

Research Article

In Vitro Antimicrobial Activity of Modified Lysozyme (Lys 90) and Herbal Extracts Against Multidrug-Resistant *Escherichia coli*

Eni Kusumaningtyas¹, Bagem BR Sembiring², Andriani¹, Widodo Suwito³, Dwi Endrawati¹, Prima Mei Widiyanti¹, Tati Ariyanti¹ and Syahrizal Nasution⁴

¹Research Center for Veterinary Science, Research Organization of Health, National Research and Innovation Agency (BRIN), Bogor, Indonesia

²Research Center for Agroindustry, Research Organization for Agricultural and Food, National Research and Innovation Agency (BRIN), Bogor, Indonesia

³Research Center for Food Technology and Processing, Research Organization for Agricultural and Food, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia

⁴Department of Food Technology, Institute Technology Sumatera, Jati Agung, South Lampung, Indonesia

Article history

Received: 24-04-2025

Revised: 24-05-2025

Accepted: 23-06-2025

Corresponding Author:

Widodo Suwito

Research Center for Food Technology and Processing, Research Organization for Agricultural and Food, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia
Email: widodo.suwito@yahoo.com

Abstract: The emergence of Antimicrobial Resistant (AMR) poses a significant challenge to the poultry farming industry. This study investigated the potential of Lysozyme (Lys), modified Lysozyme (Lys 90), an herbal extract, and their combination (herbs + Lys 90) as alternative antimicrobial agents against Multidrug-Resistant *Escherichia coli* (MDR-*E. coli*). Lys 90 was prepared by heating Lys at 90 °C for 20 minutes. The herbal extract consisted of garlic (*Allium sativum*), lempuyang (*Zingiber aromaticum*), ginger (*Zingiber officinale*), and turmeric (*Curcuma domestica*) in a ratio of 4:2:1:1. Antibacterial activities of Lys, Lys 90, herbal extract, and their combination (herbs + Lys 90) against MDR-*E. coli* were evaluated using agar well diffusion method. Morphological effects of these treatments on MDR-*E. coli* cells were examined using Scanning electron microscopy (SEM). The results indicated that combination (herbs + Lys 90) produced a significantly larger inhibition zone against MDR-*E. coli* compared to Lys, Lys 90, and the herbal extract alone. The SEM analysis revealed that MDR-*E. coli* cells treated with Lys, Lys 90, the herbal extract, and combination (herbs + Lys 90) exhibited notable morphological changes, including cell elongation, pore formation, and structural damage. Combination of (herbs + Lys 90) represents a promising initial strategy for combating MDR-*E. coli*. However, this approach is limited to in vitro studies. Therefore, further in vivo investigations and comprehensive toxicity assessments are necessary to evaluate the safety and efficacy of this combination before it can be considered for application in the treatment of MDR-*E. coli* infections in poultry.

Keywords: Modified Lysozyme; Herbal Extracts; Antimicrobial Resistance

Introduction

Antimicrobial Growth Promoters (AGPs) are commonly used in poultry farming; however, their use has raised public concern due to their contribution to Antimicrobial Resistant (AMR) in livestock-derived food products. The AMR can subsequently be transmitted to humans or the environment (Paul *et al.*, 2022). Moreover, bacterial infections in poultry can lead to significant

economic losses on chicken farms, primarily due to increased mortality rates and reduced productivity. Antibiotics are essential for the treatment of bacterial infections; however, their inappropriate or excessive use contributes to the rise of antibiotic-resistant bacterial strains (Sakr *et al.*, 2020).

The emergence of Anti Microbial-Resistant (AMR) bacteria presents a significant threat to public health. Consequently, there is an urgent need for novel and



effective strategies to combat AMR. Identifying viable alternatives to antibiotics is a crucial first step in improving livestock production. These alternatives must be practical, suitable for large-scale use in livestock populations, and non-toxic to humans. Anti Microbial Peptides (AMPs), derived from plants, probiotics, and bacteriophages, represent a promising and safe approach to combating AMR. This innovative strategy offers significant potential in addressing the growing challenge of antimicrobial resistance (Łojewska and Sakowicz, 2021).

Natural antimicrobials, such as plant extracts, have been used for centuries to combat infections. Plants possess a remarkable ability to synthesize a wide range of secondary metabolites, including phenolics and polyphenols, alkaloids, terpenoids, essential oils, and various other bioactive compounds (Abdallah *et al.*, 2023). Spices, which are plant derived substances with aromatic or potent properties, play a pivotal role not only in enhancing food flavor but also in providing medicinal, preservative, and antioxidant benefits. The antimicrobial properties of spices, extensively documented in ancient literature, continue to attract significant research interest. Numerous spices, including clove, thyme, cinnamon, oregano, coriander, turmeric, star anise, garlic, and black pepper, exhibit notable antimicrobial activities (Khatri *et al.*, 2023).

Modified Lysozyme (Lys 90) has emerged as a promising antimicrobial agent. This naturally occurring enzyme, in its native monomeric form, targets Gram-positive bacteria by cleaving β -(1-4) glycosidic bonds, thereby effectively killing them. However, Lys 90 induces a transformation into a dimeric form, which confers entirely new beneficial properties, including bacteriostatic effects against Gram-negative bacteria and enhanced medicinal properties (Leśnierowski *et al.*, 2021). Strategies that increase lysozyme's cationic charge and hydrophobicity have been shown to enhance its antibacterial efficacy. Furthermore, heat and enzymatic treatments have proven effective in broadening the antibacterial spectrum of hen egg white lysozyme (Nawaz *et al.*, 2022).

The use of herbal treatments for infectious diseases, particularly in poultry, has been increasing. This trend is largely driven by the emergence of Multidrug-Resistant *Escherichia coli* (MDR-*E. coli*), which exhibit resistance to multiple antibiotics. The combination of herbal extracts comprising garlic (*Allium sativum*), lempuyang (*Zingiber aromaticum*), ginger (*Zingiber officinale*) and turmeric (*Curcuma domestica*) with Lys 90 is hypothesized to be effective against MDR-

E. coli. However, this approach has not been extensively studied or reported in the literature. Therefore, the present study offers a novel contribution compared to previous research. Accordingly, the aim of this study was to evaluate the antibacterial activities of Lys 90, herbal extracts, and their combination against MDR-*E. coli*.

Materials and Methods

Ethical Approval

The Secretariat of the Research Ethics Committee of the National Research and Innovation Agency (BRIN) determined that ethical clearance was not required for this study, as the researchers did not have direct contact with human participants or animals.

Bacterial Strains

The study involved *E. coli* ATCC 25922, and an MDR-*E. coli* isolate associated with poultry disease, obtained from the BBLitvet Culture Collection (BCC) in Bogor, Indonesia. *E. coli* ATCC 25922 is commonly used as a standard reference strain in antimicrobial susceptibility testing due to its genetic stability, which ensures more accurate and reliable test results. Moreover, this strain has well characterized sensitivity to a wide range of antimicrobial agents, making it suitable as a positive control in antimicrobial susceptibility testing (CLSI, 2020).

Lysozyme and Lys 90

Commercial chicken Egg White Lysozyme (HEWL) (PC0710-5g, Vivantis Technologies Sdn Bhd, Revongen Corporation, Center, Malaysia) was used as the starting material for the preparation of Lys 90. To modify the lysozyme, a 1 mg/mL solution of HEWL in 10 mM phosphate-buffered saline (PBS) (w/v), pH 6.8, was heated at 90 °C for 20 minutes (Tomezyk *et al.*, 2023). Following heating, the solution was centrifuged at 3000×g for 15 minutes to remove insoluble aggregates. The resulting supernatant contained Lys 90.

Herbs

Fresh rhizomes of garlic (*A. sativum*), lempuyang (*Z. aromaticum*), ginger (*Z. officinale*), and turmeric (*C. domestica*) were obtained from traditional markets in Bogor, West Java, Indonesia. The herbal materials were thoroughly washed with water to remove impurities, then drained and cut into approximately 1 cm pieces. Subsequently, the samples were dried in an oven at 40 °C for 24 hours. After drying, the materials were ground using a blender and sifted through a 60 mesh sieve. Based on the preliminary study, an herbal mixture consisting of garlic (*A. sativum*), lempuyang (*Z. aromaticum*), ginger (*Z. officinale*), and turmeric (*C. domestica*) in a ratio of 4:2:1:1 was prepared, followed by extraction. The extraction was performed through maceration with a 96% ethanol solvent. One hundred grams of the herbal blend was combined with 300 mL of ethanol and allowed to macerate for 24 hours at room temperature. The solvent was replaced every 24 hours until the solution became clear, indicating that all active compounds had been

extracted. The supernatant was then filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator at 40

°C to obtain the crude extract. Screening of the herbal blend extract was conducted to identify the presence of alkaloids, hydroquinone, flavonoids, tannins, saponins, steroids, and triterpenoids (Fasya *et al.*, 2020; Haida *et al.*, 2021). A quantitative analysis of the content of alkaloids, hydroquinone, flavonoids, tannins, saponins, steroids, and triterpenoids in herbal blends was conducted using the UV-Vis spectrophotometry method.

Antibacterial Activity

The antibacterial activities of Lys, Lys 90, herbs, and combination of (herbs + Lys 90) against MDR-*E. coli* were assessed using the agar well diffusion method, as described by Scieuzo *et al.* (2023). MDR-*E. coli* isolate was cultured on Blood agar (CM 0055B; Oxoid Ltd., Basingstoke, United Kingdom), and incubated for 24 hours at 37 °C. MDR-*E. coli* was standardized with the McFarland 0.5 turbidity, and was spread onto Mueller-Hinton agar (CM 0337; Oxoid Ltd., Basingstoke, United Kingdom) using a sterile bacterial cell spreader. After a five minute period, four wells (0.5 cm in diameter) were perforated in the MHA using a sterile cork borer. Each well was filled with one of the following treatments, dissolved in dimethyl sulfoxide (DMSO) at concentrations of 0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL: Lys, Lys 90, herbs, and (herbs + Lys 90). Each test was conducted in technical triplicate to ensure the reliability and reproducibility of the results. One hundred µL of each treatment was added to the corresponding well, and the plates were incubated for 24 hours at 37 °C. Positive controls included penicillin (10 U), enrofloxacin (5µg), streptomycin (10µg), colistin sulfate (10µg), cefotaxime (30µg), and gentamicin (10µg), while DMSO was used as the negative control. The antibacterial activity of Lys, Lys 90, herbs, and combination of (herbs + Lys 90) against MDR-*E. coli* was assessed by measuring the diameter of the inhibition zone (mm) (CLSI, 2020). The largest zone of inhibition was considered to represent the most potent antibacterial activity.

Scanning electron microscopy (SEM)

Effects of Lys, Lys 90, herbs, and combination of (herbs + Lys 90) on the morphology of MDR-*E. coli* cells were evaluated using SEM, following the protocol described by Novikov *et al.* (2019). Bacterial suspension was standardized to a 0.5 McFarland turbidity standard. Bacterial cells were treated with Lys, Lys 90, herbs, and combination of (herbs + Lys 90) at a concentration of 1 mg/mL, each in a 1:1 ratio. Untreated MDR-*E. coli* served as the negative control. Each treatment mixture was incubated for 2 hours at 37 °C. After centrifugation at 2500× g, the bacterial pellets were washed with distilled

water and dehydrated through a graded ethanol series (50% ethanol for 5 minutes). Following dehydration, the samples were rinsed with saline and fixed with a 2.5% buffered glutaraldehyde solution in 0.1 M Phosphate-Buffered Saline (PBS) (pH 7) at 4 °C for 18 hours. The samples were then post-fixed in 2% tannic acid for 18 hours, washed four times with cacodylate buffer (5 minutes each), and rinsed with distilled water. Dehydration was continued with an ethanol series: 50% ethanol for 5 minutes (repeated four times), followed by 70%, 85%, and 95% ethanol for 20 minutes each, and finally absolute ethanol for 10 minutes (twice). The samples were further dehydrated in tert-butanol for 10 minutes (twice) and then freeze-dried. The dehydrated samples were mounted onto specimen stubs and coated with gold using an ion sputter coater. Imaging was conducted using a JSM IT200 SEM.

Statistical Analysis

The phytochemical composition of the herbal mixture, along with the antibacterial activities and SEM effects of Lys, Lys 90, the herbal extract, and their combination (herbs+Lys 90) against MDR-*E. coli*, are presented descriptively.

Results

Herbs

The combination of garlic (*A. sativum*), lempuyang (*Z. aromaticum*), ginger (*Z. officinale*), and turmeric (*C. domestica*) in a ratio of 4:2:1:1 contains significant levels of bioactive compounds, including flavonoids (2.15 mg/mL), saponins (2.67 mg/mL), hydroquinone (2.79 mg/mL), and triterpenoids (1.28 mg/mL), as presented in (Table 1).

Antibacterial

Antibacterial activities of Lys, Lys 90, herbs, and combination of (herbs + Lys 90) were evaluated at concentrations of 0.1 mg/mL, 1 mg/mL, 10 mg/mL, and 100 mg/mL by measuring diameter of the inhibition zones (mm) against *E. coli* ATCC 25922 and MDR-*E. coli*. At a concentration of 0.1 mg/mL, (herbs + Lys 90) combination produced a larger inhibition zone against both *E. coli* ATCC 25922 and MDR-*E. coli* compared to Lys, Lys 90, or herbs alone (Fig. 6). This trend persisted at concentrations of 1 mg/mL, 10 mg/mL, and 100 mg/mL, where the combination consistently resulted in greater inhibition zones than any of the individual treatments (Fig. 7-9).

SEM

surface roughening, cytoplasmic morphological changes in MDR-*E. coli* were examined using electron microscopy following treatment with Lys, Lys 90, herbs, and combination of (herbs + Lys 90), as shown in Fig. 1-5.

Untreated MDR-*E. coli* cells exhibited smooth surfaces and normal morphology. In contrast, cells treated with Lys showed significant alterations, including leakage, cell elongation, and membrane damage.

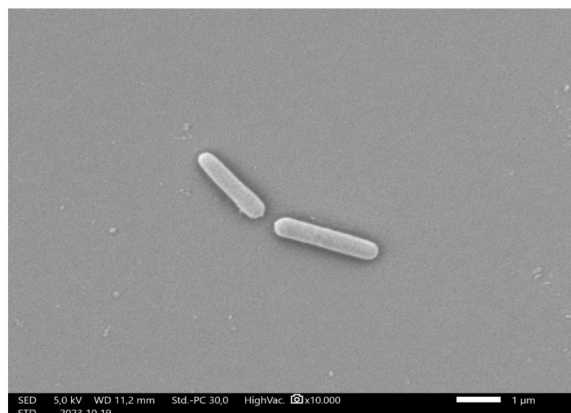


Fig. 1: Scanning electron microscopy (SEM) image of MDR-*E. coli* cells before treatment captured at 10,000 × magnification

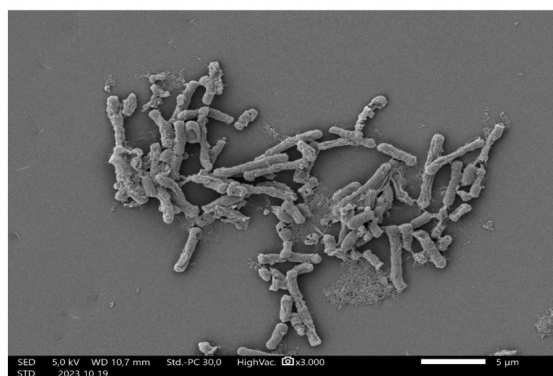


Fig. 2: Scanning electron microscopy (SEM) image of MDR-*E. coli* cells following treatment with herbs at a concentration of 0.1 mg/mL, captured at 3,000 × magnification

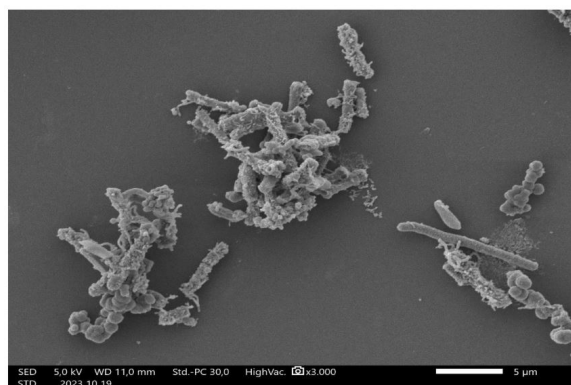


Fig. 3: Scanning electron microscopy (SEM) image of MDR-*E. coli* cells following treatment with Lys at a concentration of 0.1 mg/mL, captured at 3,000 × magnification

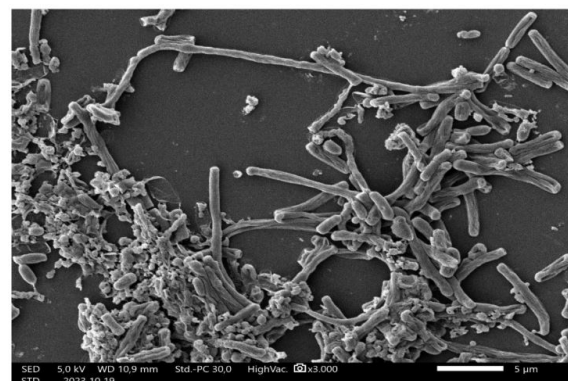


Fig. 4: Scanning electron microscopy (SEM) image of MDR-*E. coli* cells following treatment with Lys 90 at a concentration of 0.1 mg/mL, captured at 3,000 × magnification

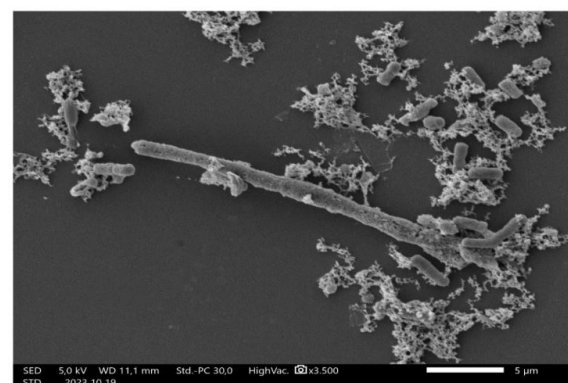


Fig. 5: Scanning electron microscopy (SEM) image of MDR-*E. coli* cells following treatment with (herb + Lys 90) at a concentration of 0.1 mg/mL, captured at 3,000 × magnification

Discussion

Lysozyme, an enzyme renowned for its antimicrobial properties, is widely distributed across the animal kingdom, serving as a natural bactericide (Nawaz *et al.*, 2022). Its importance extends beyond immune defense, as it plays a crucial role in protecting and supporting the growth of developing embryos. In hen egg white, a primary source of lysozyme, this enzyme comprises approximately 3.5% of the total protein content (Li *et al.*, 2022). Lysozyme also enhances the efficacy of antibiotics, thereby contributing significantly to the overall strengthening of the immune response. This multifaceted function underscores lysozyme's pivotal role in both innate immunity and broader defense mechanisms within the organism.

Lysozyme activity, which is influenced by pH, plays a critical role in its antibacterial function (Nawaz *et al.*,

2022). Similarly, Khorshidian *et al.* (2022) emphasized that pH is a key factor in the formation of new lysozyme oligoforms, which in turn significantly impacts its antibacterial activity. Under normal conditions, native hen egg white lysozyme exists in a dimeric form at pH 7. According to Catalini *et al.* (2021), dimer formation may result from lysozyme denaturation induced by factors such as pH changes, variations in lysozyme concentration, duration of denaturation, and fluctuations in temperature and/or the surrounding medium.

The bioactive compounds present in the herbal mixture used in this study are believed to possess antimicrobial properties that may contribute to combating AMR. Ajanaku *et al.* (2022) reported that bioactive compounds present in herbs possess considerable potential as natural therapeutic agents. Curcumin, a bioactive compound in turmeric (*C. domestica*), has been shown to be effective against *Staphylococcus aureus* (*S. aureus*) and *E. coli* (Hussain *et al.*, 2022). Although curcumin demonstrates promising antibacterial potential, it also exhibits selectivity toward specific microorganisms (Adamczak *et al.*, 2020). Ginger (*Z. officinale*) has been reported to contain a diverse range of phytochemicals that contribute to its broad-spectrum antimicrobial activity against various pathogens (Shalaby *et al.*, 2023). Similarly, garlic (*A. sativum*) contains bioactive compounds such as allicin, and allyl sulfides, which exhibit notable antibacterial properties (Bhatwalkar *et al.*, 2021). Comparable antimicrobial constituents are also found in lempuyang (*Z. aromaticum*).

Antibacterial activity of Lys 90 is enhanced compared to that of Lys. This increased efficacy may not be solely attributed to its enzymatic function, but rather to the involvement of an alternative mechanism of action. These insights provide new perspectives on the complex mechanisms underlying the enhanced antibacterial activity of Lys 90, highlighting structural adaptations as a critical factor in their increased efficacy. Our findings are consistent with those of Delbue *et al.* (2023), who reported that heat treatment can modify both the peptide sequence and composition of lysozyme derived products, thereby influencing their antimicrobial activity. It is noteworthy that the conventional understanding posits that heating lysozyme results in enzyme inactivation. However, several studies have intriguingly demonstrated that controlled heating at specific temperatures can enhance lysozyme's antibacterial activity (Huang *et al.*, 2023; Khorshidian *et al.*, 2022; Leśnierowski *et al.*, 2021; Wang *et al.*, 2020).

Combination of herbs and Lys 90 enhances antimicrobial activity by facilitating improved penetration into bacterial cell walls. Additionally, antimicrobial properties of herbs are attributed to the presence of active

compounds such as flavonoids, alkaloids, tannins, saponins, phenols, steroids, and triterpenoids (Table 1). Our findings are consistent with those of Wei and Yi (2023), who reported that combination of herbs and Lys 90 significantly enhances antimicrobial efficacy. This formulation may represent a promising strategy for combating MDR *E. coli* infections. Antimicrobial properties of AMPs and combination of herbs and Lys 90 can be compared from multiple perspectives. AMPs primarily exert their effects by disrupting microbial cell membranes, ultimately leading to cell death (Gagandeep *et al.*, 2024). AMPs exhibit broad-spectrum activity and are effective against a wide range of microorganisms, including bacteria, viruses, and fungi. In addition to their potent antimicrobial effects, AMPs generally possess good stability, although they may be vulnerable to enzymatic degradation. In contrast, combination of herbs and Lys 90 exerts its antimicrobial action primarily through the hydrolytic activity of lysozyme on bacterial cell walls. The herbal components may enhance this effect through various mechanisms, including anti-inflammatory, antioxidant, and direct antimicrobial activities (Nawaz *et al.*, 2022).

The antimicrobial spectrum of the herbs combined with Lys 90 at various concentrations was broader and more potent than that of Lys, Lys 90, or herbs alone (Fig. 6-9). This enhanced activity is likely due to the additional bioactive properties of the herbal components. However, the overall stability of this combination is influenced by the intrinsic stability of both lysozyme and the specific herbal constituents used (Ferraboschi *et al.*, 2021). It is also important to note that the efficacy and safety of both AMPs and the herbs + Lys 90 combination are affected by several factors, including the type of target microorganism, the concentration of the antimicrobial agent, and environmental conditions. Therefore, continued research is essential to comprehensively evaluate the effectiveness and safety of these antimicrobial strategies across diverse clinical and environmental contexts. For example, a study by Liu *et al.* (2020) demonstrated that Phylloseptin Peptide (PPV1), a potent AMP from the phylloseptin family, effectively inhibited *S. aureus* growth both in vitro and in vivo without inducing toxicity. These findings highlight the therapeutic potential of PPV1 and support its further investigation as a promising antimicrobial agent.

Table 1: Phytochemical and level of herbs

Phytochemical	Present in extract	Content (mg/mL)
Flavonoids	+	2.15
Alkaloids	-	-
Tanin	-	-
Saponins	+	2.67
Hydroquinone	+	2.79
Steroid	-	-
Triterpenoids	+	1.28

+ = Positive; - = Negative

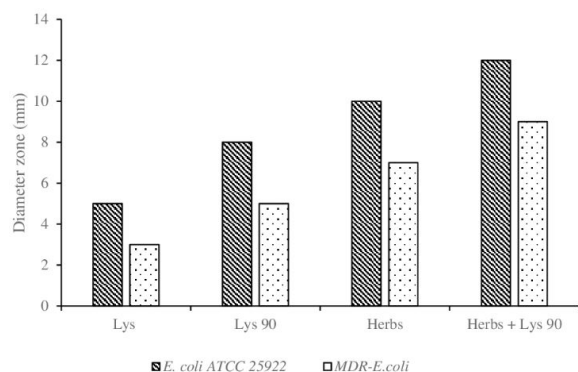


Fig. 6: Diameters inhibition zones Lys,Lys 90,herbs,and (herbs+lys 90) against MDR-*E. coli* and *E. coli* ATCC 25922 at concentration of 0.1 mg/mL

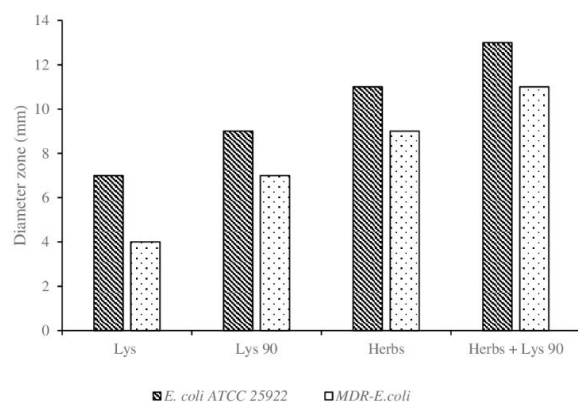


Fig. 7: Diameters inhibition zones Lys,Lys 90,herbs,and (herbs+lys 90) against MDR-*E. coli* and *E. coli* ATCC 25922 at concentration of 1 mg/mL

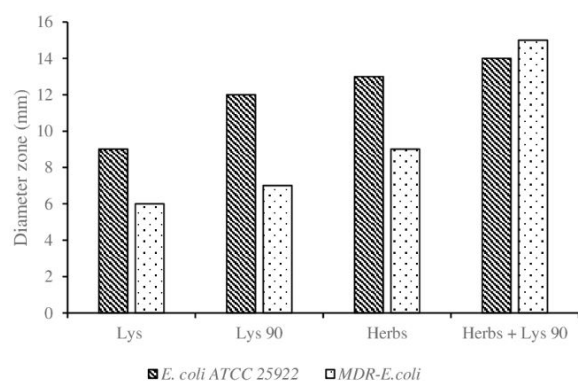


Fig. 8: Diameters inhibition zones Lys,Lys 90,herbs,and (herbs+lys 90) against MDR-*E. coli* and *E. coli* ATCC 25922 at concentration of 10 mg/mL

This study was conducted under in vitro conditions; therefore, further in vivo research is necessary to validate the findings. Additionally, the herbal materials used were sourced from traditional markets and consisted of unidentified varieties, which may have influenced their

phytochemical composition. As noted by Mwamatope *et al.* (2021), the phytochemical profile of medicinal plants is influenced by factors such as plant variety and seasonal variation. Furthermore, the antimicrobial activity of herbs and Lys 90 combination has thus far been evaluated only against MDR-*E. coli*, with no data currently available regarding its efficacy against multidrug-resistant Gram-positive bacteria. Such information is crucial to determine whether the herbs and Lys 90 combination exhibits broad-spectrum activity against both Gram-negative and Gram-positive multidrug-resistant pathogens. Preliminary evidence suggests that this formulation may possess broad-spectrum antimicrobial potential, highlighting the need for further comprehensive studies.

The SEM revealed distinct morphological changes in cells treated with Lys, including the formation of blisters, dimples, and slight cell elongation Fig. (1-5). In contrast, treatment with Lys 90 induced more pronounced features, such as wrinkling, tearing along the cell surface, and significant cell elongation. These observations are consistent with the findings of Mojsoska (2022) regarding the effects of peptoid treatment on *E. coli* cells. Cell elongation or filamentation is a well-documented bacterial stress response, often triggered by certain antibiotics like beta-lactams. Beta-lactams induce filamentation by interfering with cell wall synthesis, binding to penicillin-binding proteins, and inhibiting peptidoglycan cross-linking, which results in biomass accumulation (Kim *et al.*, 2023). Furthermore, bacterial filamentation can be initiated by the activation of the Save-Our-Soul (SOS) response, a mechanism involved in deoxyribonucleic acid (DNA) damage repair. The lack of cell division during elongation can lead to the accumulation of crosslink defects, forming pores that ultimately cause cell bulging and lysis (Mojsoska, 2022; Kim *et al.*, 2023).

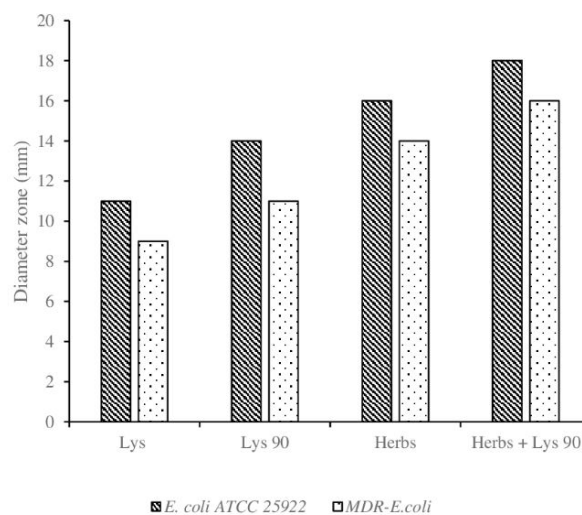


Fig. 9: Diameters inhibition zones Lys,Lys 90,herbs,and (herbs+lys 90) against MDR-*E. coli* and *E. coli* ATCC 25922 at concentration of 100 mg/mL

Future research on herbs and Lys 90 combination should prioritize in vivo studies in poultry farms to evaluate its practical effectiveness under real-world conditions. In particular, challenge trials using MDR *E. coli* isolates are necessary to determine whether administration of the formulation confers protective effects against infection. Additionally, to maintain its antimicrobial efficacy, it is crucial to ensure the stability of herbs and Lys 90 formulation, which may be enhanced through the application of nanoparticle-based delivery systems.

Conclusion

Combination of herbs and Lys 90 represents a promising strategy for combating MDR-*E. coli*, demonstrating greater potential than the use of either Lys 90 or herbs alone. However, further research is necessary to assess the in vivo antimicrobial efficacy of this combination, particularly against both Gram-positive and Gram-negative multidrug-resistant bacteria.

Acknowledgement

This research was supported by research facilities and technical support from the Cibinong Advanced Characterization Integrated Bioproduct Laboratory at the National Research and Innovation Agency (BRIN). The authors would like to thank Susan Maphilindawati Noor, Raphaella Widiastuti, and Dyah Haryuningtyas Sawitri for their valuable assistance in reviewing the manuscript and providing helpful feedback during its preparation.

Funding Information

This research scheme, Riset dan Inovasi untuk Indonesia Maju (RIIM), was supported by funds from National Research and Innovation Agency (BRIN) of Indonesia and Educational Fund Management Institution (LPDP), Ministry of Finance. No 82/II.7/2022.

Author's Contributions

Eni Kusumaningtyas, Widodo Suwito, Andriani, Bagem BR Sembiring, Syahrizal Nasution: Designed and coordinated the research and drafted the initial manuscript.

Dwi Endrawati, Prima Mei Widiyanti, Tati Ariyanti: Performed data analysis.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all coauthors have reviewed and approved the manuscript. Additionally, there are no ethical issues associated with this publication.

References

- Abdallah, E.M., Alhatlani, B.Y., de Paula Menzes, R., & Martins, C.H. (2023). Back to nature: medicinal plants as promising sources for antibacterial drugs in the post-antibiotic Era. *Plants*, 12:1-27. <https://doi.org/10.3390/plants12173077>
- Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2020). Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, 13(7), 153. <https://doi.org/10.3390/ph13070153>
- Ajanaku, C. O., Ademosun, O. T., Atohengbe, P. O., Ajayi, S. O., Obafemi, Y. D., Owolabi, O. A., Akinduti, P. A., & Ajanaku, K. O. (2022). Functional bioactive compounds in ginger, turmeric, and garlic. *Frontiers in Nutrition*, 9. <https://doi.org/10.3389/fnut.2022.1012023>
- Bhatwalkar, S. B., Mondal, R., Krishna, S. B. N., Adam, J. K., Govender, P., & Anupam, R. (2021). Antibacterial properties of organosulfur compounds of garlic (*Allium sativum*). *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.613077>
- Catalini, S., Perinelli, D. R., Sassi, P., Comez, L., Palmieri, G. F., Morresi, A., Bonacucina, G., Foggi, P., Pucciarelli, S., & Paolantoni, M. (2021). Amyloid self-assembly of lysozyme in self-crowded conditions: The formation of a protein oligomer hydrogel. *Biomacromolecules*, 22(3), 1147–1158. <https://doi.org/10.1021/acs.biomac.0c01652>
- CLSI. (2020). *Clinical and Laboratory Standards Institute* (pp. 15–20).
- Delbue, S., Pariani, E., Parapini, S., Galli, C., Basilico, N., D'Alessandro, S., Pellegrino, S., Pini, E., Ciceri, S., Ferraboschi, P., & Grisenti, P. (2023). Heat-treated lysozyme hydrochloride: A study on its structural modifications and anti-SARS-CoV-2 activity. *Molecules*, 28(6), 2848. <https://doi.org/10.3390/molecules28062848>
- Fasya, A. G., Amalia, S., Megawati, D. S., Salima, F., Kusuma, V. A., & Purwantoro, B. (2020). Isolation, identification, and bioactivity of steroids isolates from *Hydrilla verticillata* petroleum ether fraction. *IOP Conference Series: Earth and Environmental Science*, 456(1), 012009. <https://doi.org/10.1088/1755-1315/456/1/012009>
- Ferraboschi, P., Ciceri, S., & Grisenti, P. (2021). Applications of lysozyme, an innate immune defense factor, as an alternative antibiotic. *Antibiotics*, 10(12), 1534. <https://doi.org/10.3390/antibiotics10121534>
- Gagandeep, K. R., Balenahalli Narasingappa, R., & Vishnu Vyas, G. (2024). Unveiling mechanisms of antimicrobial peptide: Actions beyond the membranes disruption. *Heliyon*, 10(19), e38079.

- <https://doi.org/10.1016/j.heliyon.2024.e38079>
- Haida, S., Bakkouche, K., Kribii, A. R., & Kribii, A. (2021). Chemical composition of essential oil, phenolic compounds content, and antioxidant activity of *Cistus monspeliensis* from Northern Morocco. *Biochemistry Research International*, 2021, 1–13. <https://doi.org/10.1155/2021/6669877>
- Huang, S., Wu, Z., Zhou, B., Jiang, X., Lavillette, D., & Fan, G. (2023). Heat-denatured lysozyme is a novel potential non-alcoholic disinfectant against respiratory virus. *Food and Environmental Virology*, 15(3), 212–223. <https://doi.org/10.1007/s12560-023-09556-1>
- Hussain, Y., Alam, W., Ullah, H., Dacrema, M., Daglia, M., Khan, H., & Arciola, C. R. (2022). Antimicrobial potential of curcumin: Therapeutic potential and challenges to clinical applications. *Antibiotics*, 11(3), 322. <https://doi.org/10.3390/antibiotics11030322>
- Khatri, P., Rani, A., Hameed, S., Chandra, S., Chang, C.-M., & Pandey, R. P. (2023). Current understanding of the molecular basis of spices for the development of potential antimicrobial medicine. *Antibiotics*, 12(2), 270. <https://doi.org/10.3390/antibiotics12020270>
- Khorshidian, N., Khanniri, E., Koushki, M. R., Sohrabvandi, S., & Yousefi, M. (2022). An overview of antimicrobial activity of lysozyme and its functionality in cheese. *Frontiers in Nutrition*, 9. <https://doi.org/10.3389/fnut.2022.833618>
- Kim, K., Wang, T., Ma, H. R., Şimşek, E., Li, B., Andreani, V., & You, L. (2023). Mapping single-cell responses to population-level dynamics during antibiotic treatment. *Molecular Systems Biology*, 19(7). <https://doi.org/10.15252/msb.202211475>
- Leśniewski, G., Yang, T., & Cegielska-Radziejewska, R. (2021). Unconventional effects of long-term storage of microwave-modified chicken egg white lysozyme preparations. *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-89849-2>
- Li, Z., Huang, X., Tang, Q., Ma, M., Jin, Y., & Sheng, L. (2022). Functional properties and extraction techniques of chicken egg white proteins. *Foods*, 11(16), 2434. <https://doi.org/10.3390/foods11162434>
- Liu, Y., Shi, D., Wang, J., Chen, X., Zhou, M., Xi, X., Cheng, J., Ma, C., Chen, T., Shaw, C., & Wang, L. (2020). A novel amphibian antimicrobial peptide, Phylloseptin-PV1, exhibits effective anti-staphylococcal activity without inducing either hepatic or renal toxicity in mice. *Frontiers in Microbiology*, 11. <https://doi.org/10.3389/fmicb.2020.565158>
- Łojewska, E., & Sakowicz, T. (2021). An alternative to antibiotics: Selected methods to combat zoonotic foodborne bacterial infections. *Current Microbiology*, 78(12), 4037–4049. <https://doi.org/10.1007/s00284-021-02665-9>
- Mojsoska, B. (2022). Solid-phase synthesis of novel antimicrobial peptoids with α - and β -chiral side chains. *Methods in Enzymology*, 327–340. <https://doi.org/10.1016/bs.mie.2021.10.010>
- Mwamatope, B., Tembo, D., Kampira, E., Maliwichi-Nyirenda, C., & Ndolo, V. (2021). Seasonal variation of phytochemicals in four selected medicinal plants. *Pharmacognosy Research*, 13(4), 218–226. <https://doi.org/10.5530/pres.13.4.14>
- Nawaz, N., Wen, S., Wang, F., Nawaz, S., Raza, J., Iftikhar, M., & Usman, M. (2022). Lysozyme and its application as antibacterial agent in food industry. *Molecules*, 27(19), 6305. <https://doi.org/10.3390/molecules27196305>
- Novikov, I., Subbot, A., Turenok, A., Mayanskiy, N., & Chebotar, I. (2019). A rapid method of whole cell sample preparation for Scanning electron microscopy using neodymium chloride. *Micron*, 124, 102687. <https://doi.org/10.1016/j.micron.2019.102687>
- Paul, S. S., Rama Rao, S. V., Hegde, N., Williams, N. J., Chatterjee, R. N., Raju, M. V. L. N., Reddy, G. N., Kumar, V., Phani Kumar, P. S., Mallick, S., & Gargi, M. (2022). Effects of dietary antimicrobial growth promoters on performance parameters and abundance and diversity of broiler chicken gut microbiome and selection of antibiotic resistance genes. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.905050>
- Sakr, S., Ghaddar, A., Hamam, B., & Sheet, I. (2020). Antibiotic use and resistance: An unprecedented assessment of university students' knowledge, attitude and practices (KAP) in Lebanon. *BMC Public Health*, 20(1). <https://doi.org/10.1186/s12889-020-08676-8>
- Scieuzo, C., Giglio, F., Rinaldi, R., Lekka, M. E., Cozzolino, F., Monaco, V., Monti, M., Salvia, R., & Falabella, P. (2023). In vitro evaluation of the antibacterial activity of the peptide fractions extracted from the hemolymph of *Hermetia illucens* (Diptera: Stratiomyidae). *Insects*, 14(5), 464. <https://doi.org/10.3390/insects14050464>
- Shalaby, E. A., Shanab, S. M. M., Hafez, R. M., & El-Ansary, A. E. (2023). Chemical constituents and biological activities of different extracts from ginger plant (*Zingiber officinale*). *Chemical and Biological Technologies in Agriculture*, 10(1). <https://doi.org/10.1186/s40538-023-00385-9>
- Tomezyk, Ł., Leśniewski, G., & Cegielska-Radziejewska, R. (2023). Lysozyme modification using proteolytic enzymes. *Molecules*, 28(17), 6260. <https://doi.org/10.3390/molecules28176260>

- Wang, Y., Li, S., Jin, M., Han, Q., Liu, S., Chen, X., & Han, Y. (2020). Enhancing the thermo-stability and anti-bacterium activity of lysozyme by immobilization on chitosan nanoparticles. *International Journal of Molecular Sciences*, 21(5), 1635. <https://doi.org/10.3390/ijms21051635>
- Wei, S. W., & Yi, T. W. (2023). Study on antibacterial activity and structure of chemically modified lysozyme. *Molecules*, 28(1), 95–101. <https://doi.org/10.3390/molecules28010095>