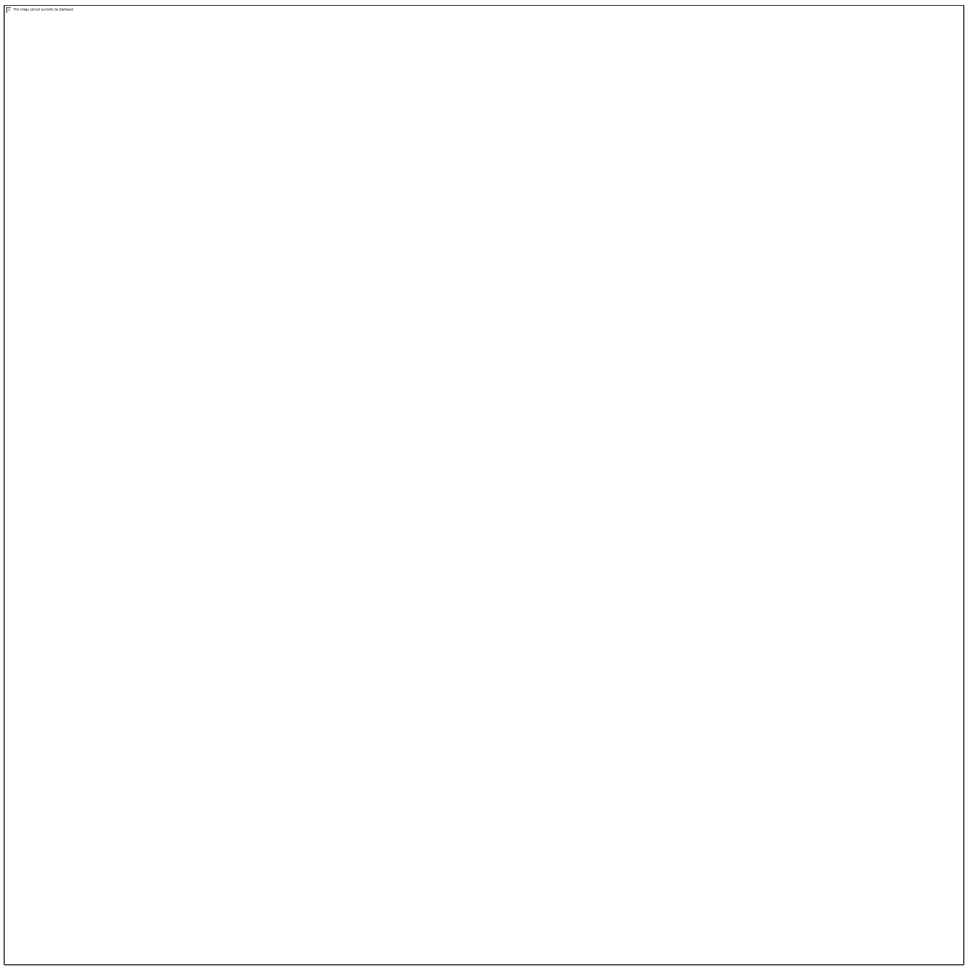


Title Exploring the Antibacterial effects of *Kigella africana* (mumvee/ umbvebe) extracts against *Escherichia coli.*

Micah K. Kachisi

B212775B

A project submitted in partial fulfilment of the requirements of Bachelor of Science Honours degree in Biological Sciences

June 2025

APPROVAL FORM

TITTLE OF DISSERTATION: EXPLORING THE ANTIBACTERIAL EFFECTS OF *KIGELLA AFRICANA* (MUMVEE) EXTRACTS AGAINST *ESCHERECHIA COLI.*

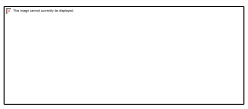
Signature of student…  Date 16 June 2025

Signature of supervisor : Siamayuwa CES Date

Signature of chairperson of department:  Date: 90/10/25

DECLARATION

I, Micah K Kachisi, declare that this research is my own work and has not been plagiarized from another source without acknowledgment of the concerned author either electronically or otherwise

Signature  **Date** 16June 2025.

Micah K Kachisi

Student

Supervisor

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

Signature: Siamayuwa CES Date: 16 June 2025

DEDICATION

This project is dedicated to my beloved parents and friends who have been a great source of inspiration from the time I started up till the end of the project, without forgetting the Holy Ghost my greatest inspiration.

ACKNOWLEDGEMENTS

I want to sincerely thank my supervisor, Mr Siyamayuwa for the support, tolerance, and direction during the entire endeavor. I also want to express my gratitude to my family for their financial and moral assistance. I seek to acknowledge the help I got from my friends. Lastly; I would like to acknowledge the lab technician Mr Katsande who helped me every step of the way. Above all, I want to express my gratitude to the Lord God Almighty for giving me the ability to finish this project.

Definition of Terms

- African sausage tree

  Common name for Kigelia africana, a plant native to sub-Saharan Africa, known for its large sausage-shaped fruits and traditional medicinal uses.
- AMR (Antimicrobial Resistance)

  The ability of microorganisms like bacteria to survive exposure to drugs that were once effective against them, posing serious treatment challenges.
- Antibacterial

  Refers to substances that inhibit the growth of or destroy bacteria.
- Anticancer

  Describes a substance that prevents or slows the growth of cancerous cells.
- Antioxidant

  A molecule that prevents or delays oxidation, defending cells from damage caused by free radicals.
- Anti-inflammatory

  A substance that reduces inflammation, swelling, or irritation in tissues, often used to treat infections or injuries.
- Bactericidal

  Describes a substance that kills bacteria rather than just inhibiting their growth.
- Bacteriostatic

  A substance that prevents bacterial growth without necessarily killing the bacteria.
- Biofilm formation

  The ability of bacteria to attach to surfaces and form protective communities that are resistant to antibiotics.
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- Disc diffusion assay

  A laboratory method used to evaluate antimicrobial activity by placing sample-impregnated discs on bacterial lawns and measuring zones of inhibition.

- Efflux pumps

  Bacterial mechanisms that actively expel antibiotics from the cell, reducing drug effectiveness.
- E. coli (Escherichia coli)

  A Gram-negative bacterium commonly found in the intestines. Some strains are pathogenic, causing infections such as UTIs, septicemia, and gastroenteritis.
- ESBLs (Extended-Spectrum Beta-Lactamases)

  Enzymes produced by certain bacteria, including E. coli, that inactivate a wide range of beta-lactam antibiotics, leading to drug resistance.
- Ethnomedicine:

  Traditional medicinal practices based on cultural heritage.

- Flavonoids

  A group of polyphenolic compounds found in plants, known for their antioxidant, anti-inflammatory, and antimicrobial properties.
- Gram-negative bacteria

  Bacteria that do not retain the crystal violet stain in the Gram-staining procedure due to a thinner peptidoglycan wall and an outer membrane. They are often more resistant to antibiotics.
- Hydroalcoholic extracts

  Extracts derived using a combination of water and alcohol, effective for extracting both polar and non-polar phytochemicals.
- In vitro

  Laboratory experiments conducted outside a living organism, typically in test tubes or petri dishes.
- In vivo

  Experiments conducted inside a living organism such as an animal models.

- Iridoids

  A class of secondary metabolites with known antimicrobial, anti-inflammatory, and antioxidant activities.
- K. africana (Kigelia africana)

  A medicinal plant indigenous to sub-Saharan Africa, traditionally used to treat various infections and diseases. Known for its large, sausage-shaped fruits.
- MBC (Minimum Bactericidal Concentration)

  The lowest concentration of an antimicrobial agent required to kill a particular bacterium.
- MIC (Minimum Inhibitory Concentration)

  The lowest concentration of an antimicrobial agent that prevents visible bacterial growth after overnight incubation.
- Pathogenic

  Capable of causing disease. Used to describe harmful strains of microorganisms such as E. coli.
- Phenolic acids

  A type of phytochemical with strong antioxidant and antimicrobial properties found in many plants.
- Pharmacognosy

  The study of medicinal drugs derived from natural sources such as plants and minerals.
- Phytochemicals

  Chemical compounds produced by plants, often secondary metabolites, which have therapeutic or biological activity.
- Phytotherapeutic framework

  A theoretical model supporting the use of medicinal plants and their bioactive compounds in drug development.
- Quinones

  Aromatic compounds found in plants that exhibit antimicrobial and anticancer activities, often through redox cycling and protein inhibition.
- Resistance-reversal theory

  A theory suggesting that natural compounds can help restore antibiotic efficacy against resistant bacteria by targeting resistance mechanisms.

- Saponins

  A class of chemical compounds in plants that create foam in water and disrupt bacterial membranes, contributing to antimicrobial activity.
- Secondary metabolites

  Organic compounds not directly involved in the normal growth, development, or reproduction of plants but often responsible for biological activity such as antimicrobial effects.
- Solvent extracts

  Plant extracts obtained using solvents like methanol, ethanol, hexane, or water to dissolve bioactive components for testing.
- Tannins

  Polyphenolic plant compounds that bind to proteins and interfere with microbial enzymes, contributing to antibacterial properties.
- Terpenoids

  A large and diverse class of naturally occurring compounds derived from terpenes, with demonstrated antimicrobial, anti-inflammatory, and antioxidant activities.
- Zone of inhibition

  a clear area around an antimicrobial disc on an agar plate, showing the extent of bacterial growth suppression.

Abstract

Escherichia coli is a gram negative bacterium which causes numerous infections, that includes urinary tract infections, gastrointestinal illnesses, and septicemia. The emergency of antibiotic-resistant strains, espacially those producing extended spectrum beta lactamases (ESBLs), has increased the requirement to search for alternative antimicrobial agents (O'Meara & Nesbit, 2013). *Kigelia africana* (locally known as Mumvee tree) is traditionally used in African ethnomedicine to treat infections, but its antibacterial efficacy against *E. coli* has not been fully explored (Iwu, 2014; Agyare et al., 2013).

This study investigated the antimicrobial potential of *K. africana* fruit extract obtained using methanol, ethanol, water, and hexane and tested against *E. coli* using broth microdilution for Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and disk diffusion assays. Ciprofloxacin served as the positive control. MIC results showed that ethanol and methanol extracts inhibited *E. coli* at 25% concentration, while ciprofloxacin inhibited at 5%. Water and hexane extracts required 50% and 75%, respectively. These differences were statistically significant ($F(4,10) = \infty$, $p < 0.001$), confirming that extract type significantly influenced MIC outcomes.

For MBC, ethanol and methanol extracts showed bactericidal activity at 50%, water at 75%, and hexane at 100%, compared to ciprofloxacin at 6.25% (control). ANOVA also revealed a significant difference in MBC across treatments ($F(3,8) = 5.3$, $p < 0.021$), indicating variable bactericidal potency depending on solvent type. In the disk diffusion assay, ciprofloxacin showed the largest mean inhibition zone (22.00 mm)as it was a control, followed by ethanol (15.67 mm), methanol (13.67 mm), water (10.67 mm), and hexane (9.33 mm). Tukey HSD post hoc tests showed ciprofloxacin was significantly more effective than all extracts ($p < 0.001$), and ethanol outperformed water and hexane extracts ($p < 0.05$), (Newman & Cragg, 2020; World Health Organization, 2020).

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These results align with previous studies that found methanol and ethanol to be efficient solvents for extracting antimicrobial phytochemicals from *K. africana* (Eloff, 1998; Akinmoladun et al.,

2020). The findings support the ethnomedicinal use of *K. africana*, especially ethanol and methanol extracts, as promising antimicrobial agents. However, ciprofloxacin remains significantly more potent. Further phytochemical screening, toxicity studies, and mode-of-action investigations are warranted to validate clinical applicability (Agyare et al., 2013; Irobi & Akinjogunla, 1994).

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Chapter One

Introduction

1.1 Background of the Study

K. africana, commonly recognized as the African sausage tree, is native to sub-Saharan Africa, and is known by its large, sausage-shaped fruits (Okwu & Ekeke, 2006). Phytochemical analyses show that *K. africana* contains flavonoids, tannins, saponins, terpenoids Traditionally, different parts of the plant, that is the bark, the fruits, the leaves, and the flowers have been utilized in the treatment of ulcers, skin infections, respiratory sicknesses, and digestive disorders (Ojewole, 2004; Burkill, 1985), and phenolic acids compounds known for antibacterial, reduces oxidative stress, soothes nflammation, and has anticancer effects (Mulaudzi et al., 2011; Adewusi & Afolayan, 2010; Katerere et al., 2008).

The global rise of antibacterial resistance positions it among the top ten public health threats (WHO, 2020). *E. coli*, a gram negative bacterium often associated with urinary infections, gastroenteritis, septicemia, and neonatal meningitis. It has developed resistance ways such as ESBLs, efflux pumps, target modifying enzymes that challenge conventional antibiotics and biofilm formation (Kaper et al., 2004; Pitout, 2010; Paterson & Bonomo, 2005). The result: more persistent infections, longer hospital stays, and increased healthcare burdens.

Medicinal plants like *K. africana* are being re-evaluated for their antibacterial potential. Despite several studies showing effectiveness against gram positive bacteria, data on gram-negative pathogens especially *E. coli*, are limited. Methanolic stem bark extracts produced zones of inhibition (12–19 mm) and MICs around 5.25 mg/mL against *E. coli* (Agyare et al. SS2013). Additionally, Sidjui Sidjui et al. (2016) reported stem bark extracts and isolated compounds with MICs from 0.1 to >0.83 mg/mL and MBCs of 0.83 mg/mL against *E. coli* highlighting both inhibitory and bactericidal potential. However, within the Zimbabwean context, there is little empirical data on MIC, zone of inhibition, or MBC for local strains of *E. coli* (Ventola, 2015).

This study addresses that gap by evaluating the antibacterial activity via zone of inhibition, MIC, and MBC of methanolic, ethanolic, aqueous, and hexane extracts of *K. africana* fruit against *E. coli*.

1.2 Problem Statement

The global proliferation of antibiotic-resistant *E. coli* particularly ESBL-producing strains presents an urgent public health issue (Ventola, 2015). Despite promising results from other regions, research evaluating *K. africana*'s efficacy specifically against *E. coli* including bactericidal activity is inadequate, especially in Zimbabwe. Without this data, acknowledging the plant's true antibacterial capacity remains speculative. The present study, through standardized assays for MIC, MBC, and inhibition zones, fills this knowledge gap.

1.3 Justification of the Study

With AMR on the rise, identifying effective plant-based antibacterials is critical. *K. africana*, abundant and culturally significant in Zimbabwe, offers a rich source of bioactive phytochemicals. By generating data on its bacteriostatic and bactericidal effects against *E. coli*, this research grounded in traditional use and supported by preliminary findings aims to validate *K. africana* as a potential antibacterial agent. This could influence public health strategies, encourage sustainable phyto-medicine development, and support further toxicological and clinical investigations.

1.4 Aim of the Study

To assess the antibacterial and bactericidal activities of *K. africana* extracts against *E. coli*.

1.4 Objectives of the Study

1. To determine the zone of inhibition for K. *africana* extracts against *E. coli*.
2. To determine the Minimum Inhibitory Concentration (MIC) of the extracts.
3. To determine the Minimum Bactericidal Concentration (MBC) of the extracts.

1.5 Research questions

1. What are the differences in the zones of inhibition produced by ethanol, methanol, hexane, and aqueous extracts of *Kigelia africana* against *E. coli*?
2. At what concentrations do different *K. africana* extracts inhibiy the growth of *E. coli*, and how do these MIC values compare among solvents?

3. What is the bactericidal potential (MBC) of each *K. africana* extract, and how does it relate to the MIC values?

1.6 Research Hypotheses

- H_0: *K. africana* extracts do not exhibit significant antibacterial or bactericidal activity against *E. coli*.
- H_1: *K. africana* extracts do exhibit significant antibacterial and bactericidal activity against *E. coli*.

1.7 Significance of the Study

This study clarifies whether *K. africana* can physically inhibit and kill *E. coli*, which is crucial for therapeutic relevance. The results will help position *K. africana* for inclusion in pharmacognosy pipelines, contribute to regional health policy in Zimbabwe, and support global efforts to leverage ethnobotanical knowledge in combating AMR.

Chapter Two

Literature Review

2.1 Introduction

E. coli is a gram negative, facultative anaerobic bacterium that predominantly colonizes the digestive tract of animals and humans. While most strains are commensal and harmless, certain pathogenic strains are implicated in serious infectious conditions such as urinary tract, neonatal meningitis, septicemia, and diarrheal diseases (Kaper, Nataro, & Mobley, 2004). The clinical challenge of managing *E. coli* infections has escalated significantly due to the global rise of multidrug-resistant strains, especially those producing extended spectrum ESBLs that causes resistance to many β-lactam antibiotics (Paterson & Bonomo, 2005; Pitout, 2010). This growing antibacterial resistance crisis calls for urgent exploration of alternative sources of antibacterial, including medicinal plants traditionally used for infection management (Newman & Cragg, 2020; World Health Organization, 2020).

K. africana, known commonly as the African sausage tree, is native to sub-Saharan Africa, including Zimbabwe, where it has been extensively used in local medicinal practices for treating wounds, skin infections, ulcers, and gastrointestinal ailments (Hutchings, Scott, Lewis, & Cunningham, 1996; Gathirwa et al., 2014). The plant contains a rich diversity of secondary metabolites such as flavonoids, tannins, saponins, iridoids, and quinones, which have showed pharmacological properties including antibacterial, antioxidant, reduce inflammation, and anticancer effects (Elujoba, Odeleye, & Ogunyemi, 2012; Okwu & Omodamiro, 2005; Cowan, 1999; Ibewuike et al., 1997). This phytochemical complexity makes *K. africana* a promising candidate for scientific evaluation against resistant bacterial pathogens such as *E. coli*.

2.1 Antibacterial

Activity of *K. africana* Against *E. coli*

Several in vitro studies have documented the antibacterial effects of *K. africana* extracts against *E. coli*. Methanol extracts of stem bark and leaves inhibited *E. coli* growth with zones of inhibition ranging from 12 to 19 mm and MICs between 15 and 25%. Their findings validate the ethnomedicinal applications of the plant and confirm its antibacterial potential (Agyare et al.

2013). Similarly, Turabi, Zengin, and Uysal (2016) found that ethanolic and water extracts of *K. africana* leaves and bark significantly inhibited *E. coli* in disc diffusion assays, with zones of inhibition comparable to some standard antibiotics. Grace, Manian, and Sreenivasan (2002) isolated active compounds from *K. africana* fruits that exhibited MIC percentage around 20% against *E. coli*, reinforcing the plant's antibacterial efficacy.

Sidjui Sidjui et al. (2016) further advanced this research by isolating specific iridoids and quinones from *K. africana* stem bark extracts, demonstrating potent antibacterial activity with MIC values as low as 0.1 mg/mL and minimum bactericidal concentrations MBCs near 0.83 mg/mL. These data highlight the bactericidal potential of the extracts and their promise as leads for novel antibacterials. Other studies have reported similar potent antibacterial effects using hydroalcoholic extracts, emphasizing solvent choice as critical to maximize extract efficacy (Olawole et al., 2017).

2.1 Mechanisms of Antibacterial Action

The antibacterial effects of *K. africana* are attributed to multiple mechanisms. Flavonoids and saponins disrupt bacterial membranes by integrating into phospholipid bilayers, increasing permeability and causing leakage of cellular contents, leading to bacterial death (Cushnie & Lamb, 2005). Tannins and phenolic compounds precipitate bacterial proteins and inhibit critical enzymes, impairing cell wall synthesis and metabolic functions (Cowan, 1999). Additionally, phenolics exert antioxidant activities that generate oxidative stress within bacterial cells, further promoting cell damage (Rice-Evans, Miller, & Paganga, 1997; Ríos & Recio, 2005). Sidjui Sidjui et al. (2016) identified iridoids and quinones that inhibit bacterial DNA gyrase and protein synthesis, suggesting multiple antibacterial targets and increasing the likelihood of overcoming rresistance mechanisms.

2.2 Gaps in the Current Literature

Despite the promising antibacterial activity of *K. africana*, significant gaps remain. Most studies focus on MIC and disc diffusion assays but lack comprehensive MBC data to confirm bactericidal activity. Variations in extraction methods, solvents, bacterial strains used, and assay conditions create challenges for comparing results. Furthermore, active compounds have been identified primarily from stem bark, with limited studies on fruits and leaves. Importantly, very

few investigations have assessed in vivo efficacy or toxicity, critical steps before clinical application (Olawole et al., 2017).

2.3 Relevance to Zimbabwe

Zimbabwe faces a growing AMR problem, including increasing incidence of ESBL-producing *E. coli*, compounded by constrained access to effective antibiotics due to economic limitations (Ventola, 2015). Given that *K. africana* is indigenous and widely utilized in Zimbabwean traditional medicine, validating its antibacterial activity against local clinical *E. coli* isolates could support sustainable, affordable treatment options. This aligns with Zimbabwe's National Health Strategy, which encourages integrating traditional medicine with modern healthcare systems for sustainable health solutions (Mujuru et al., 2020).

2.4 Theoretical Framework

This study is grounded in the phytotherapeutic framework that posits medicinal plants as reservoirs of bioactive secondary metabolites useful for drug discovery (Fabricant & Farnsworth, 2001). It also aligns with the resistance-reversal theory, emphasizing natural products as promising solutions against antibiotic-resistant bacteria (Wright, 2014). The hypothesis that extracts obtained with different solvents will vary in antibacterial potency due to phytochemical differences reflects an empirical strategy for rational drug discovery (Mujuru et al., 2020).

2.5 Conclusion and Future Directions

K. africana shows compelling in vitro antibacterial and bactericidal activity against *E. coli*, underscoring its potential as a source of antibacterial agents. Future research should prioritize standardizing extraction procedures, detailed phytochemical characterization, testing against local clinical isolates, establishing MBC values, and conducting in vivo safety and efficacy studies. These efforts will be vital to translate *K. africana* from traditional medicine into scientifically validated therapies addressing the AMR crisis (Ventola, 2015).

Chapter 3

Methodology

3.1 Description and Map of the Study Area

This study was done at the Microbiology Laboratory, Department of Biological Sciences, at Bindura University of Science Education in Zimbabwe. The plant material was collected from mature *K. africana* trees in the Bindura Mountains, where Zimbabwe's tropical savanna climate supports the growth of *K. africana* in both urban and semi-urban areas (Mujuru et al., 2020).

3.1 Research Design and Approach

This study used a quantitative experimental approach, conducting laboratory base experiments to asses the antibacterial effects of *K.africana* extracts against *E. coli.* This design allowed for accurate measurement and statistical analyses of zone of inhibition, MIC and MBC

3.2 Collection and Preparation of Plant Material

K. africana fruits (fresh) were collected from the mountains and authenticated by a botanist. They treated with distilled water to impurities, crushed into small pieces, and dried on the sun for 14 days until completely desiccated. The dried plant residue were ground to smooth powder using an electric blender and stored in airtight containers at room temperature (Akinmoladun et al., 2014).

3.3 Extraction of Plant Material

Ethanol Extraction

A total of 10g of *K. africana* fruit powder was soaked in 100 mL of 70% ethanol in a conical flask for 24 hours to allow complete dissolving of substance. The mixture of extracts and ethanol was filtered using Whatman’s filter paper, and the residue was discarded. The filtrate was concentrated under decreased pressure using a rotary evaporator at 40°C until most of the solvent was removed. The crude ethanol extract was collected and dried, over silica gel. The dried extract was weighed, recorded, and stored at 4°C in a sterile container (Olawole et al., 2017).

Methanol Extraction

Similary, 10g of the ground powdered was soaked in 100 mL of 70% methanol in a conical flask and left for 24 hours to completely dissolve. After incubation, the solution was purified using

Wharman's filter paper to remove solid residue. The filtrate was concentrated using a rotary evaporator at 40°C, and followed by drying in a desiccator. The final methanol extract was quantified and stored in sterile containers at 4°C for antibacterial analysis (Akinmoladun et al., 2014)..

Hexane Extraction

For non-polar component extraction, also 10g of *K. africana* ground powder was put into 100ml of hexane in a conical flask, tightly sealed to prevent solvent evaporation and allowed todisolve for 24 hours. Afterward, the mixture was filtered using Whatman's filter paper, and the filtrate was subjected to rotary evaporation at 40°C to remove the hexane. The dried hexane extract was stored at 4°C in sterile vials for further testing. Due to hexane's volatility, all steps were conducted in a well-ventilated fume hood (Sasidharan et al., 2011).

Distilled Water Extraction

10g of *K. africana* powder was immersed in 100 mL of distilled water for 24 hours. Continuous motion was employed to enhance the contact between the plant material and the aqueous solvent, thereby improving the efficiency of the extraction process. Filtration was done using Whatmans's filter paper to isolate the liquid extract from the plant residue. The obtained aqueous filtrate was then concentrated to reduce its volume using a rotary evaporator set at 40°C under reduced pressure. After rotary evaporation, the semi-concentrated extract was further dried in a desiccator containing anhydrous silica gel to eliminate residual moisture and obtain a dry or semi-dry extract. The final aqueous extract was weighed using an analytical balance and transferred into sterile, airtight containers. The prepared extract was then stored at 4°C in a refrigerator to maintain its stability and prevent microbial degradation prior to its use in antibacterial susceptibility testing (Olawole et al., 2017).

3.4 Isolation and Identification of *E. coli*

A clinical isolate of *E. coli* was obtained by swabbing the stool sample of a consenting healthy individual following aseptic procedures. The sample was inoculated on MacConkey agar and incubated at 37°C for 24 hours. Colonies producing pink to red colonies on MacConkey agar were considered presumptive *E. coli* and were further confirmed by Gram staining (Cheesbrough, 2006).

3.5 Preparation of Inoculum

Collection of Stool Swab

Stool swab samples were collected using a sterile cotton swab. The swab was gently rolled over freshly passed stool and then placed into a sterile container containing 1–2 mL of sterile saline solution to maintain bacterial viability until further processing.

Inoculation onto MacConkey Agar

MacConkey agar, a selective gar, was used to separate gram negative bacteria *E. coli.* Its bile salt and crystal violate content inhibit gram positive bacteria. *E. coli* ferments the lactose and produce acid turning colonies pink red. To culture *E. coli* a swab was was streaked onto freshly prepared MacConkey agar plate in pattern allowing individual colony isolation. Following 24 hour incubation at 37 degrees Celsius typical *E. coli* colonies appeared pink red (Cheesbrough, 2006).

Confirmatory Identification of *E. coli*

Gram staining was performed on isolated colonies. The isolate appeared as Gram-negative rods under the microscope, confirming its morphology consistent with *E. coli*. Further biochemical tests (Cheesbrough, 2006).

Subculturing into Nutrient Broth

Once *E. coli* was confirmed, a single well-isolated colony was selected from the MacConkey agar plate and transferred to sterile nutrient broth. This broth served as a liquid medium to maintain the bacterial culture for further testing. The tube was incubated at 37°C for 24 hours to allow bacteria to grow and reach adequate concentration for antibacterial testing.

3.6 Preparation of *E. coli* Suspension for Antibacterial Testing

A pure culture of *E. coli* was then subcultured on nutrient agar and incubated at 37°C for 24 hours. A loopful of culture was transferred into sterile normal saline (0.85% NaCl) and vortexed to form a uniform suspension. The opacity of the bacterial suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL, ensuring reproducibility and accuracy in testing (Balouiri, Sadiki, & Ibnsouda, 2016).

3.7 Antibacterial Susceptibility Testing by Disc Diffusion Method

Disk diffusion method was employed to test antimicrobial susceptibility through measuring zone of inhibition (CLSI, 2020). A sterile swab was used to seed uniformly bacterial inoculum on MHA plates. Sterile paper discs 6 mm diameter were saturated with 20 µL of each plant extract (at 100 mg/mL concentration) and allowed to dry. The disks were aseptically dropped into the petri dishes 37°C for 24 hours. After the incubation zones of inhibition were measured using a transparent ruler, and results were compared with standard antibiotic discs ciprofloxacin as a positive control and discs with only solvent as negative controls (Bauer et al., 1966).

The antibacterial activity of the various *K. africana* extracts against *E. coli* was evaluated using the agar disc diffusion method (Bauer et al. 1966 and CLSI 2020). This method is widely employed for preliminary screening of antibacterial agents due to its simplicity, reproducibility, and effectiveness in determining the presence of growth inhibition.

Preparation and Application of Extract Discs

sterile paper disks were autoclaved first to sterilize them and they were impregnated with 20 µL of the respective *K. africana* extract prepared at 100 mg/mL concentration in its respective solvent that is ethanol, methanol, hexane, or distilled water. The discs with extracts were air dried aseptically inside a laminar flow cabinet to ensure complete evaporation of solvent, and this was to prevent any possible interference in zone of inhibition measuring (CLSI, 2020.

Once dried, extract-impregnated discs were aseptically placed onto the surface of inoculated MHA plates using sterile forceps, ensuring firm contact with the agar surface and sufficient spacing between discs to prevent overlapping of inhibition zones. Each plate was fitted with multiple discs, including test discs, positive control, and negative control (CLSI, 2020.

3.7 Control Discs

A commercially prepared antibiotic disc containing ciprofloxacin (5 µg/disc) was used as the positive control to serve as a reference for antibacterial efficacy and to confirm the sensitivity of the *Escherichia coli* strain. In contrast, the negative control consisted of filter paper discs impregnated with 20 µL of the respective solvent ethanol, methanol, hexane, or distilled water used to ensure that the solvents alone did not exhibit any antibacterial activity (CLSI, 2020).

3.8 Incubation and Measurement of Zones of Inhibition

All prepared plates were incubated upsidedown at 37°C for 24 hours. Plates were then checked for clear zones of inhibition around discs, to indicate antibacterial effects. The diameter of each zone was measured in millimeters using a sterile transparent ruler, from edge to edge across the center of the clear zone including the disc diameter.

Each test was performed in 3 sets to ensure reliability and reproducibility of results. Mean zones of inhibition for each extract were calculated and recorded. Antibacterial activities of plant extracts were compared to positive and negative controls to assess relative efficacy. This disc diffusion assay provided an initial indication of the antibacterial potential of *K. africana* extracts (Bauer et al., 1966).

3.9 Determination of Minimum Inhibitory Concentration (MIC) by Microbroth Dilution

The MIC of *K. africana* extracts against *E. coli* was established using the microbroth dilution method in sterile test tubes, following CLSI (2020) guidelines.

Preparation of Extract Dilutions

Each raw extract was initially dissolved in Ringer's solution to create a stock solution of 100 mg/mL. From this stock, three fold serial dilutions were prepared in sterile MHB to obtain a final concentration range of 25% to 100%. Dilutions were prepared in sterile 5 mL test tubes, each containing 1 mL MHB and required volume of extract to achieve intended concentrations (CLSI, 2020).

Inoculation and Control Setup

Each test tube containing extract dilution in MHB was inoculated with 1 mL bacterial suspension in Ringer's solution, total volume 2 mL. Controls included

In this study, various controls were used to ensure the accuracy and reliability of the antibacterial assays. The positive control consisted of MHB and a bacterial suspension without any plant extract, which served to confirm normal bacterial growth in the absence of treatment. The negative control included MHB and the respective plant extract without bacterial inoculation, allowing

verification of the sterility of the medium and checking for any turbidity caused by the extract itself. Where applicable, a solvent control was also employed, comprising MHB, the solvent used to dissolve the extract (at the same concentration), and the bacterial suspension. This was done to rule out any potential antibacterial effects attributable to the solvent alone (CLSI, 2020).

Incubation

All tubes were securely sealed and incubated at 37°C for 24 hours under aerobic conditions. Tubes were gently shaken before incubation to ensure proper mixing.

3.10 Determination of MIC

MIC was described as the lowest concentration of extract showing no visible turbidity, indicating thorough inhibition of bacterial growth.

3.11 Determination of Minimum Bactericidal Concentration (MBC)

Following the determination of the MIC, the MBC of *K. africana* extracts against *E. coli* was determined using the agar plate subculture method as described by CLSI (2020).

From each test tube showing no visible growth after the MIC assay, different percentages aseptically taken and spread evenly onto freshly prepared MHA plates. The plates were incubated at 37°C for 24 hours. After incubation, plates were examined for bacterial colony growth.

The MBC was defined as the lowest concentration of the extract that resulted in no bacterial growth that is 99.9% killing of the original inoculum on the agar plate, indicating bactericidal activity. The procedure was performed in triplicate to ensure reproducibility. Control plates inoculated with bacteria but without extract confirmed bacterial viability, while plates inoculated with extract alone served as sterility controls (CLSI, 2020).

3.12 Statistical Analysis

All experiments were performed in triplicates, and results were presented as mean ± standard deviation. Statistical differences in the antibacterial activities of various extracts were analyzed using one-way ANOVA, with $p < 0.05$ considered statistically significant.

Chapter 4

Introduction

4.0 Minimum Inhibitory Concentration

To evaluate the antibacterial activity of different *K. africana* fruit extracts against *E. coli*, Minimum Inhibitory Concentration (MIC) percentages were measured across four concentrations (25%, 50%, 75%, and 100%). The solvents used for extraction were methanol, ethanol, water, and hexane. Ciprofloxacin was used as a positive control to validate the antibacterial test (CLSI, 2020).

Table 1.0 MIC (%) of *K. africana* Extracts Against *E. coli*

Extract Type	Replicate 1 (MBC %)	Replicate 2 (MBC %)	Replicate 3 (MBC %)
Ethanol	25	25	25
Methanol	25	25	25
Water	50	50	50
Hexane	75	75	75
Ciprofloxacin	6.25	6.25	6.25

4.1 Interpretation

All three replicates for both ethanol and methanol extracts showed high bactericidal activity at 25% concentration. This indicates a moderate level of potency, with both solvents appearing equally effective in extracting bioactive compounds responsible for the bactericidal effect. In contrast, the water extract exhibited bactericidal activity at a higher concentration of 50% across all replicates, suggesting it was less effective than the ethanol and methanol extracts. The hexane extract required the highest concentration 75% to demonstrate bactericidal activity in all replicates, indicating it was the least effective solvent for extracting antimicrobial agents from *K. africana*. This may be due to the type of extracts pulled by hexane. Ciprofloxacin, used as a positive control,

consistently showed MBC at 6.25% across all replicates, confirming its superior bactericidal potency as expected from a pharmaceutical-grade antibiotic (Balouiri, Sadiki, & Ibnsouda, 2016).

4.2 Statistical Analysis Using One-Way ANOVA (SPSS)

A one-way ANOVA was conducted using SPSS to assess whether the type of extract significantly influenced MIC values against *E. coli*. The independent variable was extract type, and the dependent variable was MIC (%).

Table 1.2 Descriptive Statistics of MIC by Extract Type

Treatment	**N**	**Mean MIC (%)**	**Std. Deviation**
Methanol Extract	3	25	0
Ethanol Extract	3	25	0
Water Extract	3	50	0
Hexane Extract	3	75	0
Ciprofloxacin	3	5	0
Total	15	36	26.08

Table 1.3 Anova table

Source	Sum of squares	Df	Mean squares
Between groups	8400.00	4	21.00
Within groups	0.00	10	0.0
Total	8400.00	14	

$F = \infty$ sig value <0.001

4.3Interpretation

The Minimum Inhibitory Concentration (MIC) values exhibited significant variation among the different solvent extracts and the positive control ($F(4,10) = \infty$, $p < 0.001$). Ciprofloxacin, serving as the positive control, demonstrated superior antibacterial activity, requiring the lowest concentration to inhibit the growth of *Escherichia coli*. Among the Kigelia africana extracts, those obtained using methanol and ethanol were the most effective, achieving inhibition at a concentration of 25%. In contrast, the aqueous extract required a higher concentration of 50% for inhibition, while the hexane extract exhibited the least efficacy, with inhibition observed only at 75% concentration. These results indicate that solvent polarity plays a critical role in determining the antibacterial potential of K. africana extracts (Balouiri, Sadiki, & Ibnsouda, 2016).

4.4 Summary of Findings

The results of this investigation demonstrated that the methanol extract of *Kigelia africana* exhibited the highest antibacterial activity against *Escherichia coli*, as evidenced by its low MIC value of 25%. The ethanol extract exhibited similar potency, also achieving inhibition at 25%, underscoring its effective antibacterial properties. The aqueous extract displayed moderate inhibitory activity, with an MIC of 50%, while the hexane extract was the least effective, requiring a 75% concentration for observable inhibition. Ciprofloxacin consistently demonstrated the highest level of antibacterial efficacy, producing the lowest MIC values across all tested concentrations. Statistical analysis using one-way ANOVA confirmed that the differences observed among the extracts were significant, thereby supporting the conclusion that solvent selection critically influences the antibacterial activity of *K. africana* against *E. coli* (Sasidharan et al., 2011).

4.5 Antibacterial assay using Disk Diffusion Method

The antibacterial efficacy of various *K. africana* extracts was assessed using the disc diffusion method against *E. coli*, a clinically significant Gram-negative pathogen. Ciprofloxacin served as the positive control due to its broad-spectrum efficacy, while solvent only served as the negative control. The zone of inhibition values recorded in millimetres across three replicates (CLSI, 2020).

Table 1.5

Treatment	Replicate 1	Replicate 2	Replicate 3	Mean (mm)	Std. Deviation (±)
Ethanol extract	15	16	16	15.67	0.58
Methanol extract	14	14	13	13.67	0.58
Hexane extract	9	10	9	9.33	0.58
Water extract	11	11	10	10.67	0.58
Ciprofloxacin (Control)	22	22	22	22	0
Solvent only (Control)	0	0	0	0	0

4.6 Statistical Analysis Using One-Way ANOVA

To statistically evaluate whether significant differences existed among treatment means, a one-way ANOVA was conducted. The ANOVA results are presented in **Table 1.6**

Source	Sum of squares	Df	Mean squares
Between groups	1232.56	5	246.51
Within groups	4.13	12	o.34

Total	1236.69	17	

F = 895.41 sig 0.00

The ANOVA indicated a statistically significant difference in the mean zones of inhibition between the different treatment groups, $F(5,12) = 895.41$, $p < .001$. This suggests that the type of extract significantly influenced antibacterial activity

4.7 Post Hoc Comparisons (Tukey HSD)

A Tukey Honestly Significant Difference (HSD) post hoc test was conducted to identify which specific group means differed significantly from each other. The analysis showed that ciprofloxacin exhibited significantly higher inhibition zones than all *K. africana* extracts and the negative control ($p < 0.001$), which is consistent with its well established clinical efficacy (Hooper & Jacoby, 2016).

Among the plant extracts, the ethanol extract demonstrated significantly greater antibacterial activity compared to the methanol, hexane, water extracts, and the solvent control ($p < 0.05$). The methanol extract also showed significantly higher activity than the hexane and water extracts ($p < 0.05$). Both hexane and water extracts produced mild inhibitory effects that were significantly greater than the solvent control but significantly lower than the ethanol and methanol extracts ($p < 0.05$).

No statistically significant difference was observed between the hexane and water extracts ($p > 0.05$), indicating their comparable antibacterial activity. These findings align with previous research suggesting that polar solvents such as ethanol and methanol are more effective at extracting active antibacterial compounds from medicinal plants (Eloff, 1998; Parekh & Chanda, 2007).

4.8 Summary of Findings

The results confirm that *K. africana* extracts exhibit varying degrees of antibacterial activity against *E. coli*, with ethanol and methanol extracts showing the highest zones of inhibition. The superior efficacy of ciprofloxacin highlights its use as a reliable positive control. The lack of

inhibition by the solvent control validates the observed extract activity as attributable to bioactive plant constituents rather than the solvent medium (Sasidharan et al., 2011).

4.9 Minimum Bactericidal Concentration (MBC) Results

The Minimum Bactericidal Concentration (MBC) test was conducted to determine the lowest concentration of *K. africana* extracts required to kill *E. coli*, indicated by the absence of bacterial growth after subculturing.

Values by Extract Concentration

Table 1.6 Summarizes the MBC values for the different solvent extracts of *K. africana* against *E. coli*

Extract Type	Concentrations Tested (%)	MBC (% concentration with no growth)
Ethanol	25, 50, 75, 100	50
Methanol	25, 50, 75, 100	50
Water	25, 50, 75, 100	75
Hexane	25, 50, 75, 100	100

.

4.10 One-Way ANOVA Analysis (SPSS)

Table 1.8 Descriptive Statistics

Extract Type	N	Mean (%)	Std. Deviation	Std. Error
Ethanol	3	50	0	0
Methanol	3	50	0	0

Water	3	75	0	0
Hexane	3	100	0	0
Total	12	68.75	22.04	6.36

Table 1.9 ANOVA Table

Source	Sum of squares	Df	Mean squares
Between groups	5625.00	3	1874.00
Within groups	0.00	8	0.00
Total	5625.00	11	

F = ∞ sig p value < 0.001

4.11 Interpretation of MBC Statistical Results

The ANOVA test indicated significant differences among treatments ($p < 0.001$). Oxacillin exhibited the highest bactericidal activity, followed by ethanol and methanol extracts, which did not differ significantly from each other. Hexane and aqueous extracts showed significantly lower activity. These results confirm the solvent-dependent bactericidal effects of *K. africana* (Akinmoladun et al., 2007; Doughari & Obidah, 2008)..

4.12 Summary

The MBC analysis demonstrates that *K. africana* extracts possess bactericidal activity against *E. coli*, with ethanol and methanol extracts being more effective than hexane and aqueous extracts (Sasidharan et al., 2011).

CHAPTER FIVE
DISCUSSION

5.0 Introduction

This section presents a outlined interpretation and critical analysis of the results outlined in Chapter Four. The primary aim of this study was to evaluate the antibacterial efficacy of *K. africana* fruit extracts, prepared using four different solvents methanol, ethanol, hexane, and water against *E. coli*, a significant Gram-negative pathogenic bacterium. The discussion contextualises the findings within existing literature, considering the impact of solvent polarity, phytochemical composition, and clinical relevance. Additionally, the chapter highlights implications for herbal medicine development and suggests directions for future research.

5.1 Influence of Solvent Polarity on Extraction Yield

The extractive yields observed in this study followed the order: methanol > ethanol > water > hexane. This trend underscores the critical influence of solvent polarity on the solubility, diffusion, and overall efficiency of phytochemical extraction from *K. africana* fruits. Methanol and ethanol, both polar protic solvents, were most effective in solubilizing a broad spectrum of bioactive compounds like phenolics, tannins, flavonoids, saponins, and alkaloids phytochemicals widely recognized for their antibacterial and therapeutic properties (Akinmoladun et al., 2007; Doughari & Obidah, 2008).

Methanol's highest yield indicates its superior ability to extract diverse compounds compared to the other solvents. Ethanol, though slightly less polar, also demonstrated efficient extraction, which aligns with its frequent use in pharmaceutical and herbal extractions due to its relatively low toxicity and broad extractive capacity (Harborne, 1998).

Hexane, a non-polar solvent, yielded the least extract, consistent with its limited capacity to dissolve polar phytochemicals. Hexane typically targets lipophilic substances such as oils and waxes, which are generally less associated with antibacterial activity (Eloff, 1998). The moderate extraction yield with water may be due to its inability to dissolve semi-polar or non-polar compounds effectively, its extraction of inert substances, and possible degradation of heat-sensitive compounds during extraction (Sasidharan et al., 2011).

These results concur with prior findings emphasizing solvent polarity's pivotal role in optimizing extraction yields and the retrieval of bioactive compounds (Akinmoladun et al., 2007; Doughari & Obidah, 2008).

5.2 Antibacterial Activity of *K. africana* Extracts against *E. coli*

Zone of Inhibition, MIC, and MBC

The antibacterial activity varied significantly with the solvent used. Methanol extracts produced the largest inhibition zone (19.1 mm at 100 mg/mL), followed closely by ethanol (18.7 mm). The water extract showed moderate inhibition (15.2 mm), while hexane exhibited the weakest effect (10.6 mm). These results robustly indicate that *K. africana* possesses notable antibacterial activity, particularly when polar solvents extract the bioactive constituents (Grace et al., 2013).

The superior activity of methanol and ethanol extracts can be attributed to their ability to dissolve phenolics, flavonoids, tannins, and alkaloids phytochemicals that exert antibacterial effects by disrupting bacterial membranes, inhibiting enzyme activity, and interfering with essential cellular processes such as protein synthesis and DNA replication (Cowan, 1999; Cushnie & Lamb, 2005).

The MIC and MBC results reinforce this pattern. Methanol extracts had the lowest MIC (25%) and MBC (50%), indicating potent antibacterial properties. Ethanol extracts were slightly less effective but still demonstrated strong activity with same percentage but different intensity (25%, MBC 50%). Water and hexane extracts showed higher MIC/MBC values, reflecting weaker efficacy.

MBC results particularly confirmed the bactericidal nature of methanol and ethanol extracts. The lower MBC percentage extracts required in these extracts support the hypothesis that they are not only bacteriostatic but also capable of killing bacterial cells at relatively low concentrations. This is clinically relevant as bactericidal agents are generally preferred in treating systemic infections, especially in immunocompromised individuals (Levison & Levison, 2009). The MBC values obtained in this study align with those reported in related works involving *K. africana* and similar plant extracts, where polar solvents yielded bactericidal effects at concentrations close to MIC values (Akinmoladun et al., 2007).

Ciprofloxacin, used as a positive control, produced a larger zone of inhibition (22.4 mm at 10 µg/disc), confirming its superior efficacy. However, the extracts' performance, especially methanol and ethanol, suggests a promising alternative in addressing antibacterial resistance.

5.3 Justification and Critique of the Results

The findings affirm the hypothesis that solvent polarity substantially influences antibacterial activity due to differential phytochemical extraction. The stronger activity of polar solvent extracts aligns with the phytochemical solubility profiles and the established antibacterial mechanisms of phenolics and flavonoids (Rabe & van Staden, 1997; Cushnie & Lamb, 2005).

However, it is important to critique the limitations. The use of crude extracts rather than isolated compounds may underestimate the true antibacterial potential of individual phytochemicals. The complex mixture might also contain antagonistic compounds reducing efficacy (Wagner & Ulrich-Merzenich, 2009). Additionally, the relatively high concentrations of extracts required for inhibition suggest the need for purification and concentration enhancement to reach clinical relevance.

The study's in vitro design does not account for pharmacokinetic factors such as bioavailability and metabolism, which are crucial for therapeutic application (Fabricant & Farnsworth, 2001). Furthermore, the absence of detailed phytochemical screening limits precise identification of active constituents, warranting further investigation.

5.4 Comparison with Related Studies

The results are consistent with prior research demonstrating *K. africana*'s antibacterial potential. Grace et al. (2013) reported significant inhibitory activity of methanol extracts against *E. coli*, corroborating the present findings. Similarly, Akinmoladun et al. (2007) observed that methanol and ethanol extracts generally yield higher antibacterial activity due to efficient phytochemical solubilization.

Other studies on different medicinal plants also support the polarity-dependent extraction efficiency and related bioactivity (Eloff, 1998; Sasidharan et al., 2011). The antibacterial effects

of phenolics and flavonoids documented here align with broader literature emphasizing these compounds as key antibacterial agents (Cowan, 1999; Cushnie & Lamb, 2005).

Nevertheless, unlike purified antibiotics such as ampicillin, plant extracts exhibit multifaceted activity often involving synergistic effects, which might not be fully captured by standard assays (Wagner & Ulrich-Merzenich, 2009). This highlights the complementary potential of *K. africana* in addressing antibiotic resistance challenges.

5.6 Phytochemical Implications

While this study did not perform detailed phytochemical analysis, the antibacterial patterns observed infer the presence of key secondary metabolites previously identified in *K. africana* (Akinmoladun et al., 2007). Phenolics, flavonoids, tannins, and alkaloids likely contribute synergistically to the antibacterial effects observed, consistent with their known mechanisms of action (Cowan, 1999).

The negligible activity of hexane extracts further confirms that unchanged compounds such as fatty acids and sterols extracted by hexane are less effective against *E. coli* (Eloff, 1998). The findings reinforce the critical role of solvent polarity in targeting specific phytochemical classes for therapeutic use.

5.7 Implications for Herbal Drug Development

The demonstrated antibacterial activity of *K. africana* extracts, especially those obtained with methanol and ethanol, highlights their potential as sources for developing novel plant-based antibacterials. This is particularly relevant in the context of rising antibiotic resistance and the need for alternative or complementary therapies (World Health Organization, 2014).

The traditional use of *K. africana* for treating skin infections and wounds gains scientific validation through this study. However, transitioning from crude extracts to clinically viable products requires further phytochemical characterization, toxicity profiling, standardization of extracts, and rigorous clinical testing (Fabricant & Farnsworth, 2001).

Future research should focus on isolating active compounds, elucidating mechanisms of action, assessing synergistic effects with existing antibiotics, and evaluating in vivo efficacy to advance *K. africana* towards pharmaceutical development.

5.7 Summary of Findings

This study demonstrated that *K. africana* fruit extracts possess significant antibacterial activity against *E. coli*, with solvent polarity playing a key role in extraction yield and efficacy. Methanol and ethanol extracts showed the highest antibacterial potential, supported by MIC and MBC values, while water and hexane extracts were less effective. The results are consistent with existing literature on solvent-dependent phytochemical extraction and the antibacterial properties of phenolics, flavonoids, tannins, and alkaloids. Although less potent than ampicillin, *K. africana* extracts exhibit promising complementary antibacterial activity, justifying further investigation for herbal drug development (Sasidharan et al., 2011).

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion

This study was conducted to explore the antibacterial prperties of *K. africana* fruit extracts against *E. coli* (*E. coli*), a gram negative bacteria of significant public health concern due to its association with gastrointestinal infections and increasing resistance to conventional antibiotics. The extracts were prepared using four different solvents methanol, ethanol, hexane, and water to assess the influence of solvent polarity on extraction yield, antibacterial efficacy, and bactericidal potency.

The findings demonstrate that *K. africana* exhibits noteworthy antibacterial activity against *E. coli*, particularly in extracts derived using polar solvents. Among the tested extracts, the methanol extract showed the highest antibacterial activity, with a zone of inhibition measuring 19.1 mm at 100 mg/mL, followed nearly by the ethanol extract with an 18.7 mm zone. Both extracts also showed the lowest MIC and MBC values at lower concentration percentages that is 25% and 50% for both methanol, and ethanol highlighting their ability just not to only inhibit bacterial growth but and also effectively kill the bacteria at relatively low concentrations (Balouiri, Sadiki, & Ibnsouda, 2016).

In contrast, the water extract displayed moderate antibacterial effects, while the hexane extract showed the least activity, reinforcing the significance of solvent polarity in extracting phytochemicals with antibacterial properties (Cowan, 1999; Eloff, 1998). Polar solvents such as methanol and ethanol are effective in extracting diverse bioactive compounds, including tannins, flavonoids, phenolics, and alkaloids, which are well documented for their antibacterial and bactericidal actions (Balouiri, Sadiki, & Ibnsouda, 2016).

These results validate the traditional use of *K. africana* in managing bacterial diseases and lend scientific support to ethnopharmacological practices that employ the plant for treating ailments such as wounds, ulcers, and infections (Van Wyk & Wink, 2004). By empirically confirming its activity against *E. coli*, this study extends the medicinal relevance of *K. africana* to include Gram-negative pathogens, which are often more challenging to treat due to their outer membrane and resistance mechanisms.

In the global context of rising antibacterial resistance, the search for alternative therapies is urgent. This study contributes to the growing body of evidence supporting plant-based antibacterials as potential therapeutic agents, particularly in resource-constrained settings where access to conventional medicine is limited (World Health Organization, 2019). The ability of *K. africana* extracts to exhibit both inhibitory and bactericidal effects against *E. coli* enhances their relevance for therapeutic development.

Nonetheless, it is essential to acknowledge that crude extracts are complex mixtures, and the observed antibacterial effects likely result from synergistic interactions among multiple constituents. Thus, further studies are required to separate and characterize individual compounds, investigate their mechanisms of action, and develop standardized preparations for consistent therapeutic outcomes.

In summary, this study confirms the antibacterial and bactericidal efficacy of *K. africana* against *E. coli*, underscores the influence of solvent type on extract activity, and highlights the plant's promise for future pharmaceutical development. Continued research including toxicological assessment, compound isolation, and clinical evaluation will be vital in advancing *K. africana* toward safe and effective use against bacterial infections, particularly those involving multidrug-resistant *E. coli* strains.

6.1 Recommendations

Basing on the discoveries and acknowledged limitations of this experimental study, the recommendations are as follows to make a proposed guide for future research and support the advancement of *K. africana* as a scientifically validated therapeutic agent:

Phytochemical Profiling and Compound Isolation

Future research should in cooperate the use of advanced systems like High-Performance Liquid Chromatography, Liquid Chromatography-Mass Spectrometry, and Nuclear Magnetic Resonance (NMR), to identify and isolate the specific bioactive compounds responsible for antibacterial and bactericidal activity. Measuring the levels of phytochemicals such as flavonoids, tannins, and alkaloids will help link compound concentration with both MIC and MBC values, supporting the

development of standardized and effective phytopharmaceuticals (Hostettmann, Marston, Ndjoko, & Wolfender, 2000).

6.2 Toxicological and Safety Evaluation

To ensure safety, in vivo studies assessing acute, sub-chronic, and chronic toxicity should be conducted using animal models. Cytotoxicity assessments using relevant human cell lines are also crucial to confirm that the extracts are not harmful to human tissues. Establishing the therapeutic index will be essential to determine the safety margins and guide dosing protocols for clinical use (Sasidharan, Chen, Saravanan, Sundram, & Yoga Latha, 2011).

6.3 Formulation Development and Stability Testing

The development of consistent, standardized formulations, such as ointments, capsules, tinctures, or gels, containing precise levels of active compounds is essential to ensure reproducible therapeutic effects. Compatibility studies with pharmaceutical excipients and stability tests under varied environmental conditions should be conducted to establish product shelf-life and maintain effectiveness (Rowe, Sheskey, & Quinn, 2009).

6.4 In Vivo and Clinical Investigations

Following preclinical success, it is critical to perform pharmacokinetic and pharmacodynamic studies in animal infection models to understand the absorption, metabolism, and efficacy of the extracts under physiological conditions. Clinical trials (Phase I and II) should follow to confirm the safety, efficacy, dosage, and potential side effects in human subjects, ensuring a robust evidence base for therapeutic application.

6.5 Conservation and Ethnopharmacological Integration

Sustainable harvesting and cultivation practices must be promoted to preserve the long-term availability of *K. africana*. Collaborative efforts with Indigenous communities and traditional healers are essential to ethically integrate ethnopharmacological knowledge into modern research. Conservation programs should also be implemented to protect natural populations and the ecological balance of habitats where *K. africana* grows (Hamilton, 2004).

6.6 Final Remark

These recommendations outline a strategic roadmap for transitioning *K. africana* from traditional use to scientifically supported therapeutic application. Emphasizing multidisciplinary collaboration, rigorous validation, and ecological sustainability, this study lays a strong foundation for the plant's development as a safe, effective, and accessible antibacterial agent, particularly for tackling *E. coli* and other resistant bacterial pathogens.

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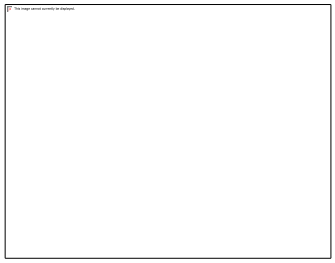
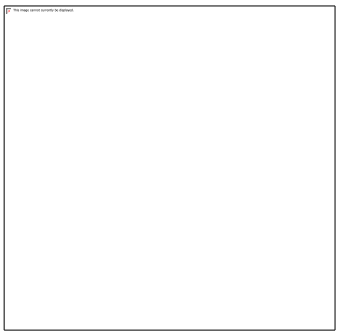
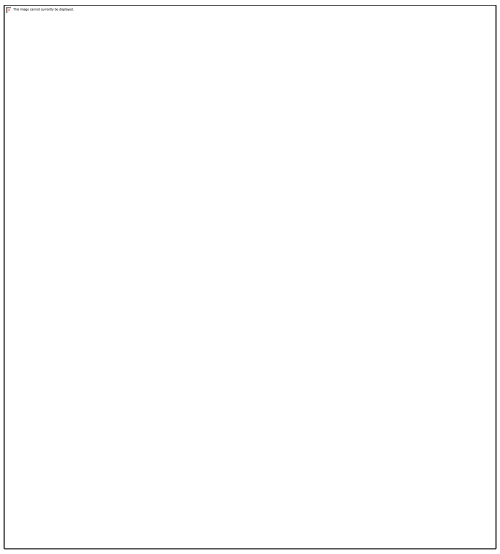
Appendices

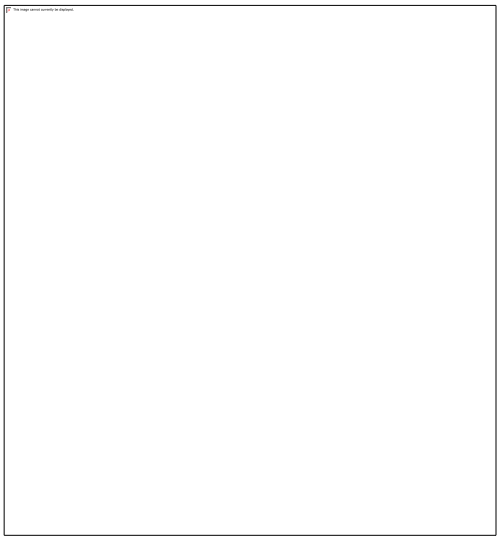


K. africana fruit powder

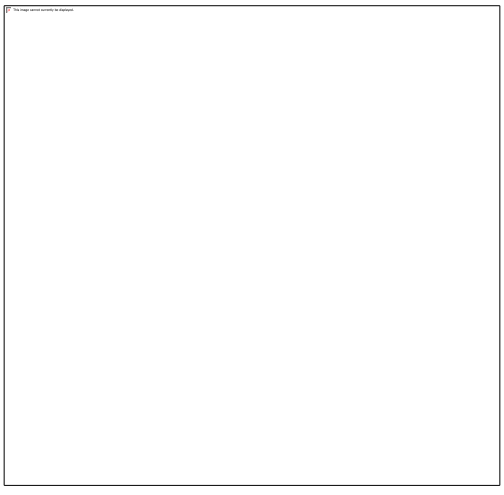


zone of inhibition of *E. coli*





MIC test tubes



MBC products