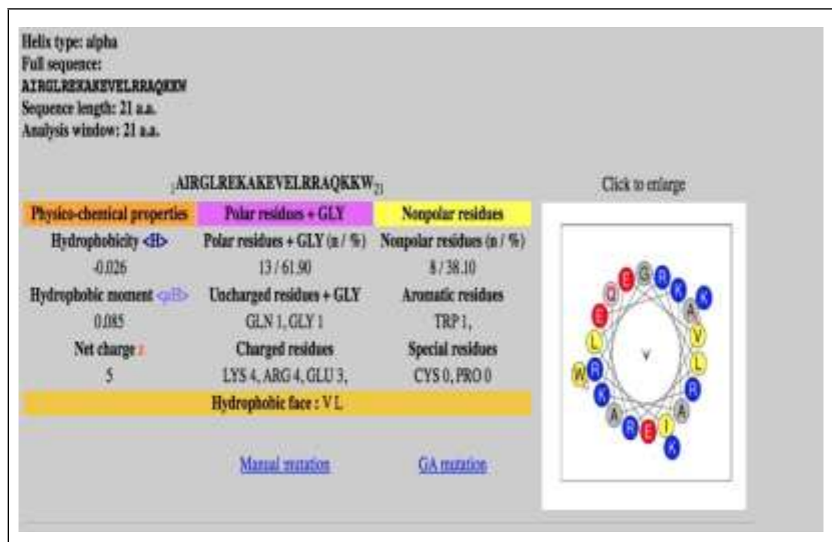

Figure 15 Physio-chemical properties for the mutant peptide.

Native peptide

High hydrophobic quantities remain representative of the helical movements of native peptide. These include the following elements: alanine, leucine, valine, arginine, tryptophan, isoleucine, glutamic acid, glutamine, glycine and threonine. The hydrophilic residues include two glycine (Figure 16).

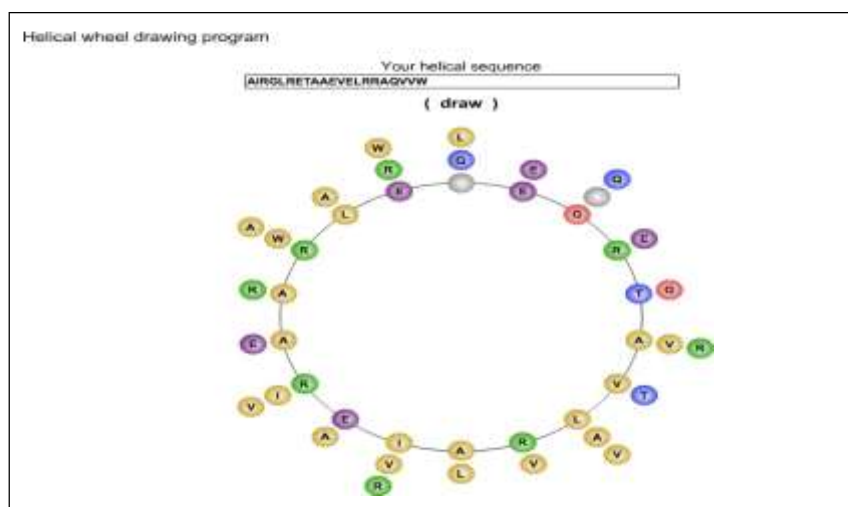

Figure 16: Helical wheel projection obtained for the native peptide.

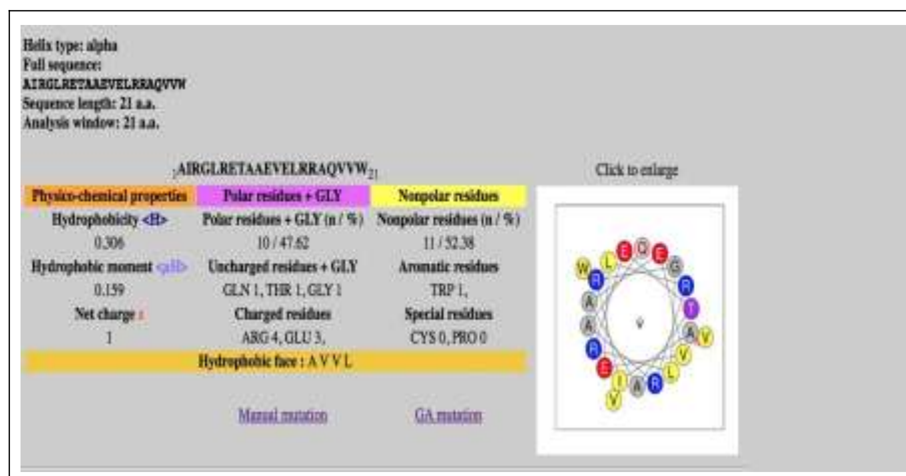

Figure 17 Physio-chemical properties for the native peptide.

GOR IV analyses

Mutant peptide

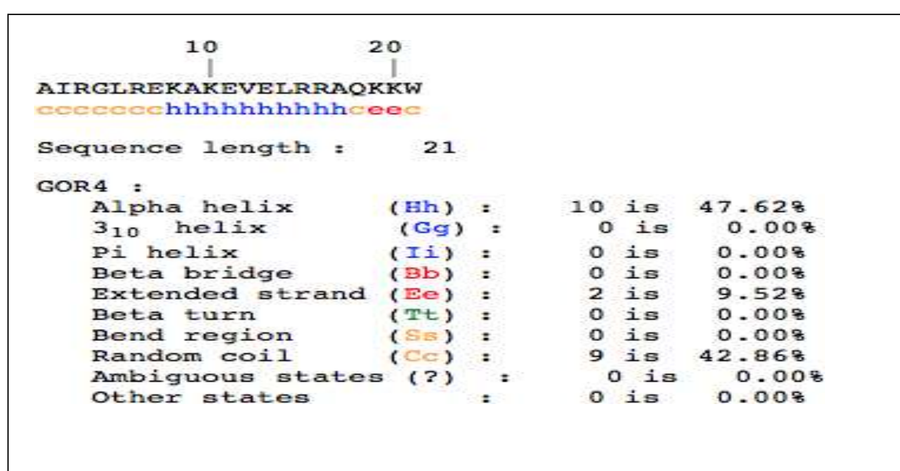

Figure 18: GOR IV prediction for the mutant peptide.

The GOR IV analysis of the mutant peptide (Figure 18) indicated a 0% beta turn, but also the presence of an extended strand (9.52%), a random coil (42.86%) and an alpha helix (47.62%).

Native peptide

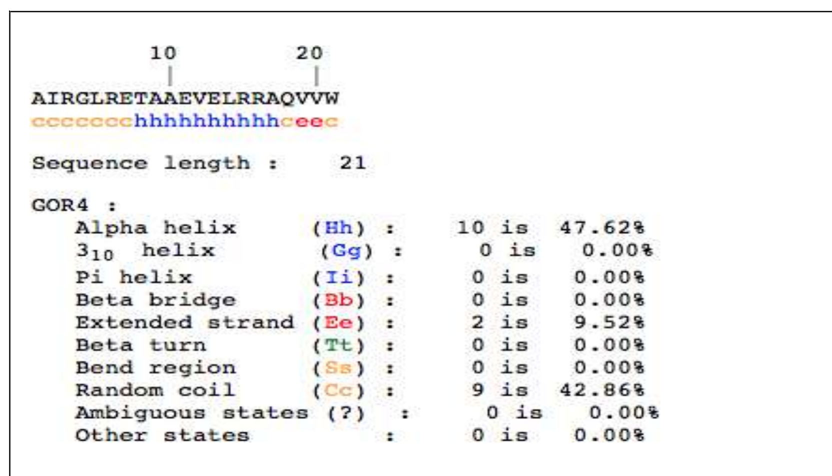

Figure 19: GOR IV prediction for the native peptide.

The GOR IV analysis of the native peptide (Figure 4.19) indicates a 0% beta turn, bend regions and a beta bridge. However, it also shown an α-helix (47.62%), an extended strand (9.52%) and a random coil (42.86%). This is similar to the GOR IV analysis.

DISCUSSION:

This study aims to ascertain the antimicrobial properties of lactoferrin and black seed oil through the application of the disk diffusion method. This study is the first of its kind to use the disk diffusion method to determine the antimicrobial activity of a mutant peptide and a native peptide. It involves the in vitro examination of the synergistic actions of the mutant peptide of camel lactoferrin and Nigella sativa oil. The evaluation of the lactoferrin's antimicrobial activity involved the analysis of the inhibition zones surrounding the substance in an in vitro culture. When there were no such inhibition zones, it was presumed that the substance had no antimicrobial activity.

Table 1 and Figure 3 detail the results of the experiment, showing the substances' antimicrobial potency against the following organisms: Streptococcus pneumonia, Streptococcus agalactiae, Streptococcus pygogenes, Staphylococcus aureus and Candida albicans. By examining the diameter of the inhibition zones in each culture, the study showed that 10µl of the mutant peptide was most effective against Streptococcus pyogenes, producing an average inhibition zone diameter of 13.60 mm. It was also found that 10µl of the native peptide was most effective against Streptococcus pneumonia, with an average inhibition zone diameter of 14.00 mm (Table 2, Figure 4).

Nevertheless, although plants which are derived from antibacterials has less ability resist infections, plants in general, have the ability to protect themselves from infections successfully.

For this reason, it would be clear that plants can adopt different strategy which synergy to be able to fight bacteria (Doble, et al., 2008).

Accordingly, in the present study, the black seed has been synergized with LC (mutant) to increase the capacity of killing the bacteria in a wide range. This can be compatible with our results. The results identify that the largest zone of inhibition (27mm diameter) was recorded in a sample of Streptococcus agalactiae, and the sample involved the in vitro Synergistic Action of both the mutant peptide and the Nigella sativa oil (5µl) (Table 3, Figure 5).

The largest zone of inhibition (27 mm diameter) was recorded in a sample of Streptococcus agalactiae that involved the in vitro synergistic action of both the mutant peptide and the Nigella sativa oil (5µl).

The results obtained for brand of the black seed oil provided the basis for a valid conclusion. Specifically, it was found that black seed oil had the most noticeable impact on Stretococcus agalactiae, since the zone of inhibition for this case had a diameter of 39.00 mm (Table 4, Figure 7).

Figures 3 and 4 describe the effect that lactoferrin had on the gram-positive bacteria. This effect may be ascribed to the electrostatic damage to the cell wall of the bacteria. Specifically, lactoferrin binds to the negatively charged lipid matrix of the bacterial cell wall and membrane, disrupting and potentially inhibiting or destroying the organism (Robert et al., 2006).

Lactoferrin's essential goal is to sequester free iron—and, in doing so, to eliminate the crucial substrate needed for bacterial development. The antibacterial activity of lactoferrin is additionally clarified by the vicinity of particular receptors on the cell surface of microorganisms. Lactoferrin ties to the lipopolysaccharide of bacterial dividers, and the oxidized iron piece of the lactoferrin oxidizes microscopic organisms through the development of peroxides. This influences the layer's porousness and causes cell breakdown (lysis) (Evans, 2003).

The cLf (native peptide) indicated a critical inhibitory impact against all microorganisms. However, this effect was more compelling in Streptococcus pneumonia than in the others (Figure 13). Lfcin and the other peptides derived from Lf or Lfcin were more potent antibacterial agents—a property exhibited by their interaction with and penetration of the bacterial membrane (Evans et al., 2010).

According to the bioinformatic tools used in this study, AMP is dependent on the total net charge of the peptide, such that a positive increase in the total net charge implies a better AMP property. The native lactoferrin peptide had a total net charge of +1, compared to that of the mutant lactoferrin peptide, which had a total net charge of +5, as shown in Table 5. Thus, theoretically, the mutant lactoferrin peptide should be a better AMP than the native lactoferrin peptide. The mutant

lactoferrin peptide also produced a larger inhibition zone than the native lactoferrin peptide. The results are in accordance with those of the bioinformatics tool, which suggested that the total net charge of the mutant lactoferrin peptide was higher (+5) than that of the native lactoferrin peptide (+1).

Water-soluble AMPs can penetrate the lipid bilayer of the microbial cell membrane through their fundamental hydrophobic property. In this study, the peptide mutant displayed a 38% hydrophobicity and a +5 total net charge, as determined by the bioinformatics analysis (Table 5). Figure 14 illustrates the seven hydrophobic residues expected to exist on the helical cylinder surface. Taken together, these findings show that the mutant peptide is amphipathic and α-helical, with antimicrobial properties.

The results obtained from the sequencing suggest that the mutant peptide contains 4% glycine residues, as shown in Table 5 also illustrates that the native peptide has a 52% hydrophobicity and a +1 total net charge. Furthermore, Figure 16 shows that the native peptide is α-helical and amphipathic due to the nine hydrophobic residues believed to exist on the surface of the α-helix. Both the native and the mutant peptides are cationic AMPs with amphipathic properties, although the former displays greater hydrophobicity (52% versus 38%).

The native and mutant peptides have Boman index values of 2.42 and 3.82 kcal/mol, respectively, as shown in Table 4.5. Figure 4.16 illustrates the 9.52% extended strand and 42.86% random coil of the mutant peptide. The wild-type sequence exhibited a 47.62% α- helix, a 9.52% extended strand and a 42.86% random coil configuration, when resolved by GOR IV (Figure 19). Taken together, these findings show that, compared to the mutant form, the native peptide displays greater antimicrobial activity and reduced side effects.

Nigella sativa oil

Nigella sativa (NSO) is an annually blossoming plant from the family Ranunculaceae. It is local to Southwest Asia, Southern Europe and North Africa; however, it has been developed and utilized in diverse parts of the world. In Arabic, this plant is known as Habbat al-barakah. In English, it is regularly known as a dark seed. N. sativa was also a conventional sauce in traditional times, and its seeds were widely used to flavour different foods.

In this study, the oil was observed to be more powerful on gram +ve than gram –ve microorganisms, as shown in Table 4 and Figure 7. These results are congruent with prior studies. Various mixes drawn from plants frequently indicate significant movement against gram +ve microbes, but not against gram –ve species. Gram-negative microbes have a viable penetrability obstruction in their external layer, which limits the infiltration of amphipathic mixes and multi-

drug resistance pumps that expel poisons over this boundary. It is conceivable that the obvious lack of effectiveness of plant antimicrobials is due to a great extent to this porousness barrier (Lindequist et al., 2001; Kahsai, 2002; Lewis et al., 2002).

As demonstrated, all ten brands of dark seed oil had antibacterial effects against Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus pneumonia, Streptococcus agalactiae and Candida albicans (Table 4). However, brand ten was found to be more viable than the others for all tested microscopic organisms. This could be may be due to differences among the sensitivity of the alkaloids, since different solvents may provide different types of alkaloids or dilute these brands (Table 4) (Akbar et al., 1988).

The antibacterial impact was proven by taking the diverse centralization of the Nigella sativa oil from the ten brands and applying it directly to the microorganisms (Table 4, Figure 7). This approach demonstrated that the impact of oil number ten had the greates inhibitory effect, brought on this oil's specific aspects. The greatest inhibition was seen for Streptococcus agalactiea (Figure 7), with a ZOI of 39 mm. No inhibition was seen for the negative form (Figure 8).

This experiment included tests outside vitro to examine the effect of the oil in inhibiting the growth of certain microorganisms (Staphylococcus aureus, Streptococcus agalactiae, Streptococcus pyogenes, Streptococcus pneumonia and Candida albecans) due to their significance for preventing the growth of these types of bacteria. The results showed that the black seed oil contained several active elements, including alkaloids, nigellimine and nigellicine. These are all presented as being responsible for the anti- bacterial activity. Moreover, the extraction activity of the black seed oil is found to stem from the existence of phenol compounds, which are active in inhibiting gram –ve and gram +ve microorganisms, as shown in Figures 9, 10, 11 and 12. This finding was determined by taking samples from many oil brands. The results confirm that, with regard to the effect of the black seed oil concentration for each brand on the bacteria, brand ten (as shown in (Table 4) had the greatest effect in inhibiting bacterial growth. The zone of inhibition reached 38 mm for the Streptococcus agalactiae bacteria, as shown in (Figure 7). A lesser zone of inhibition was found for Streptococcus pneumonia (9.5mm).

Nigella sativa has an inhibitory impact on methicillin-resistant Staphylococcus aureus (MRSA). This discovery warrants further examination of the results for pharmaceutical purposes (Hanan et al., 2008). Dark cumin or Nigella sativa seeds have numerous therapeutic properties, including bronchodilator, hypotensive, antibacterial, antifungal, pain-relieving, mitigating and immune-potentiating characteristics, and they are acknowledged as all-around panaceas (Khan, 1999). It has

also been noted that Nigella sativa oil has a defensive effect against murine cytomegalovirus contamination (Salem and Hossain, 2000).

Additional research conducted by Benjar et al. (2004) explored black seed oil and illustrated a sizeable impact on standard Staphylococcus aureus.

CONCLUSION

This research study applied a lab setting to discern the potential efficacies of novel AMPs in lactoferrin. The lab context allowed AMP components to be located within the given protein.

The study developed and maintained hypotheses related to the complexity of Lf. It also showed that native and mutant aspects (with the exception of iron-chelating) have direct effects on bacteria. The binding of lactoferrin to the lipids within lipopolysaccharides, through the initialisation of the immune system, prevents the binding of additional LPS parts within bacteria. The cell membrane's structural integrity is subsequently lost, resulting in total cell deterioration.

This study has achieved promising outcomes related to the analysis of the mutant peptide (or the synergistic action of camel lactoferrin); however, further work is required to better understand the mechanisms and processes involved. Research on molecular-level cloning is also needed in order to better ascertain camel lactoferrin gene behaviour and properties.

Using the disc diffusion method, crucial areas of inhibition against tested oral streptococci were identified in black seed oil. The present research has uncovered the effects of various pharmaceutical iterations of alkaloids on the antibacterial properties of Nigella sativa oil in relation to gram-positive bacteria. It was shown that these bacteria had greater sensitivity than gram-negative bacteria, with well-defined inhibition zones.

Limitations

The time frame and resource pressures (i.e., the lack of microorganism types) weighed heavily on this research study, such that Lf behaviour was observed in just five microbe types. Such a narrow range of type-testing reduces the possibility of uncovering anti- microbe behaviours. Therefore, a broader range of bacteria and viruses should analysed to sketch a more finely grained and detailed understanding than has been achieved here.

Additionally, constraints on available resources caused a lack of available Lfs for use in the microbe experimentation. This resulted in findings with limited scope. As has been mentioned, only five types of microbes were used in the peptide experiments—and, in some cases, native peptides remained undissolved, despite the addition of ethanol. This resulted in mixtures that retained their turbidity.

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