

FACULTY OF SCIENCE

BIOLOGICAL SCIENCES DEPARTMENT

Combined Phytogenic Effects of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*) and chilli (*Capsicum annuum*) on broiler chicken gut pathogens, *Escherichia coli* and *Salmonella*

By

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APPROVAL FORM

The undersigned certify that they have read the dissertation titled '**Combined Phytogenic Effects of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*)) and chilli (*Capsicum annuum*) on broiler chicken gut pathogens, *Escherichia coli* and *Salmonella'*** and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

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Signature of Chairperson of Department:  Date: 13 August 2026

DECLARATION

I, Jacqueline Matambanadzo (B223382B) declare that this research herein is my own work and has not been plagiarized from another source(s) without acknowledgement of the concerned author(s) either electronically or otherwise.

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I, J. Ndava, declare that I have supervised this thesis and I am satisfied that it can be submitted to the Biological Sciences Department, Faculty of Science and Engineering, at Bindura University of Science Education.

Signature:  Date:15/06/26

DEDICATION

I dedicate this project to my parents, siblings and all the Matambanadzo and Matumbarira families, for their unwavering support during my academic career.

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my gratitude to the Almighty Lord for guiding me in this research project. I would also like to thank my research supervisor Mr Ndava who diligently guided me throughout this project. Additionally, I also like to thank my parents and relatives for their constant support financially and emotionally during my academic career. My special appreciation goes to the Biological Sciences laboratory staff who assisted me in carrying out this project and also to the Mutare Veterinary lab technicians who made me equipped to carry out the procedures of this project during the attachment period.

LIST OF ABBREVIATIONS AND ACROYNMS

ANOVA	Analysis of Variance
ATP	Adenosine trio-phosphate
AMR	Antimicrobial Resistance
BPW	Buffered Peptone Water
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
EMB	Eosin Methylene Blue
E. coli	Escherichia coli
MIC	Minimum Inhibitory concentration
MBC	Minimum Bacterial concentration
NaCl	Sodium chloride
FICI	Fractional Inhibitory Concentration Index
SPSS	Statistical Package for Social Sciences
XLD	Xylose-Lysine-Deoxycholate
STD	Standard deviation
Combo	combination of garlic, rosemary and chilli
g	grams
mm	millimetres

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Abstract

The rise in antimicrobial resistance and the restriction of antibiotic use in broiler production have necessitated effective phytogenic alternatives to control *Escherichia coli* and *Salmonella*. This study investigated the combined antimicrobial effects of garlic (*Allium sativum*, Amaryllidaceae), rosemary (*Rosmarinus officinalis*, Lamiaceae) and chilli (*Capsicum annuum*, Solanaceae) extracts against these key broiler chicken pathogens addressing a critical knowledge gap. Agar well diffusion assessed antibacterial activity of the plant extracts and the amoxicillin followed by broth microdilution method for the MIC/MBC determination. Synergy testing was performed using the fixed ratio combination broth microdilution method. Garlic had the highest extraction yield, followed by rosemary and chilli. Amoxicillin produced largest inhibition zones overall. The 1.1.1 combination of garlic, rosemary and chilli extracts exhibited greater antibacterial activity against both pathogens than individual extracts at 75%, 50% and 25% concentrations, with maximum zones of 13.00±1.00mm (*E.coli*) and 12.00±1.00mm (*Salmonella*) at 75%, indicating potential synergy .The combined extracts enhanced potency against *E.coli* with a mean MIC of 2.08mg/ml lower than individual garlic (2.60mg/ml), rosemary (3.65mg/ml) and chilli (5.21mg/ml). Amoxicillin had a MIC below 0.39mg/ml (lowest tested) for both pathogens, showing greater potency. The combination (G+R+C) showed the lowest MIC of 2.08mg/ml compared to rosemary (4.17mg/ml), chilli (5.21mg/ml) and garlic (8.33mg/ml). All plant extracts and combinations were bactericidal against both pathogen*s*. The FICI index was 0.59 against *E. coli* (additive effect) and 0.38 against *Salmonella* (synergistic effect). The combination was statistically significantly more effective than individual extract against both *E. coli* and *Salmonella* ($p<0.05$). Further research is recommended on other multidrug-resistant clinical isolates. The study concludes that conventional antibiotics are the most potent followed by the garlic-rosemary-chilli combination, while individual extracts are the least effective against *E. coli* and *Salmonella.*

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Poultry farming, particularly broiler production, is a major contributor to global food security and economic development. However, the industry faces significant challenges from pathogenic bacteria, notably *Escherichia coli* and *Salmonella*, which can compromise poultry health, reduce productivity, and pose serious public health risks through zoonotic transmission (Abd El-Hack et al., 2022). Traditionally, antibiotics have been widely used in poultry diets as growth promoters and disease preventives. However, the extensive and often indiscriminate use of antibiotics has contributed to the emergence of antimicrobial-resistant (AMR) strains, prompting global efforts to reduce antibiotic use in livestock (World Health Organization [WHO], 2017). This shift has led to growing interest in natural, plant-based alternatives known as phytogenic (Dhama et al., 2018). Phytogenic feed additives, including herbs, spices, and plant extracts, possess antimicrobial, antioxidant, and immunomodulatory properties (Zeng et al., 2020). Garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), and chilli (*Capsicum annuum*) are well-known for their potent bioactive compounds such as allicin, rosmarinic acid, and capsaicin, respectively. Individually, these phytochemicals have demonstrated antimicrobial effects against various pathogens (Kaefer & Milner, 2008 and Zhang et al., 2024). Garlic contains allicin which inhibits enzyme activity in *E. coli* and *Salmonella* thus disrupting critical metabolic processes in the bacteria. Rosemary is rich in potent phenolic acid called carsinosic acid and volatile compounds that contribute to strong antimicrobial and antioxidant activities against these bacteria. Chilli contains capsaicin that demonstrated antimicrobial effects against gram negative bacteria by interfering with cell membrane integrity and ATP production (Burt, 2004). However, studies examining the combined effects of these plants against poultry gut pathogens are limited thus there is a significant gap in knowledge of their potential synergistic, additive antimicrobial effects of combining these three plants against *E.*

coli and *Salmonella.*

1.2 Problem Statement

Escherichia coli and *Salmonella* infections remain major concerns in broiler production (Ssemakade et al., 2025). These infections reduce the efficiency and profitability of poultry operations through slower growth rates, reduced performance, higher mortality, and increased veterinary costs, leading to economic losses. These infections also pose significant public health risks through the potential transmission of pathogens via contaminated poultry meat (Andino & Hanning, 2015). Antibiotics have been widely used for managing these infections. The increasing restriction on antibiotic use in animal feeds, necessitated by concerns over the global public health threat of antimicrobial resistance (AMR), requires the development of effective natural alternatives (Vandeplas et al., 2010). While individual plant-based phytogenics like garlic, rosemary, and chilli have demonstrated antimicrobial properties, there is a critical gap regarding their combined effects on these specific gut pathogens in poultry. The lack of comprehensive studies on this combined approach represents a gap in knowledge and limits the development of effective phytogenic feed alternatives, leaving the poultry industry vulnerable and without a viable, sustainable solution.

1.3 Rationale for the Study

The poultry industry plays a critical role in providing affordable animal protein, particularly in developing countries where broiler production is a major source of income and nutrition. *Escherichia coli* and *Salmonella* pathogens also pose significant public health risks due to their potential transmission to humans through the food chain. Traditionally, antibiotic growth promoters and therapeutic antibiotics have been widely used to control bacterial infections in poultry production. However, the extensive and sometimes inappropriate use of antibiotics has contributed to the emergence of antibiotic-resistant bacterial strains, prompting global and national restrictions on antibiotic use in animal feeds (McEwen & Collignon, 2018). This situation has created an urgent need for effective, safe, and sustainable alternatives to conventional

antibiotics for controlling gut pathogens in poultry. Phytogenic feed additives derived from medicinal plants such as garlic *(Allium sativum*), rosemary (*Rosmarinus officinalis)*, and chilli *(Capsicum annuum)* have been reported to possess antimicrobial, antioxidant, and immunomodulatory properties (Zeng et al., 2020). Individually, these plants have demonstrated inhibitory effects against enteric pathogens, but limited studies have investigated their combined (synergistic) effects on poultry gut pathogens under controlled experimental conditions. The interaction of multiple phytochemicals may enhance antimicrobial efficacy through complementary mechanisms of action, potentially offering improved pathogen suppression compared to single-plant treatments (Mushemesh et al., 2023). Furthermore, there is a lack of locally relevant data on the effectiveness of combined phytogenic formulations in broiler production systems, particularly in contexts where access to commercial feed additives is limited and farmers may rely on readily available plant resources.

Therefore, this study is designed to evaluate the combined antimicrobial effects of garlic, rosemary, and chilli extracts against *E. coli* and *Salmonella* associated with broiler gut infections, thereby addressing an important research gap in alternative poultry disease management strategies.

1.4 Justification of the Study

The justification of this study lies in addressing the need to identify safe, cost-effective, and sustainable alternatives to antibiotics in poultry production systems thereby reducing the dependence on synthetic antibiotics and mitigating the spread of antimicrobial resistance (Robinson et al., 2016). By developing natural antimicrobial feed additives from medicinal plants, the research aims to provide poultry farmers especially small scale and resource limited producers with evidence based phytogenic strategies that improve bird health, reduce disease-related losses, and enhance productivity. This plant based additives also enable the better control of E. *coli* and *Salmonella* pathogens thus enhancing food safety by reducing bacterial contamination of poultry products, thereby protecting consumers and public health (Gadde et al., 2017).

Academically and scientifically, the study will contribute to existing knowledge on phytogenic feed additives by providing data on potential synergistic antimicrobial interactions among commonly available medicinal plants generating valuable data for further research into plant-based antimicrobial formulations and functional feeds in animal biotechnology and aligning with global efforts to promote sustainable agricultural practices(Landoni & Albarellos, 2015).The results may inform future poultry health management policies and support innovation in natural product-based animal health interventions by offering evidence based guidelines for integrating plant derived antimicrobials into poultry production, which can drive policy updates and foster new, sustainable health solutions for animal agriculture. Therefore, the study holds significance for poultry production, public health, scientific research and sustainable agricultural development.

1.5 Aim of the Study

The aim of this study was to investigate the combined antimicrobial effects of garlic, rosemary, and chilli extracts against broiler chicken pathogens *Escherichia coli* and *Salmonella.*

1.6 Main Objectives

The objectives of this study were to:

- Determine the percentage extraction yield of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), and chilli (*Capsicum annuum*) using ethanol.
- Determine the individual antimicrobial effects of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), and chilli (*Capsicum annuum*) extracts against *E. coli* and *Salmonella*.
- Evaluate the combined antimicrobial effects of garlic, rosemary, and chilli on *E. coli* and *Salmonella.*
- Compare the effectiveness of the combined phytogenics to a conventional antibiotic treatment.

1.7 Research Questions

- What is the percentage extraction yield of garlic (*Allium sativum*), rosemary

(*Rosmarinus officinalis*) and chilli (*Capsicum annuum*) using ethanol?

- What are the individual antimicrobial effects of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), and chilli *(Capsicum annuum*) extracts on the growth of *Escherichia coli* and *Salmonella spp.*?
- Does the combined application of garlic, rosemary, and chilli extracts produce greater antimicrobial inhibition of *Escherichia coli* and *Salmonella spp*. compared to their individual effects?
- How does the antimicrobial effectiveness of the combined phytogenic extracts compare with that of a conventional antibiotic treatment against *Escherichia coli* and *Salmonella* spp.?

1.8 Hypothesis

- Null Hypothesis (H_0): There is no significant difference between the antimicrobial effectiveness of the combined phytogenic extracts and that of the conventional antibiotic against *Escherichia coli* and *Salmonella spp*.
- Alternative Hypothesis (H_1): The combined phytogenic extracts are as effective as or more effective than the conventional antibiotic in inhibiting *E. coli* and *Salmonella spp.*

1.9 Significance of the Study

The study is expected to provide direct evidence of measurable pathogen inhibition by measuring zones of inhibition and determining minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC) in laboratory assays. The study will advance scientific knowledge by elucidating the synergistic interaction among three plant extracts, quantifying how their combined bioactive compounds enhance antimicrobial activity beyond individual effects, thereby improving understanding of multi-herbal phytogenic synergy. Finally, the investigation will generate data for optimizing a garlic-rosemary and chilli extract formulation that maximizes inhibition of the target pathogens, informing the development of evidence based natural antimicrobial agents for potential application in poultry production with benefits for

researchers and poultry producers seeking scientifically validated phytogenic mixtures to control bacterial pathogens.

1.10 Limitations of the Study

This study has several limitations. First, the investigation is conducted entirely under in vitro laboratory conditions rather than in live broiler chickens. Consequently, the results may not fully reflect the vivo situation, where factors such as absorption, metabolism of bioactive compounds, immune responses, and the complex interactions within the gut environment may influence antimicrobial effectiveness. Secondly, bacterial isolates were obtained from cloacal swabs collected from a limited number of small-scale farms, which may not represent the full diversity of *E. coli* and *Salmonella* strains present in larger commercial poultry production systems, therefore, the generalizability of the findings to broader poultry populations may be limited. Lastly, efficiency associated with the use of ethanol and water as solvents may have affected the concentration and stability of bioactive compounds in the plant extracts which could influence consistency and reproducibility of antimicrobial activity observed in the assays.

1.11 Delimitations of the Study

Furthermore, in vivo feeding trials, assessment of growth performance, and evaluation of physiological responses in broiler chickens were beyond the scope of this research. The study focused on two bacterial pathogens, *Escherichia coli* and *Salmonella* species which are major enteric pathogens in broiler chickens. The phytogenic agents investigated were restricted to garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*), and chilli (*Capsicum annuum*). Only in vitro antimicrobial assays, specifically agar well diffusion, broth microdilution for determination of MIC and MBC, and checkerboard assays for evaluating combined effects were employed to assess antimicrobial activity. Furthermore, biofilm analysis was not used. In vivo feeding trials, assessment of growth performance, and evaluation of physiological responses in broiler chickens are beyond the scope of this research. In vivo feeding trials and assessment of broilers' growth performance and evaluation of physiological

responses in broiler chickens were beyond the scope of this study.

1.12 Definition of Terms

Phytogenics

Plant-derived compounds derived from herbs, spices or extracts like garlic, rosemary and chilli used as feed additives for their beneficial properties, such as antimicrobial, antioxidant, or growth-promoting effects (Zeng et al., 2020).

Antimicrobial Resistance (AMR)

The ability of microorganisms (like bacteria) to survive or grow in the presence of a drug (e.g., an antibiotic) that would normally kill them or inhibit their growth (WHO, 2017).

Minimum Inhibitory Concentration (MIC)

The MIC is the lowest concentration of an antimicrobial agent like antibiotic, antiseptic or phytogenic compound that visibly inhibits the growth of a microorganism after overnight incubation (CLSI, 2018).

Minimum Bactericidal Concentration (MBC)

The MBC is the lowest concentration of an antimicrobial agent required to kill 99.9% of a specific initial bacterial inoculum within a standardized time frame usually 24hrs (CLSI, 2018).

Synergistic Effect

An interaction between two or more agents (e.g., plant extracts) where their combined effect is greater than the sum of their individual effects. This is typically indicated by a Fractional Inhibitory Concentration Index (FICI) of ≤ 0.5 (Odds, 2003).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Poultry Production and Gut Health in Broiler Chickens

2.1.1 Importance of Gut Health in Broiler Productivity

Gut health is essential in today's broiler farming, as it plays a key role in how well birds absorb nutrients, use feed efficiently, support their immune system, and perform overall.

The gastrointestinal tract is a constantly changing system filled with microbes that play a key role in keeping the intestines healthy. These microbes help by preventing harmful bacteria from taking over, influencing how the immune system responds, and making substances like short-chain fatty acids that aid in absorbing nutrients (Diaz Carrasco et al., 2019). A healthy and varied microbiota lowers the energy needed for the immune system to respond and helps the body grow well, which leads to better efficiency in converting feed into weight gain and higher overall productivity. Changes in diet or heat stress can lead to gut dysbiosis due to the disruption of balance in the gut. This condition makes animals more likely to get infections in the digestive tract and can also affect how well they grow (Naeem & Bourassa, 2025).

2.1.2 Relationship Between Gut Microbiota and Disease Susceptibility

The bacteria in the gut act as a defense system that prevents harmful germs from taking hold. Beneficial microbes, especially lactic acid bacteria, help by keeping harmful germs away, support the gut's mucus layer, and help the immune system grow stronger. A disrupted balance of gut bacteria, known as dysbiosis, usually comes before more diseases, slower weight gain, and poorer feed efficiency in broilers. A healthy microbial ecosystem helps break down nutrients and also prevents the body from using too much energy to fight harmful bacteria, which keeps energy available for growth and other important functions (Diaz Carrasco et al., 2019).

2.2 Escherichia coli and Salmonella as Major Broiler Gut Pathogens

2.2.1 Biology and Pathogenesis of Escherichia coli in Poultry

Escherichia coli is a type of bacteria that is Gram-negative and usually found in the intestines of animals but certain harmful strains, known as avian pathogenic *E. coli* or APEC, can lead to serious infections called colibacillosis in birds. APEC strains have different factors that help them grow in various tissues, which can cause diseases like airsacculitis, pericarditis, perihepatitis, omphalitis, and septicemia in broilers (Kathayat et al., 2021). The process of disease development includes sticking to the cells lining

the gut, avoiding the body's natural defenses, and spreading to areas outside the intestine, which can lead to illness, death, and financial loss. APEC is also becoming a bigger worry because it is linked to antimicrobial resistance and has the ability to spread to animals. APEC has several genes that help it stick to, enter, and survive in host tissues, leading to colibacillosis. This disease causes high death rates and poor performance in broiler chickens. APEC can pass from parent birds to their chicks through eggs and also spread between birds in a flock, making it a serious problem for young chicks. There is also evidence of antibiotic resistance, including strains that are resistant to many different types of antibiotics, which makes treating and controlling the disease more difficult (Rahim et al., 2024).

2.2.2 Biology and Pathogenesis of Salmonella spp. in Poultry

Salmonella bacteria are types of pathogens that affect the intestines and can cause illness in both chickens and people who eat contaminated food. Non-typhoidal serovars such as *S. Enteritidis* and *S. Typhimurium* settles in the bird's digestive system and are major causes of salmonellosis. The infection starts when the pathogen is swallowed through contaminated food, water, and some are found in the environment (Vica, 2025). It survives in the stomach and then enters the cells lining the intestines and this happens because of special proteins called fimbrial adhesins and other harmful factors that help the bacteria invade the cells. These harmful factors are carried by specific genetic regions known as *Salmonella* pathogenicity islands SPI-1 and SPI-2 (Hansen-Wester and Hensel, 2001). Once it is established, *Salmonella* can live inside cells, spread throughout the body, and create strong biofilms in the environment, making it hard to eliminate. One of the harmful traits of *Salmonella* is that it only needs a small amount to infect young chicks. Pathogenic mechanisms involve the ability of pathogens to stick to surfaces, enter cells, survive inside them, and spread throughout the body. Biofilm formation helps bacteria stay in the environment longer and makes them harder to kill with disinfectants. Even with existing methods such as vaccines and feed additives, it is still difficult to achieve a lasting decrease in their presence (Shaji et al., 2023).

2.2.3 Transmission Pathways and Food Safety Implications

Transmission Pathways

E. coli and *Salmonella* are both spread in broiler production systems through two main ways which are vertical and horizontal transmission. Vertical transmission occurs when these harmful bacteria move from breeder hens to their chicks via infected eggs. This is especially true for APEC, which can lower the survival rate of chicks and affect their early growth. Horizontal transmission occurs when infected birds come into direct contact with each other, or when they touch contaminated materials like litter, water, feed, tools, or the environment. Diaz Carrasco et al. in 2019 showed that this kind of spread happens across different stages of poultry farming, including hatchery, grow-out houses, and processing areas. These harmful bacteria can stay in the environment for a long time even with proper management.

Food Safety Implications

These pathogens in broiler meat and eggs can lead to serious health problems for the public. *Salmonella* species are one of the main reasons for food poisoning around the world, usually because food is not handled properly, is not cooked enough, or gets contaminated through cross-contact during preparation. *E. coli*, especially the strains that produce shiga toxin, can lead to serious stomach and intestine problems in humans when food safety measures are not followed properly. The existence of strong and antibiotic-resistant bacteria in poultry products is a worry for how well treatments work and for long-term food safety plans (Shaji et al., 2023).

2.3 Use of Antibiotics in Poultry Production and Emerging Challenges

2.3.1 Role of Antibiotics in Growth Promotion and Disease Control

Antibiotics have been used in chicken farming for a long time for various reasons such as helping animals grow faster, stopping diseases before they start, and treating sick birds when they are already ill. Using lower than recommended amounts of antibiotics can help animals use their food more efficiently and grow better. This happens because these smaller doses reduce hidden infections and change the bacteria in the gut (Mak et

al., 2022). In the traditional approach, antibiotics including bacitracin, salinomycin, aminoglycosides such as gentamicin, beta-lactams like penicillin and amoxicillin, sulfonamides, and tetracyclines are commonly added to the diets of birds to improve their health and overall performance. This practice played a major role in boosting the production of poultry meat and lowering the costs of production, particularly in intensive farming setups in areas with high demand (Mak et al., 2022). Using antibiotics too much has caused big worries about harmful chemicals left in chicken meat that people eat. These chemicals could affect people's health in bad ways, like making them sick or harming their immune system (Abou-Jaoudeh et al., 2024).

2.3.2 Development of Antimicrobial Resistance (AMR)

The heavy use of antibiotics in raising poultry has been connected to the growth and spread of antimicrobial resistance. When people are exposed to antibiotics over a long time, even in small amounts, it creates an environment where bacteria that are resistant to these drugs can thrive. This makes it easier for these resistant bacteria to share genes that help them survive, like plasmids, integrons, and transposons (Mak et al., 2022). Several harmful bacteria found in poultry, such as *Escherichia coli,* different types of *Salmonella*, *Campylobacter jejuni* and *coli*, *Enterococcus, Staphylococcus*, and *Clostridium perfringens*, have been found to carry many resistance genes. This reduces the effectiveness of several antibiotic types in treating infections. Additionally, *Salmonella* strains linked to poultry have shown resistance to **β**-lactam antibiotics, tetracyclines, and aminoglycosides, which creates a threat to food safety (Mak et al., 2022).

Antibiotic residues found in poultry products, combined with weak regulations in developing countries, increase worries about antibiotic resistance. Abou-Jaoudeh et al. (2024) point out that the absence of strong monitoring systems, the availability of inexpensive antibiotics, and the rising demand for poultry protein have all contributed to the improper use of antibiotics. This has led to the spread of resistance genes among bacteria linked to poultry farming. These resistant strains can spread to humans through food or the environment, making it harder to treat infections and raising public health

concerns (Abou-Jaoudeh et al., 2024).

2.3.3 Regulatory Restrictions and the Post-Antibiotic Era

To combat the increasing problem of antibiotic resistance, several countries have introduced rules limiting the use of antibiotics in food animal farming, especially in poultry production. Some places, like the European Union, have stopped using antibiotics to help animals grow faster and now require vets to oversee their use for treating illnesses. Denmark and Sweden were among the first countries to ban antibiotics that promote growth in animals (Maron et al. 2013). The Globalization and Health survey found that at least six major regions completely ban the use of growth promoters and require a prescription for giving antimicrobials to animals raised for food, while other areas have some limits on their use (Maron et al., 2013). These rules are meant to reduce the development of drug-resistant bacteria and ensure that antibiotics remain effective for use in both human and animal health.

Even though some wealthy countries have made advances in regulations, the actual carrying out and making sure these rules are followed still varies a lot around the world. Many low and middle income countries do not have full policies or systems in place to track and manage how antibiotics are used, which lets the unnecessary use of antibiotics in poultry continue (Abou-Jaoudeh et al., 2024). The WHO Global Action Plan on Antimicrobial Resistance highlights the need to cut down on antibiotic use in healthy animals, especially those that are important for medicine, and focuses on finding better alternatives and improving how antibiotics are managed to slow down the growth of drug-resistant bacteria (World Health Organization, 2017). The discussion among scientists about a future without effective antibiotics shows a world where everyday infections, which were once simple to treat, are no longer manageable because of widespread antibiotic resistance. This situation calls for changes in how antibiotics are made, better ways to monitor resistance, and new approaches to treating diseases.

2.4 Phytogenic Feed Additives as Alternatives to Antibiotics

2.4.1 Definition and Classification of Phytogenics

Phytogenic feed additives (PFAs), also known as phytobiotics or botanicals, are compounds taken from plants that are added to the diets of poultry. Their purpose is to enhance productivity, promote better health, and improve how efficiently the birds use their feed, as noted in Windisch et al. (2008) study. PFAs consist of herbs, spices, essential oils, and oleoresins that come from various parts of plants and have a wide range of chemical compounds like flavonoids, terpenoids, alkaloids, and phenolic acids. These additives can be added to feed in different forms, such as whole dried plants, ground powders, extracts, essential oils made by distillation, or oleoresins taken out using solvents. Usually, PFAs are grouped based on where they come from, like herbs and spices, and their active form, such as essential oils or oleoresins. This classification is important because each type might affect the digestive system and gut bacteria in poultry differently, as noted by Gadde et al. (2017). Another important study by Abdelli et al. (2021) describes PFAs as secondary metabolites and essential oils that have active biological effects. These compounds can improve digestive secretions, help the body absorb nutrients better, and support the immune system.

2.4.2 Mechanisms of Antimicrobial Action of Plant-Derived Compounds

Phytogenic compounds have antimicrobial properties that work in different ways compared to traditional antibiotics. Aminullah et al. (2025) explained that PFAs have antimicrobial effects by breaking down bacterial cell membranes, stopping the function of important enzymes inside cells, and creating oxidative stress in bacteria. Phenolic substances like carvacrol, thymol, and flavonoids can damage the walls and membranes of bacteria, leading to the loss of internal materials and lessening the ability of harmful pathogens to survive. These compounds also affect the enzymes that bacteria use for breathing and making energy, which causes the harmful organisms to lose their energy. Additionally, PFAs can change the types of bacteria in the gut by supporting good bacteria like *Lactobacillus* species and reducing the growth of bad bacteria such as

Escherichia coli and *Clostridium perfringens*. This modulation helps in competitive exclusion, which lowers the risk of infection and boosts intestinal health (Oni & Oke, 2025). Phytogenics also have antioxidant and anti-inflammatory effects, which can strengthen the immune system by reducing oxidative stress and inflammation in the intestines, thus offering more protection against harmful microbes and supporting general well-being.

2.4.3 Advantages and Limitations of Phytogenic Additives in Poultry

Advantages

Phytogenic additives in poultry offer a benefit by lowering the risk of antibiotic resistance, unlike single-target antibiotics. PFAs are complex mixtures of active substances that work in various ways to fight microorganisms, making it less likely for harmful bacteria to develop resistance (Aminullah et al., 2025). Studies show that PFAs can improve the structure of the gut by increasing the height of the villi and boosting enzyme release, which helps in better digestion and absorption of nutrients. They also help maintain a healthy balance of gut bacteria, leading to improved feed efficiency and growth (Abdelli et al., 2021). These compounds also have antioxidant properties and can strengthen the immune system, allowing birds to better handle stress and be less likely to get sick (Oni & Oke, 2025). As more regulations limit the use of antibiotics in food production, PFAs are a good fit for antibiotic-free and organic poultry systems, satisfying consumer preferences for safer products with fewer chemical residues).

Limitations

A big challenge with PFAs is that the mix of active ingredients can vary depending on the plant species, how the plants are harvested, and the methods used to extract them. This makes it hard to ensure consistent results and set standard dosages. Also, differences in the chemical makeup of these compounds and how poultry process them can influence how well they work in the body, making it difficult to predict how different groups of birds or farming systems will respond. While many studies show positive effects of PFAs, there are not enough large-scale tests in real-world settings, which leaves some uncertainty for farmers thinking about using them in various

production situations (Oni & Oke, 2025).

2.5 Antimicrobial Properties of Selected Phytogenic Plants

2.5.1 Antimicrobial Activity of Garlic (*Allium sativum*)

Bioactive Compounds and Mechanisms of Action

Garlic, which is scientifically known as *Allium sativum*, contains a variety of organosulfur compounds. Some of these include allicin, ajoene, diallyl sulfide, diallyl disulfide, and diallyl trisulfide. Among these, allicin is seen as the main substance that fights microbes. It is made when garlic is crushed, and the enzyme alliinase turns alliin into allicin (Ankri and Mirelman, 1999).

Allicin fights bacteria by stopping enzymes that contain sulfhydryl groups, messing up how bacteria make energy, blocking the creation of DNA and proteins, changing the way the bacterial cell membrane works, causing the cells inside stuff to leak out, and also stopping bacteria from communicating with each other, which makes them less harmful. Garlic contains organosulfur compounds which contribute to its broad antimicrobial activity. These mechanisms enable garlic to fight both types of bacteria, Gram-positive and Gram-negative, even those that are resistant to antibiotics (Singh et al., 2025).

Evidence Against Enteric Pathogens

Many laboratory tests done in a controlled environment and real-life animal studies have shown that garlic works well against common disease-causing bacteria in poultry, including *Escherichia coli, Salmonella* types, *Clostridium perfringens*, and *Campylobacter jejuni*. Ankri and Mirelman (1999) found that allicin stopped the growth of *E. coli* and *Salmonella enterica* by interfering with important enzymes needed for their metabolism. In studies on poultry nutrition, adding garlic powder or extracts to the diet greatly lowered the number of *E. coli* and *Salmonella* in the intestines, and also helped improve the structure of the gut and strengthen the immune system .Garlic has been found to work well against enteric pathogens by interfering with the enzymes

needed for metabolism. Ankri and Mirelman (1999) reported that allicin can inhibit RNA synthesis and disrupts thiol-containing enzymes that are essential for bacterial growth and metabolism thus reducing bacterial growth and limiting the development of antibiotic resistance.

2.5.2 Antimicrobial Activity of Rosemary (*Rosmarinus officinalis*)

Phenolic Diterpenes and Antioxidant Properties

Rosemary, which is known as *Rosmarinus officinalis,* has a lot of phenolic diterpenes, especially carnosic acid, carnosol, and rosmarinic acid. These compounds are known for their powerful ability to fight against oxidation and stop the growth of microbes. These compounds have two important functions, they help protect the body's tissues from damage caused by harmful substances and also fight off harmful microorganisms (Sienkiewicz et al., 2013).Rosemary phenolics work against microbes by breaking down their cell membranes, stopping the production of ATP, binding to important metal ions, and turning off key enzymes in microbes. Its antioxidant properties also help fight microbes by making it harder for harmful bacteria to survive through an environment that is less favorable for their growth.

Activity Against Gram-Negative Bacteria

Gram-negative bacteria have an outer membrane made of lipopolysaccharides that makes them resistant to many plant extracts, so rosemary essential oil has been found to inhibit Gram-negative bacteria like *E. coli* and *Salmonella typhimurium* (Sienkiewicz et al., 2013). Research shows that rosemary extracts help reduce bacterial growth by making the bacterial membranes more permeable, which causes the contents inside the bacteria to leak out. Olivas-Mendez et al. (2022) showed that rosemary essential oil, especially when mixed with garlic and chili extracts, worked well in stopping the growth of *Salmonella,* coliform bacteria, and *Staphylococcus aureus* in food, which hints at its possible use in keeping poultry gut health in check. Also, using rosemary has been linked to better balance in the gut microbiota, supporting good bacteria like lactic acid bacteria and reducing harmful bacteria.

2.5.3 Antimicrobial Activity of Chilli *(Capsicum annuum)*

Capsaicinoids and Antimicrobial Mechanisms

Chilli peppers, which are scientifically known as *Capsicum annuum*, contain compounds called capsaicinoids, with capsaicin and dihydrocapsaicin being the main ones. These substances are what give chilli peppers their spicy taste and also help them fight off harmful microbes. Capsaicin has both bacteriostatic and bactericidal effects, which depend on its concentration and the type of bacteria involved (Dorantes et al., 2000). The antimicrobial actions of capsaicinoids involve breaking down bacterial cell membranes, preventing the formation of biofilms, disrupting communication systems used by bacteria, and reducing the production of harmful toxins. These actions lower the ability of bacteria to survive and cause disease instead of just slowing their growth.

Effects on Gut Pathogens

Capsaicin has been shown to inhibit the growth of important stomach bacteria like *E. coli*, *Salmonella* types, and *Helicobacter pylori*. Dorantes et al. (2000) found that capsaicin extracts effectively reduced the growth of food pathogens like *Salmonella enterica*. More recent research shows that capsaicin also helps improve gut health indirectly by changing the balance of bacteria in the intestines, encouraging the growth of good bacteria and reducing harmful ones. In poultry, adding capsaicin has been linked to less intestinal inflammation and better protection of the gut lining, which helps prevent harmful bacteria from taking hold (Nunes et al., 2025).

2.6 Combined Phytogenic Formulations and Synergistic Effects

2.6.1 Concept of Synergy in Antimicrobial Combinations

Synergy in antimicrobial combinations refers to a situation where using two or more agents together results in an effect that is more significant than what would be expected if each agent were used alone. This phenomenon is usually assessed using the fractional inhibitory concentration index (FICI), where a value of FICI ≤ 0.5 suggests synergy,

0.5–1 indicates additive effects, and a value greater than 1 means indifference or antagonism (Jeong et al., 2023). Mechanistically, synergy happens when different agents that target separate or related processes work together, such as one affecting the cell membrane and another disrupting internal metabolic processes (Abass et al., 2022). These interactions can lead to better effectiveness, less need for high doses, and a lower chance of resistance forming when compared to using a single treatment alone, as shown in Jeong et al.'s 2023 study.

2.6.2 Evidence of Synergistic Effects Among Plant Extracts

Numerous empirical studies demonstrate synergistic antimicrobial activities when plant extracts are used in combination either with each other or with conventional antibiotics:

Synergy Among Plant Extract Combinations

Donkor et al. (2023) tested different mixtures of *Senna alata*, *Ricinus communis*, and *Lannea barteri* against several types of bacteria and a fungus, including *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Candida albicans*. They discovered that certain combinations of *L. barteri* aqueous extract and *S. alata* ethanol extract worked together better than either one alone to fight microbes, with a combined minimum inhibitory concentration that was much lower than when each extract was used separately. Jeong et al. (2023) showed that using extracts from *Sanguisorba officinalis (SO)* and *Uncaria gambir Roxb (UG)*. together greatly lowered the minimum inhibitory concentrations against methicillin-resistant *Staphylococcus aureus (*MRSA*)*, and this combined effect was confirmed through checkerboard assays, which showed a FICI value of 0.5. The combination of SO and UG showed continued ability to kill bacteria and stopped the growth of resistant strains even after multiple passages.

Synergy Between Plant Extracts and Antibiotics

Donkor et al. (2024) also looked into using plant extracts along with antibiotics such as ampicillin or fluconazole to fight clinical strains of *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*. They reported that ethanol extracts of *S. alata* worked better together with ampicillin and amoxicillin, as shown by the FICI values.

Additional evidence comes from studies combining plant extracts like *Camellia sinensis*, *Thymus vulgaris, Rosmarinus officinalis,* and *Salvia officinalis* with clindamycin, producing synergy against methicillin-resistant *S. aureus* (FICI < 0.5) in vitro (Mayyas et al., 2021). Research on *Acinetobacter baumannii* showed that combinations of plant-active ingredients such as geraniol and linalool with antibiotics like colistin yielded strong synergistic interactions (FICI = 0.12–0.5), drastically lowering antibiotic MICs and inhibiting biofilm formation (Aslan and Alim, 2025). Together, these studies illustrate that phytogenic combinations can enhance antimicrobial potency against resistant pathogens, often surpassing the efficacy of individual constituents.

2.6.3 Advantages of Multi-Component Phytogenic Additives

Using combinations of two or more plant extracts or phytochemicals as multi-component phytogenic additives provides several benefits. They provide a wider range of antimicrobial effects, and by combining extracts from various plants, they can target several different pathways in microbes. This increases the chance of stopping the growth of both Gram-positive and Gram-negative bacteria, as well as fungi. Donkor et al. (2023) showed this in their research on the extract pairs of *S. alata*, *R. communis*, and *L. barteri*.

Synergistic interactions can also help reduce the amount of each ingredient needed, which lowers the risk of harmful effects and side effects, making it especially important in clinical or feed additive uses. Multi-component formulations often contain different substances that work in various ways, such as breaking down cell membranes or blocking enzymes, which makes it harder for harmful organisms to become resistant. Jeong et al. (2023) found that there was no increase in MRSA resistance even after repeated use of the SO plus UG mix, which highlights its benefit. Plant-based combinations can break down biofilm communities that shield bacteria from antibiotics and also affect the pumps that help bacteria resist drugs. Sarigiannis and Papaneophytou,

(2024) pointed out how phytochemicals play a role in processes like stopping efflux pumps and disrupting quorum sensing. In the fields of animal science and nutrition, using mixtures of plant-based additives has been connected to better gut health, stronger immune systems, and less need for antibiotics to boost growth. This helps improve the overall performance and sustainability of livestock production.

Although the potential of phytogenic synergy is generally recognized, studies also highlight the importance of standardization, detailed research into how these effects work, and testing in real-life situations to turn lab results into practical applications in medicine or industry.

2.7 Methods for Evaluating Antimicrobial Activity of Phytogenics

2.7.1 In Vitro Antimicrobial Assays (Agar Diffusion, MIC, MBC)

A variety of lab-based tests are used to check the antimicrobial effects of plant-based compounds in a controlled environment. These methods offer reliable and fairly quick early results for activity, but they vary in how sensitive they are and how accurately they can measure the amount.

Agar diffusion methods, including disc-diffusion and well-diffusion, are commonly used for the initial testing of plant extracts and purified plant compounds. In the disc-diffusion method, also known as the Kirby-Bauer test, a filter disk soaked in the plant-based extract is put onto an agar plate that has been evenly spread with the bacteria being tested; the size of the area where bacterial growth is stopped shows how strong the antimicrobial effect is (Bauer, Kirby, & Sherris, 1996). Agar-well diffusion works on the same principle but involves creating wells in the agar that is already filled with the extract solution. These methods are commonly used in studies involving plant extracts because they are easy to use and cost-effective, but the way certain plant compounds spread can sometimes make it hard to interpret the results (Balouiri et al., 2015).

The minimum inhibitory concentration (MIC) is a more precise way to measure how effective an antimicrobial agent is. It is the lowest amount of the antimicrobial needed to stop the growth of microbes when they are allowed to grow in a controlled environment (Balouiri et al., 2015). MICs are usually found by testing the extract in 96-well microplates using a series of two-fold dilutions, along with a fixed number of bacteria, as recommended by the Clinical and Laboratory Standards Institute guidelines. Using indicators such as resazurin helps make it easier to see the difference between growth and no growth outcomes.

The minimum bactericidal concentration is determined by taking samples from the MIC wells that show no visible growth and culturing them on fresh agar. The lowest concentration that kills at least 99.9% of the bacteria is considered the MBC. The MIC/MBC ratio helps determine whether an antimicrobial is bacteriostatic or bactericidal. Along with agar diffusion and MIC tests, the MBC assay offers a solid method for early testing of phytogenics as antimicrobials, but it is important to consider how sensitive each test is and how they are standardized (Balouiri et al., 2015).

2.7.2 Checkerboard Method and FICI for Synergy Testing

Once individual activity is identified, researchers usually look into how different plant-based compounds or plant extracts work together with known antimicrobial agents. The checkerboard microdilution method, which was first designed to test how antimicrobial drugs interact, is now commonly used to study combinations of phytogenics (Odds, 2003). In a checkerboard plate, serial dilutions of two agents are spread out across the rows and columns of a microtiter plate. After exposing the bacteria to a standard number of bacteria, the minimum inhibitory concentrations when used together are noted. The level of synergy is measured using the Fractional Inhibitory Concentration Index (FICI). FICI=MIC of agent A in combination / MIC of agent A alone + MIC of agent B in combination/MIC of agent B alone

A FICI value of 0.5 or lower is usually seen as showing synergy, while a value above 1

indicates antagonism, according to Balouiri et al., 2015. Emmanuel, Elabor, and Limangba, (2025) applied this method to show how *Calotropis procera* extracts work together with ciprofloxacin to fight *E. coli* and *Staphylococcus aureus*, as shown by their FICI values. Due to the complexity of the checkerboard method when more than two agents are involved, alternative approaches such as the fixed ratio combination broth microdilution method can be used to determine the Fractional Inhibitory Concentration Index (Jacobs, 1996).

2.7.3 Limitations of In Vitro Models in Predicting In Vivo Outcomes

In vitro tests are useful for initial screening but cannot completely mimic real-life conditions inside the body. These lab-based systems do not include immune reactions, metabolic processes, or how drugs are absorbed and moved in the body, which are important for knowing how well treatment works in animals or people (Balouiri et al., 2015). Standard lab environments have consistent nutrients and pH levels, which are very different from the complex and varied conditions found in tissues or infection areas. These differences can change how microbes respond to treatments and how stable extracts remain. Some plant-based compounds might work well in lab tests but are not easily taken in or broken down quickly in the body, which limits their effectiveness as treatments. Checkerboard or MIC synergy seen in lab tests might not lead to better results in real biological environments, where the active ingredients could fight for the same enzymes or be removed from the body in different ways. Different in vitro methods such as disk diffusion, broth microdilution, and checkerboard can produce varying results, making it difficult to draw conclusions without supporting in vivo data (Hossain, 2024). Thus, in vitro findings act as an initial indication of antimicrobial activity, but thorough pharmacological research involving animal models is necessary to verify effectiveness, safety, and appropriate dosing before moving to clinical use.

2.8 Knowledge Gaps and Justification for the Current Study

Limited Studies on Garlic–Rosemary–Chilli Combinations

There is a lot of research on the antimicrobial effects of individual plant extracts such as garlic, rosemary, and chilli, but limited studies look into how these three plants work together against common foodborne bacteria. A significant study conducted by Olivas-Mendez et al. (2022) looked into how effective rosemary essential oil, garlic essential oil, and chipotle pepper oleoresin are at preventing the growth of bacteria and reducing oxidation in beef hamburgers, both on their own and when used together. They discovered that each extract, either by itself or when used together, slowed the growth of aerobic bacteria, *Salmonella* species, coliforms, and other microorganisms, showing that the treated meat products had better microbial quality.

Although this study looked at combinations like rosemary, garlic, and chilli oleoresin, it mainly focused on preserving the product rather than thoroughly testing how effective these spices are at fighting *E. coli* and *Salmonella* at the cell or molecule level showing a lack of research that specifically targets these three spices together. Research on combining essential spice oils shows that mixing certain herbs like bay leaf, black pepper, garlic, and others can greatly improve their antibacterial power. However, chilli is usually left out of these studies. Also, how garlic, rosemary, and chilli work together is still not well understood. Olivas-Mendez et al. (2022) tested combinations of essential oils against foodborne bacteria like *E. coli* and *Salmonella*. Their findings indicated that mixtures of these oils can inhibit both *Escherichia coli* and *Salmonella typhimurium*. However, they did not specifically test the combination of the three spices of interest together. Existing research highlights the general antimicrobial properties of garlic, rosemary, and chili but shows a lack of detailed information on how these three together works against important gut pathogens, which justifies the need for this current study.

Need for Locally Relevant Data

Most research on antimicrobial properties of spice extracts is done in developed countries under controlled lab environments, where they use standard bacterial strains and processed food matrices like meat products or eggs (Sienkiewicz et al., 2013).This

creates a gap for locally relevant data in Sub-Saharan Africa, where environmental conditions, native bacterial strains and traditional spice varieties differ significantly from controlled lab settings (Mashabela et al., 2022). Torres et al. (2024) found that aqueous garlic extract showed limited efficacy against E. *coli* and *Salmonella,* in the laboratory conditions, highlighting that antimicrobial activity is highly context dependent. Several locally used spices have demonstrated antimicrobial potential against poultry pathogens in Sub-Saharan Africa. Zimbabwe ethnomedical plants like Combretum and Pteleopsis species showed antimicrobial activity against *E. coli* and *Salmonella* isolated from chickens with MIC ranging from 1.9-1.56µg/ml (Silen et al., 2023). These findings suggest that results from one region may not directly apply to another due to different plant phytochemistry, bacterial resistance profiles and food systems (Oni and Oke, 2025). Therefore, gathering information about locally grown garlic, rosemary, and chilli along with native bacterial strains is important to support antimicrobial claims in a food safety setting that is relevant to the local area.

Rationale for Targeting *E. coli* and *Salmonella.*

E. coli and *Salmonella* are some of the most common foodborne pathogens worldwide and are frequently used as standard test organisms in studies about antibiotics. Research shows that garlic extracts can stop the growth of *E. coli* and *Salmonella* strains, especially those found in poultry, which prove they are good choices for testing how effective antimicrobial substances are. Torres et al. in 2024 found that garlic crude extract can stop the growth of multidrug-resistant *E. coli* and *Salmonella* bacteria that come from poultry. Rosemary essential oils are known to fight different types of bacteria because they contain chemicals like carnosic acid and rosmarinic acid as shown by Sienkiewicz et al. (2013) and Altemimi et al. (2017). However, there is limited information on how effective they are specifically against *E. Coli* and *Salmonella*. It is important to study these bacteria because they are major causes of foodborne sickness and have a big impact on public health. They are also commonly involved in outbreaks linked to contaminated food. Their gram-negative cell walls make them more resistant to natural antimicrobials compared to many gram-positive bacteria, which is why they

are often used as tough test subjects to check how well new antimicrobial mixtures work.

CHAPTER 3

MATERIALS AND METHODS

3.1 Research Design

An experimental laboratory-based research design was employed in this study. A cross-sectional sampling approach was used to isolate and identify bacterial pathogens from broiler chicken cloacal swabs. The controlled in-vitro experiments were conducted to evaluate the antimicrobial activity of individual plant extracts, their combined formulations and to compare their effectiveness with that of a conventional antibiotic. All culture media, biochemical reagents and laboratory consumables used in this study were sourced from laboratory stock and prepared according to manufacturers' instructions.

3.2 Sample Collection and Plant Extract Preparation

3.2.1 Plant Material Processing

Fresh garlic (*Allium sativum*) bulbs, rosemary (*Rosmarinus officinalis*) leaves and chilli (*Capsicum annum*) fruits were collected from local home gardens in Bindura, Zimbabwe. The collected fresh plant materials were thoroughly washed with distilled water to remove debris and air-dried under shade at ambient temperature (approximately 25°C) in a well-ventilated area for two weeks. The dried materials were separately milled into fine powder using an electric blender and stored in sterile, airtight

containers until extraction.

3.2.2 Maceration Extraction Method

Crude extracts were prepared using the maceration technique. Twenty-five grams (25g) of each powdered plant material were soaked in 250ml of 70% ethanol and allowed to macerate for 7 days with periodic agitation. The use of 70% ethanol was selected due to its efficiency in extracting both polar and moderately polar phytochemicals (Zeng et al., 2020). The mixtures were filtered using Whatman No. 1 filter paper, and the filtrates were concentrated using a temperature-controlled water bath at 60 ^{0}C (Appendix A). The concentrates were further dried in a desiccator to obtain crude extracts. Percentage yield was calculated using the formula:

Extract Yield (%) = (Weight of dried extract / Weight of dry plant powder) × 100

Extraction yield was determined to assess the efficiency of ethanol as a solvent and to standardize the amount of plant material used for antimicrobial testing. The dried extracts were weighed to determine percentage yield and then reconstituted in distilled water to prepare stock solutions of 250mg/ml, which was defined as 100% concentration. Working concentrations of 75%, 50% and 25% were prepared from the stock by dilution with sterile distilled water corresponding to 187.5 mg/ml, 125 mg/ml and 62.5 mg/ml of dried extract respectively.

3.2.3 Sample Collection and Enrichment

A total of 50 cloacal swab samples were aseptically collected from broiler chickens reared on small-scale family farms in the Bindura area. The swabs were immediately placed in Amies transport medium and transported to the laboratory under cold conditions within 2 hours of collection. Each swab was inoculated into 10 ml of Buffered Peptone Water (BPW) and incubated at 37 °C for 24 hours for bacterial enrichment.

3.2.4 Isolation and Presumptive Identification

Following enrichment, aliquots were streaked onto selective and differential media (Appendix B). MacConkey Agar and Eosin Methylene Blue (EMB) Agar were used for the isolation of *E. coli*, indicated by the pink colonies on MacConkey Agar and metallic

green shine colonies on EMB Agar. Xylose Lysine Deoxycholate (XLD) Agar was used for *Salmonella* isolation, where red colonies with black centers were considered presumptive *Salmonella*. All the plates were incubated at 37 °C for 24 hours.

3.2.5 Confirmation of Isolates

Presumptive colonies were sub-cultured on Nutrient Agar to obtain pure isolates. Gram Staining was performed to confirm Gram-negative rod morphology. Biochemical tests including Indole, citrate, catalase, and urease tests were conducted to confirm the identities of *E. coli* and *Salmonella* isolates.

3.3 Antimicrobial Susceptibility Testing

3.3.1 Preparation of Bacterial Inoculum

Confirmed isolates were suspended in sterile saline, and turbidity was adjusted to match the 0.5 McFarland standards corresponding to approximately 1.5×10^8 CFU/mL.

3.3.2 Agar Well Diffusion Assay

Antimicrobial activity was initially assessed using the agar well diffusion method. Mueller-Hinton Agar (MHA) plates were inoculated with standardized bacterial suspensions using sterile swabs. Wells of 6 mm diameter were aseptically punched into the agar and filled with 100 µL of individual or combined plant extract solutions at 75%, 50% and 25% concentrations .For the combination assay, the three extracts were mixed in the 1.1.1 ratio by equal volumes prior to dilution so that each extract contributed to one third of the total concentration at each level. Amoxicillin discs and distilled water served as positive and negative controls respectively. The plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimetres. All assays were performed in triplicate (Appendix D).

3.3.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth microdilution method in 96-well microtiter plates according to CLSI (2018) guidelines (Appendix C). The crude extracts stock

concentrations of 250 mg/ml were first diluted with sterile Mueller-Hinton broth to obtain working concentrations of 100 mg/ml. Garlic, rosemary and chilli extracts were mixed at 1:1:1 ratio before dilution so that each extract contributed one-third of the total concentration at each level. Two-fold serial dilutions were then carried across the microtiter plate to obtain final extract concentrations ranging from 100 mg/ml to 0.39 mg/ml. Each well contained 50 µl of extract dilution and 50 µl of standardized *E.coli* and *Salmonella* inoculum, giving a final volume of 100 µl per well. Amoxicillin was used as a positive control. A growth control containing bacteria and broth only and a sterility control containing broth only were included. The plates were incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration showing no visible bacterial growth.

For MBC determination, 10 µl of aliquots from wells with no visible growth were sub-cultured onto MHA plates and incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration showing no bacterial growth on agar, indicating $\geqslant$99.9% bacterial killing.

3.4 Assessment of Combined Effects (Synergy Testing)

The fractional inhibitory concentration index (FICI) of the combined garlic, rosemary and chilli extracts was determined against *E.coli* and *Salmonella* using the fixed ratio combination broth microdilution method at 1:1:1 ratio by equal volume. The minimum inhibitory concentrations (MICs) of each extract were first determined individually for both bacterial strains using two-fold broth microdilution method in 96-well microplates as described in the MIC determination section. The three extracts were mixed in a 1:1:1 ratio by equal volume to make a combination assay. The total concentration of the combined extract mixture was prepared and its MIC was determined using the same two-fold broth microdilution procedure. The concentration of each extract in the combination was one-third of the total combination MIC. Fractional Inhibitory Concentration Index (FICI) was calculated as:

$$FICI = \frac{MIC_{Garlic\ (combination)}}{MIC_{Garlic\ (alone)}} + \frac{MIC_{Rosemary\ (combination)}}{MIC_{Rosemary\ (alone)}} + \frac{MIC_{Chilli\ (combination)}}{MIC_{Chilli\ (alone)}}$$

Interpretation followed Odds (2003):

• Synergy: FICI ≤ 0.5

• Additivity: 0.5 < FICI ≤ 1.0

• Indifference: 1.0 < FICI ≤ 4.0

• Antagonism: FICI > 4.0

3.5 Comparison with Conventional Antibiotic

The antimicrobial effectiveness of individual plant extracts (garlic, rosemary and chilli) and their 1:1:1 combinations were compared to amoxicillin (positive control) using zone of inhibition diameters and MIC values against both *E. coli* and *Salmonella* isolates. Mean zone of inhibition diameters for each extract treatment, their combination and amoxicillin were presented in tabular format for direct visual comparison for each bacterial species. Mean MIC values of the plant extracts and their combination were compared to amoxicillin MIC using graphical presentation to illustrate relative potency against both test organisms. Statistical comparison of mean zone diameters between all treatment groups (individual extracts, combined extracts and amoxicillin) was performed using two way ANOVA with Tukey's post-hoc test ($p < 0.05$), as detailed in section 3.6 .

3.6 Data Analysis

All experiments were conducted in triplicates, and results were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS software. Data was first tested for normality using Shapiro-Wilk. Differences among treatment groups were analysed using two-way Analysis of Variance (ANOVA). Two way ANOVA was used because the study involved two independent variables extract type and concentration. Tukey's post-hoc test was applied to determine specific group differences where significant differences ($p<0.05$) were observed.

3.7 Ethical Considerations

Informed consent was obtained from poultry owners prior to sample collection. Cloacal swabs sampling was conducted humanely and with minimal handling to avoid distress to the birds.

CHAPTER 4

RESULTS

4.1 Extraction yield

The yields of extraction were expressed as the percentages of initial mass of the sample macerated from single extractions. Ethanol was used as a solvent for extraction. Garlic produced the highest extraction yield, followed by rosemary and chilli (Table 1).

Table 1: Percentage extraction yield from single extraction

Extract	Original Mass(g)	Final mass of extract(g)	Percentage yield (%)
Garlic	25	5	20
Rosemary	25	4	16
Chilli	25	3.75	15

4.2 Antibacterial screening

4.2.1 Activity against *Escherichia coli*

Amoxicillin (positive control) consistently produced the largest zones of inhibition, while water (negative control) showed no activity. The combined extract (garlic + rosemary + chilli in a 1:1:1 ratio) produced larger inhibition zones than individual extracts at all concentrations. The mean zones of inhibition against *E.coli* differed

among the three concentrations. The antibacterial activity of the extracts increased with concentration (75% > 50% > 25%). Amoxicillin, a positive control consistently produced the largest zones of inhibition at all concentrations, while water (negative control) showed no activity. Standard deviation indicated moderate variability across replicates (Table 2).

Data on the zones of inhibition against *E. coli* were normally distributed for all extract groups, according to Shapiro-Wilk test ($p > 0.05$).Two-way ANOVA showed significant effects of extract type and concentration on the zone of inhibition, with no significant interaction ($p=0.760$). Post hoc Tukey tests showed that amoxicillin was significantly more effective than all plant extracts. The combination of garlic, rosemary and chilli was significantly more effective than garlic and rosemary alone, but not different from chilli. The 75% concentration produced significantly larger inhibition zones than both 50% and 25%, while there was no difference between 50% and 25%. These results support the hypothesis that both extract type and concentration influence antibacterial activity, with the combinations showing enhanced effects relative to individual extracts, though the effect of concentration is consistent across all extracts (Appendix E).

Table 2: Mean Zones of Inhibition of *E.coli (mm)*

	Mean ± STD in mm		
Test Agent	75%concentration	50% concentration	25% concentration
Garlic	11.33±1.53	8.67±1.15	7.33±1.15
Rosemary	11.00±1.00	8.00±2.00	7.00±1.00
Chilli	11.00±1.00	9.33±0.58	8.00±1.00
Combo	13.00±1.00	11.00±1.00	9.33±0.58
Amoxicillin (+ve)	17.67±1.53	16.00±2.65	16.33± 1.53
Water (-ve)	00.00	00.00	00.00

4.2.2 Activity against *Salmonella*

A similar trend was observed for *Salmonella*, where antibacterial activity increased with concentration (Table 3). The combined extract again showed greater activity than individual extracts.

Data on the zones of inhibition against *Salmonella* were normally distributed for any extract group, according to Shapiro-Wilk test ($p > 0.05$).Two-way ANOVA showed significant effects of extract type and concentration on the zone of inhibition, with no significant interaction ($p=0.545$). Post hoc Tukey tests showed that amoxicillin was significantly more effective than all plant extracts. The combination of garlic, rosemary and chilli was significantly more effective than garlic and rosemary alone, but not different from chilli. The 75% concentration produced significantly larger inhibition zones than both 50% and 25%, while there was no difference between 50% and 25%. These results support the hypothesis that both extract type and concentration influence antibacterial activity, with the combinations showing enhanced effects relative to individual extracts, though the effect of concentration is consistent across all extracts (Appendix F).

Table 3: Mean Zones of Inhibition of *Salmonella*

	Mean± STD in mm		
Test agent	75%concentration	50%concentration	25%concentration
Garlic	10.67±1.15	7.67±1.52	6.00 ± 2.00
Rosemary	10.00±1.00	7.67±0.58	5.33±1.53
Chilli	9.33±1.15	7.67±1.53	6.67±1.15
Combo	12.00±1.00	9.68±0.58	8.00±1.00
Amoxicillin(+ve)	17.67±1.52	16.67±2.08	16.33±1.53
Water(-ve)	0.00	0.00	0.00

4.3 The Minimum Inhibitory Concentration and Minimum Bacterial

Concentration

4.3.1 MIC of extracts against *Escherichia coli*

Amoxicillin showed the highest potency, with an MIC below the lowest tested concentration (<0.39 mg/ml). Among plant extracts, the combination (combo) that contained 1:1:1 garlic, rosemary and chilli had the lowest MIC (2.08 mg/ml), followed by individual plant extracts garlic (2.60 mg/ml), chilli (3.65 mg/ml), and rosemary (5.21 mg/ml) (fig1).

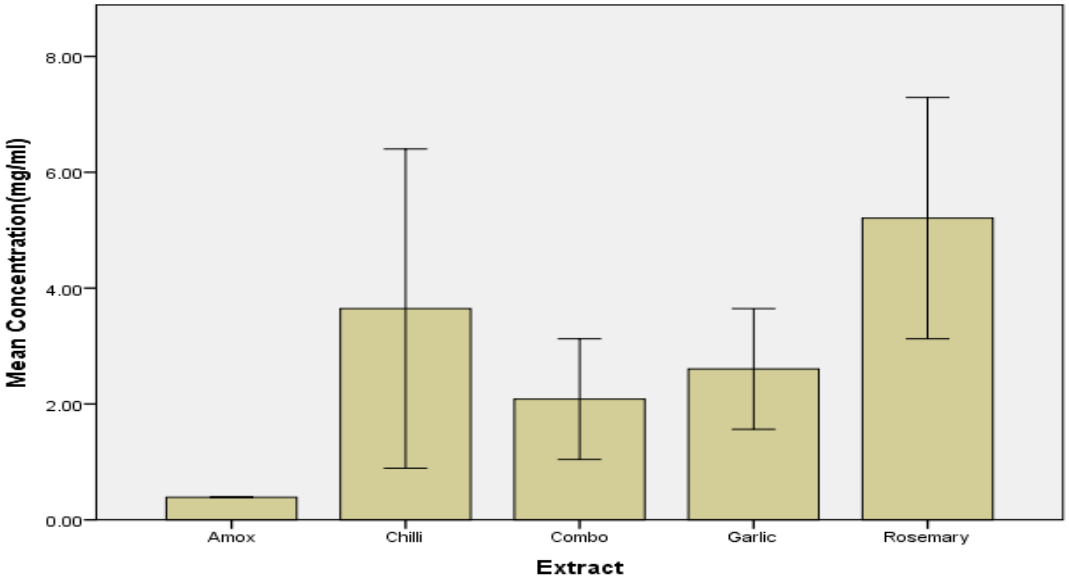

Figure 1: Minimum Inhibitory Concentration (MIC) of extracts against *Escherichia coli.*

4.3.2 MIC of extracts against *Salmonella*

Amoxicillin again showed the lowest (< 0.39 mg/mL). The combination extract showed the greatest activity (2.08mg/mL), followed by rosemary (4.17mg/mL), chilli (5.21 mg/mL), and garlic (8.33 mg/mL) (Fig 2).

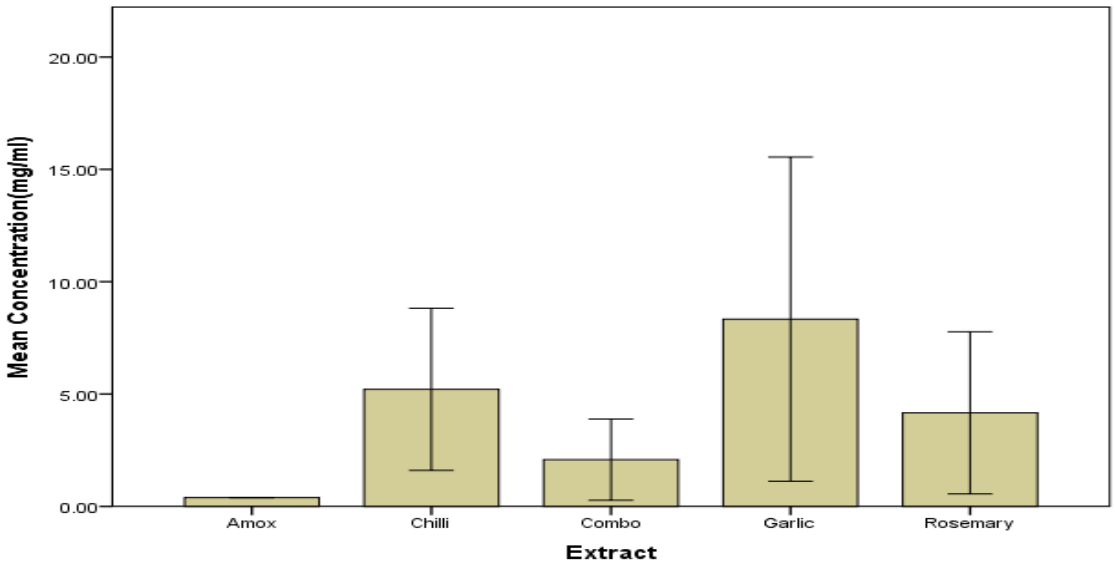

Figure 2: Minimum Inhibitory Concentration (MIC) of extracts against *Salmonella.*

4.3.3 MBC and MIC against *E.coli*

The triple combination of garlic, rosemary and chilli showed the lowest MIC, followed by garlic, chilli and lastly rosemary. The MBC values ranged from 3.13 mg/ml (combo and garlic) to 6.25 mg/ml (rosemary and chilli). The MBC/MIC ratios for all extracts were below 4 confirming the activity of being bactericidal rather than bacteriostatic under the tested conditions (Table 4).

Table 4: MIC/MBC determination against *E.coli*

Test Agent	MIC mg/ml	MBC mg/ml	MBC/MIC	Activity
Garlic	2.60	3.13	1.20	Bactericidal
Rosemary	5.21	6.25	1.20	Bactericidal
Chilli	3.65	6.25	1.71	Bactericidal
Combo(G+R+C)	2.08	3.13	1.50	Bactericidal

4.3.4 MIC/MBC against *Salmonella*

The mean MIC and MBC of four test agents were determined against *Salmonella*. The triple combination (G+R+C) showed the lowest MIC, followed by rosemary, chilli and lastly by garlic. The MBC values ranged from 3.13 mg/ml (combo) to 12.5 mg/ml (garlic). All extracts had MBC/MIC ratios below 4 showing bactericidal activity rather than bacteriostatic against Salmonella under the tested conditions (Table 5).

Table 5 :MIC/MBC determination against *Salmonella*

Test Agent	MIC mg/ml	MBC mg/ml	MBC/MIC	Activity
Garlic	8.33	12.5	1.50	Bactericidal
Rosemary	4.17	6.25	1.50	Bactericidal
Chilli	5.21	6.25	1.20	Bactericidal
Combo(G+R+C)	2.08	3.13	1.50	Bactericidal

4.4: Synergy Testing

4.4.1: Fractional inhibitory concentration index (FICI) of plant extracts against *E. coli*

The combo (garlic + rosemary +chilli) extracts produced a mean MIC of 2.08 mg/ml where each individual extract had MIC values of 0.69 mg/ml in the Combo. Garlic produced a FICI value of 0. 27, rosemary had 0.13 and chilli had 0.19 and their total produced a FICI index of 0.59. The FICI of 0.59 indicated an additive effect, approaching the synergy threshold of ≤0.5 (Table 6).

Table 6 Fractional Inhibitory Concentration Index (FICI) determination against *E. coli*

Plant extract	Individual MIC mg/ml	MIC in combo mg/ml	FICI Value
Garlic	2.60	0.69	0.27
Rosemary	5.21	0.69	0.13
Chilli	3.65	0.69	0.19
FICI Index(total)			0.59

4.4.2: Fractional inhibitory concentration index (FICI) of plant extracts against *Salmonella*

FICI Index produced was 0.38 which lied within the synergistic effect range which is ≤0.5, meaning that the combination was significantly more potent than the sum of its parts *Salmonella* (Table 7).

Table 7: Fractional Inhibitory Concentration (FICI) determination against *Salmonella*

Plant Extracts	Individual MIC mg/ml	MIC in combo mg/ml	FICI Values
Garlic	8.33	0.69	0.08
Rosemary	4.17	0.69	0.17
Chilli	5.21	0.69	0.13
FICI Index			0.38

CHAPTER 5

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Extraction yields

The ethanol yields were within expected ranges for ethanol extraction. Fadiatussalma (2025) observed a much lower garlic yield of 1.16% using 96% ethanol using high performance liquid chromatography (HPLC). This suggested that extraction duration, particle size and cultivar differences highly influence efficiency. Rosemary extraction yielded a moderate amount of product, likely due to non-optimised experimental conditions. Previous studies indicate that ethanol concentration plays a key role in determining the recovery of phenolic acids such as carnosic and rosmarinic acid (Pizani et al., 2024). Similarly, Cuong et al. (2025) demonstrated that ultrasound-assisted extraction increased chilli yield to 25.01% compared to 15% obtained through conventional maceration, highlighting the limitation of passive solvent diffusion

through rigid cell wall structure of chilli. Overall, optimization of factors such as temperature, solvent concentration and extraction method could improve the efficiency and yield of the extracts.

5.1.2 Antibacterial screening

5.1.2.1 Mean zones of inhibition against *E. coli*

The antibacterial activity of the tested plant extracts against *Escherichia coli* was concentration- dependent. The combined extract (G+R+C) produced the largest zones of inhibition at all concentrations with a maximum of 13.00mm at 75%. Garlic extract showed moderate activity (11.33 mm at 75%). This is lower than the findings of Akullo et al. (2022), who reported inhibition zones of 19.67mm using a methanolic garlic extract at a lower concentration of 25mg/ml, suggesting that both solvent type and garlic variety influence antimicrobial activity. Awurum (2020) reported a 19.67mm inhibition for aqueous chilli extract against *E. coli*, showing good inhibitory activity. The comparatively lower activity observed in this study may be attributed to the moderate extract concentration used (25%-75%). The combined extract demonstrated greater antibacterial activity than the individual extracts, suggesting a possible synergistic effect. This is consistent with Amirian et al., (2017), who reported synergistic effects between *Rosmarinus officinalis* and *Salvia officinalis* against *E.coli* isolated from urinary tract infections, with FICI indices indicating synergy. The positive control, amoxicillin, produced inhibition zones of 17.67 mm at 75 %, which falls within standard susceptibility ranges compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines for *E. coli*.

Two-way ANOVA revealed significant main effects of both extract type and concentration on *E. coli* inhibition, with no significant interaction between the two factors (Ebrahimi et al., 2008). Post-hoc Tukey testing analysis indicated that amoxicillin was the most effective treatment. The combined extract was significantly more effective than garlic and rosemary individually, but not significantly different from chilli alone. An increase in concentration from 25% to 75% resulted in a marked increase in antibacterial activity, suggesting a threshold dose effect. These findings are

consistent with Nair et al. (2020), who reported additive effects of spice combinations against Gram-negative bacteria.

5.1.2.2 Mean zones of inhibition against *Salmonella*

The antibacterial activity of the tested plant extracts against *Salmonella* was also concentration dependent. The combined extracts showed the highest inhibition, with a zone of 12.00mm at 75%.Garlic extract exhibited moderate activity (10.67mm at 75 %), which is comparable to the findings of Mushemesh et al . (2023) who reported a 14.18mm zone against *Salmonella spp*. Rosemary extract produced an inhibition zone of 10.00 mm, which is consistent with previous studies indicating that rosemary has antibacterial properties against *Salmonella,* although effectiveness varies depending on extraction method and concentration (Stojanovic-Radic et al., 2018). Chilli extract showed the lowest activity (9.33mm at 75%). This is lower than reports on jalapeno pepper extracts against *Salmonella enterica*, where ethanol-based extracts demonstrated greater antibacterial activity than aqueous extracts (Bacon et al., 2017).

The combination extracts produced larger zones compared to individual extracts, indicating a possible synergistic interaction among the components. This observation is consistent with Stojanovic-Radic et al. (2018) who reported that combined spice extracts exhibit enhanced antimicrobial activity against Salmonella compared to single extracts. Amoxicillin (positive control) produced a larger zone of inhibition at 75% concentration. According to Clinical and Laboratory Standards Institute, (2012) breakpoint criteria for Salmonella spp, inhibition zones of 14-17 mm indicate intermediate susceptibility, while zones ≥18mm indicate full susceptibility. Based on these criteria, the Salmonella strain tested in this study can be classified as intermediately susceptible to amoxicillin. The negative control (water) showed no zone of inhibition, confirming the absence of contamination and validating the experimental results.

On data analysis for Salmonella, the combined extract showed higher antibacterial activity than garlic and rosemary individually but was not significantly different from chilli alone (Nair et al., 2020). The concentration increases from 25% to 75% showed

significant increase on inhibition indicating a concentration-dependent effect and the non-linear dose response characteristic of phytochemicals (Ebrahimi et al., 2008). The overall synergy (FICI 0.38) and lower individual MIC values supported the combination increased efficacy even though it was not statistically different from chilli alone. Two-way ANOVA with post-hoc Tukey testing offered a strong statistical framework to validate these concentration-extract interactions and the three separate extracts showed similar activity against Salmonella and did not differ significantly from one another.

5.1.3 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

5.1.3.1 Minimum inhibitory concentration determination against *Eschericia coli*

The combination of garlic, rosemary and chilli demonstrated enhanced antibacterial activity against *E.coli* with a MIC of 2.08 mg/ml, compared to individual extracts. This supports previous findings that mixtures of plant extracts can produce additive or synergistic antimicrobial effects (Maharjan et al., 2019). Amoxicillin exhibited the highest efficacy, with a MIC value below 0.39 mg/mL (the lowest concentration tested), which is consistent with the known effectiveness of conventional antibiotics against susceptible bacterial strains. Sonme and Ogbu (2026) similarly reported a larger inhibition zone of 33.0mm for amoxicillin against *E. coli*, exceeding the activity observed for the plant extracts. The high efficacy of amoxicillin is due to its specific mechanism of action, which involves inhibition of penicillin-binding proteins during bacterial cell wall synthesis. Among the individual plant extracts, garlic showed the strongest antibacterial activity (MIC 2.60mg/ml), likely due to the presence of processes organosulfur compounds that interfere with bacterial metabolic processes. However, Yadav et al. (2019) reported a higher inhibitory activity for ethanolic garlic extract against *E. coli*, suggesting that variations in extraction methods, plant source, and bacterial strains may influence results.

Rosemary exhibited the weakest antibacterial activity with a MIC of 5.21mg/ml. This may be attributed to the protective outer membrane of Gram-negative bacteria, which can limit the penetration of bioactive compounds. Previous studies by Al-Tayawi et al. (2024) reported MIC values ranging from 25 to 100mg/ml for rosemary extracts, depending on concentration. The moderate activity of rosemary is associated with phenolic compounds such as rosmarinic acid and carnosic acid, although higher concentrations are often required to achieve bactericidal effects against Gram-negative organisms (Kesici et al., 2023). Wide variability observed in chilli and rosemary results may be due to experimental factors such as extraction solvent type and differences in phytochemical composition.

5.1.3.2 Minimum inhibitory concentration determination against *Salmonella*

Amoxicillin showed a MIC value against *Salmonella* of less than 0.39 mg/ml, which is consistent with established antimicrobial efficacy, although resistance remains a concern (Burch and Sperling, 2018). The combination of garlic, rosemary and chilli also demonstrated enhanced activity (MIC=2.08 mg/ml) compared to individual extracts supporting findings by Olivas-Mendez et al. (2022) that combined plant extracts may exhibit synergistic effects. In contrast to the *E. coli* results, rosemary showed the lowest MIC (4.17mg/ml) followed by chilli (5.21mg/ml) and garlic (8.33mg/ml), indicating species-specific differences in susceptibility. The moderate activity of chilli against *Salmonella* is supported by studies by Xie et al. (2025), who observed that *Salmonella* was less resistant in chilli and Magar & Shrestha, (2023), which reported inhibition zones ranging from 10 to 28mm against *Salmonella typhi.* Abed et al. (2025) reported stronger antibacterial effects of garlic against food-borne pathogens, including *Salmonella typhimurium,* suggesting that differences in bacterial strains and extraction techniques may influence antimicrobial performance (Gonelimali et al., 2018).

5.1.3.3 MIC/MBC determination for *E. coli*

The plant extracts and their combination demonstrated bactericidal activity against *E. coli* as indicated by the MBC/MIC ratio ranging from 1.20 to 1.71. These values are

below the threshold of ≤ 4, which is used to define bactericidal action (Makade et al., 2024). This finding is consistent with other studies showing MBC/MIC ratios below 4 for plant-derived antimicrobials, confirming their bactericidal rather than bacteriostatic effects (Makade et al., 2024). The combined extracts showed the greatest potency, with the lowest MIC while maintaining bactericidal activity. This supports the potential of combination treatments to enhance antimicrobial effectiveness against foodborne pathogens. The low MBC/MIC ratios observed across all treatments indicate bacterial killing occurred at concentrations close to those required for growth inhibition.

5.1.3.4 MIC/MBC determination for *Salmonella*

The tested plant extracts and their combination also demonstrated bactericidal activity against *Salmonella* with MBC/MIC ratio ranging from 1.20 and 1.50, which are below the threshold for bactericidal classification (Voss-Rech et al., 2011).The combination extract(G+R+C) showed the lowest MIC and MBC values, indicating enhanced antibacterial potency compared to individual extracts. This is supported by Olivas-Mendez et al., (2022), who demonstrated that combined plant extracts can effectively inhibit the growth of *Salmonella spp* in food systems. Rosemary exhibited the lowest MBC/MIC ratio among the individual extracts suggesting strong bactericidal efficiency. These findings are consistent with Ouattara et al. (2013) who reported MBC/MIC ratios for various plant extracts bactericidal against *Salmonella serovars*. Overall, the superior activity of the combined extract highlights the synergistic potential of these plant components, with effective bacterial killing achieved at lower concentrations than when the extracts are used individually (Olivas-Mendez et al., 2022).

5.1.4 Synergy Testing

5.1.4.1 Fractional Inhibitory concentration (FICI) against *E.coli*

The combination of garlic, rosemary and chilli demonstrated an additive antibacterial effect against *E. coli*, with a FICI value of 0.59. Although this value is slightly above the threshold for synergy (< 0.5), it is close to the synergistic range and indicates enhanced activity when the extracts are combined. The MIC of the combined extract

(0.69mg/ml) was substantially lower than the MICs of the individual extracts (2.60-5.21mg/ml), demonstrating that the combination achieved effective inhibition at reduced concentrations. This observation is consistent with studies by Wesseling & Martin, (2022) which report improved antimicrobial efficiency in combined antibiotics similarly for plant extracts.

Olivas-Mendez et al. (2022) also found that combinations of garlic and rosemary essential oils combined with chilli oleoresin produced enhanced antimicrobial effects, with some mixtures showing synergistic interactions (FICI<0.5) against Gram-negative bacteria. The additive interaction observed in this study suggests that such combinations may still be beneficial in reducing required dosages while maintaining efficacy and potentially lowering cytotoxic effects.

5.1.4.2 Fractional Inhibitory concentration against *Salmonella*

The combination of garlic, rosemary and chilli exhibited strong synergistic activity against *Salmonella,* with a FICI value of 0.38, which is below the synergy threshold (≤ 0. 5). Individual FIC values ranged from 0.08 to 0.17 and the combined MICs (0.69 mg/ml) were significantly lower than the MICs of the individual extracts (4.17-8. 33 mg/ml). These findings are consistent with Olivas-Mendez et al. (2022) who demonstrated synergistic antimicrobial effects of similar plant extract combinations against *Salmonella* in food systems. The observed synergy may be explained by complementary mechanisms of action, including disruption of the Gram-negative outer membrane, inhibition of the efflux pump, and multi-target antimicrobial activity. Together, these mechanisms reduce the concentration required to inhibit bacterial growth (Wesseling and Martin, 2022).

5.2 CONCLUSIONS

The ethanolic extracts of garlic (*Allium sativum*), rosemary (*Rosmarinus officinalis*) and chilli (*Capsicum annuum*) exhibited concentration-dependent antibacterial activity against *Escherichia coli* and *Salmonella* isolated from broiler chickens. Amoxicillin a conventional antibiotic demonstrated the greatest antibacterial activity against *E. coli*

and *Salmonella* as compared to plant extracts and their combinations. The combination extracts consistently produced the lowest MIC values and the largest zones of inhibition for both bacteria, indicating greater potency than individual plant extracts. All individual plant extracts and their 1:1:1 combinations were bactericidal. The combination showed synergism against *Salmonella* and an additive effect against *E. coli.* Two way ANOVA confirmed that both extract and concentration significantly affected inhibition zones where 75% concentration yielded the highest activity. These findings conclude that combining garlic, rosemary and chilli extracts enhance antibacterial activity, suggesting effective potential natural alternatives for controlling bacterial infections in poultry.

5.3 RECOMMENDATIONS

- Further studies are recommended to optimize the dose and formulation of garlic, rosemary and chilli extract combinations to establish the maximum effective and non-cytotoxic concentration for use in poultry feed and drinking water.

- Phytochemical profiling using HPLC/GC-MS and time kill assays should also be conducted to identify the specific bioactive compounds and their concentrations in the plant extracts responsible for antimicrobial activity and also to clarify the mechanisms underlined in the synergistic against *Salmonella* and additive effects against *E. coli*.

- Checkerboard assays should be conducted using combinations of plant extracts and poultry conventional antibiotics to assess their potential to reduce antibiotic dosages and reduce resistance development.

- Further in-vivo studies are required to confirm efficacy and safety of these plants beyond the in-vitro findings.

- The study recommends future studies on comparing the phytochemical profile and antimicrobial activity of garlic, rosemary and chilli extracts sourced from different geographical locations within Zimbabwe and this will determine the consistency and reproducibility of their efficacy against poultry pathogens.

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APPENDICES

Appendix A : Filtration of extracts

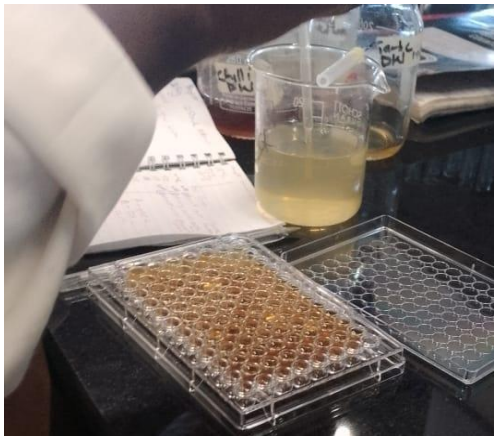


Appendix B: Media preparation and plating

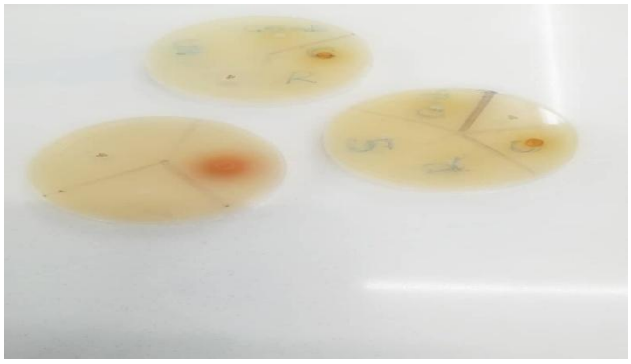


Appendix 3: MIC determination

Appendix C: MIC determination



Appendix D : Zones of inhibition



Appendix E Data analysis for *E. coli*

Normality testing data for *Escherichia coli*

Tests of Normality

	Extracts	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Zone	Amox	.206	9	.200*	.926	9	.442
	Chilli	.171	9	.200*	.972	9	.915
	Combo	.180	9	.200*	.936	9	.542
	Garlic	.258	9	.085	.925	9	.433
	Rosemary	.176	9	.200*	.928	9	.462

TWO-WAY ANOVA for *E. coli*

Tests of Between-Subjects Effects

Dependent Variable: Zone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	481.333[a]	14	34.381	18.868	.000	.898
Intercept	5445.000	1	5445.000	2988.110	.000	.990
Extracts	392.000	4	98.000	53.780	.000	.878
Concentration	80.400	2	40.200	22.061	.000	.595
Extracts * Concentration	8.933	8	1.117	.613	.760	.140
Error	54.667	30	1.822			
Total	5981.000	45				
Corrected Total	536.000	44				

Tukey Post Hoc for *E. coli*

Multiple Comparisons

Dependent Variable: Zone

Tukey HSD

(I) Extracts	(J) Extracts	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Amox	Chilli	7.2222*	.63635	.000	5.3764	9.0680
	Combo	5.5556*	.63635	.000	3.7098	7.4014
	Garlic	7.5556*	.63635	.000	5.7098	9.4014
	Rosemary	8.0000*	.63635	.000	6.1542	9.8458
Chilli	Amox	-7.2222*	.63635	.000	-9.0680	-5.3764
	Combo	-1.6667	.63635	.092	-3.5125	.1791
	Garlic	.3333	.63635	.984	-1.5125	2.1791
	Rosemary	.7778	.63635	.739	-1.0680	2.6236
Combo	Amox	-5.5556*	.63635	.000	-7.4014	-3.7098
	Chilli	1.6667	.63635	.092	-.1791	3.5125
	Garlic	2.0000*	.63635	.029	.1542	3.8458
	Rosemary	2.4444*	.63635	.005	.5986	4.2902
Garlic	Amox	-7.5556*	.63635	.000	-9.4014	-5.7098
	Chilli	-.3333	.63635	.984	-2.1791	1.5125
	Combo	-2.0000*	.63635	.029	-3.8458	-.1542
	Rosemary	.4444	.63635	.955	-1.4014	2.2902
Rosemary	Amox	-8.0000*	.63635	.000	-9.8458	-6.1542
	Chilli	-.7778	.63635	.739	-2.6236	1.0680

	Combo	-2.4444*	.63635	.005	-4.2902	-.5986
	Garlic	-.4444	.63635	.955	-2.2902	1.4014

Multiple Comparisons

Dependent Variable: Zone

Tukey HSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
25.00	50.00	-1.0000	.49291	.123	-2.2152	.2152
	75.00	-3.2000*	.49291	.000	-4.4152	-1.9848
50.00	25.00	1.0000	.49291	.123	-.2152	2.2152
	75.00	-2.2000*	.49291	.000	-3.4152	-.9848
75.00	25.00	3.2000*	.49291	.000	1.9848	4.4152
	50.00	2.2000*	.49291	.000	.9848	3.4152

Appendix F: Data analysis for *Salmonella*

Tests of Normality

	Extract	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Zone	Amox	.264	9	.069	.860	9	.096
	Chilli	.212	9	.200*	.863	9	.104
	Combo	.143	9	.200*	.980	9	.964
	Garlic	.149	9	.200*	.972	9	.913
	Rosemary	.161	9	.200*	.971	9	.907

Two-ANOVA for *Salmonella*

Tests of Between-Subjects Effects

Dependent Variable: Zone

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	652.311[a]	14	46.594	25.262	.000	.922
Intercept	4580.356	1	4580.356	2483.325	.000	.988
Extract	548.089	4	137.022	74.289	.000	.908
Concentration	91.244	2	45.622	24.735	.000	.622
Extract * Concentration	12.978	8	1.622	.880	.545	.190
Error	55.333	30	1.844			
Total	5288.000	45				
Corrected Total	707.644	44				

Multiple Comparisons

Dependent Variable: Zone

Tukey HSD

(I) Extract	(J) Extract	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Amox	Chilli	9.0000*	.64022	.000	7.1430	10.8570
	Combo	7.0000*	.64022	.000	5.1430	8.8570
	Garlic	8.7778*	.64022	.000	6.9208	10.6348
	Rosemary	9.2222*	.64022	.000	7.3652	11.0792
Chilli	Amox	-9.0000*	.64022	.000	-10.8570	-7.1430
	Combo	-2.0000*	.64022	.030	-3.8570	-.1430
	Garlic	-.2222	.64022	.997	-2.0792	1.6348
	Rosemary	.2222	.64022	.997	-1.6348	2.0792
Combo	Amox	-7.0000*	.64022	.000	-8.8570	-5.1430
	Chilli	2.0000*	.64022	.030	.1430	3.8570
	Garlic	1.7778	.64022	.066	-.0792	3.6348
	Rosemary	2.2222*	.64022	.013	.3652	4.0792
Garlic	Amox	-8.7778*	.64022	.000	-10.6348	-6.9208
	Chilli	.2222	.64022	.997	-1.6348	2.0792
	Combo	-1.7778	.64022	.066	-3.6348	.0792
	Rosemary	.4444	.64022	.956	-1.4126	2.3015

Rosemary	Amox	-9.2222*	.64022	.000	-11.0792	-7.3652
	Chilli	-.2222	.64022	.997	-2.0792	1.6348
	Combo	-2.2222*	.64022	.013	-4.0792	-.3652
	Garlic	-.4444	.64022	.956	-2.3015	1.4126

Multiple Comparisons

Dependent Variable: Zone

Tukey HSD

(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
25.00	50.00	-1.4000*	.49591	.022	-2.6226	-.1774
	75.00	-3.4667*	.49591	.000	-4.6892	-2.2441
50.00	25.00	1.4000*	.49591	.022	.1774	2.6226
	75.00	-2.0667*	.49591	.001	-3.2892	-.8441
75.00	25.00	3.4667*	.49591	.000	2.2441	4.6892
	50.00	2.0667*	.49591	.001	.8441	3.2892