AFRICA UNIVERSITY

(A United Methodist-Related Institution)

SENSITIVITY PATTERNS OF LOZENGE FORMULATION AGAINST SELECTED BACTERIAL AND FUNGAL ISOLATES ASSOCIATED WITH THROAT INFECTIONS

BY

SIMBISAI CLOTILDA MANGWIRO

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF MEDICAL LABORATORY SCIENCES IN THE COLLEGE OF HEALTH, AGRICULTURE, AND NATURAL SCIENCES

2025

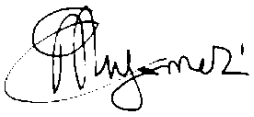
Abstract

The increasing prevalence of antimicrobial resistance in common oral pathogens has necessitated the development of alternative therapeutic approaches. Lozenge formulations containing natural ingredients with antimicrobial properties offer potential treatment options for oral and pharyngeal infections. This study aimed to evaluate the sensitivity patterns of a lozenge formulation developed at Africa University against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans*. A laboratory-based experimental design was used to demonstrate in vitro antimicrobial activity, determine minimum inhibitory concentrations (MICs), and compare sensitivity patterns with known antibiotics. The disc diffusion method was used to determine and measure zones of inhibition, while the broth micro dilution method was used to determine MICs. Statistical analysis included Dunnett's Multiple Comparisons Test which was used to compare the zones of inhibition of the lozenge formulation against each known antibiotic. The lozenge formulation exhibited significant antimicrobial activity against *S. aureus* and *K. pneumoniae* with mean zones of inhibition of 13.0±2.1 mm and 16.4±2.4 mm, respectively, but showed no activity against *C. albicans* (0.0±0.0 mm). MIC values were determined to be 40.0% for *Staphylococcus aureus* and 50.0% for *Klebsiella pneumoniae*, with *Candida albicans* showing no inhibition at concentrations of up to 50.0%. Statistical analysis revealed significant differences in MIC values between *Staphylococcus aureus* and *Candida albicans* ($p=0.019$), confirming the greater susceptibility of *Staphylococcus aureus* to the lozenge formulation. Comparison with known antibiotics demonstrated that the lozenge formulation performed better than the known antibiotics, with relative potency values ranging from 86.7% to 328.0%. The lozenge formulation showed significantly higher inhibition against *Staphylococcus aureus* than all tested known antibiotics except tetracycline, while it demonstrated significantly higher inhibition than all tested antibiotics except ciprofloxacin against *Klebsiella pneumoniae*. The findings indicated that the lozenge formulation has potential as an alternative or adjunctive therapy for bacterial infections in the oral cavity, particularly those caused by *Staphylococcus aureus* and *Klebsiella pneumoniae*, which have shown increasing resistance to conventional antibiotics. However, the lack of activity against *Candida albicans* indicated that the formulation should not be used as a sole treatment for fungal infections in the oral cavity, and combination therapy with antifungal agents may be necessary. Further in vivo studies are recommended to evaluate the clinical efficacy and safety of the formulation, along with investigations into its chemical composition to identify the specific compounds responsible for its antimicrobial activity.

Keywords: Lozenge formulation, antimicrobial activity, minimum inhibitory concentration, *Staphylococcus aureus, Klebsiella pneumoniae*, *Candida albicans*, sensitivity pattern, antibiotic resistance

Declaration

I, ……, student number …….. do hereby declare that this dissertation is my original work except where sources have been cited and acknowledged. The work has never been submitted, nor will it ever be submitted to another university for the award of a Bachelor of Science degree.

Simbisai Clotilda Mangwiro	 14/04/2025
Student's Full Name	Student's Signature (Date)
Prof. Eltony Mugomeri	 14/04/2025
Main Supervisors Full Name	Main Supervisor's Signature (Date)

Copyright

Acknowledgements

I would like to express my immense gratitude to my academic supervisor Professor E. Mugomeri for guiding, advising and supporting me with this dissertation from project formulation till completion. I would also like to thank Mr Zibako for assisting with laboratory experiments which formed the basis for all data collected for this dissertation. I would also like to acknowledge my family, for their constant prayers, support and drive throughout my academic journey.

Dedication

I dedicate this dissertation to my parents Mr and Mrs Mangwiro and my siblings, as well as the growing body of people who are trying to constantly find alternative ways to fight AMR and provide healthy, affordable and sustainable medical therapies.

List of Acronyms and Abbreviations

AMR- Antimicrobial Resistance

ESBL- Extended Spectrum Beta Lactamases

MIC- Minimum Inhibitory Concentration

MRSA- Methicillin-Resistant *Staphylococcus Aureus*

WHO- World Health Organization

Definition of Terms

Anti-microbial Resistance (AMR)- when microorganisms (bacteria, viruses, fungi, and parasites) develop the ability to survive or grow in the presence of antibiotics or other antimicrobial agents that would normally kill or inhibit their growth (WHO, 2023).

In-vitro- experiments or biological studies conducted in a controlled laboratory environment outside of a living organism (Tortora, et al, 2019)

Minimum Inhibitory Concentration (MIC)- the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism (Kononen & Muller, 2021)

Zone of Inhibition- the clear area around an antimicrobial agent where no bacterial growth occurs, used to measure the effectiveness of an antimicrobial substance (Tortora, et al, 2019)

Tables of Contents

List of Tables

List of Figures

List of Appendices

CHAPTER 1: INTRODUCTION

1.1 Introduction

The oral cavity hosts a wide range of microorganisms that commonly cause infections such as sore throat, and oral candidiasis. Prevalent pathogens responsible for these infections include *Klebsiella pneumoniae, Staphyloccocus aureus and Candida albicans* which are known to cause severe infections, including pneumonia and oral thrush and present significant threats to oral and pharyngeal health particularly in immunocompromised patients (Li et al., 2022). Most of these pathogens show an increased rate of anti-microbial resistance to on-the-market antibiotics, which has paved the way for the development of alternative methods to combat bacterial infections. This involves the formation and development of lozenge formulations, which are dosage forms that are intended to dissolve slowly in the mouth for local and specified delivery toward target areas in the oral cavity (Mastropietro et al., 2017).

These lozenges contain natural ingredients such as garlic, ginger, propolis, and essential oils, all of which exhibit antimicrobial effects and can combat various pathogens (EsentüRK Güzel et al., 2024). However, there is a pressing need for comprehensive data on the sensitivity patterns of lozenge formulations being developed against a broad spectrum of pathogens. Understanding these sensitivity patterns is essential for determining dosage and enhancing treatment outcomes. As a result, this study aimed to assess the sensitivity pattern of a lozenge formulation developed at Africa University, against *S. aureus, K. pneumoniae, and C. albicans.*

1.2 Background to the study

The increasing prevalence of antimicrobial resistance of common oral cavity pathogens has highly threatened the currently available treatment related to bacteria. This has led to prolonged illnesses, higher mortality rates, and increased healthcare costs which has made the healthcare diagnostics and medicine delivery system inefficient. This situation has driven several medical practitioners as well as Big Pharma to think of other unconventional methods of medicine to target several bacteria-caused infections. Some of these unconventional methods include the development of lozenge formulations which are used specifically for the local treatment of mouth and throat infections.

The pathogens targeted in this study are responsible for a spectrum of infections:

- *Staphylococcus aureus:* Associated with pneumonia; notable for methicillin-resistant *Staphylococcus aureus* (MRSA) strains.
- *Klebsiella pneumoniae*: Causes pneumonia, with increasing reports of multidrug-resistant strains.
- *Candida albicans*: A fungal pathogen responsible for oral thrush, particularly in immunocompromised individuals.

Exploring the antimicrobial efficacy of lozenge formulations against these pathogens could provide valuable insights into alternative treatment options, especially in the context of rising antimicrobial resistance.

1.2.1 Overview of Lozenge Formulations

Lozenges are various-shaped, solid dosage forms usually containing a medicinal agent and a flavoring substance, intended to be dissolved slowly in the oral cavity for localized or

systemic effect (R & Agarwal, 2022). They are also called troches or pastilles and are widely used for the symptomatic relief of sore throats and other oral and pharyngeal conditions. They function by delivering active ingredients directly to the site of infection or irritation, providing both local and systemic effects. Several studies showed that natural nectars included in lozenge formulations have antimicrobial properties, with a study on the antimicrobial activity of Manuka honey indicating that Manuka honey has a relatively high MGO concentration which correlates positively with antimicrobial efficacy, making it effective against Gram-positive bacteria like *Staphylococcus aureus*. In addition to that, natural ingredients included in several lozenges such as garlic, ginger, and propolis further enhance their antimicrobial properties, making them a viable alternative or adjunctive therapy for managing oral infections (Schmitt et al., 2021).

1.2.2 Prevalence and Impact of Target Pathogens

1.2.2.1 Staphylococcus aureus

Staphylococcus aureus is a common bacterium, with studies showing that it colonizes 30% of the human population. It is a leading causative agent in pneumonia and other respiratory tract infections, surgical sites, prosthetic joints, and cardiovascular infections, as well as nosocomial bacteremia (Cheung et al., 2021). A study on Predictors of Mortality in *Staphylococcus aureus* bacteremia estimated that *S. aureus* bacteremia has an incidence rate ranging from 20 to 50 cases/100,000 per year, and 10% to 30% of these patients will die from the infection (Cheung et al., 2021b). This was highly attributed to increased hospital infections and invasive procedures which pave the way for the spread of the bacteria.

Methicillin-resistant *Staphylococcus aureus* has also shown an upward trend over the past decade, with a study of MRSA prevalence showing an increase of about 33.7% in a space of three years. The study attributed this rising trend to a lack of regulated infection control practices in certain facilities, overuse, and misuse of antibiotics (Lohan et al., 2021). A study on the prevalence and antibiotic resistance of *Staphylococcus aureus* in infections highlighted that a substantial proportion (24.6%) of *S. aureus* exhibited multidrug resistance, with the highest resistance being observed for penicillin (91.2%). This study aimed to determine the burden and antimicrobial susceptibility pattern of *S. aureus* and MRSA and concluded that continuous drug monitoring would be essential in monitoring drug resistance (Sahle & Merid, 2024).

1.2.2.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is one of the most prevalent bacterial pathogens, often linked to a healthcare origin. The organism is a key etiologic factor in healthcare-associated infections such as pneumonia, bloodstream infection, and urinary tract infection. Literature indicated that between 3% to 5% of cases of community-acquired pneumonia can be caused by *Klebsiella pneumoniae*, though this percentage increases to 15% in developing areas with increased exposure to risk factors of the bacterium. Scientific studies indicated that *Klebsiella pneumoniae* is responsible for almost 12% of hospital-associated infections, especially among patients receiving ventilation and are highly susceptible to such infections (Ashurst JV, 2023).

Several reports have expressed concern over the antimicrobial resistance of *Klebsiella pneumoniae,* pointing out the high incidence of multidrug resistance and the existence of

extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* bacterium. An analysis of studies of hospital-associated carbapenem resistance attributed to *Klebsiella pneumoniae* estimated that carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is present in approximately 28.69% of infected patients across the globe, though there is a geographic variance (Lin et al., 2023). Throat infections can also be caused by *Klebsiella pneumoniae*, with a high risk of transmission to other anatomical locations, potentially causing serious complications such as bacteremia and sepsis if interventions that address such complications are not taken.

1.2.2.3 *Candida albicans*

Candida albicans is a fungus that can be found in the oral cavity and mucosal membranes. It is highly pathogenic in individuals with immunocompromised individuals due to immune suppression. *Candida albicans* is mostly isolated from the oral cavity, with infections manifesting as oral candidiasis (thrush) or denture stomatitis. The prevalence of *Candida albicans* is said to be extremely high in diabetic patients due to the knit-tied relationship between sugar and bacteria, with a meta-analysis showing a prevalence rate of about 87.5% of *Candida albicans* in the oral cavity of diabetes mellitus patients (Nouraei et al., 2021). This was crucial in identifying diabetes as a risk factor in the transmission of *Candida albicans*.

Anti-fungals have been largely utilized in the treatment and management of *Candida albicans.* Several *Candida* species have built resistance mechanisms against antifungals, however, *Candida albicans* was stated to show little to no resistance, with The Centre for Disease Control indicating that resistance in *C. albicans,* particularly to azole antifungals,

has been minimal and should continue to be monitored (2024). There are also alternative treatments that have been suggested, however, continuous surveillance would be important for monitoring issues.

1.3 Statement of the Problem

There has been an increased use of lozenges due to their antimicrobial therapeutic nature, especially on pathogens like *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Candida albicans* which target the oral cavity. Although the antimicrobial properties of the contents of some lozenge formulations are known, their efficacy and the extent to which they can fight off microbial infections, and even cause antimicrobial resistance is not widely known. This has affected how these lozenges are administered as dozes might be too much or too little. Additionally, various lozenges, especially those formulated in developing countries are known to provide cures and relief but have not been tested against potential pathogens to determine details of prescriptions. As a result, this study aimed to fill that gap by evaluating the sensitivity patterns of a lozenge formulation made at Africa University, against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*.

1.4 Research Objectives

1.4.1 General Objective

To evaluate the sensitivity patterns of a lozenge formulation against *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Candida albicans.*

1.4.2 Specific Objectives

1. Demonstrate in vitro antimicrobial activity of the lozenge formulation
2. Determine minimum inhibitory concentrations of the lozenge formulation
3. To compare the sensitivity patterns of the pathogens to the lozenge formulation with those of standard antibiotics

1.5 Research Questions

This study aims to address the following research questions:

1. What is the in vitro antimicrobial activity of the lozenge formulation against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans*?
2. What are the minimum inhibitory concentrations (MICs) of the lozenge formulation against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans*?
3. How does the sensitivity pattern of the pathogens to the lozenge formulation compare with that of standard antimicrobial agents?

1.6 Hypotheses

H_0: The lozenge formulation shows no significant antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans*

H_1: The lozenge formulation showed significant antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumoniae,* and *Candida albicans*.

1.7 Justification of the Study

While some lozenge formulations are well known for their antimicrobial properties, there is insufficient information on whether these formulations have undergone sensitivity testing or not. This can lead to misinformed dosing where dosages may be too high or too low, thus compromising the lozenge formulation's treatment outcomes, potentially causing antimicrobial resistance. Understanding how these formulations perform against select pathogens is important for optimizing their use and ensuring the correct dosages are taken. This study sought to fill this gap by evaluating the sensitivity patterns of a lozenge formulation developed at Africa University against clinically significant pathogens. Establishing evidence-based guidelines for using these formulations can enhance public health outcomes.

1.8 Limitations of the Study

At the time of the study, the components of the lozenge formulation were not disclosed to the researcher. As a result, the researcher could not assess key components of the formulation which might have been more helpful in assessing antimicrobial activity and mechanism of the formulation.

1.9 Study Delimitations

The study was conducted in-vitro (a controlled laboratory environment) which might not have been indicative of the human oral cavity where several factors can influence the action of the lozenge e.g. saliva, pH variations, immune responses, and microbial interactions.

Additionally, the use of specific clinical isolates of the pathogens may not have been reprehensive of the full genetic diversity or resistance profiles of these species

1.10 Chapter Summary

This chapter was opened with an introduction, a background to the study, a problem statement, and objectives and justification of the study. It was concluded by the limitations of the study. The following chapter reviews the literature for this study.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter analyzed recent literature on lozenge formulations and the pathogens of interest in this study. It highlighted different studies and perceptions brought about by scholars who participated in studies and experiential work on lozenge formulations. Additionally, it also gave a basis for the theoretical framework for this study.

The literature reviewed in this chapter addressed the following topics:

1. Overview of pathogens under study
2. Antimicrobial properties of lozenge formulations
3. Lozenge formulations and pathogen sensitivity
4. Factors Influencing Antimicrobial Sensitivity Patterns

2.2 Theoretical Framework

The theoretical framework which guided this study encompassed the principles of antimicrobial sensitivity and the mechanisms by which lozenge formulations exert their effects.

2.2.1 Antimicrobial Sensitivity Principle

Antimicrobial sensitivity measures the susceptibility of microorganisms to specific antimicrobial agents. It is regarded as the susceptibility of actinobacteria to antibiotics. Bacterial isolates are either observed as sensitive (S), intermediate (I), or resistant to an antibiotic (Hazarika & Thakur, 2020). Standardized laboratory ways such as disk diffusion or the Kirby Bauer method and broth dilution tests are used to determine the sensitivity of

antimicrobial agents against pathogens. A quantitative method is then used to determine the minimum inhibitory concentration of an antibiotic against select pathogens (Shen & Zhang, 2022).

2.2.2 Bioactive Compound Delivery and Efficacy Theory

The efficacy of lozenge formulations depends on dissolution kinetics, local concentration achievement and contact time optimization (Pandey et al., 2021). Lozenge formulations deliver active ingredients directly to the oral cavity, allowing for localized treatment of infections or symptoms and this largely depends on their rate solubility and dissolution. The slow dissolution of lozenges ensures a prolonged release of active compounds, maintaining therapeutic concentrations at the site of action. This localized delivery is particularly beneficial for treating oral and pharyngeal infections, as it maximizes the contact time between the antimicrobial agents and the pathogens leading to the initial disruption of pathogen cell membranes (Pandey et al., 2021b).

2.3 Relevance of the Theoretical Framework to the Study

The theoretical framework outlined in this study, comprising the antimicrobial sensitivity principle and mechanisms of lozenge formulations is important in understanding the mechanism by which the efficacy of the lozenge formulation was determined. The antimicrobial sensitivity principle formed the foundation for how select pathogens responded to the antimicrobial agents in the lozenges. The Kirby Bauer method was used to determine the minimum inhibitory concentration against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*, which would be essential in determining the dosage required to ascertain the efficacy of this lozenge formulation.

The Bioactive Compound Delivery and Efficacy Theory provides the rationale for investigating lozenges as a delivery mechanism for antimicrobial compounds and informs the interpretation of observed inhibitory effects (Pandey et al., 2021). The theory describes how lozenges achieve targeted delivery to the infection site. When a lozenge disintegrates, it delivers active ingredients into the forming saliva with a reservoir of antimicrobial ingredients to diffuse through oropharynx and oral cavity (Elvan et al., 2021). Local delivery offers maximum concentration at the infection site while minimizing both systemic absorption and chances of side effects. This is particularly important for pathogens like *S. aureus, K. pneumoniae and C. albicans* which infect pharyngeal tissue and oral mucosa, where targeted delivery significantly enhances therapeutic effectiveness.

2.4 Overview of Pathogens Under Study

2.4.1 Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium commonly isolated from the skin, nasal cavity, and oral cavity, where it can cause both localized and systemic infections. While the anterior nares were traditionally recognized as the primary colonization site, recent studies have highlighted the oral cavity and oropharynx as significant reservoirs for *S. aureus*.

Hanson et al. (2018) reported that 37.9% of adults in their study carried *S. aureus* in the oropharynx, indicating that the throat is a notable colonization site. Risk factors for *S. aureus* colonization include weakened immune systems, hospitalization exposure, poor oral hygiene, and use of medical devices. Mertz et al. (2019) found that younger individuals, especially those under 30, have a higher likelihood of throat carriage of *S. aureus*. They also noted that

individuals without exposure to healthcare settings were more likely to be exclusive throat carriers—a group at high risk for community-onset methicillin-resistant *Staphylococcus aureus* (MRSA. The presence of MRSA strains in the oral cavity is particularly concerning due to their antimicrobial resistance to commonly used antibiotics such as penicillin and methicillin.

Lozenge formulations containing antiseptic agents like chlorhexidine and cetylpyridinium chloride have shown efficacy in reducing *S. aureus* bacterial load in the oral cavity. Dudek-Wicher et al. (2022) demonstrated that cetylpyridinium chloride-containing agents can reduce biofilm formation and bacterial adhesion, thereby decreasing the prevalence of oral pathogens, including *S. aureus*. Donkor and Kotey (2020) suggested that incorporating natural antimicrobial agents like essential oils or plant-based extracts into lozenges could provide alternative therapeutic options for combating resistant strains of *S. aureus*.

2.4.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative, encapsulated, non-motile bacterium with high potential for antibiotic resistance. While primarily associated with hospital-acquired infections, research also indicates its presence in the oral cavity and oropharynx, particularly in immunocompromised individuals.

K. pneumoniae colonization in the oropharynx can serve as a reservoir for future infections, especially in patients with compromised immune systems or underlying health conditions. Gorrie et al. (2018) A study on antimicrobial-resistant *Klebsiella pneumoniae* carriage found out that the prevalence of *K. pneumoniae* throat carriage was 4.1%, while rectal carriage was

10.8%. Their study identified the patient's gut as the primary source of *K. pneumoniae*, with other risk factors playing insignificant roles in infection. The major concern with *K. pneumoniae* is its resistance to antibiotics, particularly through mechanisms such as extended-spectrum beta-lactamases (ESBL) and carbapenemases.

Lozenge formulations containing broad-spectrum antiseptic agents may provide an alternative approach to managing *K. pneumoniae* oral and throat infections, potentially circumventing the challenge of antibiotic resistance. These formulations deliver antiseptic agents directly to the site of infection, maximizing local efficacy while minimizing systemic exposure.

2.4.3 *Candida albicans*

Candida albicans is a common fungal organism that causes oral candidiasis, one of the most prevalent fungal infections affecting the oral mucosa. Studies indicate that *C. albicans* is part of the normal flora, with approximately 30% to 40% of people carrying the organism, and carriage rates increasing with age (Daniel et al., 2023).

Although *C. albicans* is present in 50% of the normal flora of healthy mouths, it causes candidiasis when increased numbers of yeast cells invade the mucosa. Patel (2022) noted that *Candida* colonization of the oral cavity increases in immunocompromised individuals, leading to the development of oral candidiasis. Risk factors associated with this colonization include xerostomia, smoking, oral prostheses, dental caries, diabetes, and cancer treatment.

Topical antifungal therapy serves as first-line treatment for uncomplicated cases of oral candidiasis, with agents including nystatin, miconazole, clotrimazole, and ketoconazole.

Despite the availability of these treatments, concerns exist about the development of resistance among fungal pathogens like *Candida*, which can complicate treatment outcomes. Lozenge formulations containing antifungal active ingredients such as clotrimazole and nystatin have demonstrated efficacy against *C. albicans*. However, Černáková et al. (2022) emphasized the importance of monitoring resistance trends to ensure effective treatment. This highlights the need for continued research on alternative antifungal approaches, including novel lozenge formulations that may overcome resistance mechanisms.

2.5 Antimicrobial Properties of Lozenge Formulations

Lozenges have proven to be efficient in delivering oral care due to their ease of administration, targeted delivery, and localized action. This has been made even better as most recent studies have been more focused on developing lozenge formulations with more powerful antimicrobial properties thus inhibiting bacterial growth. This has been possible due to the presence of active ingredients in the lozenge formulations which exhibit strong antimicrobial properties. For example, a study assessing the effectiveness of an enhanced lorodent probiotic lozenge demonstrated that the formulation could effectively reduce the presence of *S. mutans*, a gram-positive coccus commonly found in the human oral cavity and also showed broader pathogen spectra to throat infections (Ebrahim et al., 2022).

2.5.1 Natural Ingredients with Antimicrobial Activity in Lozenge Formulations

Several natural ingredients have been utilized in various lozenge formulations for their antimicrobial properties. Honey bees' main products are known to be honey, propolis, and Perga. These compounds contain many bioactive components that give antioxidant,

antibacterial, anti-inflammatory, and wound-healing properties (Larsen & Ahmed, 2022). Plant-derived compounds are mostly secondary metabolites and contain oxygen-substituted derivatives that are responsible for antimicrobial activity against pathogens. Most of these include phenolic acids, quinones, saponins, and flavonoids, which play a significant role in cell lysis (Gyawali & Ibrahim, 2014).

In addition to that, essential oils have also demonstrated antimicroial properties. Studies reported that oils from Lippia javanica (zumbani), seaweed (Chondrus crispus), pine turpentine, juniper, eucalyptus, sage, lemon, and tea tree exhibit antimicrobial activity that can be harnessed in lozenge formulations (EsentüRK Güzel et al., 2024b). However, Han & Parker (2017) in their study, noted that pine turpentine oil and juniper oil exhibit stronger antibacterial, antifungal, antiseptic, and anti-inflammatory mechanisms compared to other essential oils, which would be essential knowledge in the development of any novel lozenge formulations.

2.5.2 Mechanisms of Antimicrobial Action of Lozenge Formulations

The antimicrobial efficacy of these natural ingredients is attributed to various mechanisms. These mechanisms include the disruption of cell membrane integrity, whose action has been evident through essential oils and phenolic compounds, which interact with the cell membranes of microorganisms, compromising their integrity and leading to leakage of cellular contents (Han & Parker, 2017). In addition to that certain compounds in natural extracts can also bind to and inhibit enzymes critical for microbial metabolism and reproduction, thereby restricting their growth and survival. This was especially evident in a study that showed the enzyme inhibitory activities of flavonoids in bacteria (Donadio et al.,

2021). Similar results were also obtained by a study that evaluated the antifungal activity against *Penicillium notatum* of the ethanoic extracts of propolis from China and United States, which were both composed mainly by flavonoids (Xiaolan Xu, 2015).

Moreover, some antimicrobial compounds contained in lozenges can also interfere with nucleic acid synthesis in microorganisms thus preventing replication and synthesis (Brudzynski, 2021). Many natural products are also said to exhibit antimicrobial activity through Ph. modulation, thus creating an environment that is unfavorable for microbial growth. For example, honey is said to have a low pH and high osmolality that contribute to its antimicrobial effects (Łyskowski et al., 2023). These mechanisms often work synergistically, enhancing the overall antimicrobial efficacy of lozenge formulations and potentially reducing the likelihood of resistance development.

2.6 Previous Studies on Lozenge Formulations and Pathogen Sensitivity

2.6.1 Natural Antimicrobial Agents in Lozenges

Several studies have evaluated the therapeutic potential of lozenge formulations containing natural antimicrobial agents against various oral and pharyngeal pathogens. Hussein et al. (2021) formulated lozenges using aqueous extracts of Miswak (Salvadora persica) and evaluated their antimicrobial activity against clinical isolates of oral pathogens. The lozenges exhibited significant antibacterial and antifungal activities, with notable inhibition zones against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus mutans*. The study found that at a concentration of 85 mg/ml, the Miswak extract produced inhibition zones of 25 ± 0.78 mm, 30 ± 0.77 mm, 21 ± 0.83 mm, and 18 ±

0.86 mm against *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. mutans*, respectively. These zones were significantly larger than those produced by antimicrobial standards like cefuroxime and chlorhexidine against the same bacterial strains, suggesting that natural lozenge formulations may offer advantages over conventional antimicrobials.

An investigation on lozenge formulations from the methanol extract of Moringa oleifera confirmed the effectiveness of these formulations against isolated cultures of *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas aeruginosa*. The study concluded that Moringa oleifera lozenges could be an option for treating mouth and upper respiratory tract infections (Agboke, 2023). However, while natural formulations have demonstrated efficacy, some researchers have noted that certain ingredients cannot act effectively on their own and need to be combined with other ingredients for optimal activity. Ranjan Sahoo et al. (2022) evaluated essential oil-based lozenges using menthol and eucalyptus and found that while they showed moderate antibacterial activity, the addition of other antibacterial components could enhance pathogen sensitivity and increase their potential for managing bacterial infections.

2.6.3 Lozenge Formulation Considerations and Efficacy

The efficacy of lozenge formulations depends on several factors, including the physicochemical properties of active pharmaceutical ingredients (APIs), excipient selection, and desired mechanism of action against target pathogens (Elvan et al., 2021). The API must exhibit appropriate solubility and stability within the lozenge matrix to ensure consistent delivery and efficacy. This can be especially cemented by a study carried out to develope a

functional lozenge with microencapsulated *Lactiplantibacillus pentosus which* found that chlorhexidine lozenges were more effective in exhibiting antimicrobial activity in the oral cavity due to their broad-spectrum activity against select pathogens (Elvan et al. 2021).

Excipient selection also significantly influences the physical stability, palatability, and controlled release of APIs in lozenge formulations. These include binders, fillers, lubricants, flavoring agents, and sweeteners, which affect dissolution release rates. They are said to help maintain the stability of APIs in lozenge formulations for extended periods, improving the shelf life of the dosage form Jadav et al. (2022). However, the literature has also emphasized that active pharmaceutical ingredients in their pure form often do not retain stability for long, resulting in denaturation before serving their intended purpose.

A sustained and controlled release of APIs is essential for ensuring the prolonged antimicrobial efficacy of lozenges. This controlled release allows for extended contact time between antimicrobial agents and target pathogens, maximizing therapeutic efficacy while minimizing the need for frequent administration.

2.8 Chapter Summary

This chapter reviewed the literature related to lozenge formulations, their antimicrobial properties and mechanisms, and the factors influencing antimicrobial sensitivity patterns. The literature review provided insights into the characteristics and prevalence of the pathogens under study: *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. It examined the antimicrobial properties of natural and synthetic ingredients used

in lozenge formulations, their mechanisms of action. Previous studies on lozenge formulations also demonstrated the potential of lozenge formulations as alternative or adjunctive therapies for managing oral and pharyngeal infections, particularly in the context of rising antimicrobial resistance. The formulation considerations highlighted the importance of API properties, excipient selection, and controlled release mechanisms in determining the efficacy of lozenge formulations. The next chapter will give the research methodology, which will give an outline of how the study will be carried out.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

This chapter outlines the methodology that was used in this research study, detailing the research design, study setting, and population, as well as the procedures and techniques used to investigate the sensitivity pattern of the lozenge formulation against *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. The research design was experimental, and focused on in vitro testing of the lozenge formulation's antimicrobial efficacy.

3.2 Research Design

This study was carried out using an experimental research design to determine the antimicrobial properties of the lozenge formulation against *Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans*. It is a laboratory-based experimental design that includes in vitro assays under a controlled environment to ensure accurate results. This research design was employed because it allowed for the objective measurement of variables and the establishment of cause-and-effect relationships between the lozenge formulation and selected pathogens.

3.3 Study Setting and Materials

3.3.1 Study Setting

The research was conducted at the Africa University Teaching Laboratory under the Medical Laboratory Science Department located in Mutare, Zimbabwe. This laboratory is equipped

with advanced microbiological equipment, including biosafety cabinets, incubators, autoclaves, and analytical instruments, providing a controlled environment for conducting the experiments.

3.3.2 Materials

3.3.2.1 Test Organisms

The following microbial strains were used in this study:

- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Candida albicans*

The bacterial strains were ATCC (American Type Culture Collection) strains obtained from Victoria Chitepo Hospital, a provincial hospital in Mutare, Zimbabwe with a functional microbiology laboratory. The fungal strain was however, obtained from Africa University Teaching Laboratory. Including a fungal pathogen alongside bacterial pathogens provided a more comprehensive assessment of the lozenge formulation's antimicrobial potential applications in treating various infections of the oral cavity. The rationale for the selection of these test organisms is as follows:

Staphylococcus aureus was selected as a representative of gram positive bacterium due to its association with oral and throat infections, with studies showing that *S. aureus* is found in approximately 30-40% of the human population's oral cavity and has been implicated in various oral infections like angular cheilitis, parotitis, and oral mucositis (Hanson et al.,

2018). Additionally, the prevalence of methicillin resistant strains (MRSA) also necessitated its relevance in the study, considering the role of lozenges in combating AMR.

Klebsiella pneumoniae was selected as a representative of gram negative bacterium and especially because of its emerging significance in oral and respiratory infections, as well as its prevalence in ventilated patients. The prevalence of extended-spectrum beta-lactamase (ESBL) producing *K. pneumoniae* especially in relation to AMR further justified its inclusion in this study.

Candida albicans was selected as a representative of fungal pathogens, especially since it's a cause of oral thrush.

3.3.2.2 Culture Media

The following media were used for cultivation and sensitivity testing: MacConkey Agar, Cystine-Lactose-Electrolyte-Deficient (CLED) Agar, Sabouraud Dextrose Agar (SDA), Peptone Water and Mueller-Hinton Agar

3.3.2.3 Antimicrobial Agents

3.3.2.3.1 Lozenge Formulations

The lozenge formulations under investigation were a readily developed and made product that was formulated by the Africa University Research and Innovation Department. The specific makeup and formulations of the lozenge were not disclosed at the time of this study.

3.3.2.3.2 Known Antibiotics

The following known antibiotics were used as controls:

- For *S. aureus*: Ampicillin, Gentamicin, Cotrimoxazole, Cloxacillin Ciprofloxacin, Clindamycin, Nitrofurantoin
- For *K. pneumoniae*: Ciprofloxacin, Gentamicin, Cloxacillin, Cotrimoxazole, Tetracycline, Clindamycin and Cefuroxime.

These antibiotics were selected based on their clinical relevance and their representative nature of various antibiotic classes and mechanisms of action. The inclusion of both narrow and broad-spectrum antibiotics provided a comprehensive basis for evaluating the relative potency of the lozenge formulation.

3.4 Sampling Techniques

3.4.1 Sample Size Determination

Purposive sampling was used to select representative clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans* as highlighted under study materials (3.3.2.1 Test Organisms). All experiments were conducted in quintuplicate to to ensure the reproducibility of results. Mean values and standard deviations were calculated to provide a measure of the consistency of the findings. Formulation concentrations of 50%, 40%, and 30% of the lozenge formulation were selected based on preliminary testing and literature review of similar natural product extracts. 50% was established as the upper limit to maintain physicochemical properties of lozenge formulation while the 10% decreasing intervals allowed for precise determination of MIC.

3.5 Data Collection Methods

This section describes the procedures that were used for the collection and culturing of bacterial and fungal strains used in the study.

3.5.1 Demonstrating in vitro antimicrobial activity of the lozenges

The following materals were used: Prepared lozenges dissolved in sterile distilled water, Mueller-Hinton Agar, CLED Agar, MacConkey Agar (for bacteria), Sabouraud Dextrose Agar (for *Candida albicans*), peptone water, sterile petri dishes, sterile swabs and inoculating loops, bacterial and fungal cultures (*S. aureus*, *K. pneumoniae*, *C. albicans*), incubator (37°C for bacteria, 25-30°C for *C. albicans*), sterile forceps or pipettes, known antibiotics, TST indicator and an autoclave.

3.5.1.1 Media Preparation

MacConkey agar, CLED agar, SDA agar, Meullar Hinton agar and Peptone water were prepared according to the manufacturer's guideline and heated with frequent agitation until complete dissolution. The medium was sterilized by autoclaving at 121°C for 15 minutes at 15 psi using a TST indicator to ensure sterility. After being cooled to 45-50°C, the medium was poured into sterile Petri dishes flaming in between to ensure sterility. The plates were allowed to solidify at room temperature and stored at 4°C.

3.5.1.2 Cultivation of Test Organisms

3.5.1.2.1 Bacterial Strain Cultivation

S. aureus was cultured on MacConkey agar plates, *K. pneumoniae* on CLED agar by streak plate method and *C. albicans* on SDA plates by streak plate method. The bacterial plates were incubated at 37°C for 24 hours, and the fungal plates at 25°C for 48 hours. The isolated colonies were selected for further testing.

3.5.1.3 Preparation of Inoculum

3.5.1.3.1 Bacterial Inoculum Preparation

About 3-5 isolated colonies of *S. aureus* and *Klebsiella pneumoniae* strains were picked separately from 24-hour cultures. The colonies were each suspended in 5 ml of sterile 0.9% peptone water. The turbidity was adjusted to match the 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/ml). The suspensions were used within 15 minutes of preparation.

3.5.1.3.2 Fungal Inoculum Preparation

C. albicans colonies from 48-hour cultures were suspended in 5 ml of sterile 0.9% saline. The suspension was then vortexed for 15 seconds to ensure dissolvance and equal distribution of fungal colonies and the turbidity was adjusted to match the 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/ml).

3.5.1.4 Antimicrobial Susceptibility Testing

3.5.1.4.1 Disc Diffusion Method

The disc diffusion method was used where Mueller-Hinton agar plates were prepared as described in section 3.5.1.1.4. The plates were allowed to thaw to room temperature before

inoculation. Sterile cotton swabs were dipped into the prepared inoculum as described in section 3.5.1.3, streaked and rotated several times, and pressed firmly against the inside wall of the tube to remove excess fluid. The swab was then streaked over the entire surface of the agar plate three times, rotating the plate approximately 60° each time to ensure even distribution of colonies across the plates. The plates were allowed to dry for 3-5 minutes. Sterile filter paper discs (6 mm diameter) were impregnated with 11.19g/25ml of the lozenge solution and allowed to soak for 30 minutes. The impregnated discs and standard antibiotic discs were placed in the Mueller-hinton agar separately using sterile forceps and the plates were incubated at 37°C for 18-24 hours for *Staphylococcus aureus* and *Klebsiella pneumoniae* and at 25°C for 24-48 hours for *C. albicans*. After incubation, the diameters of inhibition zones were then measured using a Vernier caliper.

3.5.2 Determination of Minimum Inhibitory Concentrations (MIC) of the Lozenge Formulation

The MICs of the lozenge extracts were determined using the broth microdilution method. Serial dilutions of the lozenge formulation were prepared in three working concentrations (50.0%, 40.0%, and 30.0%) from the stock solution. 3-5 isolated colonies were then transferred into 5 ml of sterile peptone water and the turbidity was adjusted to match the 0.5 McFarland standard (approximately 1.5 × 10^8 CFU/ml) by visual comparison. For each test organism, 10 sterile test tubes were prepared and labeled accordingly: three tubes for each concentration (50.0%, 40.0%, and 30.0%), one positive control tube (inoculum without lozenge formulation) and one negative control tube (lozenge formulation without inoculum). The inoculated concentrations were then streaked on MacConkey agar for *Staphylococcus*

aureus and *Klebsiella pneumoniae*, and SDA for *Candida albicans*. The plates were then incubated at 37°C for 18-24 hours in the case of bacteria, and 24-48 hours for fungi. The MIC was then determined as the lowest concentration of the extract that inhibited visible growth.

3.5.3 Comparison of Sensitivity Patterns with Standard Antibiotics

The comparison was done by carrying out the disc diffusion method as outlined in 3.5.1.4.1 and comparing the results for the known antibiotics against those of the lozenges.

3.6 Data Analysis

The data obtained from the antimicrobial assays was analyzed using descriptive, inferential statistical methods and pictorial visuals for the zones of inhibition. Mean zones of inhibition and MIC values were tabulated for the lozenge formulation against each pathogen. Dunnett's Multiple Comparisons Test was used to compare the zones of inhibition of the lozenge formulation with each antibiotic. Statistical analyses were performed using GraphPad Prism version 9.0.

3.7 Ethical Considerations

Permission to carry out the study and ethical clearance was obtained from the Africa University Research Ethics Committee (AUREC). The key ethical principles of research relevant to this study were taken cognizance of throughout the research.

3.8 Summary

This chapter outlined the methodology that was used to determine the sensitivity patterns of lozenge formulations against *Staphylococcus aureus*, *Klebsiella pneumoniae,* and *Candida albicans*. The use of standardized in vitro assays and rigorous data analysis methods ensured the reliability and validity of the findings, contributing valuable insights to the field of antimicrobial therapy.

CHAPTER 4: RESULTS AND ANALYSIS

4.1 Introduction

This chapter presents the results of the experimental investigation on the in vitro antimicrobial activity of the lozenge formulation against three pathogens: *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Candida albicans*. The results are organized according to the study objectives: demonstration of in vitro antimicrobial activity, determination of minimum inhibitory concentrations (MICs), and comparison of sensitivity patterns with standard antibiotics.

4.2 In Vitro Antimicrobial Activity of the Lozenge Formulation

The initial screening of antimicrobial activity was conducted using the Meullar Hinton agar disc diffusion method. The zones of inhibition were measured in millimeters (mm) and recorded for each test organism.

4.2.1 Known Antibiotics Antimicrobial Activity

To validate the testing methods and establish antimicrobial performance, known antibiotics were first tested against each test organism. Figure 1 and Tables 1 and 2 present the zones of inhibition observed for the known antibiotic against the test organisms.

Figure 1: Zones of inhibition for known antibiotics against Staphylococcus aureus and Klebsiella pneumoniae

Table 1:Zones of inhibition (mm) for known antibiotics against Staphylococcus aureus

Antimicrobial Agent	Abbreviation	Zone of Inhibition (mm)	WHO Breakpoints (mm)	Interpretation
Ampicillin (10µg)	AMP	0	S: ≥15, I: 12-14, R: ≤11	Resistant
Erythromycin (5µg)	ERY	0	S: ≥23, I: 14-22, R: ≤13	Resistant
Ciprofloxacin (5µg)	CPR	5	S: ≥21, I: 15-20, R: ≤14	Resistant
Clindamycin (2µg)	CLN	10	S: ≥21, I: 14-18, R: ≤13	Resistant
Nitrofurantoin (10µg)	NIT	5	S: ≥17, I: 15-16, R: ≤14	Resistant
Tetracycline (10µg)	TET	15	S: ≥19, I: 15-18, R: ≤14	Intermediate
Cotrimoxazole (25µg)	COT	0	S: ≥16, I: 11-15, R: ≤10	Resistant
Cloxacillin (5µg)	CXC	0	S: ≥18, I: 13-17, R: ≤12	Resistant

S: Susceptible, I: Intermediate, R: Resistant

The results demonstrated that *S. aureus* exhibited resistance to most standard antibiotics tested. Only tetracycline showed intermediate activity with a zone of inhibition of 15 mm, while other antibiotics either showed no activity (Ampicillin, Gentamicin, Cotrimoxazole, Cloxacillin) or minimal activity insufficient to classify the strain as susceptible (Ciprofloxacin, Clindamycin, Nitrofurantoin). This resistance profile indicated that the *S. aureus* strain used in this study represented a clinically relevant challenge for antimicrobial testing against the lozenge formulation.

Table 2: Zones of inhibition (mm) for known antibiotics against Klebsiella pneumoniae

Antimicrobial Agent	Abbreviation	Zone of Inhibition (mm)	WHO Breakpoints (mm)	Interpretation
Ciprofloxacin (5µg)	CPR	18	S: ≥21, I: 16-20, R: ≤15	Intermediate
Gentamicin (10µg)	GEN	0	S: ≥15, I: 13-14, R: ≤12	Resistant
Cloxacillin (5µg)	CXC	0	S: ≥18, I: 13-17, R: ≤12	Resistant
Cotrimoxazole (25µg)	COT	0	S: ≥16, I: 11-15, R: ≤10	Resistant
Tetracycline (10µg)	TET	0	S: ≥19, I: 15-18, R: ≤14	Resistant
Clindamycin (2µg)	CLN	5	S: ≥21, I: 15-20, R: ≤14	Resistant
Cefuroxime (30µg)	CRX	0	S: ≥18, I: 15-17, R: ≤14	Resistant

**S: Susceptible, I: Intermediate, R: Resistant*

For *K. pneumoniae*, resistance was observed against most of the standard antimicrobial agents, with ciprofloxacin showing intermediate activity with a zone of inhibition of 18 mm. The lozenge formulation exhibited a mean zone of inhibition of 16.4 mm, which would be classified as intermediate sensitivity if compared to the breakpoints for conventional antimicrobials. This antimicrobial activity qualified the strain for testing against the lozenge.

4.2.2 Lozenge Formulation Antimicrobial Activity

Following the evaluation of standard antimicrobial agents, the lozenge formulation was tested against all three test organisms. Table 3 presents the zones of inhibition observed for the lozenge formulation.

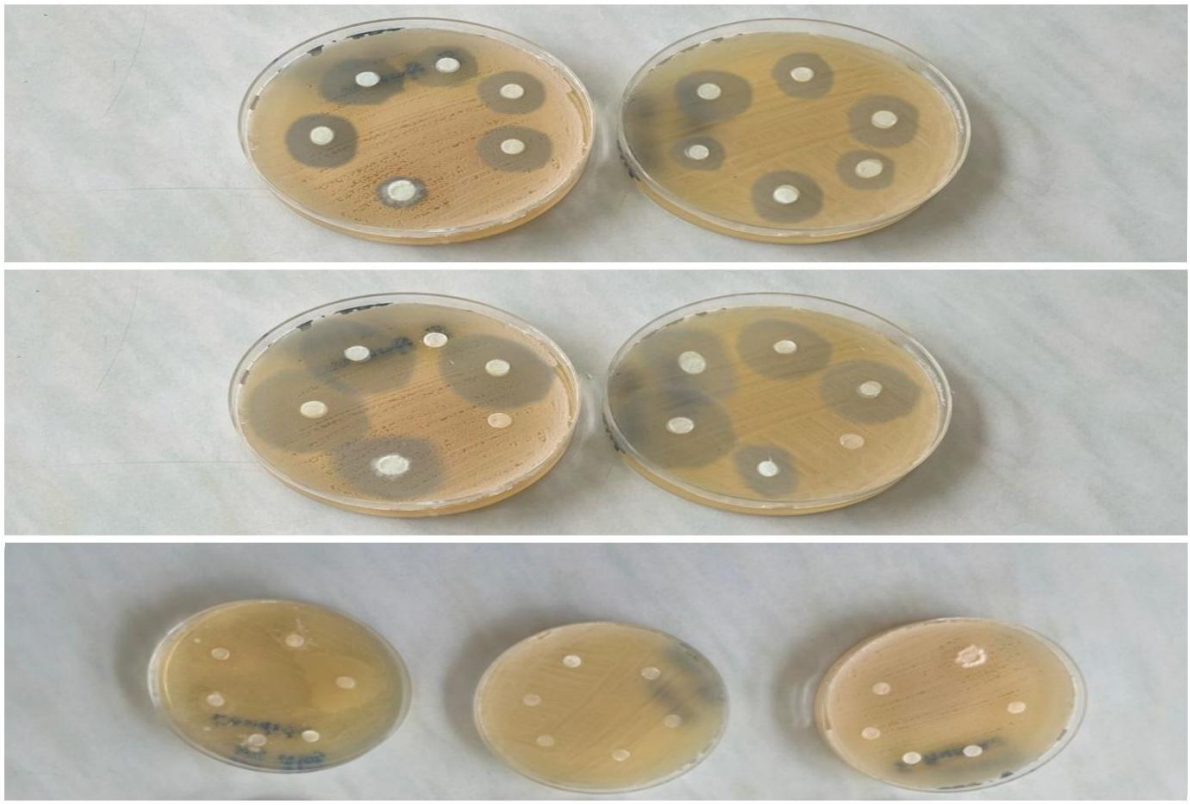


Figure 2: Zones of inhibition for lozenge formulation against Staphylococcus aureus, Klebsiella pneumoniae and Candida albicans respectively (from top to bottom)

Table 3: Zones of inhibition (mm) for lozenge formulation against test organisms

Test organism	**Replicate 1**	**Replicate 2**	**Replicate 3**	**Replicate 4**	**Replicate 5**	**Mean ± SD**
S. aureus	13	15	10	12	15	13.0 ± 2.1
K. pneumoniae	19	15	13	17	18	16.4 ± 2.4
C. albicans	0	0	0	0	0	0.0 ± 0.0

The results indicate that the lozenge formulation exhibited significant antimicrobial activity against *S. aureus* with a mean zone of inhibition of 13.0 ± 2.1 mm and against *K. pneumoniae* with a mean zone of inhibition of 16.4 ± 2.4 mm. However, no inhibitory effect was observed against *C. albicans*, as evidenced by the absence of any zone of inhibition across all replicates (0.0 ± 0.0mm). These results suggest that the antimicrobial compounds in the lozenge formulation lack antifungal properties and that the active ingredients in the formulation may target bacterial cell structures or metabolic pathways that are absent in fungal cells, such as peptidoglycan synthesis, bacterial protein synthesis, or bacterial-specific enzyme systems

4.2.2 Comparison with Antibiotic Controls

The antimicrobial activity of the lozenge formulation was compared with that of standard antimicrobial agents against *S. aureus* and *K. pneumoniae*. The results of these comparative analyses are presented in Figure 3.

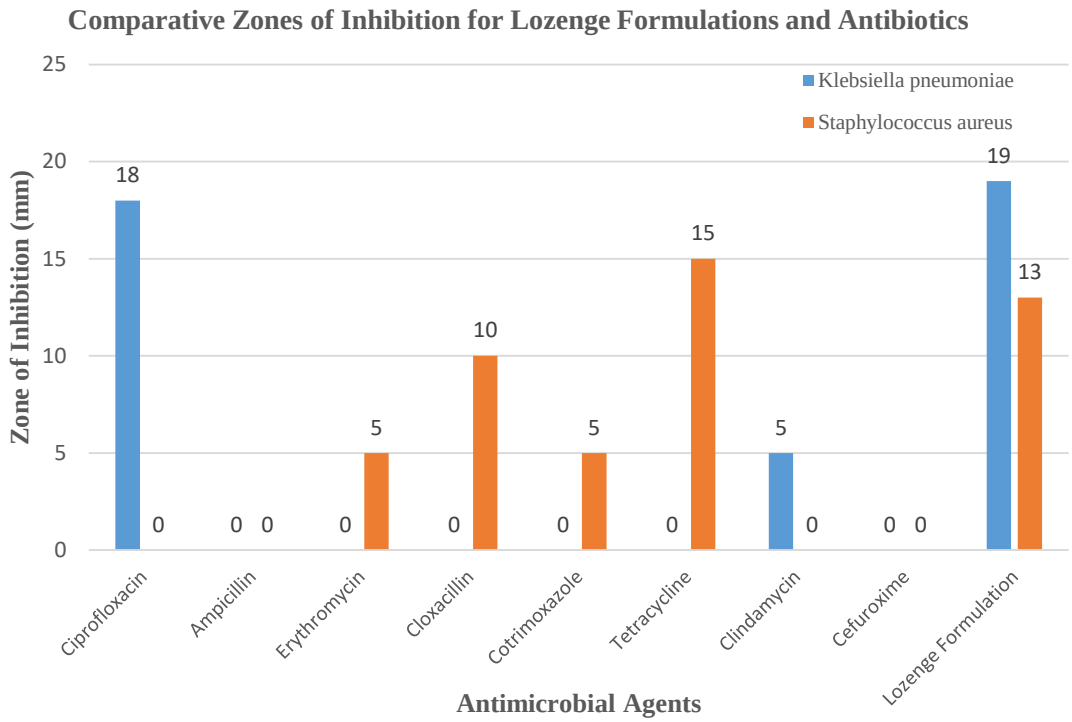


Figure 3: Comparative Zones of Inhibition for Lozenges and Known Antibiotics

The comparison reveals that *S. aureus* exhibited resistance to most of the standard antimicrobial agents tested, with only tetracycline showing intermediate activity. The lozenge formulation demonstrated a zone of inhibition of 13 mm, which would be interpreted as intermediate resistance if compared to the breakpoints for conventional antimicrobials. For *K. pneumoniae*, resistance was observed against most of the standard antimicrobial agents, with ciprofloxacin showing intermediate activity with a zone of inhibition of 18 mm. The lozenge formulation exhibited a mean zone of inhibition of 16.4 mm, which would be classified as intermediate sensitivity if compared to the breakpoints for conventional antimicrobials.

4.3 Minimum Inhibitory Concentrations (MICs) of the Lozenge Formulation

The minimum inhibitory concentrations were determined using the broth micro dilution method. Three concentrations of the lozenge formulation (50.0%, 40.0%, and 30.0%) were tested against the three test organisms.

4.3.1 MIC Determination

Figures 9, 10 and 11 show the growth patterns observed at different concentrations, while table 4 presents them.

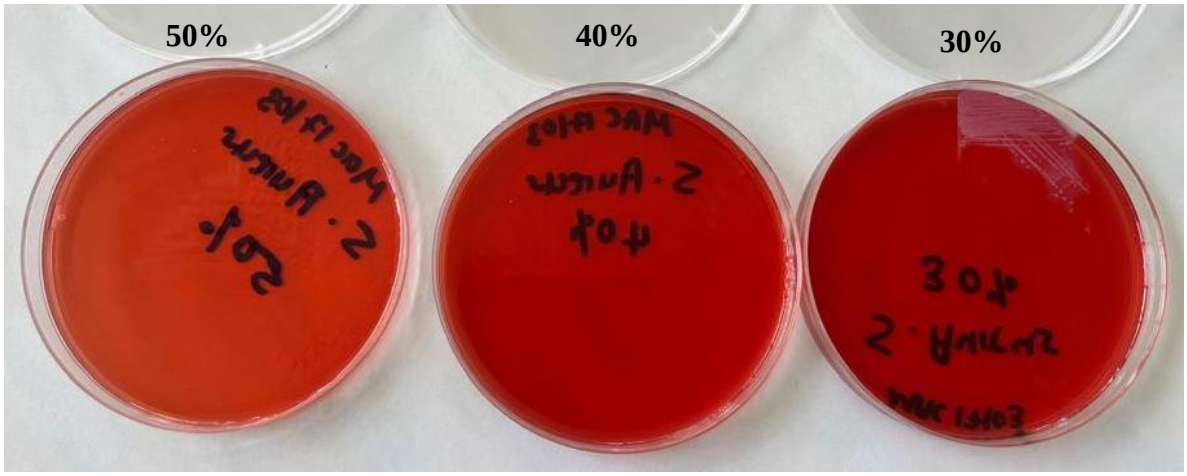


Figure 4: Minimum Inhibitory Concentration for S. aureus

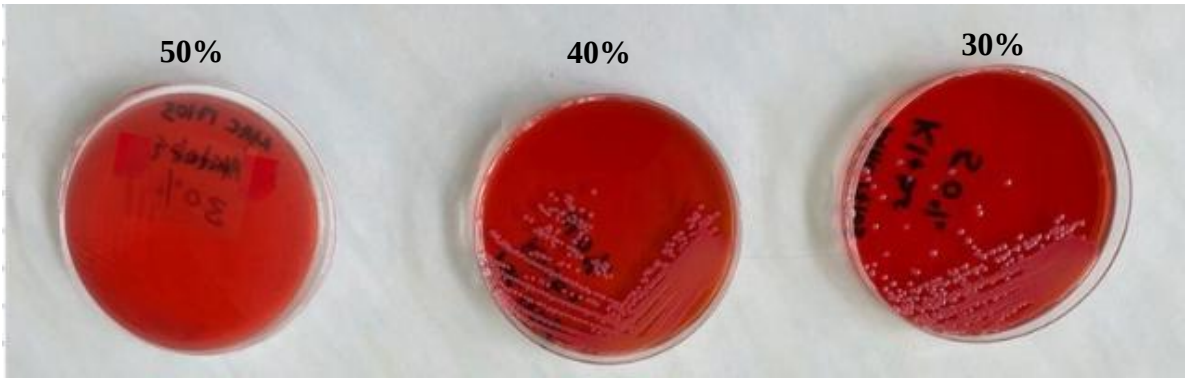


Figure 5: Minimum Inhibitory Concentration for K. pneumoniae

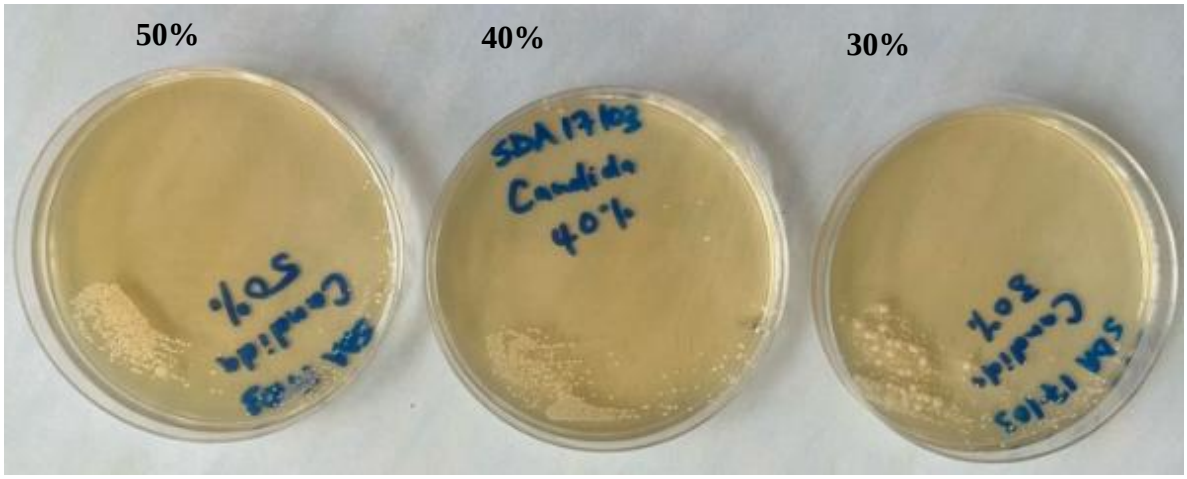


Figure 6: Minimum Inhibitory Concentration for Candida albicans

Table 4: Growth patterns at different concentrations of lozenge formulation

Test Organism	50.0% Concentration	40.0% Concentration	30.0% Concentration
Staphylococcus aureus	No Growth	No Growth	Growth
Klebsiella pneumoniae	No Growth	Growth	Growth
Candida albicans	Growth	Growth	Growth

**"Growth" indicates visible microbial growth in the test medium after 24 hours of incubation at 37*°C*

"No Growth" indicates complete inhibition of visible growth.

Based on these observations, the MIC values for the test organisms were determined as shown in Table 5.

Table 5: Minimum inhibitory concentrations (MICs) of the lozenge formulation

Test Organism	MIC (% Concentration)
Staphylococcus aureus	40.0
Klebsiella pneumoniae	50.0
Candida albicans	>50.0*

**>50.0% indicates that growth was observed at all tested concentrations, suggesting the MIC is higher than the maximum concentration tested (50.0%).*

The results indicate that *S. aureus* responded more to the lozenge formulation with an MIC of 40.0%, while *K. pneumoniae* required a higher concentration of 50.0% for inhibition. The lozenge formulation did not inhibit the growth of *C. albicans* at the tested concentrations, suggesting an MIC value greater than 50.0%. The inability of the lozenge formulation to inhibit *C. albicans* even at the highest tested concentration (50.0%) confirms the lack of antifungal activity as observed in the disc diffusion assay.

4.4 Comparison of Sensitivity Patterns with Known Antibiotics

4.4.1 Relative Potency Analysis and Dunnett's Multiple Comparisons Test

The relative potency of the lozenge formulation compared to known antibiotics was calculated based on the zones of inhibition. The potency ratio was determined using the following formula:

$$\text{Relative Potency} = \frac{\text{Zone of inhibition of lozenge formulation (mm)}}{\text{Zone of inhibition of known antibiotic (mm)}} \times 100\%$$

Table 6: Relative potency of lozenge formulation compared to antibiotics against S. aureus and K. pneumoniae respectively

	Antibiotic	Zone of Inhibition (mm)	Relative Potency (%)
S. aureus			
	Ciprofloxacin	5	260.0
	Cloxacillin	10	130.0
	Nitrofurantoin	5	260.0
	Tetracycline	15	86.7
K. pneumoniae			
	Ciprofloxacin	18	91.1
	Clindamycin	5	328.0

**A high relative potency does not necessarily indicate clinical superiority, as many factors including pharmacokinetics, bioavailability, and target site concentration affect clinical efficacy. The high relative potency values observed against resistant strains suggest that the lozenge formulation may offer alternative antimicrobial mechanisms that could be valuable in addressing antibiotic resistance.*

The relative potency analysis revealed substantial efficacy of the lozenge formulation when compared with conventional antibiotics. For *S. aureus*, the lozenge formulation showed 260.0% relative potency compared to both Ciprofloxacin and Nitrofurantoin, and 130.0% relative potency against Cloxacillin. Only Tetracycline showed marginally greater antimicrobial activity than the lozenge, with the formulation demonstrating 86.7% potency relative to this antibiotic. For *K. pneumoniae*, the lozenge formulation exhibited slightly greater activity than Ciprofloxacin (91.1% relative potency) and remarkably higher efficacy against Clindamycin, with a relative potency of 328.0%.

4.4.2 Statistical Comparison of Antimicrobial Activity

Dunnett's multiple comparisons test was used to compare the zones of inhibition of the lozenge formulation with each antibiotic. Table 9 shows results for the Dunnett’s test.

Table 7: Dunnett's test results for comparison of lozenge formulation with antibiotics against S. aureus and K. pneumoniae respectively

	Comparison	Mean Difference	95% CI	P Value	Significance
S. aureus					
	Lozenge vs. AMP	13.00	[11.29, 14.71]	<0.001	Significant
	Lozenge vs. ERY	13.00	[11.29, 14.71]	<0.001	Significant
	Lozenge vs. CPR	8.00	[6.29, 9.71]	<0.001	Significant
	Lozenge vs. CLN	3.00	[1.29, 4.71]	0.002	Significant
	Lozenge vs. NIT	8.00	[6.29, 9.71]	<0.001	Significant
	Lozenge vs. TET	-2.00	[-3.71, -0.29]	0.025	Significant
	Lozenge vs. COT	13.00	[11.29, 14.71]	<0.001	Significant
	Lozenge vs. CXC	13.00	[11.29, 14.71]	<0.001	Significant
K. pneumoniae					
	Lozenge vs. CPR	-1.60	[-3.31, 0.11]	0.067	Not significant
	Lozenge vs. GEN	16.40	[14.69, 18.11]	<0.001	Significant
	Lozenge vs. CXC	16.40	[14.69, 18.11]	<0.001	Significant
	Lozenge vs. COT	16.40	[14.69, 18.11]	<0.001	Significant
	Lozenge vs. TET	16.40	[14.69, 18.11]	<0.001	Significant
	Lozenge vs. CLN	11.40	[9.69, 13.11]	<0.001	Significant
	Lozenge vs. CRX	16.40	[14.69, 18.11]	<0.001	Significant

The Dunnett's test results indicate that for *S. aureus*, the lozenge formulation showed significantly higher inhibition (p=<0.001) than all tested antibiotics except tetracycline (p=0.025), which exhibited slightly higher activity. For *K. pneumoniae*, the lozenge

formulation demonstrated significantly higher inhibition ($p=<0.001$) than all tested antibiotics except ciprofloxacin, where the difference was not statistically significant ($p=0.025$).

4.5 Chapter Summary

This chapter presented the results obtained from experiments carried out to determine the sensitivity patterns of the lozenge formulation against *S. aureus, K. pneumoniae* and *C. albicans.* The formulation was effective at inhibiting the growth of these bacteria at specific concentrations, with MIC values of 40.0% for *S. aureus* and 50.0% for *K. pneumoniae*. However, no antimicrobial activity was observed against the fungal pathogen *C. albicans*. This chapter also compared the antimicrobial activity of the lozenge formulation against that of known antibiotics. The selective antimicrobial activity that was observed suggests that the lozenge formulation may contain antibacterial compounds that target bacterial structures and biochemical pathways which are absent in fungi.

These findings partially support the alternative hypothesis (H_1), that the lozenge formulation shows significant antimicrobial activity against the tested pathogens, though the lack of activity against *C. albicans* indicates that the antimicrobial spectrum is limited to bacterial pathogens rather than extending to fungal organisms. However, the null hypothesis (H_0) cannot be fully rejected, as the lozenge formulation showed no antimicrobial activity against the fungal pathogen *C. albicans*. The results obtained from this chapter shall be discussed in chapter 5 together with any recommendations.

CHAPTER 5: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter interprets and discusses the findings reported in Chapter 4, drawing connections between the results and existing literature. The chapter also provides conclusions on the effectiveness of the lozenge formulation against the selected pathogens, discusses the implications of these findings, offers recommendations for practical applications, and suggests gaps for future research.

5.2 Discussion of Results

5.2.1 In Vitro Antimicrobial Activity of the Lozenge Formulation

The results of the experiments revealed that the lozenge formulation showed antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* with 13.0±2.1 mm and 16.4±2.4 mm zones of inhibition, respectively. No inhibitory activity was observed against *Candida albicans*. Variable antimicrobial activity against the three pathogens is in line with the observation of Hussein et al. (2021), where they reported variable antimicrobial activity of Miswak extract lozenges against oral pathogens of varied identity, and Agboke (2023) who found similar variability from lozenge extracts of Moringa oleifera against microbial species of varied identity.

The potentially significant activity against *K. pneumoniae* (19 mm) is also applicable with the emerging trend of antimicrobial resistance in *Klebsiella pneumoniae*. Lin et al. (2023) mentioned that carbapenem-resistant *K. pneumoniae* is found in about 28.69% of patients

infected globally, and thus alternative antimicrobial drugs are required. The activity of the lozenge preparation against *K. pneumoniae* suggests its potential as an adjunctive treatment of *K. pneumoniae* infection.

The absence of activity against *C. albicans* contrasts with findings by Larsen and Ahmed (2022), who reported antifungal activities of honey-derived lozenges against Candida species, suggesting that the current formulation may lack antifungal compounds or that *C. albicans* possesses inherent resistance mechanisms against the active ingredients.

5.2.2 Minimum Inhibitory Concentrations (MICs)

The MIC test revealed *S. aureus* was most susceptible to the lozenge formulation (MIC: 40.0%), compared to the higher concentration required for *K. pneumoniae* (50.0%). This susceptibility pattern is consistent with the findings of Dudek-Wicher et al. (2022), who reported varying susceptibilities of oral pathogens to antimicrobial agents used in oral health prophylaxis, with *S. aureus* showing greater susceptibility than other antimicrobial agents that were under study.

The relatively high MIC (40.0%; 50.0%) suggest that antimicrobial agents of the lozenge product may be present in minimal conditions, or that their efficacy is influenced by the physical and chemical properties of the lozenge matrix This is because the action of lozenge products is highly depending on several factors, such as the physicochemical nature of the active ingredients and the nature of the excipients Elvan et al. (2021).

5.2.3 Comparison with Known Antibiotics

The lozenge formulation outperformed most antibiotics against both bacterial pathogens, with relative potency values ranging from 130.0% to 380.0% compared to various antibiotics. For *S. aureus*, the formulation showed higher inhibition than all tested antibiotics except tetracycline, while for *K. pneumoniae*, it demonstrated significantly higher inhibition than all antibiotics except ciprofloxacin. These findings are consistent with a study on Miswak extract lozenges which exhibited inhibition zones significantly higher than those of known antibiotics like cefuroxime and chlorhexidine against various bacterial strains (Hussein et al. 2021).

A high relative potency does not necessarily indicate clinical superiority, as many factors including pharmacokinetics, bioavailability, and target site concentration affect clinical efficacy. However, the high relative potency values observed against resistant strains suggest that the lozenge formulation may offer alternative antimicrobial mechanisms that could be valuable in addressing antibiotic resistance.

5.3 Limitations of the Study

While the study has provided valuable, several limitations should be acknowledged:

1. The study was conducted in vitro, and the results may not directly translate to in vivo conditions due to various factors such as the presence of saliva, varying pH levels, and the complex microbial community in the oral cavity.
2. The strains of the selected pathogens, which may not fully represent the genetic diversity and variable resistance patterns found in clinical isolates.

5.4 Conclusions

Based on the findings of this study, the following conclusions were drawn:

1. The lozenge formulation demonstrated significant antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* but showed no activity against *Candida albicans*. This suggests that the formulation has a selective antimicrobial spectrum, primarily targeting bacterial pathogens.
2. The minimum inhibitory concentrations of the lozenge formulation were determined to be 40.0% for *Staphylococcus aureus* and 50.0% for *Klebsiella pneumoniae*, indicating moderate potency of the antimicrobial compounds in the formulation. The higher MIC for *K. pneumoniae* suggests that this organism is slightly less susceptible to the formulation compared to *S. aureus*.
3. Comparison with known antibiotics showed that the lozenge performed better than most antibiotics against both bacterial pathogens, with significantly higher zones of inhibition and high relative potency values. This suggests that the formulation could be a valuable alternative or adjunct to conventional antibiotics.
4. The research hypothesis was partially supported by the experimental findings. The alternative hypothesis (H_1) that the lozenge formulation would show significant antimicrobial activity against the selected pathogens was confirmed for the bacterial pathogens *Staphylococcus aureus* and *Klebsiella pneumoniae*, but rejected for the fungal pathogen *Candida albicans*. This selective antimicrobial activity suggests that the lozenge formulation contains compounds that specifically target bacterial structures or metabolic pathways absent in fungal cells."

5.5 Implications of the Study

The findings of this study have several implications for clinical practice, public health, and future research:

5.5.1 Clinical Implications

The demonstrated antimicrobial efficacy against *S. aureus* and *K. pneumoniae* positions the lozenge formulation as a potential alternative or adjunctive therapy for bacterial infections in the oral cavity, particularly where conventional antibiotics may be less effective due to resistance. However, its lack of activity against *C. albicans* indicates it should not be used as a sole treatment for fungal infections. The high relative potency compared to several antibiotics suggests potential for reducing systemic antibiotic use, potentially decreasing adverse effects.

5.5.2 Public Health and Research Implications

1. The development of effective antimicrobial lozenges could contribute to reducing the burden of oral and pharyngeal infections, which are common public health problems with significant impact on quality of life and healthcare costs.
2. The differential susceptibility observed among the tested pathogens suggests the presence of specific antimicrobial compounds in the formulation, warranting further investigation into their identities and mechanisms of action.

5.6 Recommendations

Based on the findings and conclusions of this study, the following recommendations are proposed:

5.6.1 Recommendations for Practice

The lozenge formulation should be considered for further development as a potential therapeutic option for bacterial infections in the oral cavity, particularly those caused by *S. aureus* and *K. pneumoniae*. Given its lack of activity against *C. albicans*, it should not be recommended as a sole treatment for fungal infections. The formulation's efficacy against resistant bacterial strains suggests that it can be used in cases where conventional antibiotics show reduced effectiveness.

5.6.2 Recommendations for Further Research

1. In vivo studies should be conducted to evaluate the efficacy, safety, and tolerability of the lozenge formulation in humans, as in vitro results may not fully predict clinical outcomes.
2. The chemical composition of the lozenge formulation should be analyzed to identify the specific compounds responsible for its antimicrobial activity, which could inform further optimization of the formulation.
3. The potential of incorporating additional antimicrobial agents, particularly those with antifungal properties, into the formulation should be explored to expand its antimicrobial spectrum.

5.7 Chapter Summary

This study demonstrated significant antimicrobial activity of the lozenge formulation against bacterial pathogens but not against the fungal pathogen, with MICs of 40.0% for *S. aureus* and 50.0% for *K. pneumoniae*. The formulation has potential as an antimicrobial agent for bacterial infections in the oral cavity, particularly those caused by *S. aureus* and *K. pneumoniae*. The selective antimicrobial spectrum underscores the need for comprehensive evaluation and potential optimization to expand its applications in oral healthcare.

APPENDICES

Appendix 1: Study Methodology

Figure 7: Lozenges Under Study

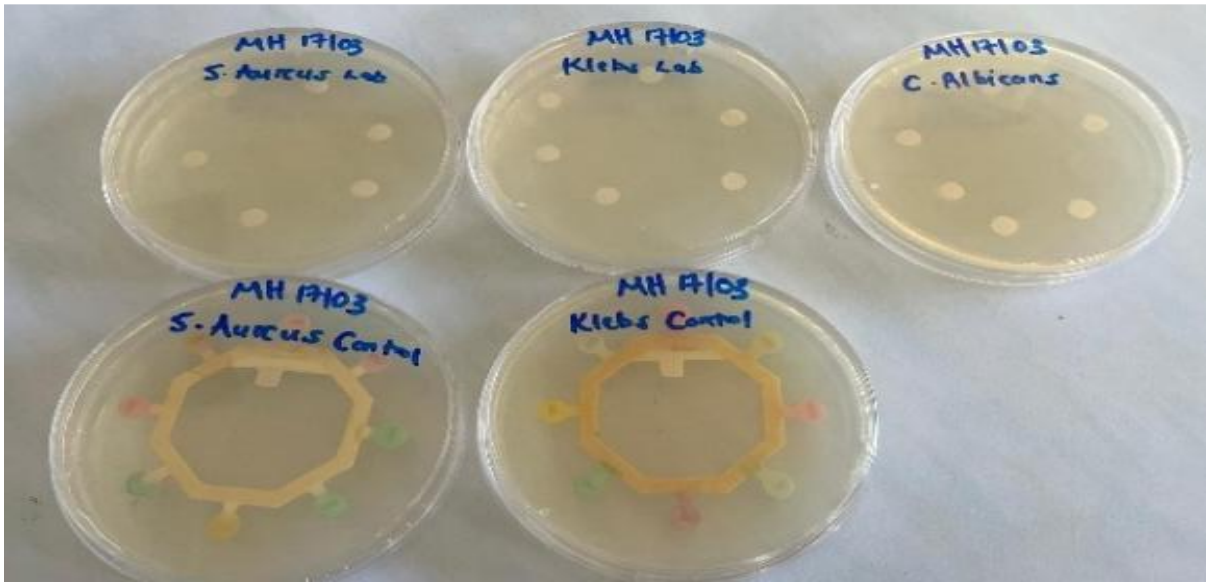


Figure 8: Determination of in-vitro antimicrobial activity

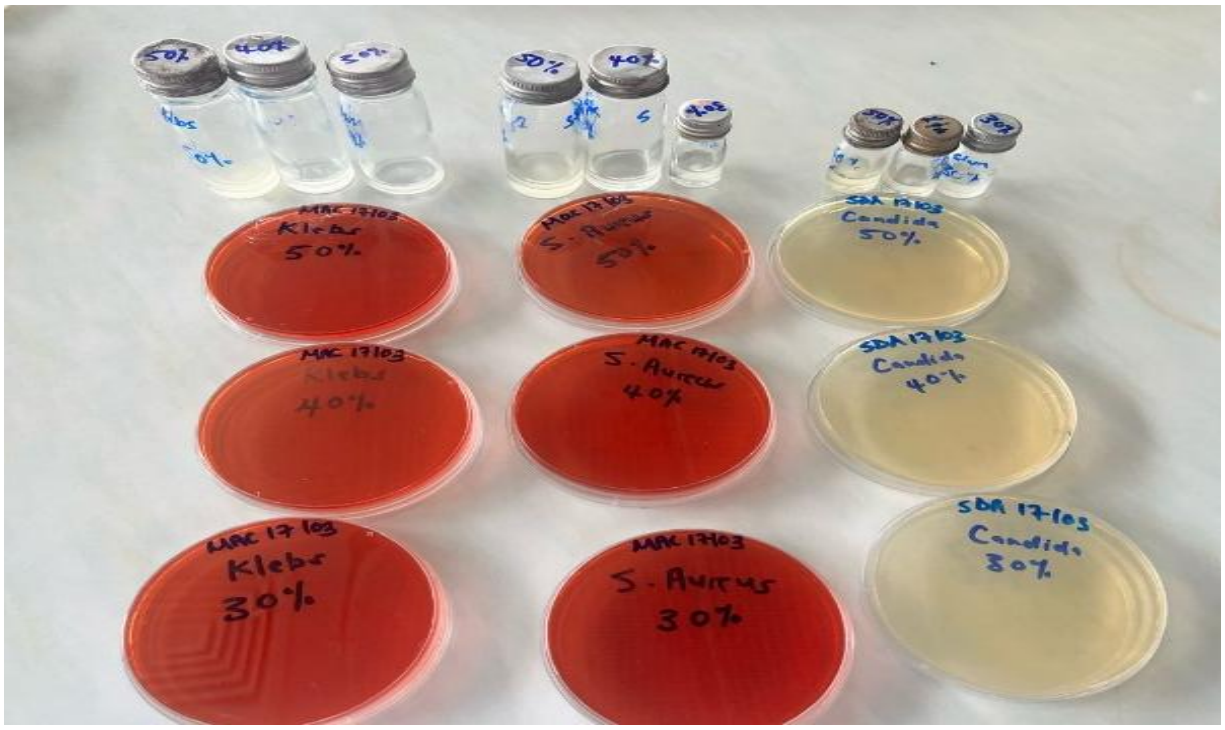


Figure 9: Determination of minimum inhibitory concentration

Appendix 2: Work Plan

Table 8: Work Plan

	Month	January				February				March				April			
	Week	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Activity																	
Preparation and submission of the proposal to AUREC																	
Pretesting																	
Experiments																	
Data processing and analysis																	
Project writing																	
Project submission to Africa University																	

Appendix 3: Budget

Table 9: Budget

ITEM	**UNIT COST**	**MULTIPLYING FACTOR**	**TOTAL COST**
Culturing Media	-	-	$100
Standard Lozenges	-	-	$10
Lozenge Nectar Formulations	-	-	$20
Laboratory Consumables	-	-	$20
Antimicrobial Agents	-	-	$15
Microbial Strains	-	-	$20
Total			**$185**

Appendix 4: AUREC Letter of Approval

"Investing in Africa's future"

AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE (AUREC)

P.O. Box 1320 Mutare, Zimbabwe, Off Nyanga Road, Old Mutare-Tel (+263-20) 60075/60026/61611 Fax: (+263 20) 61785 Website: www.africau.edu

Ref: AU 3621/25

11 March, 2025

SIMBISAI CLOTILDA MANGWIRO
C/O Africa University
Box 1320
MUTARE

RE: ***SENSITIVITY PATTERN OF LOZENGE NECTOR FORMULATIONS AGAINST STAPHYLOCOCCUS AUREUS, KLEBSIELLA PNEUMONIAE, AND CANDIDA ALBICANS***

Thank you for the above-titled proposal you submitted to the Africa University Research Ethics Committee for review. Please be advised that AUREC has reviewed and approved your application to conduct the above research.

The approval is based on the following.

a) Research proposal

- **APPROVAL NUMBER** AUREC 3685/25
  This number should be used on all correspondences, consent forms, and appropriate document
- **AUREC MEETING DATE** NA
- **APPROVAL DATE** March 11, 2025
- **EXPIRATION DATE** March 11, 2026
- **TYPE OF MEETING**: Expedited
  After the expiration date, this research may only continue upon renewal. A progress report on a standard AUREC form should be submitted a month before the expiration date for renewal purposes.
- **SERIOUS ADVERSE EVENTS** All serious problems concerning subject safety must be reported to AUREC within 3 working days on the standard AUREC form.
- **MODIFICATIONS** Prior AUREC approval is required before implementing any changes in the proposal (including changes in the consent documents)
- **TERMINATION OF STUDY** Upon termination of the study a report has to be submitted to AUREC.

Yours Faithfully

MARY CHINZOU
FOR CHAIRPERSON
AFRICA UNIVERSITY RESEARCH ETHICS COMMITTEE

REFERENCES

Jadav, M., Patel, Dr. J., Dr, & Upadhyay, Dr. U., Dr. (2022). Pharmaceutical excipients. *National Journal of Pharmaceutical Sciences*, *2*(2). https://doi.org/10.22271/27889262.2022.v2.i2a

Agboke, A. A. (2023). Formulation of Lozenge of Methanol Root Extract of Moringa oleifera Lam (Moringaceae) and its Efficacy against some Clinically isolated Pathogens. *Nigerian Journal of Pharmaceutical and Applied Science Research*, *12*(4), 19–27. https://doi.org/10.60787/nijophasr-v12-i4-526

Azeem, K., Fatima, S., Ali, A., Ubaid, A., Husain, F. M., & Abid, M. (2025). Biochemistry of bacterial biofilm: Insights into antibiotic resistance mechanisms and therapeutic intervention. *Life*, *15*(1), 49. https://doi.org/10.3390/life15010049

Behbahani, S. B., Kiridena, S. D., Wijayaratna, U. N., Taylor, C., Anker, J. N., & Tzeng, T.-R. J. (2022a). pH variation in medical implant biofilms: Causes, measurements, and its implications for antibiotic resistance. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1028560

Behbahani, S. B., Kiridena, S. D., Wijayaratna, U. N., Taylor, C., Anker, J. N., & Tzeng, T.-R. J. (2022b). pH variation in medical implant biofilms: Causes, measurements, and its implications for antibiotic resistance. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.1028560

Belay, W. Y., Getachew, M., Tegegne, B. A., Teffera, Z. H., Dagne, A., Zeleke, T. K., Abebe, R. B., Gedif, A. A., Fenta, A., Yirdaw, G., Tilahun, A., & Aschale, Y. (2024). Mechanism of antibacterial resistance, strategies and next-generation antimicrobials to contain antimicrobial resistance: A review. *Frontiers in Pharmacology*, *15*.

https://doi.org/10.3389/fphar.2024.1444781

Biała, M., Babicki, M., Malchrzak, W., Janiak, S., Gajowiak, D., Żak, A., Kłoda, K., Gibas, P., Ledwoch, J., Myśliwiec, A., Kopyt, D., Węgrzyn, A., Knysz, B., & Leśnik, P. (2024). Frequency of group A streptococcus infection and analysis of antibiotic use in patients with pharyngitis—a retrospective, multicenter study. *Pathogens*, *13*(10), 846. https://doi.org/10.3390/pathogens13100846

Brudzynski, K. (2021). Honey as an ecological reservoir of antibacterial compounds produced by antagonistic microbial interactions in plant nectars, honey and honey bee. *Antibiotics*, *10*(5), 551. https://doi.org/10.3390/antibiotics10050551

Cdc. (2024, May 22). *Clinical considerations for group A streptococcus*. Group A Strep Infection. https://www.cdc.gov/group-a-strep/hcp/clinical-guidance/index.html

Černáková, L., Líšková, A., Lengyelová, L., & Rodrigues, C. F. (2022). Prevalence and Antifungal Susceptibility Profile of Oral Candida spp. Isolates from a Hospital in Slovakia. *Medicina*, *58*(5), 576. https://doi.org/10.3390/medicina58050576

Cheung, G. Y. C., Bae, J. S., & Otto, M. (2021). Pathogenicity and virulence of *Staphylococcus aureus*. *Virulence*, *12*(1), 547–569. https://doi.org/10.1080/21505594.2021.1878688

Chew, S. C., Yam, J. K. H., Matysik, A., Seng, Z. J., Klebensberger, J., Givskov, M., Doyle, P., Rice, S. A., Yang, L., & Kjelleberg, S. (2018). Matrix Polysaccharides and SiaD Diguanylate Cyclase Alter Community Structure and Competitiveness of Pseudomonas aeruginosa during Dual-Species Biofilm Development with *Staphylococcus aureus*. *mBio*, *9*(6). https://doi.org/10.1128/mbio.00585-18

Daniel, K., Greenberg, R. G., Boutzoukas, A., & Katakam, L. (2023). Updated perspectives on the diagnosis and management of neonatal invasive candidiasis. *Research and Reports in Neonatology*, *Volume 13*, 45–63. https://doi.org/10.2147/rrn.s409779

Donkor, E. S., & Kotey, F. C. (2020). Methicillin-Resistant *Staphylococcus aureus* in the Oral Cavity: Implications for Antibiotic Prophylaxis and Surveillance. *Infectious Diseases: Research and Treatment*, *13*. https://doi.org/10.1177/1178633720976581

Dudek, B., Tymińska, J., Szymczyk-Ziółkowska, P., Chodaczek, G., Migdał, P., Czajkowska, J., & Junka, A. (2023). In vitro activity of octenidine dihydrochloride-containing lozenges against biofilm-forming pathogens of oral cavity and throat. *Applied Sciences*, *13*(5), 2974. https://doi.org/10.3390/app13052974

Dudek-Wicher, R., Junka, A. F., Migdał, P., Korzeniowska-Kowal, A., Wzorek, A., & Bartoszewicz, M. (2022). The antibiofilm activity of selected substances used in oral health prophylaxis. *BMC Oral Health*, *22*(1). https://doi.org/10.1186/s12903-022-02532-4

Ebrahim, F., Malek, S., James, K., MacDonald, K., Cadieux, P., Burton, J., Cioffi, I., Lévesque, C., & Gong, S.-G. (2022). Effectiveness of the Lorodent Probiotic Lozenge in Reducing Plaque and Streptococcus mutans Levels in Orthodontic Patients: A Double-Blind Randomized Control Trial. *Frontiers in Oral Health*, *3*. https://doi.org/10.3389/froh.2022.884683

Egorov, A. M., Ulyashova, M. M., & Rubtsova, M. Yu. (2018). Bacterial enzymes and antibiotic resistance. *Acta Naturae*, *10*(4), 33–48. https://doi.org/10.32607/20758251-2018-10-4-33-48

Elvan, M., Baysal, A. H., & Harsa, S. T. (2021). Developing a functional lozenge with

microencapsulated Lactiplantibacillus pentosus to improve oral and dental health. *Food Bioscience, 40,* 100883. https://doi.org/10.1016/j.fbio.2021.100883

EsentüRK Güzel, İ., Abdo, L., Topuzoğlu, S., Yildiz, C., Yilmaz, F. N., & Döşler, S. (2024a). Natural ingredients included antimicrobial lozenge formulations for oral care. *Journal of Research in Pharmacy*, *28(1)*(28(1)), 248–257. https://doi.org/10.29228/jrp.692

EsentüRK Güzel, İ., Abdo, L., Topuzoğlu, S., Yildiz, C., Yilmaz, F. N., & Döşler, S. (2024b). Natural ingredients included antimicrobial lozenge formulations for oral care. *Journal of Research in Pharmacy*, *28(1)*(28(1)), 248–257. https://doi.org/10.29228/jrp.692

Gorrie, C. L., Mirceta, M., Wick, R. R., Judd, L. M., Wyres, K. L., Thomson, N. R., Strugnell, R. A., Pratt, N. F., Garlick, J. S., Watson, K. M., Hunter, P. C., McGloughlin, S. A., Spelman, D. W., Jenney, A. W. J., & Holt, K. E. (2018). Antimicrobial-Resistant *Klebsiella pneumoniae* Carriage and Infection in Specialized Geriatric Care Wards Linked to Acquisition in the Referring Hospital. *Clinical Infectious Diseases, 67*(2), 161–170. https://doi.org/10.1093/cid/ciy027

Group A streptococcus. (2024, February 1). National Collaborating Centre for Infectious Diseases. https://nccid.ca/debrief/group-a-streptococcus/

Gyawali, R., & Ibrahim, S. A. (2014). Natural products as antimicrobial agents. *Food Control, 46*, 412–429. https://doi.org/10.1016/j.foodcont.2014.05.047

Han, X., & Parker, T. L. (2017). Anti-inflammatory activity of Juniper (Juniperus communis) berry essential oil in human dermal fibroblasts. *Cogent Medicine, 4*(1), 1306200. https://doi.org/10.1080/2331205x.2017.1306200

Hanson, B. M., Kates, A. E., O'Malley, S. M., Mills, E., Herwaldt, L. A., Torner, J. C., Dawson, J. D., Farina, S. A., Klostermann, C., Wu, J. Y., Quick, M. K., Forshey, B. M., & Smith, T. C. (2018). *Staphylococcus aureus*in the nose and throat of Iowan families. *Epidemiology and Infection*, *146*(14), 1777–1784. https://doi.org/10.1017/s0950268818001644

Hazarika, S. N., & Thakur, D. (2020). Actinobacteria. In *Beneficial Microbes in Agro-Ecology* (pp. 443–476). Elsevier. https://doi.org/10.1016/b978-0-12-823414-3.00021-6

Hedin, K., Thorning, S., & van Driel, M. L. (2023). Different antibiotic treatments for group A streptococcal pharyngitis. *Cochrane Database of Systematic Reviews*, *2023*(11). https://doi.org/10.1002/14651858.cd004406.pub6

Hussein, E. F., Abdelgadir, W. A., Osman, R. M., Waddad, A. Y., & Abdelgadir, A. A. (2021). Formulation and Antimicrobial Evaluation of Miswak (Salvadora persica L.) Chewing Stick Aqueous Extract Lozenges. *Drug Des*, *10*, 182.

Johnston, M., McBride, M., Dahiya, D., Owusu-Apenten, R., & Singh Nigam, P. (2018). Antibacterial activity of Manuka honey and its components: An overview. *AIMS Microbiology*, *4*(4), 655–664. https://doi.org/10.3934/microbiol.2018.4.655

Kononen, E., & Muller, H.-P. (2021) Microbiology of the gingival sulcus and the adjacent oral mucosa. Pathogens, 10 (2), 165. https://doi.org/10.3390/pathogens10020165

Larsen, P., & Ahmed, M. (2022). Evaluation of biological activities and medicinal properties of honey drops and honey lozenges. *Nutrients*, *14*(22), 4738. https://doi.org/10.3390/nu14224738

Li, X., Liu, Y., Yang, X., Li, C., & Song, Z. (2022). The oral microbiota: Community

composition, influencing factors, pathogenesis, and interventions. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.895537

Lin, X., Li, C., Zhang, S., Yang, X., & Jiang, M. (2023). The Global and Regional Prevalence of Hospital-Acquired Carbapenem-Resistant *Klebsiella pneumoniae* Infection: A Systematic Review and Meta-analysis. *Open Forum Infectious Diseases*, *11*(2). https://doi.org/10.1093/ofid/ofad649

Lohan, kirti, Sangwan, J., Mane, P., & Lathwal, S. (2021). Prevalence pattern of MRSA from a rural medical college of North India: A cause of concern. *Journal of Family Medicine and Primary Care*, *10*(2).

Łyskowski, A., Miłek, M., & Dżugan, M. (2023). Assessing the antimicrobial properties of honey protein components through in silico comparative peptide composition and distribution analysis. *Antibiotics*, *12*(5), 830. https://doi.org/10.3390/antibiotics12050830

Mastropietro, D., Park, K., & Omidian, H. (2017). 4.23 Polymers in oral drug delivery. In *Comprehensive Biomaterials II* (pp. 430–444). Elsevier. https://doi.org/10.1016/b978-0-12-803581-8.09291-2

Mertz, D., Frei, R., Periat, N., Zimmerli, M., Battegay, M., Flückiger, U., & Widmer, A. F. (2019). Exclusive *Staphylococcus aureus* Throat Carriage: At-Risk Populations. *Archives of Internal Medicine*, *169*(2), 172–178. https://doi.org/10.1001/archinternmed.2008.536

Nigussie, D., Davey, G., Legesse, B. A., Fekadu, A., & Makonnen, E. (2021). Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. *BMC Complementary*

Medicine and Therapies, *21*(1). https://doi.org/10.1186/s12906-020-03183-0

Nouraei, H., Jahromi, M. G., Jahromi, L. R., Zomorodian, K., & Pakshir, K. (2021). Potential pathogenicity of candida species isolated from oral cavity of patients with diabetes mellitus. *BioMed Research International*, *2021*(1). https://doi.org/10.1155/2021/9982744

Patel, M. (2022). Oral Cavity and *Candida albicans*: Colonisation to the Development of Infection. *Pathogens*, *11*(3), 335. https://doi.org/10.3390/pathogens11030335

Peterson, E., & Kaur, P. (2018). Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02928

R, H., & Agarwal, D. (2022). Formulation and Evaluation of meclofenamate fast dissolving tablet. *Asian Journal of Pharmaceutical Research and Development*, *10*(2), 138–145. https://doi.org/10.22270/ajprd.v10i2.1044

Ranjan Sahoo, M., Srinivasan Umashankar, M., & Raghava Varier, R. (2022). Development and Evaluation of Essential Oil-based Lozenges using Menthol and Eucalyptus and in vitro Evaluation of their Antimicrobial activity in S.aureus and E.coli. *Research Journal of Pharmacy and Technology*, 5283–5288. https://doi.org/10.52711/0974-360x.2022.00890

Richards, R. M. E., Xing, J. Z., & Weir, L. F. C. (2020). *Pharmaceutical Research*, *13*(4), 583–587. https://doi.org/10.1023/a:1016002322692

Sahle, B., & Merid, Y. (2024). Prevalence and antibiotic resistance of *Staphylococcus aureus* in wound infections: A hospital study in Hawassa, Ethiopia. *The Journal of Infection*

in Developing Countries, *18*(10), 1530–1538. https://doi.org/10.3855/jidc.19023

Schmitt, A., Roy, R., & Carter, C. J. (2021a). Nectar antimicrobial compounds and their potential effects on pollinators. *Current Opinion in Insect Science*, *44*, 55–63. https://doi.org/10.1016/j.cois.2021.03.004

Schmitt, A., Roy, R., & Carter, C. J. (2021b). Nectar antimicrobial compounds and their potential effects on pollinators. *Current Opinion in Insect Science*, *44*, 55–63. https://doi.org/10.1016/j.cois.2021.03.004

Sedlock, D. M., & Bailey, D. M. (2019). Microbicidal activity of octenidine hydrochloride, a new alkanediylbis[pyridine] germicidal agent. *Antimicrobial Agents and Chemotherapy*, *28*(6), 786–790. https://doi.org/10.1128/aac.28.6.786

Shen, C., & Zhang, Y. (2022). Antibiotic susceptibility testing and evaluation of antiseptics/disinfectants. In *Introductory Microbiology Lab Skills and Techniques in Food Science* (pp. 129–136). Elsevier. https://doi.org/10.1016/b978-0-12-821678-1.00028-9

Stan, D., Enciu, A.-M., Mateescu, A. L., Ion, A. C., Brezeanu, A. C., Stan, D., & Tanase, C. (2021). Natural compounds with antimicrobial and antiviral effect and nanocarriers used for their transportation. *Frontiers in Pharmacology*, *12*. https://doi.org/10.3389/fphar.2021.723233

Steyer, A., Marušić, M., Kolenc, M., & Triglav, T. (2021). A throat lozenge with fixed combination of cetylpyridinium chloride and benzydamine hydrochloride has direct virucidal effect on sars-cov-2. *COVID*, *1*(2), 435–446. https://doi.org/10.3390/covid1020037

Tortora, G. J., Funke, B. R., & Case, C. L. (2019). Microbiology: An introduction (13th ed,,

pp. 610-612). Pearson

Wessels, M. R. (2022). Streptococcus pyogenes Pharyngitis and Scarlet Fever. *Streptococcus Pyogenes: Basic Biology to Clinical Manifestations*.