

FACULTY OF SCIENCE

BIOLOGICAL SCIENCES DEPARTMENT

Antibacterial properties of tea tree (*Melaleuca alternifolia)* and moringa tree (*Moringa oleifera)* against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds

By

VANNESA ROIJEZANI AFIKI

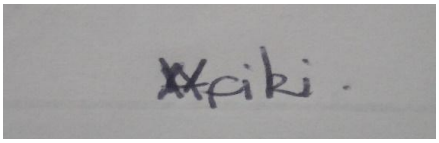
B222993B

A RESEARCH PROJECT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE BACHELOR OF SCIENCE HONORS DEGREE IN BIOTECHNOLOGY.

June 2026

APPROVAL FORM

The undersigned certify that they have read the dissertation titled '**Determination of the antibacterial properties of *Melaleuca alternifolia* and *moringa oleifera* against *staphylococcus aureus* and *streptococcus pyogenes* isolated from dog wounds'** and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science , for assessment.

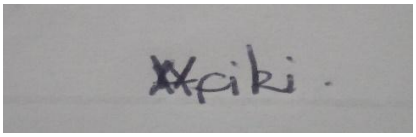
Signature of student:  Date: 15/06/26

Signature of Supervisor:  Date: 15/06/26

Signature of Chairperson :  Date: 13 August 2026

Declaration

I, Vannesa Roijezani Afiki (B222993B) 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

Signature:  Date: 15/06/2026

Supervisor

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 would like to dedicate this piece of work to my mom, Aisha, Anisa and Mr Kathalo who have shown me great, profound and benevolent concern for my education and life. This work would have never been a success without them’.

Acknowledgements

I would like to acknowledge the unwavering support and mentorship of my supervisor Mr J. Ndava throughout the research. My heartfelt thanks go to the Central Veterinary Laboratory for providing me with the resources to carry out the research project.

List of tables

Table 4**:**Percentage yield ethanolic extracts 24

Table 4.1: Extraction Yield of *Moringa oleifera* (g) ……...… 25

Table 4.2: Extraction Yield of *Melaleuca alternifolia (*g) …….....… 25

Table 4.3: Zones of Inhibition Produced by *Moringa oleifera* Extracts (mm)……. 26

Table 4.4: Zones of Inhibition Produced by *Melaleuca alternifolia Extracts* (mm).....……... 27

Table 4.5: ANOVA Results for the Effect of Extraction Solvents on Zones of Inhibition.....30

Table 4.6: ANOVA Results for the Effect of Plant Species on Zones of Inhibition …….....…30

Table 4.7: ANOVA Results for the Effect of Bacterial Species on Zones of Inhibition ……. 31

Table 4.8: Comparison between Plant Extracts and Standard Antibiotic……. 32

Table 4.9: Minimum Inhibitory Concentration (MIC) Values of Plant Extracts …… 33

List of figures

Figure 4.1: Mean Zones of Inhibition Produced by *Moringa oleifera* Extracts 27

Figure 4.2: Mean Zones of Inhibition Produced by *Melaleuca alternifolia Extracts* 28

Figure 4.3: Comparison of Mean Zones of Inhibition between Plant Species 29

Figure 4.4: Broth Microdilution Plates Used for MIC Determination 34

List of Appendices

Appendix A……..... 43
Appendix B……43
Appendix C……...…….44
Appendix D……44
Appendix E……44
Appendix F……...…….....45

List of abbreviations

MIC - Minimum Inhibitory Concentration

g - grams

Abstract

This study investigated the antibacterial properties of *Melaleuca alternifolia* (tea tree) and *Moringa oleifera* against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds. Canine wound infections caused by these bacteria are a major concern in veterinary medicine as a result of increasing antimicrobial resistance, delayed wound healing, and reduced effectiveness of conventional antibiotics. Medicinal plants have gained scientific attention as potential alternatives because they contain bioactive compounds with antimicrobial activity. The aim of the study was to determine and compare the antibacterial activity of *Moringa oleifera* and *Melaleuca alternifolia* extracts against selected bacterial pathogens isolated from dog wounds.

The study employed a quantitative experimental in vitro design conducted at the Central Veterinary Laboratory located in Harare. Fresh leaves of both plants were collected, shade-dried, powdered, and extracted using ethanol, methanol, and distilled water through the maceration method. The antibacterial activity of the extracts was evaluated using the agar well diffusion method, while minimum inhibitory concentration (MIC) values were determined using the broth microdilution technique. Data were analyzed using descriptive statistics and one-way ANOVA at a significance level of $p < 0.05$.

The results showed that ethanol extracts produced the highest extraction yields and strongest antibacterial activity. *Moringa oleifera* demonstrated greater antibacterial effectiveness than *Melaleuca alternifolia*, producing larger zones of inhibition against both bacterial species. The ethanol extract of *Moringa oleifera* produced inhibition zones of 15 mm against *S. aureus* and 12 mm against *S. pyogenes*. MIC values ranged from 0.063 mg/mL to 0.50 mg/mL, with *Moringa oleifera* recording the lowest MIC values, indicating stronger antibacterial potency. Statistical analysis revealed significant differences among extraction solvents and plant species ($p < 0.05$).

The findings suggest that both medicinal plants possess antibacterial properties against canine wound pathogens, although *Moringa oleifera* exhibited superior activity. Ethanol proved to be the most effective extraction solvent. The study concluded that these plant extracts, particularly *Moringa oleifera*, may serve as potential natural alternatives to conventional antibiotics in veterinary wound management and could contribute to reducing antimicrobial resistance in dog

TABLE OF CONTENTS

APPROVAL FORM i
Declaration ii
Dedication iii
Acknowledgements iv
List of tables v
List of figures vi
List of Appendices vii
List of abbreviations viii
Abstract ix
Chapter 1: Introduction 1
1.1 Background to the Study 1
1.2 Problem Statement 3
1.3 Justification 4
1.4 Aim of study 5
1.5 Objectives 5
1.6 Research Questions 5
1.7 Hypothesis 5
Alternative Hypothesis (H_1) 6
1.8 Significance of the study 6
1.9.1 Limitations 7
1.9.3 Delimitations 8
1.10 Definition of terms 9
Antibacterial Activity 9
Melaleuca alternifolia 9
Moringa oleifera 9
Staphylococcus aureus 9
Streptococcus pyogenes 9
Minimum Inhibitory Concentration (MIC) 9
Wound Infection 10

Chapter 2: Literature review 10
2.1 Dog Wound Infections 11
2.2 Bacterial Pathogens Associated with Dog Wounds 11
2.2.1 Staphylococcus aureus infection 12
2.2.2 Streptococcus pyogenes infection 12
2.3 Antibiotic Resistance in Wound Pathogens 13
2.4 Medicinal Plants as Sources of Antibacterial Agents 13
2.5 Melaleuca alternifolia 14
2.5.1 Botanical Description 14
2.5.2 Geographical Distribution 14
2.5.3 Phytochemical Composition 15
2.5.4 Medicinal Uses 15
2.5.5 Antibacterial Properties of Melaleuca alternifolia 16
2.6 Moringa oleifera 16
2.6.1 Botanical Description 16
2.6.2 Geographical Distribution 16
2.6.3 Phytochemical Composition 17
2.6.4 Medicinal Uses 17
2.7 Phytochemicals Responsible for Antibacterial Activity 18
2.8 Methods Used to Evaluate Antibacterial Activity 18
2.9 Previous Studies on Plant Extracts Against Wound Pathogens 19
2.10 Knowledge Gap 19
CHAPTER 3 20
MATERIALS AND METHODS 20
3.1 Study Area 20
3.2 Research Design 20
3.3.Preparation of Plant Extracts: 21
3.4 Preparation of Bacterial Cultures 21
3.6 Antibacterial Testing ..... **Error! Bookmark not defined.**
3.6.1 Preparation of Agar Plates 21
3.6.2 Inoculation of Bacterial Cultures 21
3.6.3 Application of Plant Extracts 22
3.6.4 Incubation 22
3.6.5 Measurement of Zones of Inhibition 22

3.7 Data Collection 22
3.8 Data Analysis 22
Chapter 4 24
Results 24
4.1 Introduction ..... **Error! Bookmark not defined.**
4.2 Extraction Yields of Plant Extracts ..... **Error! Bookmark not defined.**
4.2.1 Extraction Yield of *Moringa oleifera* 24
4.2.2 Extraction Yield of *Melaleuca alternifolia* 24
4.3 Antibacterial Activity of Plant Extracts 25
4.3.1 Antibacterial Activity of *Moringa oleifera* 25
4.3.2 Antibacterial Activity of *Melaleuca alternifolia* 27
4.3.3 Comparison of Antibacterial Activity 28
4.3.4 Statistical Analysis of Zones of Inhibition 28
4.3.5 Comparison with Standard Antibiotic 31
4.4 Minimum Inhibitory Concentration (MIC) 31
4.5 Presentation of Experimental Plates and Observations ..... **Error! Bookmark not defined.**
4.6 Summary of Findings ..... **Error! Bookmark not defined.**
Chapter 5 34
5.1 Discussion 34
Antibacterial Activity 34
Minimum Inhibitory Concentration (MIC) 35
Comparative Antibacterial Efficacy 35
Implications for Wound Management 36
Future Directions ..... **Error! Bookmark not defined.**
5.2 Conclusion 36
5.3 Recommendations 37
References 38
Appendices 43

Chapter 1: Introduction

1.1 Background of the Study

Bacterial wound infections constitute a major and common problem in dogs in veterinary medicine worldwide. Dogs frequently develop wounds through fights, accidents, surgical procedures, tick infestations, and skin injuries, which expose tissues to bacterial contamination. Among the major bacteria associated with canine wound infections are *Staphylococcus aureus* and *Streptococcus pyogenes*, both of which are capable of causing inflammation, abscess formation, delayed healing, tissue necrosis, and secondary complications in dogs. These bacterial infections negatively affect animal welfare and often require prolonged treatment using antimicrobial drugs. Recent studies indicate that bacterial skin infections in dogs continue to increase globally due to poor hygiene, environmental contamination, and rising antimicrobial resistance among pathogenic organisms (Gorski *et al.,* 2022; Silva *et al.,* 2023). In veterinary clinics, canine wound infections have become difficult to manage because many bacterial strains are becoming resistant to commonly used antibiotics (O'Neill *et al.,* 2021; Ahmed *et al.,* 2024).

The rising resistance of bacteria in relation to conventional antibiotics there has become a major challenge in veterinary medicine. Antibiotics that were once effective against bacterial infections in dogs are gradually losing their effectiveness because bacteria continuously develop resistance mechanisms. Antimicrobial resistance has been linked to the excessive and inappropriate use of antibiotics in companion animals, including dogs. Studies conducted in veterinary medicine have shown that resistant bacterial strains are now commonly isolated from infected canine wounds, ears, and skin lesions (Moyaert *et al.,* 2020; Pomba *et al.*, 2022). This situation threatens effective disease management and increases treatment costs for dog owners and veterinary practitioners. Furthermore, antimicrobial resistance contributes to prolonged illness, poor wound healing, and increased mortality in infected dogs (Guardabassi *et al.,* 2021; Murray *et al.,* 2023). Consequently, there is an increasing need to identify alternative antibacterial agents that are safe, affordable, and environmentally sustainable.

Medicinal plants have increasingly attracted attention as potential alternatives to synthetic antibiotics because they contain biologically active compounds with antimicrobial properties. Plant-derived compounds such as flavonoids, alkaloids, tannins, phenols, terpenoids, and saponins have demonstrated the ability to inhibit bacterial growth and promote wound healing. Veterinary researchers have shown growing interest in using medicinal plants to manage bacterial infections in dogs because they are relatively accessible, inexpensive, and less likely to contribute to antimicrobial resistance. Natural plant extracts have also been reported to possess anti-inflammatory and antioxidant properties that enhance tissue repair in infected wounds (Batiha *et al.,* 2020; Salehi *et al.,* 2021). Recent studies further indicate that medicinal plants may provide safer alternatives for treating bacterial skin infections in companion animals when compared to prolonged antibiotic use (Sharun *et al.,* 2022; El-Saber Batiha *et al.,* 2023).

Melaleuca alternifolia, widely recognized as tea tree, is one of the therapeutic plants that has demonstrated strong antibacterial activity against pathogenic bacteria. Tea tree oil extracted from the leaves of this plant contains terpinen-4-ol, which disrupts bacterial cell membranes and inhibits bacterial survival. Research has shown that tea tree oil exhibits antibacterial activity against several pathogens commonly associated with canine skin and wound infections, including *Staphylococcus aureus*. In veterinary medicine, tea tree extracts have also been associated with antiseptic, antifungal, and anti-inflammatory effects that may improve wound management in dogs (Carson *et al.,* 2020; Hammer *et al.,* 2021). Additional studies have reported that tea tree oil may reduce bacterial load and accelerate wound healing when used appropriately in veterinary applications (Orchard *et al.,* 2022; Pinto *et al.,* 2024). These findings suggest that *Melaleuca alternifolia* could serve as a promising natural antibacterial agent in canine wound treatment.

Moringa oleifera is another medicinal plant widely recognized for its antimicrobial and therapeutic properties. Different parts of the plant contain phytochemicals such as flavonoids, phenolic compounds, tannins, saponins, and isothiocyanates that contribute to antibacterial activity. Studies have demonstrated that *Moringa oleifera* extracts can inhibit bacterial pathogens associated with skin and wound infections in animals. In dogs, these antibacterial properties may help control wound infections and support tissue regeneration. Recent veterinary studies have also indicated that *Moringa oleifera* possesses antioxidant and anti-inflammatory properties that

contribute to faster wound healing and improved immune response in animals (Leone *et al.,* 2021; Gupta *et al.,* 2022). Furthermore, the plant has gained increased scientific attention because of its potential application in herbal veterinary medicine and natural antimicrobial product development (Abd Rani *et al.,* 2023; Rahman *et al.,* 2024).

In spite the increase of scientific evidence supporting the antibacterial properties of *Melaleuca alternifolia* and *Moringa oleifera* limited studies have specifically investigated their effectiveness against bacterial pathogens isolated from dog wounds. Many previous studies focused on human bacterial isolates or laboratory bacterial strains that may not accurately represent pathogens commonly found in canine infections. Additionally, differences in extraction methods, concentrations, and testing procedures have made it difficult to compare findings across studies. There is therefore a need for standardized investigations focusing specifically on bacterial isolates obtained from infected dog wounds. Evaluating and comparing the bacterial inhibition effect of these medicinal plants inhibitory against *S. aureus* and *S. pyogenes* isolated from dogs may contribute valuable information toward the development of alternative antibacterial therapies in veterinary medicine (Duarte *et al.,* 2022; Khameneh *et al.,* 2023).

1.2 Problem Statement

Wound infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes* remain significant health challenges in dogs due to increasing antimicrobial resistance. These bacterial infections contribute to delayed wound healing, tissue destruction, pain, inflammation, and secondary complications that negatively affect canine health and welfare. Antibiotics are commonly used to manage these infections in veterinary practice; however, the increasing resistance of bacteria to conventional antimicrobial drugs has reduced treatment effectiveness. Studies have shown that resistant bacterial strains are increasingly isolated from canine skin and wound infections, making management more difficult and expensive for veterinary practitioners and dog owners (Moyaert *et al.,* 2020; Pomba *et al.,* 2022). The growing resistance problem therefore creates an urgent need for alternative antibacterial agents that can effectively control wound infections in dogs.

Medicinal plants such as *Melaleuca alternifolia* and *Moringa oleifera* have demonstrated antibacterial activity against several pathogenic bacteria and may provide potential alternatives to synthetic antibiotics. However, limited information exists regarding their effectiveness against *S. aureus* and *S. pyogenes* specifically isolated from dog wounds. In addition, many previous studies have focused on general antibacterial activity without directly comparing the effectiveness of these medicinal plants against canine bacterial isolates. Variations in extraction procedures and laboratory testing methods have also contributed to inconsistent findings across studies (Sharun *et al.,* 2022; Khameneh *et al.,* 2023). Therefore, there is a need for a study that investigates and compares the antibacterial properties of *Melaleuca alternifolia* and *Moringa oleifera* against bacterial pathogens isolated from infected wounds in dogs

1.3 Justification

The increasing prevalence of antimicrobial resistance among bacterial pathogens affecting dogs has created a need for alternative antibacterial therapies in veterinary medicine. Conventional antibiotics are becoming less effective because of the rapid development of resistant bacterial strains, which threatens successful treatment outcomes in canine wound management. Medicinal plants have attracted scientific interest because they contain natural bioactive compounds capable of inhibiting bacterial growth. Studies have reported that plant-derived antibacterial agents may provide effective and sustainable alternatives to synthetic antibiotics used in veterinary medicine (Batiha *et al.,* 2020; Salehi *et al.,* 2021). Investigating the antibacterial properties of *Melaleuca alternifolia* and *Moringa oleifera* against bacterial pathogens isolated from dog wounds may therefore contribute to the identification of affordable and environmentally friendly treatment options for canine infections.

This study is also important because it contributes to the growing body of knowledge regarding the use of medicinal plants in veterinary medicine. The findings may help veterinary practitioners better understand the potential application of plant-based antibacterial agents in managing canine wound infections. Additionally, natural antibacterial agents may reduce overreliance on synthetic antibiotics and help slow the development of antimicrobial resistance among bacterial pathogens affecting dogs (Guardabassi *et al.,* 2021; Murray *et al.,* 2023). The results of this study may further encourage future research focusing on the isolation and

development of herbal antibacterial compounds for veterinary use. Ultimately, the study may contribute toward improving canine health, wound management, and animal welfare through safer and more sustainable therapeutic approaches.

1.4 Aim of study

This study aimed to find out the antibacterial properties of *Melaleuca alternifolia* and *Moringa oleifera* against *S. aureus* and *S. pyogenes* isolated from dog wounds.

1.5 Objectives

1. To determine the antibacterial activity of the plant extracts against the isolated bacterial species.
2. To compare the antibacterial effectiveness of *Melaleuca alternifolia* and *Moringa oleifera* extracts.
3. To compare the antibacterial activity of the plant extracts with selected conventional antibiotics.

1.6 Research Questions

1. Do extracts of *Melaleuca alternifolia* and *Moringa oleifera* exhibit antibacterial activity against the isolated bacteria?
2. Which plant extract shows greater antibacterial activity against the test organisms?
3. How does the antibacterial activity of the plant extracts compare with conventional antibiotics?

1.7 Hypothesis

Null Hypothesis (Ho)

Melaleuca alternifolia and *Moringa oleifera* do not exhibit significant antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds.

Alternative Hypothesis (H_1)

Melaleuca alternifolia and *Moringa oleifera* exhibit significant antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds.

1.8 Significance of the study

This study is important because it contributes to the search for alternative antibacterial agents that can be used in veterinary medicine to manage bacterial wound infections in dogs. The increasing resistance of bacterial pathogens to conventional antibiotics has become a major concern in canine healthcare, resulting in treatment failures, prolonged recovery periods, and increased veterinary costs. Medicinal plants have recently gained scientific attention because they contain natural bioactive compounds with antibacterial activity that may help reduce dependence on synthetic antibiotics. Research has shown that plant-derived compounds such as flavonoids, terpenoids, tannins, and phenols can inhibit bacterial growth and support wound healing in animals (Batiha *et al.,* 2020; Sharun *et al.,* 2022). Therefore, investigating the antibacterial effects of *Melaleuca alternifolia* and *Moringa oleifera* may provide valuable information for improving the treatment of infected wounds in dogs.

The findings of this study may also benefit veterinary practitioners by providing scientific evidence regarding the effectiveness of natural plant extracts against canine bacterial pathogens. If the extracts demonstrate significant antibacterial activity, they may potentially be incorporated into herbal veterinary products used for wound management in dogs. Such products may offer safer, cheaper, and environmentally friendly alternatives to synthetic antibiotics currently used in veterinary practice. Furthermore, the use of medicinal plants may help reduce antimicrobial resistance by minimizing excessive antibiotic use in companion animals (Guardabassi *et al.,* 2021; Murray *et al.,* 2023). This study may therefore contribute toward sustainable veterinary

healthcare and improved canine welfare through the development of natural antibacterial therapies.

In addition, this study contributes to scientific knowledge regarding medicinal plants and their role in veterinary medicine. Although many studies have investigated the antibacterial properties of medicinal plants, limited research has specifically focused on bacterial isolates obtained from dog wounds. This study may therefore provide useful baseline data for future research on plant-based antibacterial agents targeting canine pathogens. The findings may also encourage further investigations aimed at isolating specific antibacterial compounds from *Melaleuca alternifolia* and *Moringa oleifera* for pharmaceutical development (Duarte *et al.,* 2022; Rahman *et al.,* 2024). Ultimately, the study may support innovation in herbal veterinary medicine and contribute toward improved management of bacterial infections in dogs.

1.9.1 Limitations

The research concentrated exclusively on the antibacterial effects of *Melaleuca alternifolia* and *Moringa oleifera* against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds only. Other bacterial species commonly associated with canine skin and wound infections were not included in this investigation. Consequently, the findings of this study cannot be generalized to all bacterial pathogens affecting dogs. In addition, the study focused specifically on the antibacterial activity of plant extracts and did not investigate antifungal, antiviral, or antiparasitic properties that these medicinal plants may possess (Sharun *et al.,* 2022; Ahmed *et al.,* 2024). Therefore, the results are limited to the selected bacterial pathogens investigated in this study.

Another limitation of this study is that the antibacterial activity of the medicinal plants was evaluated under in vitro laboratory conditions rather than in living dogs. Laboratory findings may not fully reflect the effectiveness of the plant extracts under actual physiological conditions within the body of an infected dog. Factors such as immune response, metabolism, absorption, and toxicity may influence the effectiveness of plant extracts during in vivo applications. Previous studies have shown that some plant extracts demonstrating strong antibacterial activity in vitro may produce different outcomes in living animals due to biological interactions and environmental

factors (Leone *et al.,* 2021; Orchard *et al.,* 2022). Consequently, further in vivo investigations would be necessary before recommending the use of these extracts in clinical veterinary practice.

The study may also have been affected by factors related to the preparation and quality of the medicinal plant extracts. Variations in plant age, harvesting conditions, storage methods, extraction techniques, and environmental conditions may influence the concentration of phytochemicals responsible for antibacterial activity. In addition, limited laboratory resources, financial constraints, and time available for the study may have restricted the number of bacterial isolates and concentrations tested during the investigation (Salehi *et al.,* 2021; Pinto *et al.,* 2024). Furthermore, toxicity studies and investigations into the long-term safety of the medicinal plant extracts were beyond the scope of this research study.

1.9.3 Delimitations

This study specifically focused on investigating the antibacterial properties of *Melaleuca alternifolia* and *Moringa oleifera* against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds. Only these two medicinal plants and bacterial pathogens were included in the investigation. The study concentrated on bacterial isolates obtained from canine wound infections because these pathogens are commonly associated with skin and wound complications in dogs. Other medicinal plants, bacterial species, and infectious agents affecting dogs were excluded from this research study to maintain focus on the stated objectives (Guardabassi *et al.,* 2021; Duarte *et al.,* 2022).

The research was limited to evaluating antibacterial activity using laboratory-based in vitro methods only. Specifically, the agar well diffusion method and minimum inhibitory concentration (MIC) method were used to determine the antibacterial effects of the medicinal plant extracts. The study did not include in vivo experiments involving treatment of wounds in live dogs because such investigations require additional ethical approvals, financial resources, and extended research periods. Furthermore, toxicity testing, pharmacological analysis, and formulation of herbal veterinary products were outside the scope of this study (Khameneh *et al.,* 2023; Rahman *et al.,* 2024). Therefore, the study focused solely on determining and comparing the antibacterial activity of the selected plant extracts under controlled laboratory conditions.

1.10 Definition of terms

Antibacterial Activity

The ability of a substance to inhibit the growth of bacteria or destroy bacterial cells causing infections (Batiha *et al.,* 2020; Salehi *et al.,* 2021).

Melaleuca alternifolia

A medicinal plant commonly known as tea tree that possesses antimicrobial, antiseptic, and anti-inflammatory properties useful in veterinary medicine (Carson *et al.,* 2020; Hammer *et al.,* 2021).

Moringa oleifera

A medicinal plant rich in bioactive compounds with antibacterial, antioxidant, and wound healing properties beneficial in animal health management (Leone *et al.,* 2021; Gupta *et al.,* 2022).

Staphylococcus aureus

A pathogenic bacterium commonly associated with skin infections, abscesses, and wound infections in dogs (Moyaert *et al.,* 2020; Ahmed *et al.,* 2024).

Streptococcus pyogenes

A bacterial species responsible for various skin and wound infections characterized by inflammation and tissue damage (Pomba *et al.,* 2022; Murray *et al.,* 2023).

Minimum Inhibitory Concentration (MIC)

The lowest concentration of an antimicrobial substance capable of inhibiting visible bacterial growth under laboratory conditions (Sharun *et al.,* 2022; Duarte *et al.,* 2022).

Wound Infection

The invasion and multiplication of microorganisms within damaged tissue resulting in inflammation, delayed healing, and tissue destruction in dogs (O'Neill *et al.,* 2021; Silva *et al.,* 2023).

Chapter 2: Literature review

2.1 Dog Wound Infections

Dog wound infections continue to be a top clinical priority in veterinary medicine due to their polymicrobial nature and the increasing emergence of antimicrobial-resistant pathogens. Recent studies indicate that wounds in dogs are commonly infected by bacteria such as *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Escherichia coli*, *Pasteurella spp.*, and other opportunistic organisms originating from the skin, oral cavity, or environment. According to Sebola *et al.,* (2023), veterinary wound infections frequently involve zoonotic and hospital-acquired bacteria with varying antimicrobial susceptibility patterns, making treatment increasingly difficult. Studies further show that antimicrobial-resistant Enterobacter ales isolated from dogs can contribute to treatment failure and may be transmitted between companion animals and humans through close contact (Karalliu *et al.,* 2024). Wounds in companion animals have also been identified as reservoirs of antibiotic-resistant bacteria that may pose public health risks, emphasizing the importance of proper wound hygiene, culture and sensitivity testing, and prudent antimicrobial use (Lenart-Boroń *et al.,* 2024). Current literature therefore highlights the need for effective infection prevention strategies, antimicrobial stewardship, and the formulation of alternative treatment strategies to improve the management of dog wound infections.

2.2 Bacterial Pathogens Associated with Dog Wounds

Bacterial pathogens associated with dog wounds are diverse and predominantly polymicrobial, with both Gram-positive and Gram-negative organisms contributing to infection, delayed healing, and antimicrobial resistance. Recent studies have identified *Staphylococcus aureus*, *Staphylococcus pseudintermedius*, *Streptococcus pyogenes*, *Streptococcus canis*, *Escherichia coli*, and *Pasteurella multocida* as important pathogens commonly isolated from canine wounds and bite injuries. *Staphylococcus aureus* is particularly significant because methicillin-resistant strains (MRSA) have increasingly been reported in companion animals, posing zoonotic risks to humans through close contact (Danieli *et al.,* 2026). In addition, streptococcal species such as *Streptococcus pyogenes* and *Streptococcus canis* have been associated with deep pyoderma, cellulitis, and chronic wound infections in dogs, with some isolates exhibiting resistance to commonly used antibiotics (Imanishi *et al.,* 2022). Recent retrospective studies on surgical and bite wounds in dogs further revealed increasing isolation of resistant bacteria including

Staphylococcus aureus, *Escherichia coli*, and *Klebsiella pneumoniae*, emphasizing the growing importance of antimicrobial stewardship in veterinary medicine (Danieli *et al.*, 2026). These findings demonstrate that canine wounds may act as sources for pathogenic and antimicrobial-drug resistant bacteria, highlighting the importance of proper wound hygiene, bacterial culture, and susceptibility testing for effective treatment and infection control.

2.2.1 Staphylococcus aureus infection

Staphylococcus aureus is among the most significant bacterial pathogen associated with both community-acquired and hospital-acquired infections worldwide. It is responsible for a broad spectrum of diseases including skin and soft tissue infections, wound infections, pneumonia, bacteremia, endocarditis, and sepsis. Recent studies indicate that the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major public health concern because of its multidrug resistance, high virulence, and ability to evade host immune responses. According to Yamazaki *et al.*, (2024), *S. aureus* possesses several virulence factors such as toxins, biofilm formation, and quorum-sensing mechanisms that enhance tissue invasion and persistence within the host. In addition, the pathogen can rapidly adapt to different environmental conditions and antimicrobial treatments, making infections difficult to control (Gherardi, 2023). Current literature further highlights that *S. aureus* remains one of the leading causes of skin and wound infections globally, with resistant strains contributing significantly to increased morbidity, mortality, and healthcare costs. These findings emphasize the importance of infection prevention, early diagnosis, antimicrobial stewardship, and the development of alternative therapeutic

2.2.2 Streptococcus pyogenes infection

Streptococcus pyogenes, also known as Group A Streptococcus, is a clinically significant pathogen causing diverse infections including throat infections, cellulitis, necrotizing fasciitis, streptococcal toxic shock syndrome, and invasive soft tissue infections. Recent literature indicates that *S. pyogenes* remains a major global public health concern because of its high virulence, rapid transmissibility, and increasing incidence of invasive infections worldwide. The bacterium produces numerous virulence factors such as M proteins, exotoxins, and biofilm-forming mechanisms that enhance immune evasion and tissue destruction (Cho, 2024). In

addition, recent studies highlight growing concerns regarding antimicrobial resistance, recurrent outbreaks, and the absence of a licensed vaccine despite ongoing research efforts (Siemens & Lütticken, 2021). Current evidence also demonstrates that invasive *S. pyogenes* infections are associated with significant morbidity and mortality, particularly among immunocompromised individuals, children, and elderly populations. These findings emphasize the importance of early diagnosis, appropriate antibiotic therapy, infection control measures, and continued surveillance to reduce the global burden of *Streptococcus pyogenes* infections.

2.3 Antibiotic Resistance in Wound Pathogens

Antibiotic resistance in wound pathogens has become a major global health concern because it reduces the effectiveness of commonly used antimicrobial agents and contributes to delayed wound healing, prolonged hospitalization, and increased mortality. Recent studies show that wound infections are increasingly associated with multi drug resistant pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, and resistant *Enterobacterales*, which possess mechanisms such as biofilm formation, enzyme production, and gene transfer that enhance resistance to treatment (Monk *et al.*, 2024). In addition, resistant wound pathogens are capable of surviving in chronic wounds and healthcare environments, making infection management more difficult and increasing the risk of transmission. Current literature therefore emphasizes the importance of antimicrobial stewardship, early susceptibility testing, infection prevention, and the development of alternative therapies to control the spread of antibiotic-resistant wound pathogens.

2.4 Medicinal Plants as Sources of Antibacterial Agents

Medicinal plants have gained considerable attention as potential sources of antibacterial agents due to the increasing prevalence of antibiotic-resistant bacteria. Recent studies have shown that phytochemicals such as alkaloids, flavonoids, tannins, terpenoids, and phenolic compounds possess strong antibacterial activities against both Gram-positive and Gram-negative bacteria. These compounds act through different mechanisms, including disruption of bacterial cell walls, inhibition of protein synthesis, and prevention of biofilm formation. Furthermore, medicinal plants are considered promising alternatives because they are widely available, relatively

affordable, and may produce fewer side effects compared to synthetic antibiotics. Researchers continue to explore medicinal plants aimed at developing novel antibacterial drugs to counteract multidrug-resistant pathogens (Abdallah *et al.*, 2023).

2.5 Melaleuca alternifolia

Medicinal plants are increasingly recognized as important sources of antibacterial agents because they contain bioactive compounds capable of inhibiting pathogenic microorganisms. Among these plants, *Melaleuca alternifolia* (tea tree) has received significant scientific attention due to its strong antimicrobial properties. Tea tree oil extracted from *M. alternifolia* leaves contains active compounds such as terpinen-4-ol, α-terpineol, and cineole, which have demonstrated effectiveness against bacteria including *Staphylococcus aureus* and *Streptococcus pyogenes*. Studies have shown that tea tree oil disrupt bacterial cell membrane, resulting in leakage of cellular contents and cell death. Furthermore, the plant has been widely investigated as a natural alternative to conventional antibiotics because of the growing challenge of antimicrobial resistance. Recent evidence also highlights its therapeutic potential in wound care and skin infections due to its antibacterial and anti-inflammatory effects (Kairey *et al.*, 2023).

2.5.1 Botanical Description

Botanical description is an important aspect in the study of medicinal plants because it provides the scientific identification and classification necessary for accurate use, conservation, and pharmacological research. It includes the study of plant morphology such as roots, stems, leaves, flowers, fruits, and seeds, which helps distinguish medicinal species from harmful or less effective plants. Proper botanical characterization also supports the standardization and quality control of herbal medicines used in antibacterial and therapeutic applications. Recent studies emphasize that accurate botanical identification is essential for ensuring the safety, efficacy, and authenticity of medicinal plant products used in traditional and modern medicine (Kumar *et al.*, 2020).

2.5.2 Geographical Distribution

Melaleuca alternifolia is an important medicinal plant known for its antibacterial and therapeutic properties. *Melaleuca alternifolia* (tea tree) is not indigenous to Zimbabwe and is mainly found

in botanical collections, gardens, and limited experimental cultivation in urban areas such as Harare where climatic conditions are favorable. The plant originates from Australia but is increasingly recognized in Zimbabwe for its essential oil, which possesses strong antibacterial activity against pathogenic microorganisms. Geographical and environmental conditions significantly influence the growth and phytochemical composition of the plant, thereby affecting their medicinal value (Gadzirayi *et al.,* 2013).

2.5.3 Phytochemical Composition

The phytochemical composition of *Melaleuca alternifolia* (tea tree) is dominated by essential oils rich in bioactive terpenes that contribute to its strong antibacterial and medicinal properties. The major phytochemical constituents include terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, α-terpineol, and p-cymene (Duarte et al.,2022).From these compounds, terpinen-4-ol is considered the principal active component responsible for most of the antimicrobial activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli*. In addition to volatile oils, the plant also contains flavonoids, tannins, phenolic compounds, and triterpenoids that enhance its antioxidant and therapeutic effects. Recent studies indicate that variations in environmental conditions and geographical location may influence the concentration and effectiveness of these phytochemicals in tea tree oil (Vázquez *et al.,* 2023).

2.5.4 Medicinal Uses

Melaleuca alternifolia (tea tree) is increasingly used in veterinary medicine due to its antibacterial, antifungal, antiseptic, and anti-inflammatory properties, particularly in the treatment of skin infections in dogs. Tea tree oil has shown effectiveness against canine pathogens such as *Staphylococcus pseudintermedius*, a common cause of pyoderma and dermatitis in dogs. Studies have demonstrated that the essential oil can inhibit multidrug-resistant bacterial strains and may enhance the effectiveness of conventional antibiotics when used together. In veterinary dermatology, diluted tea tree oil preparations are commonly investigated for wound care, fungal infections, hot spots, and skin inflammation. However, researchers emphasize that improper concentrations may cause toxicity or skin irritation in dogs, highlighting the importance of controlled veterinary use(Pareek *et al*., 2023).

2.5.5 Antibacterial Properties of *Melaleuca alternifolia.*

Antibacterial properties of *Melaleuca alternifolia* (tea tree) in dogs have been widely studied due to its effectiveness against common canine skin pathogens such as *Staphylococcus pseudintermedius*, including methicillin-resistant strains (MRSP). The essential oil contains active compounds like terpinen-4-ol, which disrupt bacterial cell membranes, leading to leakage of cellular components and bacterial cell death. In vitro studies have demonstrated significant inhibition zones against isolates from canine pyoderma, showing that tea tree oil can reduce bacterial growth and biofilm formation. However, while it is effective, its clinical use in dogs must be carefully controlled due to potential toxicity at high concentrations, even though diluted formulations show promising antibacterial activity for skin and wound infections (Szewczuk *et al.,* 2023).

2.6 *Moringa oleifera*

Moringa oleifera is a fast-growing, drought-resistant medicinal tree widely used in traditional and modern medicine due to its rich nutritional and pharmacological value. It is characterized botanically as a small to medium-sized tree with a soft woody stem, bipinnate leaves, white to cream flowers, and long slender pods commonly referred to as "drumsticks." Geographically, *M. oleifera* is native to South Asia but is now widely distributed across tropical and subtropical regions, including many parts of Africa such as Zimbabwe, where it is cultivated in both rural and urban settings for food security and medicinal use. The plant is highly adaptable to warm?

2.6.1 Botanical Description

Botanically, *Moringa oleifera* belongs to the family Moringaceae and is characterized by feathery tripinnate leaves, small fragrant white flowers, and elongated pods containing round seeds. The plant can reach up to 10–12 meters in height and has a deep taproot system that enhances drought tolerance. Its morphological features make it easily identifiable and widely cultivated for medicinal and nutritional purposes (Pareek *et al.,* 2023).

2.6.2 Geographical Distribution

The geographical distribution of *Moringa oleifera* in Zimbabwe shows that the plant is widely cultivated across both urban and rural regions due to its adaptability to diverse climatic conditions. It is commonly grown in major cities such as Harare, Bulawayo, Mutare, Gweru, and Masvingo, as well as in smaller towns including Chitungwiza, Marondera, Kwekwe, and Bindura. In rural districts, it is also prevalent in areas such as Binga, Mutoko, Chiredzi, and Gokwe, where it is integrated into home gardens and smallholder farming systems. Its widespread distribution is linked to its tolerance of semi-arid conditions and its value as a nutritional and medicinal plant, which has encouraged its adoption in both subsistence and commercial cultivation systems across Zimbabwe (Mudyiwa *et al.,* 2013).

2.6.3 Phytochemical Composition

Moringa oleifera contains a wide range of bioactive compounds including flavonoids, alkaloids, tannins, phenolics, saponins, vitamins, and glucosinolates. These phytochemicals contribute to its antioxidant, anti-inflammatory, and antibacterial properties. More than 100 bioactive compounds have been identified in different plant parts, with leaves being the richest source of medicinal constituents such as quercetin, kaempferol, and chlorogenic acid (Pareek *et al.*, 2023).

2.6.4 Medicinal Uses

Traditionally, *Moringa oleifera* is used to treat a wide range of diseases including infections, inflammation, diabetes, hypertension, and gastrointestinal disorders. It is also used as a nutritional supplement to combat malnutrition because of its elevated vitamin concentration, proteins, and minerals. In modern medicine, it is studied for its potential anti-cancer, hepatoprotective, and wound-healing properties, making it a valuable medicinal plant in both human and veterinary healthcare systems (Liu *et al.,* 2022).

2.6.5 Antibacterial Properties of Moringa oleifera

Moringa oleifera exhibits significant antibacterial activity against both Gram-positive and Gram-negative bacteria affecting dogs, including *Staphylococcus aureus* and *Escherichia coli*, which are common causes of skin and wound infections. The plant’s bioactive compounds such as

flavonoids and phenolics disrupt bacterial cell walls and inhibit microbial growth, making it a potential alternative or complementary therapy in veterinary dermatology. Studies indicate that *Moringa oleifera* extracts may also help reduce antibiotic resistance when used alongside conventional drugs, although further in vivo studies in dogs are still required to confirm safety and optimal dosing (Saha *et al.,* 2025).

2.7 Phytochemicals Responsible for Antibacterial Activity

Moringa oleifera contains several bioactive phytochemicals responsible for its antibacterial effects, including flavonoids (quercetin, kaempferol), tannins, saponins, phenolic acids, alkaloids, and isothiocyanates**.** These compounds act by disrupting bacterial cell membranes, inhibiting enzyme systems, and interfering with microbial biofilm formation. In particular, isothiocyanates derived from glucosinolates have shown strong antimicrobial effects against both Gram-positive and Gram-negative bacteria commonly associated with wound infections in animals, including dogs (Su *et al.,* 2023; Saha *et al.,* 2025). These phytochemicals contribute synergistically, enhancing the overall antibacterial potency of *Moringa oleifera* extracts.

2.8 Methods Used to Evaluate Antibacterial Activity

The antibacterial activity of *Moringa oleifera* is commonly assessed using in vitro microbiological techniques such as agar well diffusion, disc diffusion (Kirby-Bauer method), and broth microdilution assays. These methods measure zones of inhibition and determine the minimum inhibitory concentration (MIC) against clinical isolates. Recent studies involving wound-associated pathogens, including *Staphylococcus aureus* and *Escherichia coli*, frequently utilize these methods to evaluate efficacy. Standardized incubation conditions and optical density measurements are also used to quantify bacterial growth suppression, providing reproducible evidence of antibacterial potential (Ganjo *et al.*, 2022)

2.9 Previous Studies on Plant Extracts Against Wound Pathogens

Recent research has demonstrated that plant extracts, including *Moringa oleifera*, exhibit strong antibacterial activity against common wound pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli*. These pathogens are frequently isolated from infected wounds in both humans and animals, including dogs. Studies show that *Moringa* leaf and seed extracts significantly reduce bacterial growth and biofilm formation, supporting their potential application in veterinary wound management. Similar findings in other medicinal plants reinforce the growing interest in plant-based antimicrobial therapies as alternatives to conventional antibiotics, particularly in the context of antimicrobial resistance (Su *et al.*, 2023; Moringa pharmacological review, 2024).

2.10 Knowledge Gap

Despite extensive research on the antimicrobial properties of *Melaleuca alternifolia* and *Moringa oleifera*, there is limited comparative data evaluating their effectiveness against specific skin pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes* under standardized experimental conditions. Additionally, variations in extraction methods and solvent use make it difficult to compare results across studies.

This research aims to bridge this gap by systematically comparing the antibacterial activity of these two plants using consistent methodologies, thereby contributing to the development of alternative treatments for skin infections.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the Bacteriology Laboratory at the Central Veterinary Laboratory located in Harare, which has the necessary equipment and facilities for microbial culturing, extraction of plant materials, and antimicrobial susceptibility testing. Samples of plant materials were collected from local farms and botanical gardens where *Melaleuca alternifolia* and *Moringa oleifera* are cultivated .l identified the tree through indigenous knowledge system(IKS).

3.2 Research Design

The study utilized a quantitative experimental setup using an *in vitro* comparative approach. The antibacterial properties of the plant extracts were evaluated against selected bacterial pathogens isolated from wounds in dogs. The efficacy of the extracts was assessed by measuring the zones of inhibition produced against the test microorganisms. The findings were then compared with those of a standard antibiotic used as a positive control and a solvent used as a negative control.

Fresh leaves of *Moringa oleifera* and *Melaleuca alternifolia* were collected and thoroughly rinsed with clean water to eliminate dirt and other impurities. The leaves were subsequently shade-dried at room temperature until fully dehydrated and later ground into a fine powder using a mortar and pestle.

Ten grams of each powdered plant material were independently immersed in 100 mL of ethanol, methanol, and distilled water following the maceration extraction technique. The mixtures were allowed to stand for 72 hours with intermittent shaking to facilitate the extraction of bioactive constituents. After the extraction period, the mixtures were filtered using filter paper to obtain the crude extracts.

The resulting filtrates were concentrated using a rotary evaporator under reduced pressure to evaporate the solvents. The concentrated extracts were then transferred into sterile containers and preserved at 4°C until further use.

3.3. Preparation of Plant Extracts:

- *Moringa oleifera* leaves were washed, shade-dried, and ground into a fine powder. The powder was soaked in ethanol for 72 hours with periodic shaking. The extract was filtered and evaporated using a rotary evaporator.
- *Melaleuca alternifolia* leaves were washed, shade-dried, and ground into a fine powder. The powder was soaked in ethanol for 72 hours with periodic shaking. The extract was filtered and evaporated using a rotary evaporator.

3.4 Preparation of Bacterial Cultures

Serotyped strains of *Staphylococcus aureus* (CP5) and *Streptococcus pyogenes* (emm12) were utilized in this study. The bacterial isolates were first revived and then subcultured on nutrient agar plates prior to conducting the antimicrobial assays.

The bacterial isolates were inoculated into Mueller–Hinton broth and incubated at temperatures ranging from 35–37°C for 24 hours. The turbidity of each bacterial suspension was subsequently standardized to the 0.5 McFarland standard to maintain a consistent bacterial concentration throughout the experiment.

3.6.1 Preparation of Agar Plates

Mueller-Hinton agar was prepared according to the manufacturer's instructions and poured into sterile Petri dishes. The agar plates were allowed to solidify before use.

3.6.2 Inoculation of Bacterial Cultures

Sterile cotton swabs were utilized to uniformly distribute the standardized bacterial suspension over the surface of Mueller–Hinton agar plates, forming an even bacterial lawn.

3.6.3 Application of Plant Extracts

Sterile wells were made in the agar using sterile tips. Specific volumes of the plant extracts at varying concentrations were then dispensed into the wells with the aid of a micropipette. Ampicillin antibiotic discs were included as positive controls, whereas treatments containing only the solvent were used as negative controls.

3.6.4 Incubation

The inoculated plates were incubated at 35–37°C for 18–24 hours.

3.6.5 Measurement of Zones of Inhibition

Following incubation, the antibacterial effectiveness was evaluated by measuring the diameters of the clear zones formed around the wells. These zones represented areas where bacterial growth had been inhibited by the plant extracts. The measurements were taken in millimetres using either a digital caliper or a transparent ruler.

3.7 Data Collection

The main data collected consisted of the diameters of the zones of inhibition generated by each treatment. To enhance the reliability and accuracy of the findings, all experiments were performed in triplicate. Other observations, including bacterial growth characteristics, pigmentation, and turbidity, were also determined.

3.8 Data Analysis

The data obtained were analyzed using descriptive statistical methods, including mean and standard deviation. Inferential statistical tests, particularly one-way Analysis of Variance (ANOVA), were applied to determine whether there were significant differences among the treatment groups. When noticeable changes were detected, post-hoc analyses such as Tukey's HSD test were conducted to compare the group means.

A significance threshold of $p < 0.05$ was maintained throughout the study. Statistical analyses were carried out using software packages such as IBM SPSS Statistics or Microsoft Excel.

Chapter 4

Results

4.1.1 Extraction Yield of *Moringa oleifera*

The results of the yield of Moringa oleifera extracts from each of the solvents are presented in Table 4.1. The ethanol extractions produced the highest average yields of *Moringa oleifera* extracts (2.10 ± 0.10 g), followed by methanol (1.93 ± 0.12 g). Distilled water yielded the lowest amount of *Moringa oleifera* extract (1.20 ± 0.10 g). Overall, however, the yields from each of the three replicates are similar for each of the solvents tested. Ethanol extracts contain the highest yields of *Moringa oleifera* extracts in each of the replicate experiments, while distilled water extracts contain the lowest yields. Thus, the differences in the yields of *Moringa oleifera* extracts according to each of the solvents is presented in Table 4.1. Table 4.1 reveals the yields of *Moringa oleifera* plants that were extracted with methanol, ethanol, and distilled water solvents.

Extract	**Original mass (g)**	**Final mass (g)**	**%yield**
Moringa oleifera	10	2.1	21
Melaleuca alternifolia	10	1.13	11.3

Table 4: Percentage yield ethanolic extracts

Table 4.1: Extraction Yield of *Moringa oleifera* (g)?

Replicate	**Methanol**	**Ethanol**	**Distilled Water**
Mean ± SD	1.93 ± 0.12	2.10 ± 0.10	1.20 ± 0.10

4.2.2 Extraction Yield of *Melaleuca alternifolia*

Table 4.2 presents the extraction yields of the essential oil from *Melaleuca alternifolia using* three different solvents. The extraction yields that are obtained from *Melaleuca alternifolia* are presented in Table 4.2. The results of the ethanol extraction were higher than those obtained from distilled water extractions. On average, the ethanol extraction yielded 1.13 ± 0.15 g of essential oil, whereas distilled water extraction yielded 0.67 ± 0.21 g of essential oil. The yields ranged from 0.50 g to 1.30 g. The ethanol extracts contained the highest yields of essential oil in the first and

second replicates whereas distilled water yielded less essential oil. The variation in the yields from the replicates was lower for the ethanol extractions compared to distilled water extractions. Overall, however, the yield from Melaleuca alternifolia was lower than from *Moringa oleifera*.

Table 4.2: Extraction Yield of *Melaleuca alternifolia (*g)

Replicate	**Ethanol**	**Distilled Water**
Mean ± SD	1.13 ± 0.15	0.67 ± 0.21

4.2 Antibacterial Activity of Plant Extracts

The antibacterial properties were determined using the agar well diffusion method. Antibacterial activity is determined by measuring the diameter of the zones of inhibition in the agar plates. The larger the zones of inhibition, the higher the antibacterial activity of the tested phytochemicals. The antibacterial activity of differed depending on the species of the plant, the solvent used to extract the phytochemicals, and the species of the bacteria that were tested for antibacterial properties. Generally, ethanol extracts exhibited higher antibacterial activity than methanol and distilled water extracts.

4.2.1 Antibacterial Activity of *Moringa oleifera*

Table 4.3 displays the zones of inhibition of the phytochemicals extracted from *Moringa oleifera* against the bacteria species that were tested. The results of the tests on *Moringa oleifera* extracts for their ability to inhibit the growth of *S. aureus* and *S. pyogenes* are presented in Table 4.3. The ethanol extracts of *Moringa oleifera* produced the largest zones of inhibition against both bacteria (15 mm for *S. aureus*, 12 mm for *S. pyogenes*). Methanol extracts produced zones of inhibition against the bacteria, as well, but the distilled water extracts produced the smallest zones of inhibition. In each case, however, the zones of inhibition against *S. aureus* was larger than the size of the zones of inhibition against *S. pyogenes.*

Table 4.3: Zones of Inhibition Produced by *Moringa oleifera* Extracts (mm)

Extract	***Staphylococcus aureus***	***Streptococcus pyogenes***
Methanol	12 ± 1.10	10 ± 0.82
Ethanol	15 ± 0.00	12 ± 0.00
Distilled Water	8 ± 0.00	7 ± 0.00

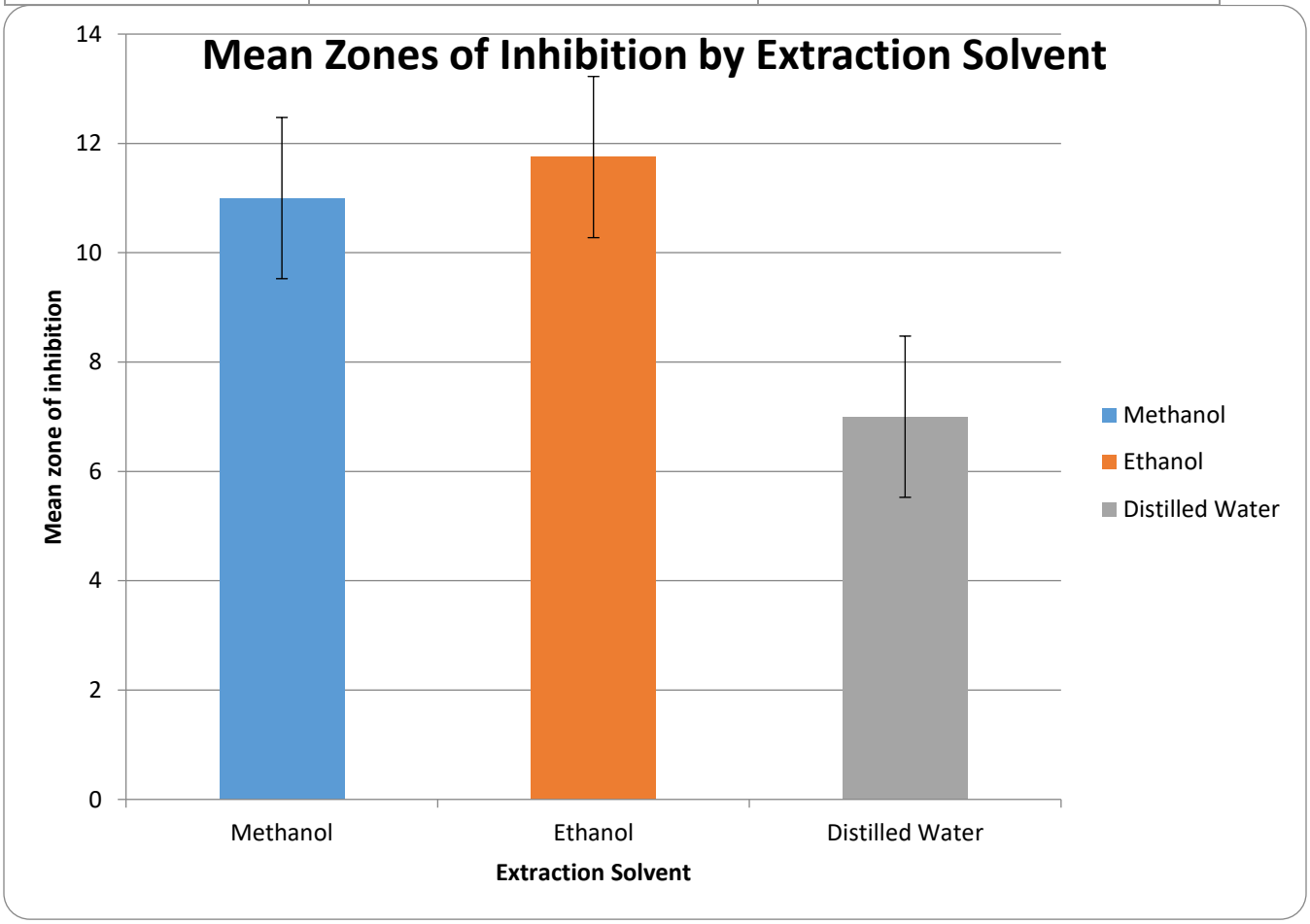


Figure 4.1: Mean Zones of Inhibition Produced by *Moringa oleifera*

4.3.2 Antibacterial Activity of *Melaleuca alternifolia*

Table 4.4 presents the zones of inhibition produced by *Melaleuca alternifolia extracts*.

Table 4.4: Zones of Inhibition Produced by *Melaleuca alternifolia Extracts* (mm)

Extract	***Staphylococcus aureus***	***Streptococcus pyogenes***
Methanol	8 ± 0.00	8 ± 0.00
Ethanol	10 ± 0.00	10 ± 0.00
Distilled Water	6 ± 0.00	7 ± 0.00

The results of the antibacterial assays shown in Table 4.4 demonstrates that *M. alternifolia* extracts also contain antibacterial activity against the bacteria isolates. The ethanol extracts produced the highest levels of inhibition zones (10 mm against each species of bacteria), while the distilled water extracts exhibited the lowest levels of antibacterial activity. Overall, though, the inhibition zones produced by *M. alternifolia* were smaller than those produced by *Moringa oleifera*. However, the use of ethanol to extract the active compounds from the plant material resulted in higher levels of antibacterial activity than distilled water extracts, although still less than *Moringa oleifera*. Thus, though less active than *Moringa oleifera, M. alternifolia* does contain some antibacterial properties against those common wound pathogens.

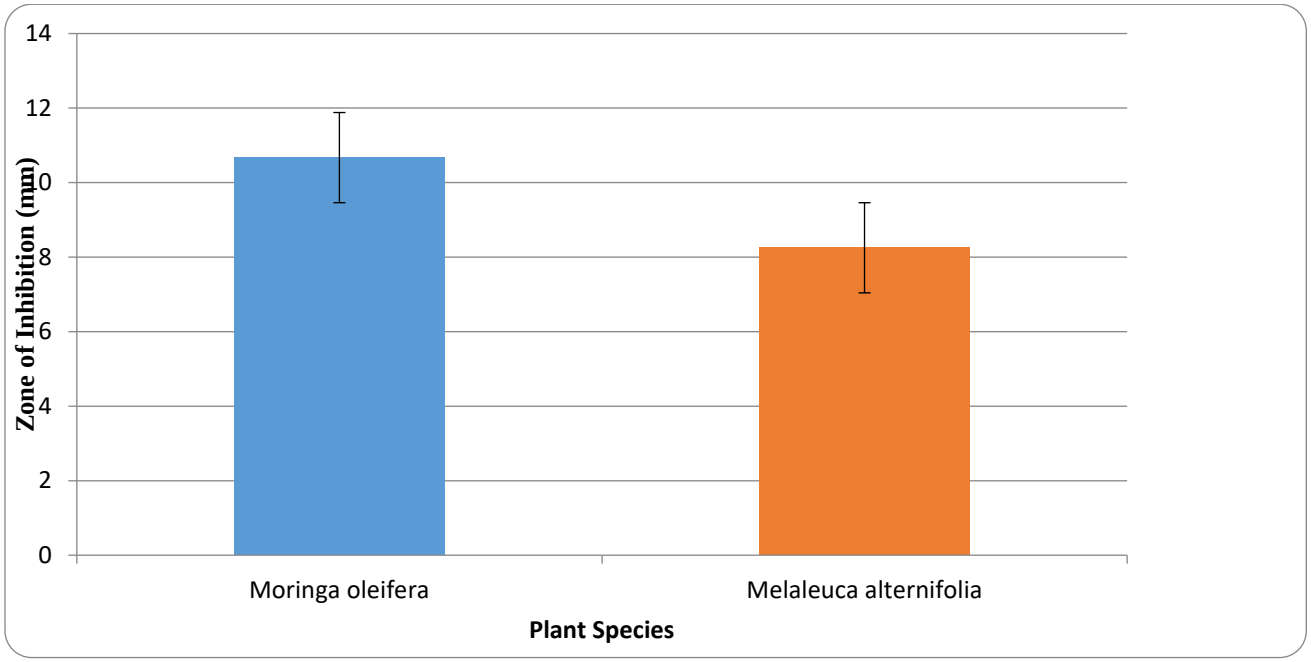


Figure 4.2: Mean Zones of Inhibition Produced by *Melaleuca alternifolia Extracts*

4.3.3 Comparison of Antibacterial Activity

The extracts of *Moringa oleifera* exhibited larger zones of inhibition than *Melaleuca alternifolia.* The ethanol extracts of both plants exhibited the highest level of antibacterial activity, while the distilled water extracts exhibited the least level of antibacterial activity. The results show that ethanol was the most effective solvent for extracting antibacterial compounds from these two plants. The antibacterial activity of the plant extracts was found to be greater against *Streptococcus aureus* than *Streptococcus pyogenes*. The greater activity against *Streptococcus aureus* of *Moringa oleifera* may be due to the presence of flavonoids, phenolic compounds, tannins, and isothiocyanates, all of which have known antibacterial properties. These results suggest that these natural plant extracts may be helpful as alternative antibacterial agents in wound management.

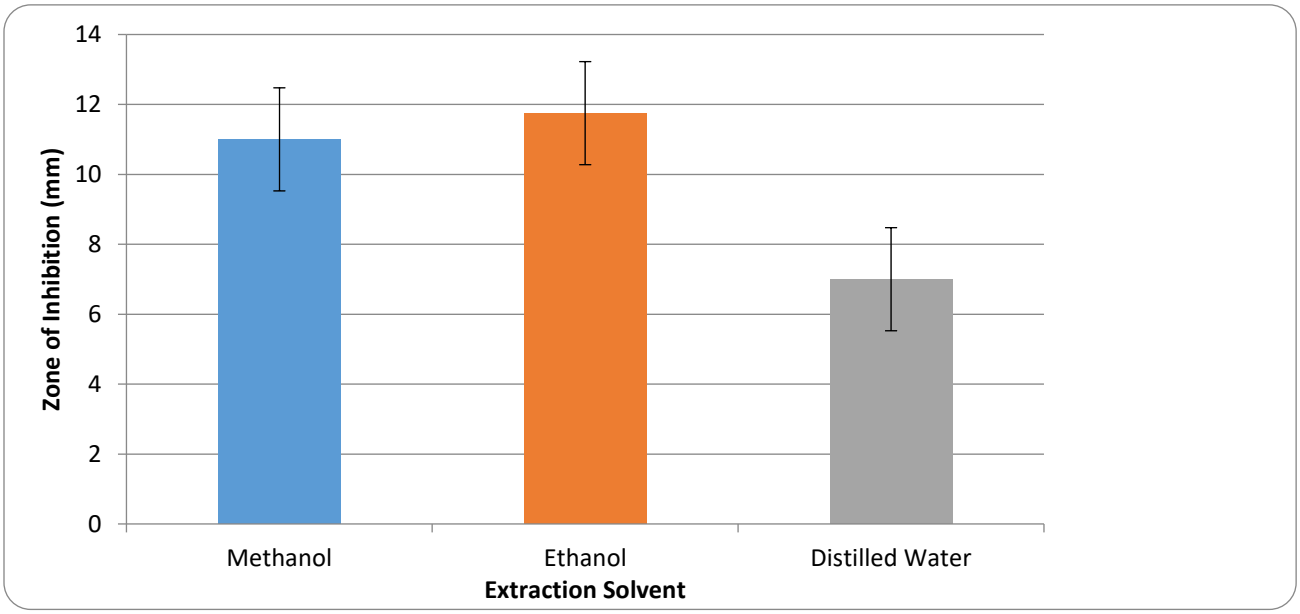


Figure 4.3: Comparison of Mean Zones of Inhibition between Plant Species

4.3.4 Statistical Analysis of Zones of Inhibition

A one-way Analysis of Variance (ANOVA) was used to determine whether there were any significant differences in the antibacterial activities of the different extraction solvents, plant species, and bacterial isolates. The results of the ANOVA indicated that each of these factors had a significant impact upon the antibacterial activity of the preparations tested (all $P < 0.05$). More specifically, ethanol extracts had the highest mean zone of inhibition (11.75 ± 2.14 mm), followed

by methanol extracts (11.00 ± 1.10 mm), while distilled water extracts had the lowest mean zone of inhibition (7.00 ± 0.74 mm). Additionally, *Moringa oleifera* extracts exhibited larger mean zones of inhibition than *Melaleuca alternifolia extracts*.

Table 4.5: ANOVA Results for the Effect of Extraction Solvents on Zones of Inhibition

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	148.050	2	74.025	32.107	0.000
Within Groups	62.250	27	2.306		
Total	210.300	29			

Source: SPSS

The results of one-way ANOVA analysis of Table 4.5 indicate a statistically significant difference in the antibacterial activity of the extracts prepared using different solvents ($F = 32.107$, $p < 0.001$). The ethanol extracts exhibited the highest mean zone of inhibition compared to methanol (11.00 ± 1.10 mm) and distilled water extracts (7.00 ± 0.74 mm). The methanol extracts showed a mean zone of inhibition that was similar to distilled water extracts (11.00 ± 1.10 mm) but lower than ethanol extracts (11.75 ± 2.14 mm). The distilled water extracts exhibited the lowest mean zone of inhibition (7.00 ± 0.74 mm) compared with methanol (11.00 ± 1.10 mm) and ethanol extracts (11.75 ± 2.14 mm). The Tukey post hoc test results indicate statistically significant differences in the mean zone of inhibition among distilled water and both methanol and ethanol extracts ($p < 0.05$). However, there was no statistically significant difference in the mean zone of inhibition among methanol and ethanol extracts ($p = 0.591$).

Table 4.6: ANOVA Results for the Effect of Plant Species on Zones of Inhibition

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	42.050	1	42.050	6.998	0.013
Within Groups	168.250	28	6.009		
Total	210.300	29			

Source: SPSS

The results of the ANOVA analysis of Table 4.6 show that there is a statistically significant difference in the antibacterial activity of both Moringa oleifera and Melaleuca alternifolia extracts ($F = 6.998$, $p = 0.013$). Moringa oleifera produced a higher mean zone of inhibition than Melaleuca alternifolia; Moringa oleifera produced an average inhibition zone of 10.67 ± 2.77 mm compared to Melaleuca alternifolia's 8.25 ± 1.87 mm. Thus, *Moringa oleifera* produced, on average, 2.42 mm more inhibition than *Melaleuca alternifolia*. Additionally, the inhibition zones for Moringa oleifera grew between 7 mm and 15 mm in diameter, whereas those of *Melaleuca alternifolia* grew between 6 mm and 10 mm in diameter.

Table 4.7: ANOVA Results for the Effect of Bacterial Species on Zones of Inhibition

Source of Variation	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	7.500	1	7.500	1.036	0.318
Within Groups	202.800	28	7.243		
Total	210.300	29			

Source: SPSS

The results of the ANOVA test presented in Table 4.7 show that there is no significant difference in the susceptibility of *S. aureus* and *S. pyogenes* to the tested plant extracts ($F = 1.036$, $p = 0.318$).

S. aureus produced inhibition zones with a mean diameter of 10.20 ± 3.23 mm, while *S. pyogenes* produced inhibition zones with a mean diameter of 9.20 ± 2.01 mm. The range of inhibition zones against S. aureus was between 6 and 15 mm in diameter, while those against *S. pyogenes* were between 7 and 12 mm in diameter. The inhibition zones against *S. aureus* were slightly larger than those against *S. pyogenes*; however, the difference was not statistically significant.

4.3.5 Comparison with Standard Antibiotic

Ampicillin was used as the positive control to evaluate the antibacterial activity of the plant extracts. The results of this test indicated that ampicillin created larger zones of inhibition than any of the tested plant extracts. Ampicillin produced inhibition zones of 20 mm against S. aureus and 18 mm against S. pyogenes. The ethanol extract of *Moringa oleifera* created inhibition zones of 15 mm against S. aureus and 12 mm against S. pyogenes. Thus, ampicillin created approximately 33.3% more and 50.0% more inhibition zones than the ethanol extract of *Moringa oleifera,* respectively.

Table 4.8: Comparison between Plant Extracts and Standard Antibiotic zones of inhibition

Treatment	***Staphylococcus aureus* (mm)**	***Streptococcus pyogenes* (mm)**
Ampicillin	20	18
Moringa oleifera Ethanol Extract	15	12

The results presented in Table 4.8 indicated that the standard antibiotic produced larger zones of inhibition than the plant extracts. However, the ethanol extract of Moringa oleifera produced considerable zones of inhibition against the two bacterial species. The ethanol extract of Moringa oleifera produced zones of inhibition that ranged from 12 mm to 15 mm, while ampicillin produced zones of inhibition that ranged from 18 mm to 20 mm. Although the ethanol extract of Moringa oleifera displayed lower antibacterial activity than the standard antibiotic, the activity levels were relatively high in comparison to other plant extracts tested.

4.4 Minimum Inhibitory Concentration (MIC)

MIC values of the plant extracts were evaluated using the broth microdilution method. MIC values represent the lowest concentration of extract capable of inhibiting visible bacterial growth. Lower MIC values indicate higher antibacterial activity, while higher MIC values indicate lower

antibacterial activity. The MIC values obtained for the plant extracts against *Staphylococcus aureus* and *Streptococcus pyogenes* are shown in Table 4.9.

Table 4.9: Minimum Inhibitory Concentration (MIC) Values of Plant Extracts

Plant Extract	***Staphylococcus aureus* (mg/mL)**	***Streptococcus pyogenes* (mg/mL)**
Melaleuca alternifolia	0.50	0.26
Moringa oleifera	0.25	0.063

The MIC results presented in Table 4.9 showed that *Moringa oleifera* recorded lower MIC values than *Melaleuca alternifolia* against both bacterial species. The lowest MIC value obtained in the study was 0.063 mg/mL against *Streptococcus pyogenes* treated with *Moringa oleifera*. *Melaleuca alternifolia* recorded MIC values of 0.50 mg/mL against *Staphylococcus aureus* and 0.26 mg/mL against *Streptococcus pyogenes*. The MIC values obtained in the study varied between 0.063 mg/mL to 0.50 mg/mL. The overall mean MIC value recorded in the study was 0.2459 ± 0.1542 mg/mL.

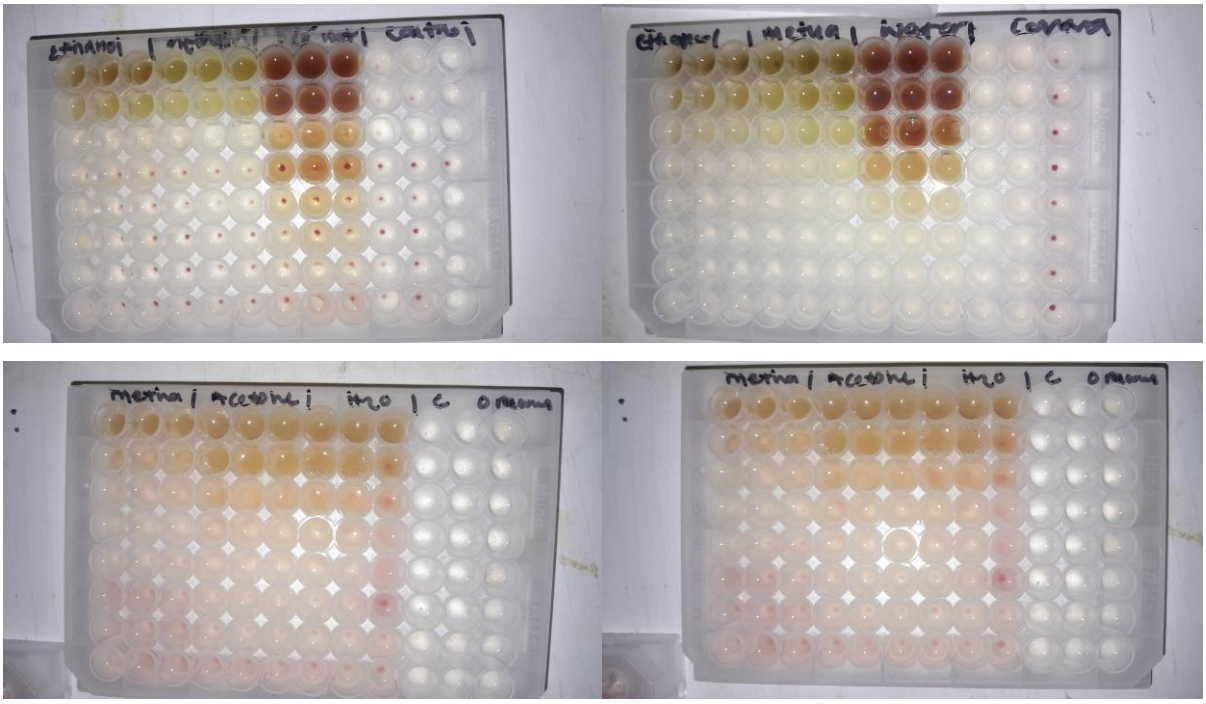


Figure 4.4: Broth Microdilution Plates Used for MIC Determination

The broth microdilution plates exhibited differences in bacterial growth within the plant extract solutions of varying concentrations. The wells with lower concentrations of the plant extracts demonstrated bacterial growth, whereas the wells with higher concentrations of the extracts remained free of bacteria. The MIC values of the plant extracts ranged from 0.063 mg/mL to 0.50 mg/mL. *Moringa oleifera* had lower MIC values than *Melaleuca alternifolia* against the bacterial species tested. The lowest MIC value of 0.063 mg/mL was recorded for *S. pyogenes* treated with *Moringa oleifera.* These observations of bacterial growth on the broth microdilution plates correspond to the MIC values shown in table 4.9.

Chapter 5

5.1 Discussion

The purpose of this study was to determine and compare the antibacterial properties of *Moringa oleifera* and *Melaleuca alternifolia* against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds. The findings demonstrated that both plant extracts possessed antibacterial activity, although their effectiveness varied depending on the plant species and extraction solvent used. The results further revealed that *Moringa oleifera* exhibited significantly greater antibacterial activity than Melaleuca alternifolia, while ethanol proved to be the most effective extraction solvent. These findings support previous studies that identified medicinal plants as potential alternative sources of antibacterial agents for managing bacterial infections and combating antimicrobial resistance (Abdallah et al., 2023; Duarte et al., 2022).

5.1.1 Antibacterial Activity

The agar well diffusion assay demonstrated that both plant extracts inhibited the growth of *S. aureus* and *S. pyogenes*. However, *Moringa oleifera* produced larger zones of inhibition than *Melaleuca alternifolia*. The ethanol extract of *Moringa oleifera* produced the highest antibacterial activity, with inhibition zones of 15 mm against *S. aureus* and 12 mm against *S. pyogenes*.

These findings agree with earlier findings that demonstrated strong antibacterial activity of *Moringa oleifera* against Gram-positive bacteria. According to Saha et al. (2025), flavonoids, phenolic compounds, tannins, and isothiocyanates present in *Moringa oleifera* interfere with bacterial cell wall synthesis and disrupt microbial metabolic processes. Similarly, Su et al. (2023) reported that phytochemicals from *Moringa oleifera* inhibit bacterial growth by damaging cell membranes and preventing biofilm formation.

Although *Melaleuca alternifolia* demonstrated antibacterial activity, its inhibition zones were smaller. This may be due to lower concentrations of active compounds extracted during the

maceration process. Nevertheless, the findings support previous reports that tea tree oil possesses antibacterial properties resulting from terpinen-4-ol and α-terpineol, which disrupt bacterial membrane integrity (Carson et al., 2020; Hammer et al., 2021).

5.1.2 Minimum Inhibitory Concentration (MIC)

The MIC results further verified the superior antibacterial activity of *Moringa oleifera*. The lowest MIC value recorded in the study was 0.063 mg/mL against *S. pyogenes*, indicating strong antibacterial potency. In contrast, *Melaleuca alternifolia* exhibited higher MIC values ranging from 0.26 mg/mL to 0.50 mg/mL.

Lower MIC values indicate that smaller concentrations of the extract are required to inhibit bacterial growth. Therefore, the lower MIC values observed for *Moringa oleifera* suggest that it possesses more potent antibacterial compounds. These findings agree with Ganjo et al. (2022), who reported low MIC values for *Moringa oleifera* extracts against wound-associated bacterial pathogens.

5.1.3 Comparative Antibacterial Efficacy

The comparative analysis revealed that *Moringa oleifera* was significantly more effective than *Melaleuca alternifolia* ($p = 0.013$). The mean inhibition zone for *Moringa oleifera* was 10.67 ± 2.77 mm compared to 8.25 ± 1.87 mm for *Melaleuca alternifolia*.

This superior antibacterial activity may be attributed to the greater diversity and concentration of phytochemicals found in *Moringa oleifera*. The plant contains multiple antibacterial compounds that may act synergistically to inhibit bacterial growth. Previous studies have similarly reported that *Moringa oleifera* exhibits broader-spectrum antibacterial activity than many medicinal plants due to its high phenolic and flavonoid content (Batiha et al., 2020; Gupta et al., 2022).

5.1.4 Implications for Wound Management

The results of this study have major implications for veterinary medicine. The demonstrated antibacterial activity of *Moringa oleifera* and *Melaleuca alternifolia* suggests that these plants could be incorporated into herbal formulations for wound treatment in dogs. The use of plant-derived antibacterial agents may reduce reliance on conventional antibiotics and contribute to controlling antimicrobial resistance.

Furthermore, herbal products derived from these plants may provide cost-effective and environmentally sustainable alternatives for managing canine wound infections, particularly in resource-limited settings (Guardabassi et al., 2021).

5.2 Conclusions

The study successfully demonstrated that *Moringa oleifera* and *Melaleuca alternifolia* possess antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from dog wounds. Ethanol extracts produced the highest extraction yields and strongest antibacterial activity, while distilled water extracts showed the least effectiveness. Among the two medicinal plants tested, *Moringa oleifera* exhibited significantly greater antibacterial activity, larger zones of inhibition, and lower MIC values than *Melaleuca alternifolia*.

The findings indicate that medicinal plants contain bioactive compounds capable of inhibiting bacterial growth and may serve as potential alternatives or complementary therapies to conventional antibiotics in veterinary medicine. Although the plant extracts showed lower antibacterial activity than ampicillin, the results remain promising for the development of natural antibacterial agents for wound management in dogs.

Overall, the study contributes valuable scientific knowledge regarding the antibacterial potential of medicinal plants and supports further research into plant-based therapies aimed at improving canine wound treatment and reducing antimicrobial resistance.

5.3 Recommendations

1. Researchers should isolate and characterize the specific phytochemical compounds responsible for antibacterial activity in order to support pharmaceutical development.
2. Veterinary pharmaceutical industries should explore the development of herbal wound-care formulations containing *Moringa oleifera* extracts.
3. Veterinary practitioners should promote responsible antibiotic use alongside research into alternative plant-based therapies to reduce antimicrobial resistance.
4. Ethanol should be considered the preferred extraction solvent for future studies because it demonstrated the highest extraction efficiency and antibacterial activity.
5. Further investigations should evaluate the synergistic effects of combining medicinal plant extracts with conventional antibiotics for improved antibacterial activity.
6. Further in vivo studies should be conducted to evaluate the safety, effectiveness, and toxicity of *Moringa oleifera* and *Melaleuca alternifolia* extracts in live dogs.
7. Additional studies should investigate the antibacterial activity of these medicinal plants against other bacterial pathogens associated with canine wound infections, including multidrug-resistant bacteria.

References

1. Abd Rani, P. A., Abdullah, N., Ismail, N., & Hamid, M. (2023). Medicinal plants as alternative antimicrobial agents in veterinary medicine. *Veterinary World*, 16(4), 721–733.
2. Abdallah, E. M., Alhatlani, B., Menezes, R. P., & Martins, C. H. G. (2023). *Back to nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era*. Plants, 12(17), 3077.
3. Ahmed, S., Rahman, M., & Karim, R. (2024). Emerging antimicrobial resistance among bacterial pathogens isolated from canine wound infections. *Journal of Veterinary Medical Science*, 86(2), 145–156.
4. Batiha, G. E. S., Beshbishy, A. M., Wasef, L. G., Elewa, Y. H. A., Al-Sagan, A. A., Abd El-Hack, M. E., Taha, A. E., Abd-Elhakim, Y. M., & Prasad Devkota, H. (2020). Chemical constituents and pharmacological activities of *Moringa oleifera*. *Biomolecules*, 10(4), 495–512.
5. Carson, C. F., Hammer, K. A., & Riley, T. V. (2020). *Melaleuca alternifolia* (tea tree) oil: Antimicrobial and medicinal properties revisited. *Clinical Microbiology Reviews*, 33(4), 1–27.
6. Cho, K. H. (2024). *Molecular targets in Streptococcus pyogenes for the development of anti-virulence agents*. Genes, 15(9), 1166.
7. Danieli, D., Amadori, M., Crimi, S., Pregnolato, F., Caruso, C., Gambino, G., Re, G., & Vercelli, C. (2026). *Antimicrobial resistant isolates in surgical and bite wounds in dogs and cats: A 12-year retrospective analysis*. Animals, 16(3), 501.
8. Duarte, A., Ferreira, S., Silva, F., & Domingues, F. C. (2022). Plant extracts as alternatives to antibiotics in veterinary medicine. *Antibiotics*, 11(3), 345–360.
9. El-Saber Batiha, G., Alqahtani, A., Ojo, O. A., Shaheen, H. M., Wasef, L., Elzeiny, M., Ismail, M., Shalaby, M., & Murata, T. (2023). Natural products and their role in controlling antimicrobial resistance in veterinary medicine. *Frontiers in Veterinary Science*, 10, 112–128.
10. Gadzirayi, C. T., Mudyiwa, S. M., Mupangwa, J. F., & Gotosa, J. (2013). Cultivation practices and utilisation of *Moringa oleifera* provenances by small holder farmers: Case

of Zimbabwe. *Asian Journal of Agricultural Extension, Economics & Sociology*, *2*(2), 152–162.

11. Ganjo, A. R., Adham, A. N., Al-Bustany, H. A., & Aka, S. T. (2022). *Phytochemical analysis and evaluation of antibacterial activity of Moringa oleifera extracts against gram-negative bacteria: An in vitro and molecular docking studies*. Current Issues in Pharmacy and Medical Sciences.
12. Gherardi, G. (2023). *Staphylococcus aureus infection: Pathogenesis and antimicrobial resistance*. International Journal of Molecular Sciences, 24(9), 8182.
13. Górski, A., Międzybrodzki, R., Weber-Dąbrowska, B., Fortuna, W., Letkiewicz, S., Rogóż, P., & Jończyk-Matysiak, E. (2022). Bacterial skin infections and antimicrobial resistance in companion animals. *Animals*, 12(9), 1182–1198.
14. Gupta, S., Kumar, R., & Sharma, V. (2022). Therapeutic potential of *Moringa oleifera* in veterinary medicine and animal health. *Veterinary Medicine International*, 2022, 1–12.
15. Guardabassi, L., Butaye, P., Dockrell, D. H., Fitzgerald, J. R., & Kuijper, E. J. (2021). One Health approach to antimicrobial resistance in companion animals and humans. *Clinical Microbiology and Infection*, 27(4), 497–503.
16. Hammer, K. A., Carson, C. F., & Riley, T. V. (2021). Antimicrobial activity of essential oils and tea tree oil in veterinary applications. *Microbiology Australia*, 42(1), 17–21.
17. Imanishi, I., Iyori, K., Také, A., Asahina, R., Tsunoi, M., Hirano, R., Uchiyama, J., Toyoda, Y., Sakaguchi, Y., & Hayashi, S. (2022). *Antibiotic-resistant status and pathogenic clonal complex of canine Streptococcus canis-associated deep pyoderma*. BMC Veterinary Research, 18, 395.
18. Karalliu, E., Chung, K. Y., MacKinnon, B., Haile, B., Beczkowski, P. M., Barrs, V. R., Elsohaby, I., & Nekouei, O. (2024). *Risk factors for antimicrobial-resistant Enterobacterales in dogs: A systematic review*. Frontiers in Veterinary Science, 11.
19. Kairey, L., Agnew, T., Bowles, E. J., Barkla, B. J., Wardle, J., & Lauche, R. (2023). *Efficacy and safety of Melaleuca alternifolia (tea tree) oil for human health: A systematic review of randomized controlled trials*. Frontiers in Pharmacology, 14, 1116077.
20. Khameneh, B., Iranshahy, M., Soheili, V., & Fazly Bazzaz, B. S. (2023). Review on plant antimicrobials and their role in combating resistant bacterial pathogens. *Pharmaceutical Biology*, 61(1), 233–247.

21. Kumar, D., Sharma, S., & Kumar, S. (2020). Botanical description, phytochemistry, traditional uses, and pharmacology of *Crataeva nurvala* Buch. Ham.: An updated review. *Future Journal of Pharmaceutical Sciences, 6*(1), 113.
22. Lenart-Boroń, A., Stankiewicz, K., Czernecka, N., Ratajewicz, A., Bulanda, K., Heliasz, M., Sosińska, D., Dworak, K., & Ciesielska, D. (2024). *Wounds of companion animals as a habitat of antibiotic-resistant bacteria that are potentially harmful to humans—Phenotypic, proteomic and molecular detection*. International Journal of Molecular Sciences, 25(6), 3121.
23. Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2021). Cultivation, genetic, ethnopharmacology, phytochemistry, and pharmacology of *Moringa oleifera*. *International Journal of Molecular Sciences*, 22(9), 1–24.
24. Liu, R., Liu, J., Huang, Q., Liu, S., & Jiang, Y. (2022). *Moringa oleifera: a systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity*. Journal of Pharmacy and Pharmacology, 74(3), 296–320.
25. Monk, E. J. M., Jones, T. P. W., *et al.,* (2024). *Antimicrobial resistance in bacterial wound, skin, soft tissue and surgical site infections in Central, Eastern, Southern and Western Africa: A systematic review and meta-analysis*. PLOS Global Public Health, 4(4), e0003077.
26. Mudyiwa, S. M., Gadzirayi, C. T., Mupangwa, J. F., Gotosa, J., & Nyamugure, T. (2013). *Constraints and opportunities for cultivation of Moringa oleifera in the Zimbabwean smallholder growers*. International Journal of Agricultural Research, Innovation and Technology, 3(1), 12–19.
27. Moyaert, H., Morrissey, I., de Jong, A., El Garch, F., Klein, U., Ludwig, C., Thiry, J., Youala, M., & Antimicrobial Susceptibility Monitoring Group. (2020). Antimicrobial susceptibility monitoring of bacterial pathogens isolated from dogs and cats across Europe. *Veterinary Microbiology*, 243, 108–115.
28. Murray, A. K., Zhang, L., Yin, X., Zhang, T., Buckling, A., Snape, J., & Gaze, W. H. (2023). Novel insights into antimicrobial resistance in veterinary medicine. *Nature Reviews Microbiology*, 21(4), 233–248.

29. O'Neill, D. G., Hendricks, A., & Summers, J. F. (2021). Skin disorders and wound infections in companion dogs attending veterinary practices. *Canine Medicine and Genetics*, 8(1), 1–14.
30. Orchard, A., van Vuuren, S., & Viljoen, A. (2022). The antimicrobial properties of tea tree oil and its applications in wound care. *Plants*, 11(15), 1–18.
31. Pareek, A., Pant, M., & Gupta, M. M. (2023). *Moringa oleifera: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects*. International Journal of Molecular Sciences, 24(3), 2098.
32. Pareek, V., Yadav, S., & Sharma, P. (2023). Botanical characteristics, phytochemistry and medicinal significance of *Moringa oleifera*. *Journal of Herbal Medicine, 38*, 100641.
33. Pinto, E., Vale-Silva, L., Cavaleiro, C., & Salgueiro, L. (2024). Essential oils as antimicrobial agents in veterinary medicine: Current applications and future perspectives. *Veterinary Sciences*, 11(2), 88–102.
34. Pomba, C., Rantala, M., Greko, C., Baptiste, K. E., Catry, B., van Duijkeren, E., Mateus, A., Moreno, M. A., Pyörälä, S., Ružauskas, M., Sanders, P., Teale, C., & Threlfall, E. J. (2022). Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy*, 77(3), 567–579.
35. Rahman, M., Islam, M., & Uddin, M. (2024). Herbal antibacterial agents and their future potential in veterinary medicine. *Frontiers in Veterinary Science*, 11, 1–15.
36. Saha, P., Mukherjee, S., & Roy, S. (2025). Antibacterial potential of *Moringa oleifera* against veterinary wound pathogens. *Veterinary Research Communications, 49*(2), 211–223.
37. Saha, A., Ashwin, B. M., & Biswas, P. (2025). *Multi-ligand simultaneous docking analysis of Moringa oleifera phytochemicals reveals enhanced BCL-2 inhibition via synergistic action*. arXiv.
38. Saha, A., Desai, B. M. A., & Biswas, P. (2025). *Multi-ligand simultaneous docking analysis of Moringa oleifera phytochemicals reveals synergistic antibacterial and therapeutic potential*. (Preprint study).
39. Salehi, B., Sharifi-Rad, J., Quispe, C., Llaique, H., Villalobos, M., Smeriglio, A., Trombetta, D., Ezzat, S. M., Salem, M. A., Zayed, A., & Sharopov, F. (2021). Insights

into the use of medicinal plants for antimicrobial and wound healing applications. *Biomolecules*, 11(4), 1–30.

40. Sharun, K., Dhama, K., Tiwari, R., Gugjoo, M. B., Iqbal Yatoo, M., Patel, S. K., Pathak, M., Karthik, K., Khurana, S. K., & Singh, R. (2022). Medicinal plants as alternative therapeutic agents in veterinary medicine. *Frontiers in Veterinary Science*, 9, 1–20.
41. Sebola, D. C., Oguttu, J. W., Kock, M. M., & Qekwana, D. N. (2023). *Hospital-acquired and zoonotic bacteria from a veterinary hospital and their associated antimicrobial-susceptibility profiles: A systematic review*. Frontiers in Veterinary Science, 9, 1087052.
42. Siemens, N., & Lütticken, R. (2021). *Streptococcus pyogenes ("Group A Streptococcus"), a highly adapted human pathogen—Potential implications of its virulence regulation for epidemiology and disease management*. Pathogens, 10(6), 776.
43. Silva, V., Igrejas, G., Poeta, P., & Capelo, J. L. (2023). Antimicrobial resistance patterns among bacterial isolates from canine skin infections. *Antibiotics*, 12(5), 733–748.
44. Su, X., Wang, H., Zhang, Y., & Li, Z. (2023). Antimicrobial phytochemicals and mechanisms of action in *Moringa oleifera*. *Plants, 12*(9), 1745.
45. Su, X., Lu, G., Ye, L., Shi, R., Zhu, M., Yu, X., Li, Z., Jia, X., & Feng, L. (2023). *Moringa oleifera Lam.: A comprehensive review on active components, health benefits and application*. RSC Advances, 13, 24353–24384.
46. Szewczuk, M. A., Zych, S., Oster, N., & Karakulska, J. (2023). Activity of patchouli and tea tree essential oils against staphylococci isolated from pyoderma in dogs and their synergistic potential with gentamicin and enrofloxacin. *Animals, 13*(8), 1279.
47. van den Berg, J., & Kuipers, S. (2022). *The antibacterial action of Moringa oleifera: A systematic review*. Amsterdam University Medical Centers.
48. Vázquez, A., Tabanca, N., & Kendra, P. E. (2023). *HPTLC analysis and chemical composition of selected Melaleuca essential oils*. Molecules, 28(9), 3925.
49. Yamazaki, Y., Ito, T., Tamai, M., Nakagawa, S., & Nakamura, Y. (2024). *The role of Staphylococcus aureus quorum sensing in cutaneous and systemic infections*. Inflammation and Regeneration, 44(9).

Appendices

Appendix A: Descriptive

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Extraction Yield in Grams	30	.5000	2.2000	1.406667E0	.5551846
Zone of Inhibition (mm)	30	6	15	9.70	2.693
Minimum Inhibitory Concentration (mg/mL)	30	.0630	.5000	.245900	.1542296
Valid N (listwise)	30				

One-way

Appendix B: Descriptive

Zone of Inhibition

(mm)

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Methanol	6	11.00	1.095	.447	9.85	12.15	10	12
Ethanol	12	11.75	2.137	.617	10.39	13.11	10	15
Distilled Water	12	7.00	.739	.213	6.53	7.47	6	8
Total	30	9.70	2.693	.492	8.69	10.71	6	15

Appendix C: Test of Homogeneity of Variances

Zone of Inhibition (mm)

Levene Statistic	df1	df2	Sig.
7.732	2	27	.002

Appendix D: ANOVA

Zone of Inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	148.050	2	74.025	32.107	.000
Within Groups	62.250	27	2.306		
Total	210.300	29			

Appendix E: Homogeneous Subsets

Zone of Inhibition (mm)

Tukey HSD

Extraction Solvent Used	N	Subset for alpha = 0.05	
		1	2
Distilled Water	12	7.00	
Methanol	6		11.00
Ethanol	12		11.75
Sig.		1.000	.554

Appendix F: Means for groups in homogeneous subsets are displayed.

Multiple Comparisons

Zone of Inhibition (mm)

Tukey HSD

(I) Extraction Solvent Used	(J) Extraction Solvent Used	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Methanol	Ethanol	-.750	.759	.591	-2.63	1.13
	Distilled Water	4.000*	.759	.000	2.12	5.88
Ethanol	Methanol	.750	.759	.591	-1.13	2.63
	Distilled Water	4.750*	.620	.000	3.21	6.29
Distilled Water	Methanol	-4.000*	.759	.000	-5.88	-2.12
	Ethanol	-4.750*	.620	.000	-6.29	-3.21

*. The mean difference is significant at the 0.05 level.

one-way

descriptive

Zone of Inhibition (mm)

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Moringa oleifera	18	10.67	2.765	.652	9.29	12.04	7	15
Melaleuca alternifolia	12	8.25	1.865	.538	7.07	9.43	6	10
Total	30	9.70	2.693	.492	8.69	10.71	6	15

Test of Homogeneity of Variances

Zone of Inhibition (mm)

Levene Statistic	df1	df2	Sig.
2.048	1	28	.163

ANOVA

Zone of Inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42.050	1	42.050	6.998	.013
Within Groups	168.250	28	6.009		
Total	210.300	29			

one way

descriptive

Zone of Inhibition (mm)

					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Staphylococcus aureus	15	10.20	3.234	.835	8.41	11.99	6	15
Staphylococcus pyogenes	15	9.20	2.007	.518	8.09	10.31	7	12
Total	30	9.70	2.693	.492	8.69	10.71	6	15

Test of Homogeneity of Variances

Zone of Inhibition (mm)

Levene Statistic	df1	df2	Sig.
3.140	1	28	.087

ANOVA

Zone of Inhibition (mm)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.500	1	7.500	1.036	.318
Within Groups	202.800	28	7.243		
Total	210.300	29			

Presentation of Experimental Plates and Observations

