

FACAULTY OF SCIENCE

BIOLOGICAL SCIENCES DEPARTMENT

A comparative investigation of the antimicrobial efficacy of ethnomedicinal oils from *Lippia. javanica* (Zumbani) leaves, *Ziziphus mauritiana* (Masau) leaves, and *Kigelia africana* fruit (mubvee) against skin-related *Candida albicans* and *Staphylococcus aureus.*

By

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B224194B

A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honors Degree in Biotechnology.

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APPROVAL FORM

Title of dissertation: A comparative investigation of the antimicrobial efficacy of ethnomedicinal oils from *Lippia javanica* (Zumbani) leaves, *Ziziphus mauritiana* (Masau) leaves, and *Kigelia. africana* fruit (mubvee) against skin-related *Candida albicans* and *Staphylococcus aureus.*

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The undersigned certified that they have read the dissertation and it is suitable for submission to the Faculty of Science and was checked for conformity with the faculty.

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Signature of Supervisor Date: 22/06/2026

Signature of Chairperson of Department: Date: 6 August 2026

DECLARATION

I, Sithole Cynthia, declared that this research 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: 22 June 2026

DEDICATION

Every challenging work needs self-effort as well as the guidance of elderly people, especially those who are very close to our hearts. My humble effort is dedicated to my loving Sithole family, whose affection, love, encouragement, and prayers day and night have enabled me to achieve such success and honor. Along with all hardworking and respected supervisors that guided me all the way, and to my friends and other loved ones who have shown me kindness during my study.

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List of abbreviation

ANOVA - Analysis Of Variance

MHA - Mueller Hinton Agar

MSA - Mannitol Salt Agar

SDA - Sabourad Dextrose Agar

SPSS - Statistical Package for the Social Sciences

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ABSTRACT

Candida albicans and *Staphylococcus aureus* are two species that cause skin infections and are important global health issues, especially because of the rising cases of antimicrobial resistance. They can cause various skin disorders and systemic diseases and the resistance to conventional antifungal and antibacterial agents has worsened the treatment strategies. Thus, the use of other therapeutic agents from ethnomedicinal plants is gaining more interest. In this study, the antimicrobial activity of macerated oils of *Lippia javanica* (Zumbani), *Kigelia africana* (Mubvee) and *Ziziphus mauritiana* (Masau) against *C. albicans* and *S. aureus* isolates was evaluated. Plant materials were gathered, shade dried and pulverized before being extracted using oil infusion method with olive oil. Phytochemical screening was performed to identify the bioactive compounds. Sterile swabs were used to collect the microorganisms from the dermal surfaces of skin and identified using cultural, Gram staining and biochemical properties. The antimicrobial activity of the plant extracts was tested using disc diffusion method on Mueller-Hinton agar against *S. aureus* and for *C. albicans*. Zones of inhibition were determined in millimetres and the efficacy of the antimicrobial activity of each of the plant extracts at different concentrations was evaluated by measuring the Zones of Inhibition. Microorganisms that have been found and tested in this work are *S. aureus* and *C. albicans* (fungal pathogen). After gram staining *S. aureus* and *C. albicans* were both seen as purple (gram positive). Biochemical tests were done for confirmation of the presence of these microorganisms. The major phytochemical constituents identified in the macerated oils extracted from *L. javanica*, *K. africana* and *Z. mauritiana* - namely alkaloids, flavonoids, tannins, saponins, phenolics and terpenoids - are known to have antimicrobial properties and may be responsible for the antimicrobial activity observed against both *S. aureus* and *C. albicans* The results indicated that both the microorganisms were inhibited by all three extracts. The antimicrobial activity of extract tested was highest for extract of *Z. mauritiana* (10.3mm) and *K. africana* (9.3mm) while the lowest was for extract of *L. javanica* (8mm). Additionally, it was found that the lower the concentration of extract the lower the antimicrobial activity. The findings of the research work can be applied in the traditional use of ethnomedicinal plants in treatment of skin infections, and their potential as alternatives to produce anti-microbial agents. The results indicate that the antimicrobial activity recorded is due to the presence of these bioactive compounds and further studies are recommended with advanced phytochemical analysis and toxicity testing for elucidating the action of these compounds. Two-way ANOVA was used for statistical analysis of the main effects and interactions. All tests were performed at $\alpha = 0.05$ significance level and Bonferroni correction was used for multiple comparison. The antimicrobial activity of three plant extracts from the genus of olive-oil, *K. africana, L. javanica and Z. mauritiana*, were statistically evaluated on *S. aureus* because of the diameter of the inhibition zone. Two-way ANOVA showed highly significant differences were obtained for extract type ($F = 26.36$, $p < 0.001$) and highly significant differences were obtained for extract concentration ($F = 27.05$, $p < 0.001$) with no significant interaction effect ($p = 0.760$).Another statistical analysis also investigates the antimicrobial

effect of three olive-oil plant extract (*K. africana, L. javanica and Z. mauritiana*) against *C. albicans* with the result in terms of diameter of inhibition zones. The two-way ANOVA showed that the effect of the type of plant extract and extract concentration were not significant ($F = 3.28$, $p = 0.0549$ and $F = 36.09$, $p < 0.001$, respectively) and that there was no significant interaction effect between the two factors ($p = 0.535$).

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CHAPTER 1: INTRODUCTION

1.1 Background of study

Candida albicans and *Staphylococcus aureus* are major pathogens that cause a variety of skin infections such as candidiasis and staph infections that result in substantial morbidity and mortality. In the U.S., antibiotic-resistant infections are estimated to cost over $20 billion and the death rate from an antibiotic-resistant infection is 20% more than from a non-drug-resistant infection. (Cassini et al., 2019). As these infections have become more prevalent, and antibiotic resistance has increased, alternative therapeutic approach is needed(Shah et al., 2022). In this context, the use of ethnomedicinal plants with antimicrobial properties has been increasing. *L. javanica* (Zumbani), *K. africana* (mubvee) and *Z. mauritiana* (Masau) are three plants that are commonly used in traditional medicine in several cultures around the world for their health benefits (Pascual et al., 2001), The antimicrobial activity of the oils extracted from the leaves of these plants are determined in the present study.

1.2 Problem Statement.

With the increasing prevalence of skin infections with *C. albicans* and *S. aureus*, the need to develop alternative treatments is imperative, especially considering the problem of antibiotic resistance. While *L. javanica*, *K. africana* and *Z. mauritiana* have been used in traditional medicine, there is scarce empirical evidence on their antimicrobial activity against these pathogens. Thus, this study seeks to fill this knowledge gap and establish a scientific basis for their use in treating skin-related infections.

Skin infection caused by *C. albicans* and *S. aureus* represents a significant global health burden, with increasing incidence reported in immunocompromised and healthy populations. The rising prevalence is compounded by the emergence of antimicrobial resistance, which has reduced the efficacy of conventional antifungal and antibacterial agents and increased treatment costs and hospitalization rates. This has created an urgent need for the development of alternative, affordable, and effective therapeutic options. While *L. javanica*, *K. africana*, and *Z. mauritiana* have a long history of use in traditional medicine across Southern Africafor the management of skin ailments, wounds, and infections, there is limited empirical and scientific evidence validating their antimicrobial efficacy against *C. albicans* and *S. aureus*. Most existing studies focus on crude extracts or other plant parts, and few have evaluated macerated oils specifically, which are the form commonly used in folk practice. Thus, there is a clear knowledge gap regarding the antimicrobial potential and mechanism of action of these plant-derived oils against common skin pathogens. This study seeks to address this gap by providing scientific evidence to support or refute their traditional use, and to establish a basis for their potential integration into modern dermatological treatments.

1.3 Aim(s):

The main aim of the study was to assess and compare the antimicrobial activity of macerated oils of *L. javanica* leaves, *K. africana* fruit and *Z. mauritiana* leaves against the skin associated *C. albicans* and *S. aureus.*

1.4 Objectives:

- To screen for bioactive compounds in macerated oils of *L. javanica, Z. mauritiana*, and *K. africana*.
- To isolate and characterize *C. albicans* and *S. aureus* from dermal surfaces of the skin
- To investigate the antimicrobial efficacy of macerated oils of *L. javanica, Z. mauritiana*, and *K. africana* against *C. albicans* and *S. aureus*

1.5 Research questions:

- What are the major bioactive compounds present in the macerated oils of *L. javanica* leaves, *Z. mauritiana* leaves, and *K. africana* fruit?
- Can *C. albicans* and *S. aureus* be isolated from dermal surfaces?
- Which of the macerated oils has the most antimicrobial activity against *C. albicans* and *S. aureus*?

1.6 Hypothesis

Null Hypothesis (Ho):

There is no significant difference in the antimicrobial activity of ethnomedicinal macerated oils of *L. javanica* leaves, *Z. mauritiana* leaves, and *K. africana* against skin-related *C. albicans* and *S. aureus*, as measured by the zone of inhibition.

H_o: $\mu 1 = \mu 2 = \mu 3$

Alternative hypothesis (H1):

There is a significant difference in the antimicrobial activity of ethnomedicinal macerated oils of *L. javanica* leaves, *Z. mauritiana* leaves, and *K. africana* against skin-related *C. albicans* and *S. aureus*, as measured by the zone of inhibition.

H_1: $\mu 1 \neq \mu 2 \neq \mu 3$

1.7 Significance:

The scientific understanding of the antimicrobial properties of *L. javanica, K. africana* and *Z. mauritiana* will be facilitated by this research. It could offer useful information for the research of potential natural alternatives to treat skin infections, ethnomedicinal plant use in modern medicine, and antibiotic resistance.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The literature shows that there are several antimicrobial properties of plant extracts like *L. javanica*, *K. africana* and *Z. mauritiana*. Potential antimicrobial constituents like terpenoids, flavonoids, in these plants have been reported(Khare et al., 2021). But limited information exists on their effectiveness against *C. albicans* and *S. aureus*. Thus, this study aims to assess these ethnomedicinal oils for their antimicrobial activity in order to fill this gap.

2.2 *Candida albicans*

Antibiotic use, diabetes (Concia et al., 2009), and weakened immune system are risk factors for candidiasis. Symptoms include itching, discharge and irritation in the vagina in the case of vaginal infections, and white patches and soreness in the mouth in the case of oral infections(Thomas-Rüddel et al., 2022). Typically, the infection is treated using antifungal medications, although there has been a great deal of interest in the use of alternative treatments, such as natural extracts and macerated oils, in recent years. Understanding *C. albicans* is needed to develop effective treatment strategies, especially in the face of the challenges.

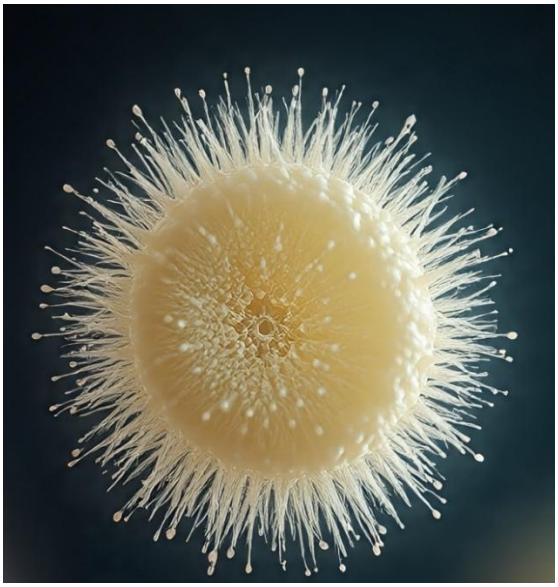

Figure 1 microscopic image of *C. albicans* on the dermal surface showing yeast cells and pseudohyphae**.**

C. albicans is a yeast which lives naturally in the body, such as in the mouth, gut and vaginal region. It is normally harmless, but when it grows too much, it can lead to infection (candidiasis). Oral thrush, vaginal yeast infections and more serious, systemic infections can occur, particularly in immune-compromised people(Poulain, 2015).

2.3 *Staphylococcus. aureus*

This bacterium has a tendency to develop resistance to antibiotics, such as methicillin-resistant *S. aureus* (MRSA) which is known to be difficult to treat. (Guo et al., 2020). Infections may present as boils, cellulitis and impetigo, with symptoms of redness, swelling, and pain(Thapaliya et al., 2019). Open wounds and weakened immune systems are risk factors for infection, as are close contact environments(Wertheim et al., 2005). *S. aureus* is still a problem today, both in healthcare and community settings because it is virulent and resistant, and therefore there is a need for treatment and prevention strategies.

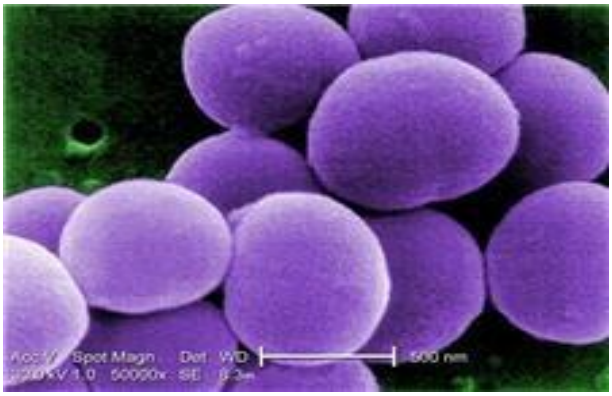

Figure 2: Scanning electron micrograph of *S. aureus* bacterium, purple colored, spherical cocci.

S. aureus is a gram-positive bacterium which is present in the nose and skin of healthy people. It's not necessarily harmful, but also a pathogen that causes various infections, such as skin infections, pneumonia and bloodstream infections(Cheung et al., 2021) .

2.4 *Lippia. javanica*

The chemical constituents of macerated oil of *L. javanica* were found to be rich and diverse with the major constituents being monoterpenes and sesquiterpenes (Maroyi, 2017). The main compounds are caryophyllene (antioxidant with anti-inflammatory properties), limonene (citrus aroma and antimicrobial activity), thymol (antiseptic and antifungal) and geraniol (shows pleasant aroma and has antioxidant activity)(Baschieri et al., 2017). The therapeutic properties of macerated oil may vary due to the geographical origin, extraction procedure and the maturity stage of the plant at the time of harvesting (Viljoen et al., 2005). The beneficial property of *L. javanica* macerated oil is due to its bioactive components that act on multiple biological pathways. The antimicrobial activity of macerated oil is mainly attributed to its ability to disrupt the microbial cell membrane, which result in microbial growth inhibition and consequently cell death in bacteria and fungi (Manenzhe et al., 2004). Furthermore, substances such as caryophyllene can regulate inflammatory processes by inhibiting the release of pro-inflammatory cytokines, thus having anti-inflammatory properties (Yeung et al., 2018). The oil also exhibits antioxidant activity. Geraniol, a major compound present in the oil, acts as a free radical scavenger and protects cells against oxidative stress.Moreover, the volatile compounds of the oil

act as good insect repellent due to their effect on the sensory perception of the insect (Samie et al., 2009). These mechanisms highlight the therapeutic potential of *L. javanica* for use in traditional and modern medicine



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Figure 3 *L. javanica* leaves showcasing vibrant green foliage with delicate white flowers

2.5 *Ziziphus mauritiana*

Z. mauritiana (jujube) contains a variety of bioactive compounds in its macerated oils which play a prominent role in therapeutic properties. Key constituents are monoterpenes like limonene and pinene which have aromatic and therapeutic properties(Prakash et al., 2021). The complex composition makes it possible to use it in traditional medicine because of its geographic origin, the maturity of the plants and the extraction methods. *Z. mauritiana* is effective due to multiple biological processes which are underlying its therapeutic properties. The macerated oils of it have excellent antioxidant activity, which helps to neutralize free radicals and reduce oxidative stress in the body (Prakash et al., 2021). Moreover, the plant exhibits anti-inflammatory effects by blocking pro-inflammatory cytokines and pathways(Khanam et al., 2025). *Z. mauritiana* has been traditionally known for its calming properties and could also be used as a sedative, possibly affecting the activity of neurotransmitters. Besides, macerated oils of it have antimicrobial properties and disrupt the cell membranes of different pathogens or block their metabolic process, thus increasing the medicinal use (Rahman et al., 2021).



Figure *4 Z. mauritiana,* displaying its glossy green leaves and clusters of small, orange-red berries that are transitioning from green to ripe red.

2.6 *Kigelia africana*

K. africana is an iconic African herbal medicinal plant species that has been pan-African in distribution and with great indigenous non-medicinal and medicinal uses. The plant is used traditionally as a remedy for numerous diseases, such as wounds, rheumatism, psoriasis, diarrhea, and stomach ailments(Abbas et al., 2024). It's also used as an aphrodisiac and as a skin care product. The fruits of *K. africana* are used in traditional African medicine as poultices, pastes and emollients in the treatment of skin irritations like eczema, psoriasis and as emollients for skin care, especially for the use of African women(Nabatanzi et al., 2020).

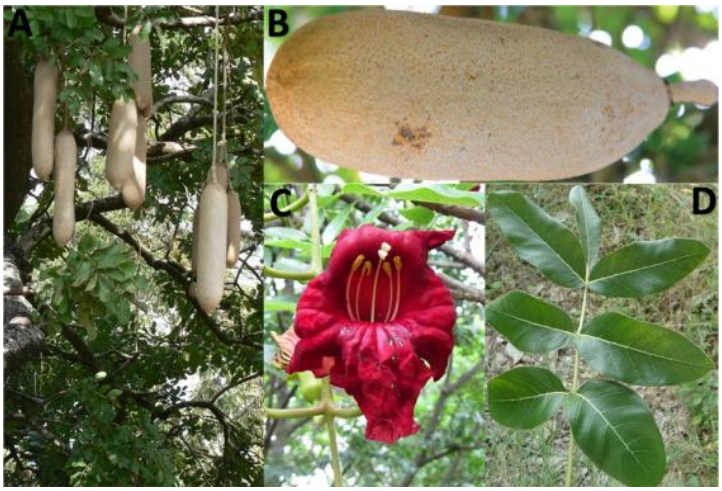

Figure *5*: *K. africana* tree with hanging fruits (A), fruit (B), flower (C), and leaves (D)

CHAPTER 3:MATERIALS AND METHODS

3.1 Study Area

The study was carried out in laboratory conditions of the Department of Biological Sciences, Bindura University of Science Education. The selected area has all the necessary facilities required for extraction and analysis of essential oils and thus creates a proper environment for carrying out the research. The laboratory is equipped for different experimental procedures to ensure accurate and reliable results for the evaluation of the antimicrobial activity of the oils obtained from *L. javanica, K. africana* and *Z. mauritiana*.
The *Z. mauritiana* (leaves) and *L. javanica* (leaves) were collected from Bindura, Zimbabwe (17.3019° S, 31.3306° E) while *K. africana* (fruit) was collected from Shamva, Zimbabwe (17.3113° S, 31.5756° E). Collection was done in the early dry season in May 2025 from 0800 to 1100 hours, to achieve the best phytochemical integrity. All the plants were collected from natural habitats where they grow in abundance under natural conditions. The gathered specimens were identified and verified botanically by a competent taxonomist at the National Herbarium and Botanic Garden of Zimbabwe with voucher specimens prepared and deposited for future reference. Identification was made from the morphological features such as fruit structure, habit of the plant and leaf shape, and verified with standard taxonomic keys and herbarium reference collections.

3.2 Research Design

A comparative experimental design was employed in this study, which included three groups for *L. javanica* oil, *K. africana* oil, and for *Z. mauritiana* oil. Both plants were subjected to oil extraction, and an evaluation of the oils was conducted in a controlled environment against both *C. albicans* and *S. aureus*.

Antimicrobial activity was evaluated using disc diffusion methods which enabled the determination of the activity of each oil in a clear manner. 3 replicates were repeated for each treatment to make 3 replications so that the results would be statistically valid and reliable. This systematic method will allow the full evaluation of the antimicrobial activity of extracts from both plants.

3.3 Research Procedure

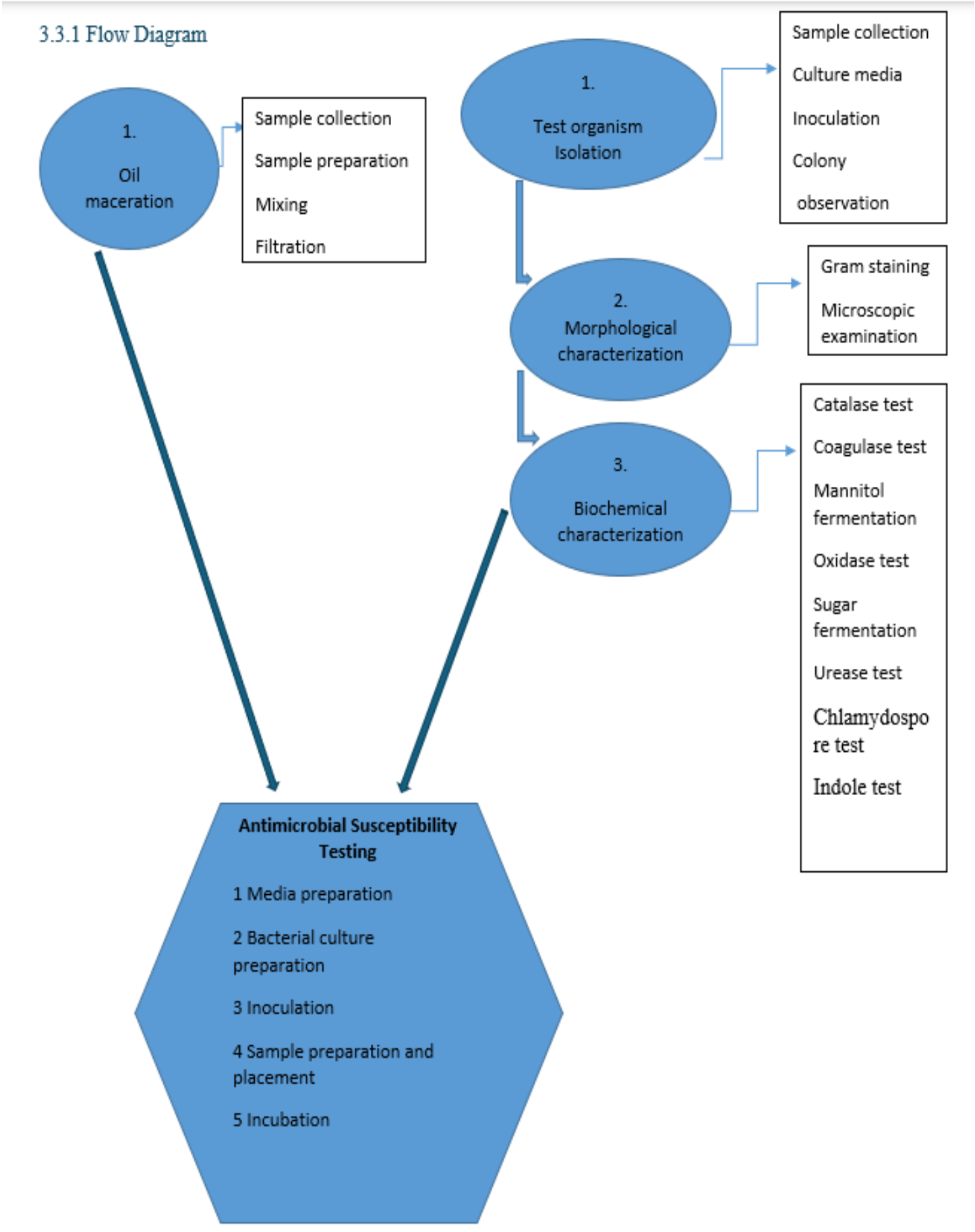

Figure 6 Flow diagram for the methodology

3.3.2 Oil maceration

Healthy and mature Masau leaves and Zumbani leaves which did not show any signs of injury or disease were taken. The leaves were cut from the stem with a sharp knife leaving a small piece of stem on each leaf. Mubvee fruits were selected that are moderately ripe with no signs of damage. Masau leaves, and mubvee fruit were washed under running water to remove any dirt. To avoid loses of bioactive compounds, the plant material was shade dried for 2 weeks. Dried plant materials were ground in a powder using a mortar and pestle.

These were all closed containers that contained 25g of dried plant material each. Olive oil was poured over the plant material in each container, so as to cover the whole plant material with 100ml of olive oil in each container. The mixture was well stirred to make sure that the plant materials were homogeneously distributed in the oil. The container was then covered with a lid and placed under shade at a room temperature (25ºC) for 3 weeks. The plant material was distributed evenly in the oil by shaking the containers every day. Oil infusion was filtered after three weeks in another glass jar. Solids were removed and the oil infusion was kept in a dark coloured container for additional uses. To avoid the loss of bioactive compounds from the oil infusion, it was stored in a cool and dark environment.



Figure 7 *Ziziphus mauritiana* maceration process



Figure 8 *Kigelia africana* maceration process

3.3.4 Isolation of *S. aureus*

Test organisms were collected from the clinic. In this procedure, test organisms such as *S. aureus* were collected from the skin using a standardized sampling method. A sterile swab was moistened with sterile saline or broth. This swab was gently rubbed over the targeted skin area, ensuring adequate coverage to collect a representative sample.

The swab was immediately placed into a sterile tube containing transport medium to preserve the viability of the organisms during transport to the laboratory. The tube was labelled with relevant information, including the site of collection and the date. The collected samples were then transported to the laboratory as quickly as possible for further processing and analysis, where they were inoculated onto appropriate culture media for the identification and characterization of *S. aureus* and any other potential pathogens.

3.3.5 Culture media preparation

Mannitol Salt Agar (MSA) was used, which is a selective medium. For this procedure 30.78 g MSA medium was dissolved in 810 mL distilled water. The mixture was shaken and heated until it was completely dissolved. The media was then autoclaved for 15 minutes at 121°C and 15 pounds of pressure to make it sterile. The medium was then transferred to sterile petri dishes and cooled down to solid state after autoclaving.

3.3.6 Inoculation and sub culturing

The streak plate method was used to streak the sample onto the agar plates. The plates were incubated at 37 ºC for 24 hours. Colonies that were golden yellow were observed on MSA due to mannitol fermentation. A colony of *S. aureus* was picked using a sterile loop and was representative. To obtain pure cultures, a streak was made on fresh MSA. These plates were incubated again at 30°C for 24 hours.

3.3.7 Morphology characterization for *S. aureus*

3.3.5.1 Gram staining

A nutrient broth culture of *S. aureus* was made and a suspension of *S. aureus* was prepared by inoculating the nutrient broth culture on a sterile loop. This was incubated for 24 hours at 37°C. A microscope slide was obtained that had been cleaned. A drop of bacterial suspension was laid on the slide and was left to dry out completely at room temperature. The slide was then quickly passed through the flame of the Bunsen burner to heat fix the bacteria to the slide. Slide was stained for 1 minute in crystal violet. Slide stained for 1 minute with crystal violet stain. Thereafter the slide was stained and rinsed off with distilled water. The slide was stained with the iodine solution for 1 minute to form the complex with the crystal violet. This slide was then washed again with distilled water. A decolorizing agent (generally ethanol or acetone) was applied dropwise to the slide to decolorize the sample until it becomes clear (10-30 seconds). Immediately after the slide was washed with distilled water. The slide was then stained for 30 seconds in safranin and then washed in distilled water. The slide was air dried or blotted with filter paper. This was viewed with a microscope using the oil immersion objective. Morphology and Gram reaction of the bacteria were recorded.

3.3.8 Biochemical characterization of *S. aureus*

3.3.8.1Catalase Test:

A catalase test was performed on the *S. aureus,* and a few drops of hydrogen peroxide were added to the colony. The presence of catalase enzyme in the organism was shown by the formation of bubbles. The process was to add a small sample of a colony of *S. aureus* to a glass slide. A few drops of hydrogen peroxide were dropped on the colony and then bubbles (positive catalase reaction) were seen.

3.3.8.2 Indole Test

The researcher aseptically transferred growth (24 hours culture) to a sterilized test tube of tryptophan broth (4mL). The tube was then incubated at 37℃ for 24 hours. 50 micro liters (0.5 mL) of kovac reagent was added to broth culture and the researcher observed for the presence or absence of a ring.

3.3.8.3 Mannitol Fermentation:

The organism was inoculated onto MSA. If *S. aureus* fermented mannitol, the agar changed colour to yellow due to acid production. A MSA plate was inoculated with a streak of *S. aureus*. The plate was incubated at 37°C for 24 hours. After incubation, a colour change of the medium to yellow indicated mannitol fermentation.

3.3.8.4 Oxidase Test:

The oxidase test was performed using filter paper soaked in an oxidase reagent. A colour change indicated the presence of cytochrome c oxidase. A piece of filter paper was soaked with the oxidase reagent, and a

colony of *S. aureus* was smeared onto the filter paper. The development of a purple colour within 30 seconds indicated a positive oxidase reaction.

3.3.8.5 Sugar Fermentation

Different sugar fermentation tests were conducted to determine if *S. aureus* could ferment glucose, lactose, and sucrose. Colour changes in the medium indicated fermentation. Glucose, lactose, and sucrose fermentation tubes were prepared with a phenol red indicator. A colony of *S. aureus* was inoculated into each tube. The tubes were incubated at 37°C for 24 hours, and any colour change indicated fermentation of the respective sugars.

3.3.8.6 Urease Test:

A urease test was performed using urea broth. A colour change indicated urease production, which helped in the identification of *S. aureus*. Urea broth was inoculated with a colony of *S. aureus*. The broth was incubated at 37°C for 24-48 hours. A colour change to pink indicated a positive urease test, confirming urease production.

3.4.1 Isolation of *C. albicans*

Test organisms were collected from the skin of different people who had filled out the consent form. In this procedure, test organisms such as *C. albicans* were collected from the skin using a standardized sampling method. First, the area of the skin was cleaned with an antiseptic solution to minimize contamination. A sterile swab was then moistened with sterile saline or broth. This swab was gently rubbed over the targeted skin area, ensuring adequate coverage to collect a representative sample. After swabbing, the swab was immediately placed into a sterile tube containing transport medium to preserve the viability of the organisms during transport to the laboratory. The tube was labelled with relevant information, including the patient's identification, the site of collection, and the date. The collected samples were then transported to the laboratory as quickly as possible for further processing and analysis, where they were inoculated onto appropriate culture media for the identification and characterization of *C. albicans* and any other potential pathogens.

3.4.2 Culture media preparation

A selective medium, SDA, was used. In this procedure, 30.78 grams of SDA medium was dissolved in 810 millilitres of distilled water. The mixture was shaken and heated until it dissolved completely. Next, the medium was autoclaved at 121°C and 15 pounds of pressure for 15 minutes to ensure sterility. After autoclaving, the medium was poured into sterile petri dishes and allowed to cool until it solidified.

3.4.3 Inoculation and Sub culturing

The streak plate method was used to streak the sample on the agar plates. These plates were then incubated for 24 hrs at 37 ºC. The plates were then observed for the presence of colonies after the incubation period. *C. albicans* formed colonies that were creamy, white or yellowish. A sterile loop was used to pick a representative colony of *C. albicans*. To obtain pure cultures the colony was streaked onto fresh SDA or Chromogenic Agars. These plates were incubated again at 37°C for 24hours. A small amount of the sub cultured colony was taken and mixed with a drop of sterile saline on a glass slide. A coverslip was then put over the mixture and the slide was checked under the microscope for the yeast cells and pseudohyphae.

3.4.4. Morphology characterization of *C. albicans*

3.4.4.1 Gram staining

A sterile loop was used to inoculate a nutrient broth culture for the preparation of a suspension of *C. albicans.* This culture was grown at 37°C for 24 hours. A microscope slide was cleaned and a few drops of the bacterial solution was dropped on the slide. The slide was left to dry at room temperature. The slide was then quickly taken through the Bunsen burner flame to heat fix the bacteria, making them stick to the slide. This stain, crystal violet, was applied to the slide for 1 minute. The slide was stained and very carefully washed with distilled water. The crystal violet was complexed with the iodine solution on the slide for 1 minute. The slide was then again rinsed with distilled water. The slide was dropwise decolorized with the decolorizing agent (typically ethanol or acetone) until the decolorizing agent ran off clear (10-30 seconds). This slide was then thoroughly washed with distilled water at once. The slide was stained with safranin for 30 seconds followed by distilled water washing the slide. The slide was air dried or gently blotted with filter paper. This was viewed with a microscope with an oil immersion objective. The morphology and Gram reaction of the bacteria was noted.

3.4.5 Biochemical Characterization of *C. albicans*

3.4.5.1 Urease Test:

A culture of *C. albicans* was inoculated into a urease broth containing urea as the sole nitrogen source. The culture was incubated at 30°C for 24 hours. After incubation, it was assessed for colour change. It was observed that *C. albicans* did not produce urease, resulting in no colour change in the medium.

3.4.5.2 Sugar Fermentation Test:

Various sugar broths, maltose and glucose were prepared and inoculated with *C. albicans*. The cultures were incubated at 30°C for 24 to 48 hours. After incubation, the presence of gas and pH indicators was checked. It was confirmed that *C. albicans* fermented certain sugars, indicated by gas production or a colour change in the medium.

3.4.5.3 Chlamydospore Formation:

C. albicans culture was spread on cornmeal agar media with 2% Tween 80. It was cultured at room temperature for 24 hours. After incubation, the presence of chlamydospores was observed under the microscope. It was confirmed that *C. albicans* produced chlamydospores, which are characteristic of this species.

3.4.5.4 Indole Test:

A culture of *C. albicans* was inoculated into a tryptophan-rich medium. After incubation, Kovac's reagent was added to the culture. It was observed that there was no colour change, confirming a negative result and indicating that *C. albicans* did not produce indole.

3.4.5.5 Citrate Test:

C. albicans was inoculated onto a citrate medium, which served as the sole carbon source. The culture was incubated and monitored for growth and colour change. It was confirmed that no growth occurred, and the medium remained unchanged, indicating a negative result for citrate utilization.

3.4.5.6 Catalase test

Hydrogen peroxide was added to a few drops to a colony of *C. albicans* to do the catalase test. The organism was able to break down hydrogen peroxide as evidenced by the bubbles. This was done by placing a small inoculum of *C albicans* on a glass slide. A few drops of hydrogen peroxide were poured into the colony, and bubbles were seen, which means that the catalase reaction was positive.

3.5. Phytochemical screening

The phytochemicals of interest in this survey were alkaloids, flavonoids, saponins and tannins. Qualitative analyses for above phytochemicals were done on the hexane, ethyl acetate and methanol extracts of all plant species as described below.

3.5.1 Alkaloids:

(a) Dragendorff test

2 mg of plant extracts were dissolved in 6 ml of ethanol. 1 ml of the ethanolic extract solution was mixed with 5 ml of distilled water and then a few drops of 2 M Hydrochloric acids were added. Then 1 ml of Dragendorff reagent was added. Formation of a yellow or orange precipitate indicates the presence of alkaloids (Alshehri et al., 2022).

(b) Mayer’s test

The Mayer’s reagent was prepared by mixing 1.36 g of mercuric chloride and 5.00 g of potassium iodide and then diluted to 100 ml with distilled water. A few drops of the Mayer’s reagent were added to the ethanolic extract. Alkaloids are detected when white or pale-yellow precipitate is formed (Bisht et al., 2024).

A 10mg of the plant extract was prepared and mixed with iodine in potassium iodide (KI) and the test used was Wagner's test. Brown/red precipitate indicates presence of alkaloids.

3.5.2 Flavonoids:

(a)Sodium hydroxide test

1 ml of the stock solution was treated with a few drops of the 1 M NaOH solution. Flavanoids are detected by the formation of intense yellow colour, which disappears on the addition of a few drops of 1 M HCl (Hossain et al., 2013).

(b)Shinoda test

A small amount of the extract was dissolved in ethanol and filtered. About 1 g of magnesium turnings were added and then a few drops of concentrated HCl were added. Formation of a pink, orange or red to purple colour indicates the presence of flavonoids (Usman et al., 2009).

3.5.3 Saponins:

10 ml of distilled water was added to 1 ml of the ethanolic solution of the plant extract. The mixture was then shaken for 10 s. Formation of foam is a positive result (Usman et al., 2009).

3.5.4 Tannins:

Ferric chloride test

A few drops of 5 % FeCl3 were added to 1 ml of the stock solution. Presence of an intense blue-black, blue green,

3.5.5 Phenolic compounds

The sample solution was placed in a clean test tube, after which 2–3 drops of neutral ferric chloride solution were added. The test tube was gently shaken to mix the contents thoroughly, and the mixture was then observed for any immediate colour change. Purple / violet / blue / green coloration shows the presents of phenolic compounds.

3.5.6 Terpenoids

About 2 mL of the sample solution was placed in a clean test tube, after which 2 mL of Chloroform was added. A few drops of concentrated Sulfuric Acid were then carefully added down the side of the test tube to form a layer. The mixture was allowed to stand and was observed for the formation of a reddish-brown coloration at the interface, indicating the presence of terpenoids

3.6 Antimicrobial Susceptibility Testing for *Z. mauritiana* oil, *K. africana*oil, and *L. javanica* oil against *S. aureus* and *C. albicans*

3.6.1 Antibacterial Screening

The antibacterial activity of plant extracts was investigated by the disk diffusion assay (Tendencia, 2004; Sandle, 2016).

3.6.2 Preparation of discs

The discs were cut in a circular form with a diameter of 5 mm each and were sterilized by autoclaving at 121℃ for 15min.

3.6.3 Media preparation (Mueller Hinton agar)

A measured 30.78g of Mueller Hinton agar medium was dissolved in 810ml of distilled water, shaken, and heated to dissolve completely. The medium was autoclaved at 121ºC and 15 pounds of pressure for 15 minutes. The medium was then poured into sterile Petri dishes and allowed to cool to solidify.

3.6.4 Broth preparation (nutrient broth)

A measured 0.702g of nutrient broth medium was dissolved in 54ml of distilled water, shaken, and heated to dissolve completely. A volume of 9ml of the dissolved medium was then distributed into glass test tubes. The medium was autoclaved at 121ºC and 15Pa for 15 minutes.

3.6.5 Bacterial culture preparation

A pure culture plate of bacteria was brought, using *C. albicans* and *S. aureus*, as we wanted to examine the antimicrobial activity of our macerated oil against these microorganisms. An inoculating loop was burned to red hot and cooled inside the biosafety cabinet. One loopful of bacterial colony was picked from the pure culture plate using the sterile inoculating loop. The fresh nutrient broth tube was inoculated with the bacteria and incubated at 36ºC for 24 hours.

3.6.6. Inoculation of culture plates

A fresh Mueller Hinton Agar plate was labelled with the sample name, bacteria name, and date. The broth culture tube was rotated several times to mix the culture using a vortex mixer. A sterile cotton swab stick was taken, and the micropipette was used to measure 10µl of broth culture using yellow tips, which was then placed on the plate containing the MHA. The MHA plate was inoculated using the swab in a zigzag style. Inoculation was done by rotating the MHA plate occasionally, and each edge of the MHA plate was also inoculated.

3.6.7 Sample preparation

Six glass test tubes were taken and labelled from A to F (original sample). Additional test tubes for serial dilution were prepared and labelled as well. A specific volume of the original sample (e.g., 1ml) was transferred into tube A10-1. A specific volume of diluent (e.g., 9ml) was added to tube A10-1, and the

mixture was mixed thoroughly (vortexed if needed). A specific volume of diluent (e.g., 9ml) was added to tube A10-2, and a specific volume (e.g., 1ml) from tube A10-1 was transferred to tube A10-2. This process was repeated for the remaining tubes.

Using sterile techniques, equal-sized disks were taken and added to all the test tubes. After 15 minutes, the disks were removed and placed on the inoculated MHA plate using forceps. Each disk was pressed on every side to ensure proper placement. A disk was picked up with the forceps and placed on the MHA surface, keeping a distance from other samples. The plate was covered but not inverted, and the plates were incubated at 35ºC for 24 hours. After 24 hours, the plates were taken out of the incubator and placed on a black background by inverting the plate. The zone of inhibition around the antibiotic and sample was observed.



Figure 9 AST process

3.7 Data Collection and Data Analysis

Data included the diameter of the zones of inhibition measured in millimetres for each oil against both pathogens. One effective analysis tool that was used to analyse the antimicrobial efficacy data from the study was the Statistical Package for the Social Sciences (SPSS). SPSS was widely used for statistical analysis in the social sciences but was also suitable for biomedical research. It provided various statistical tests, including ANOVA, t-tests, and regression analysis, which helped assess the differences in antimicrobial activity between the essential oils from the various plant sources and their effectiveness against the two microorganisms, *C. albicans* and *S. aureus*.

CHAPTER 4:

4.0: Introduction

This is the chapter in which data collected from experimental tasks are presented.

4.1 Isolation of test organisms



Figure 4.1.1: Isolates of *S. aureus* in Mannitol Salt Agar



Figure 4.1.2: Isolate of *C. albicans* in Potato Dextrose Agar

4.2: Morphology Characterization

Table 4.2:1: Colony Morphology in Mannitol Salt Agar

Characteristic	Isolate			
	A	B	C	D
Color	purple	purple	yellow	cream
Texture	shiny	shiny	shiny	smooth
Elevation	flat	elevated	flat	flat
Size	big	small	big	big
Edges	Smooth	Smooth	Smooth	Rough
Media color	pink	pink	pink	yellow

Table 4.2.2: Colony Morphology in potato dextrose agar

Characteristic	Isolate		
	E	F	G
color	Cream	white	white
shape	Mat like	circular	circular
texture	smooth	rough	smooth
size	big	small	small
elevation	flat	flat	elevated
edges	Rough edges	Rough edges	Rough edges

4.3: Gram Staining Results

Table 4.3.1: Microscopic examination of *S. aureus*

Isolate	Gram positive/negative	Shape
A	-	micrococci
B	+	micrococci
C	-	cocci
D	+	cocci

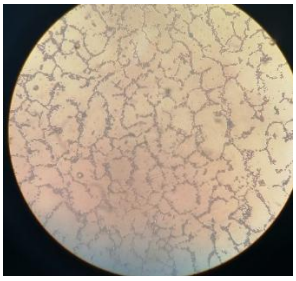

Figure 10 Isolate D

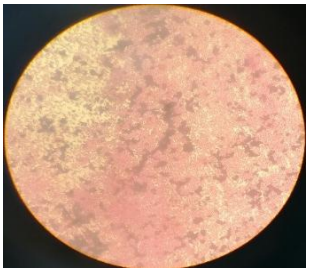

Figure 11 Isolate A

Isolates D and B were Gram-positive, spherical coccus that appeared purple under a microscope and were typically arranged in grape-like clusters. Due to their thick peptidoglycan cell walls, they retained the crystal violet stain after decolorization and were characterized as Gram-positive cocci in clusters. Isolates A and C were Gram-negative and appeared pink under a microscope.

Table 4.3.2: Microscopic Examination of *C. albicans*

isolate	Gram positive/ negative	shape
E	+	oval
F	+	micrococci
G	+	Oval

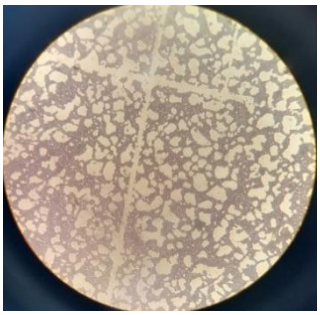

Figure 12 Iisolate G

The purple clustered oval structures corresponded to yeast cells of *C. albicans*. The dense network or chain-like patterns likely represented pseudohyphae, which were characteristic when *Candida* began filamentous growth. The lighter background corresponded to the stained slide and the surrounding medium. The large number of cells indicated heavy yeast presence in the sample.

4.4 Biochemical characterization of S. auerus.

Table 4.4.1: Biochemical Test Results for Four *S. aureus* Isolates

Biochemical Test	Isolate A	Isolate B	Isolate C	Isolate D
Catalase Test	+	+	+	+
IndoleTest	+	+	+	+
Mannitol Fermentation	_	_	+	+
Oxidase Test	−	−	−	−
Urease test	+	+	+	+
Sugar fermentation	−	−	−	−
Glucose fermentation	+	+	_	+
Sucrose fermentation	+	_	+	+
Fructose fermentation	_	+	+	+

The four *S. aureus* isolates showed consistent results in catalase (+), indole (+), urease (+), and were oxidase-negative (−). They differed mainly in carbohydrate fermentation: mannitol was fermented by isolates C and D only, glucose was fermented by isolates A, B, and D but not C, sucrose was fermented by isolates A and C only, and fructose was fermented by isolates B and C only. Overall, core enzymatic traits were similar, while sugar fermentation patterns distinguished the isolates.

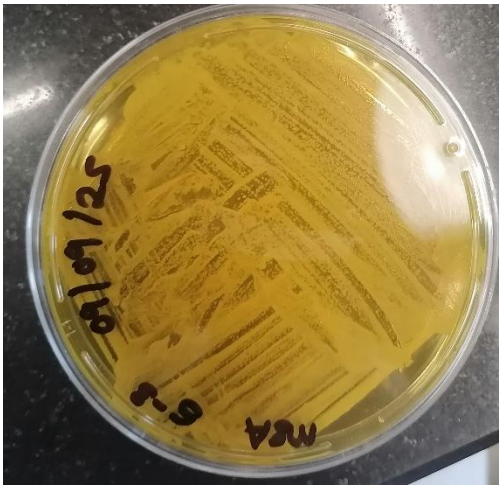

Figure 13 Mannitol test for isolate D



Figure 14 Mannitol fermentation for other isolates

Table 4.4.2: Biochemical and Phenotypic Test Results for Three *C. albicans* Isolates

Biochemical Test	Isolate E	Isolate F	Isolate G
Catalase Test	+	+	+
Citrate Utilization	–	–	+
Glucose fermentation	+	+	+
Maltose fermentation	–	–	+
Indole Test	+	–	–

Biochemical Test	Isolate E	Isolate F	Isolate G
Catalase Test	+	+	+
Citrate Utilization	_	_	+
Chlamydospore Formation	+	_	+
Urease Test	_	_	_

Key

- + = Positive reaction / observed
- − = Negative reaction / not observed

Isolates E, F, and G are all positive for the catalase test and glucose fermentation, and all are negative for the urease test. In the citrate utilization test, isolates E and F are negative while isolate G is positive. For maltose fermentation, isolates E and F are negative, while isolate G is positive. In the indole test, isolate E is positive, isolate F shows a weak/variable (±) reaction, and isolate G is negative. For chlamydospore formation, isolates E and G are positive, while isolate F shows a weak/variable (±) result.



Figure 15 Chlamydospore formation for isolate F and G, Catalase Test

4.5: Phytochemical screening tests

Table 4.4.2: Biochemical and Phenotypic Test Results for Three *C. albicans* Isolates

Phytochemical / Biochemical Test	*K. africana*	*Z. mauritiana*	*L. javanica*
Alkaloids (Dragendorff)	+	+	±

Phytochemical / Biochemical Test	*K. africana*	*Z. mauritiana*	*L. javanica*
Flavonoids (Shinoda test)	+	+	+
Tannins (Ferric chloride test)	±	+	±
Saponins (Foam test)	–	±	–
Phenolic compounds	+	+	+
Terpenoids	+	+	+

Key:

- ++ = strongly present
- + = present
- ± = trace / weak presence
- – = absent

K. africana, *Z. mauritiana*, and *L. javanica* all tested positive for flavonoids, phenolic compounds, and terpenoids. In the alkaloid test (Dragendorff), *K. africana* and *Z. mauritiana* were positive, while *L. javanica* showed a weak (±) reaction. For tannins (Ferric chloride test), *Z. mauritiana* was positive, while *K. africana* and *L. javanica* showed weak (±) results. In the saponin (foam) test, *K. africana* and *L. javanica* were negative, while *Z. mauritiana* showed a weak (±) reaction. Overall, *Z. mauritiana* shows the strongest presence of the tested phytochemicals compared to the other two plants.

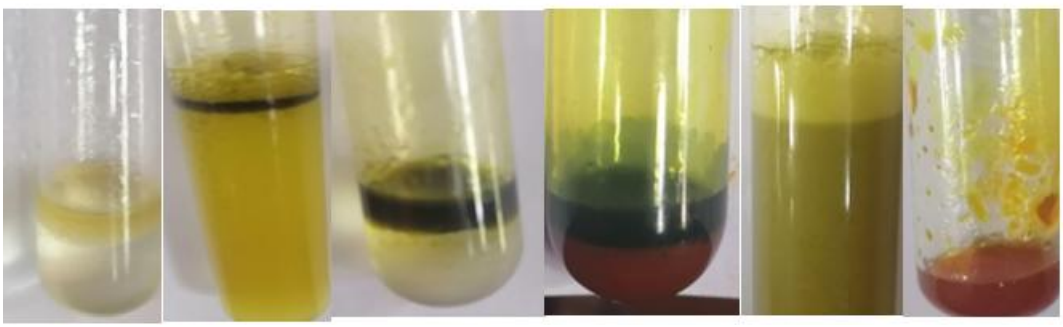

Figure 16 Phytochemical screening for the plant-olive oil extract.

4.6: Antimicrobial Susceptibility Testing for olive-oil extracts of *Z. mauritiana* oil, *K. africana* oil, and *L. javanica* oil against *S. aureus* and *C. albicans*

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Table 4.6.1: Average zones of inhibition (mm) of olive-oil plant extracts against *S. aureus*

Plant extract	100% (mm)	75% (mm)	50% (mm)	25% (mm)	+ve control (Ciprofloxacin (5 µg)	_ve control (Distilled water	_ve relative control(olive oil)
K. africana	9.3 ± 0.5774	8.3 ±0.5774	7.3± 0.5774	6.3± 0.5774	7± 0	_	_
L. javanica	8 ± 1	7 ± 0	6 ± 0	6± 0	7 ± 0	_	_
Z. mauritiana	10.3 ±1.1547	9 ±1	8.3± 0.5774	7.3± 0.5774	7 ± 0	_	_

	Mean ± Std?						
Plant extract	100%	75%	50%	25%	+ve control Ciprofloxacin **(**5 µg**)**	-ve control (distilled water)	Relative control (olive oil)
K. africana	9.3 ± 0.5774	8.3 ±0.5774	7.3± 0.5774	6.3± 0.5774	7± 0	_	_
L. javanica	8 ± 1	7 ± 0	6 ± 0	6± 0	7 ± 0	_	_
Z. mauritiana	10.3 ±1.1547	9 ±1	8.3± 0.5774	7.3± 0.5774	7 ± 0	_	_

The average zones of inhibition produced by the olive-oil plant extracts against *S. aureus* increased with concentration. At 100%, *K. africana*, *L. javanica*, and *Z. mauritiana* produced inhibition zones of 9.3 mm, 8 mm, and 10.3 mm, respectively. At 75%, the zones decreased to 8.3 mm (*K. africana*), 7 mm (*L. javanica*), and 9 mm (*Z. mauritiana*); at 50%, they further reduced to 7.3 mm, 6 mm, and 8.3 mm; and at 25%, they were the lowest at 6.3 mm, 6 mm, and 7.3 mm. Across all concentrations, *Z. mauritiana* showed the greatest inhibitory activity, followed by *K. africana*, while *L. javanica* had the lowest activity. The positive control

showed a 7 mm zone, while the negative controls (−ve and _ve relative control) produced no inhibition, confirming that the observed effects were due to the plant extracts.

The statistical analysis examines the antimicrobial efficacy of three olive-oil plant extracts *K. africana, L. javanica,* and *Z. mauritiana* against *S. aureus* using inhibition zone diameter as the primary outcome measure. Two-way ANOVA revealed highly significant effects for both plant extract type ($F = 26.36$, $p < 0.001$) and extract concentration ($F = 27.05$, $p < 0.001$), with no significant interaction effect ($p = 0.760$).

Z. mauritiana demonstrated superior antimicrobial activity, producing a mean inhibition zone of 8.8 mm across all concentrations, significantly exceeding *L. javanica* (6.7 mm, $p = 0.001$). All extracts exhibited dose-dependent responses, with inhibition zones decreasing proportionally as concentration decreased from 100% to 25%.

Table 4.6.1: Average zones of inhibition (mm) of olive-oil plant extracts against *C. albicans*

Plant extract	100% (mm)	75% (mm)	50% (mm)	25% (mm)	+ve control (Nystatin (100 Units)	_ve control (distilled water)	_ve relative control (olive oil)
K. africana	7.6 ± 0.5774	7 ± 0	6 ± 0	5.6 ± 0.5774	7 ± 0	_	_
L. javanica	7.6 ± 0.5774	7.3 ± 0.5774	6 ± 0	6 ± 0	7 ± 0	_	_
Z. mauritiana	8 ± 0	7 ± 0	6.6 ± 0.5774	6.3 ± 0.5774	7 ± 0	_	_

	Mean ± Std						
Plant extract	100%	75%	50%	25%	+ve control (Nystatin (100 Units)	-ve control (distilled water)	Relative control (olive oil)
K. africana	7.6 ± 0.5774	7 ± 0	6 ± 0	5.6 ± 0.5774	7 ± 0	_-	_-
L. javanica	7.6 ± 0.5774	7.3 ± 0.5774	6 ± 0	6 ± 0	7 ± 0	_-	_-

Z. mauritiana	8 ± 0	7 ± 0	6.6 ± 0.5774	6.3 ± 0.5774	7 ± 0	_	_

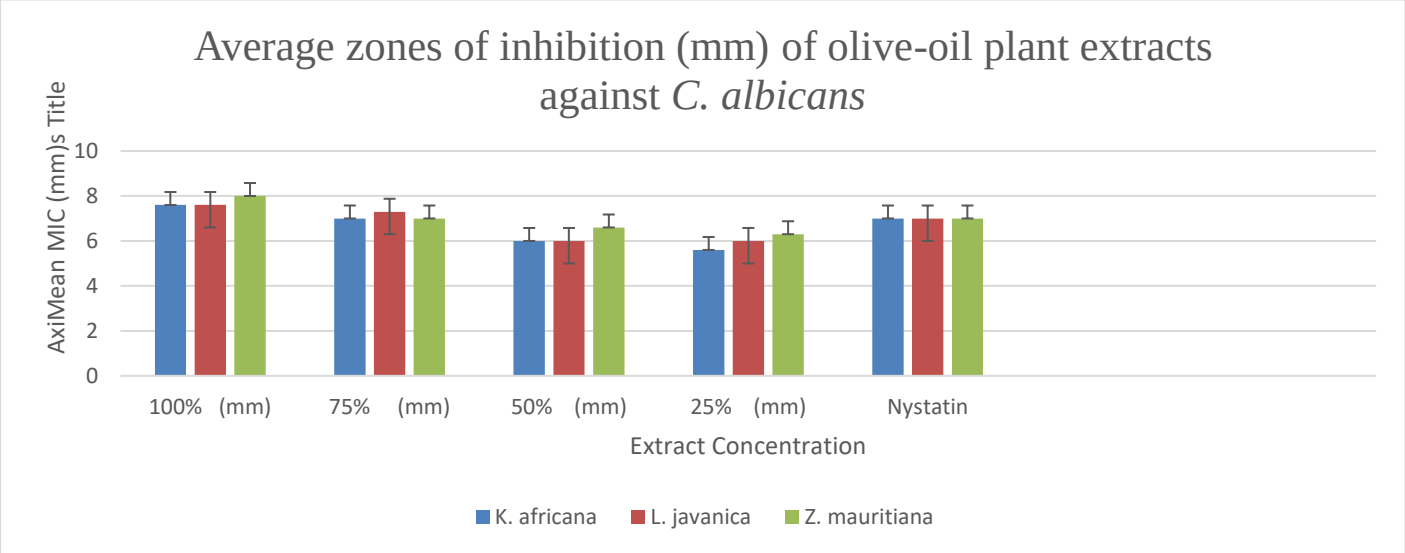

Figure 17 Average zones of inhibition (mm) of olive-oil plant extracts against *C. albicans*

The olive-oil plant extracts inhibited *C. albicans* with the greatest activity at 100% concentration and a general decrease as the concentration reduced to 25%. For *K. africana*, the zones of inhibition were 7.6 mm (100%), 7 mm (75%), 6 mm (50%), and 5.6 mm (25%), showing a clear concentration-dependent reduction. For *L. javanica*, the zones were 7.6 mm (100%), 7.3 mm (75%), 6 mm (50%), and 6 mm (25%), indicating relatively stable activity at mid to low concentrations compared with *K. africana*. For *Z. mauritiana*, the zones were 8 mm (100%), 7 mm (75%), 6.6 mm (50%), and 6.3 mm (25%), making it the most effective extract overall. The positive control produced a 7 mm inhibition zone, while the negative and negative relative controls showed no inhibition, indicating that the antifungal activity observed was due to the plant extracts.

The statistical analysis examines the antimicrobial efficacy of three olive-oil plant extracts, *africana, L. javanica, and Z. mauritiana* against *Candida albicans* using inhibition zone diameter as the primary outcome measure. Two-way ANOVA revealed non-significant effects for plant extract type ($F = 3.28$, $p = 0.0549$) and highly significant effects for extract concentration ($F = 36.09$, $p < 0.001$), with no significant interaction effect ($p = 0.535$).

CHAPTER 5: DISCUSSION

5.1 Introduction

This chapter interprets and discusses the findings presented in Chapter Four in relation to the study objectives, research questions, and existing literature. The discussion focuses on the isolation and characterization of *S. aureus* and *C. albicans*, phytochemical screening of the olive-oil extracts, and the antimicrobial activity of *K. africana*, *Z. mauritiana*, and *L. javanica* against the two pathogens.

5.2 Isolation and Identification of Test Organisms

If *S. aureus* is isolated on Mannitol Salt Agar (MSA) and *C. albicans* is isolated on Potato Dextrose Agar (PDA), then the dermatology surfaces are contaminated with these organisms. MSA is selective and differential medium which favors the growth of halotolerant bacteria like *S. aureus* and differentiates on the basis of mannitol fermentation(Talukdar et al., 2021). Yellow colonies in some isolates suggest fermentation of mannitol which is a characteristic property of pathogenic *S. aureus*. The results are in line with the previous findings that *S. aureus* can be isolated from skin samples because it is naturally found on the skin and in the nose (Sharara et al., 2021).

Likewise, the creamy to white colonies that were seen for *C. albicans* is similar to the typical yeast morphology. The successful isolation also suggests that the skin is a reservoir for opportunistic pathogens, especially in the presence of favorable conditions, such as compromised skin barriers or immunity. The successful isolation also indicates that the skin can serve as a reservoir of opportunistic pathogens, especially when immunity is lowered or skin barriers are compromised (Talapko et al., 2021).

5.3 Morphological and Microscopic Characterization

5.3.1 *Staphylococcus aureus*

Gram staining indicated that isolates B and D showed purple colour and appeared cocci under the microscope and in clusters. It is a 'clumping' pattern typical of Staphylococcus species. The cell wall was thick and retained the crystal violet stain, which was indicative of gram-positive bacteria(Becerra et al., 2016). Isolates A and C gave gram negative reactions which is not in line with the known characteristics of Staphylococcus aureus which is gram positive. This discrepancy could be due to experimental error during Gram staining (e.g., over-decolorization) or to possible contamination and the resulting mixed culture. Based on this and biochemical features, the Gram-positive cocci in clusters, isolates B and D are more confidently identified as *S. aureus*. Definitive identification would require further confirmatory tests including coagulase test and/or DNase test.

5.3.2 *Candida albicans*

The yeast cells were oval in shape with pseudohyphae formation observed under the microscope in *C. albicans*. The capacity to switch between the yeast and filamentous forms is one of the virulence properties of *C. albicans* and is demonstrated by the presence of pseudohyphae. This morphological flexibility is likely to have a role in pathogenicity and penetration of host tissues.

Heavy counts of yeast indicate high level colonization, consistent with an opportunistic pathogen nature of yeast in skin infections (Robbins & Cowen, 2023).

5.4 Biochemical Characterization

5.4.1 *Staphylococcus aureus*

Biochemically, the four isolates have a similar pattern that is consistent with the genus Staphylococcus: all of them are catalase positive and oxidase negative, indole positive and urease positive, with the sugars showing a strong relatedness (Table 5), with Isolates A and B being mannitol-negative and Isolates C and D mannitol-positive, A showing glucose and sucrose fermentation, no fructose fermentation, B showing glucose and fructose fermentation, no sucrose fermentation, C showing sucrose and fructose fermentation, no glucose fermentation, and D showing glucose and sucrose fermentation, no fructose fermentation. The results of mannitol fermentation (A/B negative vs C/D positive) are the most significant difference in the result, so C/D is more consistent with the typical mannitol fermenting phenotype assigned to presumptive identification of *S. aureus*, while A/B may indicate an atypical mannitol fermenting phenotype, test variation, or even be consistent with a related catalase-positive species, so the mannitol fermentation result should be treated as supportive rather than confirmatory and further species identification (e.g., coagulase and/or DNase) would help confirm the conclusions.

Most isolates were urease positive and oxidase negative, which are typical biochemical characteristics of *S. aureus*. Isolates differed in their ability to ferment mannitol: some isolates produced acid (yellow medium) and others did not. This is a variation that suggests strain differences, typical of clinical isolates(Kenny et al., 2013). All the isolates grew positive with indole, a result which does not support the general biochemical characteristics of *S. aureus*, which is normally indole negative. This error may be procedural, or it could have been caused by contamination or by the inaccuracy of the reagents used. Thus, more emphasis is given to the more reliable identification tests in the confirmation of *S. aureus* isolates which include catalase activity, Gram staining and mannitol fermentation.

5.4.2 *Candida albicans*

100% of all isolates were catalase positive and urease negative, consistent with the characteristics of typical *C. albicans*. Glucose can be metabolized by fermentation, indicating metabolic activity. The citrate

utilization and indole reaction results along with the differences in the maltose fermentation may be due to strain variability.

The formation of chlamydospores in both isolates E and G supports species identification as *C. albicans* is known for producing chlamydospores(Whiteway & Bachewich, 2007). These results confirm the proper identification of the fungus isolates.

5.5 Phytochemical Screening

All three plant extracts contained compounds with biological activity as revealed through phytochemical analysis. *K. africana*, *Z. mauritiana* and *L. javanica* were found to contain flavonoids, phenolic compounds and terpenoids. The antimicrobial, antioxidant and anti-inflammatory properties of these compounds is well established (Abbas et al., 2024)

The *Z. mauritiana* had the highest overall phytochemical content, especially for alkaloids and tannins. The higher antimicrobial activity recorded (Alli & Nnodu, 2020) is its ability to interfere with the cell wall and enzymes of microbes. The detection of terpenoids in all the extracts is very important as terpenoids are known to cause leakage of cellular contents and cell death due to their ability to disrupt microbial membranes (Qi et al., 2020)The three plant oils could differ in antimicrobial activity due to the different intensities of phytochemicals.

5.6 Antimicrobial Activity Against *S. aureus*

Results of disc diffusion activity showed that all olive-oil extracts had antibacterial activity against *S. aureus*. The zones of inhibition were reduced with decreasing concentration, which showed a dose-dependent response. The higher concentration means that there are more compounds that are active enough to inhibit the bacteria growth. *Z. mauritiana* possessed the best antibacterial activity, with *K. africana* having a higher activity than *L. javanica* which had the least inhibitory activity. The higher activity of *Z. mauritiana* might be due to its more potent phytochemical composition, notably alkaloids and tannins. The presence of measurable inhibition zones compared to the controls indicates that the antimicrobial effect is related to the antimicrobial plant extracts and not the solvent. These are findings that corroborate long-standing use of these plants for the treatment of bacterial skin infections.

5.7 Antifungal Activity Against *Candida albicans*

All extracts were found to have antifungal activity against *C. albicans*, with smaller inhibition zones than those seen for *S. aureus*. This indicates that under the tested situations these oils were more effective against bacteria than fungi. However, *Z. mauritiana* was the most active antifungal agent closely followed by *L. javanica* and *K. africana*. The antifungal activity could possibly be related to flavonoids and terpenoids that

are able to damage fungal cell membranes and inhibit enzyme activity (Hsu et al., 2021). The zone size is also reduced with the decrease in concentration, which further attests a concentration dependent inhibition.

Statistical Analysis

The differences in antimicrobial activity between the plant extracts are not chance ($p < 0.05$). This reinforces the conclusion that *Z. mauritiana* has better antimicrobial activity than other plants tested. The differences in activity can be explained by the differences in phytochemical compositions with higher levels of alkaloids and tannins.

5.8 Comparison with Previous Studies

Other ethnomedicinal plants like *Lippia javanica*, *Ziziphus mauritiana* and *Kigelia africana* have also been reported to possess antimicrobial activity (Abbas et al., 2024).

The results obtained from this study are in accordance with the previous study on identification of bioactive compounds that inhibit the growth of microbes in these plants. For instance, (Asowata-Ayodele et al., 2016)found that extracts of *Lippia javanica* have antimicrobial and antifungal properties, with the presence of flavonoids and terpenoids. Similarly, research on *Kigelia africana* has also demonstrated that extracts from the fruit exhibit antibacterial properties against several Gram-positive bacteria, such as *Staphylococcus aureus* (Khan, 2025). The disrupted cell walls of bacteria and the microbial metabolic processes are thought to be the cause of the activity exhibited by these compounds(Chilala et al., 2025). Furthermore, earlier studies have shown that *Ziziphus mauritiana* possesses high antimicrobial and antioxidant properties, which are attributed to its alkaloids, tannins and phenolic compounds(Alli & Nnodu, 2020). The findings of the present study corroborate these results with the *Z. mauritiana* having the maximum ZOI against both *S. aureus* and C. *albicans*. (Prakash et al., 2021).

The inhibition zones in this study were comparatively small, however, when compared to other studies that employed organic solvent extracts. The difference may be attributed to the use of olive oil as an extraction solvent as it may not extract as many active compounds as other solvents, such as methanol or ethanol.

5.9 Limitations of the Study

This work was successfully able to demonstrate the antimicrobial activity of the selected plant oils but there were some limitations.

Firstly, the study employed olive oil as an extraction medium, and this could have led to the choice of less efficient solvent (olive oil) to extract all the bioactive compounds. Secondly, there was only the disc

diffusion method used to test the antimicrobial activity. Other techniques, such as determining the minimum inhibitory concentration (MIC) of the extracts, might yield a more accurate picture of the antimicrobial power of the extracts.

A further drawback is that the study was conducted with a restricted number of microbial isolates, which might not reflect the true diversity of clinical isolates. In addition, the plant material used can vary in phytochemical content based on such environmental factors as maturity and geographical location, and drying conditions. Although these constraints existed, the study revealed fruitful initial findings in support of the antimicrobial activity of the plants used in the ethnomedicinal practices.

CHAPTER 6: CONNCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study aimed to evaluate and compare the antimicrobial efficacy of olive-oil macerated extracts of *K. africana*, *Z. mauritiana*, and *L. javanica* against *S. aureus* and *C. albicans*, which are important causative agents of skin infections. The findings of this research provide scientific evidence supporting the traditional medicinal use of these plants in managing microbial infections.

The successful isolation and biochemical confirmation of *S. aureus* and *C. albicans* from dermal samples confirmed the presence of these pathogens on skin surfaces, highlighting their relevance in skin-related infections. Morphological, Gram staining, and biochemical test results were consistent with standard identification characteristics, validating the reliability of the experimental procedures.

Phytochemical screening revealed that all three plant extracts contained bioactive compounds, particularly flavonoids, phenolic compounds, and terpenoids, which are known for their antimicrobial properties. *Z. mauritiana* demonstrated a comparatively stronger presence of several phytochemicals, including alkaloids and tannins, which may have contributed to its higher antimicrobial performance.

The antimicrobial susceptibility testing using the disc diffusion method showed that all extracts exhibited inhibitory activity against both microorganisms. The zones of inhibition were concentration-dependent, with higher concentrations producing larger inhibition zones. Among the three plant extracts, *Z. mauritiana* consistently demonstrated the highest antimicrobial activity against both *S. aureus* and *C. albicans*, followed by *K. africana*, while *L. javanica* showed comparatively lower but still measurable activity. Overall, the results indicate that the olive-oil macerated extracts possess significant antibacterial and antifungal properties, with greater effectiveness observed against *S. aureus* than *C. albicans*. The findings suggest that these ethnomedicinal plants have potential as alternative or complementary therapeutic agents in the treatment of skin infections, particularly in the context of increasing antimicrobial resistance.

Therefore, the study concludes that there is evidence of antimicrobial activity among the tested plant oils, supporting the alternative hypothesis that significant differences exist in their antimicrobial effects. These results contribute valuable scientific data to validate traditional knowledge and encourage further pharmacological exploration of these medicinal plants.

6.2 Recommendations

Based on the findings of this study, the following recommendations are made:

1. Future research should include minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) assays to determine the precise potency of the extracts.
2. Active compounds responsible for antimicrobial activity should be isolated, purified, and structurally characterized using advanced techniques such as GC-MS, HPLC, or NMR analysis.
3. Animal model studies should be conducted to evaluate the safety, toxicity, and therapeutic effectiveness of these extracts before clinical application.
4. Comprehensive toxicity testing is necessary to ensure the safety of long-term use, especially for topical applications.
5. The development of standardized topical formulations (e.g., creams, ointments, or gels) containing these plant extracts should be explored for potential commercial and clinical use.
6. Further research should evaluate the effectiveness of these extracts against antibiotic-resistant strains such as methicillin-resistant *S. aureus* (MRSA) and drug-resistant *Candida* species.
7. Studies with larger sample sizes and multiple clinical isolates should be conducted to enhance the reliability and generalizability of the findings.

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Appendix

Table 4.3.1: Microscopic examination of *S. aureus*

A	B	C	D
Pink	purple	pink	purple
Scattered	scattered	Net like	netlike
micrococci	micrococci	cocci	cocci
chained	chained	chained	chained

Table 4.3.2: Microscopic Examination of *C. albicans*

E	F	G
Purple	Purple	purple
micrococci	micrococci	micrococci
chained	chained	chained

Table 4.6.1: Antimicrobial test results for *S. aureus* showing zones of inhibition, mean, stanfard deviations

Plant extract	100%	75%	50%	25%	+ve control	_ve control	_ve relative control
K. africana 1	10mm	8mm	7mm	6mm	7mm	_	_
K. africana 2	9mm	8mm	8mm	6mm	7mm	_	_
K. africana 3	9mm	9mm	7mm	7mm	7mm	_	_
mean	9.33333333	8.33333333	7.33333333	6.33333333	7		
Standard deviation	0.57735027	0.57735027	0.57735027	0.57735027	0		
L. javanica 1	7mm	7mm	6mm	6mm	7mm	_	_
L. javanica 2	8mm	7mm	6mm	6mm	7mm	_	_
L. javanica 3	9mm	7mm	6mm	6mm	7mm	_	_
mean	8	7	6	6	7		
Standard deviation	1	0	0	0	0		
Z. mauritiana 1	11mm	10mm	9mm	8mm	7mm	_	_
Z. mauritiana 2	9mm	8mm	8mm	7mm	7mm	_	_
Z. mauritiana 3	11mm	9mm	9mm	7mm	7mm	_	_

mean	10.3333333	9	8.66666667	7.33333333	7		
Standard deviation	1.15470054	1	0.57735027	0.57735027	0		

Table 4.6.1: Antimicrobial test results for *C. albicans* showing zones of inhibition, mean and standard deviatiins

.

Plant extract	100%	75%	50%	25%	+ve control	_ve control	_ve relative control
K. africana 1	8mm	7mm	6mm	5mm	7mm	_	_
K. africana 2	8mm	7mm	6mm	6mm	7mm	_	_
K. africana 3	7mm	7mm	6mm	6mm	7mm	_	_
mean	7.66666667	7	6	5.66666667	7		
Standard deviation	0.57735027	0	0	0.57735027	0		
L. javanica 1	8mm	7mm	6mm	6mm	7mm	_	_
L. javanica 2	7mm	7mm	6mm	6mm	7mm	_	_
L. javanica 3	8mm	8mm	6mmm	6mm	7mm	_	_
mean	7.66666667	7.33333333	6	6	7		
Standard deviation	0.57735027	0.57735027	0	0	0		
Z. mauritiana 1	8mm	7mm	7mm	6mm	7mm	_	_
Z. mauritiana 2	8mm	7mm	7mm	7mm	7mm	_	_

Z. mauritiana 3	8mm	7mm	6mm	6mm	7mm	_	_
mean	8	7	6.66666667	6.33333333	7		
Standard deviation	0	0	0.57735027	0.57735027	0		

Statistical Summary Table

Parameter	S. aureus	C. albicans
Extract Effect (ANOVA)	F = 26.36, p < 0.001 ***	F = 3.28, p = 0.055 (ns)
Concentration Effect (ANOVA)	F = 27.05, p < 0.001 ***	F = 36.09, p < 0.001 ***
Interaction Effect (ANOVA)	F = 0.557, p = 0.760 (ns)	F = 0.865, p = 0.535 (ns)
Mean Inhibition Range	6.75–8.88 mm	6.55–6.975 mm
Regression R^2 Range	0.695–0.849	0.718–0.829
Significant Pairwise Differences	Z. mauritiana vs L. javanica (p = 0.001)	None (all p > 0.05)
Positive Control	7.0 ± 0 mm	7.0 ± 0 mm
Negative Control	No inhibition	No inhibition

Extract Performance Summary

Staphylococcus aureus (Mean ± SD, mm)

Extract	100%	75%	50%	25%	Mean
K. africana	9.3 ± 0.577	8.3 ± 0.577	7.3 ± 0.577	6.3 ± 0.577	7.8 ± 1.20
L. javanica	8.0 ± 1.000	7.0 ± 0.000	6.0 ± 0.000	6.0 ± 0.000	6.75 ± 0.90
Z. mauritiana	10.3 ± 1.155	9.0 ± 1.000	8.3 ± 0.577	7.3 ± 0.577	8.88 ± 1.30

Candida albicans (Mean ± SD, mm)

Extract	100%	75%	50%	25%	Mean
K. africana	7.6 ± 0.577	7.0 ± 0.000	6.0 ± 0.000	5.6 ± 0.577	6.55 ± 0.80
L. javanica	7.6 ± 0.577	7.3 ± 0.577	6.0 ± 0.000	6.0 ± 0.000	6.725 ± 0.70
Z. mauritiana	8.0 ± 0.000	7.0 ± 0.000	6.6 ± 0.577	6.3 ± 0.577	6.975 ± 0.80

Linear Regression Parameters

Staphylococcus aureus

Extract	Slope	Intercept	R^2	p-value
K. africana	0.0400	5.30	0.849	2.06×10^{-5}
L. javanica	0.0280	5.00	0.717	5.11×10^{-4}
Z. mauritiana	0.0388	6.30	0.695	7.51×10^{-4}

Candida albicans

Extract	Slope	Intercept	R^2	p-value
K. africana	0.0280	4.80	0.829	3.86×10^{-5}
L. javanica	0.0244	5.20	0.718	5.04×10^{-4}
Z. mauritiana	0.0220	5.60	0.723	4.58×10^{-4}

Post-hoc Tukey HSD Test Results

Staphylococcus aureus

Comparison	Mean Difference	p-adjusted	Significant
K. africana vs *L. javanica*	−1.05 mm	0.0996	No
K. africana vs *Z. mauritiana*	−0.925 mm	0.1621	No
L. javanica vs *Z. mauritiana*	−1.975 mm	0.0010	Yes

Candida albicans

Comparison	Mean Difference	p-adjusted	Significant
K. africana vs *L. javanica*	0.175 mm	0.8649	No
K. africana vs *Z. mauritiana*	0.425 mm	0.4334	No
L. javanica vs *Z. mauritiana*	0.25 mm	0.7447	No