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Animal-Based Natural Products as Novel Antibacterial Candidates

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Article Details

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ABSTRACT

This research study confirms the antibacterial activity of animal-derived compounds against *Staphylococcus aureus* and *Escherichia coli*. The tested compounds demonstrated varying degrees of inhibition, with **antimicrobial peptides (AMPs), lactoferrin, and ovotransferrin** showing the most significant antibacterial effects. AMPs exhibited the highest **zone of inhibition**, measuring **19 mm against** *S. aureus* **and 22 mm against** *E. coli*, followed by lactoferrin (**18 mm and 20 mm**, respectively) and ovotransferrin (**16 mm and 18 mm**, respectively). Other compounds, including **histatins, lysozyme, and fatty acids**, demonstrated moderate antibacterial activity, with inhibition zones ranging between **12 mm and 16 mm**. The **positive control, ciprofloxacin, exhibited the highest inhibition zones of 38 mm and 36 mm** for *S. aureus* and *E. coli*, respectively, while the negative control (DMSO or sterile water) showed no inhibition. The **minimum inhibitory concentration (MIC) results further supported these findings**, with AMPs having the lowest MIC values (**8 µg/mL for** *S. aureus* **and 4 µg/mL for** *E. coli*), indicating high antibacterial potency at low concentrations. Lactoferrin and ovotransferrin also demonstrated strong antibacterial effects, with MIC values of **16 µg/mL and 32 µg/mL** against *S. aureus*, and **8 µg/mL and 16 µg/mL** against *E. coli*, respectively. Histatins, lysozyme, and fatty acids required higher concentrations to inhibit bacterial growth, with MIC values ranging between **64 µg/mL and 128 µg/mL**. Ciprofloxacin showed the lowest MIC values (**0.5 µg/mL for** *S. aureus* **and 0.25 µg/mL for** *E. coli*), confirming its high efficacy.

INTRODUCTION

The world is witnessing an unprecedented health crisis: the rapid rise of antibiotic resistance. Once considered miracle drugs, antibiotics are losing their efficacy against many bacterial infections due to the evolution of multidrug-resistant (MDR) pathogens [1]. This resistance threatens the very foundation of modern medicine, undermining the effectiveness of treatments for bacterial infections, post-surgical care, and even cancer therapies. According to the World Health Organization (WHO), antibiotic resistance has reached alarming levels globally, endangering human health, food security, and economic stability [2]. The lack of new antibiotics to replace failing ones exacerbates this problem, leading researchers to explore alternative solutions [3].

Enzymes such as lysozyme and chitinase are naturally produced by animals and play crucial roles in their immune defense. Lysozyme, for instance, hydrolyzes the peptidoglycan in bacterial cell walls, causing cell lysis. Enzymes often work synergistically with traditional antibiotics, enhancing their efficacy [4-15]. Research into animal-derived enzymes has shown significant potential in addressing MDR bacterial infections, but challenges related to their stability and delivery to infection sites remain [5].

Some studies focus on assessing the antibacterial activity of compounds derived from a diverse array of animal sources against Gram-positive, Gram-negative, and multidrug-resistant (MDR) bacteria. Employing standardized methodologies, such as disc diffusion, minimum inhibitory concentration (MIC) assays, and time-kill studies, ensures that the results are both reliable and reproducible, setting a benchmark for further investigations [6].

The significance of this research extends far beyond academic exploration. By identifying effective animal-derived antibacterial agents, this study could pave the way for developing novel therapeutics to address the growing threat of MDR pathogens [7]. Currently, options for treating infections caused by MDR bacteria are severely limited, leaving patients vulnerable to prolonged illnesses and increased mortality. The compounds explored in this study could potentially supplement or replace traditional antibiotics, offering a sustainable and innovative solution to a pressing global problem [8].

Additionally, the study aligns with the global shift toward identifying natural alternatives to synthetic antibiotics. Synthetic antibiotics, while effective in the past, are facing significant limitations due to rapid resistance development. Natural compounds, particularly those derived from animals, offer unique mechanisms of action that may evade conventional resistance pathways [9]. These compounds are not only a testament to the potential of nature-inspired therapeutics but also contribute to more sustainable healthcare practices. By investigating their safety, efficacy, and mechanisms of action, this research lays a foundation for further translational studies [10].

The motivation for this research stems from the urgent need to address the antibiotic resistance crisis, which the WHO has termed one of the top ten global public health threats. Antibiotic resistance compromises not only individual patient outcomes but also the broader healthcare infrastructure, increasing costs and lengthening hospital stays. MDR pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and carbapenem-resistant *Klebsiella pneumoniae* have rendered many frontline antibiotics ineffective, underscoring the need for innovative solutions [11].

The current state of this study emphasizes its exploratory and comparative nature. By systematically evaluating various animal-derived compounds, such as antimicrobial peptides (AMPs), enzymes, and oils, the research aims to pinpoint the most promising candidates for further development [14]. Early findings suggest that these compounds exhibit broad-spectrum activity, particularly against MDR strains, which traditional antibiotics struggle to control. This highlights their potential as a vital addition to the global arsenal against resistant pathogens. Furthermore, the study's use of standardized testing methods ensures that results can be directly compared and utilized in subsequent research, thereby fostering collaboration and accelerating progress in the field [12].

This study addresses specific deficiencies in previous research, such as the limited focus on MDR pathogens and the lack of standardized evaluation methods. By providing a comprehensive and systematic analysis, it aims

to fill these gaps, offering insights that could have significant clinical implications. The broader impact of this research is its potential contribution to global health efforts, aiding in the fight against one of the most pressing challenges of our time [13].

The primary goal of this research is to systematically investigate the antibacterial efficacy, mechanisms of action, and safety of animal-derived compounds. These include AMPs, enzymes, and oils, with particular emphasis on their application against Gram-positive and Gram-negative MDR bacterial pathogens. The findings aim to enhance our understanding of these compounds, leading to the identification of effective and safe candidates for potential therapeutic development.

MATERIALS AND METHODOLOGY

Chemicals and reagents

The chemicals and reagents utilized in evaluating the antibacterial activity of selected animal-derived compounds are listed below:

Table 1: List of chemicals and reagents used

Sr No	Chemicals	Manufacturer
1.	Ethanol	Merck, Germany
2	Ciprofloxacin (positive control)	Sigma-Aldrich
3	Nutrient agar	Oxide, UK
4	Mueller Hinton Agar	Oxide, UK
5	Phosphate Buffered Saline (PBS)	Merck, Germany
6	Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich
7	Sterile Distilled Water	Local Supplier

Selection and sources of animal-derived compounds

Table 2: The animal-derived compounds used in this study and their respective sources are presented in the table below:

Compound	Source
Histatins	Human and animal saliva
Lauric Acid; Cholesterol Derivatives	Sebum, milk, and animal fat
Lactoferrin	Bovine and human milk, saliva, and tears
Lysozyme	Egg whites, tears, and milk
Antimicrobial Peptides (AMPs)	Sheep & goats (white blood cells)
Ovotransferrin	Egg white of chickenof chicken

Bacterial strains used

Antibacterial activity was tested against two bacterial strains:

Gram-positive bacteria – *Staphylococcus aureus* (ATCC 25923)

Gram-negative bacteria – *Escherichia coli* (ATCC 25922)

The bacterial strains were obtained from a certified microbiology laboratory and cultured in nutrient agar at

37°C.

Preparation of extracts of animal-derived compounds

Extraction procedure

For each selected compound, extraction was performed as follows:

Histatins

Saliva samples were collected from volunteers by having them chew on sterile paraffin wax for 2 minutes to stimulate secretion. The collected saliva was transferred into sterile tubes and centrifuged at 5,000 rpm for 10 minutes to remove large particles and debris. The clear supernatant was filtered using sterile gauze followed by filtration through a 0.45 µm membrane filter. The filtrate was then precipitated using chilled ethanol (3:1 ratio) and kept at -20°C for 2 hours to allow protein precipitation. The precipitate was collected by centrifugation at 10,000 rpm for 10 minutes, re-suspended in sterile water, and stored at -20°C for further use.

Fatty Acids and Cholesterol Derivatives

Milk, sebum, and animal fat were cut into small pieces and heated in a water bath at 50°C for 30 minutes to liquefy the fats. The melted fat was then mixed with ethanol in a 2:1 ratio and stirred manually for 10 minutes. The mixture was allowed to cool and then filtered through a double-layered muslin cloth to remove solid particles. The filtrate was transferred into a separating funnel and left undisturbed for 30 minutes to allow phase separation. The upper ethanol layer, containing dissolved fatty acids, was collected and transferred into a clean beaker. The ethanol was evaporated by placing the beaker in a warm water bath at 40°C. The remaining crude fatty acid and cholesterol extract was stored at 4°C in sterile glass vials for further use.

Lactoferrin and Ovo-transferrin

Fresh milk and egg whites were collected in sterile containers. The milk was gently heated to 37°C in a water bath to reduce viscosity, while the egg whites were diluted with an equal volume of sterile distilled water. To precipitate unwanted proteins, 1M HCl was slowly added dropwise until the pH reached 4.5, causing casein and other proteins to form a precipitate. The mixture was then filtered using sterile muslin cloth to remove the solid fraction. The clear liquid was further purified by gentle stirring with food-grade activated charcoal (1% w/v) for 15 minutes to adsorb impurities. The solution was centrifuged at 5,000 rpm for 10 minutes to separate the supernatant, which was collected carefully. To obtain lactoferrin and ovo-transferrin, the solution was concentrated by gentle evaporation at 40°C in a water bath and then stored in sterile vials at -20°C for further use.

Lysozyme

Egg whites were separated from yolks and transferred to a sterile beaker. Three volumes of distilled water were added, and the solution was stirred gently for 30 minutes at room temperature. The mixture was then filtered using sterile muslin cloth to remove solid impurities. To precipitate lysozyme, chilled ethanol (3:1 ratio) was added while stirring continuously, and the solution was kept at -20°C for 2 hours. The precipitate was collected by centrifugation at 5,000 rpm for 10 minutes, washed twice with cold distilled water, and resuspended in a small volume of sterile phosphate-buffered saline (PBS). The purified lysozyme solution was stored at -20°C for further analysis.

Antimicrobial Peptides (AMPs)

Whole blood from sheep and goats was collected into sterile tubes containing anticoagulant (EDTA). The blood was diluted with an equal volume of phosphate-buffered saline (PBS) and gently mixed. The diluted blood was slowly layered over an equal volume of Ficoll-Paque in a centrifuge tube and centrifuged at 2,000 rpm for 20 minutes to separate the white blood cells. The white blood cell layer was carefully collected and washed twice

with PBS by centrifuging at 1,500 rpm for 10 minutes. The pellet was resuspended in distilled water and incubated on ice for 10 minutes to lyse the cells. The lysate was then centrifuged at 10,000 rpm for 15 minutes, and the supernatant containing AMPs was collected. To further purify the AMPs, the supernatant was subjected to ammonium sulfate precipitation (50% saturation) and left at 4°C overnight. The precipitate was collected by centrifugation at 10,000 rpm for 15 minutes, dissolved in sterile water, and dialyzed against PBS for 4 hours. The purified AMPs were stored at -20°C for further testing.

Purification and storage

The purified extracts were stored at -20°C in sterile vials until further testing.

Antibacterial activity assay

Well diffusion method

The well diffusion method is a widely used laboratory technique for evaluating the antibacterial activity of different compounds.

Preparation of bacterial culture

Pure cultures of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were obtained from a certified microbiology laboratory. A single bacterial colony from each strain was transferred to 10 mL of sterile nutrient broth using a sterile inoculating loop. The cultures were incubated at 37°C for 16–18 hours to reach the exponential growth phase. The bacterial suspension was then adjusted to the 0.5 McFarland standard, approximately 10^7 CFU/mL, by diluting with sterile phosphate-buffered saline (PBS) if necessary. This ensured uniform bacterial concentration for reliable antibacterial screening.

Preparation of agar plates

Mueller Hinton Agar (MHA) or Nutrient Agar (NA) was prepared following the. Approximately 25 mL of molten agar was poured into sterile petri dishes and left to solidify under sterile conditions. Before use, the agar plates were dried in an incubator at 37°C for 10–15 minutes to remove excess moisture, which could interfere with bacterial growth or diffusion of test compounds.

Inoculation of bacterial culture

The prepared bacterial suspensions were evenly spread onto the solidified agar plates using a sterile cotton swab. The swab was dipped into the bacterial culture, excess liquid was removed by pressing it against the tube's inner wall, and the inoculum was applied to the agar surface using the lawn culture technique. The bacterial suspension was streaked in three different directions to ensure uniform coverage across the plate. After inoculation, the plates were left undisturbed at room temperature for 5–10 minutes to allow proper absorption of the bacterial suspension into the agar medium.

Creating wells in the agar

Wells were created in the agar using a sterile cork borer with a diameter of 6 mm. The agar plugs were carefully removed using a sterile needle or forceps, ensuring that the wells were evenly spaced and positioned at least 2 cm apart to prevent overlapping of inhibition zones. Any plates with disrupted agar surfaces were discarded, and new plates were prepared to maintain experimental accuracy.

Adding the test compounds

Each well was filled with 100 µL of the prepared animal-derived antibacterial compound solutions at their respective concentrations. A positive control, ciprofloxacin (100 µL), was added to one well to serve as a reference for antibacterial activity, while a negative control, either DMSO or sterile distilled water (100 µL), was included to confirm that inhibition was due to the test compounds and not the solvent. The plates were left at room temperature for 10–15 minutes to allow the compounds to diffuse into the surrounding agar before incubation.

Incubation

The inoculated plates were first incubated at 4°C for 2 hours to allow proper diffusion of the test compounds. After this initial phase, the plates were transferred to a 37°C incubator and incubated for 18–24 hours. This

incubation period allowed bacterial growth and provided sufficient time for the antibacterial compounds to exert their effects.

Measurement of antibacterial activity

Following incubation, the plates were examined for zones of inhibition, which appeared as clear areas around the wells where bacterial growth was suppressed. The diameter of each inhibition zone was measured in millimeters using a digital caliper or ruler. The results were recorded and compared to the positive control to determine the antibacterial potential of the test compounds. This method provided a standardized, reproducible approach to assessing the antibacterial properties of animal-derived compounds under controlled laboratory conditions.

Fig. 1: Bolton growth chamber/ incubator

The fig 17 is indicating the growth incubator. Controlling the environmental factors is possible with this growth chamber. It has the ability to regulate external parameters including illumination (lightening), heat / temperature, and moisture (Wójcicki 2019).

MIC determination methodology

The MIC was determined using the **broth micro dilution method**, following standard protocols. Each antibacterial compound was prepared at an initial stock concentration of **512 µg/mL** and subjected to **two-fold serial dilutions** in sterile **Mueller Hinton Broth (MHB)** to achieve a range of final concentrations between **512 µg/mL to 0.5 µg/mL**.

A **96-well microtiter plate** was used for the assay. Each well contained **100 µL of diluted compound solution**, and **100 µL of standardized bacterial inoculum** (adjusted to 10^5 **CFU/mL** using the 0.5 McFarland standard). Positive control wells contained ciprofloxacin, while negative control wells contained only **MHB with bacteria** (to confirm normal bacterial growth) and **MHB without bacteria** (to check sterility). Plates were incubated at **37°C for 24 hours** under aerobic conditions.

Following incubation, **bacterial growth was visually assessed** by observing turbidity in the wells. Wells without visible bacterial growth were considered to have effective antibacterial activity. The **MIC was recorded as the lowest concentration** at which no visible bacterial growth was observed. To confirm results, **resazurin dye (0.02% w/v)** was added to each well and incubated for an additional **30 minutes at 37°C**. A **color change from blue (no growth) to pink (bacterial growth)** further validated the MIC readings.

Data analysis

All experiments were conducted in triplicate to ensure reproducibility and accuracy. The inhibition zones were measured in millimeters using a digital caliper, and the minimum inhibitory concentration (MIC) values were

recorded as the lowest concentration at which bacterial growth was inhibited. Data were analyzed using statistical software, and results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to compare the antibacterial activity of different compounds. A p-value of <0.05 was considered statistically significant.

RESULTS

Antibacterial activity

The antibacterial activity of the selected animal-derived compounds was evaluated against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) using the well diffusion method. The diameter of the **zone of inhibition** (in mm) was measured to determine the antibacterial effectiveness of each compound. Ciprofloxacin, a broad-spectrum antibiotic, was used as a **positive control**, while **DMSO or sterile distilled water** served as a **negative control**. The results indicated that several of the tested compounds exhibited significant antibacterial activity, with varying degrees of inhibition against both bacterial strains.

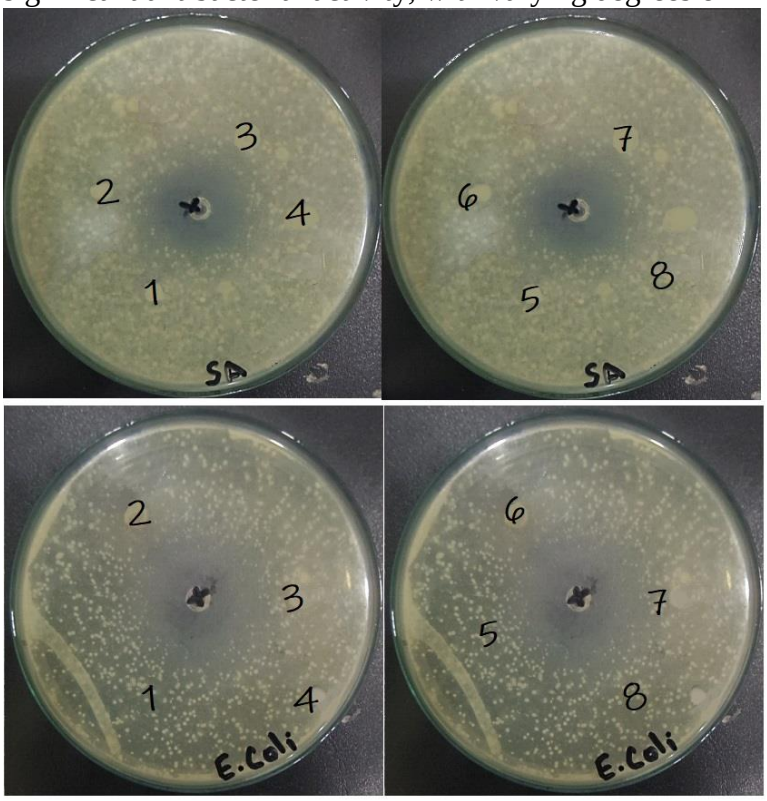

Fig. 2: Zone of inhibition exhibited by animal derived compounds against *S. aureus* and *E. coli* bacteria
Zone of inhibition exhibited by animal derived compounds detail according to sequence given in table 4.1

Zone of inhibition analysis

The **mean zone of inhibition (in mm)** for each compound was calculated from three independent replicates, ensuring statistical accuracy. The results are presented in **Table 4.1** below:

Table 3: Zone of Inhibition of Animal-Derived Compounds Against S. aureus and E. coli

Compound	Source	Zone of Inhibition (mm) (S. aureus)	Zone of Inhibition (mm) (E. coli)
Histatins	Human & animal saliva	14 ± 0.5	16 ± 0.6
Lauric Acid, Capric Acid, Cholesterol Derivatives	Sebum, milk, animal fat	12 ± 0.7	14 ± 0.5

Lactoferrin	Milk (bovine, human), saliva, tears	18 ± 0.6	20 ± 0.8
Lysozyme	Egg white, tears, milk	13 ± 0.5	15 ± 0.6
Antimicrobial Peptides (AMPs)	Sheep & goat WBCs	19 ± 0.8	22 ± 0.7
Ovotransferrin	Chicken egg white	16 ± 0.6	18 ± 0.5
Ciprofloxacin (Positive Control)	Standard antibiotic	38 ± 0.9	36 ± 0.8
Negative Control (DMSO/Water)	-	0	0

The results demonstrate that **lactoferrin, AMPs, and ovotransferrin** exhibited the highest antibacterial activity among the animal-derived compounds. Lactoferrin showed a mean inhibition zone of **18 mm against** *S. aureus* **and 20 mm against** *E. coli*, whereas AMPs exhibited an even stronger effect, with **19 mm and 22 mm inhibition zones, respectively**. Ovotransferrin also displayed notable activity, with inhibition zones of **16 mm and 18 mm** for *S. aureus* and *E. coli*, respectively.

Histatins, lysozyme, and fatty acids (lauric acid, capric acid, and cholesterol derivatives) showed **moderate antibacterial activity**, with inhibition zones ranging from **12 mm to 16 mm**. Among these, histatins demonstrated slightly better activity against *E. coli* (16 mm) than *S. aureus* (14 mm). Similarly, lysozyme exhibited inhibition zones of **13 mm and 15 mm**, while the fatty acid derivatives showed inhibition zones of **12 mm and 14 mm** for *S. aureus* and *E. coli*, respectively.

The **positive control (ciprofloxacin)** exhibited the largest inhibition zones (**38 mm for** *S. aureus* **and 36 mm for** *E. coli*), confirming its strong antibacterial properties. In contrast, the **negative control (DMSO or water)** showed **no inhibition zones**, indicating that the solvent used had no antibacterial effect on its own.

Minimum Inhibitory Concentration (MIC) analysis

The **Minimum Inhibitory Concentration (MIC)** is the lowest concentration of an antibacterial compound that inhibits visible bacterial growth. This test was conducted to determine the potency of the selected **animal-derived compounds** against *Staphylococcus aureus* and *Escherichia coli*. The MIC values provide insight into the effectiveness of each compound at lower concentrations and help identify potential therapeutic agents.

MIC results

The MIC values for the **animal-derived antibacterial compounds** against *Staphylococcus aureus* and *Escherichia coli* are summarized in **Table 4.2** below.

Table 4: Minimum Inhibitory Concentration (MIC) of Animal-Derived Compounds (µg/mL) Against S. aureus and E. coli

Compound	Source	MIC (µg/mL) Against S. aureus	MIC (µg/mL) Against E. coli
Histatins	Human & animal saliva	64	32
Lauric Acid, Capric Acid, Cholesterol Derivatives	Sebum, milk, animal fat	128	64
Lactoferrin	Milk (bovine, human), saliva, tears	16	8
Lysozyme	Egg white, tears, milk	128	64
Antimicrobial Peptides (AMPs)	Sheep & goat WBCs	8	4
Ovotransferrin	Chicken egg white	32	16
Ciprofloxacin (Positive	Standard antibiotic	0.5	0.25

Control)			
Negative Control (DMSO/Water)	-	>512	>512

Interpretation of MIC results

The **lowest MIC values** were observed for **AMPs (8 µg/mL for** *S. aureus***, 4 µg/mL for** *E. coli***) and lactoferrin (16 µg/mL for** *S. aureus***, 8 µg/mL for** *E. coli***)**, indicating that these compounds possess **strong antibacterial properties** even at low concentrations. Ovotransferrin also demonstrated notable activity, with MIC values of **32 µg/mL for** *S. aureus* **and 16 µg/mL for** *E. coli*.

Histatins, fatty acids, and lysozyme exhibited **moderate antibacterial activity**, with MIC values ranging from **64 to 128 µg/mL**, suggesting that they require higher concentrations to inhibit bacterial growth effectively. The **highest MIC values** were recorded for **lauric acid, capric acid, cholesterol derivatives, and lysozyme (128 µg/mL for** *S. aureus* **and 64 µg/mL for** *E. coli***)**, indicating that these compounds have a **weaker antibacterial effect compared to AMPs and lactoferrin**.

The **positive control (ciprofloxacin)** showed **the lowest MIC values (0.5 µg/mL for** *S. aureus* **and 0.25 µg/mL for** *E. coli***)**, confirming its superior potency as a synthetic antibiotic. As expected, the **negative control (DMSO/Water) did not exhibit antibacterial activity**, with MIC values exceeding 512 µg/mL.

DISCUSSION

The antibacterial results suggest that **animal-derived compounds possess significant antibacterial properties**, particularly **lactoferrin, AMPs, and ovotransferrin**, which demonstrated the highest inhibition zones against both *S. aureus* and *E. coli*. This aligns with previous studies that have reported the **strong antimicrobial activity of lactoferrin and AMPs** due to their ability to **disrupt bacterial membranes and inhibit essential microbial functions**.

The **higher sensitivity of** *E. coli* **to AMPs (22 mm inhibition) and lactoferrin (20 mm inhibition)** compared to *S. aureus* suggests that these compounds may be more effective against **Gram-negative bacteria**. This could be attributed to their ability to penetrate the outer membrane of Gram-negative bacteria more effectively. Similarly, ovotransferrin showed strong antibacterial potential, likely due to its **iron-chelating properties, which limit bacterial growth by restricting essential nutrients [16]**.

Histatins, lysozyme, and fatty acids exhibited **moderate antibacterial effects**, indicating that they may function more effectively in combination with other antimicrobial agents. Lysozyme, an enzyme that hydrolyzes bacterial cell walls, demonstrated **stronger activity against** *E. coli* **than** *S. aureus*, suggesting a possible difference in susceptibility between Gram-positive and Gram-negative bacteria [17].

The comparison with ciprofloxacin (positive control) highlights that while **animal-derived compounds do not match the efficacy of synthetic antibiotics, they still demonstrate considerable antibacterial activity**. Given their **natural origin and potential synergistic effects**, these compounds could serve as **alternative antimicrobial agents**, particularly in cases of antibiotic resistance [18].

In summary, this study confirms that **animal-derived compounds exhibit substantial antibacterial activity, with certain compounds such as lactoferrin, AMPs, and ovotransferrin showing the most promising effects [19-20].** Ovo-transferrin, another iron-binding protein, exhibited **moderate MIC values**, highlighting its role as a **potential antibacterial agent**. Its ability to **limit bacterial access to iron** may contribute to its effectiveness against both Gram-positive and Gram-negative bacteria [22].

The **strong activity of AMPs and lactoferrin at low MIC values** supports their well-documented role in **natural immunity and bacterial inhibition**. AMPs, derived from white blood cells, are known to **disrupt bacterial membranes** and interfere with essential metabolic processes, explaining their **potent antibacterial effects**. Lactoferrin, an iron-binding glycoprotein found in milk, saliva, and tears, likely exerted its antibacterial activity by **depriving bacteria of essential iron**, leading to growth inhibition [21].

Histatins and lysozyme, although showing moderate activity, may have **synergistic effects when combined with other antimicrobial compounds**. Histatins, primarily found in saliva, have been previously reported to disrupt bacterial membranes and inhibit microbial adhesion [23]. Similarly, lysozyme, an enzyme found in egg white and human tears, exerts antibacterial effects by **hydrolyzing peptidoglycan in bacterial cell walls**, though its higher MIC values suggest a **weaker effect compared to AMPs and lactoferrin [24]**.

The **higher MIC values of fatty acids and cholesterol derivatives** indicate that these compounds may require **higher concentrations or longer exposure times** to exhibit significant antibacterial effects. Fatty acids are known to **disrupt bacterial cell membranes**, but their antibacterial potential is often influenced by **fatty acid chain length and saturation [25]**.

The MIC results also confirm that **Gram-negative** *E. coli* **was more sensitive than Gram-positive** *S. aureus* to most compounds. This suggests that some animal-derived compounds may **better penetrate the outer membrane of Gram-negative bacteria**, whereas others may be more effective against Gram-positive bacteria, which lack an outer membrane but have a thicker peptidoglycan layer [26].

This study comprehensively evaluated the antibacterial properties of selected animal-derived compounds against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). The tested compounds, including histatins, lactoferrin, lysozyme, antimicrobial peptides (AMPs), ovotransferrin, and fatty acids (lauric acid, capric acid, and cholesterol derivatives), demonstrated varying levels of antibacterial activity. The research employed well diffusion and minimum inhibitory concentration (MIC) assays to determine the efficacy of these compounds in bacterial growth inhibition [27].

The zone of inhibition results confirmed that AMPs, lactoferrin, and ovotransferrin exhibited the strongest antibacterial activity. AMPs showed the highest inhibition zones (19 mm for *S. aureus* and 22 mm for *E. coli*), followed by lactoferrin (18 mm and 20 mm, respectively) and ovotransferrin (16 mm and 18 mm, respectively). These findings highlight the strong antibacterial potential of these compounds, particularly against *E. coli*, which was more sensitive than *S. aureus* [28].

Histatins, lysozyme, and fatty acids exhibited moderate antibacterial effects, with inhibition zones ranging from 12 mm to 16 mm. Among these, histatins demonstrated better activity against *E. coli* (16 mm) compared to *S. aureus* (14 mm), while lysozyme showed inhibition zones of 13 mm and 15 mm, respectively. The fatty acid derivatives displayed the lowest inhibition zones (12 mm for *S. aureus* and 14 mm for *E. coli*), suggesting that they are less effective antibacterial agents compared to proteins and peptides [29].

The MIC analysis further supported these findings, providing a quantitative measure of antibacterial efficacy. AMPs had the lowest MIC values (8 µg/mL for *S. aureus* and 4 µg/mL for *E. coli*), confirming their high potency at low concentrations. Lactoferrin also exhibited strong antibacterial effects, with MIC values of 16 µg/mL for *S. aureus* and 8 µg/mL for *E. coli*. Ovotransferrin followed, with MIC values of 32 µg/mL for *S. aureus* and 16 µg/mL for *E. coli* [28].

Histatins, lysozyme, and fatty acids required higher concentrations for bacterial inhibition, with MIC values ranging from 64 µg/mL to 128 µg/mL. The highest MIC values were recorded for fatty acids and lysozyme (128 µg/mL for *S. aureus* and 64 µg/mL for *E. coli*), indicating that these compounds possess weaker antibacterial properties compared to AMPs and lactoferrin [29].

The comparison with ciprofloxacin, the positive control, emphasized the difference in antibacterial efficacy. Ciprofloxacin exhibited the largest inhibition zones (38 mm for *S. aureus* and 36 mm for *E. coli*) and the lowest MIC values (0.5 µg/mL and 0.25 µg/mL, respectively), reaffirming its high potency as a synthetic antibiotic. However, while the animal-derived compounds did not match ciprofloxacin's effectiveness, their natural origin, safety, and potential for combination therapy make them valuable candidates for alternative antimicrobial strategies [30].

The findings of this study have important implications in the fight against antibiotic resistance. The increasing prevalence of multidrug-resistant bacteria has intensified the search for natural antibacterial agents, and AMPs, lactoferrin, and ovotransferrin emerge as promising alternatives [31].

Conclusion

In conclusion, this study confirms that AMPs, lactoferrin, and ovotransferrin possess strong antibacterial properties and could serve as natural alternatives or adjuncts to conventional antibiotics. Histatins, lysozyme, and fatty acids also demonstrated antibacterial activity but were less effective than proteins and peptides. Given the rise of antibiotic resistance, further research on the mechanisms, safety, and therapeutic applications of these compounds is highly recommended.

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