UNISA | college of agriculture and environmental sciences

Identification of Bioactive compounds and Evaluation of antibacterial activity of selected medicinal plants against *Vibrio cholerae*

By

JERIKIAS MARUMURE

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the subject

LIFE SCIENCES

Department of Life and Consumer Sciences

College of Agriculture and Environmental Sciences

University of South Africa

Supervisor: DR. M.A.Nyila

2024

UNISA college of agriculture and environmental sciences

DECLARATION

I, **Jerikias Marumure** hereby declare that the dissertation/thesis, with the title; '**Identification of Bioactive compounds and Evaluation of antibacterial activity of selected medicinal plants against *Vibrio cholerae***' which I hereby submit for the degree of Doctor of Philosophy at the University of South Africa, is my own work and has not previously been submitted by me for a degree at this or any other institution.

I declare that the dissertation /thesis does not contain any written work presented by other persons whether written, pictures, graphs or data or any other information without acknowledging the source.

I declare that where words from a written source have been used the words have been paraphrased and referenced and where exact words from a source have been used the words have been placed inside quotation marks and referenced.

I declare that I have not copied and pasted any information from the Internet, without specifically acknowledging the source and have inserted appropriate references to these sources in the reference section of the dissertation or thesis.

I declare that during my study I adhered to the Research Ethics Policy of the University of South Africa, received ethics approval for the duration of my study prior to the commencement of data gathering, and have not acted outside the approval conditions.

I declare that the content of my dissertation/thesis has been submitted through an electronic plagiarism detection program before the final submission for examination.

Student signature: Jerikias Marumure

Date: 14 November 2024

ACKNOWLEDGEMENTS

The successful completion of this project would have been unattainable without the invaluable help and unwavering patience of several people and organisations. Therefore, the author would like to offer sincere gratitude to the following individuals:

I am grateful to Dr. MA. Nyila, my supervisor, for his sagacity, direction, and motivation. I am grateful for his unwavering support and his readiness to put in extra effort to ensure the success of the project. He bolstered my optimism and morale throughout moments of being inundated with stress and obstacles. The critical feedback he provided during the entire process of developing and writing my thesis was invaluable and very much appreciated. I am sincerely grateful to him for his contributions. Working under him was a prestigious and exhilarating experience.

I would like to express my gratitude to the Head of the Department of Life and Consumer Sciences, as well as all the members of the department, for providing me with the valuable chance to pursue my PhD studies and for facilitating my access to knowledge in the department.

The Masters & Doctoral Bursary department at UNISA provided the funding for tuition fees and research expenses.

I would like to express my gratitude to Mr. Brian Mapurisa, the former head of the Department of Physics, Geography, and Environmental Science, for permitting me to carry out some experimental activities in the Biology laboratories of Great Zimbabwe University.

The laboratory technicians, specifically Mr. Mukwamba and Mr. Muza, from the Biology Department at Great Zimbabwe University, were instrumental in promptly providing the necessary apparatus and equipment for certain experimental tasks.

The experiment would not have been completed without the aid of Mrs. Epiphania Chitsamatanga and Mr. Simbarashe Hatidani, (Biology department, University of Zimbabwe). They played a crucial role in offering some assistance in carrying out certain experimental tasks for the investigation.

I would like to express my appreciation to Mr. Christopher Chapano, the Head of the National Herbarium and Botanic Garden in Harare, for his meticulous authentication of the plant species used in this study.

I would also like to express my gratitude to my wife, Tracy Marumure, for her unwavering support and encouragement, as well as to my children, Mutsawashe and Munashe, for their patience and understanding throughout this endeavour.

I would also like to express my gratitude to my relatives and friends, especially Zakio Makuvara, Jonathan Marumure, Agrippa Dube, and Dr. Ngavaite Chigede, for their assistance.

Lastly, I would like to express my gratitude to the Divine Being for granting me the comprehension and determination to accomplish this thesis.

SUMMARY

Background: Cholera has remained a significant public health concern in sub-Saharan Africa since its emergence and is still endemic today. Despite the manageable nature of the disease, cholera in Zimbabwe continues to pose a major public health challenge due to various overwhelming factors. Medicinal plants are effective options for such infections due to their diverse bioactive compounds with notable therapeutic potential. Thus the study focused on identifying medicinal plants utilised in traditional cholera treatment and validating their medicinal use through analysis of bioactive compounds, cytotoxicity assessment, and evaluation of antibacterial activity against *Vibrio cholerae.*

Methodology: A literature study approach was employed, to identify and select medicinal plants. The agar well diffusion method was applied to evaluate the antibacterial efficacy of these selected plants against *V. cholerae,* while the brine shrimp lethality assay was conducted to assess their cytotoxic activity. Finally, gas chromatography-mass spectrometry (GC-MS) analysis was performed to determine the bioactive compounds present in the selected medicinal plants.

Results: *Ziziphus mucronata* Willd., *Kirkia acuminata* Oliv., *Sclerocarya birrea* Hochst., and *Crossopteryx febrifuga* Benth. were the most utilised by several ethnic groups. Significant anti-*Vibrio cholerae* activity was demonstrated by *K. acuminata*, *S. birrea*, and *Z. mucronata*, with minimum inhibitory concentrations (MIC) of stembark extracts at $\leq$ 1 mg/mL. Extracts of *Z. mucronata* and *K. acuminata* showed no toxic effects, with lethality concentrations (LC_{50}) of 1023.58 μg/mL and 1583.34 μg/mL, respectively. The GC-MS analysis identified 23 distinct phytochemical constituents in the extracts of *K. acuminata* and 22 in *Z. mucronata* stembarks, including bioactive compounds with established antimicrobial, antifungal, and antioxidant properties such as behenic alcohol, 1-dodecanol, and octacosanol, as well as pharmacologically underexplored hydrocarbons like tetrapentacontane and tetracontane, albeit in varying concentrations.

Conclusion: *K. acuminata* and *Z. mucronata* were successfully recognised as medicinal plants suitable for incorporation into cholera management. Their significant antibacterial and non-cytotoxic activities, along with a wide range of bioactive compounds revealed through GC-MS analysis, validate their use in traditional medicine and offer important insights into novel drug

development. However, it is essential to highlight that these findings should not be viewed as a complete substitute for the established mainstream approaches to cholera treatment or prevention.

Keywords: medicinal plants, *Kirkia acuminata*, *Ziziphus mucronata,* antibacterial activity, cholera, gas chromatography-mass spectrometry, bioactive compounds, traditional medicine, cytotoxic activity, cholera management

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

WHO	World Health Organisation
CT	Cholera toxin
QS	Quorum sensing
ZINATHA	Zimbabwe National Traditional Healers' Association
STIs	Sexually Transmitted Infections
SSA	Sub-Saharan Africa
DRC	Democratic Republic of Congo
CFR	Case fatality rate
CFU	Colony forming units
cAMP	cyclic Adenosine monophosphate
AMR	Antimicrobial resistance
MIC	Minimum inhibitory concentration
BSLA	Brine Shrimp Lethality Assay
LC_{50}	Lethal Concentration 50
GC-MS	Gas Chromatography-Mass Spectrometry
RMA	Resistance-modifying agents
MSF	Médecins Sans Frontières
WASH	Water, Sanitation, and Hygiene
NGOs	non-governmental organisations
UNICEF	United Nations Children's Fund
OVCs	Oral cholera vaccines
iNOS	Inducible Nitric Oxide Synthase
PPAR-γ	Peroxisome Proliferator-activated Receptor Gamma
COX-2	Cyclooxygenase-2
CRP	C-reactive protein

CHAPTER 1: INTRODUCTION

1.1 Background

Nature consistently exemplifies the extraordinary quality of friendship, particularly towards humanity (Verma and Singh, 2008). From the dawn of human societies in ancient times, people have taken advantage of the 'elements' present in their surroundings to treat several diseases (Jamshidi-Kia et al., 2018; Petrovska, 2012; Verma and Singh, 2008). Until now, these 'elements' have been utilised within the framework of traditional medicine, even with the rise of synthetic alternatives. Significantly, through a process of trial and error, individuals across almost every continent have discovered methods to derive medicines from certain plant species. The understanding of these 'medicinal plants' has been continuously transmitted through traditional healers and elders over the years (Jamshidi-Kia et al., 2018; Khan, 2014). Medicinal plants possess substances in one or more of their parts that can be utilised for therapeutic applications, or that serve as precursors for the scientific synthesis of beneficial pharmaceuticals (Sofowora et al., 2013). These parts are integral to traditional medicine, consisting of natural compounds that emerge from various cultural contexts. More importantly, in numerous regions of the developing world today, a significant percentage of the population, ranging from 70 to 95%, still depends on plants as their main source of medicine (Adeleye et al., 2021). Furthermore, various countries have incorporated traditional plant-based remedies into their mainstream healthcare systems through regulatory frameworks (Chassagne et al., 2021). Moreover, it has been observed that plant medicines tend to be harmonious with the human body, resulting in little to no adverse reactions (Prasathkumar et al., 2021).

Sub-Saharan Africa (SSA) is facing ongoing cholera outbreaks, with the region accounting for over 86% of reported cases and deaths globally. For example, in 2017 and 2018, cholera outbreaks were reported in seven countries in Sub-Saharan Africa: Cameroon, the Democratic Republic of Congo, Tanzania, Kenya, Mozambique, Zimbabwe, and Zambia (Gwenzi and Sanganyado, 2019). The most devastating outbreak occurred in Zimbabwe in 2008, marked by a case-fatality ratio (CFR) that escalated to 20% in rural regions, leading to a rapid increase in cases and fatalities within a matter of days (Gwenzi and Sanganyado, 2019). Actually, in Zimbabwe, cholera has been a persistent issue, with the latest outbreak commencing in February 2023. The problem is escalating, as evidenced by the 35,000 cases and 600 fatalities recorded in 2024 alone (Médecins Sans Frontières, 2024).

Since the 2018 outbreak, the prevalent strain in Zimbabwe has been identified as *V. cholerae* O1 serotype Ogawa. The strain exhibits multi-drug resistance, unexpectedly high level of resistance to ciprofloxacin (96.7%) and ceftriaxone (99.6%), and currently remains endemic (Mashe et al., 2020). Additionally, existing literature indicates that resistance in SSA is primarily observed against trimethoprim-sulphamethoxazole in 50% of the studies, ampicillin in 43.3% of the studies, chloramphenicol in 43.3% of the studies, and streptomycin in 30% of the studies (Mohammed et al, 2018). Furthermore, the findings indicate that the bacterium is progressively enhancing its resistance to antimicrobial drugs over time (Miwanda et al., 2015). It has been observed that the case fatality rates among untreated patients may exceed 70% as a result of severe watery diarrhoea (1 L per hour) and circulatory collapse (Davies et al.. 2017). The primary virulence factor of *V. cholerae* is the synthesis of heat-labile enterotoxins, which results in water efflux into the lumen of the small intestine, ultimately leading to circulatory collapse. The neutralisation of cholera toxins (CT) immediately upon their secretion by the bacteria is one of the targets of natural compounds (Komiazyk et al., 2019).

The effectiveness of infection treatments worldwide is increasingly jeopardised by antimicrobial resistance (AMR). *Vibrio cholerae* presents a significant challenge in certain developing countries, particularly due to the emergence of drug-resistant strains (Gupta et al., 2016). Confronted with the escalating challenge of antibiotic resistance and the urgent demand for novel therapeutic agents, there is a growing interest in plant extracts and other natural compounds that may provide essential solutions. Similarly, it has been noted that plants possess a range of secondary metabolites that serve as defence mechanisms against damage caused by invading microorganisms and pests (Chassagne et al., 2021). The development of novel antibacterial agents appears to be unappealing due to the costly processes involved and the rapid onset of signs and symptoms following pathogen entry into the body (Komiazyk et al., 2019). This evidence supports the use of plant extracts as alternatives to conventional antibiotics, despite their reported lower efficacy than synthetic options. Several studies have shown that plant extracts and isolated phytochemicals can effectively suppress the growth of *V. cholerae* (Jantapaso et al., 2024; Saha et al., 2022; Manjunath et al., 2022). Additionally, these substances inhibit specific stages in the lifecycle of *V. cholerae* even within the patient, thereby reducing the severity of diarrhoea and other related symptoms (Komiazyk et al., 2019). The documented folkloric applications of specific plant species for cholera management (Maroyi, 2017; Kigen et al., 2016; Chigora et al., 2007; Gelfand et al., 1985) have ignited fresh initiatives to integrate these practices into the ongoing fight against cholera in Zimbabwe. Additionally, Dettweiler et al. (2020) indicated that specific phytochemical products and plant extracts may function as resistance-modifying agents (RMA) and could thus be beneficial in addressing multi-drug resistant bacteria. The capacity of plant extracts to function as RMAs holds significant potential for reinstating the effectiveness of antibiotics against resistant pathogenic bacteria (do Socorro Costa et al., 2024; Anokwah et al., 2023; Rawangkan et al., 2022). For instance, a chequerboard assay demonstrated that the combination of the water extract from coffee beans and tetracycline exhibited greater efficacy than either treatment alone against drug-resistant *V. cholerae* (Rawangkan et al., 2022).

While existing literature sources provide extensive lists of medicinal plants utilised for cholera and other diarrhoeal diseases within certain cultural groups in Zimbabwe (Maroyi, 2011, 2016), there are no investigations to verify the antimicrobial and cytotoxic properties of these plants and their bioactive compounds. This type of investigation is crucial for advancing drug discovery from herbal sources and should be leveraged for cholera management. Moreover, the application of plant extracts as remedies is deeply rooted in African culture, and any favourable outcomes from the study will be embraced and implemented without reservation.

1.2 Problem Statement

Cholera is a highly feared disease, leading to outbreaks that result in considerable illness and mortality if not promptly well managed. In several epidemic and endemic regions, the disease remains inadequately managed, especially in nations with elevated human poverty indices (Ganesan et al., 2020). For example, seven countries in Sub-Saharan Africa have recently faced repeated cholera outbreaks, representing over 86% of the globally reported cases and fatalities (Gwenzi and Sanganyado, 2019). Zimbabwe, like other countries in Sub-Saharan Africa, reports cholera cases frequently and annually. For example, the nation, faced a significant outbreak in 2008, exhibiting a case-fatality ratio (CFR) reaching 20% in rural areas, with a surge in cases and fatalities occurring within a matter of days (Gwenzi and Sanganyado, 2019). Furthermore, the latest outbreak is currently ongoing, with 35,000 reported cases and 600 fatalities recorded in 2024 (Médecins Sans Frontières, 2024).

Managing cholera in developing countries presents significant challenges. Cholera in Zimbabwe is predominantly found in urban regions, primarily caused by overburdened and malfunctioning water and sanitation infrastructure, inadequate water supply, contaminated water sources, and poor water storage practices. Additionally, cultural practices like improper corpse handling during burials contribute to the array of risk factors (Chimusoro et al., 2018). Antimicrobial resistance poses a significant threat to the effectiveness of infection treatments globally. Reports of drug resistance to various antibiotics are emerging from numerous countries experiencing endemic cholera, highlighting the escalating issue of drug resistance in *V. cholerae*, particularly in developing nations (Gupta et al., 2016). Antimicrobial-susceptibility data from 65% of the confirmed cases during the 2018 Zimbabwe cholera outbreak revealed that the *V. cholerae* O1 serotype Ogawa isolates exhibited multidrug resistance, demonstrating an unexpectedly high level of resistance to ciprofloxacin and ceftriaxone. It is noteworthy that azithromycin demonstrated efficacy against the isolates. Nevertheless, the occasional detection of azithromycin-resistant genes, including *mph*(A) or *mph*(E), in isolates collected from Zimbabwe, the Democratic Republic of Congo (DRC), and Kenya between 2010 and 2015 (Mashe et al., 2020) indicates that the management and control of cholera could be particularly challenging in certain situations. Additionally, genes conferring resistance to tetracycline, specifically *tet*[A], along with mutated genes associated with ciprofloxacin resistance (*gyr*A and *par*C), as well as the resistance genes aac[6′]-Ib-cr, were identified in certain clinical isolates (Mashe et al., 2020). Furthermore, numerous studies have indicated that the isolates of *V. cholerae* are increasingly developing resistance to antimicrobial drugs over time (Irenge et al., 2020; Miwanda et al., 2015). Considering this context, the pursuit of suitable medications for cholera treatment must persist, and botanical sources seem to present a promising solution. Plants contain secondary metabolites that serve as defence mechanisms against invading microorganisms and pests (Chassagne et al., 2021). It is essential to identify and scientifically evaluate plants that possess inhibitory properties against *V. cholerae* for their application in primary health care. In Zimbabwe, the information regarding medicinal plants and their characteristics lacks regulation and, consequently, is inadequately incorporated into formal primary health care systems.

1.3 Justification of study

In Africa's primary healthcare system, herbal medicine continues to be the most affordable and accessible form of therapy, as many individuals are unable to afford the prohibitive costs associated with modern medications (Maroyi, 2013). Historically, rural communities have used plants as remedies for cholera and other related diseases. This is because some plant extracts could either completely eradicate or severely impede the growth of *V. cholerae.* Accurately forecasting cholera outbreaks presents a significant challenge because of the intricate relationships among the various risk factors for epidemics in Africa. The traditional focus on ensuring safe water and enhancing hygiene practices has been insufficient for managing cholera, with cases now arising frequently in Africa, particularly in Zimbabwe (von Seidlein et al., 2013). Moreover, the deterioration of health-care infrastructure in developing nations, the concerning rise of antibiotic resistance in *V. cholerae*, and ongoing political unrest complicate efforts to treat and eliminate diseases such as cholera. Given the circumstances, individuals, especially in rural regions, find themselves compelled to utilise and embrace medicinal plants as fundamental sources of primary health care. Therefore, this investigation focusses on identifying medicinal plants with vibriocidal properties. According to Sharma et al. (2009), modern synthetic and traditional therapies often exist side by side, yet they seldom work together. This is because certain individuals link the latter to mysticism and otherworldly spiritual abilities. As a result, this research will focus on scientifically rationalising the treatment of cholera using medicinal herbs. Achieving this requires consistency in traditional medicine regarding the sourcing of raw materials, the processes of manufacturing, and the quality control of the final products. Moreover, it is essential to assess the safety and efficacy of plant extracts utilised in the treatment of diarrhoea and cholera.

1.4 Significance of the Study

With the increasing prevalence of microbial resistance to synthetic medications, there is a noticeable shift in drug research towards natural products. Bioactive compounds present in anti-diarrhoeal medicinal plants have been shown to effectively combat bacteria, including pathogenic *Vibrio* strains (Jantapaso et al., 2024; Saha et al., 2022; Manjunath et al., 2022). Nonetheless, since most results were derived from unprocessed plant extracts, the specific bioactive components in these therapeutic plants need identification. The native plants in southern Africa have not received sufficient research focus (Maroyi, 2017). The phytochemical analysis and pharmacological activities of these plants could lead to the development of novel drugs (Mgbeahuruike et al., 2017) that address therapeutic needs (Prasathkumar et al., 2021), particularly considering the concerning rise in issues related to modern synthetic anti-diarrhoeal drugs (Suleiman et al., 2017), such as antibiotic resistance. Furthermore, it is important to highlight that antibacterial activity has garnered greater focus in studies concerning plant extracts (Zewdie et al., 2021; Mahmood et al., 2019; Tradtrantip et al., 2014; Nyila et al., 2012). Recognising plants with these properties is an essential component of conventional disease treatment.

The extracts from medicinal plants have the potential to be utilised in the formulation of cholera therapies, or they may work alongside existing medications that are effective against *V. cholerae* resistance. Plant extracts can be employed to make teas or poly-herbal formulations that may be consumed regularly or immediately by individuals suspected of cholera. This could inhibit specific phases of the bacterial infection cycle, reducing the severity of diarrhoea and its associated effects, and ultimately enhancing the likelihood of patient survival. These teas or concoctions would likely be embraced due to their dual benefits: therapeutic properties and potential for generating income. Furthermore, the investigation would additionally support the safeguarding of traditional knowledge regarding Zimbabwean medicinal plants utilised for the treatment of diarrhoeal diseases, especially cholera. The transmission of this knowledge to future generations is essential, particularly within the framework of African culture. Ultimately, the investigation aims to systematically dismantle the misconceptions that hinder individuals from exploring traditional medicine as a viable treatment alternative.

1.5 Aim of the study

The aim of this study is to select medicinal plants and evaluate their antibacterial activity against *V. cholerae*. In addition, it seeks to assess the cytotoxic activity of the plant extracts as well as identify their phytochemical components.

Objectives of the study were:

- To identify plant species used in the treatment of cholera/ diarrhoea in Zimbabwe.

- To investigate the antimicrobial efficacy of commonly used medicinal plants to clinical *V. cholerae* isolates.
- To assess the cytotoxic activity of selected medicinal plants used against diarrhoea/cholera.
- To determine the bioactive compounds from selected medicinal plants used against diarrhoea/ cholera.

1.6 Scope of the thesis

The background, literature review, an experimental chapter, a study results presentation chapter, and finally general discussion and a study conclusion comprise the thesis.

Chapter 1: This chapter provides an overview of cholera, discussing its recurrence, management strategies, and the traditional use of medicinal plants for its treatment. The chapter additionally provides a framework for understanding the significance of the study in tackling the issue at hand.

Chapter 2: This chapter presents a literature review that concentrates on cholera as a disease, examining its epidemiology, the factors influencing its spread, and the challenges faced in controlling it in Zimbabwe. Additionally, it offers background information on the medicinal plants traditionally used in the treatment of cholera and other related diarrhoeal diseases.

Chapter 3: Chapter 3 presents a research on compilation of medicinal plants traditionally employed in the treatment of cholera and other related diarrhoeal diseases in Zimbabwe.

Chapter 4: Chapter 4 is an investigation on the antimicrobial efficacy of selected medicinal plants against *V. cholerae.*

Chapter 5: Chapter 5 is an assessment of cytotoxic activity of selected medicinal plants used for cholera and other related diarrhoeal diseases treatment using the Brine Shrimp Lethality Assay.

Chapter 6: Chapter 6 presents the determination of bioactive compounds in selected plant extracts used for the traditional treatment of diarrhoea or cholera.

Chapter 7: Chapter 7 focuses on the general discussion and conclusion.

Appendices and other related information.

References

Adeleye, O. A., Femi-Oyewo, M. N., Bamiro, O. A., Bakre, L. G., Alabi, A., Ashidi, J. S., ... & Fakoya, G. (2021). Ethnomedicinal herbs in African traditional medicine with potential activity for the prevention, treatment, and management of coronavirus disease 2019. Future Journal of Pharmaceutical Sciences, 7, 1-14.

Anokwah, D., Asante-Kwatia, E., Asante, J., Obeng-Mensah, D., Danquah, C. A., Amponsah, I. K., ... & Mensah, A. Y. (2023). Antibacterial, Resistance Modulation, Anti-Biofilm Formation, and Efflux Pump Inhibition Properties of *Loeseneriella africana* (Willd.) N. Halle (Celastraceae) Stem Extract and Its Constituents. Microorganisms, 12(1), 7.

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J.T., Dettweiler, M., Marquez, L., Salam, A.M., Shabih, S., Farrokhi, D.R., & Quave, C.L. (2021). A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective. Frontiers in Pharmacology, p.2069.

Chigora, P., Masocha, R., & Mutenheri, F. (2007). The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: the case of Mutirikwi communal lands. Journal of Sustainable Development in Africa, 9(2), 26-43.

Chimusoro, A., Maphosa, S., Manangazira, P., Phiri, I., Nhende, T., Danda, S., ... & Nabyonga-Orem, J. (2018). Responding to cholera outbreaks in Zimbabwe: building resilience over time. Current Issues in Global Health, 45-64.

Davies, H. G., Bowman, C., & Luby, S. P. (2017). Cholera–management and prevention. Journal of Infection, 74, S66-S73.

Dettweiler, M., Melander, R. J., Porras, G., Risener, C., Marquez, L., Samarakoon, T., ... & Quave, C. L. (2020). A clerodane diterpene from *Callicarpa americana* resensitizes methicillin-resistant *Staphylococcus aureus* to β-lactam antibiotics. ACS Infectious Diseases, 6(7), 1667-1673.

do Socorro Costa, M., da Silva, A. R. P., Araújo, N. J. S., Paulo, C. L. R., Ribeiro, P. R. V., Tavares, J. F., ... & Coutinho, H. D. M. (2024). UPLC–QTOF-MS/MS analysis of saponin-enriched fractions from *Calliandra umbellifera* Benth. and evaluation of antibacterial activity against multidrug-resistant bacteria. Phytochemistry Letters, 59, 64-68.

Ganesan, D., Gupta, S. S., & Legros, D. (2020). Cholera surveillance and estimation of burden of cholera. Vaccine, 38, A13-A17.

Gelfand, M., Mavi, S., Drummond, R.B. and B. Ndemera. (1985). The Traditional Medicinal Practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe.

Gupta, P. K., Pant, N. D., Bhandari, R., & Shrestha, P. (2016). Cholera outbreak caused by drug resistant *Vibrio cholerae* serogroup O1 biotype ElTor serotype Ogawa in Nepal; a cross-sectional study. Antimicrobial Resistance & Infection Control, 5, 1-5.

Gupta, P., Modgil, V., Kant, V., Kaur, H., Narayan, C., Mahindroo, J., ... & Taneja, N. (2022). Phenotypic and genotypic characterization of antimicrobial resistance in clinical isolates of *Vibrio cholerae* over a decade (2002-2016). Indian Journal of Medical Microbiology, 40(1), 24-29.

Gwenzi, W., & Sanganyado, E. (2019). Recurrent cholera outbreaks in sub-Saharan Africa: moving beyond epidemiology to understand the environmental reservoirs and drivers. Challenges, 10(1), 1.

Irenge, L. M., Ambroise, J., Mitangala, P. N., Bearzatto, B., Kabangwa, R., Durant, J. F., & Gala, J. L. (2020). Genomic analysis of pathogenic isolates of *Vibrio cholerae* from eastern Democratic Republic of the Congo (2014-2017). PLoS Neglected Tropical Diseases, 14(4), e0007642. https://doi.org/10.1371/journal.pntd.0007642.

Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. Journal of Herbmed Pharmacology, 7(1).

Jantapaso, H., Aksonkird, T., & Mittraparp-arthorn, P. (2024). Characteristics of *Vibrio cholerae* isolates obtained from shrimp supply chains and inhibitory activities of rambutan (*Nephelium lappaceum* L. cv. Rong Rian) peel aqueous extract. Food Control, 158, 110231.

Khan, H. (2014). Medicinal plants in light of history: recognized therapeutic modality. Journal of Evidence-based Complementary & Alternative Medicine, 19(3), 216-219.

Kigen, G., Maritim, A., Some, F., Kibosia, J., Rono, H., Chepkwony, S., ... & Wanjoh, B. (2016). Ethnopharmacological survey of the medicinal plants used in Tindiret, Nandi County, Kenya. African Journal of Traditional, Complementary and Alternative Medicines, 13(3), 156-168.

Komiazyk, M., Palczewska, M., Sitkiewicz, I., Pikula, S., & Groves, P. (2019). Neutralization of cholera toxin by Rosaceae family plant extracts. BMC Complementary and Alternative Medicine, 19(1), 1-14.

Mahmood, N., Nazir, R., Khan, M., Khaliq, A., Adnan, M., Ullah, M., & Yang, H. (2019). Antibacterial activities, phytochemical screening and metal analysis of medicinal plants: traditional recipes used against diarrhea. Antibiotics, 8(4), 194.

Manjunath, G. B., Awasthi, S. P., Zahid, M. S. H., Hatanaka, N., Hinenoya, A., Iwaoka, E., ... & Yamasaki, S. (2022). Piperine, an active ingredient of white pepper, suppresses the growth of multidrug-resistant toxigenic *Vibrio cholerae* and other pathogenic bacteria. Letters in Applied Microbiology, 74(4), 472-481.

Maroyi A (2017) Exotic Plants in Indigenous Pharmacopoeia of South-Central Zimbabwe: Traditional Knowledge of Herbal Medicines. Research Journal of Botany, doi: 10.3923/rjb.2017.46.52.

Maroyi, A. (2011). An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. Journal of Ethnopharmacology, 136(2), 347-354.

Maroyi, A. (2013). Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. Journal of Ethnobiology and Ethnomedicine, 9(1), 1-18.

Maroyi, A. (2016). Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. African Journal of Traditional, Complementary and Alternative Medicines, 13(6), 5-10.

Mashe, T., Domman, D., Tarupiwa, A., Manangazira, P., Phiri, I., Masunda, K., Chonzi, P., Njamkepo, E., Ramudzulu, M., Mtapuri-Zinyowera, S., & Smith, A.M. (2020). Highly resistant cholera outbreak strain in Zimbabwe. New England Journal of Medicine, 383(7), 687-689.

Médecins Sans Frontières (2024). Why cholera is on the rise in Zimbabwe. [Internet]. Available from: https://www.doctorswithoutborders.org/latest/why-cholera-rise-zimbabwe. [Accessed 22 July 2024].

Mgbeahuruike, E. E., Yrjönen, T., Vuorela, H., & Holm, Y. (2017). Bioactive compounds from medicinal plants: Focus on Piper species. South African Journal of Botany, 112, 54-69.

Miwanda, B., Moore, S., Muyembe, J. J., Nguefack-Tsague, G., Kabangwa, I. K., Ndjakani, D. Y., ... & Piarroux, R. (2015). Antimicrobial drug resistance of *Vibrio cholerae.* Democratic Republic of the Congo. Emerging Infectious Diseases, 21(5), 847.

Mohammed, Y., Aboderin, A. O., Okeke, I. N., & Olayinka, A. T. (2018). Antimicrobial resistance of *Vibrio cholerae* from sub-Saharan Africa: A systematic review. African Journal of Laboratory Medicine, 7(2), 1-7.

Nyila, M. A., Leonard, C. M., Hussein, A. A., & Lall, N. (2012). Activity of South African medicinal plants against *Listeria monocytogenes* biofilms, and isolation of active compounds from Acacia karroo. South African Journal of Botany, 78, 220-227.

Petrovska, B. B. (2012). Historical review of medicinal plants usage. Pharmacognosy Reviews, 6(11), 1.

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomedicine Plus, 1(2), 100029.

Rawangkan, A., Siriphap, A., Yosboonruang, A., Kiddee, A., Pook-In, G., Saokaew, S., ... & Duangjai, A. (2022). Potential antimicrobial properties of coffee beans and coffee by-products against drug-resistant *Vibrio cholerae.* Frontiers in Nutrition, 9, 865684.

Saha, P., Banerjee, A., Banerjee, G., & Bag, P. K. (2022). Inhibitory activities of *Typhonium trilobatum* (L.) Schott on virulence potential of multi-drug resistant toxigenic *Vibrio cholerae.* Microbial Pathogenesis, 165, 105485.

Sharma, A., Patel, V.K., & Chaturvedi, A.N. (2009). Vibriocidal activity of certain medicinal plants used in Indian folklore medicine by tribals of Mahakoshal region of central India. Indian Journal of Pharmacology, 41(3), 129.

Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. The Journal of Alternative and Complementary Medicine, 2(3), 365-372.

Suleiman, M. M., Oyelowo, B. B., Abubakar, A., Mamman, M., & Kamar-deen, T. B. (2017). A controlled study to investigate anti-diarrhoeal effect of the stem-bark fractions of *Terminalia avicennioides* in laboratory animal models. International Journal of Veterinary Science and Medicine, 5(1), 14-22.

Tradtrantip, L., Ko, E. A., & Verkman, A. S. (2014). Antidiarrheal efficacy and cellular mechanisms of a Thai herbal remedy. PLoS Neglected Tropical Diseases, 8(2), e2674.

Verma, S., & Singh, S.P. (2008). Current and future status of herbal medicines. Veterinary world, 1(11), 347.

Von Seidlein, L., Jiddawi, M., Grais, R. F., Luquero, F., Lucas, M., & Deen, J. (2013). The value of and challenges for cholera vaccines in Africa. The Journal of Infectious Diseases, 208(suppl_1), S8-S14.

Zewdie, K. A., Bhoumik, D., Wondafrash, D. Z., & Tuem, K. B. (2020). Evaluation of in-vivo antidiarrhoeal and in-vitro antibacterial activities of the root extract of *Brucea antidysenterica* JF Mill (Simaroubaceae). BMC Complementary Medicine and Therapies, 20, 1-11.

CHAPTER 2: LITERATURE REVIEW

2.1 Cholera as a disease and the causative agent

Cholera, which lay people commonly diagnose it by observing key symptoms such as severe, watery diarrhoea, vomiting, and dehydration, is caused by *Vibrio cholerae* (Ojeda Rodriguez et al., 2024). The organism comprises approximately 200 serogroups, encompassing both pathogenic and non-pathogenic strains (Montero et al., 2023). The disease primarily results from the Inaba and Ogawa serotypes, along with two biotypes, classical and El Tor, of the O1 and O139 serogroups (Somboonwit et al, 2017). Current outbreaks are attributed to the O1 and El Tor biotypes, while previous epidemics were linked to O1 classical biotypes. Notably, the El Tor biotypes exhibit lower virulence than the classical biotypes (Lloyd et al, 2021). Nonetheless, various serogroups were associated with gastrointestinal problems (Dutta et al., 2013).

The majority of *V. cholerae* are eliminated by stomach acids following the consumption of contaminated food or water. The remaining bacteria penetrate the small intestine and produce cholera toxins, which serve as the main virulence factor for pathogenic strains of *V. cholerae*. The infectious dosage of *V. cholerae* O1 varies between 10^3 and 10^8 CFU, with symptoms manifesting within a period of 12 hours to 5 days (Chowdhury et al., 2022). In the ileum or colon, *V. cholerae* utilise their specialised adherence factors to attach to the microvilli surface and release cholera enterotoxins into the intestinal epithelial cell. Cholera toxin stimulates the production of cyclic adenosine monophosphate (cAMP) in epithelial cells, which sets off a series of events that culminate in severe diarrhoea (Snider et al., 2010). This occurs as water diffuses into the intestinal lumen (Ojeda Rodriguez et al., 2024).

Identifying solutions before the onset of diarrhoea symptoms can mitigate the adverse effects of the infection, thereby enhancing the likelihood of patient survival. Cholera, like other diseases, presents potential molecular targets that could be inhibited, leading to the prevention of cholera toxin internalisation by epithelial cells (Monserrat-Martinez et al., 2019). A variety of secondary metabolites present in plants have demonstrated the ability to mitigate the cytopathic effects of cholera toxins (Reddy et al., 2013). Nonetheless, if the cholera toxins remain unneutralised, the manifestations of the illness intensify significantly. In addition to the onset of diarrhoea, the patient experiences abdominal pain, bowel rumbling, and vomiting (Goffman, 2024). Consequently, excessive watery diarrhoea reaching up to 1 litre per hour can lead to hypotensive shock and potentially result in death within hours of the initial symptom ("cholera gravis"). Untreated patients suffering from severe cholera can experience death rates exceeding 70% (Ojeda Rodriguez et al., 2024).

When did cholera first emerge, and what were the circumstances surrounding its initial appearance? The complexity of these issues arises from the diverse clinical symptoms of cholera, which can be challenging to differentiate from other diarrhoeal disorders (Barua, 1992). Nonetheless, features in Sanskrit indicated that the illness was present as early as the 5th century BC and was believed to have been prevalent for centuries on the Indian subcontinent. From 1817 to 1923, cholera expanded beyond the Indian subcontinent, leading to a series of pandemics and epidemics across Asia, Africa, Europe, and Latin America. Cholera reemerged in Africa in 1970 and has continued to pose a significant public health challenge, characterised by a substantial disease burden, recurrent outbreaks, ongoing endemicity, and elevated case fatality rates, especially in the central African Great Lakes region, which may serve as reservoirs for cholera (Mengel et al., 2014), as well as in Sub-Saharan African nations (Gwenzi and Sanganyado, 2019).

2.2 Cholera in Sub-Saharan Africa

In many underdeveloped areas of the world, cholera, an old illness, continues to be a public health issue. Since the beginning of the first cholera pandemic in 1817, there have been seven pandemics. High population density, poverty, and a lack of proper sanitary and water facilities are more likely to be the root causes of cholera epidemics (Deen et al., 2020). Between 1972 and 1991, the seventh cholera epidemic, which we are currently experiencing, spread to Africa, and the illness persisted and continued to evolve. In the past, cholera outbreaks in vulnerable populations, transmission through food and water, and the establishment of resistant strains have all been observed in Africa. For instance, a cholera outbreak occurred among refugees in Malawi in 1990 (Hatch et al., 1994). The pathogen was discovered in water storage tanks (Patel and Isaäcson, 1989). After El Nino rains in the late 1990s, there were more widespread cholera epidemics in Uganda (Bwire et al., 2018), and the discovery of resistant isolates in Tanzania in 1977 (Swerdlow and Isaäcson, 1994).

Up until 2017, the WHO has received more than 4 million reports of cholera infections from the whole continent in only around 50 years. Most cases have been recorded from Africa, particularly Sub-Saharan Africa (SSA) (**Fig. 2.1**) (Mengel et al., 2014).

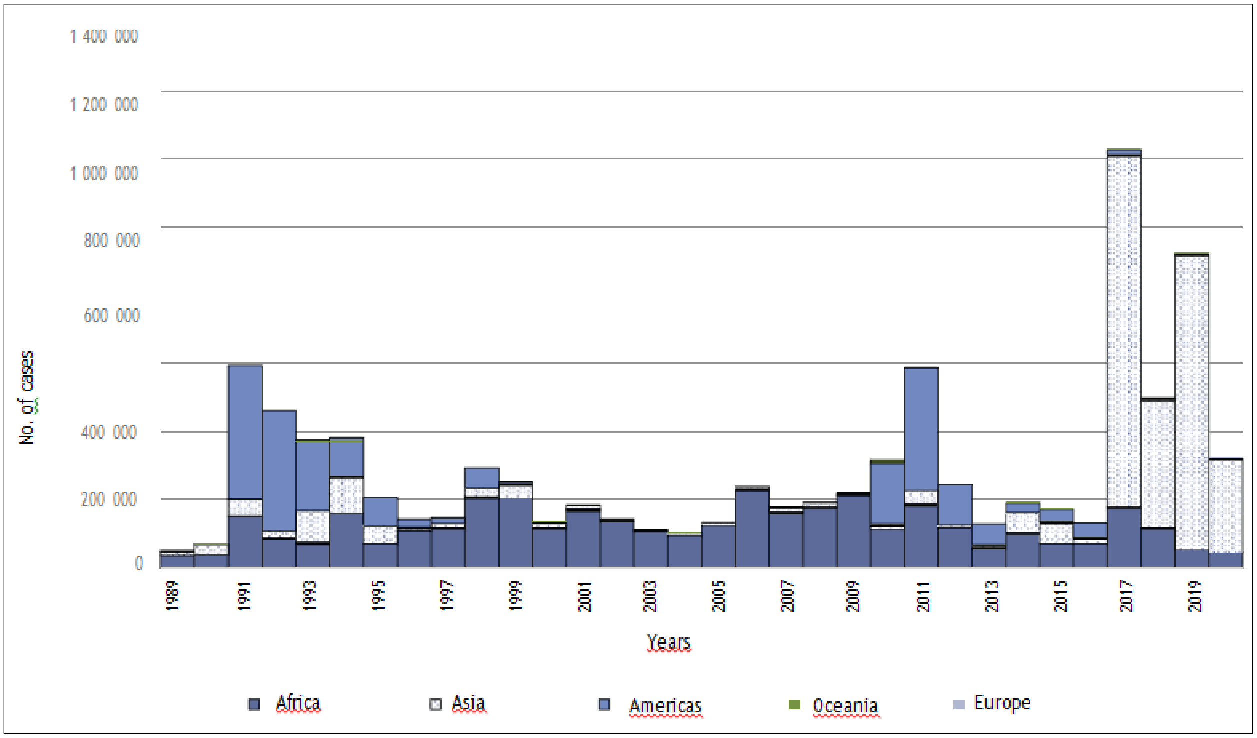

Figure 2.1: Cholera cases reported to WHO by year and by continent, 1989–2020 (Source: WHO Cholera Annual Report, 2020).

Nearly half of the reported cholera cases globally are found in Sub-Saharan Africa, which also experiences the highest case-fatality rates. The actual number of cholera cases in Africa is likely substantially higher due to underreporting and insufficient surveillance systems (Deen et al., 2020). The paucity of identification of cholera outbreaks in rural places and the political expediency of states avoiding the potential political and economic repercussions of disclosing such information were cited by the WHO as the causes of underreporting. Cases reported as "acute diarrhoeal syndrome" and "acute watery diarrhoea" could be potential cholera outbreaks (Rieckmann et al., 2019).

Currently, communities suffering from famine and clean water shortages, particularly in SSA, are more likely to suffer from cholera than other places with poor sanitation. The main way that cholera spreads is when sick individuals contaminate food or drink with their faeces, which carry the bacteria (Ojeda Rodriguez et al., 2024). Along with the apparent risk factors (such as contaminated food and water), it is believed that complex interactions among *V. cholerae* natural reservoirs, human activities, and climate risk factors control cholera outbreaks in some SSA countries (Gwenzi and Sanganyado, 2019). For instance, hot spots near water sources were the source of the repeated cholera epidemics that occurred in the Democratic Republic of the Congo (DRC) from 2008 to 2017 and resulted in 5,231 deaths. The Great Lakes, the Congo River, and its branches were identified as the outbreaks' incubators in the 10-year retrospective investigation of the outbreaks (Ingelbeen et al., 2019). Additionally, brackish water ecosystems in nature are rife with *V. cholera* (Vezzulli et al., 2013). Recent studies have shown that *V. cholerae* is present in a variety of Nile ecosystems, including in seafood (such as fish, crustaceans, and ducks), and that its pattern of distribution and transmission between diverse niches are both mysterious and interlinked (Abdel-moneam et al., 2021; Ismail et al, 2021). Fish were reported as being one of the main hosts for *V. cholerae* among various seafood (Laviad-Shitrit et al., 2019). Consequently, between 2011 and 2015, 58% of all recorded cholera cases in Uganda were in fishing settlements (Bwire et al., 2017). Furthermore, several studies reported antimicrobial resistant and virulent *Vibrio* strains isolated from diverse types of seafood (Mok et al., 2019, 2021; Ahmed et al., 2018).

In SSA countries, recent cyclical cholera epidemics have primarily affected urban or peri-urban areas (Mbala-Kingebeni et al., 2021; Penrose et al., 2010). For instance, since the 1980s, Ghana has continued to report sporadic cholera outbreaks in densely populated areas close to urban centres, where it is estimated that more than 50% of Ghanaians live. Due to a lack of access to potable water, the presence of slums, unsanitary habits, poor personal hygiene, indiscriminate waste disposal, and street vendors selling tainted food or water, these areas are always the most vulnerable to being afflicted (Mireku-Gyimah et al., 2018; Osei et al., 2008). Similarly, the recent outbreak in Zambia took place in peri-urban regions (Mwaba et al., 2020), and even Zimbabwe's 2008–2009 cholera epidemic, which is still regarded as the deadliest in Africa, mostly took place in urban areas (Gwenzi and Sanganyado, 2019).

The continent of Africa has historically had several extended and severe droughts, which have led to cholera risk factors (such as malnutrition, inadequate access to water, sanitation, and hygiene, and population relocation) (Charnley et al., 2022). Certain cholera outbreaks in Africa have been associated with droughts, even though they are not recognised as risk factors for cholera in the

WHO guidelines (Rieckmann et al., 2019). A total of 276 cholera outbreaks occurred during 118 droughts, according to data gathered from 1990 to 2010 in 41 sub-Saharan nations. The same authors contend that population hygiene suffers because of droughts, which raises the danger of diarrhoeal disease epidemics, including cholera. In Africa, cholera epidemics can be short-lived or seasonal. Instead of the sudden nature of outbreaks elsewhere, like those recorded in Zimbabwe between 2008 and 2009 and Mozambique from 2009 to 2011, which had an average length of 5 months and 7.2 weeks, respectively, in the Democratic Republic of Congo (DRC), cases occur year-round (Deen et al., 2020). This could be caused by diverse environmental and climatic conditions in various regions (Emch et al., 2008).

2.3 Cholera outbreaks in Zimbabwe

In Zimbabwe, the first cholera case was documented in 1972. Several notable outbreaks have been documented since then (**Table 2.1**). In late 1992, cholera cases were confirmed, and they were connected to an influx of migrants escaping cholera-stricken Mozambique (Bradley et al., 1996). Since 2008, El T or the O1 biotype of *V. cholerae* has been the most common in Zimbabwe (Munyenyiwa et al., 2022). Between 2008 and 2009, Zimbabwe experienced one of the deadliest cholera epidemics in Africa, with 98,585 reported cases and 4287 deaths (Mukandavire et al., 2011). A decade later, in 2018, Zimbabwe experienced another severe cholera epidemic (Mukandavire et al., 2020). Until now, cholera has remained endemic, with isolated cases reported around the country, particularly in urban areas or communities with vulnerable members of the community. For example, cholera cases have been reported in people displaced by the Tokwe-Mukosi disaster in 2014 and Cyclone Idai in March 2019 (Chen and Azman, 2019). Furthermore, most cholera cases are reported first from metropolitan regions, which are the primary epicenters (**Fig. 2.2**). For example, both the 2008 and 2018 epidemics began in Harare, the city of two million people and the hub of the entire country, and expanded into the countryside (Mukandavire et al., 2011).

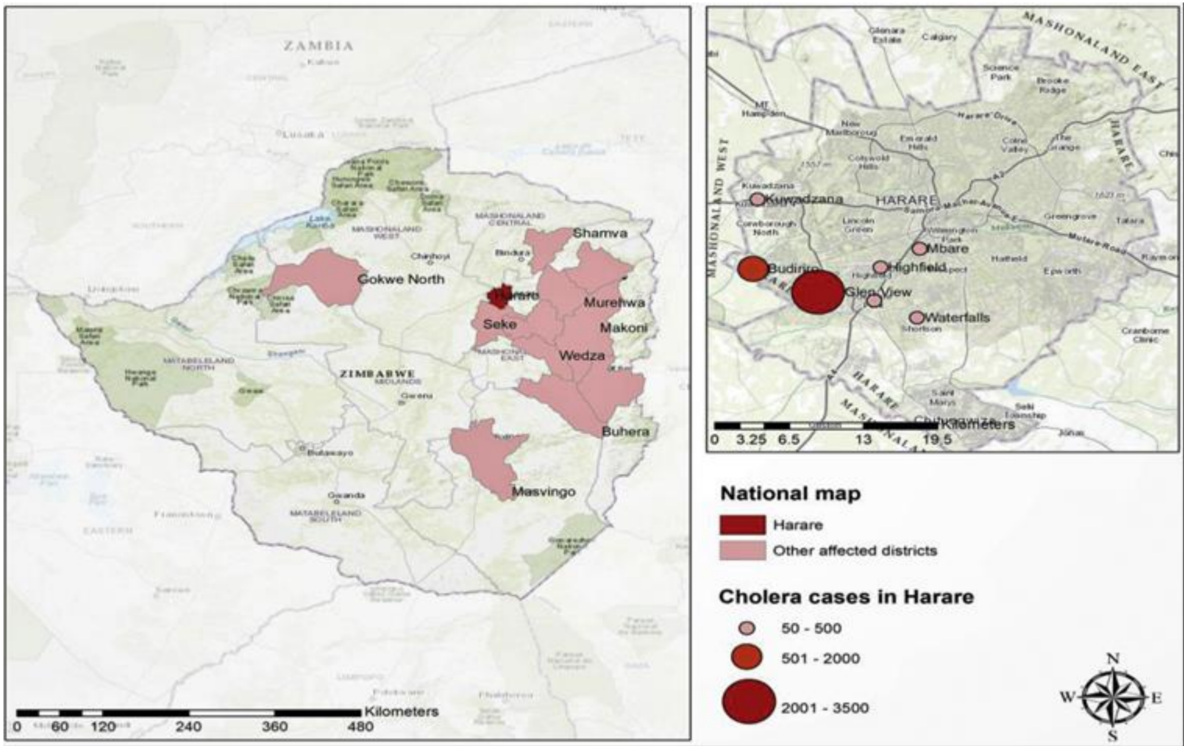

Figure 2.2: Map showing the cholera epidemic in Harare, the epicentre (Source: Mukandavire et al., 2020).

Since the 2018 epidemic, cholera has remained endemic in Zimbabwe, with the prevalence and severity of the disease decreasing significantly. Notably, recently there has been a cumulative of 28 974 cholera cases with 603 deaths (CFR 2.0%) as of 17 March 2024. All ten provinces are affected with the majority of the cases reported from Manicaland, Harare, Mashonaland West, and Mashonaland Central provinces (WHO, 2024a). However, some cases are not reported, with considerable anecdotal evidence pointing to scattered cases of cholera across the country. In 2022, numerous African nations reported cholera outbreaks, including Cameroon, Nigeria, Ethiopia, Benin, Malawi, Zimbabwe, the DRC, South Sudan, Zambia, Mozambique, Kenya, and Tanzania. Most nations provided the number of cases and the CFR as of May 2022. However, no updates were provided in Zimbabwe and a few other nations, including Mozambique, Uganda, and Togo (Reda et al., 2022). Failure to disclose such information could be due to a lack of proper reporting and surveillance systems (Khan et al., 2022), political expediency of states avoiding the potential political and economic ramifications of disclosing such information, and reporting cholera outbreaks as "acute diarrhoeal syndrome" or "acute watery diarrhoea," particularly in rural areas (Rieckmann et al., 2019).

Table 2.1: Cholera outbreaks in Zimbabwe

Year	**Number of reported cases**	**Case Fatality Rate (%)**	**References**
1972	Not known	Not known	Mashe et al, 2020
1992	7433	5.8	Cuneo et al., 2017
1999	5637	6.8	Cuneo et al., 2017
2002	3125	6.1	Cuneo et al., 2017
2008	98592	4.3	Cuneo et al., 2017; Winstead et al., 2020
2010	1220	3.3	Maponga et al., 2015
2018-2019	10730	0.64	Winstead et al., 2020
2022	135	1.5	Reda et al., 2022
2023-2024	28 974	2.0	WHO, 2024a; Olatunji et al., 2024
2024 (alone)	35 000	1.7	Médecins Sans Frontières, 2024

2.4 Drivers contributing to the recurring cholera epidemics in Zimbabwe.

Zimbabwe has seen cholera outbreaks since 1972 (**Table 2.1**). Until now, cholera has been endemic; the most current outbreak began in February 2023, and cases are steadily growing, with 35,000 reported cases and 600 deaths in 2024 alone (Médecins Sans Frontières, 2024). **Figure 2.3** shows some of the drivers of cholera outbreaks in Zimbabwe.

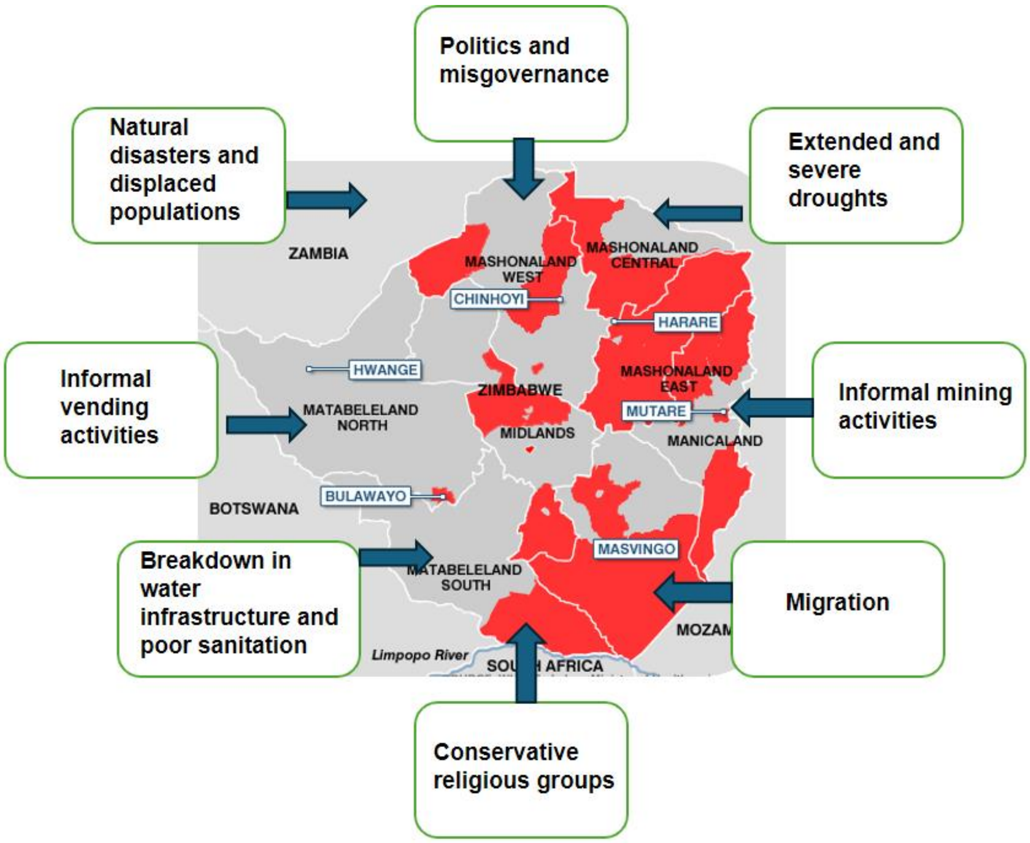

Figure 2.3: An overview of typical drivers for the repeated occurrences of cholera outbreaks in Zimbabwe (Map of Zimbabwe adapted from BBC News, 2008).

2.4.1 Breakdown in water infrastructure and poor sanitation

Zimbabwe's recurring challenges from the past outbreaks (Mashe et al., 2023), have paved the way for recent and ongoing cholera outbreaks (Olatunji et al., 2024). According to Doctors Without Borders/Médecins Sans Frontières (MSF) Southern Africa, both rural and urban areas are experiencing increased transmission risks due to vulnerabilities in water, sanitation, and hygiene facilities (Médecins Sans Frontières, 2024). In Harare, contaminated water from boreholes and wells was suspected to be the source of the outbreaks (WHO, 2018), as drinking water from some boreholes is contaminated by sewage leaking from broken pipes (The Guardian, 2018). Recently, the shortage of potable water was linked to the loss of mega volumes of treated water every day due to the bursting of old water pipes, leaving residents with not enough water, and hence resorting to alternative sources which may be unsafe (Zhakata, 2024). A number of townships in Harare and other cities have been reported to go for weeks, or even months, without a regular supply of water from the City Council (Sevenzo, 2023). Furthermore, anecdotal evidence has revealed that most Harare residents have stopped drinking water from the taps, terming it 'green' and they have resorted to drinking water sourced from boreholes and shallow wells, which could be contaminated. Moreover, the situation is compounded by raw sewage becoming a common sight in townships, uncollected garbage, and unsafe hygiene practices mostly in major cities and towns. In rural areas, recent findings indicate that most boreholes have become inoperable, leaving people with no choice but to consume unsafe water from nearby streams. This water source is often shared with both wild and domestic animals (Médecins Sans Frontières, 2024).

The dilapidated water and sewage system, in Zimbabwe, have been a persistent problem for almost 2 decades now. According to Muronzi (2024), the current sewage system is part of its inherited colonial infrastructure and has been overwhelmed as the population grew from about 3.7 million in 1960 to 17 million today. As Zimbabwe is experiencing or recovering from a debilitating social and economic crisis, which began in the last two decades, it is failing to invest in maintaining critical infrastructure for water and sewerage reticulation, resulting in frequent bursts and collapse of the systems (Mukandavire et al., 2020). Even with assistance from non-governmental organisations (NGOs) and international agencies (e.g., The United Nations Children's Fund (UNICEF)), the country is failing to meet the required US$ 8,047,500 to provide enough essential Water, Sanitation, and Hygiene (WASH) services, among other services, to combat the current and ongoing outbreak (Olatunji et al., 2024; UNICEF, 2023). Along with this background, for months now, Zimbabwe has been battling to stem the spread of deadly cholera in its cities and villages because the country simply lacks clean water, and proper sewage disposal (Sevenzo, 2023).

2.4.2 Natural disasters and displaced populations

Several studies have demonstrated how natural disasters (floods, earthquakes, tropical cyclones) cause significant population displacement and transmission of diseases (Hadeed et al., 2023; Kouadio et al., 2012). Shannon et al. (2019) found that displaced populations are often susceptible to a range of infectious diseases, such as cholera. This is because natural disasters have the potential to contaminate or destroy water sources and sanitary infrastructure, leading to the rapid spread of the disease (Watson et al., 2007). Although natural disasters in Zimbabwe are rare, a few studies established a connection between the occurrence of cholera and these disasters. For example, in 2014, there was an outbreak of diarrhoeal sickness among 1,500 people who were homeless and relocated due to the floods caused by the Tokwe-Mukosi Dam partial collapse (Muchapireyi, 2018). Consequently, the absence of safe sources of water people to depend on stagnant water, which presented a potential hazard to their health (Hove, 2016). Similarly, Cyclone Idai, which impacted the Southern Hemisphere between March 4 and 21, 2019, resulted in the loss of lives and displacement of numerous families, particularly in Zimbabwe, Mozambique, and Malawi (Chen and Azman, 2019). In Zimbabwe, specifically in the Chimanimani and Chipinge districts, a total of 51,000 individuals were rendered homeless. This led to the occurrence of diarrhoeal diseases, including cholera, in the campsites and collective centres due to the lack of access to clean water and proper sanitation facilities (Chatiza, 2019).

2.4.3 Extended and severe droughts

Several studies have established a connection between droughts and cholera, particularly in sub-Saharan Africa (Mavhura and Aryal, 2023; Charnley et al., 2022, 2021; Rieckmann et al., 2019). Charnley et al. (2022) discovered that prolonged and severe droughts have resulted in several factors that increase the risk of cholera, including malnutrition, insufficient access to water, sanitation, and hygiene, and population displacement. Although droughts are not explicitly identified as risk factors for cholera in the WHO guidelines, a study by Rieckmann et al. (2019) demonstrated that there were 276 cholera outbreaks during 118 droughts between 1990 and 2010 in 41 sub-Saharan countries. Thus, droughts play a significant role in contributing to the occurrence of cholera. Furthermore, the same authors contended that droughts can negatively affect population hygiene, resulting in a heightened susceptibility to diarrhoeal outbreaks, such as cholera. Zimbabwe has experienced numerous droughts in the past. Significant and prolonged periods of drought have been observed in the following years: 1972-1973 (Ndlovu and Mjimba, 2021), 1991-1992, 1994-1995, 2002-2003, 2015-2016, 2018-2019 (Mupepi and Matsa, 2022; Frischen et al., 2020), and also in 2023-2024 (The Guardian, 2024; Mugiyo et al., 2023). The droughts have led to water scarcity, reduced agricultural productivity, and occurrences of food insecurity, thus leading to economic downturns (Frischen et al., 2020) and a potential vulnerability to cholera transmission. Previous cholera outbreaks in Zimbabwe (Table 2.1) have co-occurred with the periods mentioned above for drought. For example, the current cholera outbreak in 2023-2024 occurred at the same time as the government's declaration of a national disaster due to drought (The Guardian, 2024).

2.4.4 Politics and misgovernance

Several studies have demonstrated that cholera epidemics in Zimbabwe have occurred due to political causes (Bhanye et al., 2023; Makoni, 2020; Masakure, 2018). In 2000, Zimbabwe had a notable shift in its political environment. An influential opposition party arose, resulting in persistent disputes. The ruling party, leveraging its powers at the national level, obstructed the opposition, which often held sway over local government (Taruvinga, 2023). Subsequently, the delivery of services, such as providing clean drinking water and sewage disposal, was impacted, leading to a rise in regular outbreaks of cholera. Following that, the government has faced consistent scrutiny from the public populace about cholera outbreaks (Chigudu, 2019). For instance, the 2008-2009 cholera epidemic, which lasted for 10 months, was highly linked to political manipulation (Mukandavire et al., 2011). Notably, the outbreak was attributed to human rights breaches and the politicisation of water, health care, aid, and information. The outbreak occurred shortly after two violent rounds of presidential elections (Cuneo et al., 2017). In addition, a subsequent wave occurred, extending until June 2011 (WHO, 2011). A decade later (i.e., 2018), another cholera outbreak coincided with the 'supposedly' coup and the contested election results, and the same concerns were raised by the citizens. Furthermore, in 2023 Zimbabwe held its elections and was seen to be back at its familiar crossroads, with cholera outbreaks occurring after disputed elections (Olatunji et al., 2024). Again, the outbreaks pointed to the government's lack of will or ability or both to stem the occurrences by providing fresh water (Sevenzo, 2023). Moreover, the cholera outbreak in Harare and other cities has been linked to the growing vulnerability of the urban poor, which is caused by their forced displacement and fueled by conflicts over economically and politically important urban areas (Birch, 2021).

2.4.5 Migration

Migrants have also been linked to cholera transmission (Deen et al., 2020). The Cholera outbreak in Zimbabwe in late 1992, was connected to an influx of migrants escaping cholera-stricken Mozambique (Bradley et al., 1996). Similarly, anecdotal evidence also pointed to migration as one of the causes of the Zimbabwean disastrous epidemic of 2008-2009, which also expanded to neighbouring Zambia and South Africa. For the recent 2023-2024 cholera outbreak, no migration from other countries was reported as the main driver for the outbreak. However, such information sometimes may not be made public, and some diarrhoeal cases which are not reported especially in rural areas could be cholera cases. However, the movement of people within the borders was linked to the spread of the outbreak. For example, the first patient was reported in Chegutu on 23 February 2023, and the numbers significantly rose as the disease spread to all geographical corners of the country (Olatunji et al., 2024). Furthermore, there was a steep increase during the holidays as people travelled and gathered to celebrate with their extended families, giving the disease new opportunities to spread (IFRC, 2024). Another key risk factor for cholera in Zimbabwe is the increase in rural-urban migration and the search for economic opportunities. As a result, this further strains the already strained water and sanitation infrastructure in most urban areas (WHO, 2018). For example, in the 2018 cholera epidemic, Glen View in Harare, an active informal trading area where people come from across the city and the rest of the country to trade, was the epicentre of the outbreak (WHO, 2018).

2.4.6 Informal mining activities

Informal mining has a historical association with the spread of cholera (Mazibuko, 2019). The mining communities in Zimbabwe have experienced a significant increase in cholera cases during the recent outbreak (WHO, 2024b). According to reports from WHO, artisanal miners obtain water from polluted sources such as abandoned mining pits and nearby rivers, which serves as a favourable environment for the spread of cholera (WHO, 2024c). In Shamva district, located in Mashonaland Central province, there were around 2,850 reported cases of cholera and 94 deaths, accounting for 19.5% of the total cases in the country. The main factors contributing to the transmission included open defecation and inadequate sanitation facilities (Médecins Sans Frontières, 2024).

2.4.7 Conservative religious groups

Certain cultural and religious beliefs hinder efforts to combat cholera and, at the same time, paradoxically contribute to the transmission of the disease. In a study conducted by Chitura (2014), it was discovered that followers of the Zionist and Apostolic church sects demonstrated a lack of adherence to measures aimed at preventing the spread of cholera, as they placed their trust in faith healing (Chitura, 2014). The recent cholera outbreak has also shown a substantial number of cases inside fundamentalist religious groups that have a stance against contemporary medical treatment. Empirical evidence indicates that they engage in unhygienic practices, such as conducting baptisms in contaminated water bodies and even engaging in open defecation during their church gatherings (Médecins Sans Frontières, 2024). Furthermore, a recent study discovered that the Johane Marange, a popular African apostolic sect that rejects Western medicine in favour of faith healing and prayer, not only contributed to the spread of cholera but also hindered ongoing vaccination efforts (Zenda, 2024). However, because of the persistent efforts of government law enforcement officers and the deaths of certain members of these religious groups, stories about these individuals have become rare. Nonetheless, it is not entirely possible to exclude the potential existence of these religious extremists who continue to preach their unwavering belief in divine healing only.

2.5 Challenges

Cholera, while treatable and manageable, can present many challenges that may hinder the process of treating, managing, and preventing the disease under particular conditions. These issues encompass a scarcity of vaccinations to adequately serve the entire population, the emergence of multi-drug-resistant strains of *V. cholerae*, and various other obstacles.

2.5.1 Emergence of multi-drug-resistant *Vibrio cholerae*

In recent decades, Zimbabwe has suffered substantial outbreaks of antibiotic-resistant *V. cholerae* strains (Kamuriwo et al., 2021; WHO, 2021), impeding efficient and timely cholera control. Notably, *V. cholerae* O1 serotype Ogawa isolates from Zimbabwe's 2018-2019 cholera outbreak showed resistance to practically all antibiotics available at the time, including ciprofloxacin, tetracycline, and ceftriaxone (Mashe et al., 2020). To address the outbreak, the patient management prescription was changed from the less expensive and more widely available ciprofloxacin to the more expensive and effective azithromycin, which was not available in health facilities (Mashe et al., 2023; Mukandavire et al., 2020). This led to increased healthcare expenses, mortality, and morbidity (WHO, 2021). According to reports from the cholera outbreak in nearby Malawi, the strain is still endemic (Chaguza et al., 2023). Moreover, Zimbabwe, a country that frequently has cholera epidemics, must always be ready to face such challenges due to the observed progressive growth in bacterial resistance to antimicrobial drugs (Miwanda et al., 2015).

2.5.2 Vaccination

The combination of cholera vaccinations with other preventive measures has been demonstrated to be critical components of disease prevention and control, particularly in cholera-endemic areas (Médecins Sans Frontières, 2024; Chowdhury et al., 2022; Pezzoli, 2020). Early introduction of vaccines during an outbreak has been shown to offer 79% protection against cholera (Chowdhury et al., 2022). According to the same perspective, several studies reached the same conclusion about the possibility that mass immunisation could have mitigated the severity of the 2008 cholera outbreak in Zimbabwe (Mukandavire et al., 2020; Clemens, 2011; Kim et al., 2011). Likewise, prompt immunisation might have contained the 2018 outbreak (Mukandavire et al., 2020). Moreover, the one-month vaccine campaign for the recent 2023–2024 outbreak solely targeted 2.3 million people, living in high-risk districts. A mere 2,303,248 vaccination doses were obtained and administered (WHO, 2024b), against the entire population of Zimbabwe which stands at 17,010,832, as of Monday, June 24, 2024 (https://www.worldometers.info/world-population/zimbabwe-population/). This low vaccination coverage has contributed to the continuous spread of cholera in the country (Olatunji et al., 2024), as have previous epidemics. All of this can be attributable to the fact that there is a global shortage of oral cholera vaccines today, and Zimbabwe does not have enough doses to reach a larger population (Médecins Sans Frontières, 2024). Furthermore, Euvichol-Plus, one of the common oral cholera vaccines (OVCs) globally (Chowdhury et al., 2022), was utilised in the most recent outbreak despite having a low and transient efficacy-two doses only offering three years of cholera protection (https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/cholera).

Furthermore, other constraints such as the necessity for a cold supply chain, production costs, and complex vaccine distribution logistics reduce the widespread use of cholera vaccines in low-income countries like Zimbabwe (Kabir, 2014).

2.6 Traditional treatment of cholera in Zimbabwe

The ongoing cholera outbreak in Zimbabwe demands a multifaceted strategy, which includes promoting traditional medicine. Improving infrastructure for clean water and acceptable sanitation during an ongoing outbreak may be difficult, especially if the country is still experiencing or recovering from an economic crisis (Mukandavire et al., 2020). As a result, this study considers the use of medicinal plants in cholera management, as plants have been identified as potential remedies for a variety of diseases (Chassagne et al., 2021; Prasathkumar et al., 2021). More importantly, this could be beneficial, especially in resource-limited endemic areas. Several studies, including surveys, reviews, and anecdotal evidence, have discovered medicinal plants employed in the folkloric treatment of cholera and other similar diarrhoeal diseases (Charla et al., 2022; Mongalo et al., 2020; Maroyi, 2013, 2016, 2017; Chigora et al., 2007; Gelfand et al., 1985). More importantly, the usage of most medicinal plant species as vibriocidal agents in traditional medicine is backed by documented data on antibacterial activity and phytochemical analysis (**Figs. 2.4 - 2.16**). However, Zimbabwe has yet to standardise and authorise the use of these therapeutic plant species in modern treatment.

2.7 Background information on the medicinal plants compiled in this study

Sixteen indigenous medicinal plant species were collected from the Mashonaland East and Masvingo provinces of Zimbabwe. The selection of medicinal plants was grounded in published literature regarding its folkloric use in treating diarrhoea, including bloody diarrhoea and cholera-related symptoms, in Zimbabwe. The plant samples collected underwent precise identification by the botanists and herbarium curators at the National Herbarium of Zimbabwe, where preserved specimens were deposited (**Table 2.2**).

Table 2.2 Voucher specimen numbers of the medicinal plants compiled in the study

Plant species	**Voucher number**	**Plant part(s) used**
Ziziphus mucronata Willd.	MJ1	Stem bark/ Leaves
Ximenia caffra Sond.	MJ5	Leaves/Roots
Vangueria infausta Burch.	MJ4	Leaves/ Stem bark
Crossopteryx febrifuga Benth.	MJ18	Stem bark
Sclerocarya birrea Hochst.	MJ9	Stem bark/Leaves
Flacourtia indica (Burm. f.) Merr.	MJ2	Leaves
Euclea divinorum Hiern	MZMJ11	Leaves/ Roots
Peltophorum africanum Sond.	MJ7	Leaves/ Stem bark / roots
Elephantorrhiza goetzei (Harms) Harms	MZMJ10	Roots / bark
Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa	MJ19	Stem bark/ Roots
Cassia abbreviata Oliv.	MJ20	Stem bark
Schotia brachypetala Sond.	MJ8	Stem bark
Grewia bicolor Juss.	MJ21	Leaves
Kirkia acuminata Oliv.	MZMJ6	Stem bark/ Roots
Ficus sur Forssk.	MJ22	Leaves
Strychnos spinosa Lam.	MJ3	Leaves

The comprehensive descriptions of the selected plants are as follows:

2.7.1 *Ziziphus mucronata* Willd.

Figure 2.4: *Ziziphus mucronata* [Photographed at Waddilove Farm, Marondera (18°17'11"S 31°34'59"E)]

Ziziphus mucronata (Fig. 2.4) belongs to Rhamnaceae family. In Zimbabwe, the plant is known as Buffalo-thorn (English) *Chinanga*, *Muchecheni* (all Shona) and *Umpasamala*, *Umphafa* (all Ndebele). The plant is native to some tropical African countries including Angola, Botswana, Eritrea, Ethiopia, Ghana, Kenya, Lesotho, Mozambique, Zambia, Namibia, Niger, Senegal, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, and Zimbabwe (Hyde et al., 2022). *Ziziphus mucronata* typically grows to be a medium-sized tree or shrub up to 9 metres tall with a crooked trunk, smooth grey-brown bark while it is young (sometimes spiky), and deeper brown, fissured bark as it ages (Orwa et al., 2009). The leaves of this species are broadly oval, noticeably asymmetrical, 3-veined from the base, varyingly close to hairless to thickly reddish hairy, particularly underside, and have finely serrated margins. In addition, the flowers are tiny, yellowish, and in axillary clusters. Finally, the plant species bears a spherical drupe that turns reddish-brown when ripe (Hyde et al., 2022).

Medicinal uses

This plant species has applications in the treatment of 46 diseases, prominently addressing gynaecological symptoms, respiratory and chest conditions, skin infections, diarrhoea, and dysentery (Mongalo et al., 2020). In Zimbabwe, a decoction or infusion of roots is employed to treat wounds, bladder infections, bilharzia, cholera, infertility in women, and abdominal pain (Maroyi, 2013; Maroyi, 2011; Gelfand et al., 1985). In other regions of Africa, various authors have documented the application of *Z. mucronata* for the treatment of diarrhoeal diseases (Chinsembu et al., 2019; Maroyi and Chekhyoussef, 2015). In South Africa, leaves are ground and boiled in water, with the resulting liquid consumed three times daily to treat diarrhoea until the infection resolves (Madikizela et al., 2012). In Kenya, the stem bark is either chewed raw or dried as a remedy for treating diarrhoea in infants (Kigen et al., 2016). Notably, bark extracts from the plant species showed non-toxicity in the brine shrimp bioassay (Khumalo et al., 2021), indicating the plant's safety for use in traditional medicine.

Phytochemistry and antimicrobial activity

Phytochemical analysis of the plant revealed the presence of several bioactive compounds such as phenolic acids, alkaloids, flavonoids, terpenoids, and quinones which are all antibacterial (Mongalo et al., 2020). Several studies reported the antimicrobial activity of *Z. mucronata* (Nemudzivhadi and Masoko, 2015; Munodawafa et al., 2013; Olajuyigbe and Afolayan, 2012). For instance, it has been reported that the plant species has potential antimicrobial activity against important pathogens, such as *Mycobacterium tuberculosis*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Escherichia coli*, *Propionibacterium acnes*, *Candida albicans*, and *Cryptococcus neoformans*, among others, with MIC of <1 mg/mL (Mongalo et al., 2020). Similarly, Coopoosamy et al. (2011) revealed substantial antimicrobial properties of *Z. mucronata* leaf, bark, and root extracts against bacteria and fungi (*Aspergillus flavus* and *Aspergillus glaucus*). More significantly, *Z. mucronata* bark extracts showed broad-spectrum activity against tested microorganisms (Khumalo et al., 2021).

2.7.2 *Ximenia caffra* Sond.

Figure 2.5: *Ximenia caffra* [Photographed at Waddilove Farm, Marondera (18°17'11"S 31°34'59"E)]

Description and distribution

Ximenia caffra (Fig 2.5) belongs to the Ximeniaceae or Olacaceae family and the species is indigenous to tropical Africa (Maroyi, 2016a). In Zimbabwe, it is known by several common names which include Munhengeni, Mutengeni, Mutsvanzva (all Shona), Sourplum (English) and Umthunduluka (Ndebele). The plant species is a deciduous tree that grows to a height of 6 m and has an untidy open crown. On younger branches, the bark is brown or green rather than dark grey and rough. Branchlets have spines at their tips. White sapwood and strong, reddish-brown heartwood can be found in trees. It has a passive root system. Fascicles, or clusters of leathery dark green leaves, are frequently found on the short spur branchlets. They are basic, measuring 60 x 25 mm. The tiny, creamy-green, sweet-scented flowers are produced in single-stem clusters in the axils of the spines or on the dwarf branchlets from August to October. They are followed by thinly fleshed, round, and appealing fruits (drupes), which are 25 mm long, shiny, deep crimson with white markings (Munodawafa, 2012; Baloyi and Reynolds, 2004).

Medicinal uses

Infusions of the roots and leaves are taken for dysentery and diarrhoea treatment (Maroyi and Chekhyoussef, 2015; Maroyi, 2016a; Munodawafa, 2012). The plant species is said to be used in traditional medicine to treat wounds, STIs, fever, eye issues, menorrhagia, malaria, intestinal worms, and coughs in addition to treating diarrhoeal disorders (Munodawafa et al., 2013). In addition, powdered roots are used to speed up the healing of wounds, are added to soup, and are an aphrodisiac when added to beer, while powdered dried leaves are taken orally for fever and infertility, gargled for tonsillitis, and used as a vermifuge. Additionally, the decoction of the roots is used to make porridge, which is consumed once daily to treat morning sickness during pregnancy. The root decoction is also used to treat infertility (Munodawafa, 2012).

Phytochemistry and antimicrobial activity

According to the results of the phytochemical analysis of *X. caffra*, the species contains a variety of chemicals, including flavonoids, phenols, phytosterols, tannins, and fatty acids (Maroyi, 2016a). The same study also demonstrated the antibacterial activity of aqueous and organic extracts of its various sections. Several investigations demonstrated potent antibacterial activity against a variety of microorganisms (Jacob et al., 2021; Chingwaru et al., 2020; Maroyi, 2016a; Munodawafa et al., 2013). For instance, Chingwaru et al. (2020) found antibacterial activity of aqueous extracts of *X. caffra* against *Shigella* spp., *Salmonella typhi*, and *Escherichia coli* O157.

2.7.3 *Crossopteryx febrifuga*

Figure 2.6: *Crossopteryx febrifuga* (Courtesy: Hyde et al., 2022).

Description and distribution

Crossopteryx febrifuga (Fig. 2.6) belongs to the Rubiaceae family. In Zimbabwe, the plant species is known by the common names: common crown-berry, crystal-bark, (all English) Mubakatirwa, Mugoko, Mukombegwa, Mukombigo, Muteyo (all Shona) and Umphokophokwana (Ndebele). The plant species is a small deciduous tree or shrub with opposite, elliptic to ovate, hairy, or glabrous leaves that have noticeable net veining on the underside. Its tiny, dense terminal heads of creamy-white flowers occasionally have pink tinges. Additionally, its fruits are ovoid capsule clusters that are capped with calyceal scars. *Crossopteryx febrifuga* is widely distributed across all of Zimbabwe's agroecological zones. The plant species' geographic range spans from Ethiopia and Senegal to much of tropical Africa and as far south as Limpopo, South Africa (Hyde et al., 2022).

Medicinal uses

Crossopteryx febrifuga is widely used in African traditional medicine to treat a variety of ailments, including dysentery and diarrhoea (Chouna et al., 2015). In Zimbabwe, preparations of the dosage involve removing the bark from the eastern and western sides of the trunk, drying and grinding it to powder and mixed with porridge and eaten (Chigora et al., 2007). The same author linked the species to the treatment of other bowel problems. The bark is dried and ground into a powder. The powder is put into a horn, inserted into the rectum and the medicine is blown into the body. In addition to treating diarrhoeal illnesses, the plant species is also used to treat wounds and other injuries as well as other illnesses like malaria, fever, painful inflammatory disorders, coughs, syphilitic ulcers, inflammation of the eyes, intestinal worms, and ophthalmia (Chigora et al., 2007; Maiga et al., 2006)

Phytochemistry and antimicrobial activity

A study on phytochemical screening and analysis studies on *C. febrifuga* extracts found tannins, alkaloids, saponins, flavonoids, steroids, carbohydrates, phenolic compounds, cardiac glycosides, and anthraquinones (Halilu et al., 2012). The presence of phytochemicals in the plant species is attributed to the species antibacterial activity. The antibacterial efficacy of plant extracts against several bacterial and fungi strains was demonstrated by Halilu et al. (2012). At doses of 50 mg/mL, 100 mg/mL, and 200 mg/mL, the extracts inhibited the growth of both gram-positive and gram-negative microorganisms, with zones of inhibition of 7 to 23 mm. Similarly, 8 mm zones of inhibition for *Aspergillus fumigatus* growth were produced by the same extracts. Furthermore, Chouna et al. (2015) isolated triterpenes from the stem bark extract of *C. febrifuga* that demonstrated antibacterial and antifungal activities comparable to or even greater than chloramphenicol and nystatin.

2.7.4 *Sclerocarya birrea* Hochst.

Figure 2.7: *Sclerocarya birrea* [Photographed at Great Zimbabwe University, Masvingo (20.1050° S, 30.8644° E)]

Description and distribution

Sclerocarya birrea (Marula) (Fig. 2.7) belongs to the Anacardiaceae family. The plant species is an African plant, native to Angola, southern Democratic Republic of Congo, Namibia, Kenya, Tanzania, Zanzibar, Botswana, Malawi, Mozambique, Zambia, Zimbabwe, South Africa, and Madagascar (Maroyi, 2013). The plant species is found in mixed deciduous forests, forested grasslands, and dry savannahs. It is primarily found in Miombo woodlands but not exclusively so. It is linked to seasonal rainfall patterns. In Zimbabwe, *S. birrea* is mostly found in Masvingo, Manicaland and Matebeleland provinces (Macheka et al., 2022). The marula is a large to medium-sized deciduous tree with a rounded crown and an upright trunk. Its leaves are imparipinnate with 7–15 pairs of ovate to elliptic leaflets and a terminal leaflet, alternating, packed near the ends of branches, and dark green above and softer bluish green below. The unisexual, unbranched blooms appear in axillary and terminal sprays and are scarlet in the bud before turning pinkish-white. The plant produces fleshy, plum-like fruits that are green while immature and turn yellow when ripe

(Hyde et al., 2022; Coates Palgrave, 1983). In Zimbabwe, *S. birrea* is known as Marula (English) Mufuna, Mupfura, Mushomo (all Shona) and Umganu (Ndebele).

Medicinal uses

Indigenous societies in Africa frequently treat bacterial-related diseases with Marula bark (Eloff, 2001). Particularly in the southern region of Africa, different communities have been employing the stembark, roots, and leaves of S. *birrea* to cure a range of diseases, such as dysentery and diarrhoea (Semenya and Maroyi, 2012). Some rural South African and Zimbabwean communities keep the plant close to their homes because of its significant economic importance (Mongalo and Makhafola, 2018). A decoction of the stem or bark of *S. birrea* is used orally in Zimbabwe for the treatment of cholera, dysentery, and diarrhoea (Masoko, 2013). In addition, rural communities in South Africa were reported to administer anally the bark and water (hot, warm, or cold) mixture three times a day, until diarrhoea subsides (Komolafe, 2014). Similarly, in Zimbabwe, the bark is crushed, mixed with water and drunk to treat stomach disorders, including "loose anal problem" (dzviti) (Maroyi, 2013). Furthermore, the species is reported to treat other diseases such as cough, pneumonia, heart pains, bilharziasis, and malaria (Maroyi, 2013). Interestingly, bark extracts of the plant species demonstrated non-toxicity against the brine shrimp bioassay (Khumalo et al., 2021), making the plant safe for use in traditional medicine.

Phytochemistry and antimicrobial activity

According to Manzo et al. (2017) and Viol et al. (2013), the phytochemical study of *S. birrea* revealed a wide range of phytochemicals, including alkaloids, flavonoids, tannins, sterol, saponin and triterpene. Most of these phytochemicals have demonstrated potent antibacterial activities (Rafey et al., 2021). *Sclerocarya birrea*'s antimicrobial properties were examined in prior investigations to support its ethnobotanical use in traditional medicine (Louis et al., 2018; Manzo et al., 2017; Njume et al., 2011). The plant extracts, for instance, significantly inhibited a variety of bacteria and fungi. The MIC range for bacterial species was 0.156 to 0.625 mg/mL, but the range for fungal species was 0.3125 to 1.25 mg/mL (Tanih and Ndip, 2012). Acetone extracts of bark and leaves were effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*, with MIC values ranging from 0.15 to 3 mg/mL

(Eloff, 2001). More significantly, *S. birrea* bark extracts showed broad-spectrum activity against the tested microorganisms (Khumalo et al., 2021).

2.7.5 *Flacourtia indica*

Figure 2.8: *Flacourtia indica* [Photographed at Waddilove Farm, Marondera (18°17'11"S 31°34'59"E)]

Description and distribution

Flacourtia indica (Fig 2.8) belongs to the Salicaceae family and in Zimbabwe the plant species is known by the common names: Batoka plum, Governor's plum (all English) Mududwe, Munhunguru, Mutombototo, Mutudza, Mutunguru (all Shona) and Umthunduluka (Ndebele). The plant species has elliptic, ovate, or nearly round leaves with an entire, scalloped, or serrated border. The plant has inconspicuous greenish-yellow flowers with short axillary or terminal sprays. The sexes of this plant are found on distinct trees. Additionally, when ripe, its fruits are juicy, nearly spherical, and purple (Hyde et al., 2022). In Zimbabwe, *F. indica* is extensively dispersed throughout the country's agroecological zones in a variety of soil types, from deep, well-drained sandy or gravelly soils to clay soils (Macheka et al., 2022). The plant species is also present in Madagascar, India, Sri Lanka, Indo-China, Indonesia, and China, in addition to tropical Africa's southern and northern parts of South Africa, and Eswatini (Hyde et al., 2022).

Medicinal uses

The leaves have been recognised for their ability to manage and control a range of stomach ailments, while the gum has been reported to be effective in treating cholera (Gelfand et al., 1985). In numerous communities across Zimbabwe, leaves are infused in cold water, and the resulting extract is consumed as a treatment for diarrhoeal diseases. Nonetheless, the method of preparing the extract recommended by certain traditional healers is distinctive in that the leaves must be browsed by mouth, chewed, and the extract ingested (Maroyi, 2011). Traditional healers in Livingstone (Zambia) also reported the practice of chewing raw leaves (Chinsembu, 2016). The rationale for browsing is shrouded in mystery and lacks a clear scientific explanation. Nonetheless, the practice of steeping plant leaves or roots remains a prevalent traditional technique for producing extracts.

Phytochemistry and antimicrobial activity

According to Maroyi (2011), the phytochemical analysis of *F. indica* revealed a wide range of phytochemicals, including flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides and phenolic compounds. Most of these phytochemicals have demonstrated potent antibacterial activities (Rafey et al., 2021). For example, Chingwaru et al. (2020) demonstrated the antibacterial activities of aqueous extracts of *F. indica* against *Shigella* spp., *Salmonella typhi* and *Escherichia coli* O157. In the same study steeping and aqueous extraction of *F. indica* leaves demonstrated significantly improved antibacterial activity as compared to other methods of extraction. This alone justifies steeping (hot or cold water) as a frequently adopted method in traditional medicine, and it should also be highly recommended as an extraction method of choice for antidiarrhoeal medicinal products.

2.7.6 *Euclea divinorum*

Figure 2.9: ***Euclea divinorum*** (Courtesy: Hyde et al., 2025).

Description and distribution

Euclea divinorum belongs to the Ebenaceae family. In Zimbabwe, the plant species is known by the following common names: Diamond-leaved euclea, Magic guarri (all English) Mubhununu, Mudziviriratsuro, Mugarazvuru, Mugurameno, Munyenya, Mushangura (all Shona) and Umtshekesane (Ndebele). *Euclea divinorum* is an evergreen shrub or small tree that can grow to a height of 9 meters. It has male and female blooms on different plants. It has a single or several stems and a circular crown. Young stems have rusty granules and are smooth and light grey. The bark is tough, longitudinally cracked, and ranges in colour from grey-brown to black (Mothogoane, 2014). Its leaves are opposite or subopposite, elliptic, and have clearly undulating edges on the upper and lower surfaces. Small, creamy-white flowers grow in short, crowded, branching inflorescences in the leaf axils. The plant species produces fleshy, single-seeded, spherical, purplish-black fruits that are around 5-7 mm in diameter (Hyde et al., 2022).

A substantial portion of tropical Africa is covered by the *E. divinorum*, which is widely distributed from South Africa through northern Namibia, Mozambique, Zimbabwe, and Botswana. It naturally grows in woodland, hillsides, thickets, thorny scrub, and bushveld (Mothogoane, 2014).

Medicinal uses

This plant has a long history of use in the treatment of numerous human ailments (Hutchings, 1996). For the treatment of diarrhoea, the roots of *E. divinorum* are crushed, boiled in water, and then consumed as a decoction (Chinsembu, 2016). Additionally, it is claimed that a fine powder made from roots and leaves is used to cure snake bites, pneumonia, tonsillitis, sore throat, constipation, and chest pain (Kipkore et al., 2014).

Phytochemistry and antimicrobial activity

According to a phytochemical analysis of an extract from the root bark of *E. divinorum*, a range of low-molecular-weight secondary metabolites, including triterpenes, sesquiterpenes, tetraterpenes, and aliphatic hydrocarbons, were present (Omara et al., 2022; Kilonzo et al., 2019). Most of these phytochemicals have demonstrated potent antibacterial activities (Rafey et al., 2021). Several studies have reported the antimicrobial properties of *E. divinorum*, hence supporting its use in traditional medicine (Girmaw and Engidawork, 2022; Omara et al., 2022; Maina and Dimba, 2021). The results of Kilonzo et al. (2019) demonstrated that root extracts had antibacterial activity against a range of bacteria and fungi and had a MIC value of 0.39 mg/mL. The extracts' antifungal activity was greater than that of fluconazole, a common antifungal drug. Likewise, the ethanolic extract of *E. divinorum* root bark showed activity with MIC values of 25, 50, 25, and 25 µg/ml for *Streptococcus pyogenes, Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, respectively (Mbabazi et al., 2020).

2.7.7 *Peltophorum africanum* Sond.

Figure 2.9: *Peltophorum africanum* (Courtesy: Hyde et al., 2022).

Description and distribution

Peltophorum africanum (Fig 2.9) is an ethnomedicinal plant in the family Fabaceae (Mazimba, 2014). In Zimbabwe, the plant species is known by the following common names: African wattle (English), Dzedze, Mudjiza, Mupumhamauva, Musambanyoka, Mutandarombo, Muzeze, Nyakambariro, Nyamanyoka, Zeze (all Shona), Umkahla and Umsehla (all Ndebele) (Hyde et al., 2022). A semi-deciduous to deciduous tree with a spreading, untidy canopy, *P. africanum* is around 15 metres tall. The bark of older trees is grooved and grey-brown, while the bark of young branches is smooth and grey. The leaves resemble acacias and are silver-grey with small hairs covering them; mature leaves turn yellow at the tips of branches. The leaves are alternating and doubly compound, with a pair of leaflets at the apex of each pinna. The leaf, stalk, and rachis are all covered in reddish-brown hairs (https://treesociety.org.zw/tree-life/380/). The plant species also features upright, spectacular inflorescences with axillary sprays of brilliant yellow flowers. Pods are indehiscent, flat, and pendulous with papery wings (Hyde et al., 2022).

The plant species is widely distributed throughout Zimbabwe's agro-ecological zones. Additionally, it spreads from the DRC down to South Africa (Hyde et al., 2022).

Medicinal uses

The plant species leaf or bark or root infusion is drunk as a remedy for diarrhoea (Chinsembu et al., 2019; Maroyi and Chekhyoussef, 2015). The plant species is also reported to treat other diseases such as syphilis, headache, toothache, sore eyes, respiratory infections, infertility, STIs, cough, joints, backache, stomach disorders, and fever (Mongalo, 2013).

Phytochemistry and antimicrobial activity

Several phytochemical screening and analysis studies on *P. africanum* extracts found several phytochemicals including tannins, saponins, flavonoids and cardiac glycosides, anthraquinone derivatives, coumarins, and alkaloids (Mongalo, 2013; Mazimba, 2014). These phytochemicals are responsible for the plant's antibacterial properties (Rafey et al., 2021). A variety of clinical infections were effectively inhibited by the stem bark's ethyl acetate extract. Bacteriostatic/fungistatic and bactericidal activities were demonstrated by the extract (Okeleye et al., 2010, 2013).

2.7.8 *Elephantorrhiza goetzei* (Harms) Harms

Figure 2.10: *Elephantorrhiza goetzei* (Hyde et al., 2022).

Description and distribution

Elephantorrhiza goetzei (Fig 2.10) belongs to the Fabaceae family. It is a 1-4 m tall deciduous shrub, infrequently a small tree. Its bipinnate leaves can have as many as 40 pairs of pinnae, each containing as many as 48 pairs of leaflets. The leaflets have a pointed apex, a midrib that begins on one side, is hairless, and can be up to 12 mm long. Flowers on axillary spikes are typically seen before the emergence of new leaves, and they are creamy-yellow with prominent stamens. Pods that are cylindrical and up to 45 cm long separate from the tenacious margins (Hyde et al., 2022). The large underground rhizome (of up to 7m) that some members of this genus sometimes possess is referred to as "elephantorrhiza," and its generic name translates as "elephant root" (Palmer and Pitman, 1972). The plant is found in Botswana, Malawi, Mozambique, Namibia, South Africa, Tanzania, Zambia, and Zimbabwe (Maroyi, 2017a). There are numerous local names for *E. goetzei* in the regions where it is found. For example, in Zimbabwe it is known by English names (long-pod cassia, narrow-rod elephant root), Ndebele names (ililamba, intolwane), and Shona names (chiurayi, mugudzuru, muzezepasi, ntorani). However, the most common name in Zimbabwe is Ntorani (Maroyi, 2017a).

Medicinal uses

Elephantorrhiza goetzei has a long history of medical use among many African ethnic groups. It has historically been used as a decoction to treat several diseases, including gastrointestinal problems (Maroyi, 2017). The root extract is consumed as a treatment for gonorrhoea, diarrhoea, and abdominal pains (Maroyi, 2011, 2013). In earlier findings by Gelfand et al. (1985), it was reported that most villages in Zimbabwe take the species bark or root decoction, infusion, or macerate orally as a diarrhoea cure. Other African nations (such as Malawi and Mozambique) have also reported using *E. goetzei* for therapeutic purposes, with the root macerate, bark or roots cooked as food, or root powder mixed into porridge and consumed orally (Viol et al., 2013; Bruschi et al., 2011; Mwafongo et al., 2010). Furthermore, *E. goetzei* is also reported to treat a variety of conditions such as malaria, pain, sores, sexually transmitted infections, microbial infections, and genito-urinary system disorders (Maroyi, 2017a; Ngarivhume et al., 2015; Viol et al., 2013; Gelfand et al., 1985).

Phytochemistry and antimicrobial activity

The phytochemical analysis of several *E. goetzei* parts revealed a variety of phytochemical classes, including phenolic substances, coumarins, flavonoids, saponins, stilbenoids, tannins, and triterpenoids (Maroyi, 2017a; Viol et al., 2013; Wanjala and Majinda, 2001). Remarkably, most of these phytochemicals have been proven to have powerful antibacterial properties (Rafey et al., 2021). Furthermore, several studies have revealed that *E. goetzei* extracts from different extraction solvents, including water, have antibacterial activities against a variety of microorganisms (Moyo et al., 1999; Mølgaard et al., 2001). For instance, *E. goetzei* extracts demonstrated antibacterial activity against *Staphylococcus aureus* with a zone of inhibition of 4.0mm (Maroyi, 2017a).

2.7.9 *Grewia bicolor*

Figure 2.11: *Grewia bicolor* (Courtesy: Hyde et al., 2022).

Description and distribution

Grewia bicolor (Fig 2.11) is a member of the Malvaceae family and has many regional common names in Zimbabwe. Among them are Mutongoro (Shona), Mwingili, Ndeywa, Ngiri (all Tonga), Sihane (Hlengwe), Umhlampunzi, Umpumpulwane (all Ndebele), and White-leaved Raisin (English).

The plant species is mostly a multi-stemmed shrub, although it can sometimes be a small tree. The juvenile branches are velvety, and the bark is dark grey and highly fissured, flaking away in strips. Its leaves are up to 7 cm long and 2 cm wide, elliptic-oblong to lanceolate, three-veined from the base, dull green above, silvery-white hairy below, and finely serrated along the border. Additionally, the axillary heads of 1.5 cm wide yellow flowers are frequently abundant. The plant species produces edible, spherical, or 2-lobed fruit with reddish-brown, 6-mm-diameter lobes (Hyde et al., 2022).

Grewia bicolor is widely distributed in Africa, Arabia, and India (Hyde et al., 2022). It is indigenous to Zimbabwe and is widely distributed throughout the country (Nyakudya et al., 2015).

Medicinal uses

The roots, bark, and leaves of *G. bicolor* are used for several purposes in traditional African ethnomedicine (Nyakudya et al., 2015). According to reports, a leaf infusion is used to cure diarrhoea in Zimbabwe (Maroyi, 2011). Decoction of the same plant species' leaves is used to treat diarrhoea in other countries, such as Zambia and South Africa (Chinsembu et al., 2019; Semenya and Maroyi, 2012). Furthermore, it has been reported that the plant's root decoctions can treat a variety of illnesses, including gonorrhoea, syphilis, abscesses, and pustulous skin sores (Arora, 2011).

Phytochemistry and antimicrobial activity

According to the findings of a phytochemical analysis of *G. bicolor*, the petroleum ether extract contained β-sitosterol, and triterpene esters, as well as the triterpenes lupeol and betulin. Three alkaloids, harman, 6-methoxyharman, and 6-hydroxyharman were found in a methanol extract, together with β-sitosterol-glucoside (Jaspers et al., 1986). These phytochemicals give the plant species its medicinal significance (Khan et al., 2022; Bildziukevich et al., 2015). *Grewia optiva* and *G. mollis*, two closely related species of *G. bicolor*, have shown antibacterial efficacy against a variety of bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* (Shagal et al., 2012; Arora, 2011). The antibacterial properties of these closely related plants support the use of G. *bicolor* leaf infusion and root decoction in the treatment of diarrhoea and other diseases (Arola, 2011; Maroyi, 2011).

2.7.10 *Kirkia acuminata* Oliv.

Figure 2.12: *Kirkia acuminata* (https://www.zimbabweflora.co.zw/speciesdata/image)

Description and distribution

The plant species known as *Kirkia acuminata*, which is a member of the Kirkiaceae family, has several common names. Mubvumira, Mutsakatidze, Mutuhwa (all Shona), Umvumile (Ndebele), White seringa and White syringa (all English) are some of the names used in Zimbabwe. The plant species is a medium-sized deciduous tree with narrowly oblong leaflets, tapering at the apex, and with finely serrated margins. The leaves are sticky when young, alternating, packed near the ends of branches, and imparipinnate. Its axillary inflorescences are branching and have small, greenish-cream blooms. They produce fruits in the form of thinly woody capsules with four valves (Hyde et al., 2022).

Kirkia acuminata is found exclusively in East, Central, and Southern Africa, with its main distribution in Angola, Botswana, DRC, Malawi, Mozambique, Namibia, Tanzania, Zambia, and South Africa. This plant species is also native to Zimbabwe and has a wide distribution across the country (Maroyi, 2017c).

Medicinal uses

Kirkia acuminata is one of the medicinal plants found in sub-Saharan Africa (Gelfand et al., 1985; Maroyi, 2011; Maroyi, 2013). More significantly, in Zimbabwe, the stem bark of *K. acuminata* is used to cure a variety of diseases like cholera, diarrhoea, dysentery, and abdominal aches (Chigora et al., 2007; Maroyi, 2013; Maroyi, 2017b). Likewise, *K. acuminata* demonstrated efficacy in the management and treatment of diarrhoea in a recent ethnobotanical survey conducted among traditional healers in Zambia. After each meal until the indications and symptoms subside, one cup of stem bark infusion is consumed (Chinsembu, 2016; Chinsembu et al., 2019). It has also been reported that the plant species can treat wounds and other conditions like backaches, constipation, coughs, snake bites, and toothaches (Maroyi, 2017b).

Phytochemistry and antimicrobial activity

Several chemicals, including alkaloids, anthraquinones, glycosides, flavonoids, phenols, and tannins, among many others, were identified through phytochemical analysis of the plant species (Maroyi, 2016). These phytochemicals give the plant species its antibacterial properties (Rafey et al., 2021). *Staphylococcus aureus* and *Mycobacterium smegmatis* were inhibited by extracts of *K. acuminata* at the lowest concentrations of 2.5 mg/ml and 1.25 mg/ml, respectively (Mabadahanye et al., 2022).

2.7.11 *Vangueria infausta*

Figure 2.13: *Vangueria infausta* (Courtesy: Hyde et al., 2022).

Description and distribution

Vangueria infausta (Fig. 2.13) is a member of the Rubiaceae family, and in Zimbabwe, it is also known as Velvet wild medlar (English), Munjiro, Munzviro, Munzviru (all in Shona), Umthofu, and Umviyo (all in Ndebele). The plant species is a small deciduous tree with velvety hairy leaves and young branches. Before the leaves have fully formed, it produces branched, densely hairy inflorescences of greenish-yellow, creamy-white flowers. Additionally, the spherical, up to 45 mm, green to yellowish brown ripe fruits are borne in pairs along the branches, held below the leaves. The fruits can be eaten (Hyde et al., 2022).

Vangueria infausta is native to Angola, Botswana, Kenya, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Tanzania, Uganda, Zambia, and Zimbabwe (Coates Palgrave, 1977).

Medicinal uses

Vangueria infausta has a long history of antidiarrhoeal usage in African countries such countries Malawi, Mozambique, South Africa, Tanzania, and Zimbabwe (Gelfand et al., 1985; Maroyi, 2011; Maroyi and Chekhyoussef, 2015), where bark, leaf and root infusion are drunk until the pain subsides (Maroyi, 2011, 2018). The importance of the species is shown by the extent that some rural communities in South Africa keep the trees around their households (Mongalo and Makhafola, 2018). Furthermore, the plant species is also used for many purposes which include malaria, pneumonia, cough, menstrual problems, aphrodisiacs, parasitic worms, chest complaints, remedy for snake bites, infertility, fever, candidiasis, and abdominal pains (Maroyi, 2018).

Phytochemistry and antimicrobial activity

Various extracts of *V. infausta* were tested in investigations for their antibacterial efficacy against a variety of medically significant bacterial species, with MIC values ranging from 0.02 to 10 mg/mL (Munodawafa et al., 2013). Additionally, phytochemical studies carried out in Zimbabwe and elsewhere discovered the presence of *V. infausta*'s biologically active flavonoids, alkaloids, anthraquinones, coumarins, glycosides, polyphenols, saponins, secoiridoids, steroids, tannins, and terpenoids (Munodawafa et al., 2013; Maroyi, 2018). The species' historic use by local communities as a treatment for diarrhoea and stomach issues is supported by the recorded phytochemistry and antibacterial activity displayed by the species (Maroyi, 2018).

2.7.12 *Ficus sur* Forssk

Figure 2.14: *Ficus sur* (Courtesy: Hyde et al., 2022).

Description and distribution

Ficus sur (Fig 2.14) is a member of the Moracea family, and in Zimbabwe, it is also known as Broom-cluster fig, Cape fig (English), and Muonde (Shona). The plant is a big, spreading tree. Its entire body exudes creamy latex. In large forest examples, the main stem may have buttresses; the bark is smooth and light grey. Its spiralled, alternating leaves have a crenate-dentate border and range in size from oval to elliptic. The underside is typically pubescent, with the upper surface being glabrous. The plant species produces huge clusters of figs carried on leafless branchlets that emerge from the main stem and more mature branches. Additionally, the figs are densely tomentose, brilliant green, and when mature, turn orange to red (Hyde et al., 2022).

From North Africa to the Western Cape in South Africa, this species is widely dispersed. It typically grows along riverbanks or in riverine forests, but it can also be found in drier forests. It only grows in locations without frost and moderate rainfall (Hankey, 2003). It is indigenous to Zimbabwe and is widely distributed throughout the country (Hyde et al., 2022).

Medicinal uses

Ficus sur roots and leaves preparations are used in the folklore treatment of diarrhoea and stomach pains in Zimbabwe (Gelfand et al., 1985; Maroyi, 2011). Similar to this, *F. sur* bark or root decoction is swallowed to treat diarrhoea in Ghana (Neuwinger, 1994). Furthermore, the plant species is reported to treat a other diseases which include gonorrhoea, sore throat, toothache, eye problems, epilepsy, leprosy, infertility, gonorrhoea and sickle cell disease (Gelfand et al., 1985).

Phytochemistry and antimicrobial activity

The traditional usage of herbal medicines is linked to the biological activities of such plants through their phytochemistry and pharmacological qualities (Maroyi, 2016). Therefore, *F. sur*'s antidiarrhoeal activities are dependent on its phytochemistry. According to Akoto et al. (2020) several chemicals, including anthraquinones, terpenoids, flavonoids, steroids, saponins, phenols and tannins, among many others, were identified through phytochemical analysis of the plant species. The same authors demonstrated the antimicrobial activity of plant extracts against bacteria and fungi with MICs ranging from 2.5- 40 mg/ mL.

2.7.13 *Strychnos spinosa*

Figure 2.15: *Strychnos spinosa* [Photographed at Waddilove Farm, Marondera (18°17'11"S 31°34'59"E)]

Description and distribution

Strychnos spinosa (monkey orange) (Fig. 2.15) is native to tropical and subtropical Africa and belongs to the Loganiaceae family (Bisset, 1970). The plant species is referred to by the following common names in Zimbabwe: Mutamba (Shona), Spiny monkey-orange (English) Umhahli and Umngono (Ndebele). The plant is a tiny deciduous tree or shrub with corky, not extensively fissured, and rough bark. Its branches frequently have hidden, curled or straight spines as a defence. Its opposite leaves are elliptic, oblong to ovate, 3–7 veined from the base, and vary in hairiness or lack of hair. Additionally, the creamy-green flowers are borne on tiny lateral spur branchlets or terminally in small, compact heads. The plant species produces spherical, up to 12 cm long, hard-shelled, green with brown spots, and edible and delectable fruits (Hyde et al., 2022).

Strychnos species are extensively spread throughout Zimbabwe's agroecological areas, growing in a variety of soil types, from deep, well-drained sandy or gravelly soils to clay soils (Macheka et al., 2022). *Strychnos spinosa*, however, is mostly found in the north, east, central south, and west (Ngadze et al., 2017). Aside from that, the plant species is said to be found throughout Africa, from Senegal to tropical Africa to South Africa (Isa et al., 2014).

Medicinal uses

Strychnos spinosa has been used traditionally for an exceedingly long time to cure a wide range of illnesses, including diarrhoea. According to reports, the Pedi tribe in South Africa's Limpopo area uses the species to manage and treat diarrhoea (Mongalo and Makhafola, 2018). In addition to treating diarrhoea, the plant species is also used to treat rheumatism, dropsy, earaches, snakebites, fever, elephantiasis, and fever (Laldinsanga et al., 2019).

Phytochemistry and antimicrobial activity

Several chemicals, including alkaloids, saponins, anthraquinones, glycosides, steroids, and terpenoids, among many others, were identified through phytochemical analysis of the plant species (Ahmad et al., 2019; Tor-Anyiin et al., 2015). The presence of these chemicals makes *S. spinosa* display a variety of biological activities against many parasites and bacteria that cause several diseases in both people and animals (Aremu and Moyo, 2022). These biological activities underpin the traditional medical application of this plant species. For instance, *S. spinosa* leaf acetone extracts showed antibacterial activity against test fungi and bacteria, with MICs ranging from 0.04 to 1.25 mg/ml (Isa et al., 2014). Comparably, *S. spinosa* and its close relatives *S.*

madagacariensis and *S. pungens* showed strong antibacterial action against pathogens like *Salmonella typhi*, *Bacillus cereus,* and *Staphylococcus aureus* (Uttu et al., 2022; Tor-Anyiin et al., 2015).

2.7.14 *Xeroderris stuhlmannii* (Taub.) Mendonca & E.P. Sousa

Figure 2.16: *Xeroderris stuhlmannii* (Courtesy: Hyde et al., 2022).

Description and distribution

Xeroderris stuhlmannii (Fig. 2.16) belongs to the Fabaceae family and in Zimbabwe the plant species is known by the common names: Muchemavanhu, Mudzugu, Mumwambizi, Muriravanhu, Murumanyama (all Shona), Umthundulu (Ndebele) and Wing pod (English). *Xeroderris stuhlmannii* is a medium- to large-sized spreading tree with densely clustered leaves. at the tips of branches. It has imparipinnate leaves with about 15 subopposite or alternate leaflets that are oblong to ovate, 5-13 cm long, green, and appear to be folded upwards along the midrib. The base of the tree is asymmetrical. White to greenish white, branching axillary and terminal sprays make up its blooms. In addition, certain plant species develop pods that are 6 to 25 cm long, thicker around the seeds, hairless, leathery and have a noticeable winged rim (Hyde et al., 2022).

The plant species is widely dispersed across all of Zimbabwe's agroecological zones, in tropical Africa, and south to Limpopo and Mpumalanga, South Africa (Hyde et al., 2022).

Medicinal uses

Xeroderris stuhlmannii was identified as one of the most significant medicinal plant species utilised as an antidiarrhoeal in traditional medicine by a recent survey carried out in Gokwe South District, Zimbabwe (Shopo et al., 2022). The plant species is also said to have a long history of being used to cure other diseases, including diabetes mellitus, coughs, malaria, colds, rheumatoid arthritis, stomachaches, dysentery, and eye infections (Nyathi et al., 2022; Ngarivhume et al., 2015; Maroyi, 2013). Furthermore, several Zimbabwean communities have reported using the same plant species to treat diarrhoea in chickens (Gobvu et al., 2022).

Phytochemistry and antimicrobial activity

Xeroderris stuhlmannii has a wide range of biological and pharmacological activities, which are widely known. Bark extracts from the plant species showed considerable antibacterial activity against various bacteria with MICs ranging from 0.23-0.80 mg/mL (Selemani et al., 2021). Similarly, old studies also concurred with the antibacterial properties of *X. stuhlmannii* (Chitemerere and Mukanganyama, 2011) even against resistant strains of *Staphylococcus aureus* of *Enterococcus faecalis* (Pereira et al., 2015). Selemani et al. (2021) also revealed that the crude bark extracts contained a variety of significant alkaloids, flavonoids, terpenoids and tri-terpenoids, polyphenols, steroids, and other phytochemicals. Most of these phytochemicals are reported to possess antibacterial properties (Rafey et al., 2021). Additionally, *X. stuhlmannii* compounds exhibit anti-parasitic, anti-cancer, and antioxidant properties (Selemani et al., 2021).

2.7.15 *Cassia abbreviata* Oliv.

Figure 2.17: *Cassia abbreviata* (Courtesy: Hyde et al., 2022).

Description and distribution

Cassia abbreviata (Fig. 2.17) belongs to the Fabaceae family and in Zimbabwe the plant species is known by the following common names: Isihaqa (Ndebele) Muremberembe, Muvheneka (all Shona) Sjambok pod, Long-tail cassia (all English). *Cassia abbreviata* is a greyish brown, longitudinally wrinkled shrub, or small, rounded tree. Its leaves are paripinnate and lack glands on the rhachis or petiole. They have 7–12 pairs of leaflets that are hairy on the underside, especially when they are young, and have an ovate–elliptic shape with a rounded tip. Before the leaves appear, the flowers are in huge, loose, terminal sprays that are yellow. In addition, the fruit is a long, 90 cm long, cylindrical brown pod that hangs from the tree for several months (Hyde et al., 2022).

The plant species is widespread throughout Zimbabwe and can be found from East Africa all the way down to South Africa (Hyde et al., 2022).

Medicinal uses

The folkloric usage of *C. abbreviata* in African traditional medicine is well recognized (Mongalo and Mafoko, 2013). The decoction of *C. abbreviata* is traditionally made by soaking fresh or dried stem bark or roots in water "overnight" (at least 12 hours) or by boiling for a while (Mulubwa and Prakash, 2015). In Zimbabwe, the roots of *C. abbreviata* are crushed, mixed with hot water, and the resulting extract is taken orally to treat several diseases, including diarrhoea (Maroyi, 2013). Other African nations like Zambia, traditional healers, employ the plant species extensively as an anti-diarrhoea medicine (Tembo, 2011). The plant species is also said to manage numerous disorders in different parts of Africa, including postpartum discomfort, menstrual cycle issues, blood vomit, close fontanelle in newborn babies, bilharzia, hernia, venereal diseases, fever, dysentery, and hernia (Mongalo and Mafoko, 2013; Bruschi et al., 2011; Ribeiro et al., 2010).

Phytochemistry and antimicrobial activity

In terms of pharmacology, *C. abbreviata* has antibacterial activities that may be linked to a range of substances, including alkaloids, tannins, anthraquinones, flavonoids, saponins, terpenoids and polyphenols (Hikaambo et al., 2022; Mongalo and Mafoko, 2013). According to several studies, the plant species has antibacterial properties that support its usage as an antidiarrhoeal in traditional medicine (Hikaambo et al., 2022; Mulubwa and Prakash, 2015). Tembo (2011) isolated compounds with noticeable anti-diarrhoeal activity from the stem bark of *C. abbreviata* extracts using chromatographic methods. Through spectrometry, it was determined that the substance was either 3-(3/-hydroxyphenyl)-2-propenoic acid or 3-(4'-hydroxy-3'-methoxyphenyl)-2-propenoic acid. In a comparable study, Chitoti et al. (2020) demonstrated the antidiarrhoeal activity of *C. abbreviata* stem bark extracts in a mouse model of castor oil-induced diarrhoea by observing a reduction in the frequency of diarrheal episodes and the volume of stools generated. Furthermore, Mongalo and Mafoko (2013) revealed that *C. abbreviata* water extracts have antibacterial efficacy against many bacterial species, including *Bacillus subtilis*, *Micrococcus kristinae*, *Enterobacter cloacae*, and *Staphylococcus aureus*. The same study showed MIC values between 1.0 mg/mL and 5.0 mg/mL. In a related study, extracts from the same plant species were effective against various bacteria, with MICs ranging from 46.88 g/ml to 93.75 g/ml (Mulubwa and Prakash, 2015).

2.7.16 *Schotia brachypetala*

Description and distribution

In Zimbabwe, *Schotia brachypetala*, a member of the Fabaceae family, is also known as Mutondochuru, Mutondoshuru, Mutondosvi, Nyamari (all Shona), Weeping boer-bean, and Weeping schotia (all English). The leaves of *S. brachypetala* have 4–7 pairs of leaflets, each with an oblique base, and the rhachis are occasionally narrowly winged. Its inflorescence is a crowded, dense panicle that is frequently carried on aged wood. Its crimson blossoms also have a glossy, moist appearance. Petals 5, with 1-4 growing normally or all reduced to linear filaments (Hyde et al., 2022). The plant species is found in Mozambique, Zimbabwe, Botswana, and South Africa (Hyde et al., 2022).

Medicinal uses

Most communities in Zimbabwe treat diarrhoea with *S. brachypetala* (Maroyi, 2016). In some regions, traditional medicine makes extensive use of the bark and roots of *S. brachypetala* as a treatment for dysentery and diarrhoea (Mlambo, 2008; Grace et al., 2003; Hutchings, 1996). Interestingly, bark extracts of the plant species demonstrated non-toxic effects against the brine shrimp bioassay (Khumalo et al., 2021), making the plant safe for use in traditional medicine. The plant species is also said to be effective in treating various conditions, such as skin conditions and heartburn (Coopoosamy and Naidoo, 2012).

Phytochemistry and antimicrobial activity

Different solvent extracts of *S. brachypetala* have been shown in numerous investigations to have antibacterial activity against a variety of bacteria with MICs ranging from 0.15 mg/mL to 3.31 mg/mL (Mathabe et al., 2006; McGaw et al., 2002). More significantly, *Schotia brachypetala* bark extracts showed broad-spectrum activity against tested microorganisms (Khumalo et al., 2021). The plant species' phytochemical analysis revealed several compounds, including seven quercetin glucoside derivatives (Du, 2011), which may be the source of its antibacterial activity (Rafey et al., 2021).

2.8 Potential Use of Plants to Decontaminate *Vibrio cholerae* from Environmental Sources

In regions with limited resources where traditional water treatment technologies are frequently unavailable or unsustainable, the use of medicinal plants for point-of-use water decontamination has attracted a lot of scholarly attention. Table 2 provides an overview of the medicinal plants studied for their efficacy in eliminating *V. cholerae* from different environmental sources, including drinking water, wastewater, seafood, and fresh ready-to-eat vegetables. A number of plant species (e.g., *Moringa oleifera*, *Strychnos potatorum*, *Vitex negundo*, and *Nephelium lappaceum*, have shown significant antibacterial, coagulant, and flocculant capabilities, resulting in microbial reductions, ranging between 75% and 100% (Jantapaso et al., 2024; Kamruzzaman et al., 2013; Yongabi et al., 2011; Sarawgi et al., 2009). Among the plant species, *M. oleifera* has shown an impressive ability, achieving as much as a 90% reduction in bacterial presence and notable turbidity improvement in both raw and river water samples (Abd Askar and Dakhil, 2023; Varkey, 2020).

A study conducted by Chakraborty (2015) highlights the potential of catechin and magnolol, derived from blackberry and magnolia species respectively, in addressing cholera-contaminated water. Likewise, in food decontamination applications, decoctions made from plants such as *Cleome gynandra*, *Tinospora cordifolia*, and *Alpinia galangal* have shown the capacity to eradicate *V. cholerae* entirely (Kumar et al., 2013). Moreover, *Vitex negundo* exhibited significant efficacy against a range of enteric pathogens, such as *V. cholerae* (Kamruzzaman et al., 2013). Furthermore, *Strychnos spinosa*, which is taxonomically related to *S. potatorum* and has demonstrated antibacterial qualities against enteric pathogens like *Salmonella typhi*, demonstrates the connection between traditional plant knowledge and contemporary research (Isa et al., 2014; Tor-Anyiin et al., 2015). This also suggests its potential as an alternative locally accessible water treatment solution.

While comprehensive investigations into cholera-specific interventions remain to be undertaken, the established phytochemical properties and demonstrated antimicrobial efficacy of Zimbabwean medicinal plants (as detailed in Section 2.7), including *S. spinosa, Z. mucronata, and S. birrea*, against diarrhoeal pathogens highlight the necessity for further inquiry into how they might be used for point-of-use water decontamination (Mongalo et al., 2020; Khumalo et al., 2021; Tanih & Ndip, 2012).

However, the combined results underscore the potential of plant-based decontaminants as sustainable, culturally accepted, and cost-effective alternatives to chemical treatments, especially in resource-limited communities facing an increased risk of cholera (Pritchard et al., 2009; Abd Askar & Dakhil, 2023).

Table 2.3: Decontamination of *Vibrio cholerae* from the Environment by Plants at Point of Use

Plant species	**Environment**	**Bacteria**	**Key findings and remarks**	**References**
Blackberry, *Magnolia* species	drinking water	*V. cholerae*	Catechin present in blackberry and magnolol present in *Magnolia* species reported to treat drinking water contaminated with cholera pathogen	Chakraborty, 2015
Cleom gynandra, Cordia obliquawillel, Sida cordifolia, Tinospora cordifolia, Moringa oleifera, Aegle marmelos, Alpinia galangal	*Chicoreus ramosus* meat	*V. cholerae*	Decoctions killed the bacteria completely during dip treatments	Kumar et al., 2013
Moringa oleifera, Jatropha curcas, Hibiscus sabdarifa	Wastewater	*Escherichia coli*	75 to 90% reductions in bacterial loads of the water samples were displayed. The extracts demonstrated both phytodisinfectant and phytocoagulant properties in water purification. The findings suggest these plants could be applied as phytodisinfectants in domestic water purification, with *M. oleifera* showing particularly promising phytodisinfection potential.	Yongabi et al., 2011
Moringa oleifera seeds	Raw water	Heterotrophic bacteria	The extract exhibited considerable microbial decontamination efficacy, attaining a removal rate of 90.0%. Although the bacteria differ, this may indicate the potential for a reduction of *V. cholerae* due to the presence of antimicrobial peptides in *Moringa* seed.	Abd Askar and Dakhil, 2023
Strychnos potatorum seeds	Drinking water	Total bacterial count and coliforms	The powder treatment resulted in 100% elimination of faecal coliforms and *Streptococcus*, therefore	Hepsibha and Kruthika, 2022

			demonstrating a great potential for microbial decontamination of water at the point of use.	
Strychnos potatorum seeds	Ground water	Heterotrophic microbial load	Seed powder at 10 mg L^{-1} reduced heterotrophic bacteria load by 99%. Ames test showed seed non-toxicity at doses evaluated. This cost-effective and efficient pre-treatment or point-of-use water disinfection method can be employed in decontamination of water.	Sarawgi et al., 2009
Nephelium lappaceum peel extract	Shrimp meat	*V. cholerae*, and other aerobic bacteria	Showed a notable decrease in total aerobic bacteria and *V. cholerae* in meat after treatment with the extract for 3 min, as the extract displayed antibacterial and antibiofilm properties.	Jantapaso et al., 2024
Vitex negundo leaf	(Environment not specified)	*Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio mimicus*, *Escherichia coli*, *Shigella* spp., and *Aeromonas* spp	The extract showed potent antibacterial activity (inhibition zone: 9.9–22.6 mm, MIC: 200–3200 µg/mL, MBC: 200–6400 µg/mL) against all the pathogenic enteric bacteria.	Kamruzzaman et al., 2013
Oregano leaves	Ready to eat fresh vegetables (cilantro, parsley, and spinach)	*Salmonella*, *Escherichia coli* O157:H7, and *Shigella sonnei*	The extract reduced the population of pathogenic bacteria on leafy greens by > 2 logs. Such decontamination can be applied to other pathogens such as *V. cholerae*.	Orue et al., 2013
Moringa seed powder	River water	*E. coli*	Acted as a natural coagulant and flocculent. Showed a decrease in turbidity to 3–5 NTU and killed *E. coli*, suggesting successful microbial decontamination and possible elimination of *V. cholerae*	Varkey, 2020
Moringa oleifera, *Jatropha curcas* and *Guar gum*	Shallow well water	Coliforms	The extracts demonstrated over 90% turbidity reduction and 80% coliform removal, underscoring the presence of natural coagulants.	Pritchard et al., 2009

Agrimonia eupatoria, Rubus fruticosus, Fragaria vesca	(Environment not specified)	*V. cholerae*	The extract exhibited bacteriostatic properties. Showcased the inhibition of *V. cholerae* through the blockage of toxin binding and internalisation, indicating potential for point-of-use preventive treatment.	Komiazyk et al., 2019

2.9 Conclusion and Way forward

The literature review presented in this chapter illustrates that cholera continues to pose a significant health challenge in Zimbabwe. Although a number of complex factors hinder its control, medicinal plants are viewed potential solutions. However, there is still a gap concerning the specific efficacy, safety, and bioactive compounds of Zimbabwean medicinal plants in relation to *Vibrio cholerae*. Moreover, the dual capability of these plants as therapeutic agents and environmental decontaminants offers a novel strategy for cholera control that necessitates thorough scientific validation. Therefore this chapter serves as a foundation for the subsequent experimental chapters, aiming to compile a comprehensive list of medicinal plants used for the treatment of cholera and other related diseases, along with investigations into their antimicrobial efficacy, cytotoxicity, and their phytochemical composition. The therapeutic potential of the selected plants can be validated through scientific methods.

References

Abd Askar, A. S., & Dakhil, A. S. D. S. (2023). Efficiency of *Moringa Oleifera* Seeds as Natural Alternative to The Chemical Coagulants in Drinking Water Treatment Process. Journal of Wildlife and Biodiversity, 7(Special Issue), 196-216.

Abdel-moneam, D. A., Ibrahim, R. A., Nashaat, M., & Shaalan, M. (2021). Multifactorial causes of mass mortality in *Oreochromis niloticus* in Kafr El-Sheikh, Egypt. Bulletin of the European Association of Fish Pathologists, 41(1), 2021.

Ahmad, G., Muhammad, A., Ahmad, K. A., & Gbeminiyi, A. (2019). Phytochemical and antimicrobial studies of the root bark extracts of *Strychnos spinosa* Lam. Bayero Journal of Pure and Applied Sciences, 12(1), 161-165.

Ahmed, H. A., El Bayomi, R. M., Hussein, M. A., Khedr, M. H., Remela, E. M. A., & El-Ashram, A. M. (2018). Molecular characterization, antibiotic resistance pattern and biofilm formation of *Vibrio parahaemolyticus* and *Vibrio cholerae* isolated from crustaceans and humans. International Journal of Food Microbiology, 274, 31-37.

Akoto, C. O., Acheampong, A., Kwame, O., & Boateng, B. A. (2020). An Assessment of the Anti-Inflammatory, Antimicrobial, and Antioxidant Activities of *Ficus sur* Stem-Bark. Organic & Medicinal Chemistry International Journal, 9(3), 116-124.

Aremu, A. O., & Moyo, M. (2022). Health benefits and biological activities of spiny monkey orange (*Strychnos spinosa* Lam.): An African indigenous fruit tree. Journal of Ethnopharmacology, 283, 114704.

Arora, S. (2011). Antibecterial, antifungal, antioxidant and phytochemical study on the leaves extract of *Grewia optiva*.

Baloyi, J.K., and Reynolds, Y (2004) Pretoria National Botanical Garden

Barua, D. (1992). History of cholera. In Cholera (pp. 1-36). Boston, MA: Springer US.

BBC News. (2008). Failing Zimbabwe: Reporter round-up. [Internet]. Available from: http://news.bbc.co.uk/1/hi/world/africa/7771184.stm [Accessed 22 July 2024].

Bhanye, J. I., Mangara, F., Matamanda, A. R., & Kachena, L. (2023). Contextualizing Harare Urban Socioeconomic Profile and History of Pandemics in the City. In COVID-19 Lockdowns and the Urban Poor in Harare, Zimbabwe: Emerging Perspectives and the Morphing of a Sustainable Urban Future (pp. 23-64). Cham: Springer Nature Switzerland.

Bildziukevich, U., Vida, N., Rárová, L., Kolář, M., Šaman, D., Havlíček, L., ... & Wimmer, Z. (2015). Polyamine derivatives of betulinic acid and β-sitosterol: A comparative investigation. Steroids, 100, 27-35.

Birch, H. (2021). Urban Governance and Disease Outbreaks: Cholera in Harare and Ebola in Monrovia. Land Issues for Urban Governance in Sub-Saharan Africa, 299-315.

Bradley, M., Shakespeare, R., Ruwende, A., Woolhouse, M. E. J., Mason, E., & Munatsi, A. (1996). Epidemiological features of epidemic cholera (El Tor) in Zimbabwe. Transactions of the Royal Society of Tropical Medicine and Hygiene, 90(4), 378-382.

Bruschi, P., Morganti, M., Mancini, M. and Signorini, M.A., 2011. Traditional healers and laypeople: a qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). Journal of Ethnopharmacology, 138(2), pp.543-563.

Bwire, G., Debes, A.K., Orach, C.G., Kagirita, A., Ram, M., Komakech, H., Voeglein, J.B., Buyinza, A.W., Obala, T., Brooks, W.A., & Sack, D.A. (2018). Environmental surveillance of *Vibrio cholerae* O1/O139 in the five African great lakes and other major surface water sources in Uganda. Frontiers in Microbiology, 9, p.1560.

Chaguza, C., Chibwe, I., Chaima, D., Musicha, P., Ndeketa, L., Kasambara, W., ... & Jere, K. C. (2023). Genomic insights into the 2022–2023 *Vibrio cholerae* outbreak in Malawi. *medRxiv*, 2023-06.

Charla, R., Patil, P. P., Bhatkande, A. A., Khode, N. R., Balaganur, V., Hegde, H. V., ... & Roy, S. (2022). In Vitro and In Vivo Inhibitory Activities of Selected Traditional Medicinal Plants against Toxin-Induced Cyto-and Entero-Toxicities in Cholera. Toxins, 14(10), 649.

Charnley, G. E., Kelman, I., & Murray, K. A. (2022). Drought-related cholera outbreaks in Africa and the implications for climate change: a narrative review. Pathogens and Global Health, 116(1), 3-12.

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J. T., Dettweiler, M., Marquez, L., Salam, A. M., Shabih, S., Farrokhi, D. R., & Quave, C. L. (2021). A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Frontiers in Pharmacology, 11, 586548. https://doi.org/10.3389/fphar.2020.586548

Chatiza, K. (2019). Cyclone Idai in Zimbabwe: An analysis of policy implications for post-disaster institutional development to strengthen disaster risk management.

Chen, W. H., & Azman, A. S. (2019). Mitigating cholera in the aftermath of cyclone Idai. The American Journal of Tropical Medicine and Hygiene, 101(5), 960.

Chigora, P., Masocha, R., & Mutenheri, F. (2007). The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: the case of Mutirikwi communal lands. Journal of Sustainable Development in Africa, 9(2), 26-43.

Chigudu, S. (2019). The politics of cholera, crisis and citizenship in urban Zimbabwe:'People were dying like flies'. African Affairs, 118(472), 413-434.

Chingwaru, C., Bagar, T. and Chingwaru, W., 2020. Aqueous extracts of *Flacourtia indica*, *Swartzia madagascariensis* and *Ximenia caffra* are strong antibacterial agents against *Shigella* spp., *Salmonella typhi* and *Escherichia coli* O157. South African Journal of Botany, 128, pp.119-127.

Chinsembu, K. C. (2016). Ethnobotanical study of plants used in the management of HIV/AIDS-related diseases in Livingstone, Southern Province, Zambia. Evidence-based Complementary and Alternative Medicine, 2016(1), 4238625.

Chinsembu, K. C., Syakalima, M., & Semenya, S. S. (2019). Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Lusaka, Zambia. South African Journal of Botany, 122, 369-384.

Chitemerere, T. A., & Mukanganyama, S. (2011). In vitro antibacterial activity of selected medicinal plants from Zimbabwe. The African Journal of Plant Science and Biotechnology, 5(1), 1-7.

Chitoti, J. L., Prashar, L., & Kaonga, P. (2020). Anti-Diarrhoeal Properties of Stem Bark of *Cassia Abbreviata* against Castor Oil-Induced Diarrhoea in Swiss Albino Mice. Medical Journal of Zambia, 47(4), 252-260.

Chitura, M. (2014). A Harzard analysis on factors associated with the cholera outbreak of January to March 2000 In Buhera district: Manicaland province, Zimbabwe. European Scientific Journal.

Chouna, J. R., Nkeng-Efouet-Alango, P., Lenta, B. N., & Sewald, N. (2015). Antimicrobial triterpenes from the stem bark of *Crossopteryx febrifuga.* Zeitschrift für Naturforschung C, 70(7-8), 169-173.

Chowdhury, F., Ross, A. G., Islam, M. T., McMillan, N. A., & Qadri, F. (2022). Diagnosis, management, and future control of cholera. Clinical Microbiology Reviews, 35(3), e00211-21.

Clemens, J. D. (2011). Vaccines in the time of cholera. Proceedings of the National Academy of Sciences, 108(21), 8529-8530.

Coates Palgrave, K. (1977). Trees of southern Africa. Veld & Flora, 63(4), 8.

Coates Palgrave, K. (1983). Trees of southern Africa, edn 2. Struik, Cape Town.

Coopoosamy, R. M., & Naidoo, K. K. (2012). An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. African Journal of Pharmacy and Pharmacology, 6(11), 818-823.

Coopoosamy, R. M., Naidoo, K. K., & Ndlazi, N. J. (2011). Cultural importance and antibacterial activity of *Ziziphus mucronata* Willd. in the Umlazi community in Durban. African Journal of Pharmacy and Pharmacology, *5*, 1979-1982.

Cuneo, C.N., Sollom, R., & Beyrer, C. (2017). The cholera epidemic in Zimbabwe, 2008–2009: a review and critique of the evidence. Health and Human Rights, 19(2), p.249.

Deen, J., Mengel, M.A., & Clemens, J.D. (2020). Epidemiology of cholera. Vaccine, 38, pp.A31-A40.

Du, K. (2011). Isolation of bioactive constituents from seeds of *Schotia brachypetala* (Fabaceae) and *Colophospermum mopane* (Fabaceae). Masters dissertation, University of the Free State.

Dutta, D., Chowdhury, G., Pazhani, G. P., Guin, S., Dutta, S., Ghosh, S., Rajendran, K., Nandy, R. K., Mukhopadhyay, A. K., Bhattacharya, M. K., Mitra, U., Takeda, Y., Nair, G. B., & Ramamurthy, T. (2013). *Vibrio cholerae* non-O1, non-O139 serogroups and cholera-like diarrhea, Kolkata, India. Emerging Infectious Diseases, 19(3), 464–467. https://doi.org/10.3201/eid1903.121156.

Eloff, J.N., 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro)(Anacardiaceae) bark and leaves. Journal of Ethnopharmacology, 76(3), pp.305-308.

Emch, M., Feldacker, C., Islam, M.S., & Ali, M. (2008). Seasonality of cholera from 1974 to 2005: a review of global patterns. International Journal of Health Geographics, 7(1), pp.1-13.

Frischen, J., Meza, I., Rupp, D., Wietler, K., & Hagenlocher, M. (2020). Drought risk to agricultural systems in Zimbabwe: A spatial analysis of hazard, exposure, and vulnerability. Sustainability, 12(3), 752.

Gelfand, M., Mavi, S., Drummond, R.B. and B. Ndemera. (1985). The Traditional Medicinal Practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe.

Girmaw, F., & Engidawork, E. (2022). In Vivo Anti-Malarial Activity of the Aqueous Root Extract of *Euclea divinorum* Hiern (Ebenaceae) against *Plasmodium berghei* ANKA. Evidence-Based Complementary and Alternative Medicine, 2022.

Gobvu, V., Pote, W., Poshiwa, X., & Benhura, M. A. (2022). Medicinal Plants Used for the Treatment of Poultry Diseases in Zimbabwe: A Systematic Review.

Goffman, L. F. (2024). Disorder and Diagnosis: Health and the Politics of Everyday Life in Modern Arabia. Stanford University Press.

Grace, O. M., Prendergast, H. D. V., Jäger, A. K., Van Staden, J., & Van Wyk, A. E. (2003). Bark medicines used in traditional healthcare in KwaZulu-Natal, South Africa: An inventory. South African Journal of Botany, 69(3), 301-363.

Gwenzi, W., & Sanganyado, E. (2019). Recurrent cholera outbreaks in sub-Saharan Africa: moving beyond epidemiology to understand the environmental reservoirs and drivers. Challenges, 10(1), 1.

Hadeed, S. J., Broadway, K. M., Schwartz-Watjen, K. T., Tigabu, B., Woodards, A. J., Swiatecka, A. L., ... & Wu, A. (2023). Notional Spread of Cholera in Haiti Following a Natural Disaster: Considerations for Military and Disaster Relief Personnel. Military Medicine, 188(7-8), e2074-e2081.

Halilu, M. E., Abubakar, A., & Garba, M. K. (2012). Antimicrobial and preliminary phytochemical studies of methanol extract of root bark of *Crossopteryx febrifuga* (Rubiaceae). Journal of Applied Pharmaceutical Science, 2(12), 066-070.

Hankey, A. (2003). Walter Sisulu National Botanical Garden

Hatch, D.L., Waldman, R.J., Lungu, G.W., & Piri, C. (1994). Epidemic cholera during refugee resettlement in Malawi. International Journal of Epidemiology, 23(6), 1292-1299.

Hikaambo, C. N. A., Chisanga, T., Kampamba, M., Akapelwa, T. M., Chimombe, T., Chulu, M., ... & Mudenda, S. (2022). Antibacterial Activity of *Cassia abbreviata* Oliv. Bark

Extract against *Escherichia coli* and *Staphylococcus aureus.* Journal of Pharmaceutical Research Science & Technology [ISSN: 2583-3332], 6(1), 76-83.

Hove, M. (2016). When flood victims became state victims: Tokwe-Mukosi, Zimbabwe. Democracy and Security, 12(3), 135-161.

Hutchings, A. (1996). Zulu medicinal plants: An inventory. University of Natal press

Hyde, M. A., Wursten, B. T., Ballings, P., & Coates Palgrave, M. (2025). Flora of Zimbabwe: Species information: individual images: *Euclea divinorum.* Retrieved May 20, 2025, from https://www.zimbabweflora.co.zw/speciesdata/image-display.php?species_id=143880&image_id=2

Hyde, M.A., Wursten, B.T., Ballings, P. & Coates Palgrave, M. (2022). Flora of Zimbabwe: Species information: *Elephantorrhiza goetzei subsp. goetzei.* https://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=126460, retrieved 20 October 2022.

Hyde, M.A., Wursten, B.T., Ballings, P. & Coates Palgrave, M. (2024). Flora of Zimbabwe: Species information: individual images: *Kirkia acuminata.* https://www.zimbabweflora.co.zw/speciesdata/image-display.php?species_id=133250&image_id=2, retrieved 15 May 2024.

IFRC (2024). A deadly start to 2024: Cholera in Zimbabwe spreads rapidly after holiday season. Available from: https://www.ifrc.org/article/deadly-start-2024-cholera-zimbabwe-spreads-rapidly-after-holiday-season [Accessed 22 July 2024].

Ingelbeen, B., Hendrickx, D., Miwanda, B., van der Sande, M. A. B., Mossoko, M., Vochten, H., Riems, B., Nyakio, J. P., Vanlerberghe, V., Lunguya, O., Jacobs, J., Boelaert, M., Kebela, B. I., Bompangue, D., & Muyembe, J. J. (2019). Recurrent Cholera Outbreaks, Democratic Republic of the Congo, 2008-2017. Emerging Infectious Diseases, 25(5), 856–864. https://doi.org/10.3201/eid2505.181141.

Isa, A. I., Awouafack, M. D., Dzoyem, J. P., Aliyu, M., Magaji, R. A., Ayo, J. O., & Eloff, J. N. (2014). Some *Strychnos spinosa* (Loganiaceae) leaf extracts and fractions have good antimicrobial activities and low cytotoxicities. BMC Complementary and Alternative Medicine, 14(1), 1-8.

Ismail, E. M., Kadry, M., Elshafiee, E. A., Ragab, E., Morsy, E. A., Rizk, O., & Zaki, M. M. (2021). Ecoepidemiology and Potential Transmission of *Vibrio cholerae* among Different

Environmental Niches: An Upcoming Threat in Egypt. Pathogens (Basel, Switzerland), 10(2), 190. https://doi.org/10.3390/pathogens10020190.

Jacob, A.D., Ogaji, O.D., Okonkwo, V.I., Abdullahi, A.S., Ocheme, G.W., Ejim, C.C. and Idris, B.A., 2021. Assessment of the Antimicrobial Activities of *Ximenia caffra* (Sour Plum) Leaf. International Journal Of Science for Global Sustainability, 7(2), pp.6-6.

Jantapaso, H., Aksonkird, T., & Mittraparp-Arthorn, P. (2024). Characteristics of *Vibrio cholerae* isolates obtained from shrimp supply chains and inhibitory activities of rambutan (*Nephelium lappaceum* L. cv. Rong Rian) peel aqueous extract. *Food Control*, *158*, 110231.

Jaspers, M. W. J. M., Bashir, A. K., Zwaving, J. H., & Malingre, T. M. (1986). Investigation of *Grewia bicolor* Juss. Journal of Ethnopharmacology, 17(3), 205-211.

Kabir, S. (2014). Critical analysis of compositions and protective efficacies of oral killed cholera vaccines. Clinical and Vaccine Immunology, 21(9), 1195-1205.

Kamruzzaman, M., Bari, S. N., & Faruque, S. M. (2013). In vitro and in vivo bactericidal activity of *Vitex negundo* leaf extract against diverse multidrug resistant enteric bacterial pathogens. Asian Pacific journal of tropical medicine, 6(5), 352-359.

Kamuriwo, A. T., Gundidza, M., & Manyuchi, P. (2021). A retrospective study of antimicrobial resistance of *Vibrio cholerae* O1 serotype Ogawa in the 2018 cholera outbreak in Harare. Journal of Pharmaceutical Sciences, 1, 1-8.

Khan, H. A., Masood, W., Siddiqui, A., Ahmad, S., Salman, Y., & Essar, M. Y. (2022). The Cholera outbreak in Karachi, Pakistan: challenges, efforts and recommendations. Annals of Medicine and Surgery, 78, 103873.

Khan, Z., Nath, N., Rauf, A., Emran, T. B., Mitra, S., Islam, F., ... & Thiruvengadam, M. (2022). Multifunctional roles and pharmacological potential of β-sitosterol: Emerging evidence toward clinical applications. Chemico-Biological Interactions, 110117.

Khumalo, G. P., Sadgrove, N. J., Van Vuuren, S. F., & Van Wyk, B. E. (2021). South Africa's Best BARK Medicines Prescribed at the Johannesburg Muthi Markets for Skin, Gut, and Lung Infections: MIC's and Brine Shrimp Lethality. Antibiotics (Basel, Switzerland), 10(6), 681. https://doi.org/10.3390/antibiotics10060681

Kigen, G., Maritim, A., Some, F., Kibosia, J., Rono, H., Chepkwony, S., ... & Wanjoh, B. (2016). Ethnopharmacological survey of the medicinal plants used in Tindiret, Nandi

County, Kenya. African Journal of Traditional, Complementary and Alternative Medicines, 13(3), 156-168.

Kilonzo, M., Rubanza, C., Richard, U., & Sangiwa, G. (2019). Antimicrobial activities and phytochemical analysis of extracts from *Ormocarpum trichocarpum* (Taub.) and *Euclea divinorum* (Hiern) used as traditional medicine in Tanzania. Tanzania Journal of Health Research, 21(2), 1-12.

Kim, S. Y., Choi, Y., Mason, P. R., Rusakaniko, S., & Goldie, S. J. (2011). Potential impact of reactive vaccination in controlling cholera outbreaks: an exploratory analysis using a Zimbabwean experience. South African Medical Journal, 101(9), 659-664.

Kipkore, W., Wanjohi, B., Rono, H., & Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. Journal of Ethnobiology and Ethnomedicine, 10(1), 1-22.

Komiazyk, M., Palczewska, M., Sitkiewicz, I., Pikula, S., & Groves, P. (2019). Neutralization of cholera toxin by Rosaceae family plant extracts. BMC complementary and alternative medicine, 19, 1-14.

Komolafe, N. T. (2014). Antimicrobial activity of some medicinal plant extracts against bacteria causing diarrhoea. Masters dissertation, University of south Africa.

Kouadio, I. K., Aljunid, S., Kamigaki, T., Hammad, K., & Oshitani, H. (2012). Infectious diseases following natural disasters: prevention and control measures. Expert Review of Anti-infective Therapy, 10(1), 95-104.

Kumar, P. A., Karpagam, P., & Patterson, J. (2013). In vitro evaluation of herbal decoctions in reducing *Vibrio cholerae* on *Chicoreus ramosus* meat. *J Med Plants Res*, *7*(45), 3298-3302.

Laldinsanga, H. S., Jahan, T., Kumar, A., & Goswami, H. K. S. (2019). Traditional anti-malarial drugs from Serchhip and Lunglei districts of Mizoram. Current Trends in Pharmaceutical Research Vol, 6(1).

Laviad-Shitrit, S., Izhaki, I., & Halpern, M. (2019). Accumulating evidence suggests that some waterbird species are potential vectors of *Vibrio cholerae*. PLoS Pathogens, 15(8), e1007814.

Lloyd, C. J., Mejia-Santana, A., Dalia, T. N., Dalia, A. B., & Klose, K. E. (2021). Natural transformation in a classical-biotype *Vibrio cholerae* strain. Applied and Environmental Microbiology, 87(10), e00060-21.

Louis, H., Linus, M. N., Israt, A., Innocent, J., Amos, P. I., & Magu, T. O. (2018). Antimicrobial activity of stem, leave and root plant extract of *Sclerocarya birrea* and *Sterculia setigera* against some selected microorganisms. World Scientific News, 92(2), 309-326.

Mabadahanye, K., Bhembe, N. L., & Green, E. (2022). Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. African Health Sciences, 22(2), 717-727.

Macheka, L., Manditsera, F. A., Ngadze, R. T., Mubaiwa, J., Nyarugwe, S., Bangira, C., ... & Kembo, G. (2022). Agro-Ecological Distribution and Consumption of Wild Harvested Edible Insects, Fruits, and Vegetables in Rural Zimbabwe. Future Foods, 100187.

Madikizela, B., Ndhlala, A. R., Finnie, J. F., & Van Staden, J. (2012). Ethnopharmacological study of plants from Pondoland used against diarrhoea. Journal of Ethnopharmacology, 141(1), 61-71.

Maiga, A., Malterud, K. E., Diallo, D., & Paulsen, B. S. (2006). Antioxidant and 15-lipoxygenase inhibitory activities of the Malian medicinal plants *Diospyros abyssinica* (Hiern) F. White (Ebenaceae), *Lannea velutina* A. Rich (Anacardiaceae) and *Crossopteryx febrifuga* (Afzel) Benth.(Rubiaceae). Journal of Ethnopharmacology, 104(1-2), 132-137.

Maina, S. W., & Dimba, E. (2021). Antimicrobial efficacy of aqueous and ethanol extracts of *Euclea divinorum* Hiern on tooth root canal microorganisms. Endodontology, 33(4), 221.

Makoni, M. (2020). Cholera: Zimbabwe's unsung political victim. The Lancet Infectious Diseases, 20(10), 1134.

Manzo, L. M., Bako, H. D., & Idrissa, M. (2017). Phytochemical Screening and Antibacterial Activity of Stem Bark, Leaf and Root Extract of *Sclerocarya birrea* (A. Rich.) Hochst. International Journal of Enteric Pathogens, 5(4), pp.127-131.

Maponga, B. A., Chirundu, D., Gombe, N. T., Tshimanga, M., Bangure, D., & Takundwa, L. (2015). Cholera: a comparison of the 2008-9 and 2010 outbreaks in Kadoma City, Zimbabwe. The Pan African Medical Journal, 20, 221. https://doi.org/10.11604/pamj.2015.20.221.5197.

Maroyi A (2016). Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. African Journal of Traditional, Complementary, and Alternative Medicines, 13(6), 5–10. https://doi.org/10.21010/ajtcam.v13i6.2

Maroyi, A. (2011). An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. Journal of Ethnopharmacology, 136(2), 347-354.

Maroyi, A. (2013) Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. Journal of Ethnobiology and Ethnomedicine 9, 31. https://doi.org/10.1186/1746-4269-9-31.

Maroyi, A. (2016). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus, Kirkia. Tropical Journal of Pharmaceutical Research, 15(11), 2507-2516.

Maroyi, A. (2016a). *Ximenia caffra* Sond. (Ximeniaceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. Journal of Ethnopharmacology. 184, 81–100. https://doi.org/10.1016/j.jep.2016.02.052.

Maroyi, A. (2017). Exotic Plants in Indigenous Pharmacopoeia of South-Central Zimbabwe: Traditional Knowledge of Herbal Medicines. Research Journal of Botany, doi: 10.3923/rjb.2017.46.52.

Maroyi, A. (2017b). Phytochemical and ethnopharmacological review of *Elephantorrhiza goetzei* (Harms) Harms. Asian Pacific Journal of Tropical Medicine, 10(2), pp.107-113.

Maroyi, A. (2017c). *Kirkia acuminata* Oliv.: a review of its ethnobotany and pharmacology. African Journal of Traditional, Complementary and Alternative Medicines, 14(2), 217-226.

Maroyi, A. (2018). Nutraceutical and ethnopharmacological properties of *Vangueria infausta* subsp. infausta. Molecules, 23(5), 1089.

Maroyi, A., & Chekhyoussef, A. (2015). A comparative study of medicinal plants used in rural areas of Namibia and Zimbabwe. Indian Journal of Traditional Knowledge.

Masakure, C. (2018). The politicisation of health in Zimbabwe: The case of the cholera epidemic, August 2008-March 2009. New Contree, 80, 24.

Mashe, T., Chaibva, B. V., Nair, P., Sani, K. A., Jallow, M., Tarupiwa, A., ... & Phiri, I. (2023). Descriptive epidemiology of the cholera outbreak in Zimbabwe 2018–2019: role of multi-sectorial approach in cholera epidemic control. BMJ Open, 13(1), e059134.

Mashe, T., Domman, D., Tarupiwa, A., Manangazira, P., Phiri, I., Masunda, K., Chonzi, P., Njamkepo, E., Ramudzulu, M., Mtapuri-Zinyowera, S., & Smith, A.M. (2020). Highly resistant cholera outbreak strain in Zimbabwe. New England Journal of Medicine, 383(7), 687-689.

Masoko, P. (2013). Ethnobotanical study of some selected medicinal plants used by traditional healers in Limpopo province (South Africa). American Journal of Research Communication.

Mathabe, M. C., Nikolova, R. V., Lall, N., & Nyazema, N. Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. Journal of Ethnopharmacology, 105(1-2), 286-293.

Mavhura, E., & Aryal, K. R. (2023). Disaster mortalities and the Sendai Framework Target A: insights from Zimbabwe. World Development, 165, 106196.

Mazimba, O. (2014). Pharmacology and phytochemistry studies in *Peltophorum africanum*. Bulletin of Faculty of Pharmacy, Cairo University, 52(1), 145-153.

Mbabazi, I., Wangila, P., & K'Owino, I. O. (2020). Antimicrobial activity of *Euclea divinorum* Hern (ebenaceae) leaves, tender stems, root bark and an herbal toothpaste formulated from its ethanolic root bark extract.

Mbala-Kingebeni, P., Vogt, F., Miwanda, B., Sundika, T., Mbula, N., Pankwa, I., Lubula, L., Vanlerberghe, V., Magazani, A., Afoumbom, M.T., & Muyembe-Tamfum, J.J. (2021). Sachet water consumption as a risk factor for cholera in urban settings: Findings from a case control study in Kinshasa, Democratic Republic of the Congo during the 2017–2018 outbreak. PLoS Neglected Tropical Diseases, 15(7), e0009477.

McGaw, L. J., Jäger, A. K., Van Staden, J., & Eloff, J. N. (2002). Variation in antibacterial activity of Schotia species. South African Journal of Botany, 68(1), 41-46.

Médecins Sans Frontières (2024). Why cholera is on the rise in Zimbabwe. [Internet]. Available from: https://www.doctorswithoutborders.org/latest/why-cholera-rise-zimbabwe. [Accessed 22 July 2024].

Mengel, M. A., Delrieu, I., Heyerdahl, L., & Gessner, B. D. (2014). Cholera outbreaks in Africa. Current Topics in Microbiology and Immunology, 379, 117–144. https://doi.org/10.1007/82_2014_369.

Mireku-Gyimah, N., Apanga, P. A., & Awoonor-Williams, J. K. (2018). Cyclical cholera outbreaks in Ghana: filth, not myth. Infectious Diseases of Poverty, 7(1), 51. https://doi.org/10.1186/s40249-018-0436-1.

Miwanda, B., Moore S., Muyembe, J.J., Nguefack-Tsague, G, Kabangwa, I.K., Ndjakani, D.Y., Mutreja, A., Thomson, N., Thefenne, H., Garnotel, E., Tshapenda, G., Kakongo, D.K., Kalambayi, G., & Piarroux, R. (2015). Antimicrobial Drug Resistance of *Vibrio cholerae*, Democratic Republic of the Congo. Emerging Infectious Diseases. doi: 10.3201/eid2105.141233. PMID: 25897570; PMCID: PMC4412219.

Mlambo, N. P. (2008). The screening of medicinal plants traditionally used to treat diarrhoea, in Ongoye area, KwaZulu Natal. Masters dissertation, University of Zululand.

Mok, J. S., Cho, S. R., Park, Y. J., Jo, M. R., Ha, K. S., Kim, P. H., & Kim, M. J. (2021). Distribution and antimicrobial resistance of *Vibrio parahaemolyticus* isolated from fish and shrimp aquaculture farms along the Korean coast. Marine Pollution Bulletin, 171, 112785.

Mok, J. S., Ryu, A., Kwon, J. Y., Park, K., & Shim, K. B. (2019). Abundance, antimicrobial resistance, and virulence of pathogenic *Vibrio* strains from molluscan shellfish farms along the Korean coast. Marine Pollution Bulletin, 149, 110559.

Mølgaard, P., Nielsen, S.B., Rasmussen, D.E., Drummond, R.B., Makaza, N., & Andreassen, J. (2001). Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. Journal of Ethnopharmacology, 74(3), 257-264.

Mongalo, N. I., & Mafoko, B. J. (2013). *Cassia abbreviata* Oliv. A review of its ethnomedicinal uses, toxicology, phytochemistry, possible propagation techniques and Pharmacology. African Journal of Pharmacy and Pharmacology, 7(45), 2901-2906.

Mongalo, N. I., Mashele, S. S., & Makhafola, T. J. (2020). *Ziziphus mucronata* Willd. (Rhamnaceae): it's botany, toxicity, phytochemistry and pharmacological activities. Heliyon, 6(4), e03708. https://doi.org/10.1016/j.heliyon.2020.e03708

Mongalo, N.I., & Makhafola, T.J. (2018). Ethnobotanical knowledge of the lay people of Blouberg area (Pedi tribe), Limpopo Province, South Africa. Journal of Ethnobiology and Ethnomedicine 14, https://doi.org/10.1186/s13002-018-0245-4

Monserrat-Martinez, A., Gambin, Y., & Sierecki, E. (2019). Thinking Outside the Bug: Molecular Targets and Strategies to Overcome Antibiotic Resistance. International Journal of Molecular Sciences, 20(6), 1255. https://doi.org/10.3390/ijms20061255.

Montero, D. A., Vidal, R. M., Velasco, J., George, S., Lucero, Y., Gómez, L. A., ... & O'Ryan, M. (2023). *Vibrio cholerae*, classification, pathogenesis, immune response, and trends in vaccine development. Frontiers in Medicine, 10, 1155751.

Mothogoane, M.S. (2014). National Herbarium, Pretoria

Moyo, F., Gashe, B. A., & Majinda, R. R. (1999). A new flavan from *Elephantorrhiza goetzei*. Fitoterapia, 70(4), 412-416.

Muchapireyi, G. (2018). An investigation into the challenges encountered by the community during the Tokwe-Mukosi rapid onset flood disaster in Zimbabwe. Masters dissertation, North-West University.

Mugiyo, H., Magadzire, T., Choruma, D. J., Chimonyo, V. G. P., Manzou, R., Jiri, O., & Mabhaudhi, T. (2023). El Niño's effects on Southern African agriculture in 2023/24 and anticipatory action strategies to reduce the impacts in Zimbabwe. Atmosphere, 14(11), 1692.

Mukandavire, Z., Liao, S., Wang, J., Gaff, H., Smith, D. L., & Morris Jr, J. G. (2011). Estimating the reproductive numbers for the 2008–2009 cholera outbreaks in Zimbabwe. Proceedings of the National Academy of Sciences, 108(21), 8767-8772.

Mukandavire, Z., Manangazira, P., Nyabadza, F., Cuadros, D. F., Musuka, G., & Morris Jr, J. G. (2020). Stemming cholera tides in Zimbabwe through mass vaccination. International Journal of Infectious Diseases, 96, 222-227.

Mulubwa, M. W. I. L. A., & Prakash, S. H. I. V. (2015). Antimicrobial activity and potency of *Cassia abbreviata* Oliv stem bark extracts. International Journal of Pharmaceutical Sciences, 7, 426-428.

Munodawafa, T. (2012). Screening of some traditional medicinal plants from Zimbabwe for biological and anti-microbial activity.

Munodawafa, T., Chagonda, L. S., & Moyo, S. R. (2013). Antimicrobial and phytochemical screening of some Zimbabwean medicinal plants. Journal of Biologically Active Products from Nature, 3(5-6), 323-330.

Munyenyiwa, A., Musuka, G., Makuvaza, M. J. M., Mucheri, T., & Midzi, N. (2022). A Review of the 2008/2009 and 2018/2019 Cholera Outbreaks in Zimbabwe: Call for Public Health Action. Journal of Infectious Diseases and Epidemiology, 8, 269.

Mupepi, O., & Matsa, M. M. (2022). Spatio-temporal dynamics of drought in Zimbabwe between 1990 and 2020: a review. Spatial Information Research, 1-14.

Mwaba J, Debes AK, Shea P, Mukonka V, Chewe O, Chisenga C, Simuyandi M, Kwenda G, Sack D, Chilengi R, & Ali M. (2020) Identification of cholera hotspots in Zambia: A spatiotemporal analysis of cholera data from 2008 to 2017. PLOS Neglected Tropical Diseases. doi: 10.1371/journal.pntd.0008227.

Mwafongo, E., Nordal, I., Magombo, Z., & Stedje, B. (2010). Ethnobotanical study of Hyacinthaceae and non-hyacinthaceous geophytes in selected districts of Malawi. Ethnobotany Research and Applications, 8, 075-093.

Ndlovu, T., & Mjimba, V. (2021). Drought risk-reduction and gender dynamics in communal cattle farming in southern Zimbabwe. International Journal of Disaster Risk Reduction, 58, 102203.

Nemudzivhadi, V., & Masoko, P. (2015). Antioxidant and antibacterial properties of *Ziziphus mucronata* and *Ricinus communis* leaves extracts. African Journal of Traditional, Complementary and Alternative Medicines, 12(1), 81-89.

Neuwinger, H. D. (1994). Fish poisoning plants in Africa. Botanica Acta, 107(4), 263-270.

Ngadze, R. T., Linnemann, A. R., Nyanga, L. K., Fogliano, V., & Verkerk, R. (2017). Local processing and nutritional composition of indigenous fruits: The case of monkey orange (*Strychnos* spp.) from Southern Africa. Food Reviews International, 33(2), 123-142.

Ngarivhume, T., van't Klooster, C.I., de Jong, J.T., & Van der Westhuizen, J.H. (2015). Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. Journal of Ethnopharmacology, 159, 224-237.

Njume, C., Afolayan, A.J., Green, E. and Ndip, R.N., 2011. Volatile compounds in the stem bark of *Sclerocarya birrea* (Anacardiaceae) possess antimicrobial activity against drug-resistant strains of *Helicobacter pylori*. International Journal of Antimicrobial Agents, 38(4), pp.319-324.

Nyakudya, T. T., Nosenga, N., Chivandi, E., Erlwanger, K. H., Gundidza, M., Gundidza, E., ... & Muredzi, P. (2015). *Grewia bicolor* seed oil: Putative pharmaceutical, cosmetic and industrial uses. South African Journal of Botany, 97, 154-158.

Nyathi, B., Bvunzawabaya, J. T., Manzombe, E., Selemani, M. A., Tsotsoro, K., Munyuki, G., & Rwere, F. (2022). Antidiabetic, antioxidant and in silico molecular docking of *Xeroderris* *stuhlmannii* (Taub.) Mendonca & EP Sousa phytochemical compounds on human pancreatic α-amylase and human lysosomal acid-α-glucosidase. *bioRxiv*.

Ojeda Rodriguez, J. A., Hashmi, M. F., & Kahwaji, C. I. (2024). *Vibrio cholerae* Infection. In StatPearls. StatPearls Publishing. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK526099/

Okeleye, B. I., Mkwetshana, N. T., & Ndip, R. N. (2013). Evaluation of the antibacterial and antifungal potential of *Peltophorum africanum*: toxicological effect on human chang liver cell line. The Scientific World Journal, 2013.

Okeleye, B. I., Samie, A., Bessong, P. O., Mkwetshana, N. F., Green, E., Clarke, A. M., & Ndip, R. N. (2010). Crude ethyl acetate extract of the stem bark of *Peltophorum africanum* (Sond, Fabaceae) possessing in vitro inhibitory and bactericidal activity against clinical isolates of *Helicobacter pylori*. Journal of Medicinal Plants Research, 4(14), 1432-1440.

Olajuyigbe, O. O., & Afolayan, A. J. (2012). Antimicrobial potency of the ethanolic crude bark extract of *Ziziphus mucronata* Willd. subsp. mucronata Willd. African Journal of Pharmacy and Pharmacology, 6(10), 724-730.

Olatunji, G., Kokori, E., Moradeyo, A., Olatunji, D., Ajibola, F., Otolorin, O., & Aderinto, N. (2024). A Perspective on the 2023 Cholera Outbreaks in Zimbabwe: Implications, Response Strategies, and Policy Recommendations. Journal of Epidemiology and Global Health, 1-6.

Omara, T., Sadİa, B. O., Mbabazi, I., & Okwir, A. Ethnobotany, Phytochemistry, Ethnopharmacology, and Toxicity of *Euclea divinorum* Hern (Ebenaceae): A Review. Journal of the Turkish Chemical Society Section A: Chemistry, 9(2), 355-372.

Orwa, C. (2009). Agroforestree Database: a tree reference and selection guide, version 4.0. http://www. worldagroforestry. org/sites/treedbs/treedatabases. asp.

Osei, F.B., & Duker, A.A. (2008). Spatial and demographic patterns of cholera in Ashanti region-Ghana. International Journal of Health Geographics, 7(1), 1-10.

Palmer, E., & Pitman, N. (1972). Trees of Southern Africa: covering all known indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho & Swaziland. Volumes 1 & 2. Trees of Southern Africa: covering all known indigenous species in the Republic of South Africa, South-West Africa, Botswana, Lesotho & Swaziland. Volumes 1 & 2.

Patel, M., & Isaäcson, M. (1989). Survival of *Vibrio cholerae* in African domestic water storage containers. South African Medical Journal= Suid-afrikaanse Tydskrif vir Geneeskunde, 76(7), 365-367.

Penrose, K., Castro, M.C.D., Werema, J., & Ryan, E.T. (2010). Informal urban settlements and cholera risk in Dar es Salaam, Tanzania. PLoS Neglected Tropical Diseases, 4(3), e631.

Pereira, F., Sancha, S., Feliciano, D., Luo, X., Mulhovo, S., Duarte, A., ... & Ferreira, M. J. U. (2015). Evaluation of the antibacterial activity of some African medicinal plants. Planta Medica, 81(16), PM_108.

Pezzoli, L. (2020). Global oral cholera vaccine use, 2013–2018. Vaccine, 38, A132-A140.

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomedicine Plus, 1(2), 100029.

Pritchard, M., Mkandawire, T., Edmondson, A., O'neill, J. G., & Kululanga, G. (2009). Potential of using plant extracts for purification of shallow well water in Malawi. Physics and Chemistry of the Earth, Parts A/B/C, 34(13-16), 799-805.

Rafey, H.A., Shah, A.H., Khalid Khan, M., Kamran, M., & Tayyab, M. (2021). Evaluation of the antibiofilm and antimicrobial activities of *Glycyrrhiza glabra* and *Viola odorata* in poly herbal formulations. Main Group Chemistry, 20(4), 557-563.

Reda, A., Sah, R., Abdelaal, A., Shrestha, S., & Rodriguez-Morales, A. J. (2022). The emergence of cholera in multiple countries amidst current COVID-19 pandemic: Situation and implications for public health and travel medicine. Travel Medicine and Infectious Disease, *49*, 102423. https://doi.org/10.1016/j.tmaid.2022.102423.

Reddy, S., Taylor, M., Zhao, M., Cherubin, P., Geden, S., Ray, S., Francis, D., & Teter, K. (2013). Grape extracts inhibit multiple events in the cell biology of cholera intoxication. PloS One, 8(9), e73390. https://doi.org/10.1371/journal.pone.0073390.

Ribeiro, A., Romeiras, M. M., Tavares, J., & Faria, M. T. (2010). Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. Journal of Ethnobiology and Ethnomedicine, 6(1), 1-15.

Rieckmann, A., Tamason, C. C., Gurley, E. S., Rod, N. H., & Jensen, P. (2018). Exploring Droughts and Floods and Their Association with Cholera Outbreaks in Sub-Saharan

Africa: A Register-Based Ecological Study from 1990 to 2010. The American Journal of Tropical Medicine and Hygiene, 98(5), 1269–1274. https://doi.org/10.4269/ajtmh.17-0778.

Sánchez, E., García, S., & Heredia, N. (2010). Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae*. Applied and environmental microbiology, *76*(20), 6888-6894.

Sarawgi, G., Kamra, A., Suri, N., Kaur, A., & Sarethy, I. P. (2009). Effect of *Strychnos potatorum* Linn. Seed extracts on water samples from different sources and with diverse properties. Asian Journal of Water, Environment and Pollution, 6(3), 13-17.

Selemani, M. A., Kazingizi, L. F., Manzombe, E., Bishi, L. Y., Mureya, C., Gwata, T. T., & Rwere, F. (2021). Phytochemical characterization and in vitro antibacterial activity of *Xeroderris stuhlmannii* (Taub.) Mendonca & EP Sousa bark extracts. South African Journal of Botany, 142, 344-351.

Semenya, S. S., & Maroyi, A. (2012). Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. Journal of Ethnopharmacology, 144(2), 395-401.

Sevenzo, F. (2023). Zimbabwe's cholera crisis fuelled by chronic water shortages: BBC News. [Internet]. Available from: https://www.bbc.co.uk/news/world-africa-67198511 [Accessed 22 July 2024].

Shagal, M. H., Kubmarawa, D., & Idi, Z. (2012). Phytochemical screening and antimicrobial activity of roots, stem-bark and leave extracts of *Grewia mollis*. African Journal of Biotechnology, 11(51), 11350-11353.

Shannon, K., Hast, M., Azman, A. S., Legros, D., McKay, H., & Lessler, J. (2019). Cholera prevention and control in refugee settings: successes and continued challenges. PLoS Neglected Tropical Diseases, 13(6), e0007347.

Sharma, L. (2020). To Determine the Potential of *Strychnos Potatorum* for Management of Portable Water.

Shopo, B., Mapaya, R. J., & Maroyi, A. (2022). Ethnobotanical study of medicinal plants traditionally used in Gokwe South District, Zimbabwe. South African Journal of Botany, 149, 29-48.

Snider, R. M., McKenzie, J. R., Kraft, L., Kozlov, E., Wikswo, J. P., & Cliffel, D. E. (2010). The effects of cholera toxin on cellular energy metabolism. Toxins, 2(4), 632–648. https://doi.org/10.3390/toxins2040632.

Somboonwit, C., Menezes, L. J., Holt, D. A., Sinnott, J. T., & Shapshak, P. (2017). Current views and challenges on clinical cholera. Bioinformation, 13(12), 405.

Swerdlow, D.L., & Isaäcson, M. (1994). The epidemiology of cholera in Africa. *Vibrio cholerae* and cholera: Molecular to global perspectives, 297-307.

Tanih, N.F. , & Ndip, R.N. (2012). Evaluation of the acetone and aqueous extracts of mature stem bark of *Sclerocarya birrea* for antioxidant and antimicrobial properties. Evidence-Based Complementary and Alternative Medicine, 2012.

Taruvinga, G. (2023). The Politicisation of Local of Government in Africa: A Zimbabwean Case Study: Politicisation of Local Government. The Strategic Review for Southern Africa, 45(2).

Tembo, M. S. (2011). Extraction, Isolation, and Identification of compound (s) responsible for anti-diarrhoeal activity of *Cassia abbreviata* Oliv. Doctoral dissertation, University of Zambia.

The Guardian (2024). Zimbabwean president declares state of disaster due to drought. [Internet]. Available from: https://www.theguardian.com/world/2024/apr/03/zimbabwean-president-declares-state-of-disaster-due-to-drought [Accessed 12 July 2024].

Tor-Anyiin, T. A., Igoli, J. O., Anyam, J. V., & Anyam, J. N. (2015). Isolation and Antimicrobial activity of sarracenin from root bark of *Strychnos spinosa*. Journal of Chemical Society of Nigeria, 40(1).

UNICEF (2023). Cholera outbreak in Eastern and Southern Africa.[Internet]. Available from: https://www.unicef.org/esa/reports/cholera-outbreak-eastern-and-southern-africa-2023#download [Accessed 22 July 2024].

Uttu, A. J., Sallau, M. S., Iyun, O. R. A., & Ibrahim, H. (2022). Antimicrobial Efficacy of Selected *Strychnos* Species: A Mini Review. Journal of Chemical Reviews, 4, 59-62.

Vezzulli, L., Colwell, R.R., & Pruzzo, C. (2013). Ocean warming and spread of pathogenic *Vibrios* in the aquatic environment. Microbial Ecology, 65(4), 817-825.

Viol, D.I., Chagonda, L.S., Moyo, S.R., & Mericli, A.H. (2013). Antibacterial, antifungal and phytochemical activities of some medicinal plants from Zimbabwe. Journal of Biologically Active Products from Nature, 3(4), 273-284.

Wanjala, C.C., & Majinda, R.R. (2001). A new stilbene glycoside from *Elephantorrhiza goetzei*. Fitoterapia, 72(6), 649-655.

Watson, J. T., Gayer, M., & Connolly, M. A. (2007). Epidemics after natural disasters. Emerging Infectious Diseases, 13(1), 1.

WHO (2011) World Health Organization, Zimbabwe weekly epidemiological bulletin. Harare.

WHO (2018). Cholera-Zimbabwe. [Internet]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/20-september-2018-cholera-zimbabwe-en [Accessed 22 July 2024].

WHO (2021). Stories from the field: How vaccines can help to prevent antibiotic resistance - Zimbabwe's response to drug-resistant outbreaks of typhoid and cholera. [Internet]. Available from: https://www.who.int/news/item/11-09-2021-stories-from-the-field-how-vaccines-can-help-to-prevent-antibiotic-resistance---zimbabwe-s-response-to-drug-resistant-outbreaks-of-typhoid-and-cholera [Accessed 22 July 2024].

WHO (2024a).Weekly Bulletin on Outbreaks and other Emergencies: Week 12: 18 - 24 March 2024. [Available from: https://reliefweb.int/report/liberia/weekly-bulletin-outbreaks-and-other-emergencies-week-12-18-24-march-2024-data-reported-1700-24-march-2024].

WHO (2024b). Ramping up response to curb Zimbabwe cholera outbreak. [Internet] Available from: https://www.afro.who.int/countries/zimbabwe/news/ramping-response-curb-zimbabwe-cholera-outbreak [Accessed 22 July 2024].

WHO (2024c). Collaborative Effort Combats Cholera Outbreak in Shamva. [Internet]. Available from: https://www.afro.who.int/countries/zimbabwe/news/collaborative-effort-combats-cholera-outbreak-shamva.

Winstead, A., Strysko, J., Relan, P., Conners, E.E., Martinsen, A.L., Lopez, V., Arons, M., Masunda, K.P., Mukeredzi, I., Manyara, J., & Duri, C. (2020). Notes from the Field: Cholera Outbreak—Zimbabwe, September 2018–March 2019. Morbidity and Mortality Weekly Report, 69(17), 527.

Yongabi, K. A., Lewis, D. M., & Harris, P. L. (2011). Application of phytodisinfectants in water purification in rural Cameroon. African Journal of Microbiology Research, 5(6), 628-635.

Zenda, C. (2024). Religious Objections To Vaccines In Africa Complicates Cholera Fight. [Internet]. Available from: https://religionunplugged.com/news/2024/2/26/religious-objectors-seen-complicating-fight-against-cholera-in-zimbabwe-and-other-african-countries.

Zhakata, I. (2024). The Herald: Harare loses US$4.8 million worth of treated water every year. [Internet]. Available from: https://www.herald.co.zw/harare-loses-us4-8-million-worth-of-treated-water-every-year/ [Accessed 22 July 2024].

CHAPTER 3

Identification of medicinal plants traditionally employed in treating cholera and other related diarrhoeal diseases in Zimbabwe

Abstract

Zimbabwe, an economically disadvantaged country, has intermittent cholera epidemics. Medicinal plants have been employed in cholera-prone areas with limited resources. This study sought to examine how medicinal plants are used for folkloric diarrhoea and cholera treatment, including the plant parts, and herbal preparation methods, along with supporting data. Electronic databases (Google Scholar, ScienceDirect, and PubMed) theses and conference papers, were used to conduct a literature search on anti-diarrhoea and anti-*Vibrio cholerae* medicinal plants used as remedies in Zimbabwe (and Limpopo province of South Africa). In addition, proper identification and naming of plant species was done as per World Flora Online. Nineteen plant species are widely used in the treatment and management of diarrhoea in Zimbabwe. Among the species, only two (*Ziziphus mucronata* Willd. and *Kirkia acuminata* Oliv.) were most popular in treating the deadly cholera disease. The leaves (75.0%), roots (58.3%) and stem bark (50.0%) are common plant parts used. Infusion is the common method of herbal preparation. The selected medicinal herbs have high inhibitory and bactericidal activity against a wide range of bacteria, with minimum inhibitory concentrations (MIC) ranging from 0.02 to 10 mg/mL. The compilation of antidiarrhoeal medicinal plants, with their corresponding antibacterial properties and phytochemical composition, confirms the traditional usage of these plants by local communities for the treatment of diarrhoea and cholera. However, further investigation is required to demonstrate their vibriocidal activity and confirm their safety.

3.1 Introduction

Gastrointestinal diseases are characterised by the presence of three or more bowel movements that exhibit abnormally loose or watery faeces (Ayalew et al., 2022). Such symptoms are related to diarrhoeal diseases (e.g., cholera, dysentery), which are a global threat and the main cause of infant death worldwide (Maroyi, 2016a). Among the diarrhoeal diseases, cholera is the most lethal and can lead to death if treatment is not provided. Cholera has been evolving continuously since its arrival in Africa in 1970, causing severe mortality and morbidity. Sub-Saharan Africa (SSA) alone accounts for half of all reported cholera cases worldwide, and the region also has the highest case-fatality rates. The number could be significantly greater than what is officially recorded due to under-reporting and insufficient surveillance systems (Deen et al., 2020). The World Health Organisation (WHO) cited the under-reporting of cholera outbreaks as a lack of detection in distant locations as well as political expediency on the part of countries seeking to avoid the potential economic and political consequences of disclosing such information (Rieckmann et al., 2019).

Diarrhoeal diseases, particularly cholera, are difficult to control in SSA due to a variety of factors. This includes: (1) poor sanitation, particularly in communities experiencing famines and clean water shortages (Ojeda Rodriguez and Kahwaji, 2020), (2) the causative agent is found in brackish water, infecting fish, crustacean seafood and water fowls (Ismail et al., 2021), (3) unsatisfactory hygienic conditions and poor clean water supply, mostly in urban or peri-urban areas (Mireku-Gyimah et al., 2018), (4) complex interactions between the natural reservoirs of *V. cholerae*, human activities and climatic risk factors (Gwenzi and Sanganyado, 2019), (5) rainy seasons, prolonged droughts and dry seasons, (6) poor politics and governance (Cuneo et al., 2017), and (7) population migration (Deen et al., 2020). Furthermore, *V. cholerae*, the causative agent, is developing resistance to some modern pharmaceuticals (Mashe et al., 2020).

All these risk factors contribute to cholera's lethal and cyclical nature in SSA nations, including Zimbabwe. As an example, the *V. cholerae* O1 serotype was linked to Zimbabwe's deadly cholera epidemic in 2008, where up to 20% of people in the rural areas who got infected died (Gwenzi and Sanganyado, 2019). This type of strain is still endemic and very resistant to ciprofloxacin and ceftriaxone (Mashe et al., 2020). Due to economic challenges, street vending of food is common in Zimbabwe's urban areas (Bobo et al., 2021; Hove et al., 2020), and this could be one of the sources of cholera transmission. Several studies have found multidrug-resistant bacteria in food sold in Harare's informal markets (Gufe et al., 2022; Gweshe et al., 2020; Gufe et al., 2019; Gadaga et al., 2008).

In Zimbabwe, cholera still wreaks havoc on vulnerable communities regularly. People affected by the Tokwe-Mukosi tragedy in 2014 and Cyclone Idai, which struck between March 4 and 21, 2019, for example, were at risk of diarrhoeal diseases, including cholera. Due to the disruption in the water and sanitation systems caused by both disasters, cholera cases were documented in camp sites and collective centres for the displaced people (Chen and Azman, 2019; UNICEF, 2019). Besides natural disasters, politics and governance also impact cholera outbreaks in the SSA. Political tentacles were observed at the centre of the 2008–2009 cholera epidemic in Zimbabwe, which was marked by a high case fatality rate (4.3%) and a 10-month duration (Mukandavire et al., 2011). The recent cholera outbreak in 2018 also coincided with the 'alleged' coup and the disputed national election results (Cuneo et al., 2017).

Control of diarrhoeal diseases, notably cholera, is difficult due to a variety of risk factors (Gufe et al., 2022; Ojeda Rodriguez and Kahwaji, 2020; Bobo et al., 2021; Ismail et al., 2021; Mireku-Gyimah et al., 2018; Gwenzi and Sanganyado, 2019; Cuneo et al., 2017; Deen et al., 2020). Furthermore, drug resistance in pathogenic bacteria is also exacerbating the problem (Mashe et al., 2020). Modern technologies (vaccines, probiotics, and phage therapy) are ineffective in the treatment and management of cholera (Hsueh and Waters, 2019). Considering this, the quest for acceptable pharmaceuticals to treat diarrhoeal diseases, particularly the devastating cholera, must continue, and plants provide possible viable alternatives. Plants contain a variety of secondary metabolites as defence mechanisms against invading microbes and pests (Chassagne et al., 2021). In Africa, the use of traditional medicinal plants to treat diarrhoea has been practised for a long time (Ayalew et al., 2022).

Zimbabwe has a varied range of plant species that have long been used in African traditional medicine to treat and control several harmful diseases that threaten human and animal health. Most medicinal plants in Zimbabwe are used to treat and control gastrointestinal ailments such as diarrhoea and cholera, according to a survey done by Maroyi (2013). Therefore, this chapter aims to compile medicinal plants utilised in traditional treatments for diarrhoea and cholera-related diseases, along with their documented biological properties. This inventory and information will significantly contribute to the treatment and eradication of cholera and other related diseases. The plants may also provide a basis for pharmaceutical research and discovery.

3.2 Materials and Methods

The inventory of medicinal plants for diarrhoea and cholera treatment and related information in this study were compiled from a literature search using various electronic databases such as Google Scholar, ScienceDirect, and PubMed. Additional information was further retrieved from various academic theses, conference papers, and reports from Non-governmental organisations (NGOs), and government health departments. Keywords such as "herbal medicines used to treat diarrhoea, gastrointestinal diseases, and cholera," "medicinal plants used to treat diarrhoea, gastrointestinal diseases, and cholera," and "traditional medicines used to treat diarrhoea, gastrointestinal diseases, and cholera" were used in the searches from literature sources. Additionally, keywords such as 'antimicrobial efficacy of medicinal plants and phytochemical analysis of medicinal plants' were also used to document related or supporting information in this study.

3.3 Results

Table 3.1 presents the medicinal plant inventory based on 46 publications that met the specified inclusion criteria. The initial search yielded over 200 publications. To enhance transparency and methodological rigour, a formal selection methodology, such as PRISMA, is recommended for future literature reviews. This study revealed the usage of 19 medicinal plant species belonging to 13 different families for the folklore treatment and management of gastrointestinal diseases, cholera, and diarrhoea in particular (**Table 3.1**). Many communities reportedly use only two plant species, *Z. mucronata* and *K. acuminata* to treat the deadly cholera disease. All the plant species were reported to have therapeutic uses other than diarrhoeal disease treatment. Different communities prepared the remedies using various parts of medicinal plants. Among plant parts, leaves were most commonly used (75.0%), followed by roots (58.3%) and bark (50.0%). Diverse ways of preparing and administering the plant parts were reported, and there was no connection to the type of plant part or species used (**Fig 3.1**). As a therapeutic method, infusing powdered plant materials (such as leaves, roots, and stembark) into water and then drinking it was popular (38.9%), whereas grinding the dried plant materials and blowing the powder anally into the rectum was least popular (5.6%).

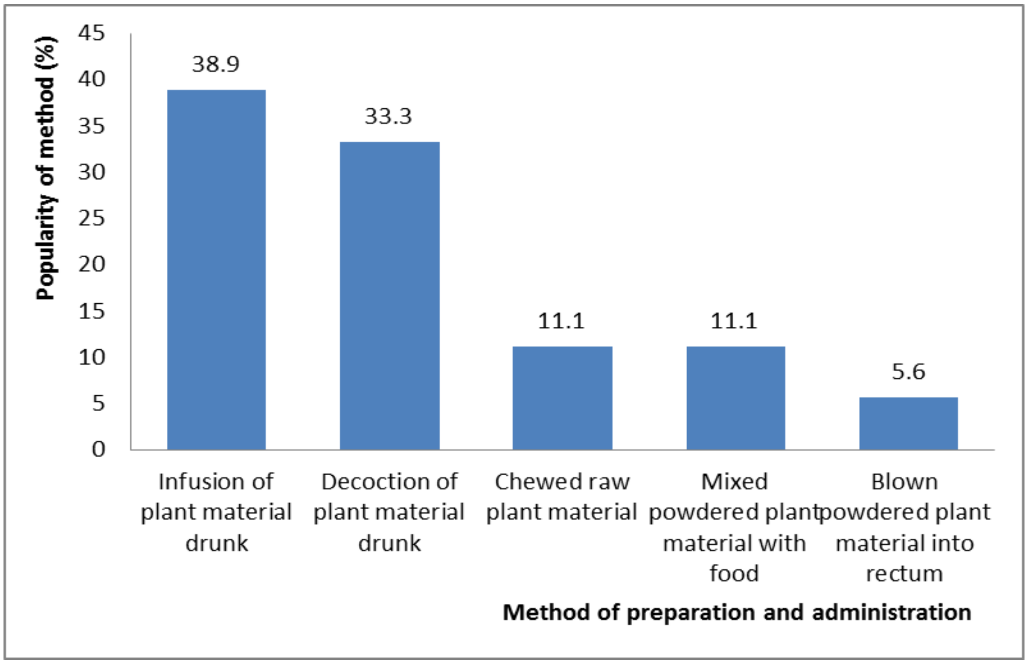

Figure 3.1 Method of preparation and administration of plant-based remedies

Table 3.1 Plant species used in Zimbabwe to treat and manage diarrhoea and cholera

Plant species	Local names	Family	Plant parts used and traditional preparation of the remedy	Reported antimicrobial activity	Phytochemical analysis	Other reported therapeutic uses	References
Ziziphus mucronata Willd.	Muchecheni (Shona), Umpasamala (Ndebele), Umlahlankosi, Umphafa (Zulu), Mokgalo (Tswana), Mutshetshete, Mukalu (Venda)	Rhamnaceae	-decoction of powdered leaves or roots drunk -raw or dried stembark chewed	Exhibited antimicrobial activity with MIC of <1 mg/ml against a wide range of pathogens	phenolic acids, alkaloids, flavonoids terpenoids, and quinones	Decoction used for STIs, sores and wounds, opportunistic infections associated with HIV/AIDS, respiratory-related infections, infertility, etc	Madikizela et al., 2012 ; Kigen et al., 2016 ; Gelfand et al., 1985; Mongalo et al., 2020 ; Maroyi, 2011
Ximenia caffra Sond.	Munhengeni (Shona), Umthunduluka-Obomvu (Zulu), Moretologa (Tswana), Mutanzwa (Venda)	Olacaceae	-Roots or leaf infusion is drunk	Root extract showed strong antibacterial activity (zone of inhibition of 10mm radius) against *Escherichia coli*	flavonoids, phenols, phytosterols, tannins and fatty acids	Used to treat wounds, STIs, infertility, fever, eye problems, menorrhagia, malaria, intestinal worms, impotence, and coughs.	Maroyi, 2011, 2016b; Maroyi and Chekhyoussef, 2015; Munodawafa et al., 2013
Vangueria infausta Burch.	Mutsvirungombe (Shona), Umviyo, Umtulwa (Zulu), Mothwani, Mmilo (Tswana), Muzwilu (Venda)	Rubiaceae	-bark or roots or leaf infusion drunk	Showed antibacterial activity against diverse range of bacterial species, with MIC values ranging from 0.02 to 10 mg/mL	flavonoids, alkaloids, anthraquinones, coumarins, glycosides, polyphenols, saponins, secoiridoids, steroids, tannins and terpenoids	Concoctions used to treat malaria, pneumonia, cough, menstrual problems, aphrodisiacs, parasitic worms, chest complaints, remedy for snake bites, infertility, fever, candidiasis, and abdominal pains	Munodawafa et al., 2013; Maroyi, 2011, 2018a

Crossopteryx febrifuga Benth.	Mukomberwa (Shona) Umphokophokwana (Ndebele), Umphokophokwana (Zulu),Mositsane (Tswana), Murombe (Venda)	Rubiaceae	-bark decoction drunk -powdered bark mixed with porridge -powdered bark put into a horn and blown into the rectum	Inhibited growth of several bacterial species, with zones of inhibition ranging from 7- 23 mm	steroids, flavonoids, terpenoids, anthraquinones, and cardiac glycosides, tannins, alkaloids, and saponins	Malaria, fever, painful inflammatory disorders, coughs, remedy for skin itching, syphilitic ulcers, inflammation of the eyes, intestinal worms, ophthalmia and wounds.	Halilu et al., 2012; Chigora et al., 2007
Sclerocarya birrea Hochst.	Mupfura (Shona), Umganu (Zulu), Morula (Tswana), Mufula (Venda)	Anacardiaceae	-decoction of crushed bark drunk, -crushed bark mixed with cold water and taken orally or anally -roots, -leaves	Strong antibacterial activity, with inhibition zones ranging from 19 to 22 mm at 100 mg/ml using various solvents.	Alkaloids, Flavonoids, Tannins, steroids, Triterpenes, and Glycosides	Bark decoctions used to treat cough, pneumonia, heart pains, bilharziasis, malaria,	Maroyi, 2013; Komolafe, 2014; Ali, 2022; Viol et al., 2013; Semenya and Maroyi, 2012
Mangifera indica L.	Mumango (Shona), Umango (Zulu), Mango (Tswana), Mango, Munangu (Venda)	Anacardiaceae	Stembark, Twigs	Demonstrated antibacterial activity against a variety of bacteria with MIC of 256 g/mL	phenolic compounds, which include phenolic acids, xanthones, benzophenones, tannins, terpenoids, flavonoids, and many other phytochemicals	Dysentery,	Tchoumba Tchoumi et al., 2021; Maroyi, 2018b; Maroyi, 2009; Kumar et al., 2021; Chitemerere and Mukanganyama, 2011
Flacourtia indica (Burm. f.) Merr.	Munhunguru (Shona), umndundela (Zulu), Moritela (Tswana), JuJhunguru (Venda)	Salicaceae	-leaves browsed by mouth and chewed -leaves steeped in cold water and extract drunk	Aqueous extraction of *F. indica* showed significant antibacterial activity	Flavonoids, saponins, alkaloids, tannins, terpenoids, glycosides and phenolic compounds.	Malaria,	Chingwaru et al., 2020; Chinsembu, 2016; Maroyi, 2011

			-leaf infusion drunk				
Euclea divinorum Hiern	Mushangura (Shona) Umtshekesane (Ndebele), Umhlangula, Ubophanyamazane, Umncafuzana (Zulu), Motlhakola (Tswana), Mutangule (Venda)	Ebenaceae	-decoction of grounded roots drunk -leaves	Demonstrated antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Escherichia coli* with MIC ranging from 25 to 50 µg/ml	anthraquinones, cardenolides, saponins, tannins, polyphenols, terpenoids, and alkaloids	Chest pain, asthma, pneumonia, tonsillitis, sore throat, constipation, gout and snake bites	Chinsembu, 2016; Nyambe, 2014; Kipkore et al., 2014; Hutchings et al., 1996; Maroyi, 2011
Peltophorum africanum Sond.	Muzeze (Shona) Umsehla (Ndebele), Umsehle (Zulu), Mosêtlha (Tswana), Musese (Venda)	Fabaceae	leaf, bark or root infusion drunk	Showed antimicrobial activity with MICs, ranging between 0.23 mg/mL and 5 mg/mL.	tannins, saponins, flavonoids, cardiac glycosides, anthraquinone derivatives, coumarins, and alkaloids	Syphilis, headache, toothache, sore eyes, respiratory infections, infertility, STIs, cough, joints, backache, stomach disorders, sore eyes and fever	Chinsembu et al., 2019; Maroyi and Chekhyoussef, 2015; Munodawafa et al., 2013; Mongalo, 2011, 2013
Elephantorrhiza goetzei (Harms) Harms	Ndorani (Shona) Intolwane (Ndebele), Intolwane (Zulu), Mositsane (Tswana), Mugudzuru (Venda)	Fabaceae	-bark or root decoction, infusion or macerate orally taken -bark or roots cooked and consumed -root powder mixed into porridge and consumed	Showed antibacterial activity against *Staphylococcus aureus* with zone of inhibition of 4.0mm	phenolic compounds, coumarins, flavonoids, saponins, stilbenoids, tannins, triterpenoids , etc	Decoction used to treat a variety of conditions, such as pain, sores, sexually transmitted infections, microbial infections, and genito-urinary system disorders.	Maroyi, 2013; Maroyi, 2011; Gelfand et al.,1985; Viol et al., 2013; Bruschi et al., 2011; Mwafongo et al., 2010; Maroyi, 2017
Xeroderris stuhlmannii (Taub.) Mendonca & E.P. Sousa	Murumanyama (Shona), Umthundulu (Ndebele), Wing pod (English)	Fabaceae	- root or bark infusion or macerate, orally taken	Bark extracts demonstrated considerable antibacterial activity against a variety of	alkaloids, flavonoids, terpenoids and tri-terpenoids,	diabetes mellitus, coughs, malaria, colds, rheumatoid arthritis, stomach-	Selemani et al., 2021; Nyathi et al., 2022; Ngarivhume et al., 2015

				bacteria, with MICs ranging from 0.23-0.80 mg/mL	polyphenols, steroids, etc	aches, dysentery, and eye infections	
Cassia abbreviata Oliv.	Isihaqa (Ndebele) Muremberembe, (Shona) Sjambok pod, Long-tail cassia (English), Isiqolo (Zulu), Molepelepe (Tswana), Muboma, Mulambivhu, Muluma-nama, Muvhonelathangu (Venda)	Fabaceae	- root or stem bark crushed, mixed with hot water, and extract taken orally	Extracts active against various bacteria, with MICs ranging from 46.88 g/ml to 93.75 g/ml	alkaloids, tannins, anthraquinones, flavonoids, saponins, terpenoids, polyphenols, etc	postpartum discomfort, menstrual cycle issues, blood vomit, closing fontanelle in newborn babies, bilharzia, hernia, venereal diseases, fever, dysentery, and hernia	Maroyi, 2013; Mulubwa and Prakash, 2015; Hikaambo et al., 2022. Mongalo and Mafoko, 2013; Bruschi et al., 2011. Ribeiro et al., 2010; Maroyi, 2011
Schotia brachypetala Sond.	Mutondochuru (Shona), Weeping boer-bean (English), Umgxamu, Uvovovo, (Zulu), Molope, Umutwa, (Tswana), Mulibi, Mununzwa, Mununzwu, Mutanswa (Venda)	Fabaceae	-bark or root extract taken orally	Strong antibacterial activity against a variety of bacteria with MICs ranging from 0.15 mg/mL to 3.31 mg/mL	Several quercetin glucoside derivatives, etc		Maroyi, 2016a; Mathabe et al., 2006; Du, 2011
Grewia bicolor Juss.	Mutongoro (Shona) Umhlampunzi (Ndebele), Umlalampunzi, Imdliwampunzi (Zulu), Mogwane (Tswana), Murabva (Venda)	Malvaceae	-Leaf decoction drunk		Triterpenes, alkaloids, etc	Root decoctions used to treat abscesses, pustulous skin lesions, syphilis and gonorrhoea	Chinsembu et al., 2019; Arora, 2011; Maroyi, 2011
Kirkia acuminata Oliv.	Mubvumira (Shona) Umvumile (Ndebele), Modumela (Tswana), Mubvumela, Mubvumala (Venda)	Kirkiaceae	-bark decoction drunk -powdered bark mixed with food and eaten -bark infusion drunk	Inhibited *Mycobacterium smegmatis* and *Staphylococcus aureus* at the lowest concentration of 2.5 mg/mL and 1.25 mg/mL, respectively.	fatty acids, flavonols, isocoumarin, lignans, neolignans, nor-carotenoids, phenols, and tannins	Used to treat abdominal pains, backache, cholera, constipation, cough, diarrhoea, dysentery, snake bites, toothache, and wounds.	Chigora et al., 2007; Maroyi, 2013; Maroyi, 2016c; Maroyi, 2017b; Chinsembu et al., 2019; Mabadahanye et al., 2022
Ficus sur Forssk.	Muonde (Shona), Umkhiwane (Zulu), Mokoyo (Tswana), Muhuyu (Venda)	Moracea	-root infusion drunk -Leaves	Showed antimicrobial activity against bacteria and fungi, with MICs ranging from 2.5- 40 mg/mL.	anthraquinones, terpenoids, flavonoids, steroids, saponins, phenols and tannins	gonorrhoea, sore throat, toothache, eye problems, epilepsy, leprosy, infertility,	Gelfand et al., 1985; Akoto et al., 2020; Maroyi, 2011

						gonorrhoea, sickle cell disease, etc	
Strychnos spinosa Lam.	Mutamba (Shona), Umhahli (Ndebele), umHlala (Zulu), Nsala, Mogorogoro (Tswana), Muramba (Venda)	Loganiaceae	Leaves	Acetone leaf extract showed antimicrobial activity against fungi and bacteria, with MICs ranging from 0.04 to 1.25 mg/ml.	alkaloids, saponins, anthraquinones, glycosides, steroids, and terpenoids	Used to treat asdropsy, earache, snakebite, fever, elephantiasis, fever, epilepsy, and rheumatism	Mongalo and Makhafola, 2018; Ahmad et al., 2019; Tor-Anyiin et al., 2015
Psidium guajava L.	Mugwavha (Shona), Ijikijolo, Ama-guava (Zulu), Moguava (Tswana), Mugwava (Venda)	Myrtaceae	Roots, Stem bark, Leaves	Ethanolic extract showed significant antibacterial activity, with zone of inhibition between 9mm and 14.50mm	tannins, flavonoids, alkaloids, saponins, glycosides, terpenoids, etc	Fever, dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief and wounds.	Kenneth et al., 2017; Maroyi, 2018b; Naseer et al., 2018; Semenya and Maroyi, 2012
Prunus persica (L.) Batsch	Mupichisi (Shona), Ipheach (Zulu), Pitshe or pijici (Tswana), Pfish (Venda)	Rosaceae	Leaves	Methanol extract showed significant antibacterial activity against several bacteria	alkaloids, glycosides, Flavonoids, steroids, tannins, phenols, etc	Malaria,	Maroyi, 2018b; Bento et al., 2020; Giday et al., 2007; Ngarivhume et al., 2015

3.4 Discussion

This study showed that 19 medicinal plant species from 13 distinct families had been used in traditional medicine to treat and control gastrointestinal illnesses, including cholera and diarrhoea (**Table 3.1**). These results, particularly regarding the kind of medicinal plants employed, the families, the plant parts used, and the preparation methods for the remedies, are almost identical to those of earlier studies (Maroyi, 2016a; Marekerah, 2015; Maroyi, 2011). A related study done in South Africa and Zimbabwe found that *M. indica*, *S. birrea*, *V. infausta*, *X. caffra*, *F. sur*, and *E. goetzei* were used as medicines to treat diarrhoea. In another study in the Nhema area of Zimbabwe, a total of 61 plant species representing 45 genera and 28 families were found to be commonly used in the treatment of 34 different human health problems. More than a third of the plant species were used for diarrhoea (Maroyi, 2011). Elsewhere, similar ethnobotanical studies revealed nearly identical outcomes (Wali et al., 2022; Madikizela et al., 2012; Appidi et al., 2008). For instance, a survey study in the Eastern Cape of South Africa found 17 plant species from 14 families that are often used to cure diarrhoea. The Fabaceae family was most common, followed by the Anacardiaceae (Appidi et al., 2008). Furthermore, a recent survey in Diamir district, Pakistan, reported a total of 61 medicinal plant species belonging to 35 families for the treatment of diarrhoeal diseases (Wali et al., 2022). However, variations in human cultural practices, geographic areas where ethnobotanical research was conducted, and data collection techniques may be the reasons why there is a disparity in the number of therapeutic plant species and the families they belong to.

Among plant parts, leaves were most commonly used (75.0%), followed by roots (58.3%) and bark (50.0%). Previous studies also confirmed the order and preference of the plant parts in the treatment of diarrhoeal diseases (Woldeab et al., 2018; Moshi et al., 2012). For instance, a recent survey conducted in Pakistan confirmed that leaves were the most often used component (Wali et al., 2022). The same authors suggested that it may be because leaves are the easiest to gather and the portions of the plant that are most readily available. In addition, leaves are the most productive plant components since they house most of the photosynthetic apparatus that produces nearly all the medicinal phytochemicals (Wali et al., 2019). However, earlier studies have shown that plant parts other than leaves are also preferred treatments for diarrhoea. For example, in related studies, roots were found to be the most preferred plant part, followed by the bark and the leaves, in the treatment of diarrhoea (Shopo et al., 2022; Semenya and Maroyi, 2012; Maroyi, 2011; Appidi et al., 2008). For example, a study conducted across South Africa and Zimbabwe, roots constituted 47.4% of reported usage, followed by bark at 26.3%, leaves at 21.1%, and rhizomes at 5.3% (Maroyi, 2016a). These results highlight regional variations in the use of plant parts as well as the possible impact of seasonal availability on harvesting methods, a topic that warrants more research in future studies. However, a related ethnobotanical survey of Zimbabwean practitioners of traditional medicine indicated that both roots and leaves are used equally frequently in traditional medicine (Matowa et al., 2020). The difference in the plant parts used in different regions could also be due to variations in cultural practices, and data collection techniques employed. There was no relationship between the type of plant part or species used in this investigation, which involved varied preparation and administration techniques used by locals (Fig 3.1). However, the most typical therapeutic method was the infusion of powdered plant materials (such as leaves, roots, and stem bark) into water and subsequent oral consumption of the solution (38.9%), then decoction and mixing powder with food (5.6%), and the least common was the anal instillation of powdered plant material into the rectum. The findings are generally consistent with traditional African medicine, which primarily relies on the preparation of remedies using straightforward hot-water extraction (steeping) and cold-water extraction techniques (Mothibe and Sibanda, 2019). The effectiveness of these preparations against bacteria that cause diarrhoea (e.g., *Salmonella typhi*) was tested by Chingwaru et al. (2020). The same authors extracted from *F. indica*, *Swartzia madagascariensis*, and *X. caffra* using the same extraction techniques. The techniques produced extracts with high antibacterial activity and chemical compositions equivalent to those of contemporary solvent extraction techniques (Chingwaru et al., 2020). Furthermore, many studies concurred with the use of decoctions and infusions in traditional medicine preparations (Maroyi, 2011; Appidi et al., 2008). For example, the indigenous inhabitants of the Eastern Cape in South Africa boil the leaves of *Z. mucronata* in water, and the decoction is drunk (Appidiidi et al., 2008), much like what is done in the majority of Zimbabwe (Gelfand et al., 1985). *Flacourtia indica* has a unique medicinal procedure in which the leaves are browsed by mouth and chewed as a diarrhoea treatment (Maroyi, 2011). A similar procedure is used in traditional medicine in other countries (Comoros and Madagascar), where raw fruits are consumed while fresh stems and leaves are chewed (Kaou et al., 2010).

Based on a literature review, all 16 medicinal plant species have enormous ethnobotanical information on how they are used by different ethnic groups across Africa and beyond as folk medicine against diarrhoea and other ailments (**Table 3.1**). For example, traditionally, *G. bicolor*, *V. infausta*, *Elephantorrhiza* spp., *F. sur*, *M. indica*, *S. birrea*, *P. guajava*, *Mangifera* *indica*, and *X. caffra* are among the medicinal plants widely used by traditional healers and individuals as antidiarrhoeals in South Africa (van Vuuren et al., 2015; Semenya and Maroyi, 2012; Maroyi, 2011; Appidi et al., 2008; Akinpelu and Onakoya, 2006). Similarly, the use of *P. africanum, E. goetzei,* and *E. divinorum* in folk medicines in other African countries is also documented (Mongalo, 2013). In Namibia, *P. africanum* bark or root decoction is used to treat diarrhoea and abdominal disorders (Chinsembu et al., 2011). Other African nations (such as Malawi and Mozambique) have also reported using *E. goetzei* for therapeutic purposes, with the root macerate, bark or roots cooked as food or the root powder mixed into porridge and consumed orally (Viol et al., 2013; Bruschi et al., 2011; Mwafongo et al., 2010). Like in Zimbabwe, locals in Kenya have employed *E. divinorum* leaves to treat numerous stomach maladies and gastrointestinal distress (Hutchings et al., 1996).

Prunus persica, *M. indica*, and *P. guajava* are the only exotic plants in Zimbabwe included in the list of medicinal plants for the treatment of diarrhoea (Maroyi, 2016; Maroyi, 2018). The use of exotic plant species in the provision of primary health care is common in other African regions (de Wet et al., 2010). However, these plant species are also used for the same purpose in other African countries (Gairola et al., 2013; Nikhal et al., 2010) as well as on other continents (de Wet et al., 2010; Ojewole et al., 2008). *Psidium guajava* and *M. indica* herbal remedies are popular in the local communities of southwestern Nigeria for treating a range of illnesses, including diarrhoea, dysentery, and gastrointestinal problems (Akinpelu and Onakoya, 2006). In addition, in India and other countries, the dried flowers of *M. indica* can be used to treat diarrhoea and chronic dysentery (Nikhal et al., 2010). Several studies elsewhere confirmed the use of *P. persica* in traditional medicine to treat diarrhoea (Wali et al., 2022; Pratama et al., 2020; Gairola et al., 2013). A survey of the Bhoxa community in India revealed that *P. persica* is one of the fifty medicinal plants used to cure dysentery and diarrhoea (Gairola et al., 2013). Similarly, in a recent survey in Diamir district, Pakistan, *P. persica* was ranked highly among the other 61 medicinal plants used in the treatment of diarrheal diseases (Wali et al., 2022). A related study revealed that the indigenous inhabitants of the Eastern Cape in South Africa boil the leaves in water and consume the decoction orally, much like what is done in the majority of Zimbabwe (Appidi et al., 2008). Additionally, the plant has long been employed in Indian folk medicine (Raturi et al., 2011).

The documented data on antibacterial activity, phytochemical analysis, and other therapeutic uses of the medicinal plant species (Table 1) support the use of the established list of antidiarrheal plants essential in traditional medicine. Several earlier studies have shown that these types of antidiarrheal plants are used in traditional medicine (Ali, 2022; Mongalo et al., 2020; Maroyi, 2018b; Maroyi, 2016a; Viol et al., 2013). Some phytochemicals that have been found in *E. goetzei*'s bark, leaves, and roots are coumarins, flavonoids, saponins, stilbenoids, tannins, and triterpenoids (Maroyi, 2017a; Viol et al., 2013; Wanjala and Majinda, 2001), to name a few. In addition to the identified components, antibacterial and cytotoxicity tests on the crude extracts have shown that the use of *E. goetzei* as a diarrhoea treatment in traditional medicine is scientifically supported (Maroyi, 2018b; Maroyi, 2017a; Viol et al., 2013; Moyo et al., 1999). Furthermore, the fact that antidiarrhoeal medicinal plants are accepted across cultural boundaries and are used in various geographic regions, in addition to their biological effects, is proof of the value of herbal remedies in basic healthcare (Maroyi, 2016a).

3.5 Conclusion

Based on a review of the literature, medicinal plant species used in Zimbabwean traditional treatment of cholera or diarrhoea were identified. The method allowed for the screening of herbal remedies that are frequently used by local populations or indigenous people to treat diarrhoea, including the deadly cholera, and provided leads to finding new and practical antidiarrhoeal therapeutic agents. The parts of the medicinal plants and their preparation techniques were established, and they agreed with information from other African nations or from overseas. Some of the biological activities of the revealed medicinal plants were uncovered, and it is without a doubt that some of the recorded species have the potential to serve as sources of pharmaceuticals for the treatment of diarrhoea and other related illnesses. As a result, the assembled list of antidiarrhoeal medicinal plants, coupled with their related antibacterial properties and phytochemical composition, verifies the traditional usage of plants in cholera and diarrhoea treatment by local communities. The next step should be to identify the precise phytochemicals responsible for the antidiarrhoeal activity, ascertain their method of action, and establish their safety and efficacy. However, the findings of the study cannot be considered entirely conclusive due to the limitations of the electronic databases utilised for data collection, which include publication bias, restricted access to full texts, and geographic and cultural constraints, among various others. Consequently, relying solely on electronic databases could lead to an overestimation of the therapeutic potential of certain plants while under-representing others that may be equally important but less studied. Moreover, methodologies like surveys and observation could be viewed as valuable additions to the use of electronic databases in future studies.

References

Ahmad, G., Muhammad, A., Ahmad, K. A., & Gbeminiyi, A. (2019). Phytochemical and antimicrobial studies of the root bark extracts of *Strychnos spinosa* Lam. Bayero Journal of Pure and Applied Sciences, 12(1), 161-165.

Akinpelu, D. A., & Onakoya, T. M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western. African Journal of Biotechnology, 5(11).

Akoto, C. O., Acheampong, A., Kwame, O., & Boateng, B. A. (2020). An Assessment of the Anti-Inflammatory, Antimicrobial, and Antioxidant Activities of Ficus sur Stem-Bark. Organic & Medicinal Chemistry International Journal, 9(3), 116-124.

Ali, M. A. E. M. (2022). Evaluation of Phytochemical and Antibacterial Activity of *Sclerocarya birrea* Stem Bark Extraction. Journal of Current Medical Research and Opinion, 5(03).

Appidi, J. R., Grierson, D. S., & Afolayan, A. J. (2008). Ethnobotanical study of plants used for the treatment of diarrhoea in the Eastern Cape, South Africa. Pakistan Journal of Biological Sciences, 11(15), 1961-1963.

Arora, S. (2011). Antibecterial, antifungal, antioxidant and phytochemical study on the leaves extract of *Grewia optiva*.

Ayalew, M., Bekele, A., Mengistie, M. G., & Atnafie, S. A. (2022). Evaluation of the antidiarrheal activity of 80% methanol extract and solvent fractions of the leaf of *Bersama abyssinica* Fresen (Melianthaceae) in mice. BMC Complementary Medicine and Therapies, 22, 1-9.

Bento, C., Goncalves, A. C., Silva, B., & Silva, L. R. (2022). Peach (*Prunus persica*): Phytochemicals and health benefits. Food Reviews International, 38(8), 1703-1734.

Bobo, T., Chirisa, I., & Toriro, P. (2021). Informal Food Spaces: Implications for Public Health. In Environmental Resilience: Food and the City-Zimbabwe (pp. 175-188). Singapore: Springer Singapore.

Bruschi, P., Morganti, M., Mancini, M., & Signorini, M. A. (2011). Traditional healers and laypeople: a qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). Journal of Ethnopharmacology, 138(2), 543-563.

Chingwaru, C., Bagar, T., & Chingwaru, W. (2020). Aqueous extracts of *Flacourtia indica, Swartzia madagascariensis* and *Ximenia caffra* are strong antibacterial agents against *Shigella* spp., *Salmonella typhi* and *Escherichia coli* O157. South African Journal of Botany, 128, 119-127.

Chitemerere, T. A., & Mukanganyama, S. (2011). In vitro antibacterial activity of selected medicinal plants from Zimbabwe. The African Journal of Plant Science and Biotechnology, 5(1), 1-7.

de Wet, H., Nkwanyana, M. N., & van Vuuren, S. F. (2010). Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu-Natal Province, South Africa. Journal of Ethnopharmacology, 130(2), 284-289.

El-Gied, A. A. A., Abdelkareem, A. M., & Hamedelniel, E. I. (2015). Investigation of cream and ointment on antimicrobial activity of *Mangifera indica* extract. Journal of Advanced Pharmaceutical Technology & Research, 6(2), 53-57.

El-Gied, A. A. A., Joseph, M. R., Mahmoud, I. M., Abdelkareem, A. M., Al Hakami, A. M., & Hamid, M. E. (2012). Antimicrobial activities of seed extracts of mango (*Mangifera indica* L.).

Fern, K., Fern, A., & Morris, R. (2014). Useful tropical plants database. Recuperado de: http://tropical. theferns. info.

Gadaga, T. H., Samende, B. K., Musuna, C., & Chibanda, D. (2008). The microbiological quality of informally vended foods in Harare, Zimbabwe. Food Control, 19(8), 829-832.

Gairola, S., Sharma, J., Gaur, R. D., Siddiqi, T. O., & Painuli, R. M. (2013). Plants used for treatment of dysentery and diarrhoea by the Bhoxa community of district Dehradun, Uttarakhand, India. Journal of Ethnopharmacology, 150(3), 989-1006.

Giday, M., Teklehaymanot, T., Animut, A., & Mekonnen, Y. (2007). Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest Ethiopia. Journal of Ethnopharmacology, 110(3), 516-525.

Gufe, C., Canaan Hodobo, T., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., ... & Machakwa, J. (2019). Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe. International Journal of Microbiology, 2019.

Gufe, C., Karembera, B., Marumure, J., & Makuvara, Z. (2022). Lead, silver nitrate and antibiotic resistance in bacteria isolated from Nile tilapia (*Oreochromis niloticus*) in anthropogenically polluted Lake Chivero, Zimbabwe. Cogent Food & Agriculture, 8(1), 2082040.

Gweshe, W. M., Muteveri, T., Gufe, C., Marumure, J., & Hodobo, T. C. (2020). Antimicrobial-resistant shiga-toxin producing *Escherichia coli* isolated from ready-to-eat meat products and fermented milk sold in the formal and informal sectors in Harare, Zimbabwe.

Halilu, M. E., Abubakar, A., & Garba, M. K. (2012). Antimicrobial and preliminary phytochemical studies of methanol extract of root bark of *Crossopteryx febrifuga* (Rubiaceae). Journal of Applied Pharmaceutical Science, 2(12), 066-070.

Hannan, A., Asghar, S., Naeem, T., Ullah, M. I., Ahmed, I., Aneela, S., & Hussain, S. (2013). Antibacterial effect of mango (*Mangifera indica* Linn.) leaf extract against antibiotic sensitive and multi-drug resistant *Salmonella typhi*. Pakistan Journal of Pharmaceutical Sciences, 26(4), 715-719.

Hove, M., Ndawana, E., & Ndemera, W. S. (2020). Illegal street vending and national security in Harare, Zimbabwe. Africa Review, 12(1), 71-91.

Kaou, A. M., Mahiou-Leddet, V., Canlet, C., Debrauwer, L., Hutter, S., Laget, M., ... & Ollivier, E. (2010). Antimalarial compounds from the aerial parts of *Flacourtia indica* (Flacourtiaceae). Journal of Ethnopharmacology, 130(2), 272-274.

Kaur, J., Rathinam, X., Kasi, M., Leng, K. M., Ayyalu, R., Kathiresan, S., & Subramaniam, S. (2010). Preliminary investigation on the antibacterial activity of mango (*Mangifera indica* L: Anacardiaceae) seed kernel. Asian Pacific Journal of Tropical Medicine, 3(9), 707-710.

Kenneth, E., Paul, T., Istifanus, N., Uba, U., Rejoice, A., Victor, O., & Mohammed, S. (2017). Phytochemical analysis and antibacterial activity of *Psidium guajava* L. leaf extracts. GSC Biological and Pharmaceutical Sciences, 1(2).

Kipkore, W., Wanjohi, B., Rono, H., & Kigen, G. (2014). A study of the medicinal plants used by the Marakwet Community in Kenya. Journal of Ethnobiology and Ethnomedicine, 10, 1-22.

Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M., ... & Mekhemar, M. (2021). Mango (*Mangifera indica* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Antioxidants, 10(2), 299.

Mabadahanye, K., Bhembe, N. L., & Green, E. (2022). Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. African Health Sciences, 22(2), 717-727.

Madikizela, B., Ndhlala, A. R., Finnie, J. F., & Van Staden, J. (2012). Ethnopharmacological study of plants from Pondoland used against diarrhoea. Journal of Ethnopharmacology, 141(1), 61-71.

Marekerah, L. (2015). A survey on the biological activities of selected plants used to manage diarrhoea and cancer in Vumba, Zimbabwe. Journal of Ethnopharmacology, 10, 123-130.

Maroyi, A. (2009). Traditional homegardens and rural livelihoods in Nhema, Zimbabwe: a sustainable agroforestry system. International Journal of Sustainable Development & World Ecology, 16(1), 1-8.

Maroyi, A. (2011). An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. Journal of ethnopharmacology, 136(2), 347-354.

Maroyi, A. (2016a). Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. African Journal of Traditional, Complementary and Alternative Medicines, 13(6), 5-10.

Maroyi, A. (2016b). *Ximenia caffra* Sond.(Ximeniaceae) in sub-Saharan Africa: A synthesis and review of its medicinal potential. Journal of Ethnopharmacology, 184, 81-100.

Maroyi, A. (2016c). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus, *Kirkia.* Tropical Journal of Pharmaceutical Research, 15(11), 2507-2516.

Maroyi, A. (2017a). *Elephantorrhiza elephantina*: Traditional uses, phytochemistry, and pharmacology of an important medicinal plant species in Southern Africa. Evidence-Based Complementary and Alternative Medicine, 2017.

Maroyi, A. (2017b). *Kirkia acuminata* Oliv.: A review of its ethnobotany and Pharmacology. African Journal of Traditional, Complementary and Alternative Medicines, 14(2), 217-226.

Maroyi, A. (2018a). Nutraceutical and ethnopharmacological properties of *Vangueria infausta* subsp. infausta. Molecules, 23(5), 1089.

Maroyi, A. (2018b). Ethnomedicinal uses of exotic plant species in south-central Zimbabwe. Journal of Ethnopharmacology, 224, 1-10.

Matowa, P. R., Gundidza, M., Gwanzura, L., & Nhachi, C. F. (2020). A survey of ethnomedicinal plants used to treat cancer by traditional medicine practitioners in Zimbabwe. BMC Complementary Medicine and Therapies, 20, 1-13.

Mongalo, N. I. (2013). *Peltophorum africanum* Sond [Mosetlha]: A review of its ethnomedicinal uses, toxicology, phytochemistry and pharmacological activities. Journal of Medicinal Plants Research, 7(48), 3484-3491.

Moshi, M. J., Otieno, D. F., & Weisheit, A. (2012). Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District. Journal of Ethnobiology and Ethnomedicine, 8, 1-11.

Mothibe, M. E., & Sibanda, M. (2019). African traditional medicine: South African perspective. Traditional and Complementary Medicine, 2019, 1-27.

Munodawafa, T., Chagonda, L. S., & Moyo, S. R. (2013). Antimicrobial and phytochemical screening of some Zimbabwean medicinal plants. Journal of Biologically Active Products from Nature, 3(5-6), 323-330.

Mushore, J., & Matuvhunye, M. (2013). Antibacterial properties of *Mangifera indica* on *Staphylococcus aureus*. African Journal of Clinical and Experimental Microbiology, 14(2), 62-74.

Mwafongo, E., Nordal, I., Magombo, Z., & Stedje, B. (2010). Ethnobotanical study of Hyacinthaceae and non-hyacinthaceous geophytes in selected districts of Malawi. Ethnobotany Research and Applications, 8, 075-093.

Nikhal, S. B., Dambe, P. A., Ghongade, D. B., & Goupale, D. C. (2010). Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion. International Journal of Pharmaceutical Sciences, 2(1), 30-32.

Nyambe, M. M. (2014). Phytochemical and antibacterial analysis of indigenous chewing sticks, *Diospyros lycioides* and *Euclea divinorum*. Doctoral dissertation, University of Namibia.

Ojewole, J. A., Awe, E. O., & Chiwororo, W. D. (2008). Antidiarrhoeal activity of *Psidium guajava* Linn.(Myrtaceae) leaf aqueous extract in rodents. Journal of Smooth Muscle Research, 44(6), 195-207.

Pratama, A. N. W., Ningsih, L. W. C., Rachmawati, E., Triatmoko, B., Tan, E. Y., & Nugraha, A. S. (2021). A study of treatment preference for diarrhea among Tengger people in Pasuruan, East Java, Indonesia. Journal of Health Research, 35(3), 202-213.

Rajan, S., Suganya, H., Thirunalasundari, T., & Jeeva, S. (2012). Antidiarrhoeal efficacy of *Mangifera indica* seed kernel on Swiss albino mice. Asian Pacific Journal of Tropical Medicine, 8(5), 630-633.

Raturi, R., Singh, H., Bahuguna, P., Sati, S. C., & Badoni, P. P. (2011). Antibacterial and antioxidant activity of methanolic extract of bark of *Prunus persica*. Journal of Applied and Natural Science, 3(2), 312-314.

Ruwanza, S., & Thondhlana, G. (2022). People’s perceptions and uses of invasive plant *Psidium guajava* in Vhembe Biosphere Reserve, Limpopo Province of South Africa. Ecosystems and People, 18(1), 64-75.

Sairam, K., Hemalatha, S., Kumar, A., Srinivasan, T., Ganesh, J., Shankar, M., & Venkataraman, S. (2003). Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica.* Journal of Ethnopharmacology, 84(1), 11-15.

Shopo, B., Mapaya, R. J., & Maroyi, A. (2022). Ethnobotanical study of medicinal plants traditionally used in Gokwe South District, Zimbabwe. South African Journal of Botany, 149, 29-48.

Tchoumba Tchoumi, L. M., Nchouwet, M. L., Poualeu Kamani, S. L., Yousseu Nana, W., Douho Djimeli, R. C., Kamanyi, A., & Wansi Ngnokam, S. L. (2021). Antimicrobial and antidiarrhoeal activities of aqueous and methanolic extracts of *Mangifera indica* Linn stem bark (Anarcadiaceae) in Wistar rats. Advances in Traditional Medicine, 21, 485-498.

Tor-Anyiin, T. A., Igoli, J. O., Anyam, J. V., & Anyam, J. N. (2015). Isolation and antimicrobial activity of sarracenin from root bark of *Strychnos spinosa*. Journal of Chemical Society of Nigeria, 40(1).

van Vuuren, S. F., Nkwanyana, M. N., & de Wet, H. (2015). Antimicrobial evaluation of plants used for the treatment of diarrhoea in a rural community in northern Maputaland, KwaZulu-Natal, South Africa. BMC Complementary and Alternative Medicine, 15, 1-8.

Viol, D. I., Chagonda, L. S., Moyo, S. R., & Mericli, A. H. (2013). Antibacterial, antifungal and phytochemical activities of some medicinal plants from Zimbabwe. Journal of Biologically Active Products from Nature, 3(4), 273-284.

Wali, R., Khan, M. F., Mahmood, A., Mahmood, M., Qureshi, R., Ahmad, K. S., & Mashwani, Z. U. R. (2022). Ethnomedicinal appraisal of plants used for the treatment of gastrointestinal complaints by tribal communities living in Diamir district, Western Himalayas, Pakistan. Plos One, 17(6), e0269445.

Wali, R., Rahman, K., Raja, N. I., Qureshi, R., & Mashwani, Z. R. (2018). A quantitative medico-botanical expedition of fairy meadows National Park, Diamir, Gilgit Baltistan, Pakistan. *BioRxiv*, 507848.

Wanjala, C. C., & Majinda, R. R. (2001). A new stilbene glycoside from *Elephantorrhiza goetzei.* Fitoterapia, 72(6), 649-655.

WFO (2022): World Flora Online. Published on the Internet; http://www.worldfloraonline.org. Accessed on: 24 Nov 2022

Woldeab, B., Regassa, R., Alemu, T., & Megersa, M. (2018). Medicinal plants used for treatment of diarrhoeal related diseases in Ethiopia. Evidence-Based Complementary and Alternative Medicine, 2018.

Yakubu, M. T., & Salimon, S. S. (2015). Antidiarrhoeal activity of aqueous extract of Mangifera indica L. leaves in female albino rats. Journal of Ethnopharmacology, 163, 135-141.

CHAPTER 4

Antimicrobial efficacy of selected medicinal plants against *Vibrio choerae*

Abstract

The medicinal plants used for gastrointestinal disorders are a panacea for problematic diarrhoeal diseases, such as cholera, as modern pharmaceuticals are expensive, and the resistance of pathogens to such pharmaceuticals is on the rise. Most developing countries treat infectious diseases using folkloric prescriptions rather than first determining antibiograms for the causative agents. This study investigated the anti-*Vibrio cholerae* activity of medicinal plants commonly used in traditional Zimbabwean medicine for the treatment of cholera. *Kirkia acuminata* Oliv., *Sclerocarya birrea* Hochst., *Ziziphus mucronata* Willd., and *Crossopteryx febrifuga* Benth. stem barks were extracted using ethanol and water. The ability of the extracts to inhibit *V. cholerae* was examined using the agar well diffusion method. In ethanol and water extraction, about 150 g of material from each plant yielded between 10 to 20.80 g of dried extract. The selected plants showed anti-*Vibrio cholerae* activity, with minimum inhibitory concentrations (MICs) of ≤ 1 mg/mL for *Z. mucronata*, 1.1–6 mg/mL for *S. birrea* and *K. acuminata*, and > 6 mg/mL for *C. febrifuga*. The anti-*Vibrio* efficacy displayed justifies their application in traditional therapy of cholera and other diarrhoeal diseases. These plant extracts can be studied further and used to design and develop less expensive pharmaceuticals that may alleviate drug resistance issues.

4.1 Introduction

Plants have been used in traditional medicine for as long as humans have existed (Adeleye et al, 2021). Currently, according to several studies (Chassagne et al., 2021; Clement et al., 2021; Kebede et al., 2021), between 70% and 95% of people in many developing countries use plants as their primary source of treatment. In most sub-Saharan African countries, several studies, including surveys, have revealed the use of various plant species to treat a wide range of diseases, including diarrhoea and cholera (Madikizela et al., 2012; Kigen et al., 2016; Tatsimo et al., 2017; Maroyi, 2018; Chinsembu et al., 2019). For example, *Senna alata* leaves are commonly used in Cameroon to treat gastrointestinal illnesses (Tatsimo et al., 2017). Similarly, a decoction of *Grewia bicolor* leaves is taken orally to treat diarrhoea in Zambia (Chinsembu et al., 2019). Furthermore, *Ziziphus mucronata* root, stem bark, and leaf extracts have been used to treat gastrointestinal symptoms in Zambia and Angola (Mongalo et al., 2020). In Zimbabwe, a considerable number of surveys revealed that a wide range of plant species are often used to treat diarrhea and cholera (Gelfand et al., 1985; Chigora et al., 2007; Maroyi, 2013; Maroyi, 2017a; Chingwaru et al., 2020). For example, *Ziziphus mucronata* (Gelfand et al., 1985), *Sclerocarya birrea* (Maroyi, 2013), *Kirkia acuminata* (Maroyi, 2017b; Chigora et al., 2007), *Crossopteryx febrifuga* (Chigora et al., 2007), and many other plant species have been reported to be utilized in folklore diarrhea and cholera treatment in Zimbabwe.

In many rural areas, where disease diagnosis often relies on traditional methods, the clinical distinction between cholera and other related diarrhoeal diseases is sometimes not evident (World Health Organisation, 2024; Ali et al., 2015). Diagnosis typically relies on observable symptoms, including 'rice water stools,' watery diarrhoea, vomiting, and signs of dehydration (Marumure and Nyila, 2024). As a result, intense episodes of diarrhoea may be mistakenly recognised as cholera and subsequently addressed with the medicinal plants that are accessible (Maroyi, 2013). A study conducted in Zimbabwe indicated that herbalists consistently categorise all acute watery diarrhoea as gastrointestinal illnesses, encompassing cholera, dysentery, and other enteric infections without differentiation (Njume and Goduka, 2012). This symptom-based diagnostic method, rather than a pathogen-specific approach, influences the interpretation of medicinal plant use in both historical and contemporary research, thereby broadening the ethnomedical application of plants categorised as cholera remedies.

The use of medicinal plants in traditional medicine is supported by scientific data showing that plants possess secondary metabolites that can destroy invading bacteria and other microorganisms (Chassagne et al., 2021). Furthermore, recent studies (Devnath et al., 2022; Kyambadde, 2022; Ssekyanzi, 2022; Setiawati et al., 2022) have documented the antibacterial activities of plants commonly employed for traditional disease treatment. For example, *K. acuminata* extracts showed antibacterial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis*, with minimum inhibitory concentrations (MICs) of 1.25 mg/ml and 2.5 mg/ml, respectively (Mabadahanye et al., 2022). To date, several studies conducted elsewhere have revealed the anti-*V. cholerae* activities of various plant extracts (Acharyya et al., 2009; Taherpour et al., 2014; Karim et al., 2021; Guo et al., 2023; Kenconojati et al., 2023;

Pangi et al., 2023). For example, *Senna alata*, *Syzygium cumini* and *Terminalia chebula* extracts, which are used in most Asian countries, have demonstrated significant bactericidal efficacy against multidrug-resistant strains of *V. cholerae*, with MIC values ranging from 0.125 to 1.5 mg/mL (Acharyya et al., 2009; Sharma et al., 2009; Tatsimo et al., 2017). However, in comparison to data on the antimicrobial activity of other plant species against other human pathogens, original studies focusing on the anti-*V. cholerae* activity of medicinal plants used in folklore cholera treatment in Zimbabwe and the entire southern region are still relatively scarce. Even though the plants and their properties remain obscure, unregulated and, consequently, poorly integrated into formal primary health care, their use is continued based on information passed from generation to generation. In addition, the limited research on anti-*V. cholerae* efficacy could be due to the perception that African traditional medicine is heavily reliant on cultural beliefs (Adeleye et al, 2021).

Despite a lack of experimental evidence to support the use of medicinal plants in folkloric diarrhea and cholera treatment, people, particularly in rural communities, continue to utilize these plants. To address this knowledge gap, the current study aimed to determine the antibacterial efficacy of the bark extracts of *K. acuminata*, *S. birrea*, *Z. mucronata*, and *C. febrifuga*, which are commonly used for the traditional treatment of diarrhoeal diseases, including cholera, in Zimbabwe. These results might lend credence to the application of these plant extracts in traditional medicine. Further research and use of these findings could address the issue of drug resistance by designing and developing more effective and affordable pharmaceuticals.

4.2 Materials and Methods

4.2.1 Collection of Plant Materials

For the purposes of this study, four medicinal plants (*K. acuminata*, *S. birrea*, *Z. mucronata*, and *C. febrifuga*) were selected based on screening criteria from the species reviewed or compiled in Chapter 2 and 3. In this study, four plants were chosen according to the screening criteria established from the 16 species detailed in Chapter 3. The rationale for the targeted selection is grounded in: (1) robust ethnobotanical literature and traditional use pertaining to Zimbabwe; (2) a scarcity of pharmacological references, particularly regarding toxicity and anti-*Vibrio cholerae* activity; (3) practical factors, such as the availability of sufficient plant material for sustainable harvesting and adequate laboratory resources; and (4) the indigenous status of the plants in Zimbabwe, particularly in cholera-endemic regions. This method facilitated a more comprehensive and significant microbiological analysis, ensuring consistency with the overarching study objectives.

Fresh samples of the stem bark of *K. acuminata*, *S. birrea*, *Z. mucronata*, and *C. febrifuga* (voucher no. MZMJ6, MJ9, MJ1, and MJ4, respectively) were collected from the National Botanic Gardens of Zimbabwe as well as the provinces of Manicaland, Mashonaland East, and Masvingo in Zimbabwe. Mr. Christopher Chapano, a certified botanist, authenticated the plant species, which were then deposited at the National Herbarium of Zimbabwe.

4.2.2 Extract Preparation

The bark samples were allowed to air dry in the shade for 72 hours at room temperature. After that, they were ground into a powder using a mortar and pestle. Large particles were removed using a 2 mm screen, and the remaining fine powder was stored at room temperature in airtight containers in the dark. Using the maceration method, approximately 150 g of the powdered samples were extracted for 48 hours in 500 mL of the solvent (absolute ethanol and water). The extracts were filtered using Whatman No. 1 filter paper (Whatman International Ltd., Cardiff, England) and concentrated with a rotary evaporator (RE 100-Pro, Biobase, Jinan, Shandong, China) under reduced pressure at 55 ± 2°C. (Fig 4.1). The crude extract was allowed to dry at room temperature until a consistent weight was achieved.

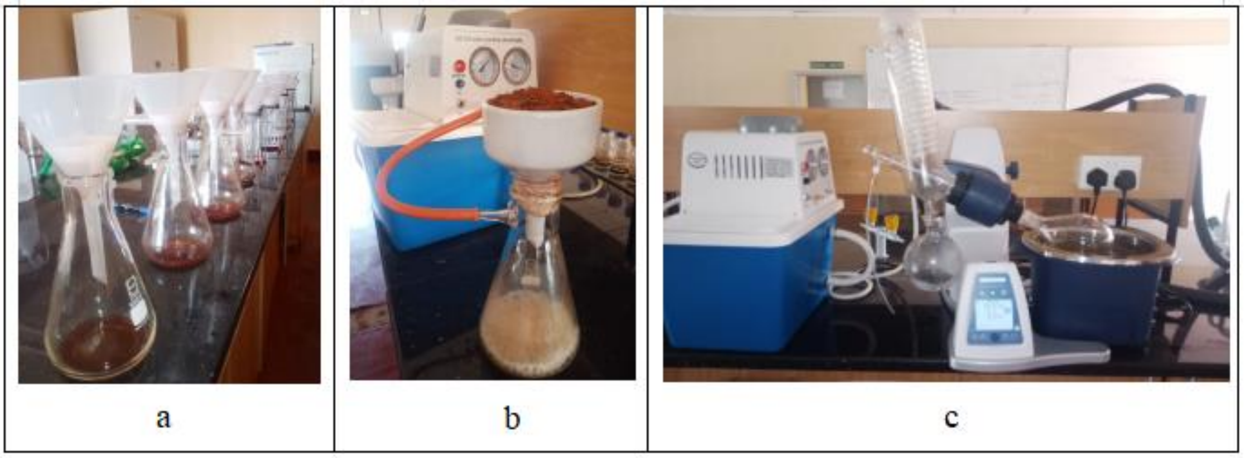

Figure 4.1: Filtration and concentration of plants extracts. (a) conventional fitration set up, (b) filtration aided by a pump, (c) concentration of filtrate using a rotary evaporator

4.2.3 Bacterial strain and preparation of inoculum

A single *V. cholerae* isolate from the 2023 cholera outbreak in Zimbabwe was used for the determination of the antibacterial activity of the selected plant species. However, no strain identification and typing was done on the isolate. The bacteria were preserved on nutrient agar before use. To prepare the bacterial cultures, one loop full of bacterial colonies was placed in a universal bottle with 10 mL of nutrient broth, and the cultures were left to grow overnight at 37 ± 2°C.

4.2.4 Screening for anti-*Vibrio cholerae* activity in selected plant species

The primary screening of the anti-*Vibrio cholerae* activity of the plant extracts (60 mg/mL) was performed via the agar well diffusion method as described previously with a few adjustments (Mehrotra et al., 2010; Balouiri et al., 2016). A standardized inoculum (100 µl) of 0.5 McFarland turbidity was evenly spread over solid Müeller-Hinton agar media using a sterile glass spreader to achieve even growth. The plates were allowed to dry, and holes with diameters of 7 mm were punched aseptically with a sterile cork borer. A volume (100 µL) of the plant extract solution at the desired concentration was introduced into the well, and streptomycin (10 µg/mL) was used as a positive control. The plates were then incubated at 37 ± 2°C for 24 hours. The antibacterial activity of the extracts was determined by measuring the diameter of the inhibition zone in millimeters using a Vernier calliper. The experiment was performed three times, and the mean values were calculated.

4.2.5 Minimum inhibitory concentration (MIC) assay

The same agar well diffusion method was used to determine the MICs of the test extracts against the *V. cholerae* isolates. Different concentrations of the test extracts (e.g., 1.0, 2.5, 6.0, and 10.0 mg/mL) were prepared by the serial dilution method using distilled water. The MIC values were used to classify the plant extracts based on the level of their antibacterial activity. According to Rios and Recio (2005) and Suliman (2010), the antimicrobial activity of plant extracts can be divided into three categories: good or potent (MIC of ≤ 1 mg/mL), moderate (MIC of 1.1 to 6 mg/mL) and poor (> 6 mg/mL) antimicrobial activity. The categories obtained were used to select test plant extracts for use in other bioassays or tests in the study.

4.3 Results

4.3.1 Extract yield of plant extracts

The extraction yield values of ethanol and water extracts of stembark of *K. acuminata*, *S. birrea*, *Z. mucronata*, and *C. febrifuga* are shown in **Table 4.1**. In general, 150 g of material from each plant yielded between 10.09 (4.44%) and 31.20 g (20.80%) of dry extract. This means that 1 kg of plant material would yield between 66.7 and 138.7 g of dry extracts. For all plant species, the extractive values of ethanol were higher than those of water.

Table 4.1 The extraction yields of the selected plant species

Voucher number	Plant species and solvent used	Extract yield (g)	Extract yield (%)
MZMJ6	Ethanol extract of *K.acuminata*	31.20	20.80
	Water extract of *K. acuminata*	15.03	10.02
MJ1	Ethanol extract of *Z. mucronata*	13.81	8.69
	Water extract of *Z. mucronata*	10.20	6.8
MJ9	Ethanol extract of *S. birrea*	16.03	10.68
	Water extract of *S.birrea*	15.04	10.04
MJ18	Ethanol extract of *C.febrifuga*	13.35	9.15
	Water extract of *C. febrifuga*	10.09	4.44

4.3.1 Anti –*Vibrio cholerae* screening of plant extracts at 60 mg/mL

All the plants screened for anti-*V. cholerae* activity were vibriocidal and significant differences ($p< 0.05$) were noted among the plant extracts. Ethanolic extracts of *K. acuminata* (bark) had a significantly higher ($p< 0.05$) zone of inhibition than all other extracts. There were no significant differences ($p> 0.05$) between zones of inhibition of aqueous *S. birrea* (bark) and *K. acuminata* (bark) extracts, and aqueous extracts of *C. febrifuga* (bark) and ethanol extracts of *S. birrea* (bark), respectively. The results are summarised in **Table 4.2**

Table 4.2 Anti –*Vibrio cholerae* screening of plant extracts (Zones of inhibition at 60 mg/mL)

Plant species	**Solvent used**	**Average zone of inhibition (mm)**	**Key remarks**
Ziziphus mucronata	Ethanol	28 ± 2.00^{ab}	Vibriocidal
Ziziphus mucronata	Water	25.6 ± 3.29^{abc}	Vibriocidal
Kirkia acuminata	Ethanol	29.4 ± 2.41^{a}	Vibriocidal
Kirkia acuminata	Water	21 ± 2.24^{d}	Vibriocidal
Sclerocarya birrea	Ethanol	21.6 ± 1.67^{cd}	Vibriocidal
Sclerocarya birrea	Water	20.4 ± 1.52^{d}	Vibriocidal
Crossopteryx febrifuga	Ethanol	24.2 ± 0.84^{bcd}	Vibriocidal
Crossopteryx febrifuga	Water	21.8 ± 1.30^{cd}	Vibriocidal
Streptomycin (control)	-	32.5 ± 1.20^{e}	Positive control
Solvent only (control)	Ethanol	0.0 ± 0.0	Negative control

***Note: Values are shown as mean ± standard deviation (n = 3). Superscript letters (a–e) denote significant differences among means within the same column according to the LSD test at p < 0.05. LSD (Least Significant Difference) = 2.620; CV% (Coefficient of Variation) = 8.5%; p-value < 0.001.** 4.3.2 Minimum inhibitory concentrations (MICs) of the plant extracts

The test extracts showed varying MIC values and anti-*V. cholerae* activity (Table 4.3 and 4.4). (Figure 2). Four extracts, that is, water and ethanol extracts of *Z. mucronata* , as well as ethanol extracts of *K. acuminata* , and *S. birrea* , had MICs ≤ 1 mg/mL and were classified as good or potent. However, both the water and ethanol extracts of *C. febrifuga* had higher MICs (> 6 mg/mL) and were classified as poor *V. cholerae* inhibitors.

4.3.2.1 Zone of inhibition (mm) and the MIC of water extracts

Significant differences (p<0.05) were noted between zones of inhibition due to different concentrations of the same plant extracts. For each plant extract, the zones of inhibition were significantly higher (p<0.05) in the high concentrations than in the lower concentrations. However, water extracts of *C. febrifuga* did not result in significant differences in zones of inhibition at concentrations 10, 5 and 1 mg/mL. Of all the plant extracts, the zones of inhibition due to *Z. mucronata* water extracts were significantly higher (p<0.05) than other plant extracts except for *S. birrea* at a concentration 5 mg/ml. Accordingly, *Z. mucronata* and *C. febrifuga* water extracts were classified as potent and poor, respectively. The results are presented in **Table 4.3.**

Table 4.3 Zone of inhibition in diameter (mm) and the minimum inhibitory concentration (MIC) of water extracts against a single *V. cholerae* isolate from the 2023 Zimbabwe cholera outbreak.

Plant extract	Zones of inhibition (diameter: mm)				MIC (mg/mL)	Key remarks on activity
	Concentration(mg/mL)					
	20	10	5	1		
Ziziphus mucronata	23.3±1.16^{b}*	20.0±1.0^{d}**	12.3±0.58^{b}***	8.3±0.58^{b}****	≤ 1	Good or potent
Kirkia acuminata	21.3±2.08^{ab}*	11.0±1.0^{b}**	8.7±0.58^{a}***	7.0±0.0^{a}****	1.1 upto 6	Moderate
Sclerocarya birrea	18.7±1.16^{a}*	14.7±1.53^{c}**	11.0±1.0^{b}***	7.0±0.0^{a}****	1.1 up to 6	Moderate
Crossopteryx febrifuga	20.0±1.00^{ab}*	7.3±0.58^{a}**	7.0±0.0^{a}**	7.0±0.0^{a}**	> 6	Poor
Streptomycin (control)	30.5±1.2^{c}	28.0±1.0^{d}	22.3±0.6^{c}	15.0±0.5^{c}	≤ 1	Positive control
Water only (control)	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	-	Negative control

Note: Each value is presented as the mean ± standard deviation (n = 3). Superscript letters denote significant differences among extracts at identical concentrations (LSD, $p < 0.05$). The MIC denotes the minimum concentration at which inhibition occurs. Streptomycin and water, are included as controls for comparative purposes. The diameter of the wells was 7.0mm and therefore the presence of this value indicates no zone.

4.3.2.2 Zone of inhibition (mm) and the MIC of the ethanol extracts

There were significant differences ($p<0.05$) between zones of inhibition due to ethanolic extracts of the same plant species at different concentrations. However, for the first three plant extracts (*Z. mucronata, K. acuminata, S. birrea*), there were no significant differences ($p>0.05$) between zones of inhibition at concentrations of 20 and 10 mg/ml. The ethanol extracts of *Z. mucronata* resulted in significantly higher zones of inhibition (at concentrations: 20, 10 and 1 mg/mL) than other plant extracts at the same concentrations. Notably, *Z. mucronata*, *K. acuminata* and *S. birrea* showed activity at a concentration of 1 mg/mL and were classified as potent extracts against *V. cholerae*. The results are summarised in **Table 4.4.**

Table 4.4 Zone of inhibition in diameter (mm) and the minimum inhibitory concentration (MIC) of ethanol extracts against a single *V. cholerae* isolate from the 2023 Zimbabwe cholera outbreak.

Plant extract	Zones of inhibition (diameter: mm)				MIC (mg/mL)	Key remarks
	Concentration(mg/mL)					
	20	10	5	1		
Ziziphus mucronata	24.7±1.15a*	22.7±1.53a*	13.3±0.58b**	9.7±0.58a***	≤ 1	Good or potent
Kirkia acuminata	22.3±1.53ab*	20.3±0.58b*	16.3±0.58a**	11.7±0.58b***	≤ 1	Good or potent
Sclerocarya birrea	19±1.0b*	18.3±0.58b*	16±1.0a**	10.3±0.58b***	≤ 1	Good or potent
Crossopteryx febrifuga	20.7±2.31b*	15.7±0.58c**	7±0.0c***	7±0.0c***	> 6	Poor inhibitor
Streptomycin (control)	30.0 ± 1.0^{c}	27.0 ± 0.9^{c}	21.0 ± 0.7^{b}	14.0 ± 0.5^{a}	≤ 1	Positive control
Ethanol only (control)	7.0±0.0	7.0±0.0	7.0±0.0	7.0±0.0	-	Negative control

Values are presented as mean ± SD (n = 3). Superscript letters denote significant differences among plant extracts at each concentration (LSD, $p < 0.05$). Streptomycin and ethanol saves as the positive and negative control, respectively. The MIC is defined as the lowest concentration at which inhibition is observed. A 7.0 mm zone indicates no inhibition, corresponding to the well diameter..

4.4 Discussion

In traditional medicine, plants have been used as a means of treatment. This practice supports the knowledge that plants possess secondary metabolites that can eliminate invasive bacteria and other microbes (Chassagne et al., 2021). The availability of such metabolites may make treating bacterial illnesses affordable and effective. Cholera is endemic in Zimbabwe, and outbreaks occur often and annually (Gwenzi and Sanganyado, 2019; The Zimbabwe Daily, 2020). Regrettably, the emergence of drug-resistant strains, weak economic performance, and high rates of inflation in Zimbabwe make it difficult to manage cholera effectively (Mashe et al., 2020). Therefore, plants are frequently used as folklore treatment. Thus, the current study investigated the antibacterial activity of stem bark extracts from *Z. mucronata*, *K. acuminata*, *S. birrea*, and *C. febrifuga*. The findings showed that the extracts had varied antibacterial activities. Notably, the inhibition was concentration-dependent, as reported in a similar study by Karim et al. (2021). More importantly, the inclusion of controls was necessary to validate the observed anti-*Vibrio cholerae* activity of the extracts in our study. Streptomycin (2 mg/mL), used as the positive control, consistently generated inhibitory zones across all tests, confirming the reliability of the testing procedures and the sensitivity of *V. cholerae*. Similarly, ethanol and water were employed as negative controls and were expected to produce no inhibition (7.0 mm, the diameter of the well), thereby confirming that the observed antibacterial activity originated from plant-derived compounds rather than the solvents. These controls lent credibility to the results by eliminating the possibility of solvent interference or procedural artifacts and allowed for a meaningful comparison of plant extract efficacy against a recognised antibacterial benchmark.

Among the selected plant species, *Z. mucronata* showed the greatest antibacterial efficacy against *V. cholerae*, as evidenced by a low MIC (≤ 1 mg/mL) for the ethanol and water extracts and zones of inhibition measuring 9.7 and 8.3 mm at a concentration of 1 mg/mL, respectively. Such MIC values (below 1 mg/mL) are classified as "active" or potent (Kokoska et al., 2019; Suliman, 2010; Rios and Recio, 2005). More importantly, the finding that only *Z. mucronata* showed such a low MIC in water extracts—the common method of herb preparation utilized in folkloric treatment—supports the possibility that *Z. mucronata* extracts could function as anti-*V. cholerae* treatments. Many investigations corroborated these results by demonstrating that *Z. mucronata* extracts are bactericidal against different bacterial (Khumalo et al., 2021; Mongalo et al., 2020; Coopoosamy et al., 2011). For example, *Z. mucronata* extracts showed similar MICs (≤1 mg/mL) against a wide range of bacterial species (Olajuyigbe and Afolayan, 2011; Nemudzivhadi and Masoko, 2015; Mongalo et al., 2020). Similarly, different zones of inhibition (between 8 and 11 mm) were detected for other *Ziziphus* species against different diarrhoeal pathogens, such as *E. coli* (Bukar et al., 2015). Furthermore, acetone and ethyl acetate samples demonstrated increased suppression of mostly gram-positive bacteria in another investigation (Coopoosamy et al., 2011).

Kirkia acuminata and *S. birrea* also showed MICs of ≤ 1 mg/mL but only in ethanol extracts. With a higher MIC of 1.1–6 mg/mL, the water extracts from both plant species exhibited moderate efficacy against *V. cholerae*. These results may point to the necessity of raising dosages during folklore prescriptions. However, other studies, which were conducted elsewhere but involving different bacteria, also support the antibacterial activity of *K. acuminata*. According to Maroyi (2017b), the extracts of *K. acuminata* demonstrated antibacterial activity against a range of bacteria. Similarly, a study conducted by Chigayo et al. (2014) revealed that aqueous extracts of *Kirkia* spp. had notable antibacterial activity against a diverse array of bacteria, including *V. cholerae*, with an average MIC of 3.445 mg/mL. Furthermore, another study reported that at the lowest concentrations of 2.5 mg/mL and 1.25 mg/mL, respectively, *K. acuminata* inhibited *Mycobacterium smegmatis* and *Staphylococcus aureus* (Mabadahanye et al., 2022). Many studies have demonstrated the antibacterial action of the bark of the marula plant, which is widely used in traditional medicine (Russo et al., 2018; Eloff, 2001). For instance, Manzo et al. (2017) showed that ethanolic extracts of *S. birrea* bark exhibited antibacterial activity against *Salmonella typhi* and *E. coli*, with zones of inhibition measuring 17 mm and 20 mm, respectively, at 200 mg/mL. Furthermore, a related study by Tanih and Ndip (2012) revealed the antibacterial activity against a variety of bacterial species, with MICs ranging from 0.156 to 1.25 mg/mL.

Notably, among the four plant species, only *C. febrifuga* had the highest MIC (> 6 mg/mL) in both the water and ethanol extracts, suggesting that this plant species is a poor inhibitor of *V. cholerae*. Nonetheless, the results indicate that the extracts, albeit at greater dosages, may be employed in the traditional treatment of cholera. Furthermore, although the results of this study indicate that only greater doses of the extracts are effective than lower doses, other studies conducted elsewhere support this antibacterial activity. For instance, the growth of gram-positive and gram-negative bacteria was inhibited when they were exposed to 50 µg/mL, 100 µg/mL, or 200 µg/mL of the methanolic root-back extract of *C. febrifuga* (Halilu et al., 2012; Chouna et al., 2015). The zones of inhibition ranged from 7 to 23 mm. The variations in effective concentrations may arise from several factors, including: the solvents utilised for extraction, the specific part of the plant employed, the geographical origin of the plant, and the season of harvesting.

Although the results are encouraging, this chapter has a significant limitation in that it only uses one *V. cholerae* isolate from the 2023 Zimbabwe cholera outbreak, which has not even been identified or typed. Siriphap et al. (2017) documented that *V. cholerae* isolates from various regions or time periods may exhibit genetic differences and varying susceptibility to antimicrobials. Therefore, these results should not be generalised to represent the antimicrobial susceptibility of all *V. cholerae* strains. Future studies should ensure the identification and typing of *V. cholerae* to establish accurate conclusions regarding the antimicrobial susceptibility of plant extracts.

4.5 Conclusion

In conclusion, our study confirmed that both ethanol and water extracts of *K. acuminata*, *S. birrea*, and *Z. mucronata* bark have antibacterial activities against a single *V. cholera* isolate from the 2023 Zimbabwe cholera outbreak. The MICs of *K. acuminata*, *S. birrea*, and *Z. mucronata*, which were ≤ 1 mg/mL, showed that the plant species are good or potent against *V. cholerae*, and these findings may lend credence to the use of the plant species as reliable sources of herbal remedies against cholera disease in Zimbabwe and other southern African countries. Despite *C. febrifuga* displaying anti- *Vibrio cholerae* activity, its MIC value (> 6 mg/mL) suggests a weak or limited antibacterial activity under the tested conditions. Therefore, it may not be a reliable choice for a standalone antibacterial therapy against *V. cholerae*. However, further investigations may be required to assess its potential synergistic activity or efficacy against other pathogens.

Even though these plant species showed positive anti-*V. cholerae* activities, additional *V. cholerae* investigations of the vibriocidal activities of all the plant extracts against different strains or isolates of *V. cholerae* are still needed.

References

Acharyya, S., Patra, A., & Bag, P. K. (2009). Evaluation of the antimicrobial activity of some medicinal plants against enteric bacteria with particular reference to multidrug resistant *Vibrio cholerae.* Tropical Journal of Pharmaceutical Research, 8(3).

Adeleye, O. A., Femi-Oyewo, M. N., Bamiro, O. A., Bakre, L. G., Alabi, A., Ashidi, J. S., Balogun-Agbaje, O. A., Hassan, O. M., & Fakoya, G. (2021). Ethnomedicinal herbs in African traditional medicine with potential activity for the prevention, treatment, and management of coronavirus disease 2019. Future Journal of Pharmaceutical Sciences, 7(1), 72. https://doi.org/10.1186/s43094-021-00223-5

Ali, M., Nelson, A. R., Lopez, A. L., & Sack, D. A. (2015). Updated global burden of cholera in endemic countries. *PLoS neglected tropical diseases*, *9*(6), e0003832.

Ali, M. A. E. M. (2022). Evaluation of Phytochemical and Antibacterial Activity of *Sclerocarya birrea* Stem Bark Extraction. Journal of Current Medical Research and Opinion, 5(03).

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 6(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Bukar, A. M., Kyari, M. Z., Gwaski, P. A., Gudusu, M., Kuburi, F. S., & Abadam, Y. I. (2015). Evaluation of phytochemical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State â€“Nigeria. Journal of Pharmacognosy and Phytochemistry, 3(5), 98-101.

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J. T., Dettweiler, M., Marquez, L., Salam, A. M., Shabih, S., Farrokhi, D. R., & Quave, C. L. (2021). A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Frontiers in Pharmacology, 11, 586548. https://doi.org/10.3389/fphar.2020.586548

Chigayo, K., Mojapelo, P. E. L., Bessong, P., & Gumbo, J. R. (2014). The preliminary assessment of antimicrobial activity of HPLC separated components of *Kirkia wilmsii*. African Journal of Traditional, Complementary and Alternative Medicines, 11(3), 275-281.

Chingwaru, C., Bagar, T., & Chingwaru, W. (2020). Aqueous extracts of *Flacourtia indica*, *Swartzia madagascariensis* and *Ximenia caffra* are strong antibacterial agents against

Shigella spp., *Salmonella typhi* and *Escherichia coli* O157. South African Journal of Botany, 128, 119-127.

Chinsembu, K. C., Syakalima, M., & Semenya, S. S. (2019). Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Lusaka, Zambia. South African Journal of Botany, 122, 369-384.

Choudhury, S., Sharan, L. and Sinha, M.P. (2014). Screening of Some Commonly Used Medicinal Plants against Enteric Human Pathogen *Vibrio holerae*. Methodology, 2014.

Chouna, J. R., Nkeng-Efouet-Alango, P., Lenta, B. N., & Sewald, N. (2015). Antimicrobial triterpenes from the stem bark of *Crossopteryx febrifuga*. Zeitschrift für Naturforschung C, 70(7-8), 169-173.

Coopoosamy, R. M., Naidoo, K. K., & Ndlazi, N. J. (2011). Cultural importance and antibacterial activity of *Ziziphus mucronata* (Willd.) in the Umlazi community in Durban. African Journal of Pharmacy and Pharmacology, 5(17), 1979-1982.

Eloff, J. N. (2001). The antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology, 76(3), 305-308.

Guo, L., Zheng, H., Lu, J., Li, S., Anchen, W., Huang, M., ... & Zhuang, L. (2023). Extraction, identification, mechanism of action, and application of Hu Zhang (*Polygonum cuspidatum*) extracts against non-O1/O139 *Vibrio cholerae* in prawn storage. Food Science and Technology, 185, 115135.

Halilu, M. E., Abubakar, A., & Garba, M. K. (2012). Antimicrobial and preliminary phytochemical studies of methanol extract of root bark of *Crossopteryx febrifuga* (Rubiaceae). Journal of Applied Pharmaceutical Science, 2(12), 066-070.

Karim, R., Begum, M. M., Jui, Y., Islam, T., Billah, M., Arafat, Y., ... & Rahman, M. S. (2021). In vitro cytotoxic and anti-*Vibrio cholerae* activities of alcoholic extracts of *Desmodium triflorum* (L.) whole plant and *Terminalia citrina* (Roxb.) fruits. Clinical Phytoscience, 7, 1-10.

Kenconojati, H., Ulkhaq, M. F., Azhar, M. H., & Rukmana, N. R. (2023). Vibriocidal Activity of Ethanol Extract of Moringa Leaves and Its Effect on the Growth of Pacific White Shrimp. Jurnal Medik Veterinar, 6(1).

Khumalo, G. P., Sadgrove, N. J., Van Vuuren, S. F., & Van Wyk, B. E. (2021). South Africa's Best BARK Medicines Prescribed at the Johannesburg Muthi Markets for Skin, Gut, and Lung Infections: MIC's and Brine Shrimp Lethality. Antibiotics, 10(6), 681.

Kokoska L, Kloucek P, Leuner O, Novy P. Plant-derived products as antibacterial and antifungal agents in human health care. Current Medicinal Chemistry. 2019 Sep 1;26(29):5501-41.

Mabadahanye, K., Bhembe, N. L., & Green, E. (2022). Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. African Health Sciences, 22(2), 717-727.

Mabadahanye, K., Bhembe, N. L., & Green, E. (2022). Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. African Health Sciences, 22(2), 717-727.

Mai, A. J., Emmanuel, M., Ayim, P., & Magaji, M. B. (2019). Evaluation of Antibacterial Activities and Cytotoxicity of *Sclerocarya birrea* Stem Bark. Open Access Library Journal, 6(9), 1-6.

Manzo, L. M., Bako, H. D., & Idrissa, M. (2017). Phytochemical screening and antibacterial activity of stem bark, leaf and root extracts of *Sclerocarya birrea* (A. Rich.) Hochst. International Journal of Enteric Pathogens, *5*(4), 127-31.

Maroyi, A. (2013). Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of ethnobiology and ethnomedicine*, *9*, 1-18.

Maroyi, A. (2017a). *Elephantorrhiza elephantina*: Traditional uses, phytochemistry, and pharmacology of an important medicinal plant species in southern Africa. Evidence-Based Complementary and Alternative Medicine, 2017.

Maroyi, A. (2017b). *Kirkia acuminata* Oliv.: A review of its ethnobotany and Pharmacology. African Journal of Traditional, Complementary and Alternative Medicines, 14(2), 217-226.

Marumure, J., & Nyila, M. A. (2024). Cholera Outbreaks in Zimbabwe: An In-Depth Analysis of Drivers, Constraints and Reimagining the Use of Medicinal Plants. *Journal of Tropical Medicine*, *2024*(1), 1981991.

Mathabe, M. C., Nikolova, R. V., Lall, N., & Nyazema, N. Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhea in Limpopo Province, South Africa. Journal of Ethnopharmacology, 105(1-2), 286-293.

Mehrotra, S., Srivastava, A. K., & Nandi, S. P. (2010). Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Journal of Medicinal Plants Research, 4(22), 2393-2398.

Mintah, S. O., Asafo-Agyei, T., Archer, M. A., Junior, P. A. A., Boamah, D., Kumadoh, D., ... & Agyare, C. (2019). Medicinal plants for treatment of prevalent diseases. Pharmacognosy-Medicinal Plants, 1-19.

Nemudzivhadi, V., & Masoko, P. (2015). Antioxidant and antibacterial properties of *Ziziphus mucronata* and *Ricinus communis* leaves extracts. African Journal of Traditional, Complementary and Alternative Medicines, 12(1), 81-89.

Njume, C., & Goduka, N. I. (2012). Treatment of diarrhoea in rural African communities: an overview of measures to maximise the medicinal potentials of indigenous plants. *International journal of environmental research and public health*, *9*(11), 3911–3933. https://doi.org/10.3390/ijerph9113911.

Olajuyigbe, O. O., & Afolayan, A. J. (2011). In vitro antibacterial activities of the methanol extract of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd. Journal of Medicinal Plants Research, 5(37913795), 18.

Pangi, V. N., Marukurti, A., Reddy, A. M., Anupoju, R., Kasi, P. B., Medapalli, S. R., ... & Farhana, S. (2023). Anti-vibriocidal activity and gas chromatography–mass spectrometry (GC–MS)-based chemical composition of *Mirabilis jalapa* leaf methanolic extract. Journal of Pharmacognosy and Phytochemistry, 12(1), 653-662.

Payne, A., Mukhopadhyay, A. K., Deka, S., Saikia, L., & Nandi, S. P. (2015). Anti-vibrio and antioxidant properties of two weeds: *Euphorbia serpens* and *Amaranthus viridis*. Research Journal of Medicinal Plant, 9(4), 170-178.

Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology, 100(1-2), 80-84.

Russo, D., Miglionico, R., Carmosino, M., Bisaccia, F., Andrade, P. B., Valentão, P., ... & Armentano, M. F. (2018). A comparative study on the phytochemical profiles and biological activities of *Sclerocarya birrea* (A. Rich.) Hochst leaf and bark extracts. International Journal of Molecular Sciences, 19(1), 186.

Sánchez, E., García, S., & Heredia, N. (2010). Extracts of edible and medicinal plants damage membranes of *Vibrio cholerae*. Applied and Environmental Microbiology, 76(20), 6888-6894.

Sharma, A., Patel, V. K., & Chaturvedi, A. N. (2009). Vibriocidal activity of certain medicinal plants used in Indian folklore medicine by tribals of Mahakoshal region of central India. Indian Journal of Pharmacology, 41(3), 129–133. https://doi.org/10.4103/0253-7613.55212

Siriphap, A., Leekitcharoenphon, P., Kaas, R. S., Theethakaew, C., Aarestrup, F. M., Sutheinkul, O., & Hendriksen, R. S. (2017). Characterization and Genetic Variation of Vibrio cholerae Isolated from Clinical and Environmental Sources in Thailand. *PloS one*, *12*(1), e0169324. https://doi.org/10.1371/journal.pone.0169324

Suliman, A. (2010). The antimicrobial activity and chemical profile of traditional medicinal plants indigenous to Southern Africa used to treat respiratory tract infections. Doctoral dissertation, University of the Witwatersrand.

Taherpour, A., Hashemi, A., Fallah, F., Erfani-Manesh, S., & Taki, E. (2014). Evaluation of Zenian and Avishan-e Shirazi antibacterial activity against *Vibrio cholerae* strains. Journal of Medical Bacteriology, 3(1-2), 17-21.

Tanih, N. F., & Ndip, R. N. (2012). The antioxidant and antimicrobial properties of acetone and aqueous extracts of the mature stem bark of *Sclerocarya birrea* were evaluated. Evidence-Based Complementary and Alternative Medicine, 2012.

World Health Organization. (2024, December 5). *Cholera*. https://www.who.int/news-room/fact-sheets/detail/cholera

Wu, Z., Raven, P.H. and Hong, D. (2003). Flora of China. Volume 5: Ulmaceae through Basellaceae. Science Press.

CHAPTER 5

Cytotoxic assessment of selected antidiarrhoeal and anti-*Vibrio cholerae* plant species in Zimbabwe

Abstract

For a very long time, many African communities have used medicinal plants as their major source of primary healthcare. However, less attention has been paid to the potential cytotoxic effects of such plants. This study aimed to investigate the cytotoxic potential of aqueous extracts of *Ziziphus mucronata* Willd. and *Kirkia acuminata* Oliv., which are extensively used in Zimbabwean folk medicine to treat cholera and diarrhoea. The Brine Shrimp Lethality Assay (BSLA) was used to examine the cytotoxicity of the extracts. Ten nauplii were introduced to three replicates of each concentration of the plant extract. After 24 hours, the surviving brine shrimp larvae were counted and the LC_{50} values were determined. The results showed that the aqueous extracts of *Z. mucronata*, and *K. acuminata* were non-toxic, with Lethal Concentration 50 (LC_{50}) values of 1023.58 µg/mL and 1583.34 µg/mL, respectively. Thus, the findings support the use of plant extracts in traditional medicine as diarrhoea and cholera treatments. In addition, this may also open the door to their possible usage in modern medicine. However, more research, such as determining any toxicological effects that can develop because of chronic or sub-chronic usage of the plant species, as well as employing other toxicological tests, is required.

5.1 Introduction

Since time immemorial, medicinal plants have been used to treat human and animal diseases (Singh, 2021; Moges and Moges, 2019; Gurib-Fakim and Mahomoodally, 2013). Today, many communities, particularly in developing countries, are increasing their use of herbal medicines for primary health care due to the following factors: (1) lower cost and availability, (2) the assumption that they are natural and thus pose no health risk (Khan and Ahmad, 2019), and (3) effectiveness as therapeutic agents (Semenya and Maroyi, 2012), and (4) their use is based on culture (Ozioma and Chinwe, 2019).

However, the assumption that medicinal plants are natural and thus pose no health risk (Khan and Ahmad, 2019), is not always true as some are cytotoxic (Chan, 2003; Fennell et al., 2004;

Kuete, 2014; Ghorani-Azam et al., 2018). Unfortunately, the potential cytotoxic effects of such plants have received little attention in traditional medicine around the world. However, with most people (80%) relying on medicinal plants for primary health care, scientists are increasingly conducting tests to assess the toxicity of traditional medicine plants (Mongalo et al., 2018). It is documented that the toxicological status of around 41% of medicinal plants used in traditional Zimbabwean medicine is unclear. As a result, such plants must go through toxicological testing (Nyagumbo et al., 2022).

The most common method used to assess the toxicity activities of African medicinal plants is the Brine Shrimp Lethality Assay (BSLA) (Nyagumbo et al., 2022). The Brine Shrimp Lethality Assay is highly preferred due to its low cost and simplicity of usage (Karim et al., 2021; Waghulde et al., 2019; Hamidi et al., 2014; Olowa and Nuñeza, 2013). Furthermore, just small quantities of test material (2–20 mg or less) are needed (Kamanja et al., 2018; Apu et al., 2013). Research has shown that BSLA correlates very well with biological and other cytotoxic aspects of plant extracts (Hamidi et al., 2014). Additionally, the assay is particularly helpful in providing a preliminary screen that can be backed by a more focused bioassay (Olowa and Nuñeza, 2013). However, in rare cases, researchers have found that the BSLA gave inconsistent cytotoxicity results on the same plant species. Naidu et al. (2014) claimed that surviving nauplii could be counted multiple times in different quadrants of the petri dish, resulting in the method's infrequent mistakes. More importantly, this can be rectified by repeating the test several times.

In BSLA, cytotoxic activity is determined by calculating the lethal concentration (LC_{50}) of the test extract. The LC_{50} of a substance is the concentration that kills 50% of the organisms in a toxicity test (Boyd, 2005). Higher LC50 values indicate that a considerable amount of the extract is required to elicit a toxic response, whereas low LC_{50} values are highly toxic and potentially harmful to human health (Okeleye et al., 2013).

In this study, aqueous extracts of *Z. mucronata*, and *K. acuminata* were examined for their cytotoxic effects against brine shrimp nauplii. These plant species, along with the bulk of other medicinal plants used in traditional Zimbabwean medicine, are utilized without first finding out their toxicological properties (McGaw et al., 2014; Kuete, 2014). The findings would be compared to their recognized folkloric medicine and validate their use. Thus, the preliminary data obtained may be used to develop novel medicinal drugs

5.2 Materials and methods

5.2.1 Plant materials

The stembarks of *Z. mucronata*, and *K. acuminata* (Table 5.1) were collected from the parent plants from the main campus of Great Zimbabwe University, Zimbabwe (20.1037° S, 30.8608° E). Qualified botanists and curators of the National Herbarium of Zimbabwe verified the authenticity of the plant species. Sample specimens were placed in the above-named herbarium with voucher numbers, MJ1 and MZMJ6, respectively. In this chapter, only these two species were chosen for cytotoxicity assessment. The reason behind this targeted selection was based on three key factors, (1) As shown in Chapter 4, both species demonstrated significant anti-*Vibrio cholerae* activity, with low MIC values (≤ 1 mg/mL) in all the tested extracts, thus suggesting strong therapeutic potential, (2) An ethnobotanical review in Chapter 3 indicated that, among the reviewed plant species, only *K. acuminata* and *Z. mucronata* are mostly used in traditional medicine for cholera treatment, and (3) focussing on these two species facilitated a more precise and resource-efficient cytotoxicity assessment, thus aligning with both scientific importance and practical limitations.

Table 5.1 Features of medicinal plants used in this study

Botanical Family	Species name	Voucher Number	Local names	Life Form	References
Kirkiaceae	*Kirkia acuminata* Oliv.	MZMJ6	mubvumira, mutsakatidze, mutuhwa, mutuva (Shona): Umvumile, umvumila,(Ndebele): musanta (Tonga)	Tree	Maroyi, 2016
Rhamnaceae	*Ziziphus mucronata* Willd.	MJ1	muchecheni, chinanga (Shona): umpasamala, umphafa (Ndebele)	Tree	Hyde, 2024

5.2.2 Stem-bark extraction

The bark samples were allowed to air dry in the shade for 72 hours at room temperature. After that, they were ground into a powder using a mortar and pestle. Large particles were removed using a 2 mm screen, and the remaining fine powder was stored at room temperature in airtight containers in the dark. Using the maceration method, about 150 g of the powdered samples were extracted over 48 hours in 500 mL of water. The extracts were filtered using Whatman no. 1 filter paper (Whatman International Ltd., Cardiff, England) and concentrated with a rotary evaporator (RE 100-Pro, Biobase, Jinan, Shandong, China) under reduced pressure at 40°C. The crude extract was allowed to dry at room temperature until a consistent weight was achieved.

5.2.3 Hatching of Brine shrimp nauplii

The eggs of brine shrimp (*Artemia salina* cysts) were hatched as described by Khumalo et al. (2021), with few modifications. Artificial saltwater was created by dissolving a mass of 16 g of sea salt in 500 mL of distilled water. After that, approximately 1 g of dried brine shrimp eggs (McMerwe, South Africa) were introduced into the artificial seawater. With a continuous light source present, a rotating pump was positioned at the bottom of the container to create aeration. This was done to mimic the natural seawater environment to ensure suitable conditions for the eggs to hatch.

5.2.4 The Brine Shrimp Lethality Assay

The BSLA was done according to the previous studies, but with some modifications (Karim et al., 2021; Waghulde et al., 2019). Ten milliliters of artificial seawater were mixed with 0.1 g of plant extract to achieve a final stock concentration of 10,000 µg/mL. Through serial dilutions, test extracts with different concentrations (1000 µg/mL, 100 µg/mL, 10 µg/mL, and 1 µg/mL) were produced.

For each dilution, 0.5 mL of test extract was mixed with 4.5 mL of seawater containing 10 nauplii in separate vials. For every concentration of the test extract, three replications (T1, T2, and T3) were employed. Distilled water was used as a control, and Vincristine sulphate as a standard. After 24 hours the number of surviving nauplii were counted and the percentage of mortality was determined.

% mortality = (No. of dead nauplii/initial no. of live nauplli) × 100

The toxicity of the plant extracts was determined based on lethal concentration for 50% (LC_{50}) of the nauplii population (Waghulde et al., 2019), and Clarkson's toxicity criterion which classifies extracts in the following order: extracts with LC_{50} above 1000 µg/mL are non-toxic, LC_{50} of 500 - 1000 µg/mL are low toxic, extracts with LC_{50} of 100 - 500 µg/mL are medium toxic, while extracts with LC_{50} of 0 - 100 µg/mL are highly toxic (Clarkson et al., 2004).

5.3 Results

The lethality concentrations (LC_{50}) of *Z. mucronata* and *K. acuminata* were found to be 1023.58 µg/mL and 1583.34 µg/mL, respectively (Table 5.2; Figs. 5.1&5.2). No mortality of brine shrimp larvae was recorded in the negative control group (water), but the positive control (Vincristine sulphate) recorded 100% mortality. This shows that the test was valid, and the results were solely dependent on the effects of the test extracts.

Table 5.2 % Mortality of shrimp nauplii after 24 hours

Plant Extracts	Concentration (µg/mL)	Number of dead Nauplii (after 24 h)			Total Number of dead Nauplii	Total Number of Nauplii Survivors	% Mortality	LC_{50} (µg/mL)
		T1	T2	T3				
Ziziphus mucronata Willd.	1	0	1	0	1	29	3.33	1023.58
	10	1	2	1	4	26	13.33	
	100	3	3	2	8	22	26.67	
	1000	3	4	4	11	19	36.67	
Kirkia acuminata Oliv.	1	0	0	1	1	29	3.33	1583.34
	10	1	1	0	2	28	6.67	
	100	1	3	1	5	25	16.67	
	1000	6	4	4	14	16	53.33	
Water (negative control)	-	0	0	0	0	30	0.00	-
Vincristine sulphate (positive control)	-	10	10	10	10	1	100	-

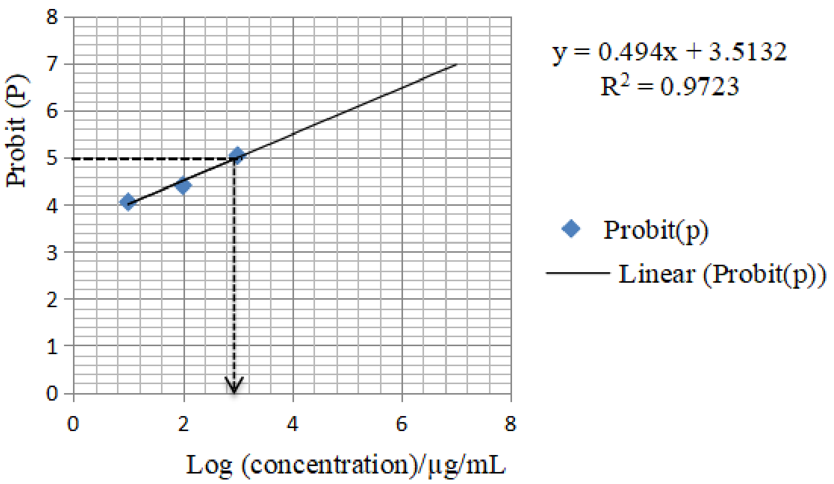

Figure 5.1: Determination of LC_{50} of *Ziziphus mucronata* Willd. bark extract

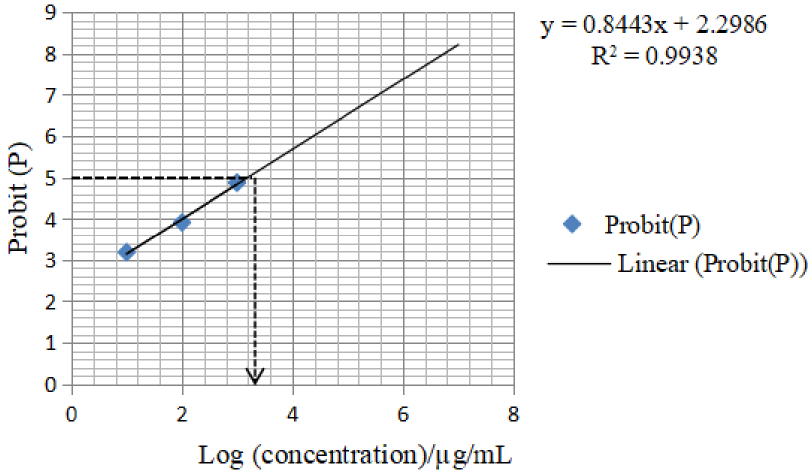

Figure 5.2: Determination of LC_{50} of *Kirkia acuminata* Oliv. bark extract

5.4 Discussion

The brine shrimp cytotoxicity assay is an appropriate method for the preliminary assessment of substance toxicity (Zimudzi, 2014). Using Clarkson et al. (2004)'s benchmark, it can be concluded that the aqueous extracts of *Z. mucronata* (1023.58µg/mL) and *K. acuminata* (1583.34µg/mL) are non-cytotoxic and are considered safe, as their respective LC_{50} values exceeded 1000 µg/mL. These results obtained on aqueous extraction are of great importance because these extracts mimic the traditional methods used in herbal remedies preparation, which typically include decoctions or infusions of plant materials (Khumalo et al., 2023).

The findings are particularly significant for *K. acuminata* because they are the first toxicological assessment of its extracts. According to review research conducted by Nyagumbo et al. (2022), no toxicological studies conducted on *K. acuminata* were documented in literature. However, Chigayo (2021) revealed that other *Kirkia* species are non-toxic. Similarly, tests conducted on other *Kirkia* species, *K. wilmsii,* demonstrated negligible toxicity at 0.80 and 1.25 mg/mL, respectively (Suleiman et al., 2010). However, despite the species closely related to *K. acuminata* based on our findings this does not apply truly to *K.acuminata*.

The lack of cytotoxic activity observed in the aqueous extract of *Z. mucronata* stembark confirms the safety of this plant species. Several studies, including reviews, have confirmed the non-cytotoxic properties of *Z. mucronata* extracts using the brine shrimp lethality assay and other toxicological techniques (Khumalo et al., 2021; Ilonga et al., 2018; Adewusia et al., 2013; Ilonga, 2012). As an example, *Z. mucronata* was categorised as non-toxic (with an LC_{50} value of > 1000 µg/mL) in a recent study conducted by Nyagumbo et al. (2022) on medicinal plants in Zimbabwe. Similarly, the use of BSLA resulted in the observation of the same level of activity for ethanol, methanol, and aqueous leaf extracts, with an LC_{50} value greater than 1000 µg/mL. The result has also been confirmed by the studies conducted by Ilonga et al. (2018), McGaw et al. (2014), Ilonga (2012), and Tafadzwa (2005). Furthermore, Adewusia et al. (2013) and Mongalo et al. (2018) additionally established the lack of toxic effects of *Z. mucronata* using MTT toxicity assays, with LC50 values greater than 100 µg/mL against SH-SY5Y cells and 220 µg/mL in Vero cells, respectively. Nevertheless, there are also few reports documenting certain *Z. mucronata* extracts exhibiting moderate toxicity, particularly with dichloromethane and hexane solvents (Shawa et al., 2015). For instance, Ilonga et al. (2018) reported LC_{50} values lower than 250 µg/mL for hexane leaf extracts. The observed variations in toxicity values could be attributed to the varied polarity of the various solvents which can lead to extraction of an array of phytochemicals. Consequently, these diverse phytochemicals may elicit different responses in the brine shrimp assay (Khumalo et al., 2021). Considering the findings with solvents with higher extraction capabilities, it is recommended to use medicines with lower doses of extracts in traditional medicine.

5.5 Conclusion

The stem-bark water extracts of *Z. mucronata* and *K. acuminata* showed no toxic activity in the Brine Shrimp Lethality Assay. The traditional methods used for preparing the herbal medication are mirrored in the water extracts. The safety and traditional usage of the plant species by Zimbabwean communities to treat diarrhoea and cholera is confirmed. However, patients and herbalists should utilise lower dosages. Nonetheless, the extracts did show more killing effects of brine shrimp larvae at higher concentrations. Future research should focus on the following to fully comprehend the cytotoxic effect of plant extracts:

- Bridging the gap between the elevated concentrations that demonstrate certain cytotoxic effects in controlled settings and the actual practices within communities, particularly given that these communities lack the means to determine the concentrations of the extracts.
- Evaluation of the relationship between the preparation of extracts (including steeping duration, temperature, and the ratios of water to plant material) to attain safer concentrations of extracts.

- Investigation of the potential toxicological effects that could result from both long-term use of the plant species.

- A comparative analysis supported by additional toxicological techniques.

The implementation of these recommendations will significantly contribute to confirming the safety and therapeutic potential of *K. acuminata* and *Z. mucronata*.

5.6 References

Adewusia, E. A., Foucheb, G., & Steenkamp, V. (2013). Effect of four medicinal plants on amyloid-β induced neurotoxicity in SHSY5Y cells. African Journal of Traditional, Complementary and Alternative Medicines, 10(4), 6-11.

Apu, A. S., Bhuyan, S. H., Khatun, F., Liza, M. S., Matin, M., & Hossain, M. F. (2013). Assessment of cytotoxic activity of two medicinal plants using brine shrimp (*Artemia salina*) as an experimental tool. International Journal of Pharmaceutical Sciences and Research, 4(3), 1125.

Chigayo, K. (2021). Phytochemical studies of extracts of the *Kirkia wilmsii* tuber. Doctoral dissertation, University of Venda.

Boyd, C. E. (2005). LC_{50} calculations help predict toxicity. Global Aquaculture Advocate, 8(1), 84-87.

Chan, K. (2003). Some aspects of toxic contaminants in herbal edicines. Chemosphere, 52(9), 1361-1371.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G., ... & Folb, P. I. (2004). In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. Journal of Ethnopharmacology, 92(2-3), 177-191.

Fennell, C. W., Lindsey, K. L., McGaw, L. J., Sparg, S. G., Stafford, G. I., Elgorashi, E. E., ... & Van Staden, J. (2004). Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology, 94(2-3), 205-217.

Ghorani-Azam, A., Sepahi, S., Riahi-Zanjani, B., Ghamsari, A. A., Mohajeri, S. A., & Balali-Mood, M. (2018). Plant toxins and acute medicinal plant poisoning in children: A systematic literature review. Journal of Research in Medical Sciences, 23(1), 26.

Gurib-Fakim, A., & Mahomoodally, M. F. (2013). African flora as potential sources of medicinal plants: towards the chemotherapy of major parasitic and other infectious diseases: A review. 8-1 ,(624)*147*□.

Hamidi, M. R., Jovanova, B., & Panovska, T. K. (2014). Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. Macedonian Pharmaceutical Bulletin, 60(1).

Hyde, M. A., Wursten, B. T., Ballings, P., & Palgrave, C. M. (2024). Flora of Zimbabwe: Species information. *Ziziphus mucronata.* (retrieved 9 April 2024).

Ilonga, S. K., Kandawa-Schulz, M. A. R. T. H. A., Lofty, H. E. S., & Lyantagaye, S. (2018). Anticancer, antioxidant and antimicrobial screening of extracts from selected medicinal plants from Oshikoto, Namibia. Asian Journal of Tropical Biotechnology, 15(2).

Ilonga, S. K. (2012). Anticancer, antioxidant and antimicrobial sreening of extracts from *Ziziphus mucronata, Heliotropium ciliatum* and *Gnidia polycephala* from the Oshikoto region of Namibia. Doctoral dissertation, University of Namibia.

Kamanja, I. T., Mbaria, J. M., Gathumbi, P. K., Mbaabu, M., Kabasa, J. D., & Kiama, S. G. (2018). Cytotoxicity of selected medicinal plants extracts using the brine shrimp lethality assay from Samburu county, Kenya. African Journal of Traditional, Complementary and Alternative Medicines, 15(5), 1-7.

Karim, R., Begum, M. M., Jui, Y., Islam, T., Billah, M., Arafat, Y., ... & Rahman, M. S. (2021). In-vitro cytotoxic and anti-*Vibrio cholerae* activities of alcoholic extracts of *Desmodium triflorum* (L.) whole plant and *Terminalia citrina* (Roxb.) fruits. Clinical Phytoscience, 7, 1-10.

Khan, M. S. A., & Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In New look to phytomedicine (pp. 3-13). Academic Press. London.

Khumalo, G. P., Van Wyk, B. E., Feng, Y., & Cock, I. E. (2023). Toxicity and phytochemical properties of southern African medicinal plants used traditionally to treat pain and inflammatory ailments. South African Journal of Botany, 160, 102-122.

Khumalo, G. P., Sadgrove, N. J., Van Vuuren, S. F., & Van Wyk, B. E. (2021). South Africa's best BARK medicines prescribed at the Johannesburg muthi markets for skin, gut, and lung infections: MIC's and brine shrimp lethality. Antibiotics, 10(6), 681.

Kuete, V. (2014). Physical, hematological, and histopathological signs of toxicity induced by African medicinal plants. In A. Kuete & E. S. Ngameni (Eds.), Toxicological survey of African medicinal plants (pp. 635-657). Elsevier.

Maroyi, A. (2016). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus, Kirkia. Tropical Journal of Pharmaceutical Research, 15(11), 2507-2516.

McGaw, L. J., Elgorashi, E. E., & Eloff, J. N. (2014). Cytotoxicity of African medicinal plants against normal animal and human cells. In V. Kuete (Eds.), Toxicological survey of African medicinal plants (pp. 181-233). Elsevier.

Moges, A., & Moges, Y. (2019). Ethiopian common medicinal plants: Their parts and uses in traditional medicine-ecology and quality control. In A. Gonzalez, M. Rodriguez, & N. G. Sağlam (Eds.), Plant science: Structure, anatomy and physiology in plants cultured in vivo and in vitro (pp. 78-101). IntechOpen.

Mongalo, N. I., Dikhoba, P. M., Soyingbe, S. O., & Makhafola, T. J. (2018). Antifungal, anti-oxidant activity and cytotoxicity of South African medicinal plants against mycotoxigenic fungi. Heliyon, 4(11).

Naidu, J. R., Ismail, R., & Sasidharan, S. (2014). Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha Spicata* L (Lamiaceae). Tropical Journal of Pharmaceutical Research, 13(1), 101-107.

Nyagumbo, E., Pote, W., Shopo, B., Nyirenda, T., Chagonda, I., Mapaya, R. J., ... & Bhebhe, M. (2022). Medicinal plants used for the management of respiratory diseases in Zimbabwe: Review and perspectives potential management of COVID-19. Physics and Chemistry of the Earth, Parts A/B/C, 128, 103232.

Okeleye, B. I., Mkwetshana, N. T., & Ndip, R. N. (2013). Evaluation of the antibacterial and antifungal potential of *Peltophorum africanum*: toxicological effect on human chang liver cell line. The Scientific World Journal, 2013, Article ID 746165.

Olowa, L. F., & Nuñeza, O. M. (2013). Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. Mortality, 1(T2), T3.

Ozioma, E. O. J., & Chinwe, O. A. N. (2019). Herbal medicines in African traditional medicine. Herbal medicine, 10, 191-214.

Semenya, S. S., & Maroyi, A. (2012). Medicinal plants used by the Bapedi traditional healers to treat diarrhoea in the Limpopo Province, South Africa. Journal of Ethnopharmacology, 144(2), 395-401.

Shawa, I. T., Mponda, J., Msefula, C., Manda, H., Gondwe, M., & Maliwichi-Nyirenda, C. (2015). Brine shrimp lethality and phytochemical determination of aqueous extracts of *Senna singueana*, *Musa paradisiaca*, and Z*iziphus mucronata* in Malawi. Journal of Basic and Applied Research, 1, 82-88.

Singh, A. G. (2021). Medicinal Plants Used in The Treatment of Gastrointestinal Disorders in Rupandehi District, Western Nepal. Butwal Campus Journal 4(1), 103-14.

Suleiman, M. M., Bagla, V., Naidoo, V., & Eloff, J. N. (2010). Evaluation of selected South African plant species for antioxidant, antiplatelet, and cytotoxic activity. Pharmaceutical Biology, 48(6), 643-650.

Tafadzwa, M. (2005). Screening of some traditional medicinal plants from Zimbabwe for biological and anti-microbial activity. DoctoralThesis , MPhil dissertation, University of Zimbabwe.

Waghulde, S., Kale, M. K., & Patil, V. (2019, November). Brine shrimp lethality assay of the aqueous and ethanolic extracts of the selected species of medicinal plants. In *Proceedings* (Vol. 41, No. 1, p. 47). MDPI.

Zimudzi, C. (2014). African potato (Hypoxis Spp): diversity and comparison of the phytochemical profiles and cytotoxicity evaluation of four Zimbabwean species. Journal of Applied Pharmaceutical Science, 4(4), 079-083.

CHAPTER 6

GC-MS-based phytoconstituent characterisation of *Kirkia acuminata* Oliv. and *Ziziphus mucronata* Willd., medicinal plants used in traditional treatment of diarrhoea and cholera.

Abstract

Kirkia acuminata and *Ziziphus mucronata* are significant medicinal plants that contribute to traditional medicine practices in Zimbabwe. The stem barks of the plants have been documented as being used in traditional medicine for addressing cholera and various diarrhoeal disorders. Nevertheless, the existing literature lacks adequate information concerning the characterisation of bioactive compounds found in the extracts obtained from the stembark of these medicinal plants. Therefore, this study analysed the bioactive compounds found in the chloroform extract of their stembarks using the Gas Chromatography-Mass Spectrometry (GC-MS) technique. The GC-MS analysis identified 23 distinct phytochemical constituents in the extracts of *K. acuminata* and 22 in *Z. mucronata* stembarks, including bioactive compounds with established antimicrobial, antifungal, and antioxidant properties such as behenic alcohol, 1-dodecanol, and octacosanol, as well as pharmacologically underexplored hydrocarbons like tetrapentacontane and tetracontane, albeit in varying concentrations. These compounds have previously been characterised as possessing antimicrobial properties. These findings indicate that the chloroform extract of *K. acuminata* and *Z. mucronata*, contain an array of bioactive compounds, which validate their therapeutic potentials. Nevertheless, we suggest conducting further comprehensive research, including the isolation, purification, and *in*-silico molecular docking investigation of the identified bioactive compounds. By doing this study, we will get insight into the mechanism by which the bioactive compounds can attach themselves to the active site of the targeted enzymes in *V. cholerae* and other human diseases. In addition, the bioactive compounds with a binding affinity that is equal to or greater than that of the usual inhibitory ligands would provide significant information for identifying possible leads in the field of cost-effective medication discovery and development.

Keywords: *Kirkia acuminata* Oliv., *Ziziphus mucronata* Willd., Gas chromatography-mass spectrometry, traditional medicine, bioactive compounds.

6.1 Introduction

Plants have been used for centuries to treat various health conditions before the development of modern medicine (Abdallah, 2011). Until now, herbal treatments are still widely used in various regions around the world, especially in areas where people do not have easy access to modern medications (Chinsembu et al., 2015). According to the World Health Organisation (WHO), a significant portion of Africa's population relies on medicinal plants for their healthcare needs, whether fully or partially (Mahomoodally, 2013). Plants possess the ability to produce secondary metabolites, which play a crucial role in protecting them from pests and microorganisms (Chassagne et al., 2021; Chinsembu et al., 2015). These compounds hold great promise for their medicinal properties. Therefore, the reason behind the traditional use of plants for medical purposes is the presence of these secondary metabolites, which possess a wide spectrum of pharmacological activities (Mariyammal et al., 2023).

Kirkia acuminata and *Z. mucronata* are two therapeutic plants that have garnered notable attention in traditional medicine, especially in southern Africa (Maroyi, 2017). *Kirkia acuminata*, commonly referred to as White Syringa, comprises a small group of three species located in East, Central, and Southern Africa (Peter, 2013). In contrast, *Z. Mucronata* is indigenous to various tropical African nations, including Angola, Botswana, Eritrea, Ethiopia, Ghana, Kenya, Lesotho, Mozambique, Zambia, Namibia, Niger, Senegal, Somalia, South Africa, Sudan, Swaziland, Tanzania, Uganda, and Zimbabwe (Hyde et al., 2022). Both species are well-known for their application in traditional medicine across Sub-Saharan Africa (SSA) (Maroyi, 2017; Gelfand et al., 1985). In Zimbabwe, *K. acuminata* and *Z. mucronata*. stembarks play a crucial role in the treatment of cholera and other related diarrhoeal diseases (Maroyi, 2017a, 2016a, 2013; Chigora et al., 2007). The conventional use of these species is similarly reflected in various other southern African nations. For instance, it has been documented that traditional healers in Zambia utilise *K. acuminata* as a treatment for diarrhoea (Chinsembu, 2016; Chinsembu et al., 2019). Additionally, in the southern African region, several authors have recorded the use of *Z. mucronata* for addressing diarrhoeal diseases (Chinsembu et al., 2019; Maroyi and Chekhyoussef, 2015). In South Africa, leaves are ground and boiled in water, and the resulting liquid is consumed three times daily to address diarrhoea until the infection resolves (Madikizela et al., 2012). In Kenya, the stem bark is either chewed raw or dried as a remedy for treating diarrhoea in infants (Kigen et al., 2016).

Hence, it is expected that medicinal plants such as *K. acuminata* and *Z. mucronata*, which have a well-established traditional usage, may contain bioactive compounds that validate their effectiveness in traditional medicine. In light of this, the identification of bioactive compounds found in medicinal plants has lately been possible through the use of gas chromatography-mass spectrometry (GC-MS) (Momin and Yeligar, 2020; Rhetso et al., 2020). However, there is a dearth of comprehensive GC-MS analysis especially for *Kirkia acuminata* Oliv. described in the existing literature. The same applies to *Ziziphus mucronata*, as the majority of GC-MS analyses have been performed on other *Ziziphus* species. Only a few studies have concentrated on qualitative phytochemistry screening approaches. For example, Mulholland et al. (2003) discovered a variety of bioactive compounds, including fatty acids, flavonols, isocoumarin, lignans, neolignans, nor-carotenoids, phenols, and tannins, in extracts of *K. acuminata* through qualitative screening methods. Similarly, Mongalo et al. (2020) identified several bioactive compounds such as phenolic acids, alkaloids, flavonoids, terpenoids, and quinones in *Z. mucronata*, all of which exhibit antibacterial properties. These findings have, however, provided partial support for the traditional medicinal applications of *K. acuminata* and *Z. mucronata* (Maroyi, 2017a), as numerous medicinal plants with similar phytochemicals are reported to exhibit antimicrobial properties against various human pathogens (Mabadahanye, 2020). For example, several studies have reported the antibacterial activity of different extracts derived from *K. acuminata* and *Z. mucronata* against a variety of bacteria (Mabadahanye et al., 2022; Maroyi, 2017b; Chigayo et al., 2014). Moreover, various research studies have provided evidence of the efficacy of isolated bioactive compounds derived from other medicinal plant species in inhibiting the growth of a wide of bacteria, including *V. cholerae* (Jantapaso et al., 2024; Saha et al., 2022; Manjunath et al., 2022). For example, in a recent study conducted by Nag et al. (2021), it was discovered that morin (3,5,7,2′,4′-pentahydroxyflavone), a type of flavonoid, exhibited significant anti-*V. cholerae* properties. Similarly, polyphenols such as catechin and isorhamnetin demonstrated noteworthy antibacterial properties when tested against *V. cholerae* (Bhattacharya et al., 2020).

Thus, this study aims to scientifically validate the therapeutic potential of the selected plants. This study was driven by various factors. Firstly, both plant species possess a rich historical background in their application for treating cholera and various human ailments. Furthermore, the current literature reveals an insufficient number of clear and comprehensive GC-MS analyses for both plant species. Consequently, the results of this study will undoubtedly contribute to the validation of *K. acuminata* and *Z. mucronata* in traditional medicine practices.This may pave the way for the formalisation and sanctioned application of this therapeutic plant in contemporary medical practices, especially beneficial in resource-constrained endemic regions. This discovery could significantly enhance our understanding and potentially reveal new targets for human diseases, particularly cholera and other related conditions.

6.2 Materials and methods

In this study, *K. acuminata* and *Z. mucronata* were selected due to their notable antibacterial and non-cytotoxic effects, as discussed in chapters 4 and 5. The procedure for the study is outlined in the schematic diagram provided in **Figure 6.1**.

Figure 6.1: An outline for the procedure of this study

6.2.1 Plant materials

The stembarks of both plants were collected from the parent plants from the main campus of Great Zimbabwe University, Zimbabwe (20.1037° S, 30.8608° E). Qualified botanists and curators (National Herbarium of Zimbabwe) verified the authenticity of the plant species. Sample specimens were deposited in the above-named herbarium with the voucher number, MZMJ6.

6.2.2 Stem-bark extraction

The stembark samples were dried naturally in the shade at room temperature and then pounded into a fine powder using a traditional home-made mortar and pestle, replicating the traditional process of grinding herbs for medicinal purposes. Large particles were removed using a 2 mm sieve, and the remaining powder was stored at room temperature in airtight containers in the dark. Using the maceration method, approximately 150 g of the powdered samples were extracted over 48 hours in 500 mL of chloroform. The extracts were filtered using Whatman no. 1 filter paper (Whatman International Ltd., Cardiff, England) and concentrated with a rotary evaporator (RE 100-Pro, Biobase, Jinan, Shandong, China) under reduced pressure at 40±2°C. The crude extract was allowed to dry at room temperature until a consistent weight was achieved.

6.2.3 Gas chromatography-mass spectrometry analysis of the extract

Gas chromatography-mass spectrometry was used to analyse volatile and semi-volatile phytoconstituents in the extracts. The analysis was conducted according to the protocol outlined by Kumar et al. (2015), with slight adjustments. The analysis was conducted using a Shimadzu GCMS-QP-2010 Ultra instrument (Japan) equipped with a ZB-5MS column (30m x 0.25mm ID x 0.25µm df) packed with 5% phenyl siloxane. The carrier gas was Helium (99.999%) at a flow rate of 1.0 mL/min and an injection volume of 2 µL was employed (Split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 2000°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The Mass spectra were taken at 70eV. The relative percentage (%) amount of each component was determined by comparing its average peak area to the overall areas. Mass spectra were processed using the software. The NIST database was used to identify the specific bioactive compounds.

6.3 Results

6.3.1 Gas chromatography-mass spectrometry analysis of the extract

The method was unable to detect non-volatile, thermally unstable, or polar bioactive compounds due to the lack of derivatisation. Therefore, only a small percentage of potentially active phytoconstituents are reflected in the findings.

The GC-MS analysis identified 23 distinct phytochemical constituents in the extracts of *K. acuminata* and 22 in *Z. mucronata* stembarks, including bioactive compounds with established antimicrobial, antifungal, and antioxidant properties such as behenic alcohol, 1-dodecanol, and octacosanol, as well as pharmacologically underexplored hydrocarbons like tetrapentacontane and tetracontane, albeit in varying concentrations (**Table 6.1; Figs. 6.2 & 6.3**). The diversification of the bioactive compounds is shown in sunburst graphs (**Figs. 6.4 and 6.5**).

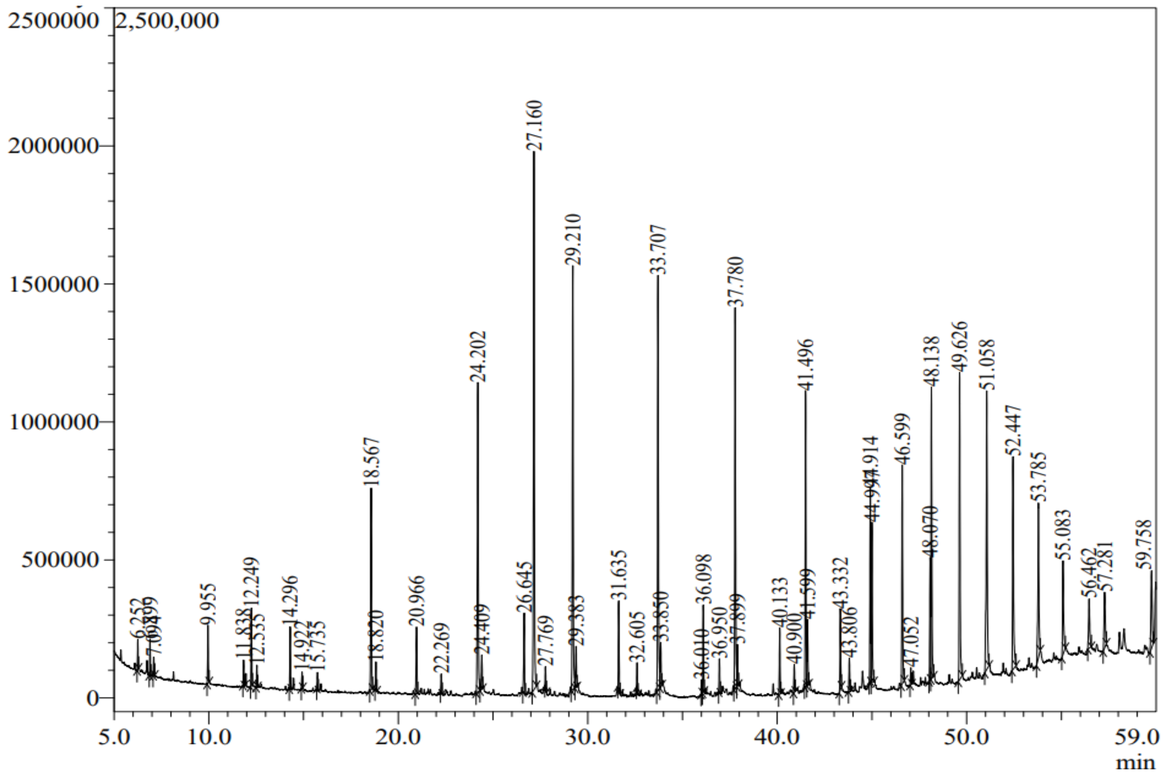

Figure 6.2: GC-MS chromatograms (abundance vs. time) of chloroform stembark extract of *K. acuminata*.

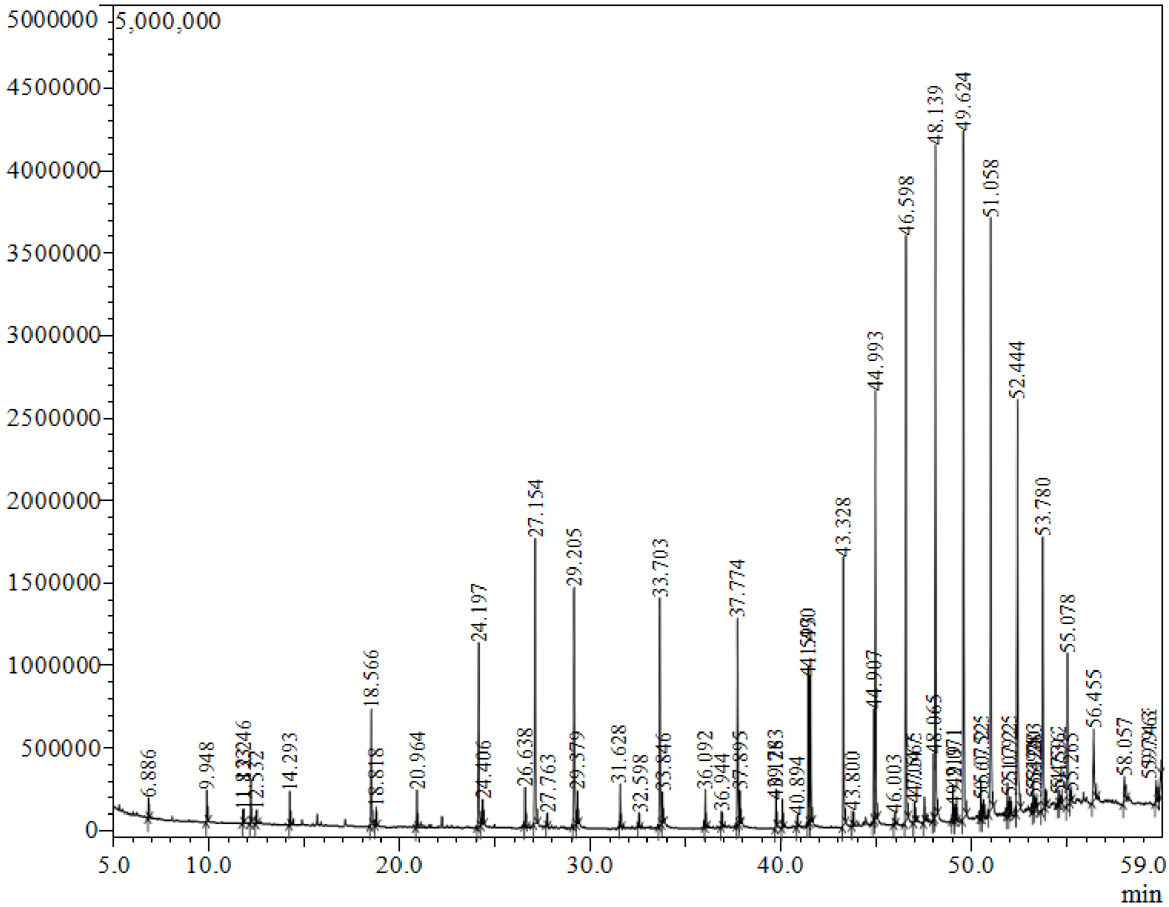

Figure 6.3: GC-MS chromatograms (abundance vs. time) of chloroform stembark extract of *Z. mucronata*.

Table 6.1 GC-MS analysis for *K. acuminata* and *Z. mucronata* chloroform stembark extract

Name of the compound	Formula	Molecular weight	Total Area%	Reported biological activity	References
***1-Dodecanol**	$C_{12}H_{26}O$	186	3.22[KA]	Suppressed *Staphylococcus aureus* growth and even potentiated the activity of norfloxacin against *Staphylococcus aureus.*	de Sousa Ferreira et al., 2023
8-Methylheptadecane		254	1.07[KA]	Linked with insecticidal properties.	Okonkwo and Udodia, 2020
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.20[KA]	Molecular docking revealed its ability to inhibit bacterial metabolism	Chinaka et al., 2023
2,4-Dimethylundecane	$C_{13}H_{28}$	184	0.20[ZM] 0.40[KA]	Not known	Not known

***Methyl 2-hydroxydecanoate**	$C_{11}H_{22}O_3$	202	0.33^{KA}	Demonstrated antimicrobial and antibiofilm properties against various human pathogens	Essghaier et al., 2023
(E)-3-Dodecene	$C_{12}H_{24}$	168	0.48^{ZM} 0.96^{KA}	Antibacterial activity documented	Sumithra and Purushothaman, 2016
Undecane	$C_{11}H_{24}$	156	0.14^{ZM} 0.27^{KA}	Showed moderate antibacterial activity.	John et al., 2008
***Hexadecane**	$C_{16}H_{34}$	226	0.39^{ZM} 0.80^{KA}	Demonstrated antibacterial activity	Rouis-Soussi et al., 2014
1-Tridecene	$C_{13}H_{26}$	182	1.66^{ZM}	Exhibited potent antibacterial activity against a wide range of bacteria	Zamakshshar et al., 2022
Tetradecane	$C_{14}H_{30}$	198	0.24^{ZM} 0.62^{KA}	Showed antibacterial and antifungal properties	Sallam and Abed, 2022
***Heneicosane**	$C_{21}H_{44}$	296	2.73^{ZM} 2.49^{KA}	Showed significant antibacterial activity against *Shigella dysenteriae* and *Proteus vulgaris*	Farzaei et al., 2014
1-Heptadecene	$C_{17}H_{34}$	238	2.85^{ZM} 5.52^{KA}	Exhibited potent antibacterial activity against a wide range of bacteria	Zamakshshar et al., 2022
***Nonadecane**	$C_{19}H_{40}$	268	0.38^{ZM} 0.85^{KA}	Demonstrated antibacterial activity	Rouis-Soussi et al., 2014
***2,4-bis(1,1-dimethylethyl)- phenol**,	$C_{14}H_{22}O$	206	4.59^{ZM} 9.49^{KA}	Demonstrated antimicrobial and antibiofilm formation properties	Fan et al., 2023; Seenivasan et al., 2022a
1-Nonadecene	$C_{19}H_{38}$	266	3.68^{ZM} 6.97^{KA}	Exhibited significant antibacterial activity	Sreenivasan et al., 2022b
***Behenic alcohol**	$C_{22}H_{46}O$	326	6.49^{ZM} 12.89^{KA}	Showed antimicrobial activity against a wide range of human pathogenic bacteria with mean inhibition zones ranging from 9.5 ± 0.91 mm to 11.3 ± 1.6 mm.	Balachandar et al., 2018
Tetracontane	$C_{40}H_{82}$	562	20.00^{ZM} 13.19^{KA}	Exhibited significant antibacterial activity against *Escherichia coli*.	Wulandari et al., 2022

Tetrapentacontane	$C_{54}H_{11}0$	758	38.54[ZM] 23.04[KA]	Demonstrated significant antibacterial activity against a wide range of bacteria, with MIC ranging from 10.93 to 43.7 µg/ml	Mariyammal et al., 2023
Docosane	$C_{22}H_{46}$	310	0.47[ZM] 1.34[KA]	Antibacterial activity displayed	Alsultan et al., 2016
***Pentacosane**	$C_{25}H_{52}$	352	2.55[ZM] 1.05[KA]	Displayed moderate antimicrobial and antifungal activities	Üçüncü et al., 2016
***Octacosanol**	$C_{28}H_{58}O$	410	3.69[ZM] 7.35[KA]	Contributed to *Cibotium barometz* rhizome extract antibacterial activity, with MIC values of 0.02–1.25 mg/mL	Heng et al., 2020
Dotriacontane	$C_{32}H_{66}$	450	8.06[ZM] 1.19[KA]	Showed low activity against the Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*	Ibrahim et al., 2017
***1-Triacontanol**	$C_{30}H_{62}O$	438	0.79[ZM] 1.72[KA]	showed significant zone of inhibition against different bacterial strains	Ali et al., 2022
Tetracosane	$C_{24}H_{50}$	338	0.12[ZM]	Antibacterial activity documented	Assefa et al., 2023.
Nonadecylcyclohexane	$C_{25}H_{50}$	350	0.18[ZM]	Detected in *Drymaria cordata* extract, which exhibited antibacterial properties.	Arya et al., 2023
***Stigmasta-5,22-diene, 3-methoxy-, (3.beta.,22E)-**	$C_{30}H_{50}O$	426	0.14[ZM] 1.33[KA]	Antimicrobial activity demonstrated	Muzahid et al., 2023

KA - phytoconstituents composition in *K. acuminata* extract, and ZM - phytoconstituents composition in *Z. mucronata* extract. Phytoconstituents in bold and with asterisk (*) are considered bioactive based on previous studies.

Figure 6.4 Sunburst chart representing classification and variation of phytoconstituents in *K. acuminata* stembark extract

Figure 6.5: Sunburst chart representing classification and variation of phytoconstituents in *Z. mucronata* stembark extract

6.4 Discussion

Kirkia acuminata and *Z. mucronata* are medicinal plants used for the treatment of various diseases, including cholera, which is endemic in many sub-Saharan African countries (Maroyi, 2017b, 2016b, 2013; Chigora et al., 2007). The therapeutic efficacy of these medicinal plants, along with other comparable plants, is linked to the composition of their bioactive compounds (Abuga et al., 2020; Seleteng-Kose et al., 2019). In accordance with this, gas chromatography and mass spectroscopy (GC–MS) has been established as a crucial method for the bioprospecting of phytoconstituents in medicinal plants (Chirumamilla et al., 2022; Momin and

Yeligar, 2020; Rhetso et al., 2020). More importantly, GC-MS analysis offers a rapid and cost-effective approach for assessing herbal products, delivering comprehensive insights into the chemical makeup of plant extracts (Lingfa et al., 2024). Thus, in this study GC-MS analysis identified 23 and 22 volatile and semi-volatile phytoconstituents in *K. acuminata* and *Z. mucronata*, respectively, with 79% of the phytoconstituents present in both medicinal plants albeit in varying quantities, some of which with confirmed or prospective bioactive characteristics (Table 6.1). Given that these plants have long been used to treat cholera and diarrhoea, these results are important for ethnopharmacological validation (Gelfand etal., 1985). Although the chromatograms (Figs. 6.2 and 6.3) showed that each plant extract contain more than 50 identifiable compounds, a number of them were not considered to be distinct phytoconstituents in our investigation. For example, contaminants and synthetic artefacts (e.g., bis(2-ethylhexyl) phthalate, hexachloroacetone, acetylene tetrachloride, and 2,2-Difluoro-1,1,1,2-tetrachloroethane), were identified and later excluded from the list of phytoconstituents. In addition, a cutoff criterion was established to exclude compounds with an area below 0.5%, unless they are known to have substantial biological importance.

The phytoconstituents that have been identified are categorised and diversified, as shown in Figures 6.4 and 6.5. The most common classification is that of hydrocarbons, which can be either alkenes or alkanes. With some exceptions, the literature on the same medicinal herbs likewise demonstrates a similar diversity (Mongalo et al., 2020; Maroyi, 2016a). However, the variations could be attributed to differences in the extraction techniques employed, especially on the type of solvent used. Therefore, employing a biphasic extraction method (e.g., chloroform + methanol/water) to capture both polar and non-polar fractions is recommended in future comparative analyses. Furthermore, GC-MS alone might have limited the findings, as it only detects volatile or semi-volatile compounds (Hübschmann, 2025). For comprehensive profiling, combining it with Liquid Chromatography-Mass Spectrometry (LC-MS) would detect both polar and non-volatile compounds (Alsehli, 2025). However, in this study, only eleven of the identified phytoconstituents were classified as ‘biologically active’: (1) 1-dodecanol, (2) methyl 2-hydroxydecanoate, (3) hexadecane, (4) heneicosane, (5) nonadecane, (6) 2,4-bis(1,1-dimethylethyl)-phenol, (7) behenic alcohol, (8) pentacosane, (9) octacosanol, (10) 1-triacontanol, and (11) stigmasta-5,22-diene, 3-methoxy-, (3.beta.,22E)-. Notably, 1-dodecanol and methyl 2-hydroxydecanoate were found exclusively in the extract of *K. acuminata*. Significantly, a majority of these phytoconstituents have established biological effects as noted in the literature (Table 6.1), Consequently, we classify them as ‘active phytoconstituents' or bioactive compounds to follow the endorsement from previous studies. However, our study did not conduct an in vitro or in vivo bioactivity testing to validate any component as genuinely bioactive.

Among the active phytoconstituents identified, behenic alcohol was found in substantial amounts as compared to others, that is, 6.49% in *Z.mucronata* and 12.89% in *K. acuminata*. Several studies on this compound, together with its derivatives, displayed significant biological activities (e.g., antibacterial, antifungal, antiviral, and antibiofilm activities) (Kim and Kim, 2024; Rav et al., 2024; Balachandar et al., 2018). For example, Rav et al. (2024) reported the strong antibacterial activity of behenic acid, a derivative of behenic alcohol, against *Vibrio parahaemolyticus* and *Aeromonas hydrophila*, with an MIC of 50 µg/mL and a zone of inhibition of 20 ± 1mm. Similarly, the same compound significantly inhibited biofilm formation of *Staphylococcus aureus* in a concentration-dependent manner, up to 20 mg/L (Kim and Kim, 2024). More importantly, a number of studies revealed that the compound has been found and isolated from other medicinal plants such as *Moringa oleifera* (Hawar et al., 2023) and *Cassia fistula* (Ferdos et al., 2021), to name but a few.

Octacosanol (7.35%), the second most abundant active phytoconstituent, demonstrated antimicrobial and antioxidant activities against a wide range of bacteria (Sengupta et al., 2018; Abubakar and Majinda, 2016) and is also present in other medicinal plants such as *Cousinia thomsonii, Albizia adianthifolia, and Origanum vulgare* (Dar et al., 2023; Abubakar and Majinda, 2016; Coccimiglio et al., 2016). Sengupta et al. (2018) showed the compound's strong antioxidant activity using different assays, including DPPH scavenging, reducing power, metal chelation, and lipid peroxidation inhibition. The same authors demonstrated the efficacy of octacosanol against *Escherichia coli* and *Pseudomonas aeruginosa* at a concentration of 2 mg/mL. More importantly, plant extracts containing octacosanol revealed the same potency of isolated octacosanol. For instance, the ethyl acetate extract from Cibotium barometz that has octacosanol showed strong activity against bacteria, and fungi (Heng et al., 2020). Furthermore, the compound also exhibited anti-inflammatory activity. Dar et al. (2023) showed that octacosanol strongly binds to several proteins involved in inflammation, like iNOS, PPAR-γ, Rel-A, COX-2, and CRP, indicating it might help reduce inflammation. Although these studies focused on various bacterial strains, they could also apply to *Vibrio cholerae* and other diarrhoeal pathogens.

The presence of 1-dodecanol and methyl 2-hydroxydecanoate only in the chemical profile of *K. acuminata extract* suggests that this plant might have more health benefits than *Z. mucronata*. These compounds, either in isolated forms or in crude extracts, demonstrated outstanding biological properties (Umoh et al., 2025; Ferreira et al., 2025; de Sousa Ferreira et al., 2023; Chanprapai et al., 2018). According to Essghaier et al. (2023), methyl 2-hydroxydecanoate showed remarkable antimicrobial efficacy, as well as anti- biofilm formation against various human pathogens. Similarly, 1-dodecanol was identified in the essential oil of *Etlingera elatior*, which demonstrated the ability to inhibit *S. aureus* even at low concentrations (de Sousa Ferreira et al., 2023). However, another study found that the same essential oil demonstrated weak antifungal activity, requiring a concentration of 60,000 µg/mL to achieve effectiveness against *Candida albicans* (Ferreira et al., 2025). This evidence indicates the limitations of crude extracts unless synergistic effects with other compounds are involved.

Another active phytoconstituent present in the extracts is heneicosane. Studies on this compound showed antimicrobial, antioxidant, and anticancer properties (Abdul et al., 2024; Sathiyaseelan et al., 2021; Vanitha et al., 2020). For example, the compound displayed remarkable antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus* with an inhibition zone of 31 ± 0.64 mm and 29 ± 0.86 mm, respectively, at a concentration of 10 µg/mL. Also, studies showed that heneicosane interacts with dihydropteroate synthase, which is a target for its antimicrobial effects (Sathiyaseelan et al., 2021). However, most of its biological activities in the current literature have been inferred from extracts containing heneicosane (Abdul et al., 2024; Bahuguna, 2021; Shah et al., 2020). For instance, *Zygophyllum luntii* leaf extract exhibited significant antibacterial efficacy (*P. aeruginosa*, zone of inhibition = 15.5 mm), though heneicosane was identified as the principal bioactive component (Shah et al., 2020). The absence of adequate experiments on pure, isolated compounds creates uncertainty regarding the biological activities of heneicosane.

Additionally, GC-MS analysis revealed the presence of certain phytoconstituents in lower quantities, which possess documented biological activities that justify their consideration for potential therapeutic applications (Table 6.1). Among them, 1-triacontanol (1.72%) has known to possess a plethora of biological properties including anti-inflammatory, antiinfertility, anticancer, cardio protective, anti-mutagenic, antifungal, antioxidant and antibacterial activities (Khan et al., 2020; Khan and Javaid, 2020; Ali et al., 2022). Pentacosane (2.55%)

was identified in the extracts; however, evidence supporting the bioactivity of its isolated form is limited. The properties are primarily derived from essential oils obtained from medicinal plants, including *Succisa pratensis*, *Pedicularis condensata*, and *Echinophora tenuifolia*, which are known to contain pentacosane. Several studies have demonstrated the antimicrobial properties of essential oils against various bacterial strains (Carev et al., 2023; Witkowska-Banaszczak and Długaszewska, 2017; Çetin et al., 2016; Üçüncü et al., 2016). In addition to having anticancer qualities, Stigmasta-5,22-diene, 3-methoxy-, (3β,22E) (1.33%) and its derivatives, such as β-sitosterol, showed antibacterial efficacy against *S. aureus*, S*higella dysenteriae*, and Candida krusei (Dube et al., 2023; Odiba et al., 2014).

In addition to the phytoconstituents with well-known bioactivities mentioned above, the phytochemical fingerprint of the *Z. mucronata* and *K. acuminata* stem bark extract also revealed the presence of other phytoconstituents with rather weak or limited bioactivities. Given their significant amounts, these compounds warrant analysis. They includes long chain alkanes and alkenes like tetracontane, tetrapentacontane, dotriacontane, 1-heptadecene, and docosane, along with cyclic derivatives such as 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (Table 6.1). Traditionally viewed as components of cuticular waxes serving structural and protective functions, recent studies indicate that these hydrocarbons possess diverse biological properties, including antimicrobial, anti-biofilm formation, and antioxidant activities. This highlights their potential significance in therapeutic applications, whether used singly or in synergy with other active phytoconstituents (Lykholat et al., 2021; Khromykh et al., 2020). Significantly, many of the compounds are present in various medicinal plants. However, the biological activities of these compounds are primarily inferred from essential oils or extracts demonstrating such activities, derived from the aforementioned medicinal plants (Fadel et al., 2024; Elkady et al., 2024; Swamy et al., 2017). For example, tetrapentacontane (38.54%), the most abundant compound, is reported to be associated with antimicrobial and antioxidant activities (Ghillace et al., 2025; Fadel et al., 2024; Geethalakshmi and Sarada, 2013). For example, the essential oil of *Leopoldia comosa*, comprising 48.25% tetrapentacontane, exhibited antioxidant potential (Fadel et al., 2024).

Tetracontane was found in various plant extracts recognised for their ethnomedicinal significance and biological properties (Wulandari et al., 2022; Rhetso et al., 2020; Hamid et al., 2017; Swamy et al., 2017). For example, Hamid et al. (2017) identified tetracontane (28.76%) as a major component in n-hexane extracts of *Thaumatococcus daniellii leaves*. The extract demonstrated antioxidant and antimicrobial properties. Dotriacontane, another phytoconstituent, has a limited range of documented antimicrobial activities (Al-Askar et al., 2024). Studies on extracts associated with this compound its biological activities (Elkady et al., 2024; Ibrahim et al., 2017; Geethalakshmi and Sarada, 2013). According to Elkady et al. (2024) dotriacontane was identified as a significant bioactive compound in the leaf extract of *Launaea nudicaulis*. The extract demonstrated antibacterial activity against *P. aeruginosa* and exhibited natural anticancer properties at safe low concentrations. Furthermore, phytoconstituents even at much lower concentrations, including 1-heptadecene and 1-tridecene, have been documented to exhibit biological properties that may provide therapeutic potential (Abdul et al., 2024; Zamakshshari et al., 2022). Several studies have demonstrated the antimicrobial properties of essential oils linked to 8-methylheptadecane, docosane, undecane, and tetradecane (Sallam and Abed, 2022; Zamakshshar et al., 2022; Sreenivasan et al., 2022b; Alsultan et al., 2016; John et al., 2008). Additionally, Chinaka et al. (2023) reported that 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione exhibited the capacity to inhibit bacterial metabolism as demonstrated through molecular docking.

While existing literature highlights the notable biological activities associated with the metabolome data in *Z. mucronata* and *K. acuminata*, it is essential to acknowledge that several limitations remain to be addressed. First, GC-MS analysis alone cannot prove the bioactivities of specific compounds as there is no testing to back it up. Most of the phytoconstituents identified are substantiated by literature from unrelated plant species and different extraction solvents, thereby complicating comparative analysis. Furthermore, a significant research gap exists in the lack of studies directly assessing antimicrobial activities against diarrhoea-causing agents such as *V. cholerae* and other related bacteria. Finally, a number of compounds, such as pentacosane and heneicosane, while frequently associated with antimicrobial extracts, have not been extensively studied in isolation, and their mechanisms of action remain unidentified. More importantly, it is essential to isolate major compounds for accurate biological evaluation.

6.5 Conclusion

The GC-MS analysis identified 23 distinct phytochemical constituents in the extracts of *K. acuminata* and 22 in *Z. mucronata* stembarks, including bioactive compounds with established antimicrobial, antifungal, and antioxidant properties such as behenic alcohol, 1-dodecanol, and octacosanol, as well as pharmacologically underexplored hydrocarbons like tetrapentacontane and tetracontane, albeit in varying concentrations. These compounds have previously been characterised as possessing antimicrobial properties. Thus, the findings indicate that the chloroform extracts of *K. acuminata* and *Z. mucronata* contain an array of bioactive compounds, which validates their usage in folkloric treatment of cholera and other related diarrhoel diseases. However, while the work offers significant insights into the composition of bioactive compounds in *K. acuminata* and *Z. mucronata*, it is important to acknowledge that it concentrated on chloroform extracts and particular bioactive molecules. Future investigations may explore other extraction methods and evaluate a broader spectrum of bioactive compounds. Furthermore, we suggest conducting further comprehensive research, including the isolation, purification, and *in*-silico molecular docking investigation of the identified bioactive compounds. By doing this study, we will get insight into the mechanism by which the bioactive compounds can attach themselves to the active site of the targeted enzymes in *V. cholerae* and other human diseases. In addition, the bioactive compounds with a binding affinity that is equal to or greater than that of the usual inhibitory ligands would provide significant information for identifying possible leads in the field of cost-effective medication discovery and development.

References

Abdallah, E. M. (2011). Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science, (Issue), 16-20.

Abdul, U., Manikandan, D. B., Arumugam, M., Alomar, S. Y., Manoharadas, S., & Ramasamy, T. (2024). GC-MS based metabolomic profiling of *Aporosa cardiosperma* (Gaertn.) Merr. leaf extracts and evaluating its therapeutic potential. Scientific Reports, 14(1), 16010. https://doi.org/10.1038/s41598-024-66491-2

Abuga, I., Sulaiman, S. F., Wahab, R. A., Ooi, K. L., & Rasad, M. S. B. A. (2020). In vitro antibacterial effect of the leaf extract of *Murraya koenigii* on cell membrane destruction against pathogenic bacteria and phenolic compounds identification. European Journal of Integrative Medicine, 33, 101010.

Al-Askar, A., Al-Otibi, F. O., Abo-Zaid, G. A., & Abdelkhalek, A. (2024). Gas chromatography-mass spectrometry comparative analysis of secondary metabolites produced by some Bacillus spp. as biocontrol agents against phytopathogenic fungi. *Egyptian Journal of Chemistry*, *67*(13), 997-1008.

Ali, J. S., Riaz, N., Mannan, A., Tabassum, S., & Zia, M. (2022). Antioxidative-, Antimicrobial-, Enzyme Inhibition-, and Cytotoxicity-Based Fractionation and Isolation of Active Components from *Monotheca buxifolia* (Falc.) A. DC. Stem Extracts. ACS Omega, 7(4), 3407–3423. https://doi.org/10.1021/acsomega.1c05647.

Alsehli, B. R. (2025). Assess the sensitivity of gas and liquid chromatography for detecting trace substances in the environment. Pure and Applied Chemistry, (0).

Alsultan, Q. M. N., Sijam, K., Rashid, T. S., & Ahmad, K. B. (2016). GC-MS Analysis and antibacterial activity of mangosteen leaf extracts against plant pathogenic bacteria. American Journal of Plant Sciences, 7(07), 1013.

Arya, A., Kumar, S., Kain, D., Ahanger, A. M., Suryavanshi, A., & Vandana. (2023). Unfolding the Chemical composition, antioxidant and antibacterial activities of *Drymaria cordata* (Linn.) Willd. against chloramphenicol-resistant *Bacillus subtilis* and β-lactams-resistant *Pseudomonas aeruginosa.* Vegetos, 1-10.

Assefa, T., Tesso, H., Abdisa, E., Guta, L., & Melaku, Y. (2023). Chemical composition and antibacterial activity of essential oils from selected species of the genus *Cucumis* in Ethiopia. Bulletin of the Chemical Society of Ethiopia, 37(3), 703-715.

Azeez, L. A., Muid, S., & Hasnul, B. M. (2016). Identification of volatile secondary metabolites from an endophytic microfungus *Aspergillus nomius* KUB105. Malaysian Journal of Analytical Sciences, 20(4), 751-759.

Balachandar, R., Karmegam, N., Saravanan, M., Subbaiya, R., & Gurumoorthy, P. (2018). Synthesis of bioactive compounds from vermicast isolated actinomycetes species and its antimicrobial activity against human pathogenic bacteria. Microbial Pathogenesis, 121, 155-165.

Bhattacharya, D., Sinha, R., Mukherjee, P., Howlader, D. R., Nag, D., Sarkar, S., ... & Gachhui, R. (2020). Anti-virulence activity of polyphenolic fraction isolated from Kombucha against *Vibrio cholerae*. Microbial Pathogenesis, 140, 103927.

Bukar, A. M., Kyari, M. Z., Gwaski, P. A., Gudusu, M., Kuburi, F. S., & Abadam, Y. I. (2015). Evaluation of phytochemical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pathogenic bacteria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State â€"Nigeria. Journal of Pharmacognosy and Phytochemistry, 3(5), 98-101.

Canli, K., Bozyel, M. E., Benek, A., Yetgin, A., Senturan, M., & Altuner, E. M. (2020). Chemical composition and in vitro antimicrobial activity of *Matthiola tricuspidata* ethanol extract. Fresenius Environmental Bulletin, 29(10), 8863-8868.

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J. T., Dettweiler, M., Marquez, L., Salam, A. M., Shabih, S., Farrokhi, D. R., & Quave, C. L. (2021). A Systematic Review of Plants With Antibacterial Activities: A Taxonomic and Phylogenetic Perspective. Frontiers in Pharmacology, 11, 586548. https://doi.org/10.3389/fphar.2020.586548.

Chigayo, K., Mojapelo, P. E. L., Bessong, P., & Gumbo, J. R. (2014). The preliminary assessment of anti-microbial activity of HPLC separated components of *Kirkia wilmsii*. African Journal of Traditional, Complementary and Alternative Medicines, 11(3), 275-281.

Chigora, P., Masocha, R., & Mutenheri, F. (2007). The role of indigenous medicinal knowledge (IMK) in the treatment of ailments in rural Zimbabwe: the case of Mutirikwi communal lands. Journal of sustainable development in Africa, 9(2), 26-43.

Chinaka, I. C. B., Oluwatosin, M. T., Ikezu, U. J., Adindu, B., & Sylvester, U. (2023). The insilico molecular docking of the compounds of the crude extract of *Dacryodes Edulis* leaves against alpha enolase to assess its activities on the treatment of bacterial pneumonia.

Chinsembu, K. C. (2016). Ethnobotanical study of plants used in the management of HIV/AIDS-related diseases in Livingstone, Southern Province, Zambia. Evidence-based Complementary and Alternative Medicine, 2016(1), 4238625.

Chinsembu, K. C., Cheikhyoussef, A., Mumbengegwi, D., Kandawa-Schulz, M., Kasanda, C. D., & Kazembe, L. (2015). Indigenous Knowledge of Namibia. University of Namibia Press.

Chinsembu, K. C., Syakalima, M., & Semenya, S. S. (2019). Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Lusaka, Zambia. South African Journal of Botany, 122, 369-384.

Chirumamilla, P., Dharavath, S. B., & Taduri, S. (2022). GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts. Bulletin of the National Research Centre, 46(1), 127.

Coopoosamy, R. M., Naidoo, K. K., & Ndlazi, N. J. (2011). Cultural importance and antibacterial activity of *Ziziphus mucronata* (Willd.) in the Umlazi community in Durban. African Journal of Pharmacy and Pharmacology, 5(17), 1979-1982.

de Sousa Ferreira, F., de Araújo Neto, J. B., de Morais Oliveira-Tintino, C. D., de Araújo, A. C. J., Ribeiro-Filho, J., Freitas, P. R., ... & Navarro, D. M. D. A. F. (2023). Chemical composition and antibacterial effects of *Etlingera elatior* (Jack) RM Smith against *Staphylococcus aureus* efflux pumps. Chemico-Biological Interactions, 386, 110751.

Elkady, F. M., Badr, B. M., Hashem, A. H., Abdulrahman, M. S., Abdelaziz, A. M., Al-Askar, A. A., ... & Hashem, H. R. (2024). Unveiling the Launaea nudicaulis (L.) Hook medicinal bioactivities: phytochemical analysis, antibacterial, antibiofilm, and anticancer activities. *Frontiers in Microbiology*, *15*, 1454623.

Essghaier, B., Mallat, N., Khwaldia, K., Mottola, F., Rocco, L., & Hannachi, H. (2023). Production and characterization of new biosurfactants/bioemulsifiers from *Pantoea alhagi* and their antioxidant, antimicrobial and anti-biofilm potentiality evaluations. Molecules, 28(4), 1912.

Fadel, H., Slougui, N., & Hazmoune, H. (2024). The first report of an Algerian Leopoldia comosa essential oil content and antioxidant assessment. *Natural Product Research*, 1-7.

Fan, K., Yu, Y., Hu, Z., Qian, S., Zhao, Z., Meng, J., Zheng, S., Huang, Q., Zhang, Z., Nie, D., & Han, Z. (2023). Antifungal Activity and Action Mechanisms of 2,4-Di-*tert*-butylphenol against *Ustilaginoidea virens*. Journal of agricultural and food chemistry, 71(46), 17723–17732. https://doi.org/10.1021/acs.jafc.3c05157.

Farzaei, M. H., Rahimi, R., Attar, F., Siavoshi, F., Saniee, P., Hajimahmoodi, M., Mirnezami, T., & Khanavi, M. (2014). Chemical composition, antioxidant and antimicrobial activity of essential oil and extracts of *Tragopogon graminifolius*, a medicinal herb from Iran. Natural Product Communications, 9(1), 121–124.

Geethalakshmi, R., & Sarada, D. V. L. (2013). Evaluation of antimicrobial and antioxidant activity of essential oil of Trianthema decandra L. *journal of pharmacy research*, *6*(1), 101-106.

Gelfand, M., Mavi, S., Drummond, R.B. and B. Ndemera. (1985). The Traditional Medicinal Practitioner in Zimbabwe. Mambo Press, Gweru, Zimbabwe

Ghavam, M., Afzali, A., Manconi, M., Bacchetta, G., & Manca, M. L. (2021). Variability in chemical composition and antimicrobial activity of essential oil of *Rosa damascena* Herrm. from mountainous regions of Iran. Chemical and Biological Technologies in Agriculture, 8, 1-16.

Ghillace, A., Chouitah, O., Kiari, F. Z., Fergoug, Z., & Daikh, Z. (2025). Phytochemical composition and antimicrobial activity of essential oil from the leaves of Peganum harmala L. in southern Algeria. *Acta Biologica Slovenica*, *68*(01).

Hamid, A. A., Aliyu, M. A., Abubakar, L. Z., Mukadam, A. A., Shehu, A., Egharevba, G., ... & Fagbohun, E. O. (2017). Thaumatococcus daniellii leaves: its chemical compositions, antioxidant and antimicrobial activities. *Ife Journal of Science*, *19*(2), 409-416.

Heng, Y. W., Ban, J. J., Khoo, K. S., & Sit, N. W. (2020). Biological activities and phytochemical content of the rhizome hairs of *Cibotium barometz* (Cibotiaceae). Industrial Crops and Products, 153, 112612.

Herwin, H. (2020). Antibacterial Activity of Bis (2-Ethylhexyl) Phthalate Leaves Fraction of *Colocacia esculenta* L against Enteric Gram Negative Bacteria. Journal of Global Pharma Technology, 13, 1-6.

Hübschmann, H. J. (2025). Handbook of GC-MS: fundamentals and applications. John Wiley & Sons.

Ibrahim, N. A., Musa, S. K., Yassin, S. M., Abuniama, N. H., Awadalkareem, S., Osama, A., & Abdalaziz, M. N. (2017). Chemical Composition and Antimicrobial Activity of Essential Oil of *Belpharis linariifolia*. International Journal of Science, Technology and Society, 5(4), 62-66.

Jantapaso, H., Aksonkird, T., & Mittraparp-arthorn, P. (2024). Characteristics of *Vibrio cholerae* isolates obtained from shrimp supply chains and inhibitory activities of rambutan (*Nephelium lappaceum* L. cv. Rong Rian) peel aqueous extract. Food Control, 158, 110231.

John, A. J., George, V., Pradeep, N. S., & Sethuraman, M. G. (2008). Composition and antibacterial activity of the leaf and fruit oils of *Pittosporum neelgherrense* Wight et Arn. Journal of Essential Oil Research, 20(4), 380-382.

Khromykh, N. O., Lykholat, Y. V., Anishchenko, A. A., Didur, O. O., Gaponov, A. A., Kabar, A. M., & Lykholat, T. Y. (2020). Diversity.Integrated Limitations and Perspective.

Khumalo, G. P., Sadgrove, N. J., Van Vuuren, S. F., & Van Wyk, B. E. (2021). South Africa's Best BARK Medicines Prescribed at the Johannesburg Muthi Markets for Skin, Gut, and Lung Infections: MIC's and Brine Shrimp Lethality. Antibiotics, 10(6), 681.

Kotan, R., Cakir, A., Dadasoglu, F., Aydin, T., Cakmakci, R., Ozer, H., Kordali, S., Mete, E., & Dikbas, N. (2010). Antibacterial activities of essential oils and extracts of Turkish *Achillea*, *Satureja* and *Thymus* species against plant pathogenic bacteria. Journal of The Science of Food and Agriculture, 90(1), 145–160. https://doi.org/10.1002/jsfa.3799.

Kumar, N. N., Ramakrishnaiah, H., Krishna, V., & Deepalakshmi, A. P. (2015). GC-MS analysis and antimicrobial activity of seed oil of *Broussonetia papyrifera* (L.) Vent. International Journal of Pharmaceutical Sciences and Research, 6(9), 3954.

Kumar, V., Sharma, A., Thukral, A. K., & Bhardwaj, R. (2016). Phytochemical profiling of methanolic extracts of medicinal plants using GC-MS. International Journal of Research and Development in Pharmacy & Life Sciences, 5(3), 2153-2158.

Lingfa, L., Tirumala, A., & Ankanagari, S. (2024). GC-MS profiling of anticancer and antimicrobial phytochemicals in the vegetative leaf, root, and stem of *Withania somnifera* (L.) Dunal. International Journal of Secondary Metabolite, 11(1), 63-77.

Lykholat, Y. V., Khromykh, N. O., Didur, O. O., Okovytyy, S. I., Sklyar, T. V., Davydov, V. R., ... & Kovalenko, I. M. (2021). Soluble cuticular wax composition and antimicrobial activity of the fruits of *Chaenomeles* species and an interspecific hybrid. Biosystems Diversity, 29(4), 334-339.

Mabadahanye, K. (2020). Isolation and analysing chemical profiles of bioactive compounds from South Africa medicinal plants with activity against pathogenic organisms. Doctoral thesis, University of Johannesburg.

Mabadahanye, K., Bhembe, N. L., & Green, E. (2022). Crude extracts activity of three selected medicinal plants from the Venda region against some pathogenic organisms. African Health Sciences, 22(2), 717-727.

Mahomoodally M.F (2013) "Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants", Evidence-Based Complementary and Alternative Medicine, https://doi.org/10.1155/2013/617459.

Manjunath, G. B., Awasthi, S. P., Zahid, M. S. H., Hatanaka, N., Hinenoya, A., Iwaoka, E., ... & Yamasaki, S. (2022). Piperine, an active ingredient of white pepper, suppresses the growth of multidrug-resistant toxigenic *Vibrio cholerae* and other pathogenic bacteria. Letters in Applied Microbiology, 74(4), 472-481.

Mariyammal, V., Sathiageetha, V., Amalraj, S., Gurav, S. S., Amiri-Ardekani, E., Jeeva, S., & Ayyanar, M. (2023). Chemical profiling of *Aristolochia tagala* Cham. leaf extracts by GC-MS analysis and evaluation of its antibacterial activity. Journal of the Indian Chemical Society, 100(1), 100807.

Maroyi, A. (2013). Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. Journal of Ethnobiology and Ethnomedicine 9, 31. https://doi.org/10.1186/1746-4269-9-31.

Maroyi, A. (2016a). Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. African journal of traditional, complementary, and alternative medicines: African Journal of Traditional, Complementary and Alternative Medicines, 13(6), 5–10. https://doi.org/10.21010/ajtcam.v13i6.2.

Maroyi, A. (2016b). Ethnomedicinal uses, phytochemistry and pharmacological properties of the genus, *Kirkia*. Tropical Journal of Pharmaceutical Research, 15(11), 2507-2516.

Maroyi, A. (2017a). Exotic Plants in Indigenous Pharmacopoeia of South-Central Zimbabwe: Traditional Knowledge of Herbal Medicines. Research Journal of Botany, doi: 10.3923/rjb.2017.46.52.

Maroyi, A. (2017b). *Kirkia acuminata* Oliv.: A review of its ethnobotany and Pharmacology. African Journal of Traditional, Complementary and Alternative Medicines, 14(2), 217-226.

Momin, Y. H., & Yeligar, V. C. (2020). Gas chromatography-mass spectrometry analysis of chloroform extract of *Coccinia grandis* Voigt. Research Journal of Pharmacy and Technology, 13(12), 5889-5892.

Mongalo, N. I., Mashele, S. S., & Makhafola, T. J. (2020). *Ziziphus mucronata* Willd.(Rhamnaceae): it's botany, toxicity, phytochemistry and pharmacological activities. Heliyon, 6(4).

Muzahid, A. A., Sharmin, S., Hossain, M. S., Ahamed, K. U., Ahmed, N., Yeasmin, M. S., ... & Bhuiyan, M. N. H. (2023). Analysis of bioactive compounds present in different crude extracts of *Benincasa hispida* and *Cucurbita moschata* seeds by gas chromatography-mass spectrometry. Heliyon, 9(1).

Nag, D., Dastidar, D. G., & Chakrabarti, G. (2021). Natural flavonoid morin showed anti-bacterial activity against *Vibrio cholera* after binding with cell division protein FtsA near ATP binding site. Biochimica et Biophysica Acta (BBA)-General Subjects, 1865(8), 129931.

Nemudzivhadi, V., & Masoko, P. (2015). Antioxidant and antibacterial properties of *Ziziphus mucronata* and *Ricinus communis* leaves extracts. African Journal of Traditional, Complementary and Alternative Medicines, 12(1), 81-89.

Okonkwo, C. O., & Udodia, P. S. (2020). Chemical Composition of Three Different Fractions Obtained from the Leaves of *Cassia occidentalis*. Annual Research & Review in Biology, 34(5), 1-6.

Olajuyigbe, O. O., & Afolayan, A. J. (2012). Antimicrobial potency of the ethanolic crude bark extract of *Ziziphus mucronata* Willd. subsp. mucronata Willd. African Journal of Pharmacy and Pharmacology, 6(10), 724-730.

Peter, M. (2013). Ethnobotanical study of some selected medicinal plants used by traditional healers in Limpopo Province (South Africa). American Journal of Research Communication, 1(8), 8-23.

Rahman, M. S., & Rashid, M. A. (2008). Antimicrobial activity and cytotoxicity of *Eclipta prostrata*. Advances in Traditional Medicine, 8(1), 47-52.

Rhetso, T., Shubharani, R., Roopa, M. S., & Sivaram, V. (2020). Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don. Future Journal of Pharmaceutical Sciences, 6, 1-9.

Rouis-Soussi, L. S., El Ayeb-Zakhama, A., Mahjoub, A., Flamini, G., Jannet, H. B., & Harzallah-Skhiri, F. (2014). Chemical composition and antibacterial activity of essential oils from the Tunisian *Allium nigrum* L. EXCLI journal, 13, 526.

Saha, P., Banerjee, A., Banerjee, G., & Bag, P. K. (2022). Inhibitory activities of *Typhonium trilobatum* (L.) Schott on virulence potential of multi-drug resistant toxigenic *Vibrio cholerae*. Microbial Pathogenesis, 165, 105485.

Sallam, K. M., & Abed, N. (2022). Evaluation of Antimicrobial Potential of Tetradecane Extracted from *Pediococcus acidilactici* DSM: 20284-CM Isolated from Curd Milk. Egyptian Journal of Chemistry, 65(3), 705-713.

Sanusi, B. S., Idris, H., Usman, A., Hamza, M., & Abdullahi, B. (2022). Phytochemical Profiling, Antimycobacterial Activity, Time-Kill Assessment and Mode of Action of the Extract of *Musa gracilis* Holttum. Tropical Journal of Natural Product Research, 6(3).

Seenivasan, A., Manikkam, R., Kaari, M., Sahu, A. K., Said, M., & Dastager, S. G. (2022). 2, 4-Di-tert-butylphenol (2, 4-DTBP) purified from *Streptomyces* sp. KCA1 from *Phyllanthus niruri*: Isolation, characterization, antibacterial and anticancer properties. Journal of King Saud University-Science, 34(5), 102088.

Seleteng-Kose, L., Moteetee, A., & Van Vuuren, S. (2019). Medicinal plants used for the treatment of sexually transmitted infections in the Maseru District, Lesotho: Antimicrobial validation, phytochemical and cytotoxicity studies. South African Journal of Botany, 122, 457-466.

Sreenivasan, A. Y. S. W. A. R. Y. A., Manikkam, R., & Manigundan, K. (2022). Isolation, Characterization and Antimicrobial Activity of Endophytic Actinobacteria from Medicinal Plants. Indian Journal of Pharmaceutical Sciences, 84(5).

Sumithra, D., & Purushothaman, D. S. (2016). Phytochemical profiling of ethanolic leaves extract of *Commelina benghalensis* L. World Journal of Pharmaceutical Research, 6, 1101-1107.

Swamy, M. K., Arumugam, G., Kaur, R., Ghasemzadeh, A., Yusoff, M. M., & Sinniah, U. R. (2017). GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian Plectranthus amboinicus leaves. *Evidence-Based Complementary and Alternative Medicine*, *2017*(1), 1517683.

Üçüncü, O., Baltacı, C., & İlter, Ş. M. (2016). Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oil from *Pedicularis condensata* BIEB. Hittite Journal of Science and Engineering, 3(2), 105-109.

Viszwapriya, D., Prithika, U., Deebika, S., Balamurugan, K., & Pandian, S. K. (2016). In vitro and in vivo antibiofilm potential of 2, 4-Di-tert-butylphenol from seaweed surface associated bacterium *Bacillus subtilis* against group A *Streptococcus*. Microbiological Research, 191, 19-31.

Wulandari, A. P., Rossiana, N., & Wandira, A. (2022). Analysis of the Bioactive Compounds and Antibacterial Test on N-Hexane Extract of Ramie (*Boehmeria nivea*). Biosaintifika: Journal of Biology & Biology Education, 14(3).

Zamakshshari, N. H., Ahmed, I. A., Didik, N. A. M., Nasharuddin, M. N. A., Hashim, N. M., & Abdullah, R. (2022). Chemical profile and antimicrobial activity of essential oil and methanol extract from peels of four *Durio zibethinus* L. varieties. Biomass Conversion and Biorefinery, 1-9.

CHAPTER 7

General Conclusion, Recommendations and Future Work

7.1 General Conclusion

There has been a significant rise in cholera outbreaks documented in various developing countries, with a specific emphasis on Africa and Asia. According to the latest data from February 2023, there have been documented reports of cholera cases in around 25 countries. Regrettably, a majority of these countries, approximately 60%, are found in sub-Saharan Africa (Erkyihun et al., 2023). According to Olatunji et al. (2024), Zimbabwe is among the countries that frequently encounter outbreaks of cholera, with a significant spike in cases occurring in 2008, when the case fatality ratio (CFR) escalated to 20% in some areas (Gwenzi and Sanganyado, 2019). Despite the known risk factors for the disease, there are still several unanswered questions that need to be addressed in Zimbabwe, and other countries in the region. Why is Zimbabwe unable to prevent cholera outbreaks? What are the reasons behind Zimbabwe's inability to contain cholera outbreaks as they occur? The current study proposes a traditional approach to complement efforts towards cholera management in Zimbabwe, considering the seemingly unavoidable cholera outbreaks in the country due to the current status quo. As a result, the integration of natural products, particularly in this resource-limited endemic country that is highly vulnerable to cholera, is recommended by the study. It has been reported that natural products, including plants, exhibit promising potential as treatments for a range of infections (Chassagne et al., 2021; Prasathkumar et al., 2021). Several studies, including surveys, reviews, and anecdotal evidence, have discovered several medicinal plants employed in the folkloric treatment of cholera and other similar diarrhoeal diseases in Zimbabwe (Maroyi, 2013, 2016). However, Zimbabwe has yet to standardise and authorise the use of such therapeutic plant species in modern treatment. In light of this context, the present study aimed to identify such medicinal plants and validate their use in traditional medicine by identifying their bioactive compound content and evaluating their antibacterial activity. Furthermore, the cytotoxic activity of the plant species was also assessed.

A preliminary literature review identified 19 medicinal plant species that Zimbabwean communities have traditionally used for managing intestinal diseases such as cholera. A considerable variety of recognised plant species fulfil comparable medicinal roles across different African countries. The investigation centres on medicinal plants that demonstrate anti-*V. cholerae* properties; nonetheless, it also includes other antidiarrhoeal plants, given that cholera and certain diarrhoeal diseases may sometimes present similar signs and symptoms. Among the documented medicinal plants, *K. acuminata*, *S. birrea*, *Z. mucronata*, and *C. febrifuga* have been recognised for their use by diverse ethnic groups across Zimbabwe. Nonetheless, only *K. acuminata* and *Z. mucronata* possess a noteworthy historical context in the management of cholera specifically. Nevertheless, the primary method of treatment consisted of infusing powdered plant materials, such as leaves, roots, and stem bark, into water, followed by oral intake of the solution. Additional techniques involved decoction and incorporating the powder into food, whereas the least commonly used method was the anal instillation of powdered plant material into the rectum. These findings correspond closely with established practices in other African medicines (Mothibe and Sibanda, 2019). However, variations in human cultural practices, the geographic distribution of plant species, the sites of ethnobotanical studies, and the methodologies used for data collection may explain the discrepancies in information concerning the plant parts utilised and the techniques of remedy preparation.

The antibacterial evaluation conducted on the three selected medicinal plants (*K. acuminata, S, birrea*, and *Z. mucronata*) demonstrated notable anti-*Vibrio cholerae* activity, with MIC values of stembark extracts at ≤ 1 mg/mL. This indicates that these plant species exhibit significant efficacy against *V. cholerae*. The findings suggest that these plants could be dependable sources for herbal treatments. Nevertheless, the fourth selected plant species, *C. febrifuga*, exhibited the highest MIC (> 6 mg/mL), and it may necessitate substantial doses. Furthermore, the water extracts of *Z. mucronata* and *K. acuminata* demonstrated no toxic activity in the Brine Shrimp Lethality Assay. The results validate the safety of their application in traditional medicine. It is recommended that patients and herbalists use lower dosages, as the extracts demonstrated a greater lethality towards brine shrimp larvae at higher concentrations. Notably, future studies should prioritise evaluating the relationship between extract preparation (including steeping duration, temperature, and water-to-plant material ratios) to achieve safer extract concentrations, as well as investigating the potential toxicological effects of prolonged use of the plant species.

More importantly, the theraupeutic potential of *K. acuminata* and *Z. mucronata* was validated by the GC-MS analysis revealing the metabolome data of the plant extracts that are responsible for their antibacterial properties. Either individual compounds or synergistic interactions among the identified long chain fatty alcohols, hydrocarbons, phenolic compounds, sterols and other component groups within the extracts could be responsible for the antibacterial activity. Long-chain fatty alcohols, for instance, found in the extracts (e.g., 1-dodecanol, behenic alcohol, octacosanol, 1-triacontanol) are known to have bactericidal effects and membrane-damaging actions on several bacteria (Shin et al., 2024; Royce et al., 2013; Togash et al., 2007). Moreover, phenolic compounds (e.g., the identified 2,4-bis(1,1-dimethylethyl)-phenol) have properties of membrane disruption and antibiofilm formation (Fan et al., 2023; Seenivasan et al., 2022; Rempe et al., 2017). Likewise surfactant properties of substances like behenic alcohol are known to prevent the formation of biofilm in common human pathogens (Kim and Kim, 2024). These findings underline the significance of searching for membrane-targeting compounds in medicinal plants used in traditional medicine to enhance our mechanistic knowledge by pointing out specific compound classes known to exhibit bioactivity. More importantly, this work provides crucial steps for mechanical validation, safety optimisation, and clinical development paths that could solve the growing burden of antimicrobial resistance in cholera treatment and lays a basis for standardised, safe, and effective antimicrobial formulations.

While the findings of this study are compelling; however, there is a limitation that should be addressed in future studies. The antimicrobial and cytotoxic assays (Chapters 4 and 5) employed water and ethanol as extraction solvents, while GC-MS analysis used chloroform as a solvent. The antibacterial activity shown by ethanolic and aqueous extracts cannot be attributed solely to the compounds discovered using GC-MS analysis, since different solvents may produce different chemical profiles and antimicrobial potencies. However, the current findings remain valid; nonetheless, a comparative analysis regarding solvents is recommended for future research.

7.2 Recommendation and Future Work

The study has provided valuable insights into the multifaceted approach to controlling and managing cholera, particularly in areas with limited resources and high endemicity. However, the results of this study, albeit significant, do not, by themselves, provide a comprehensive response to every point that has been raised concerning the recurrence and management of cholera in Zimbabwe. As a result, our study suggests the following recommendations:

- In this study, the selection of medicinal plant species was based on the plant species documented in the existing literature. Therefore, it is recommended that future studies should also consider conducting interviews with elderly individuals and traditional herbalists belonging to various ethnic groups in Zimbabwe.
- The extraction methods employed in this study were adapted from techniques designed to mimic the preparation of traditional herbal remedies. It is crucial to acknowledge that this approach may possess specific limitations concerning the quantity and variety of bioactive compounds that can be extracted. In future research, it is recommended to explore other alternative methods of extraction and solvents. This study will enable a more comprehensive evaluation of antibacterial and cytotoxic activity and a detailed analysis of the profile of bioactive compounds.
- Investigating *in vivo* models, such as animal models, is necessary for gaining a deeper understanding of the activity of the identified bioactive compounds as well as that of crude extracts.
- Research on potential toxicological consequences that may arise from the long-term or short-term use of the plant species in traditional medicine should be prioritised in future studies.
- Isolation, purification, and *in*-silico molecular docking investigation of the identified bioactive compounds and comparison of their binding affinity with existing drugs are highly recommended in future studies.
- Moreover, it is recommended that future research endeavours focus on exploring the possible potentiation of commonly used drugs already resisted by *V. cholerae* and other pathogens.

Implementing the recommendations provided above can be a valuable step in identifying potential leads for the design and development of cost-effective medicines. For instance, through the process of isolating, purifying, and conducting *in*-silico molecular docking, researchers can gain a deeper understanding of how bioactive compounds can bind to the active site of targeted enzymes in *V. cholerae* and other human pathogens. Furthermore, it is worth noting that the presence of bioactive compounds exhibiting a binding affinity that is equivalent to or surpasses that of the typical inhibitory ligands would yield valuable insights in the search for potential candidates for the development of new drugs. Moreover, individuals living in rural areas where cholera occurs frequently could benefit from the validation of the traditional use of *K. acuminata* and *Z. mucronata* in managing cholera. In the interim, the infusion or decoction of stembarks may be taken into consideration as a substitute for contemporary medications, which in certain cases are not easily accessible because of their prohibitive cost or restricted availability to medical facilities, especially in rural areas with inadequate transportation infrastructure. By incorporating *K. acuminata* and *Z. mucronata* in food, either singly or in combination (e.g., teas or poly-herbal formulations) that can be taken regularly or right away by cholera suspects, or individuals living in such areas are likely to be greatly appreciated.

References

Chassagne, F., Samarakoon, T., Porras, G., Lyles, J. T., Dettweiler, M., Marquez, L., ... & Quave, C. L. (2021). A systematic review of plants with antibacterial activities: A taxonomic and phylogenetic perspective. Frontiers in Pharmacology, 11, 586548.

Erkyihun, G. A., Asamene, N., & Woldegiorgis, A. Z. (2023). The Threat of Cholera in Africa. Zoonoses, 3(1), 20230027.

Gwenzi, W., & Sanganyado, E. (2019). Recurrent cholera outbreaks in Sub-Saharan Africa: moving beyond epidemiology to understand the environmental reservoirs and drivers. Challenges, 10(1), 1.

Maroyi, A. (2013). Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. Journal of Ethnobiology and Ethnomedicine, 9, 1-18.

Maroyi, A. (2016). Treatment of diarrhoea using traditional medicines: contemporary research in South Africa and Zimbabwe. African Journal of Traditional, Complementary and Alternative Medicines, 13(6), 5-10.7.2

Mothibe, M. E., & Sibanda, M. (2019). African traditional medicine: South African perspective. Traditional and Complementary Medicine, 2019, 1-27.

Olatunji, G., Kokori, E., Moradeyo, A., Olatunji, D., Ajibola, F., Otolorin, O., & Aderinto, N. (2024). A Perspective on the 2023 Cholera Outbreaks in Zimbabwe: Implications, Response Strategies, and Policy Recommendations. Journal of Epidemiology and Global Health, 14(1), 243-248.

Prasathkumar, M., Anisha, S., Dhrisya, C., Becky, R., & Sadhasivam, S. (2021). Therapeutic and pharmacological efficacy of selective Indian medicinal plants–a review. Phytomedicine Plus, 1(2), 100029.

Kim, S., & Kim, T. J. (2024). Inhibitory Effect of Moringa oleifera Seed Extract and Its Behenic Acid Component on Staphylococcus aureus Biofilm Formation. Antibiotics, 14(1), 19.

Togashi, N., Shiraishi, A., Nishizaka, M., Matsuoka, K., Endo, K., Hamashima, H., & Inoue, Y. (2007). Antibacterial activity of long-chain fatty alcohols against Staphylococcus aureus. Molecules, 12(2), 139-148.

Shin, S., Yu, J., Tae, H., Zhao, Y., Jiang, D., Qiao, Y., ... & Cho, N. J. (2024). Exploring the Membrane-Active Interactions of Antimicrobial Long-Chain Fatty Acids Using a

Supported Lipid Bilayer Model for Gram-Positive Bacterial Membranes. ACS Applied Materials & Interfaces, 16(42), 56705-56717.

Royce, L. A., Liu, P., Stebbins, M. J., Hanson, B. C., & Jarboe, L. R. (2013). The damaging effects of short chain fatty acids on Escherichia coli membranes. Applied microbiology and biotechnology, 97, 8317-8327.

Rempe, C. S., Burris, K. P., Lenaghan, S. C., & Stewart Jr, C. N. (2017). The potential of systems biology to discover antibacterial mechanisms of plant phenolics. Frontiers in microbiology, 8, 422.

Fan, K., Yu, Y., Hu, Z., Qian, S., Zhao, Z., Meng, J., Zheng, S., Huang, Q., Zhang, Z., Nie, D., & Han, Z. (2023). Antifungal Activity and Action Mechanisms of 2,4-Di-tert-butylphenol against Ustilaginoidea virens. Journal of agricultural and food chemistry, 71(46), 17723–17732. https://doi.org/10.1021/acs.jafc.3c05157.

Seenivasan, A., Manikkam, R., Kaari, M., Sahu, A. K., Said, M., & Dastager, S. G. (2022). 2, 4-Di-tert-butylphenol (2, 4-DTBP) purified from Streptomyces sp. KCA1 from Phyllanthus niruri: Isolation, characterization, antibacterial and anticancer properties. Journal of King Saud University-Science, 34(5), 102088.

APPENDICES

Appendix A: Ethics Clearance Certificate

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE

Date: 06/12/2021

NHREC Registration # : REC-170616-051
REC Reference # : 2021/CAES_HREC/188
Name : Mr J Marumure
Student #: 11774231

Dear Mr Marumure

Decision: Ethics Approval from 02/12/2021 to 30/11/2026

Researcher(s): Mr J Marumure
11774231@mylife.unisa.ac.za; +263-712529331

Supervisor (s): Dr MA Nyila
nyilama@unisa.ac.za; 011-471-2294

Working title of research:

Identification of bioactive compounds and evaluation of antibacterial activity of selected medicinal plants against Vibrio colerae

Qualification: PhD Life Science

Thank you for the application for research ethics clearance by the Unisa-CAES Health Research Ethics Committee for the above mentioned research. Ethics approval is granted for five years, **subject to further clarification and submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

The researcher is cautioned to adhere to the Unisa protocols for research during Covid-19.

Due date for progress report: 30 November 2022

The progress report is available on the college ethics webpage: https://w2.unisa.ac.za/www.unisa.ac.za/sites/corporate/default/Colleges/Agriculture-%26-Environmental-Sciences/Research/Research-Ethics.html

Please note the points below for further action:

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

1. Please identify the plant species involved - are they threatened or not?
2. The scope of the research is very broad – will it not be better to focus on one or two plant species rather than six, and to have two or three objectives rather than five? It is up to the supervisor to decide whether the scope should be decreased.
3. Please provide more detail on data analysis: will the researcher use one-way or two-way Anova, and why? Also define the variables. Furthermore, regressions will be used – please state the model and the variables.

*The **low risk application** was **reviewed** by the UNISA-CAES Health Research Ethics Committee on 02 December 2021 in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

The proposed research may now commence with the provisions that:

1. The researcher will ensure that the research project adheres to the relevant guidelines set out in the Unisa Covid-19 position statement on research ethics attached.
2. The researcher(s) will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.
3. Any adverse circumstance arising in the undertaking of the research project that is relevant to the ethicality of the study should be communicated in writing to the Committee.
4. The researcher(s) will conduct the study according to the methods and procedures set out in the approved application.
5. Any changes that can affect the study-related risks for the research participants, particularly in terms of assurances made with regards to the protection of participants' privacy and the confidentiality of the data, should be reported to the Committee in writing, accompanied by a progress report.
6. The researcher will ensure that the research project adheres to any applicable national legislation, professional codes of conduct, institutional guidelines and scientific standards relevant to the specific field of study. Adherence to the following South African legislation is important, if applicable: Protection of Personal Information Act, no 4 of 2013; Children's act no 38 of 2005 and the National Health Act, no 61 of 2003.
7. Only de-identified research data may be used for secondary research purposes in future on condition that the research objectives are similar to those of the original research. Secondary use of identifiable human research data require additional ethics clearance.

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

8. No field work activities may continue after the expiry date. Submission of a completed research ethics progress report will constitute an application for renewal of Ethics Research Committee approval.

Note:
*The reference number **2021/CAES_HREC/188** should be clearly indicated on all forms of communication with the intended research participants, as well as with the Committee.*

Yours sincerely,

Prof MA Antwi
Chair of UNISA-CAES Health REC
E-mail: antwima@unisa.ac.za
Tel: (011) 670-9391

Prof SR Magano
Executive Dean : CAES
E-mail: magansr@unisa.ac.za
Tel: (011) 471-3649

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Appendix B: Permit to collect medicinal plants for the study

MINISTRY OF LANDS, AGRICULTURE, FISHERIES, WATER AND RURAL DEVELOPMENT

DEPARTMENT OF RESEARCH & SPECIALIST SERVICES (DR&SS)

All Communications to be addressed to
"THE HEAD"

Telephone: 263-242-708938
E-mail: nhbg@drss.gov.zw
Fax:

NATIONAL HERBARIUM & BOTANIC GARDEN
Box A889
Avondale, Harare, Zimbabwe

24 November 2021

Great Zimbabwe University
P.O. Box 1235
Masvingo
Zimbabwe
22 November 2021

Dear Mr. Marumure

RE: COLLECTION AND IDENTIFICATION OF PLANT SPECIES FOR RESEARCH: NATIONAL HERBARIUM AND BOTANIC GARDEN

Jerikias Marumure, a lecturer at Great Zimbabwe University and doctoral student at University of South Africa is one of the researchers carrying out a research titled **'Identification of Bioactive compounds and Evaluation of antibacterial activity of selected medicinal plants against *Vibrio cholerae*'**. I am kindly requesting for assistance in collection and identification of medicinal plant parts commonly used for the treatment of diarrhoea and cholera. No tree will be uprooted or destroyed. The parts will be used for scholarly research only and any information obtained will be availed to the responsible stakeholders.
The choice for the actual plant species to be used in the study will be based on number of references/ citations, reported usage in other countries, reported bioactive compounds composition and roles, and researchers experience with the plant species. The plant parts species to be collected will include, among others, (1) *Ziziphus mucronata*, (2) *Mangifera indica*, (3) *Ximenia caffra*, (4) *Vangueria infausta*, (5) *Sclerocarya birrea*, (6) *Flacourtia indica*, (7) *Euclea divinorum*, (8) *Peltophorum africanum*, (9) *Grewia bicolor*, and (10) *Crossopteryx febrifuga*.

Yours sincerely,

C. Chapano (Mr.)
Head
National Herbarium and Botanic Garden,
0773 209 095 / 0714 025 235

Appendix C: List of Publications

Marumure, J., & Nyila, M.A. (2024). Cholera outbreaks in Zimbabwe: An in-depth analysis of drivers, constraints, and reimagining the use of medicinal plants. Journal of Tropical Medicine, 45-67, https://doi.org/10.1155/jotm/1981991.

Marumure, J., & Nyila, M. A. (in press). Chemical profiling of *Kirkia acuminata* Oliv. stembark extracts by GC-MS analysis and evaluation of its antibacterial and cytotoxic activity. Natural Product Communications. https://doi.org/10.1177/1934578X251357459.