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IN-VITRO ANTIBACTERIAL ACTIVITY OF BIOMOLECULES FORMUALTIONS (LYSOZYME) COMPARISON WITH MARKETED PRODUCTS

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ABSTRACT

The increased resistance of bacteria against conventional pharmaceutical dosage forms, the antibiotics, has raised serious health concerns. This has stimulated interest in the development of bio-based therapeutics with limited resistance, namely, proteins, enzymes, and peptides having antibacterial activity. This study envisaged the evaluation of the antimicrobial efficacy of selected biomolecules formulations, namely Lysozyme 1mg tablet and Lysozyme powder for oral solution 1mg/mL against bacteria commonly associated to nosocomial infections: *Staphylococcus aureus*. The antibiotic Streptomycin tablet was used as control compounds for comparison purposes. The sensitivity testing of the Lysozyme powder for oral solution 1mg/mL and Lysozyme 1mg tablet were done using the agar well diffusion method. The antibacterial activity of lysozyme can be attributed to its ability to hydrolyze the peptidoglycan layer of bacterial cell walls, leading to cell lysis and death. The results support the potential use of lysozyme powder for oral solution and Lysozyme 1mg tablet for managing mild antibacterial infection particularly those involving gram-positive bacteria.

KEYWORDS: Antibacterial biomolecules and Lysozyme.

1. INTRODUCTION

The antibiotic has created a lot of medical miracles and made a lot of diseases disappear, for instance, pneumonia, meningitis, puerperal fever, septicemia, tuberculosis, etc. Today in the 21st century, the development of drug-resistant bacteria makes people shocked. So-called drug-resistance bacteria is the resisting of the medicine produced by a bacterium.[3] After the bacterium kept in touch with the medicine many times, the bacterium showed a low susceptibility to the medicine. The recombinant human lysozyme is a well-known bacteriolytic enzyme whose name is 1,4-β-N-lysozyme or peptidoglycan N-acetyl muramyl hydrolase. It hydrolyzes β-1,4 glycoside bonds between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of the bacterial cell wall. Because of its bactericidal activity, lysozyme has been of interest as an anti-virus, anti-tumor anti-inflammation, and immunological regulation agent in medicine.[9] Stabilization of protein/enzymes during storage is important as maintaining their native structure represents a critical challenge in protein formulation. The selected biomolecule lysozyme is stable at -20°C with retention of maximum antibacterial activity. Lysozyme is often regarded as a potential help to overcome the problem of traditional antibiotic resistant bacterial infections. This interest explains the extensive research of lysozyme modifications to improve the applications in medicine, veterinary, crop production, feed, and food preservation.[9] The enzymes are quite stable in aqueous solutions for short periods but the pharmaceutical product must have adequate stability over storage periods of several months or years. The present invention has developed a Lysozyme complex which is difficult to administer in dosage form. Researchers overcome this disadvantage by making a drug delivery system comprising an oral tablet or reconstitutable powder composition for an oral liquid formulation which is easy to administer, more patient compliant, offers immediate effects, and has high physical stability and longer shelf life.[7]

2. MATERIALS AND METHODS

2.1.1. Materials of Lysozyme powder for oral solution 1mg/mL

Lysozyme (3X crystal) Egg white (Muramidase) HSN 35079099 from Sisco Research Laboratories pvt Ltd. Micrococcus lysodeikticus ATCC No.4698 from Sigma-Aldrich, Sodium phosphate (monobasic) from Merck Ltd. Sucrose was purchased from M.B. Sugar and

pharmaceutical Ltd and polyvinylpyrollidone (PVK K-30) was purchased from BASF Ltd. Tutti-Frutti Flavor from Firmenich. Water was deionized and double distilled.

2.1.1 Methods of Lysozyme powder for oral solution 1mg/mL

The antibacterial activity estimated by comparing the inhibition of growth of sensitive micro-organism (Staphylococcus aureus ATCC No.6538) produced by known concentration of lysozyme powder for oral solution 5mg/mL to be examined against a reference standard (Streptomycin). A concentration of 1 g/ml of the Lysozyme powder for oral solution was designed from the stock solution for agar well diffusion assay. Cultures of S.aureus, was inoculated separately on the surface of agar plates by surface spreading using a sterile cotton swab and the bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. The sensitivity testing of the Lysozyme powder for oral solution was done using the agar well diffusion method. whereby, wells of 6 mm to 8mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. About 50µl of the lysozyme powder for oral solution, of the concentration 1 g/ml, was dispensed into respective wells and 10µg streptomycin was used as a positive control since it is a broad-spectrum antibiotic. Physiological saline/Dimethyl sulfoxide (DMSO) was used as a negative control. The test was run in triplicates for quality results. The setup was incubated for 24 hours at 37°C Twenty-four (24) hours. Later, the zones of inhibition were measured using a ruler (AIM®) and a pair of dividers then results were reported in millimeters (mm).[2,3,5]

2.2.1 Materials of Lysozyme 1mg Tablet

Lysozyme (3X crystal) Egg white (Muramidase) HSN 35079099 from Sisco Research Laboratories pvt Ltd. Micrococcus lysodeikticus ATCC No.4698 from Sigma-Aldrich, Sodium phosphate (monobasic) from Merck Ltd. Sucrose was purchased from M.B. Sugar and pharmaceutical Ltd and polyvinylpyrollidone (PVK K-30) was purchased from BASF Ltd. Lactose monohydrate (Pharmatose DCL-11) was purchased from DFE, sodium starch glycolate purchased from JRS, Colloidal Silicon dioxide (Aerosil-200) purchased from Evonik and Magnesium stearate (Ligamed) purchased from peter greven. Water was deionized and double distilled.

2.2.2 Methods of Lysozyme 1mg Tablet

The antibacterial activity was estimated by comparing the inhibition of growth of sensitive micro-organisms (Staphylococcus aureus ATCC No.6538) produced by a known concentration of lysozyme 1mg tablet to be examined against a reference standard (Streptomycin).[1] A concentration of 1g/ml of the Lysozyme tablet was designed from the stock solution for agar well diffusion assay. Cultures of Staphylococcus aureus were inoculated separately on the surface of agar plates by surface spreading using a sterile cotton swab and bacterium evenly spread over the entire surface of agar plate to obtain a uniform inoculum. The sensitivity testing of the Lysozyme tablet was done using the agar well diffusion method. whereby, wells of 6 mm to 8mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer. About 50µl of the lysozyme solution, of the concentration 1g/ml, was dispensed into respective wells, and 10µg streptomycin was used as a positive control since it is a broad-spectrum antibiotic. Physiological saline/Dimethyl sulfoxide (DMSO) was used as a negative control. The test was run in triplicates for quality results. The setup was incubated for 24 hours at 37°C twenty-four (24) hours. Later, the zones of inhibition were measured using a ruler (AIM®) and a pair of dividers then results were reported in millimeters (mm).[2,3,5]

3. RESULT AND DISCUSSION

3.1 Lysozyme powder for oral solution 1mg/mL

The antibacterial activity of lysozyme powder for oral solution 5mg/5ml against Staphylococcus aureus was evaluated using agar well diffusion method. The results are presented in Table 3.1 and figure 3.1 and 3.2.

Table 3.1 In-vitro antibacterial study data of Lysozyme powder for oral solution.

Sr.No.	Batch Number	Condition	Concentration	Zone of Inhibition (mm)
1	-	Control	-	-
2	Standard Streptomycin	Initial	500µg/mL	21 ±1.2
			1000µg/mL	36 ±1.2
3	LU/SPMP/01/03	Initial	500µg/mL	16 ± 1.5
			1000µg/mL	29 ± 1.5
4	LU/SPMP/01/03	6M 25°C/60% RH	500µg/mL	15 ± 1.8
			1000µg/mL	22 ± 1.5

Figure 3.1 In-vitro antibacterial activity of initial samples.

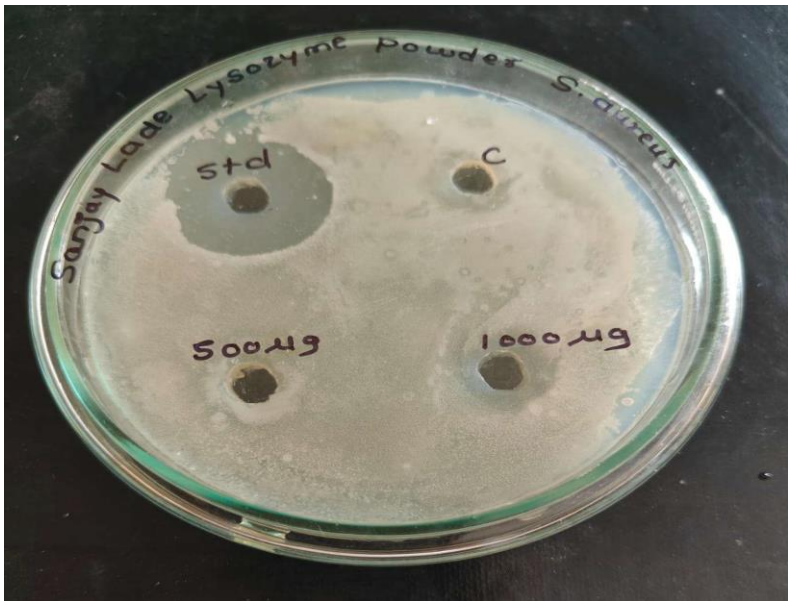

Figure 3.2 In-vitro antibacterial activity of stability samples (6M-25°C/60% RH).

The results of this study demonstrate the antibacterial activity of lysozyme against Staphylococcus aureus using the agar diffusion method. The zone of inhibition increased with increasing concentration of lysozyme, indicating a dose-dependent antibacterial effect. The antibacterial activity of lysozyme can be attributed to its ability to hydrolyze the peptidoglycan layer of bacterial cell walls, leading to cell lysis and death. The positive control, streptomycin showed a larger zone of inhibition compared to lysozyme powder for oral solution indicating its higher antibacterial potency. The negative control distilled water showed no zone of inhibition confirming the specificity of the antibacterial activity of lysozyme powder for oral solution. The results support the potential use of initial as well as 6M-RT stability samples of lysozyme powder oral solution for managing mild antibacterial infections, particularly those involving gram-positive bacteria. It may not be as potent as some conventional antibiotics. However, this can be expected due to the natural enzyme with a different mechanism of action compared to synthetic antibiotics.

3.2 Lysozyme 1mg tablet

The antibacterial activity of lysozyme 1mg Tablet against Staphylococcus aureus was evaluated using agar well diffusion method. The results are presented in table 3.2 and Figure. 3.3 and 3.4.

Table 3.2 In-vitro antibacterial study data Lysozyme 1mg Tablet.

Sr.No.	Batch Number	Condition	Pack	Concentration	Zone of Inhibition (mm)
1	-	Control	-	-	-
2	Standard Streptomycin	Initial	Marketed Blister Pack	500µg/mL	21 ±1.2
				1000µg/mL	36 ±1.2
3	LT/1/09-23/03A	Initial	Alu-Alu Pack	500µg/mL	19 ± 1.5
				1000µg/mL	28 ± 1.5
4	LT/1/09-23/03W	6M 25°C/60% RH	Alu-Alu pack	500µg/mL	17 ± 1.8
				1000µg/mL	27 ± 1.5

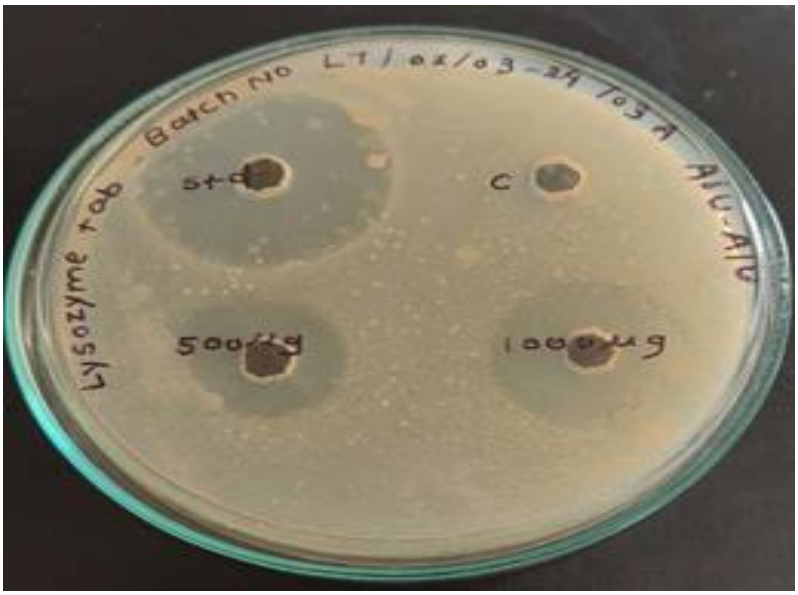

Figure 3.3 In-vitro antibacterial activity of Lysozyme 1mg tablet (Initial Samples).

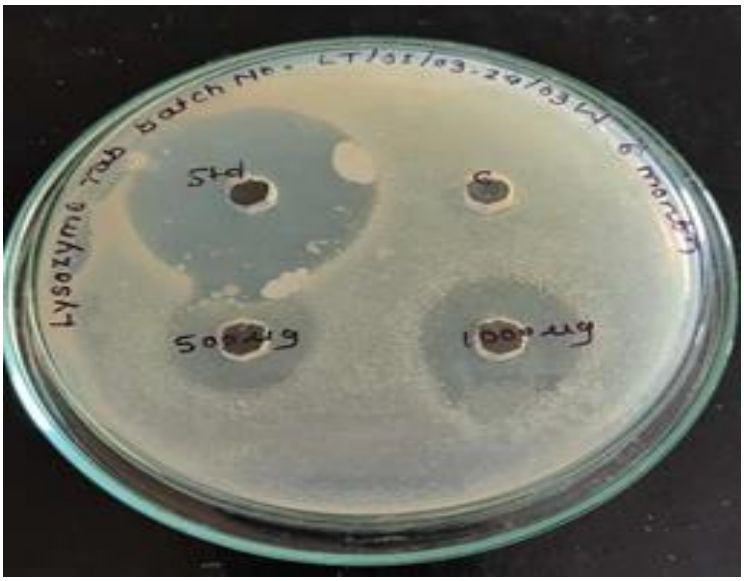

Figure 3.4 In-vitro antibacterial activity of Lysozyme 1mg tablet (Stability samples -6M-25°C/60% RH)

The results of this study demonstrate the antibacterial activity of lysozyme against Staphylococcus aureus using agar diffusion method. The zone of inhibition increased with increasing concentration of lysozyme, indicating a dose-dependent antibacterial effect. The antibacterial activity of lysozyme can be attributed to its ability to hydrolyze the peptidoglycan layer of bacterial cell walls, leading to cell lysis and death. The positive control, streptomycin showed a larger zone of inhibition compared to lysozyme powder for oral solution indicating its higher antibacterial potency. The negative control distilled water showed no zone of inhibition confirming the specificity of the antibacterial activity of the lysozyme 1mg Tablet. The results support the

potential use of initial as well as 6M-RT stability samples of lysozyme 1mg Tablet for managing mild antibacterial infections, particularly those involving gram-positive bacteria. It may not be as potent as some conventional antibiotics. However, this can be expected due to the natural enzyme with a different mechanism of action compared to synthetic antibiotics.[7.8]

1. CONCLUSION

From the results of the present study, it was concluded that Lysozyme powder for oral solution and Lysozyme 1mg tablet have significant antibacterial activity. Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobials from natural origin biomolecules. To further exploit the in vivo antibacterial activities of this formulation of biomolecules and to come up with a potent, safe, and economically affordable formulation, further investigations are to be required.

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