EVALUATION OF ANTIMICROBIAL EFFICACY OF *HIBISCUS SABDARIFFA* ON MULTIDRUG RESISTANT *ESCHERICHIA COLI* FROM URINE ISOLATES

BY

UGE, OGHENEMARHO CALEB

BMS2005052

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

SCHOOL OF BASIC MEDICAL SCIENCES

COLLEGE OF MEDICAL SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

THIS PROJECT IS SUBMITTED TO:

THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE,

SCHOOL OF BASIC MEDICAL SCIENCES

UNIVERSITY OF BENIN IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF MEDICAL LABORATORY SCIENCE DEGREE

SUPERVISOR:

DR. (MRS) A. O. ITEMIRE

AUGUST, 2025

CERTIFICATION

This is to certify that this project work was carried out by **UGE OGHENEMARHO CALEB** with matriculation number **BMS2005052** in partial fulfilment of the requirements for the award of Bachelor of Medical Laboratory Science (BMLS) from the University of Benin, Benin City, Edo State, Nigeria.

DR. (MRS) ANNE O. ITEMIRE	**DATE**
(Project Supervisor)	
DR. (MRS) Z. OMORUYI	**DATE**
(Head of Department)	
External Examiner	**DATE**

DEDICATION

This work is dedicated to my Heavenly Father who is the source of all knowledge and wisdom and to my wonderful parents for their unwavering love and support.

ACKNOWLEDGEMENTS

I acknowledge the all-wise, all-knowing, all-intelligent, and Almighty God from whom wisdom and knowledge come. I recognise the professional supervision given to me by my supervisor, Dr (Mrs) Anne O. Itemire, during this work. Special gratitude to the Head of Department, Dr (Mrs) Z. Omoruyi for creating a conducive environment for learning. To all my wonderful lecturers, it was indeed a privilege to learn under your supervision. I appreciate the Head of the Department of Pharmaceutical Microbiology's permission to use the laboratory. I sincerely appreciate Mr Wilfred Aisagbonbuomwan who guided and assisted me during this research work in the Laboratory. I want to specially appreciate my parents Mr and Mrs Uge for their unwavering support, encouragement and belief in me, from their sacrifices to their words of wisdom, I am grateful for their guidance and patience throughout this research work. I also want to extend my heartfelt thanks to my siblings, Avwerosuoghene Uge, Oghenemaga Uge for their support in both big and small ways, and for their constant encouragement and understanding.

Table of Contents

CERTIFICATION ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

LIST OF TABLES viii

LIST OF FIGURES x

ABSTRACT xi

CHAPTER ONE 1

INTRODUCTION 1

1.1 BACKGROUND OF THE STUDY 1

1.2 STATEMENT OF THE PROBLEM 2

1.3 JUSTIFICATION OF THE STUDY 3

1.4 AIM OF STUDY 4

1.5 RESEARCH OBJECTIVES 5

1.6 RESEARCH QUESTIONS 5

1.7 HYPOTHESES 5

1.8 LIMITATIONS OF THE STUDY 6

CHAPTER TWO 8

LITERATURE REVIEW 8

2.1 *Hibiscus sabdariffa* Plant 8

2.1.1 Botanical Description Of Hibiscus sabdariffa 8

2.1.2 Geographical Distribution 9

2.1.3 Taxonomy 9

2.1.4 Phytochemical constituents of *Hibiscus sabdariffa* 12

2.1.5 Pharmacological and Antimicrobial Activities of *Hibiscus sabdariffa* 14

2.2 ANTIBIOTIC RESISTANCE TRENDS AND LOCAL DATA 19

2.3 OVERVIEW OF URINARY TRACT INFECTIONS (UTIS) 21

2.4 UPEC VIRULENCE AND RESISTANCE MECHANISMS 22

2.5 CURRENT TREATMENT APPROACHES AND LIMITATIONS 24

2.6 PLANT-BASED ANTIMICROBIALS 25

2.6.1 Traditional Use of Medicinal Plants 25

2.6.2 Advantages of Plant-Based Antimicrobials 25

2.6.3 Mechanisms of Action of Plant-Based Antibacterial Agents 26

2.7 Methods for Assessing Antimicrobial Activity 29

Agar Well Diffusion Method 29

Minimum Inhibitory Concentration/ Minimum Bactericidal Concentration 30

Time-Kill Kinetics (Time-Kill Curve) 31

CHAPTER THREE 32

METHODOLOGY

Error! Bookmark not defined.

3.1 Study Area 32

3.2 Materials 32

3.2.1 Microbiological Media 32

3.2.2 Equipment and Apparatus 32

3.2.3 Glassware 32

3.2.4 Chemicals and Reagents 32

3.2.5 Clinical Isolates 33

3.3 Methods 33

3.3.1 Collection and Identification of *Hibiscus sabdariffa* 33

3.3.2 Preparation of Crude Extract 33

3.3.3 Extract Yield 33

3.4 Specimen Collection 33

3.4.1 Preparation of Test Organisms 34

3.5 Antimicrobial Assays 34

3.5.1 Determination of Minimum Inhibitory Concentration (MIC) 34

3.5.2 Determination of Minimum Bactericidal Concentration (MBC) 35

3.5.3 Determination of Time-Kill Kinetics 35

3.6 Preparation of McFarland Standard 36

3.7 Statistical Analysis 36

CHAPTER FOUR 37

RESULTS 37

4.1 Extract Yield and properties 37

4.2 Minimum Inhibitory Concentration (MIC) 37

4.3 Minimum Bactericidal Concentration (MBC) 37

4.4 Bacterial Time-Kill Kinetics 38

CHAPTER FIVE 84

5.1 Discussion
Error! Bookmark not defined.

5.2 Conclusion 86

5.3 Recommendations 86

REFERENCES 87

APPENDIX 93

LIST OF TABLES

Table 1: Zone of inhibition before plasmid curing

Table 2: Zone of inhibition after plasmid curing

Table 3: Minimum bactericidal concentration before plasmid curing

Table 4: Minimum bactericidal concentration after plasmid curing

Table 5 : Time kill kinetics of aqueous and ethanol extracts (pre-curing)

Table 6: Time kill kinetics of aqueous and ethanol extracts (post-curing)

Table 7: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts before plasmid curing

Table 8: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts after plasmid curing

Table 9: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before and after plasmid curing

Table 10: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before and after plasmid curing

Table 11: MIC and MBC of the ethanol and the aqueous extracts of *Hibiscus sabdariffa* with multi-drug resistant uropathogenic *Escherichia coli*

Table 12: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* before plasmid curing

Table 13: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* after plasmid curing

Table 14: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* before plasmid curing

Table 15: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* after plasmid curing

LIST OF FIGURES

Figure 1: Image of *Hibiscus sabdariffa* plant

Figure 2: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts before plasmid curing

Figure 3: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts after plasmid curing

Figure 4: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before and after plasmid curing

Figure 5: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before and after plasmid curing

Figure 6: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before plasmid curing

Figure 7: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract after plasmid curing

Figure 8: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before plasmid curing

Figure 9: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract after plasmid curing

ABSTRACT

The rising prevalence of multidrug-resistant uropathogenic *Escherichia coli* (UPEC) highlights the urgent need for alternative antimicrobial agents. This study aimed to evaluate the antibacterial activity, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill kinetics of ethanol and aqueous extracts of *Hibiscus sabdariffa* calyces against multidrug resistant UPEC isolates before and after plasmid curing. Antibacterial activity was determined using agar well diffusion, MIC and MBC by broth dilution, and time-kill assays by plate count method. The ethanol extract consistently showed stronger antibacterial activity than the aqueous extract, with lower MIC and MBC values and faster bacterial elimination in time-kill assays. Plasmid curing enhanced the susceptibility of UPEC to both extracts. Conclusively, *Hibiscus sabdariffa*, particularly the ethanol extract, demonstrated promising antibacterial potential against MDR UPEC, warranting further studies on dosage, and safety.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Urinary tract infections (UTIs) remain among the most prevalent bacterial infections globally, contributing to significant morbidity and healthcare costs in both developed and developing countries. Recent epidemiological evidence indicates a 66.45% increase in cases between 1990 and 2021, with an estimated 4.49 billion infections recorded during this period (He *et al*., 2025). The persistence of UTIs as a major public health concern is partly explained by their high recurrence rates and their impact on women, who are disproportionately affected due to anatomical and physiological factors (Hu *et al*., 2025).

Projections further suggest that the burden of UTIs will continue to escalate, particularly among older women, where incidence is expected to rise steadily until 2040. This increase has been linked to population aging, hormonal changes, and comorbidities that predispose elderly women to recurrent infections (Hu *et al*., 2025). Such findings highlight the urgent need for tailored preventive and therapeutic strategies that account for vulnerable populations.

The global burden of antimicrobial resistance (AMR) has also complicated the management of UTIs, with Gram-negative uropathogens, especially *Escherichia coli*, showing increasing levels of resistance to commonly used antibiotics. Studies have demonstrated that drug-resistant UTIs contribute substantially to the wider AMR crisis, positioning them as one of the most urgent threats to effective infectious disease management (Hu *et al*., 2025). Moreover, recent systematic analyses confirm that AMR trends are projected to worsen by 2050, with urinary tract pathogens

among the top contributors to resistant infections worldwide (GBD 2021 Antimicrobial Resistance Collaborators, 2024).

Given these challenges, attention has turned toward alternative therapeutic options, including medicinal plants, as viable candidates for addressing the shortcomings of current antibiotic therapy. *Hibiscus sabdariffa* (*H. sabdariffa*) in particular, has emerged as a promising plant with bioactive compounds that display notable antibacterial activity against multiple pathogens. Both *in vitro* and in silico studies have revealed the multitarget potential of *H. sabdariffa* extracts, providing strong justification for their exploration as adjuncts or alternatives to conventional antibiotics (Ghaly *et al*., 2025). Similarly, broader reviews of *Hibiscus* species have confirmed significant antimicrobial effects against *Escherichia coli* and other clinically relevant bacteria, further supporting its potential role in combating multidrug-resistant infections (Alharbi *et al*., 2024).

1.2 STATEMENT OF THE PROBLEM

The rapid escalation of antimicrobial resistance (AMR) represents one of the most pressing challenges to global health, particularly in the context of urinary tract infections (UTIs). According to Murray *et al*. (2022), resistance to key antibiotic classes such as fluoroquinolones and β-lactams accounts for over 70% of global AMR-associated deaths. This statistic highlights the alarming inefficacy of conventional antibiotics in managing infections caused by multidrug-resistant organisms, including uropathogenic *Escherichia coli* (UPEC), the predominant cause of UTIs worldwide. The consequences are dire, especially in low- and middle-income countries where diagnostic limitations and unregulated antibiotic usage accelerate the development and spread of resistance.

At the same time, there is a critical need to identify alternative treatment options that are both effective and accessible. Natural products, particularly medicinal plants, have historically contributed to antimicrobial drug discovery. *Hibiscus sabdariffa*, a plant widely cultivated in tropical regions, has long been used in traditional medicine for managing bacterial infections. Recent reviews emphasize its broad phytochemical profile including anthocyanins, organic acids, and flavonoids which confer promising antibacterial activity against resistant pathogens (Kokare *et al*., 2025). Despite these reports, there are still very few thorough studies that compare how well *H. sabdariffa* works against drug-resistant UPEC bacteria compared to standard antibiotics used in hospitals. This lack of research shows why it's urgent to study *H. sabdariffa* as a possible treatment option for the growing problems of UTIs and antibiotic resistance.

1.3 JUSTIFICATION OF THE STUDY

The global rise in antibiotic-resistant bacterial infections has created an urgent need for alternative treatment options, especially for common but potentially severe infections like urinary tract infections (UTIs). Uropathogenic *Escherichia coli* (UPEC) remains the leading cause of UTIs, and this highly adaptive pathogen presents significant treatment challenges rooted in a complex interplay of molecular factors that allow UPEC to evade host defences, persist within the urinary tract, and resist antibiotic therapy (Whelan *et al*., 2023).

High recurrence rates and increasing antimicrobial resistance among uropathogens threaten to greatly reduce the efficacy of antibiotic treatments (Zhou *et al*., 2023), particularly in low-resource settings like Nigeria. increased antibiotic resistance of UPEC isolates has been demonstrated in systematic reviews, suggesting a need for reassessment of empirical therapies in urinary tract infections treatment (Bunduki *et al*., 2021). The situation is further compounded by

the overuse and misuse of antibiotics, which drive resistance in both community and hospital settings, with recurrent UTIs frequently causing the use of various antibiotic regimens, which can generate bacterial multidrug resistance (Flores-Oropeza *et al*., 2023).

While the study of resistance mechanisms and gene transfer remains essential to controlling the spread of resistant pathogens, there is also a growing need to investigate safe, affordable, and accessible alternatives to conventional antibiotics. Traditional medicinal plants have bioactive metabolites that can significantly affect the growth rate, cell survival, and pathogenicity of antibiotic-resistant bacteria (Ghaly *et al*., 2025). *Hibiscus sabdariffa* has shown promise due to its phytochemical composition and reported antimicrobial activities against a wide range of bacteria, yeast, and fungi (Hamrita *et al*., 2022). Evaluating the effect of *Hibiscus sabdariffa* on resistant UPEC isolates will provide useful insights into its potential as a complementary or alternative therapeutic agent, particularly in environments with limited access to newer antibiotics.

This study is justified by the need to address the escalating problem of antibiotic resistance using plant-based solutions, and provide local data on the efficacy of *Hibiscus sabdariffa* against resistant UPEC strains. The findings could inform future research, guide phytotherapy-based interventions, and contribute to the global search for new antimicrobial agents with real-world relevance.

1.4 AIM OF STUDY

The aim of this study is to evaluate the antibacterial activity of aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* against resistant *Escherichia coli* isolates obtained from urinary samples in a tertiary healthcare institution in Benin City, Edo State.

1.5 RESEARCH OBJECTIVES

The specific objectives of this study are to:

1. evaluate the antibacterial activity of aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* against resistant uropathogenic *Escherichia coli* (UPEC) isolates.

2. assess the antibacterial activity of these extracts before and after plasmid curing.

3. determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts.

4. evaluate the bactericidal activity of *Hibiscus sabdariffa* extracts over time using time-kill kinetics.

1.6 RESEARCH QUESTIONS

1. What is the antibacterial activity of aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* against resistant uropathogenic *Escherichia coli* (UPEC) isolates?

2. How does the antibacterial activity of these extracts differ before and after plasmid curing?

3. What are the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the extracts against test organisms

4. What is the bactericidal effect of *Hibiscus sabdariffa* extracts over time as determined by time-kill kinetics?

1.7 HYPOTHESES

1.7.1 Null Hypotheses (H_0):

1. Aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* have no significant antibacterial activity against resistant uropathogenic *Escherichia coli* (UPEC) isolates.

2. There is no significant difference in the antibacterial activity of the extracts before and after plasmid curing.

3. There is no significant difference in the MIC and MBC values between aqueous and ethanolic extracts.

4. The extracts do not show bactericidal activity over time as determined by time-kill kinetics.

1.7.2 Alternative Hypotheses (H_1):

1. Aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* exhibit significant antibacterial activity against resistant uropathogenic *Escherichia coli* (UPEC) isolates.

2. There is a significant difference in the antibacterial activity of the extracts before and after plasmid curing.

3. There is a significant difference in the MIC and MBC values between aqueous and ethanolic extracts.

4. The extracts show bactericidal activity over time as determined by time-kill kinetics.

1.8 LIMITATIONS OF THE STUDY

This study is limited to laboratory-based analysis and does not include *in vivo* testing or clinical trials, which may affect the direct application of the findings to human treatment. The research focuses only on multidrug resistant uropathogenic *Escherichia coli* isolates collected from a

single tertiary healthcare institution, which may not capture the full spectrum of uropathogens present in other regions or populations.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Hibiscus sabdariffa* Plant

2.1.1 Botanical Description Of *Hibiscus sabdariffa*

Hibiscus sabdariffa is an annual herbaceous shrub that typically reaches a height of 1.5–2.5 m, though it can grow up to 3.5 m under favorable conditions. The plant develops an erect, branching stem that is initially green but becomes woody and reddish-brown with maturity. The stem surface is smooth to slightly pubescent with prominent nodes and internodes (Ouangraoua *et al*., 2022). The leaves are alternate, simple, and exhibit considerable morphological variation. Lower leaves are typically broad and ovate, measuring 8–15 cm in length and 6–12 cm in width, while upper leaves become progressively more lobed and palmate, often with 3–5 deep divisions. The leaf margins are serrated, and both surfaces are covered with fine hairs, giving them a slightly rough texture (Riaz and Chopra, 2018).

The flowers are solitary, axillary, and measure 4–6 cm in diameter when fully opened. Each flower consists of five pale yellow to cream-colored petals with a distinctive dark red or purple center, and a prominent red staminal column that extends beyond the petals. The most economically important feature is the fleshy, bright red calyx that surrounds each flower and fruit. These calyces, commonly known as sepals, are thick, succulent, and measure 2–3 cm in length, forming a cup-like structure that becomes the primary harvested product (Riaz and Chopra, 2018).

2.1.2 Geographical Distribution

The major areas of production of *Hibiscus sabdariffa* are located in Africa, particularly Sudan, Nigeria, and Senegal, which remain leading producers of the plant for both local consumption and export (Bilali, 2024). In Asia, significant cultivation is recorded in China and Thailand, where large-scale plantations are established to meet industrial demand for calyces in the food, beverage, and nutraceutical sectors (Tao *et al*., 2024). Smaller but notable production regions include Egypt, Ghana, and Cameroon in Africa, and Mexico and Jamaica in the Americas, where the crop is integrated into traditional diets and cultural practices (Alharbi *et al*., 2024).

The species thrives in tropical and subtropical climates, preferring sandy loamy or well-drained soils under moderate to high rainfall conditions. It typically grows in areas with temperatures ranging from 18–35 °C and is often cultivated in rain-fed systems, although irrigation is practiced in intensive farms to support commercial yield (Almajid *et al*., 2023). In Asia, particularly in Hunan Province of China, molecular breeding programs have been developed to enhance yield and adaptability, reflecting the plant's expanding agronomic base beyond its African origin (Tao *et al*., 2024).

Due to its economic, nutritional, and medicinal value, *Hibiscus sabdariffa* is increasingly cultivated in diverse regions across the tropics, with global demand driving expansion beyond traditional zones of production. Its cultivation now spans Africa, Asia, the Caribbean, and Latin America, highlighting its transition from a regional crop to an internationally traded commodity (IndustryARC, 2022; Bilali, 2024).

2.1.3 Taxonomy

Hibiscus sabdariffa L., commonly known as roselle, Jamaica sorrel, Indian sorrel, or karkade, is a shrub species belonging to the family Malvaceae. Its taxonomic classification is as follows:

Taxonomic Rank	Classification
Kingdom	Plantae
Phylum	Tracheophyta (vascular plants)
Class	Magnoliopsida (dicotyledons)
Order	Malvales
Family	Malvaceae
Genus	*Hibiscus*
Species	*Hibiscus sabdariffa* L.

This classification is consistently recognized in major repositories such as the NCBI Taxonomy Browser (NCBI Taxonomy n.d.). Furthermore, *Hibiscus sabdariffa* exhibits significant genetic and morphological variability, with two major cultivated varieties described: *Hibiscus sabdariffa* var. *sabdariffa* (red phenotype) and *Hibiscus sabdariffa* var. *altissima* (white phenotype), which are widely distributed across tropical and subtropical regions (Sanou *et al*., 2022).

Figure 2.1 *Hibiscus sabdariffa* plant showing the characteristic red calyces and pale pink flower morphology, noted for their cardiovascular therapeutic potential (Sapian *et al*., 2023)

2.1.4 Phytochemical constituents of *Hibiscus sabdariffa*

Phytochemicals are naturally occurring bioactive compounds synthesized by plants, and in *Hibiscus sabdariffa* they are largely responsible for its therapeutic, nutritional, and functional properties. Recent analyses highlight *Hibiscus sabdariffa* calyces, leaves, and seeds as rich sources of diverse secondary metabolites, including flavonoids, phenolic acids, anthocyanins, alkaloids, tannins, saponins, steroids, and organic acids (Almajid *et al*., 2023; Alharbi *et al*., 2024). These compounds form the basis of the plant's wide-ranging pharmacological effects, especially its antioxidant, antimicrobial, antihypertensive, and anti-inflammatory activities.

Advanced chromatographic and spectrometric studies have consistently revealed high concentrations of anthocyanins, with delphinidin-3-sambubioside and cyanidin-3-sambubioside identified as the dominant pigments in the calyces. These anthocyanins contribute not only to the deep red coloration but also to significant antioxidant capacity and antimicrobial action against Gram-positive and Gram-negative bacteria (Alharbi *et al*., 2024). Alongside anthocyanins, abundant flavonoids such as quercetin and kaempferol derivatives, as well as phenolic acids including protocatechuic, chlorogenic, and caffeic acid, have been detected in both aqueous and ethanolic extracts (Almajid *et al*., 2023).

Recent solvent-extraction studies confirm that ethanolic and methanolic solvents yield higher total phenolic content compared to aqueous extracts, enhancing antibacterial efficacy (Tao *et al*., 2024). Quantitative analyses report total phenolic contents exceeding 200 mg gallic acid equivalents per gram (GAE/g) in ethanolic calyx extracts, with flavonoid levels also markedly higher compared to water-based extracts (Almajid *et al*., 2023).

In addition to phenolics, phytosterols, terpenoids, and glycosides have been reported across different plant parts. Seeds are notable for containing fixed oils rich in linoleic and oleic acids, which add nutritional value and may contribute to hypolipidemic effects (Alharbi *et al*., 2024). Alkaloids and saponins present in the leaves and calyces further enhance the antimicrobial profile by disrupting microbial membranes and modulating immune responses.

The therapeutic potential of *Hibiscus sabdariffa* is thus strongly attributed to the synergistic action of its phytochemical groups, each contributing distinct biological activities. A brief account of the most relevant constituents is presented below:

Anthocyanins (Delphinidin-3-sambubioside, Cyanidin-3-sambubioside)

These pigments dominate the calyces, acting as potent antioxidants and antimicrobial agents. They scavenge free radicals and inhibit bacterial growth by damaging cell membranes and interfering with biofilm formation (Alharbi *et al*., 2024).

Flavonoids and Phenolic Acids

Quercetin, kaempferol, protocatechuic acid, and chlorogenic acid are among the key compounds. They exert strong antioxidant effects, modulate inflammatory cascades, and contribute to antibacterial action by suppressing microbial enzyme activity and disrupting metabolic pathways (Almajid *et al*., 2023).

Tannins

Detected in leaves and calyces, tannins exert antimicrobial effects through protein precipitation, disruption of microbial adhesion, and inhibition of enzymatic function, thereby reducing colonization and persistence of pathogens (Alharbi *et al*., 2024).

Saponins and Alkaloids

These metabolites enhance antimicrobial potency by increasing microbial membrane permeability and synergizing with polyphenols to amplify bactericidal activity. Alkaloids also play a role in modulating host immune responses (Almajid *et al*., 2023).

Organic Acids (*Hibiscus* acid, Citric acid, Malic acid)

Characteristic of *Hibiscus*. *sabdariffa* calyces, these acids contribute to the sour taste of *hibiscus* beverages while also possessing antimicrobial and antihypertensive properties. *Hibiscus* acid in particular has been linked to modulation of calcium influx in smooth muscle, which helps explain some of the plant's cardiovascular effects (Tao et al., 2024).

2.1.5 Pharmacological and Antimicrobial Activities of *Hibiscus sabdariffa*

Hibiscus sabdariffa L. (roselle) has long been recognized in ethnomedicine for its multipurpose therapeutic potential, and contemporary research has increasingly validated these traditional claims by linking them to a wide spectrum of bioactive phytochemicals, particularly anthocyanins, flavonoids, phenolic acids, saponins, tannins, and organic acids. These compounds collectively contribute to a wide range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, antihypertensive, antidiabetic, hepatoprotective, and cardioprotective effects (Almajid *et al*., 2023; Alharbi *et al*., 2024). The pharmacological significance of *Hibiscus sabdariffa* is especially evident in the growing body of experimental and clinical studies that examine its impact on both infectious and non-communicable diseases,

where it demonstrates notable bioactivity against resistant microorganisms, oxidative stress and metabolic dysfunction.

Antibacterial Effects

Among its most extensively studied properties is the antibacterial activity of *Hibiscus sabdariffa*, with calyx, leaf, and seed extracts showing broad-spectrum inhibitory effects against both Gram-positive and Gram-negative pathogens. Recent *in vitro* studies have demonstrated that ethanolic and methanolic extracts exert significant zones of inhibition against *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, with activity attributed primarily to anthocyanins such as delphinidin-3-sambubioside and cyanidin-3-sambubioside, along with phenolic acids like protocatechuic acid (Alharbi *et al*., 2024). The antimicrobial mechanism involves disruption of bacterial cell membranes, inhibition of key metabolic enzymes, interference with nucleic acid synthesis, and suppression of biofilm formation, processes that collectively reduce bacterial viability and prevent persistence in host tissues. Importantly, recent evidence highlights that *Hibiscus sabdariffa* extracts retain antimicrobial potency even against multidrug-resistant bacterial strains, positioning it as a promising adjunct or alternative in the face of escalating antibiotic resistance crises (Almajid *et al*., 2023).

Antifungal Activity

The antifungal efficacy of *Hibiscus sabdariffa* has also been increasingly investigated, although to a lesser extent compared to antibacterial studies. Extracts rich in anthocyanins, flavonoids, and organic acids have demonstrated inhibitory effects on *Candida albicans and Aspergillus niger*,

both of which are opportunistic fungal pathogens commonly associated with human disease. The mechanism is believed to involve destabilization of fungal cell membranes and inhibition of ergosterol biosynthesis, similar to the antifungal action of conventional azole drugs (Alharbi *et al*., 2024). These findings are of particular importance given the rising global burden of fungal infections and the limited arsenal of effective antifungal therapies.

Anti-inflammatory Activity

The anti-inflammatory potential of *Hibiscus* sabdariffa has been demonstrated in both *in vitro* and *in vivo* models. Experimental findings show that calyx extracts markedly reduce inflammation by downregulating pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, while also modulating cyclooxygenase and lipoxygenase pathways (Almajid *et al*., 2023). In rodent models, treatment with aqueous or ethanolic extracts significantly reduced carrageenan-induced paw edema in a dose-dependent manner, achieving inhibition rates comparable to those of non-steroidal anti-inflammatory drugs. The observed effect is attributed not only to anthocyanins but also to hibiscus acid and flavonoid constituents that exert immunomodulatory effects, reducing oxidative stress and inflammatory mediator synthesis.

Antipyretic and Analgesic Activity

Athough fewer studies have explored this compared to its anti-inflammatory effects, early evidence suggests that *Hibiscus* sabdariffa extracts may also have antipyretic activity by inhibiting prostaglandin E2 synthesis in the hypothalamus, helping to regulate body temperature. Its analgesic potential has also been noted in animal models, where extracts reduced pain

responses in both hot plate and acetic acid–induced writhing assays, pointing to an influence on both central and peripheral pain pathways (Alharbi *et al*., 2024).

Antioxidant Activity

The antioxidant capacity of *Hibiscus sabdariffa* is widely acknowledged and is attributed primarily to its anthocyanins, phenolic acids, and flavonoids. Recent assays, including total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), and DPPH radical scavenging activity, have consistently shown high antioxidant indices for both aqueous and alcoholic extracts, though alcoholic solvents yield higher concentrations (Tao *et al*., 2024). Anthocyanins such as delphinidin derivatives provide potent hydrogen-donating ability, neutralizing free radicals and preventing lipid peroxidation, which is a critical factor in the pathogenesis of cardiovascular, metabolic, and neurodegenerative disorders. The antioxidant activity of *Hibiscus sabdariffa* is notably dose-dependent, and its strength can vary with factors such as solvent extraction, geographic origin, and cultivation conditions, highlighting the plant's chemotypic diversity (Tao *et al.,* 2024).

Antidiabetic and Hypolipidemic Effects

Several in vivo studies have confirmed that *Hibiscus sabdariffa* exhibits antidiabetic properties through modulation of carbohydrate and lipid metabolism. Ethanolic calyx extracts have been shown to inhibit α-amylase and α-glucosidase enzymes, reducing postprandial glucose levels, while simultaneously enhancing peripheral glucose uptake and improving insulin sensitivity in streptozotocin-induced diabetic rats (Almajid *et al*., 2023). In addition, polyphenolic compounds contribute to hypolipidemic effects by lowering serum total cholesterol, triglycerides, and LDL-

C while elevating HDL-C. These outcomes are linked to the combined action of flavonoids and organic acids in modulating lipid metabolism and preventing oxidative modification of lipoproteins (Almajid *et al*., 2023).

Hepatoprotective and Nephroprotective Activity

The hepatoprotective properties of *Hibiscus sabdariffa (H. sabdariffa)* have been reported in models of chemically induced hepatic injury. Administration of aqueous or alcoholic extracts significantly restored serum biomarkers such as ALT, AST, and ALP to near-normal levels, while histopathological analysis revealed preservation of hepatocyte architecture, reduction of necrosis, and improved antioxidant enzyme activity, including SOD, CAT, and glutathione peroxidase (Alharbi *et al*., 2024). Similarly, nephroprotective effects have been demonstrated in models of renal toxicity, where *H*. *sabdariffa* reduced urea and creatinine levels while improving renal histology, effects attributed to its antioxidant and anti-inflammatory constituents (Alharbi *et al*., 2024).

Cardioprotective and Antihypertensive Activity

Perhaps the most well-documented clinical benefit of the flower is its antihypertensive effect, which has been confirmed in both animal models and human trials. Aqueous calyx infusions significantly reduced systolic and diastolic blood pressure, an effect attributed primarily to hibiscus acid, which modulates vascular smooth muscle contractility by inhibiting calcium influx, as well as to anthocyanins that enhance endothelial function through increased nitric oxide bioavailability (Tao *et al*., 2024). These mechanisms collectively improve vascular reactivity, reduce arterial stiffness, and protect against hypertension-induced organ damage, underscoring

Hibiscus sabdariffa's potential as a functional food and supportive agent in cardiovascular health management (Tao *et al*., 2024).

2.2 ANTIBIOTIC RESISTANCE TRENDS AND LOCAL DATA

The problem of antibiotic resistance is not only global but is also strongly evident in the West African sub-region, where both healthcare-associated and community-acquired infections show alarming levels of resistance across multiple classes of antibiotics. Recent systematic evaluations have helped to map this burden more clearly (Diop *et al*., 2025). African healthcare and community settings, demonstrated that resistance levels remain consistently high, particularly among Gram-negative organisms such as *Escherichia coli* and *Klebsiella pneumoniae*. The study highlighted that over 60% of isolates from clinical settings showed resistance to at least three antibiotic classes, with significant implications for treatment outcomes and patient safety. Importantly, the authors noted that the persistence of multidrug resistant pathogens in community-acquired infections suggests that antibiotic resistance is not restricted to hospital environments but is increasingly a public health issue affecting the wider population. This trend underscores the importance of examining resistance data both regionally and locally in order to understand the epidemiology of urinary tract pathogens such as uropathogenic *Escherichia coli* (UPEC) in Nigeria.

Zooming in on Nigeria specifically, sentinel surveillance data from Lagos has provided further insight into resistance dynamics (Chukwu *et al*., 2022). In a surveillance study conducted on clinical isolates obtained from tertiary healthcare facilities, researchers reported an alarmingly high prevalence of resistance to third-generation cephalosporins which are antibiotics commonly used for empirical therapy in cases of severe urinary tract infections. The study emphasized that

resistance to cephalosporins, particularly ceftriaxone and ceftazidime, was widespread and often associated with extended-spectrum beta-lactamase (ESBL) production, thereby severely limiting available treatment options (Chukwu *et al*., 2022). The Lagos study also revealed that carbapenem resistance, though less frequent, is beginning to emerge in Nigeria, raising concerns about the potential spread of carbapenem-resistant Enterobacteriaceae (CRE) in the near future. Such findings not only reflect the pressure exerted by inappropriate antibiotic use in healthcare facilities but also highlight the lack of robust antibiotic stewardship frameworks in many Nigerian hospitals (Ajuga *et al*., 2021).

Local data from outside Lagos further illustrates the scale of the resistance problem. A study on isolates from Aba metropolis in southeastern Nigeria found that 55.4% of *Escherichia coli* isolates demonstrated multidrug resistance, with significant proportions producing ESBL enzymes (Ajuga *et al*., 2021). The study reported that resistance was particularly pronounced against penicillins and cephalosporins, with relatively better susceptibility observed only to aminoglycosides and carbapenems. The high rates of ESBL production reported in Aba are consistent with observations from Lagos, reinforcing the view that ESBL-mediated resistance is now entrenched among clinical *Escherichia coli* populations in Nigeria. Notably, the Aba study included isolates from a variety of sources, including urine samples, which provides valuable context for understanding the burden of resistance in urinary tract infections specifically (Ajuga *et al*., 2021).

Together, these studies demonstrate that Nigeria is experiencing a critical phase in the antibiotic resistance crisis, characterized by high prevalence of multidrug *Escherichia coli*, widespread ESBL production, and emerging carbapenem resistance. The convergence of these findings from

regional (West Africa), national (Lagos), and local (Aba) data sources creates a strong evidence base to suggest that treatment of UPEC infections with conventional antibiotics is becoming increasingly unreliable. This has significant implications for public health, as it necessitates the urgent search for alternative treatment options, including plant-derived antimicrobials such as *Hibiscus sabdariffa*, which may provide new hope for managing multidrug-resistant UPEC infections in Nigeria and beyond (Ajuga *et al*., 2021).

2.3 OVERVIEW OF URINARY TRACT INFECTIONS (UTIS)

Urinary tract infections (UTIs) remain among the most prevalent bacterial infections worldwide, significantly impacting both community and hospital settings. They are broadly classified into lower UTIs, such as cystitis affecting the bladder, and upper UTIs, such as pyelonephritis, which involves the kidneys and can lead to more severe complications (Mancuso *et al*., 2023). Recurrent UTIs represent another clinical challenge, particularly in women, where anatomical and physiological factors predispose them to repeated infections (Mancuso *et al*., 2023).

The causes of UTIs are largely bacterial, with uropathogenic *Escherichia coli* (UPEC) identified as the predominant etiological agent across diverse populations (Mancuso *et al*., 2023; Sujith *et al*., 2024). Other causative organisms include *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Enterococcus* species, though their prevalence varies regionally and is often associated with healthcare-related infections (Sujith *et al*., 2024).

Several risk factors contribute to UTI occurrence, including urinary retention, vesicoureteral reflux, sexual activity, family history, and the presence of comorbidities such as diabetes mellitus (Mancuso *et al*., 2023). Indwelling catheters and other forms of urinary instrumentation also increase susceptibility, especially in hospitalized patients (Sujith *et al*., 2024)).

The clinical presentation of UTIs depends on the site of infection. Common symptoms include dysuria, urinary frequency, urgency, suprapubic pain, and hematuria in lower UTIs, whereas upper UTIs may present with flank pain, fever, chills, and systemic signs of infection (Sujith *et al*., 2024). If untreated, UTIs can progress to severe complications such as renal scarring, sepsis, and chronic kidney disease, emphasizing the importance of timely diagnosis and effective management (Sujith *et al*., 2023).

2.4 UPEC VIRULENCE AND RESISTANCE MECHANISMS

Uropathogenic *Escherichia coli* (UPEC) are recognized as the primary cause of urinary tract infections (UTIs), and their persistence in clinical settings is largely due to their diverse virulence arsenal and ability to acquire resistance mechanisms. These strains are often classified into phylogroups that not only determine their evolutionary lineage but also influence their virulence factor expression and antimicrobial resistance profile (Mahshouri *et al*., 2025). A recent study comprehensively analyzing phylogroups, biofilm formation, and molecular typing of UPEC highlighted that particular phylogenetic clusters are strongly associated with virulence determinants and multi-drug resistance, suggesting that pathogenicity and resistance often co-evolve within the same bacterial lineages (Mahshouri *et al*., 2025).

One of the central features of UPEC pathogenicity is biofilm formation. Biofilms provide a physical barrier that protects bacterial communities from host immune responses while simultaneously reducing the effectiveness of antimicrobial therapies (Mahshouri *et al*., 2025). The ability to form robust biofilms has been consistently linked to higher levels of resistance in UPEC isolates. For instance, research conducted in Egyptian hospitals revealed a significant correlation between biofilm production, the presence of virulence determinants, and

antimicrobial resistance patterns, further confirming that biofilm-mediated protection is a crucial factor driving UPEC persistence and treatment failure (Alshaikh *et al*., 2024).

In addition to biofilms, UPEC carry a wide range of virulence genes that facilitate adherence, invasion, iron acquisition, and immune evasion. Genes encoding adhesins such as fimH, toxins like hlyA, and siderophore systems such as iutA are frequently reported in resistant isolates, suggesting a close interplay between virulence capacity and drug resistance (Alshaikh *et al*., 2024). A recent investigation focusing on resistant *Escherichia coli* strains showed that isolates harboring multiple virulence factor genes were also those most frequently associated with biofilm formation and multidrug resistance, demonstrating that virulence and resistance often accumulate within the same bacterial populations (Mumin *et al*., 2025).

Systematic reviews and meta-analyses reinforce these findings, highlighting that UPEC possess a broad array of virulence factors in parallel with a concerning rise in antibiotic resistance worldwide. This evidence illustrates how the combination of virulence and resistance mechanisms shapes the epidemiology of UTIs, especially in regions where inappropriate antimicrobial use accelerates resistance development (Bunduki *et al*., 2021). The convergence of these traits presents significant challenges to conventional treatment, thereby underscoring the urgent need to explore alternative strategies, including plant-derived antimicrobials. In line with this, natural product-based approaches are gaining significant attention. Medicinal plants have shown promising inhibitory effects against UPEC strains, including those resistant to conventional antibiotics. Extracts from plants like *Hibiscus sabdariffa* exhibit activity not only against planktonic bacterial cells but also against biofilm-associated UPEC, suggesting that phytochemicals can disrupt both resistance mechanisms and virulence expression (Marouf *et al*.,

2022). This highlights the relevance of plant-derived therapies as a dual-action strategy targeting both pathogenic traits and antimicrobial resistance in UPEC.

2.5 CURRENT TREATMENT APPROACHES AND LIMITATIONS

The current standard of care for urinary tract infections relies heavily on a range of antimicrobial agents (Mancuso *et al*., 2023). While often effective in the acute phase, this approach is facing increasing limitations, primarily driven by the global rise of antibiotic resistance (Mancuso *et al*., 2023). The widespread and sometimes inappropriate use of antibiotics has accelerated the emergence of multi-drug resistant (MDR) UPEC strains, making treatment more difficult and increasing the risk of therapeutic failure (Mancuso *et al*., 2023). This escalating resistance is a serious threat, as the development of new antibiotics has not kept pace with the evolution of resistance mechanisms in bacteria (Stracy *et al*., 2022).

Beyond the issue of resistance, other limitations of current treatments include the negative impact on the patient's native microbiome and the failure to effectively eradicate the biofilm-associated bacteria responsible for recurrent infections. The inability of many antibiotics to penetrate the biofilm matrix means that while acute symptoms may resolve, persistent bacterial reservoirs can remain, leading to relapse. Consequently, there is a pressing need to develop and implement alternative therapeutic strategies future approaches as outlined by various experts, are shifting towards non-antibiotic treatments, such as vaccine development, anti-adhesion molecules, and strategies that target biofilm formation, with the ultimate goal of minimizing the emergence of antibiotic resistance while effectively managing UPEC infections (Mancuso *et al*., 2023).

2.6 PLANT-BASED ANTIMICROBIALS

2.6.1 Traditional Use of Medicinal Plants

Medicinal plants have historically played an important role in human health, particularly in communities where access to conventional medicine was either limited or nonexistent. Across various cultures, plants have been employed for centuries as primary therapeutic agents in the treatment of infectious diseases, wounds, and systemic illnesses. This reliance on herbal medicine is not only due to accessibility but also to the accumulated traditional knowledge passed down through generations. For instance, indigenous African communities have long depended on botanicals with antimicrobial and antioxidant properties, highlighting their relevance in managing both communicable and non-communicable diseases (Gichuru *et al*., 2025).

The medicinal use of plants has historically gone far beyond simple symptom relief, encompassing broader roles such as epidemic control and wound management. Historical records reveal that many plants with antibacterial properties were incorporated into healthcare systems for their therapeutic value long before the formal discovery of antibiotics (Panda *et al*., 2025). These traditional practices have since gained scientific support, as modern pharmacological studies confirm that many of the uses observed in folk medicine correspond with measurable antibacterial activity in laboratory settings (Breijyeh *et al*., 2021). Collectively, this evidence demonstrates that traditional medicine serves not only as a cultural legacy but also as a valuable foundation for the identification and development of novel bioactive compounds.

2.6.2 Advantages of Plant-Based Antimicrobials

In recent years, there has been renewed interest in plant-based antimicrobials due to the alarming rise in antimicrobial resistance (AMR) and the stagnation of new antibiotic discovery. Unlike

conventional antibiotics, which often target specific bacterial processes and are vulnerable to resistance development, plants produce a diverse array of bioactive metabolites that act through multiple mechanisms, making resistance less likely to develop rapidly (Woo *et al*., 2023).

Another advantage of plant-derived antimicrobials lies in their accessibility, affordability, and general acceptance across diverse populations. Herbal remedies are often cheaper than synthetic drugs and are available in regions with poor healthcare infrastructure, giving them an important role in community-level healthcare (El-Saadony *et al*., 2025). Furthermore, many of these plants offer additional health benefits due to their antioxidant, anti-inflammatory, and immune-modulating properties, making them multifunctional agents in both preventive and curative medicine (Li *et al*., 2024).

Importantly, the therapeutic value of plant-based compounds is increasingly being recognized, not only as part of traditional medicine but also as potential adjuncts or alternatives to conventional antibiotics. This relevance is especially critical in the treatment of infections caused by multidrug-resistant organisms, where standard therapies are losing effectiveness. With the global antibiotic pipeline narrowing and resistance escalating, the exploration of natural antimicrobials offers a promising and urgently needed pathway in the ongoing battle against resistant pathogens (Angelini, 2024).

2.6.3 Mechanisms of Action of Plant-Based Antibacterial Agents

The antibacterial activity of plant-derived compounds is mediated through multiple mechanisms, which enhances their therapeutic value. Unlike single-target synthetic drugs, phytochemicals often exert broad-spectrum effects that disrupt bacterial survival at different levels. For instance, many phenolic and flavonoid compounds are known to interfere with bacterial cell wall and

membrane integrity, leading to increased permeability and eventual cell lysis (Zouine *et al*., 2024).

Other plant metabolites act by inhibiting bacterial protein synthesis or interfering with nucleic acid replication, thus preventing growth and proliferation (Arip *et al*., 2022). Some compounds also disrupt bacterial quorum sensing and biofilm formation, two processes critical for the persistence of pathogens such as *Escherichia coli*. By targeting these virulence mechanisms, plant-derived antimicrobials not only inhibit bacterial growth but also reduce pathogenicity (Woo *et al*., 2023).

Recent reviews emphasize that the chemical diversity of medicinal plants translates into a wide range of antibacterial strategies, including enzyme inhibition, oxidative stress induction, and interference with essential metabolic pathways (Vaou *et al*., 2021). This multiplicity of actions underscores their potential as both standalone treatments and synergistic agents alongside conventional antibiotics

Mechanisms of Action of *Hibiscus sabdariffa*

The antimicrobial potential of *Hibiscus sabdariffa* is largely attributed to its diverse array of phytochemicals, among which anthocyanins, flavonoids, phenolic acids, organic acids such as hibiscus acid, and essential oils play a critical role. These compounds act through multiple, often synergistic, mechanisms that target bacterial survival pathways, ranging from disruption of membrane integrity to inhibition of biofilm formation and modulation of protein targets. Recent advances in both *in vitro* experimentation and *in silico* molecular docking have expanded the mechanistic understanding of these effects, showing that *Hibiscus sabdariffa* exerts antimicrobial

activity through complex multitarget interactions rather than a single pathway (Ghaly *et al*., 2025).

One of the most consistently reported mechanisms involves disruption of bacterial membranes, where phytochemicals such as *hibiscus* acid and anthocyanins destabilize lipid bilayers, leading to leakage of intracellular contents and eventual cell death (Calcáneo-Martínez *et al*., 2025). This membrane perturbation is frequently coupled with increased permeability, making bacteria more susceptible to other antimicrobial agents, thereby positioning *Hibiscus sabdariffa* extracts as potential adjuvants in the management of multidrug resistant pathogens. Complementary to this, recent *in silico* studies have demonstrated that hibiscus-derived compounds interact with bacterial proteins through molecular docking approaches, suggesting a multitarget mode of action that includes interference with enzymes involved in cell wall biosynthesis and nucleic acid function (Ghaly *et al*., 2025).

Another important mechanism lies in the modulation of bacterial biofilms, which are critical in chronic and recurrent infections. Biofilm inhibition and disruption have been documented in essential oil and phenolic-rich fractions of *Hibiscus sabdariffa*, with studies showing significant anti-biofilm activity against both Gram-positive and Gram-negative organisms (Hamrita *et al*., 2022). Similarly, Calcáneo-Martínez *et al*. (2025) reported that hibiscus acid suppressed biofilm formation in *Campylobacter jejuni*, highlighting the relevance of this mechanism in pathogenic bacteria known for their persistence and antibiotic tolerance. Such findings align with broader evidence that phenolic compounds in *Hibiscus sabdariffa* not only kill planktonic bacterial cells but also compromise the structural stability of biofilms, an effect that is crucial for therapeutic relevance.

Anthocyanins and flavonoids, which contribute to the plant's characteristic deep red pigmentation, have been shown to possess antibacterial and antioxidant effects that complement their membrane-disrupting activities. These compounds exert significant antibacterial activity through oxidative stress induction and inhibition of bacterial growth, particularly against both Gram-positive and Gram-negative species (Ghaly *et al*., 2025). These activities are complemented by phenolic acids, which interact with microbial enzymes and impair metabolic processes, as demonstrated by Hamrita *et al*. (2022) in their combined *in vitro* and *in silico* modeling study. Such results suggest that the synergy of multiple classes of phytochemicals underlies the broad-spectrum antimicrobial action observed in roselle extracts.

In addition, recent reviews have emphasized that the antimicrobial efficacy of *Hibiscus sabdariffa* cannot be attributed to a single class of compounds, but rather to the collective interplay of flavonoids, anthocyanins, organic acids, and other bioactive molecules working synergistically to overcome bacterial resistance mechanisms (Mancuso *et al*., 2023). This multifaceted approach positions *Hibiscus sabdariffa* as a promising candidate for addressing the growing challenge of antimicrobial resistance in clinical settings.

2.7 Methods for Assessing Antimicrobial Activity

Assessing the antibacterial activity of various compounds, especially novel therapeutic agents, is a fundamental step in antimicrobial research. Several standardized methods are employed to determine the efficacy of an antimicrobial agent against bacterial pathogens. The major methods are the diffusion and dilution methods, some examples include:

Agar Well Diffusion Method

The agar well diffusion method is a commonly utilized technique for assessing the antimicrobial properties of plant or microbial extracts. This method is similar to the disk-diffusion approach.

Initially, the surface of an agar plate is inoculated by evenly spreading a microbial inoculum across it. Next, a sterile cork borer or tip is used to aseptically create a hole with a diameter of 6 to 8 mm, into which a volume of 20 to 100 ml of the antimicrobial agent or extract solution at the desired concentration is added. The agar plates are then incubated under appropriate conditions based on the specific microorganism being tested. As the antimicrobial agent diffuses through the agar medium, it inhibits the growth of the tested microbial strain. (Gonzalez-Pastor *et al*., 2023).

Minimum Inhibitory Concentration/ Minimum Bactericidal Concentration

The tube dilution method is mainly used in determining minimum inhibitory concentration (MIC) which is the least concentration of antimicrobial agent required to inhibit microbial growth as well as determination of minimum bactericidal concentration (MBC) which is the least concentration of antimicrobial agent required to kill organisms. In carrying out the MIC test, a serial dilution of antibiotic agents is prepared in sterile nutrient broth within test tubes. A standardized inoculum is then introduced into each test tube containing 1 ml of the different dilutions of the test substance (antibiotics agents). The test tubes are then incubated at 37°C for a duration of 18 to 24 hours, after which they are assessed for any signs of growth or turbidity. After establishing the MIC using broth dilution techniques, samples from wells without visible growth *i.e*., at and above the MIC are subcultured onto antibiotic-free agar plates such as Mueller-Hinton agar. These plates are then incubated under standard conditions. The MBC is identified as the lowest concentration at which no bacterial colonies grow or at which there is a ≥99.9% reduction in colony-forming units (Gajic *et al*., 2022).

Time-Kill Kinetics (Time-Kill Curve)

The time-kill assay is a widely used *in vitro* method to evaluate the bactericidal or fungicidal activity of antimicrobial agents. It provides critical insights into the dynamic interactions between an antimicrobial substance and a microbial strain, allowing determination of whether the agent exhibits time-dependent or concentration-dependent killing effects (Ahmad *et al*., 2022).

For bacterial assessments, the assay typically involves preparing a standardized inoculum in a suitable broth medium. Tubes containing the microbial suspension are treated with different concentrations of the test substance, while a separate tube serves as a growth control. Samples are taken at predefined time points commonly 0, 4, 6, 8, 10, 12, and 24 hours and viable cells are enumerated using the agar plate count method. The percentage reduction in colony-forming units (CFU/mL) compared to the control is calculated to determine bactericidal activity (Odonkor *et al*., 2022).

Typically, a bactericidal effect is indicated by a lethality rate of 90% after 6 hours, which corresponds to a 99.9% lethality rate after 24 hours. (Ahmad *et al*., 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was carried out in pharmaceutical microbiology laboratory, University of Benin, Edo state.

3.2 Materials

3.2.1 Microbiological Media

Nutrient agar, Nutrient broth and Mueller Hinton agar

3.2.2 Equipment and Apparatus

Portable autoclave, weighing balance, hot air oven, mortar, pestle, mechanical grinding machine, micropipette, incubator, Whatman filter paper, cotton wool, pipette tip, a corkborer (10mm in diameter), a transparent millimetre rule, grease pencil, sterile swab sticks, tripod stand, Bunsen burner, foil paper, dried plant material.

3.2.3 Glassware

Conical flask, bottles (MacCartney, universal and Bijou) as well as test tubes, pipettes, glass stirrers, porcelain dish, pestle, maceration jars, glass funnels, beakers, measuring cylinders, and Petri dishes.

3.2.4 Chemicals and Reagents

Tween 80, distilled water. Disinfectant: Purit, soap, detergent.

3.2.5 Clinical Isolates

The isolates used include uropathogenic *Escherichia coli* (UPEC), obtained from urine samples of patients clinically diagnosed with urinary tract infections.

3.3 Methods

3.3.1 Collection and Identification of *Hibiscus sabdariffa*

The dried calyces of *Hibiscus sabdariffa* (Roselle) were purchased from Ring Road Market in Oredo Local Government Area, Edo State, Nigeria. Identification of the plant material was carried out by careful physical examination of the morphological features, including the characteristic deep red calyces and funnel-shaped flowers, and comparison with standard descriptions in relevant botanical literature (Caballero *et al.,* 2024).

3.3.2 Preparation of Crude Extract

The *Hibiscus sabdariffa* calyces were obtained in dried form and were visually inspected before processing. Approximately 250g of the calyces were macerated in 1 L of 70% ethanol, while 250 g were macerated in 2L of distilled water for 72 hours. They were stirred at intervals to allow for proper permeation of the extraction solvent. A double filtration using Whatman filter paper was carried out, and the filtrate was concentrated in a hot air oven at 50^{o}C. The extracts were weighed and refrigerated at 4^{o}C in an airtight container to preserve them for use.

3.3.3 Extract Yield

% Yield = (Weight of dry extract / Initial weight of plant material) × 100

3.4 Specimen Collection

Microorganisms used in this study were isolates obtained from sensitivity plates of already confirmed *Escherichia coli* cultures at the Medical Microbiology laboratory of the University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. A total of eight isolates, each derived from separate sensitivity plates, were collected. The isolates were preserved on Nutrient agar

slants for subsequent use. Selection was based on antimicrobial susceptibility profiles, with only multidrug-resistant (MDR) isolates.

3.4.1 Preparation of Test Organisms

The eight *Escherichia coli* isolates obtained were first maintained on Nutrient agar slants at 4 °C until required for experimentation. For long-term preservation, aliquots of the isolates were also stored in 20% glycerol broth and frozen.

Prior to antimicrobial testing, each isolate was sub-cultured onto freshly prepared Mueller Hinton agar plates and incubated at 37 °C for 18–24 hours. Distinct colonies from these overnight cultures were suspended in sterile nutrient broth and incubated for 12 hours. The resulting bacterial suspensions were standardized to match the 0.5 McFarland turbidity standard, corresponding to an inoculum density of approximately 1.0×10^7 CFU/mL. This standardized suspension was further diluted 1:100 in sterile broth to obtain a working inoculum of approximately 1.0×10^5 CFU/mL.

All antimicrobial tests were carried out in two stages. In the first stage, the extracts were tested against the resistant *Escherichia coli* isolates, where resistance was assumed to be plasmid-mediated. In the second stage, the same isolates were tested again after plasmid curing, when resistance was expected to be lost.

3.5 Antimicrobial Assays

3.5.1 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration was carried out using the agar well diffusion method (Itemire and Idu, 2024) with some modifications. Sterile Mueller Hinton agar was prepared and 30 ml was poured into Petri dishes aseptically and allowed to solidify. The Petri dishes were dried in a hot air oven at 40°C for about 10 minutes. The dried agar surface was then streaked with the

isolates using a swab stick aseptically. A sterile cork borer (10mm) was used to bore 5 wells in each agar plate. The base of the wells were sealed with 0.02 ml of molten agar. The wells were filled with 0.25 ml of 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml of the extract. The plates were incubated at 37°C for 24 hours. The inhibition zone diameters (IZD) were measured using a ruler in millimeters.

3.5.2 Determination of Minimum Bactericidal Concentration (MBC)

Minimum bactericidal concentration) of the aqueous and ethanol extracts was determined using a modified broth dilution method. Serial dilutions of the extracts were prepared in nutrient broth at concentrations of 200 mg/ml, 100 mg/ml, and 50 mg/ml. Each dilution was inoculated with the bacterial suspension and incubated at 37 °C for 24 hours. After incubation, 0.1 ml of broth from each well was aseptically streaked onto the surface of fresh sterile nutrient agar plates. The plates were then incubated at 37 °C for 48 hours and examined for bacterial growth. The lowest concentration of the extract at which no bacterial colonies were observed on the agar surface was recorded as the MBC.

3.5.3 Determination of Time-Kill Kinetics

Time-kill kinetics of ethanol and aqueous extracts of *Hibiscus sabdariffa* leaves were carried out following the procedure described by Rodríguez-Melcón *et al*. (2022). Concentrations equal to four times the MIC of the extracts were prepared in sterile nutrient broth and inoculated with 0.02 ml of 10^6 CFU/ml test bacterial isolates. The test isolates in late logarithmic growth phase was used for the assay. Aliquots of 20 µL of the medium were taken at time intervals of 0 min, 30 min, 1 hr, 2 hrs, 4 hrs, 6 hrs, and 24 hrs and plated out aseptically on sterile nutrient agar plates. The plates were incubated at 37°C for 24 hrs. A control test was performed for the organisms without the extracts. The colony forming units (CFU) of the bacteria after incubation

were counted and compared with control in terms of CFU/mL. A graph of the $\log_{10}$ of CFU/mL was plotted against time.

3.6 Preparation of McFarland Standard

A 0.5 McFarland standard solution was prepared by adding 0.5ml of 1.175 % (weight/volume). Barium Chloride dihydrate salt ($BaCl_2.2H_2O$) to 9.95 ml of 1 % Sulfuric acid (H_2SO_4). The two solutions were mixed completely to form a turbid suspension in a test tube which is then placed in a test tube rack and kept at room temperature before use.

3.7 Statistical Analysis

Statistical analysis was conducted using the GraphPad Prism software version 10.2.2. A two-way ANOVA was performed to compared between the zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts both before and after plasmid curing. A nonlinear regression analysis was performed to analyse the time-kill kinetics of the *Hibiscus sabdariffa* ethanol and aqueous extracts on the multi-drug resistant uropathogenic *Escherichia coli*. Results are presented as mean ± SEM in tables and plotted on bar charts and line graphs.

CHAPTER FOUR

RESULTS

4.1 Extract Yield and properties

The yield for ethanol extract is 4% while the yield for aqueous extract is 44.8%. The ethanol extract was dark reddish-brown, slightly viscous extract, whereas the aqueous extract was dark red and more viscous.

4.2 Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) of *Hibiscus sabdariffa* aqueous and ethanol extracts against the multidrug resistant uropathogenic *Escherichia coli* isolates showed that the ethanol extract had the lowest MIC value of 25.00 ± 0.00 mg/ml both before and after plasmid curing, while the aqueous extract recorded a higher MIC value of 100.00 ± 0.00 mg/ml before plasmid curing. However, plasmid curing significantly improved the antimicrobial potency of the aqueous extract, reducing the MIC to 50.00 ± 0.00 mg/ml.

4.3 Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of *Hibiscus sabdariffa* extracts against multidrug resistant uropathogenic *Escherichia coli* isolates showed that the ethanol extract recorded the lowest MBC value of 50.00 ± 0.00 mg/ml both before and after plasmid curing, while the aqueous extract had a higher MBC value of 200.00 ± 0.00 mg/ml before plasmid curing. Plasmid curing enhanced the bactericidal activity of the aqueous extract, reducing the MBC to 100.00 ± 0.00 mg/ml.

4.4 Bacterial Time-Kill Kinetics

The time-kill kinetics showed that both aqueous and ethanol extracts of *Hibiscus sabdariffa* exhibited bactericidal effects against multidrug resistant uropathogenic *Escherichia coli*. The ethanol extract reduced bacterial counts significantly within 6 hours, while the aqueous extract showed a slower effect, with reduction observed up to 24 hours, as shown in Figure 5.

Table 4.1: ZONE OF INHIBITION BEFORE PLASMID CURING (mm)

The antibacterial activity of *Hibiscus sabdariffa* extracts before plasmid curing showed noticeable variation across different concentrations and extract types. Table 4.1 presents the mean zones of inhibition (mm) produced by the ethanolic and aqueous extracts against MDR *E. coli* isolates. The ethanolic extract exhibited higher inhibitory activity compared to the aqueous extract across all concentrations. At 200 mg/mL, inhibition zones ranged between 25 mm and 30 mm for most isolates, while moderate activity was observed at 100 mg/mL (20–26 mm). Lower concentrations (50–12.5 mg/mL) generally showed reduced or no inhibitory effect, indicating a dose-dependent response. In contrast, the aqueous extract demonstrated weaker antibacterial activity, with measurable inhibition mainly at 200 mg/mL and 100 mg/mL, and no zones of inhibition (NZ) at lower concentrations. The minimum inhibitory concentration (MIC) values were lower for the ethanolic extract (25–100 mg/mL) compared to the aqueous extract (100–200 mg/mL), suggesting greater potency of the ethanolic fraction.

Table 4.1: ZONE OF INHIBITION BEFORE PLASMID CURING (mm)

	ETHANOL EXTRACT						AQUEOUS EXTRACT					
	200 mg/ml	**100 mg/ml**	**50mg/ml**	**25mg/ml**	**12.5 mg/ml**	**MIC mg/ml**	**200 mg/ml**	**100 mg/ml**	**50mg/ml**	**25mg/ml**	**12.5 mg/ml**	**MIC mg/ml**
1	30	25	15	NZ	NZ	50	20	16	NZ	NZ	NZ	100
2	28	24	14	NZ	NZ	50	17	14	NZ	NZ	NZ	100
3	30	26	16	NZ	NZ	50	17	14	NZ	NZ	NZ	100
4	25	22	NZ	NZ	NZ	100	20	18	NZ	NZ	NZ	100
5	26	22	12	NZ	NZ	50	17	14	NZ	NZ	NZ	100
6	27	24	12	NZ	NZ	50	16	13	NZ	NZ	NZ	100
7	25	20	NZ	NZ	NZ	100	12	NZ	NZ	NZ	NZ	200
8	30	25	16	12	NZ	25	17	13	NZ	NZ	NZ	100

KEY

NZ = No zone of inhibition

Table 4.2: ZONE OF INHIBITION AFTER PLASMID CURING (mm)

After plasmid curing, the antibacterial activity of *Hibiscus sabdariffa* extracts showed improved inhibition patterns across most isolates. Table 4.2 presents the zones of inhibition (mm) for both ethanolic and aqueous extracts following plasmid removal. The ethanolic extract maintained strong antibacterial activity, with inhibition zones ranging from 25–30 mm at 200 mg/mL and 23–26 mm at 100 mg/mL. Notably, measurable inhibition was also observed at 50 mg/mL and occasionally at 25 mg/mL, while no inhibition occurred at the lowest concentration (12.5 mg/mL). The aqueous extract, though generally less active, showed a slight increase in inhibition compared to pre-curing values, particularly at 200 mg/mL and 100 mg/mL, with inhibition zones between 15–22 mm. Lower concentrations produced little or no inhibition (NZ). The MIC values decreased for most isolates post-curing, with the ethanolic extract showing MICs as low as 25 mg/mL and the aqueous extract ranging between 50–200 mg/mL, suggesting that plasmid curing enhanced susceptibility of the bacterial isolates to the plant extracts.

Table 4.2: ZONE OF INHIBITION AFTER PLASMID CURING (mm)

	ETHANOL EXTRACT						AQUEOUS EXTRACT					
	200 mg/ ml	100 mg/ ml	50m g/ml	25m g/ml	12.5 mg/m l	MIC mg /ml	**200m g/ml**	**100m g/ml**	**50m g/ml**	**25m g/ml**	**12.5 mg/m l**	**MIC mg /ml**
1	28	23	15	12	NZ	25	22	18	NZ	NZ	NZ	100
2	28	24	14	11	NZ	25	20	18	14	NZ	NZ	50
3	28	23	15	11	NZ	25	20	17	NZ	NZ	NZ	100
4	28	24	14	NZ	NZ	50	20	16	NZ	NZ	NZ	100
5	28	26	14	NZ	NZ	50	19	15	NZ	NZ	NZ	100
6	29	25	15	NZ	NZ	50	20	16	NZ	NZ	NZ	100
7	30	25	15	12	NZ	25	15	NZ	NZ	NZ	NZ	200
8	30	26	16	12	NZ	25	19	14	NZ	NZ	NZ	100

KEY

NZ = No zone of inhibition

Table 4.3: MINIMUM BACTERICIDAL CONCENTRATION BEFORE PLASMID CURING

The minimum bactericidal concentration (MBC) values of *Hibiscus sabdariffa* extracts before plasmid curing are presented in Table 4.3. The results showed that the ethanolic extract demonstrated stronger bactericidal activity compared to the aqueous extract. For the ethanolic extract, no bacterial growth (NG) was observed at concentrations of 50 mg/mL and above for all isolates, giving a consistent MBC value of 50 mg/mL. This indicates that the ethanolic extract could completely eliminate viable bacterial cells at relatively low concentrations. In contrast, the aqueous extract showed visible bacterial growth (G) at most concentrations, with only a few isolates exhibiting complete inhibition. The MBC values for the aqueous extract ranged between 50 mg/mL and 200 mg/mL, suggesting a weaker bactericidal effect.

Table 4.3: MINIMUM BACTERICIDAL CONCENTRATION BEFORE PLASMID CURING

	ETHANOL EXTRACT				AQUEOUS EXTRACT			
	200mg/ml	**100mg/ml**	**50mg/ml**	MBC mg/ml	**200mg/ml**	**100mg/ml**	**50mg/ml**	**MBC mg/ml**
1	NG	NG	NG	50	NG	NG	G	100
2	NG	NG	NG	50	NG	NG	G	100
3	NG	NG	NG	50	NG	NG	G	100
4	NG	NG	NG	50	NG	G	G	200
5	NG	NG	NG	50	NG	NG	G	100
6	NG	NG	NG	50	NG	NG	NG	50
7	NG	NG	NG	50	NG	NG	NG	50
8	NG	NG	NG	50	NG	NG	G	100

KEY

NG: No growth

G: Growth

Table 4.4: MINIMUM BACTERICIDAL CONCENTRATION AFTER PLASMID CURING

Following plasmid curing, the minimum bactericidal concentration (MBC) results of *Hibiscus sabdariffa* extracts are shown in Table 4.4. The ethanolic extract maintained consistent bactericidal activity across all isolates, with no bacterial growth (NG) observed at 50 mg/mL and higher concentrations, resulting in uniform MBC values of 50 mg/mL. This indicates that the curing process did not diminish the efficacy of the ethanolic extract. In contrast, the aqueous extract displayed slight improvement compared to pre-curing results, as several isolates that previously showed growth (G) at lower concentrations now exhibited no growth at 50 mg/mL or 100 mg/mL. The MBC values for the aqueous extract ranged between 50 mg/mL and 100 mg/mL, reflecting increased susceptibility of the cured isolates to the extract.

Table 4.4: MINIMUM BACTERICIDAL CONCENTRATION AFTER PLASMID CURING

	ETHANOL EXTRACT				AQUEOUS EXTRACT			
	200mg/ml	**100mg/ml**	**50mg/ml**	MBC mg/ml	**200mg/ml**	**100mg/ml**	**50mg/ml**	**MBC mg/ml**
1	NG	NG	NG	50	NG	NG	G	100
2	NG	NG	NG	50	NG	NG	G	100
3	NG	NG	NG	50	NG	NG	G	100
4	NG	NG	NG	50	NG	NG	NG	50
5	NG	NG	NG	50	NG	NG	G	100
6	NG	NG	NG	50	NG	NG	NG	50
7	NG	NG	NG	50	NG	NG	NG	50
8	NG	NG	NG	50	NG	NG	NG	50

NG: No growth, G: Growth

Table 4.5 : TIME KILL KINETICS OF AQUEOUS AND ETHANOL EXTRACTS (PRE-CURING)

The time–kill kinetics of *Hibiscus sabdariffa* extracts before plasmid curing are presented in Table 4.5. The results illustrate the rate at which the ethanolic and aqueous extracts reduced viable bacterial counts over a 24-hour period at different concentrations. Both extracts exhibited progressive bactericidal activity with increasing exposure time. At 0 hour, bacterial growth was too numerous to count (TNTC) across all concentrations, but a gradual decline in colony counts was observed from 30 minutes to 4 hours. The ethanolic extract showed slightly faster killing rates than the aqueous extract, particularly at higher concentrations (100–200 mg/mL). Complete bacterial elimination (no detectable growth) occurred at 6 hours for both extracts, and this effect was maintained through 24 hours, indicating time- and concentration-dependent bactericidal activity of the plant extracts before plasmid curing.

Table 4.5 : TIME KILL KINETICS OF AQUEOUS AND ETHANOL EXTRACTS (PRE-CURING)

	Ethanol		Aqueous		
Time/Concentration	**50**	**100**	**100**	**200**	**Control**
0 hours	**TNTC**	**TNTC**	**TNTC**	**TNTC**	**TNTC**
30 mins	**100**	**82**	**80**	**72**	
1 hour	**64**	**56**	**60**	**58**	**TNTC**
2 hours	**50**	**48**	**50**	**40**	
4 hours	**18**	**15**	**18**	**15**	
6 hours	**0**	**0**	**0**	**0**	
24 hours	**0**	**0**	**0**	**0**	

TNTC= Too numerous to count

Table 4.6: TIME KILL KINETICS OF AQUEOUS AND ETHANOL EXTRACTS (POST-CURING)

The time–kill kinetics of *Hibiscus sabdariffa* extracts after plasmid curing are shown in Table 4.6. The results demonstrate enhanced bactericidal activity of both ethanolic and aqueous extracts against the cured *E. coli* isolates. At the onset (0 hour), bacterial counts were markedly lower compared to pre-curing values, indicating increased susceptibility following plasmid removal. A rapid reduction in viable cell counts was observed within the first 30 minutes to 2 hours, especially at higher extract concentrations (100–200 mg/mL). The ethanolic extract achieved complete bacterial elimination earlier than the aqueous extract, with no detectable growth recorded from the 4th hour onward. Both extracts maintained total kill up to 24 hours, confirming time-dependent bactericidal action and greater overall effectiveness after plasmid curing.

Table 4.6: TIME KILL KINETICS OF AQUEOUS AND ETHANOL EXTRACTS (POST-CURING)

	Ethanol		Aqueous		
Time/Concentration	**50**	**100**	**100**	**200**	**Control**
0 hours	**6**	**3**	**102**	**2**	**TNTC**
30 mins	**2**	**1**	**2**	**2**	
1 hour	**1**	**1**	**2**	**2**	
2 hours	**1**	**1**	**2**	**0**	
4 hours	**1**	**1**	**0**	**0**	**TNTC**
6 hours	**0**	**0**	**0**	**0**	
24 hours	**0**	**0**	**0**	**0**	

TNTC= Too numerous to count

Table 4.7: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts before plasmid curing

Table 4.7 presents the mean zones of inhibition (mm) of *Hibiscus sabdariffa* ethanol and aqueous extracts against multidrug-resistant *Escherichia coli* isolates before plasmid curing. The results reveal that the ethanolic extract exhibited stronger antibacterial activity compared to the aqueous extract at all tested concentrations. At 200 mg/mL, the ethanol extract produced a mean inhibition zone of 28.50 ± 0.72 mm, whereas the aqueous extract recorded 17.33 ± 0.56 mm. Similarly, at 100 mg/mL, inhibition by the ethanol extract (24.33 ± 0.56 mm) was greater than that of the aqueous extract (14.00 ± 0.45 mm). This pattern indicates a concentration-dependent antibacterial effect, with the ethanolic extract demonstrating higher efficacy against the resistant *E. coli* isolates prior to plasmid curing.

Table 4.7: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts before plasmid curing

Concentration	Ethanol extract	Aqueous extract
200 mg/mL	28.50 ± 0.72	17.33 ± 0.56
100 mg/mL	24.33 ± 0.56	14.00 ± 0.45

Table 4.8: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts after plasmid curing

Table 4.8 shows the mean zones of inhibition (mm) of *Hibiscus sabdariffa* ethanol and aqueous extracts against multidrug-resistant *Escherichia coli* isolates after plasmid curing. The results indicate that antibacterial activity improved slightly following plasmid removal, particularly for the aqueous extract. At 200 mg/ml, the ethanol extract produced a mean inhibition zone of 28.43 ± 0.30 mm, while the aqueous extract recorded 20.00 ± 0.38 mm. Similarly, at 100 mg/mL, inhibition zones of 24.43 ± 0.48 mm and 16.29 ± 0.57 mm were observed for the ethanol and aqueous extracts, respectively. Although both extracts remained effective, the ethanolic extract continued to exhibit superior antibacterial potency, confirming its higher efficacy against the cured *E. coli* isolates.

Table 4.8: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts after plasmid curing

Concentration	Ethanol extract	Aqueous extract
200 mg/mL	28.43 ± 0.30	20.00 ± 0.38
100 mg/mL	24.43 ± 0.48	16.29 ± 0.57

Table 4.9: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before and after plasmid curing

Table 4.9 compares the mean zones of inhibition (mm) produced by the ethanolic extract of *Hibiscus sabdariffa* against multidrug-resistant *Escherichia coli* isolates before and after plasmid curing. The results show minimal variation in antibacterial activity between the two conditions. At 200 mg/mL, inhibition zones remained almost identical (28.50 ± 0.72 mm before curing and 28.50 ± 0.34 mm after curing). Similarly, at 100 mg/mL and 50 mg/mL, only slight increases in inhibition zones were observed after curing, from 24.33 ± 0.56 mm to 24.50 ± 0.56 mm, and from 14.17 ± 0.75 mm to 14.83 ± 0.31 mm, respectively. These findings suggest that plasmid curing had little effect on the antibacterial potency of the ethanolic extract, indicating that the observed activity may not be strongly influenced by plasmid-mediated resistance.

Table 4.9: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before and after plasmid curing

Concentration	Before plasmid curing	After plasmid curing
200 mg/mL	28.50 ± 0.72	28.50 ± 0.34
100 mg/mL	24.33 ± 0.56	24.50 ± 0.56
50 mg/mL	14.17 ± 0.75	14.83 ± 0.31

Table 4.10: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before and after plasmid curing

Table 4.10 presents the mean zones of inhibition (mm) of *Hibiscus sabdariffa* aqueous extract against multidrug-resistant *Escherichia coli* isolates before and after plasmid curing. The results indicate a noticeable increase in antibacterial activity following plasmid removal. At 200 mg/mL, the mean inhibition zone increased from 17.71 ± 0.61 mm before curing to 20.00 ± 0.38 mm after curing, while at 100 mg/mL, it rose from 14.57 ± 0.69 mm to 16.29 ± 0.57 mm. This improvement suggests that the bacterial isolates became more susceptible to the aqueous extract after plasmid curing, implying that plasmid-mediated resistance previously reduced the extract's effectiveness.

Table 4.10: Zone of inhibition (in mm) of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before and after plasmid curing

Concentration	Before plasmid curing	After plasmid curing
200 mg/mL	17.71 ± 0.61	20.00 ± 0.38
100 mg/mL	14.57 ± 0.69	16.29 ± 0.57

Table 4.11: MIC and MBC of the ethanol and the aqueous extracts of *Hibiscus sabdariffa* with multi-drug resistant uropathogenic *Escherichia coli*

Table 4.11 summarizes the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic and aqueous extracts of *Hibiscus sabdariffa* against multidrug-resistant *Escherichia coli* isolates before and after plasmid curing. The ethanolic extract maintained consistent activity, with MIC and MBC values of 25 mg/mL and 50 mg/mL, respectively, both before and after curing. In contrast, the aqueous extract showed improved efficacy following plasmid removal, as the MIC decreased from 100 mg/mL to 50 mg/mL and the MBC reduced from 200 mg/mL to 100 mg/ml. These results indicate that the ethanolic extract exhibited strong and stable antibacterial potency, while the aqueous extract became more effective against the cured isolates, suggesting partial involvement of plasmid-mediated resistance in limiting its activity prior to curing.

Table 4.11: MIC and MBC of the ethanol and the aqueous extracts of *Hibiscus sabdariffa* with multi-drug resistant uropathogenic *Escherichia coli*

Type of extract	Parameter	Before plasmid curing	After plasmid curing
Ethanol extract	MIC	25 mg/Ml	25 mg/mL
	MBC	50 mg/mL	50 mg/mL
Aqueous extract	MIC	100 mg/mL	50 mg/mL
	MBC	200 mg/mL	100 mg/mL

Table 4.12: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* before plasmid curing

Table 4.12 presents the time–kill kinetics of multidrug-resistant *Escherichia coli* treated with the ethanolic extract of *Hibiscus sabdariffa* before plasmid curing. The results show a gradual reduction in viable bacterial counts over time, indicating time-dependent bactericidal activity. At both 50 mg/mL and 100 mg/mL concentrations, bacterial populations decreased steadily from 30 minutes to 4 hours, with corresponding declines in log CFU/mL values. Complete elimination of viable cells was achieved by the 6th hour at both concentrations, demonstrating the extract's strong antibacterial potential and confirming that its efficacy increases with prolonged exposure

Table 4.12: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* before plasmid curing

	100 mg/mL concentration		**50 mg/mL concentration**	
Elapsed time (h:mm:ss)	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***
0:30:00	82	1.914	100	2.000
1:00:00	56	1.748	64	1.806
2:00:00	48	1.681	50	1.699
4:00:00	15	1.176	18	1.255
6:00:00	0		0	

Table 4.13: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* after plasmid curing

Table 4.13 shows the time-kill kinetics of multi-drug resistant *Escherichia coli* isolates after plasmid curing when exposed to ethanol extract of *Hibiscus sabdariffa* at concentrations of 100 mg/mL and 50 mg/mL. At the start of the experiment (0 hour), viable counts of 3 CFU/mL and 6 CFU/mL were observed for the 100 mg/mL and 50 mg/mL concentrations respectively, corresponding to logarithmic values of 0.477 and 0.778. After 30 minutes of exposure, the viable counts reduced to 1 CFU/mL (log 0) and 2 CFU/mL (log 0.301) respectively, showing a marked decline in bacterial load.

At 1 hour and beyond (2, 4, and 6 hours), no further increase in viable count was recorded for either concentration, indicating that bacterial growth was effectively inhibited. Complete elimination of viable cells was observed at the 6-hour mark for both concentrations, confirming the bactericidal effect of the extract against the plasmid-cured multi-drug resistant *E. coli.*

Table 4.13: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with ethanol extract of *Hibiscus sabdariffa* after plasmid curing

	100 mg/mL concentration		**50 mg/mL concentration**	
Elapsed time (h:mm:ss)	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***
0:00:00	3	0.477	6	0.778
0:30:00	1	0	2	0.301
1:00:00	1	0	1	0
2:00:00	1	0	1	0
4:00:00	1	0	1	0
6:00:00	0		0	

Table 4.14: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* before plasmid curing

Table 4.14 shows the time kill kinetics of the aqueous extract of *Hibiscus sabdariffa* against multi-drug resistant *Escherichia coli* before plasmid curing at concentrations of 200 mg/mL and 100 mg/mL. At the start of exposure (0:30:00), viable counts of 72 CFU/mL and 80 CFU/mL were recorded for the 200 mg/mL and 100 mg/mL concentrations respectively, corresponding to log values of 1.857 and 1.903. After 1 hour, a gradual decrease was observed with 58 CFU/mL (log 1.763) and 60 CFU/mL (log 1.778) respectively. Further reduction occurred at 2 hours with 40 CFU/mL (log 1.602) and 50 CFU/mL (log 1.699). By 4 hours, viable counts dropped to 15 CFU/mL (log 1.176) and 18 CFU/mL (log 1.255). Complete elimination of viable *E. coli* cells was achieved after 6 hours of exposure for both concentrations, indicating time-dependent bactericidal activity of the aqueous extract.

Table 4.14: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* before plasmid curing

	200 mg/mL concentration		**100 mg/mL concentration**	
Elapsed time (h:mm:ss)	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***
0:30:00	72	1.857	80	1.903
1:00:00	58	1.763	60	1.778
2:00:00	40	1.602	50	1.699
4:00:00	15	1.176	18	1.255
6:00:00	0		0	

Table 4.15: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* after plasmid curing

Table 4.15 presents the time kill kinetics of the aqueous extract of *Hibiscus sabdariffa* against multi-drug resistant *Escherichia coli* after plasmid curing at concentrations of 200 mg/mL and 100 mg/mL. At time 0, viable counts were 2 CFU/mL (log 0.301) and 102 CFU/mL (log 2.000) for 200 mg/mL and 100 mg/mL respectively. After 30 minutes, bacterial counts dropped to 2 CFU/mL (log 0.301) for both concentrations, and by 1 hour, the viable counts further reduced to 1 CFU/mL (log 0) at 200 mg/mL while remaining at 2 CFU/mL (log 0.301) at 100 mg/mL. Complete bacterial elimination occurred at 2 hours for the 200 mg/mL concentration, whereas total kill was observed at 4 hours for the 100 mg/mL concentration. These results indicate a faster and stronger bactericidal effect of the aqueous extract following plasmid curing, suggesting that plasmid removal enhanced the susceptibility of the uropathogenic *E. coli* to *Hibiscus sabdariffa* extract.

Table 15: Time kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* treated with aqueous extract of *Hibiscus sabdariffa* after plasmid curing

	200 mg/mL concentration		**100 mg/mL concentration**	
Elapsed time (h:mm:ss)	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***	**Number of viable *Escherichia coli* (CFU/mL)**	**Log of viable *Escherichia coli***
0:00:00	2	0.301	102	2.000
0:30:00	2	0.301	2	0.301
1:00:00	1	0	2	0.301
2:00:00	0		2	0.301
4:00:00	0		0	
6:00:00	0		0	

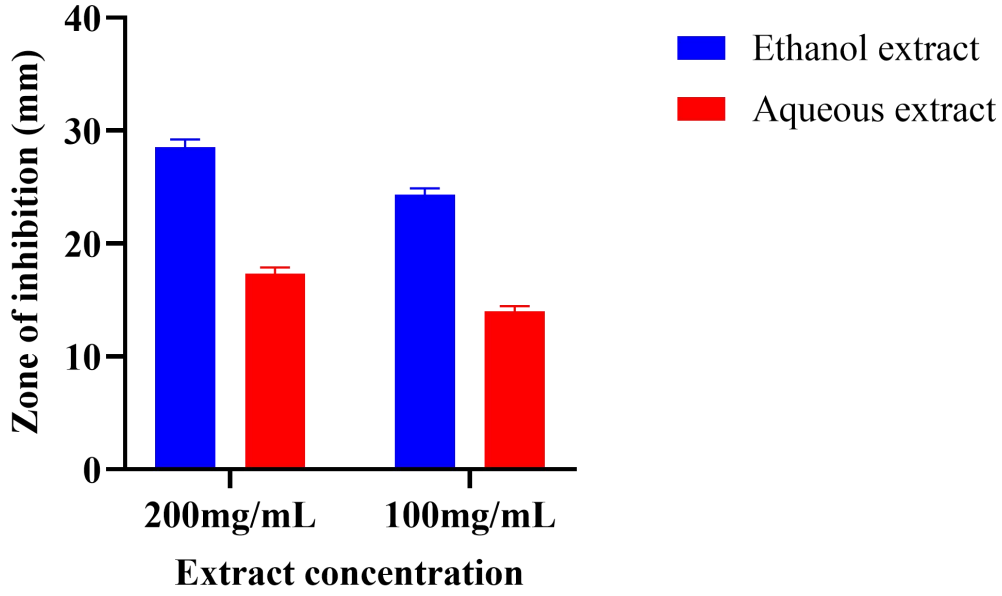

Figure 1: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts before plasmid curing

Higher concentrations of *Hibiscus sabdariffa* extract, irrespective of extract type, produced greater zones of the inhibition of *Escherichia coli* than the lower concentrations before plasmid curing, $F(1, 20) = 42.01$, $P < 0.0001$. 200 mg/mL of *Hibiscus sabdariffa* ethanol extract produced greater zones of inhibition of *Escherichia coli* than the 100 mg/mL concentration of the same extract. Likewise, 200 mg/mL of *Hibiscus sabdariffa* aqueous extract produced greater zones of inhibition of *Escherichia coli* than the 100 mg/mL concentration of the same extract.

However, the ethanol extract of *Hibiscus sabdariffa* produced greater zones of inhibition than the aqueous extract *Hibiscus sabdariffa* at the same concentrations, $F(1, 20) = 345.2$, $P < 0.0001$. 200 mg/mL of *Hibiscus sabdariffa* ethanol extract produced a greater zone of inhibition of *Escherichia coli* than that produced by the 200 mg/mL of *Hibiscus sabdariffa* aqueous extract. Likewise, 100 mg/mL of *Hibiscus sabdariffa* ethanol extract produced a greater zone of inhibition of *Escherichia coli* than those produced by the 100 mg/mL of *Hibiscus sabdariffa* aqueous extract.

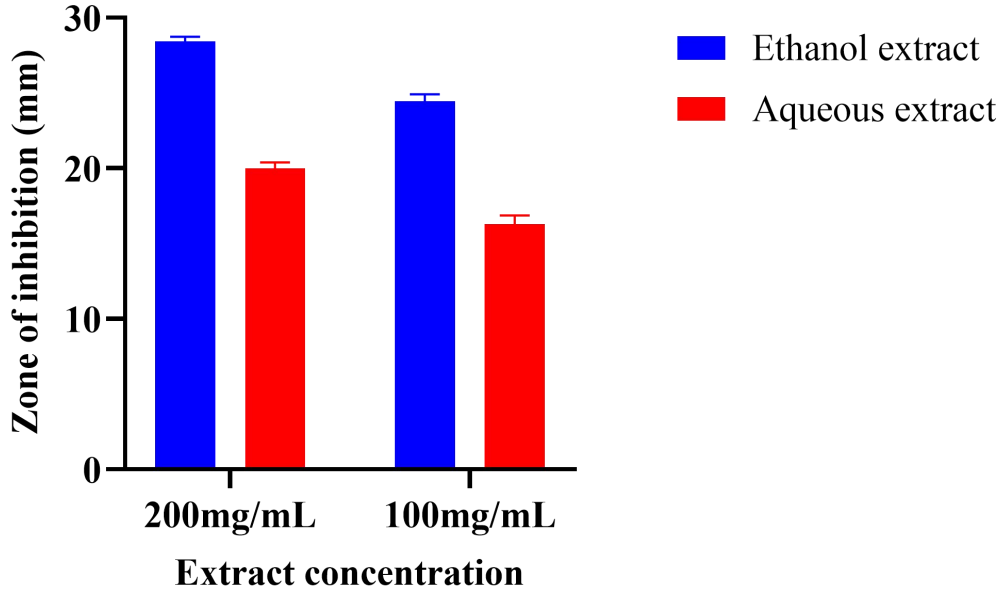

Figure 2: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol and aqueous extracts after plasmid curing

Higher concentrations of *Hibiscus sabdariffa* extract, irrespective of extract type, produced greater zones of the inhibition of *Escherichia coli* than the lower concentrations after plasmid curing, $F(1, 24) = 76.07$, $P < 0.0001$. 200 mg/mL of *Hibiscus sabdariffa* ethanol extract produced a greater zone of inhibition of *Escherichia coli* than the 100 mg/mL concentration of the same extract. Likewise, 200 mg/mL of *Hibiscus sabdariffa* aqueous extract produced a greater zone of inhibition of *Escherichia coli* than the 100 mg/mL concentration of the same extract.

However, the ethanol extract of *Hibiscus sabdariffa* produced greater zones of inhibition than the aqueous extract *Hibiscus sabdariffa* at the same concentration, $F(1, 24) = 351.0$, $P < 0.0001$. 200 mg/mL of *Hibiscus sabdariffa* ethanol extract produced greater zone of inhibition of *Escherichia coli* than that produced by the 200 mg/mL of *Hibiscus sabdariffa* aqueous extract. Likewise, 100 mg/mL of *Hibiscus sabdariffa* ethanol extract produced greater zones of inhibition of *Escherichia coli* than those produced by the 100 mg/mL of *Hibiscus sabdariffa* aqueous extract.

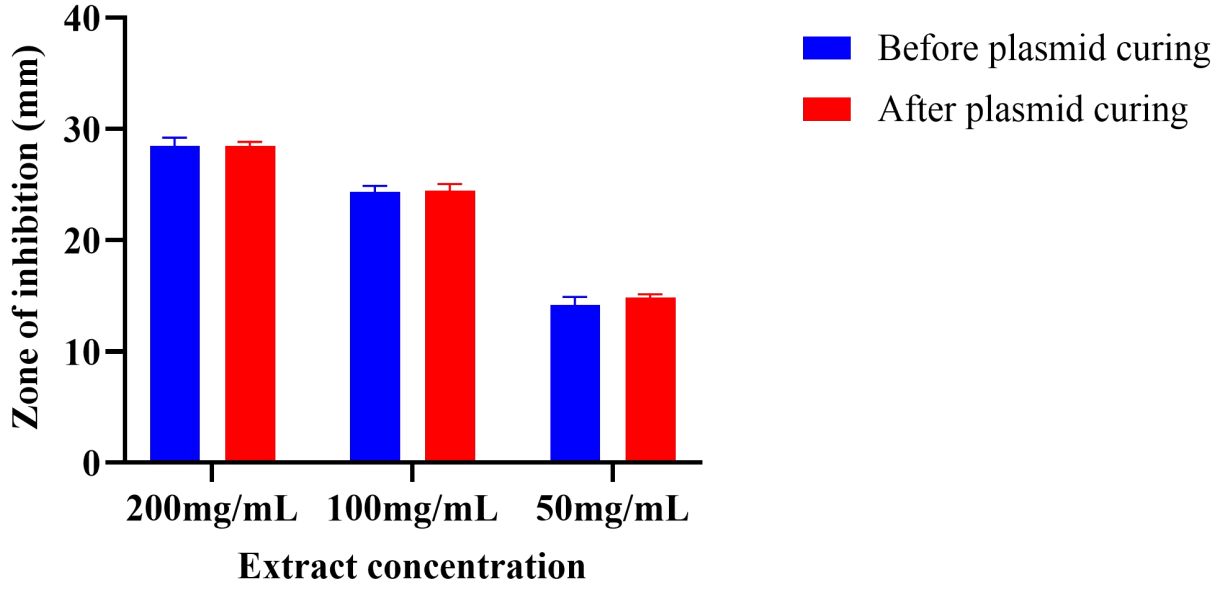

Figure 3: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before and after plasmid curing

There was no significant difference between zones of inhibition of *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before plasmid curing compared to the zones of inhibition of *Escherichia coli* by the same concentrations after plasmid curing, $F(1, 30) = 0.3623$

$P = 0.5517$.

However, higher concentrations of either of the ethanol or aqueous extract of *Hibiscus sabdariffa* produced greater zones of the inhibition than the lower concentrations of the same extract, $F(2, 30) = 324.5$, $P < 0.0001$. Before plasmid curing, 200 mg/mL of *Hibiscus sabdariffa* ethanol extract produced a greater zone of inhibition of *Escherichia coli* than the 100 mg/mL and

50mg/mL concentrations of the same extract, while 100 mg/mL of *Hibiscus sabdariffa* ethanol extract produced a greater zone of inhibition of *Escherichia coli* than the 50mg/mL concentration. The same applied to zone of inhibition of *Escherichia coli* after plasmid curing.

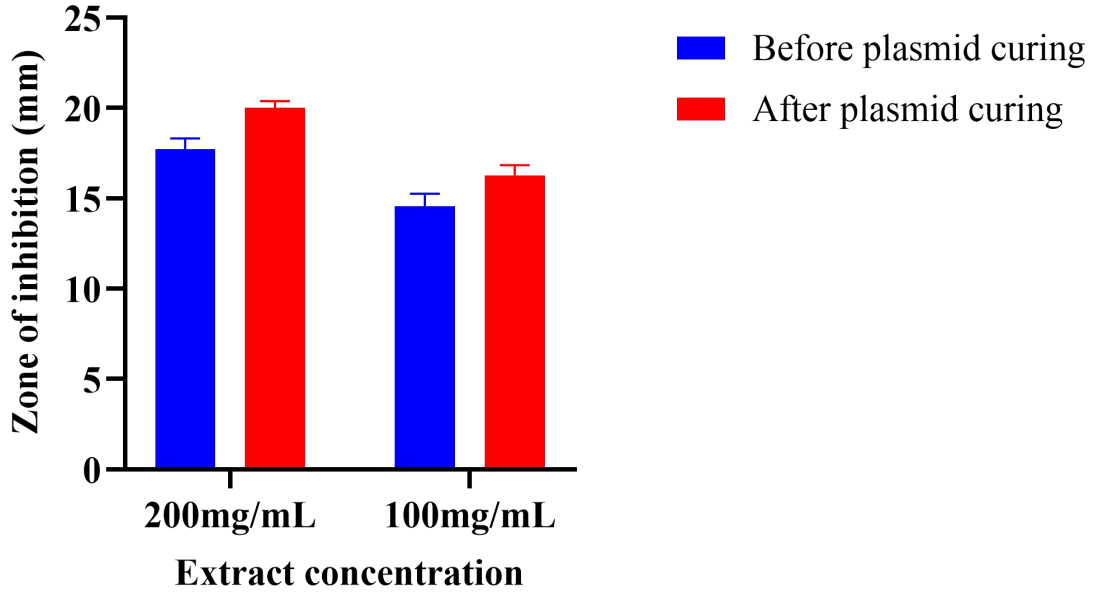

Figure 4: Bar graph showing zones of inhibition of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before and after plasmid curing

Zones of inhibition of *Escherichia coli* by 200 mg/mL and 100 mg/mL concentrations of *Hibiscus sabdariffa* aqueous extract after plasmid curing were greater than those produced by the same concentrations of the extract respectively before plasmid curing, $F(1, 24) = 12.31$, $P = 0.0018$.

Also, 200 mg/mL of *Hibiscus sabdariffa* aqueous extract produced a greater zone of inhibition of *Escherichia coli* than that produced by the 100 mg/mL concentration of the same extract before and after plasmid curing respectively, $F(1, 24) = 36.19$, $P < 0.0001$.

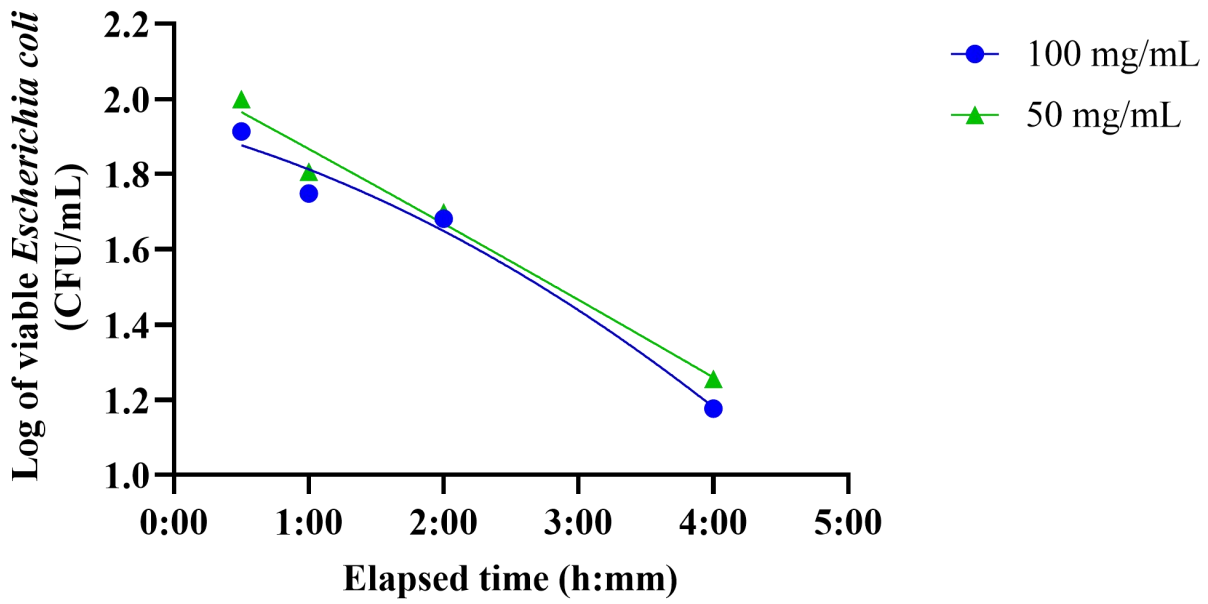

Figure 5: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract before plasmid curing

Second-order polynomial nonlinear regression model was used to evaluate the time-kill kinetics of the extract at 50mg/mL and 100 mg/mL. Both models showed excellent fit.

At 50 mg/mL: Log CFU/mL = 2.062 - 0.1930t - 0.001896t^2 (R^2 = 0.9803)

At 100 mg/mL: Log CFU/mL = 1.929 - 0.09329t - 0.02348t^2 (R^2 = 0.9783)

Due to excessively high, bacterial load at 0-hour, colony counts exceeded the countable limit (TNTC). As a result, precise log reductions from baseline could not be determined. However, subsequent time points allowed comparison of bacterial survival and extract efficacy between concentrations. At 50 mg/mL, the extract demonstrated a rapid initial kill-rate but a modest

cumulative effect. At 100 mg/mL, however, the initial kill was slower, but the overall bactericidal activity was stronger and more sustained over time. This indicates a dose-dependent effect, with 100 mg/mL showing superior bacterial reduction due to more pronounced time-related kill.

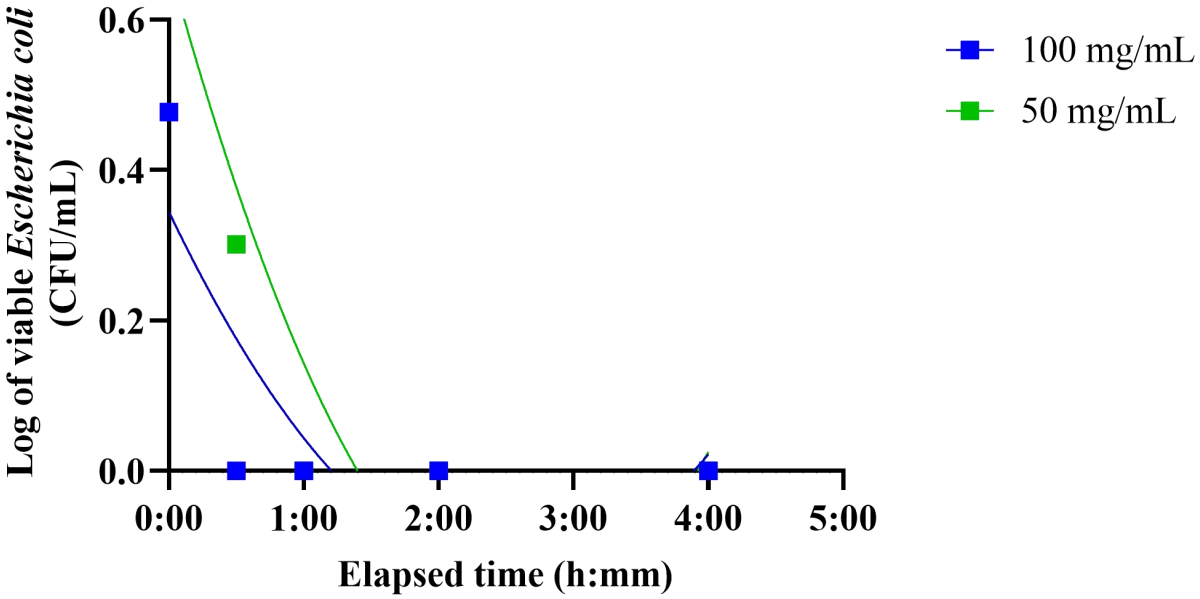

Figure 6: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* ethanol extract after plasmid curing

Second-order polynomial nonlinear regression model was used to evaluate the time-kill kinetics of the extract at 50mg/mL and 100 mg/mL.

At 50 mg/mL: Log CFU/mL = $0.6738 - 0.6530t + 0.1227t^2$ ($R^2 = 0.8747$)

At 100 mg/mL: Log CFU/mL = $0.3450 - 0.3744t + 0.07340\ t^2$ ($R^2 = 0.6538$)

At 50 mg/mL, the curve indicates a rapid initial decline in bacterial load, reaching zero log CFU/mL by 1 hour. The curve suggests a rapid kill phase followed by a plateau, indicating no regrowth over the remaining period. At 100 mg/mL, a similar trend was observed with complete kill achieved within 0.5 hour. However, the model's lower R^2 suggests greater variability, possibly due to early data convergence at zero, limiting the curve discrimination. The extract

exhibited a time- and concentration-dependent bactericidal activity. The higher 100 mg/mL concentration achieved complete microbial kill in half the time compared to 50 mg/mL. The absence of regrowth suggests a sustained antimicrobial effect at both concentrations.

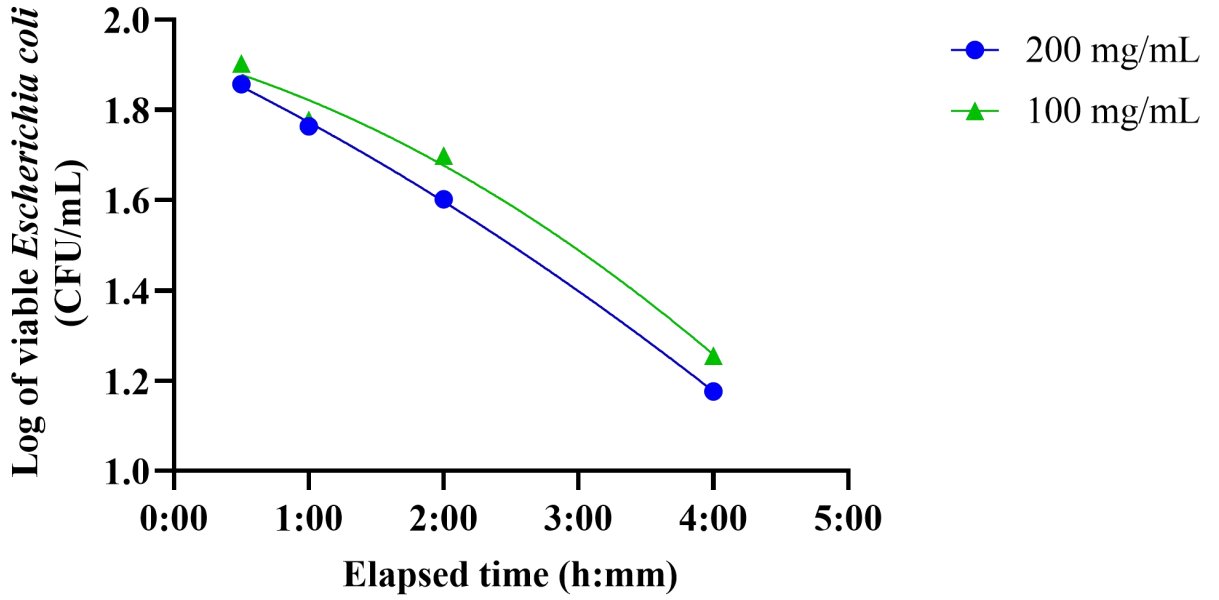

Figure 7: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract before plasmid curing

Second-order polynomial nonlinear regression model was used to evaluate the time-kill kinetics of the extract at 100mg/mL and 200 mg/mL. Both models showed excellent fit.

At 100 mg/mL: Log CFU/mL = $1.924 - 0.08017t - 0.02153t^2$ ($R^2 = 0.9873$)

At 200 mg/mL: Log CFU/mL = $1.925 - 0.1408t - 0.0115t$; ($R^2 = 0.9995$)

Due to excessively high, bacterial load at 0-hour, colony counts exceeded the countable limit (TNTC). As a result, precise log reductions from baseline could not be determined. However, subsequent time points allowed comparison of bacterial survival and extract efficacy between concentrations. Both concentrations achieved complete bacterial elimination by 6 hours. Comparatively, the higher concentration (200 mg/mL) demonstrated a more rapid and sustained

bactericidal effect, as indicated by the steeper negative linear coefficient (B_1) and near-perfect fit. These findings suggest a concentration dependent killing effect, with enhanced efficacy at higher doses.

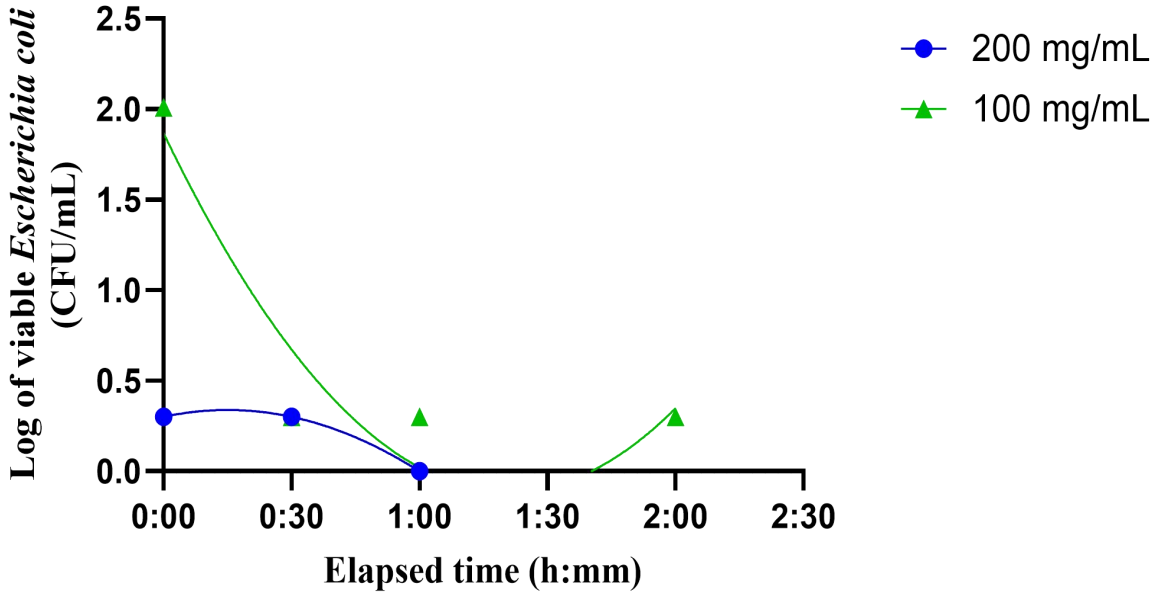

Figure 8: Line graph showing time-kill kinetics of multi-drug resistant uropathogenic *Escherichia coli* by *Hibiscus sabdariffa* aqueous extract after plasmid curing

Second-order polynomial nonlinear regression model was used to evaluate the time-kill kinetics of the extract at 100mg/mL and 200 mg/mL. Both models showed excellent fit.

At 100 mg/mL: Log CFU/mL = $1.869 - 0.934t + 1.087t^2$ ($R^2 = 0.9873$)

At 200 mg/mL: Log CFU/mL = $0.3010 + 0.3010t - 0.6021t^2$ ($R^2 = 1.000$)

At 100 mg/mL, the curve initially demonstrated a decline in bacterial count, but the positive B_2 indicates a curvature upward, suggesting potential bacterial regrowth or reduced bactericidal activity at later time points. At 200 mg/mL, however, the negative B_2 indicates a sustained bactericidal effect, with continued reduction in viable count over time and no regrowth, highlighting enhanced and sustained efficacy at this higher concentration. Compared to 100 mg/mL, the 200 mg/mL concentration exhibited more consistent bacterial killing, achieving greater log reduction with no rebound in bacterial growth, suggesting a dose-dependent antimicrobial activity.

CHAPTER FIVE

5.1 Discussion

Hibiscus sabdariffa is widely recognized for its diverse pharmacological properties, including antimicrobial, antioxidant, and anti-inflammatory activities. In this study, the antibacterial efficacy of the aqueous and ethanol calyx extracts of *Hibiscus sabdariffa* was evaluated against multidrug-resistant uropathogenic *Escherichia coli* (UPEC). Both pre- and post-plasmid curing isolates were tested to assess the possible role of plasmid-mediated resistance in affecting susceptibility to plant-based antimicrobial agents.

The results revealed that both aqueous and ethanol extracts exhibited inhibitory and bactericidal activity, confirming the therapeutic potential of *Hibiscus sabdariffa*. Statistical analysis showed that inhibition zones increased significantly with extract concentration ($p < 0.0001$). For instance, at 200 mg/mL, aqueous extract produced mean zones of inhibition of 17.71 ± 0.61 mm before curing and 20.00 ± 0.38 mm after curing, while ethanol extract recorded 28.50 ± 0.72 mm and 28.43 ± 0.30 mm, respectively. These results suggest that both solvent systems contain active phytochemicals capable of suppressing UPEC growth, though plasmid curing enhanced the susceptibility of isolates particularly to the aqueous extract. This observation highlights the importance of plasmid-mediated resistance in modulating bacterial response to plant preparations (Giamarellou, 2005).

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays further confirmed the efficacy of both extracts. Aqueous extract showed MIC and MBC values of 100 mg/mL and 200 mg/mL before curing, which improved to 50 mg/mL and 100

mg/mL after curing. Ethanol extract maintained consistently lower MIC and MBC values of 25 mg/mL and 50 mg/mL, both before and after curing. These results indicate that both extracts were effective in inhibiting and killing UPEC, but curing significantly enhanced the performance of the aqueous fraction while having minimal impact on the ethanol fraction. This trend agrees with earlier reports suggesting that ethanol-based extracts often retain stronger activity due to better solubility of bioactive phytochemicals such as flavonoids and phenolics (Almajid *et al*., 2023; Balouiri *et al*., 2016).

Time-kill kinetics experiments validated the bactericidal potential of the extracts. Both aqueous and ethanol extracts significantly reduced bacterial counts over time, with complete elimination of viable cells achieved within 6 hours at most concentrations tested. Regression analysis confirmed dose-dependent reductions, with strong fits observed for most conditions (R^2 ranging from 0.65 to 1.00). After plasmid curing, killing effects were accelerated, particularly at higher concentrations, showing that the extracts are capable of rapidly reducing bacterial populations once resistance barriers are minimized. These results are consistent with findings that flavonoid-rich plant extracts can disrupt bacterial membranes, leading to rapid leakage of intracellular contents and death (Otto, 2013).

Overall, the study demonstrates that both aqueous and ethanol extracts of *Hibiscus sabdariffa* possess significant antimicrobial activity against multidrug resistant UPEC. The improvement observed after plasmid curing underscores the role of plasmid-encoded resistance determinants in reducing susceptibility. This provides new evidence that *Hibiscus sabdariffa* extracts, irrespective of solvent system, hold promise as potential alternatives or complementary options in managing resistant urinary tract infections.

5.2 Conclusion

In conclusion, this study confirms the significant antibacterial activity of aqueous and ethanolic calyx extracts of *Hibiscus sabdariffa* against multidrug-resistant uropathogenic *Escherichia coli* (UPEC) isolates. The ethanol extract demonstrated significantly higher antibacterial activity than the aqueous extract, as reflected in lower MIC and MBC values, larger inhibition zones, and faster bactericidal activity in time-kill assays. Plasmid curing increased susceptibility of UPEC isolates to both extracts, suggesting that plasmid-mediated resistance may play a key role in affecting extract efficacy.

Therefore, the *Hibiscus sabdariffa* extracts especially the ethanolic extract appears to be more promising as a potential source of antibacterial compounds for treatment of infections caused by multidrug-resistant UPEC.

5.3 Recommendations

Further phytochemical analysis should be carried out to isolate and identify the specific bioactive compounds in the ethanol extract that are responsible for the observed antimicrobial activity. In addition, *in vivo* studies are necessary to confirm both the efficacy and safety of *Hibiscus sabdariffa* extract in the treatment of urinary tract infections caused by multidrug resistant uropathogenic *Escherichia coli*. Comparative studies with conventional antibiotics are also recommended to determine whether synergistic effects exist between the plant extract and standard drugs. Moreover, future research should focus on elucidating the mechanism of action of the ethanol extract, particularly how its phytochemicals interact with bacterial cell membranes and resistance determinants. Since plasmid curing enhanced susceptibility to the extracts, further molecular studies are advised to investigate specific plasmid-encoded resistance genes and their possible interactions with plant-derived compounds.

REFERENCES

Ahmad, S., Alam, K., Ahmad, I., Raza, K. and Hafeez, B. B. (2022). In vitro and in silico approaches for the evaluation of antimicrobial activity, time-kill kinetics, and anti-biofilm potential of thymoquinone against selected human pathogens. *Antibiotics,* 11(1): 79-90.

Ajuga, M. U., Otokunefor, K. and Agbagwa, O. E. (2021). Antibiotic resistance and ESBL production in *Escherichia coli* from various sources in Aba metropolis, Nigeria. *Bulletin of the National Research Centre,* 45(1): 173-180.

Alharbi, A. E., AlHussaini, A. M. and Alshami, I. (2024). A comprehensive review of the antimicrobial effects of hibiscus species. *Cureus,* 16(11), e73062.

Almajid, A., Bazroon, A., AlAhmed, A. and Bakhurji, O. (2023). Exploring the health benefits and therapeutic potential of Roselle (*Hibiscus sabdariffa*) in human studies: A comprehensive review. *Cureus,* 15 (11), e49309.

Angelini, P. (2024). Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. *Antibiotics,* 13(8):746-758.

Arip, M., Selvaraja, M., R, M., Tan, L. F., Leong, M. Y., Tan, P. L., Yap, V. L., Chinnapan, S., Tat, N. C., Abdullah, M., K, D. and Jubair, N. (2022). Review on plant-based management in combating antimicrobial resistance - mechanistic perspective. *Frontiers in Pharmacology,* 13, 879495.

Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis,* 6(2): 71–79.

Bilali, H. E. (2024). State and contours of research on roselle (*Hibiscus sabdariffa* L.) in Africa. *Open Agriculture,* 9(1) 202-214.

Bonkat, G., Bartoletti, R., Bruyère, F., Cai, T., Geerlings, S. E., Köves, B. and Wagenlehner, F. (2023). The current scenario and future prospects of urinary tract infections. *Pathogens,* 12(4): 549-562.

Breijyeh, Z. and Karaman, R. (2024). Antibacterial activity of medicinal plants and their role in wound healing. *Future Journal of Pharmaceutical Sciences,* 10(1): 68-79.

Bunduki, G. K., Heinz, E., Phiri, V. S., Noah, P., Feasey, N. and Musaya, J. (2021). Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. *BMC Infectious Diseases,* 21(1): 753-768.

Caballero, P. C., Enciso Garay, C. R., Macchi L, G., Nakayama, H. D., González C, M. C., Santacruz O, V. R., Cantero G, I., Ríes, A., and Samudio Oggero, A. (2024). Dataset on the qualitative characteristics of roselle varieties (*Hibiscus sabdariffa* L.). *Data in Brief,* 54: 110480.

Calcáneo-Martínez, J. C., Becerra-Morales, P. A., Beristain-Bauza, S. C., Escobedo-Reyes, A., Welti-Chanes, J., Jacobo-Velázquez, D. A. and Ramos-Parra, P. A. (2025). Antimicrobial mechanisms of *Hibiscus sabdariffa* extracts and hibiscus acid against *Campylobacter jejuni*: Membrane disruption, biofilm modulation, and antibiotic resistance implications. *Journal of Food Safety,* 45(1): e70016.

Chukwu, E. E., Awoderu, O. B., Enwuru, C. A., Afocha, E. E., Lawal, R. G., Ahmed, R. A., Olanrewaju, I., Onwuamah, C. K., Audu, R. A. and Ogunsola, F. T. (2022). High prevalence of resistance to third-generation cephalosporins detected among clinical isolates from sentinel healthcare facilities in Lagos, Nigeria. *Antimicrobial Resistance and Infection Control,* 11(1): 134.

Diop, M., Bassoum, O., Ndong, A., Wone, F., Ghogomu Tamouh, A., Ndoye, M., Youbong, T., Mbacké Daffé, S. M., Radji, R. O., Gueye, M. W., Lakhe, N. A., Fall, B., Ba, P. S. and Faye, A. (2025). Prevalence of multidrug-resistant bacteria in healthcare and community settings in West Africa: systematic review and meta-analysis. *BMC Infectious Diseases,* 25(1): 292.

Donkor, A.-M., Ahenkorah, B., Wallah, T. A. and Yakubu, A. (2023). Evaluation of extracts from *Sida acuta, Phyllanthus amarus, Parkia biglobosa* and their herbal ointment for therapeutic and biological activities. *Heliyon*, 9(9): e19316.

El-Saadony, M. T., Saad, A. M., Mohammed, D. M., Korma, S. A., Alshahrani, M. Y., Ahmed, A. E., Ibrahim, E. H., Salem, H. M., Alkafaas, S. S., Saif, A. M., Elkafas, S. S., Fahmy, M. A., Abd El-Mageed, T. A., Abady, M. M., Assal, H. Y., El-Tarabily, M. K., Mathew, B. T., AbuQamar, S. F., El-Tarabily, K. A. and Ibrahim, S. A. (2025). Medicinal plants: Bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits - a comprehensive review. *Frontiers in Immunology*, 16: 1491777.

Flores-Oropeza, M. A., Ochoa, S. A., Cruz-Córdova, A., Chavez-Tepecano, R., Martínez-Peñafiel, E., Rembao-Bojórquez, D., Zavala-Vega, S., Hernández-Castro, R., Flores-Encarnacion, M., Arellano-Galindo, J., Vélez, D. and Xicohtencatl-Cortes, J. (2023). Comparative genomic analysis of uropathogenic *Escherichia coli* strains from women with recurrent urinary tract infection. *Frontiers in Microbiology,* 14: 1340427.

Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Ranin, L. and Opavski, N. (2022). Antimicrobial Susceptibility Testing: A Comprehensive Review of Currently Used Methods. *Antibiotics*, 11(4): 427-445.

GBD 2021 Antimicrobial Resistance Collaborators. (2024). Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *The Lancet,* 404(10459): 1199–1226.

Ghaly, H. K. F., Younis, F. A. A.-Y., Soliman, A. M. and El-Sabbagh, S. M. (2025). Phytochemical and antibacterial properties of calyces *Hibiscus sabdariffa* L.: an in vitro and in silico multitarget-mediated antibacterial study. *BMC Complementary Medicine and Therapies*, 25(1): 62-75.

Gichuru, V., Sbrocca, I., Molinari, M., Tonto, T. C., Locato, V., Cimini, S. and De Gara, L. (2025). Exploring the antioxidant and antimicrobial properties of five indigenous Kenyan plants used in traditional medicine. *Scientific Reports*, 15(1): 1459-1472.

Giamarellou, H. (2005). Multidrug resistance in Gram-negative bacteria that produce extended-spectrum β-lactamases (ESBLs). *Clinical Microbiology and Infection,* 16(8): 1126–1133.

Gonzalez-Pastor, R., Carrera-Pacheco, S. E., Zúñiga-Miranda, J., Rodríguez-Pólit, C., Mayorga-Ramos, A., Guamán, L. P. and Barba-Ostria, C. (2023). Current landscape of methods to evaluate antimicrobial activity of natural extracts. *Molecules*, 28(3): 1068.

Hamrita, B., Emira, N., Papetti, A., Badraoui, R., Bouslama, L., Ben Tekfa, M.-I., Hamdi, A., Patel, M., Elasbali, A. M., Adnan, M., Ashraf, S. A. and Snoussi, M. (2022). Phytochemical analysis, antioxidant, antimicrobial, and anti-swarming properties of *Hibiscus sabdariffa* L. calyx extracts: In vitro and in silico modelling approaches. *Evidence-Based Complementary and Alternative Medicine*, 2022: 1252672.

He, Y., Zhao, J., Wang, L., Han, C., Yan, R., Zhu, P., Qian, T., Yu, S., Zhu, X. and He, W. (2025). Epidemiological trends and predictions of urinary tract infections in the global burden of disease study 2021. *Scientific Reports*, 15(1): 4702-4715

Hu, Y., Ma, W., Tang, K., Qi, Q., Xu, W., Hu, R., Liu, S., Zhang, K., Chen, J. and Liang, C. (2025). Global burden of urinary tract infections in older women from 1990 to 2021 with projections to 2040: a trend analysis of the Global Burden of Disease Study 2021. *Frontiers in Cellular and Infection Microbiology*, 15:1577777.

IndustryARC. (2022). Roselle Market Size Report, 2022–2027.

Itemire, A.O and Idu, M. (2024). Antibacterial Activity of Biherbal Root Extracts on Bacterial Isolates. *African Scientist,* 25(3): 258–264.

Kokare, S. A., Kalamb, V. S., Bakal, R. L. and Hatwar, P. R. (2025). *Hibiscus sabdariffa*: A comprehensive review of its phytochemistry, traditional uses and therapeutic potentials. *International Journal of Herbal Medicine,* 13(4): 37-41.

Li, S., Jiang, S., Jia, W., Guo, T., Wang, F., Li, J. and Yao, Z. (2024). Natural antimicrobials from plants: Recent advances and future prospects. *Food Chemistry*, 432(137231): 137231.

Mahshouri, P., Alikhani, M. Y., Momtaz, H. E., Doosti-Irani, A. and Shokoohizadeh, L. (2025). Analysis of phylogroups, biofilm formation, virulence factors, antibiotic resistance and molecular typing of uropathogenic *Escherichia coli* strains isolated from patients with recurrent and non-recurrent urinary tract infections. *BMC Infectious Diseases*, 25(1): 267-281

Manandhar, S., Luitel, S. and Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of Tropical Medicine*, 2019, 1895340-1895348.

Mancuso, G., Midiri, A., Gerace, E., Marra, M., Zummo, S. and Biondo, C. (2023). Urinary tract infections: The current scenario and future prospects. Pathogens, 12(4): 549-562.

Marouf, R. S., Mbarga, J. A. M., Ermolaev, A. V., Podoprigora, I. V., Smirnova, I. P., Yashina, N. V., Zhigunova, A. V. and Martynenkova, A. V. (2022). Antibacterial Activity of Medicinal Plants against Uropathogenic *Escherichia coli*. *Journal of Pharmacy & Bioallied Sciences*, 14(1): 1–12.

Mumin, Y. M., Yüksel, G., and Özad Düzgün, A. (2025). Investigation of virulence factor genes and biofilm formation of antibiotic resistant clinical *E. coli* isolates. Microbial Pathogenesis, 199(107257):107257.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Hay, S. I. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, 399(10325): 629–655.

NCBI Taxonomy Browser. (n.d.). *Hibiscus sabdariffa*. National Center for Biotechnology Information.

Odonkor, G., Gyasi, R. K., Osei, J. H. N., Amoa-Bosompem, M., Osei-Djarbeng, S. and Kwarteng, A. (2022). Fluoroquinolone antibiotics: In vitro antibacterial and time-kill bactericidal evaluation against etiology of bacteremia in HIV-infected patients. *Bulletin of the National Research Centre*, 46, 140-149.

Ouangraoua, W. J., Traore, R. E., Kabore, B., Sawadogo, P. and Sawadogo, M. (2022). Agromorphological characterization of *Hibiscus sabdariffa* L. collection from Burkina Faso. *European Scientific Journal*, ESJ, 18(27): 184–184.

Otto, M. (2013). Community-associated MRSA: What makes them special? *International Journal of Medical Microbiology*, 303(6–7): 324–330.

Panda, S. R., Meher, A., Prusty, G., Behera, S. and Prasad, B. R. (2025). Antibacterial properties and therapeutic potential of few medicinal plants: current insights and challenges. *Discover Plants,* 2(1): 1-12.

Riaz, G. and Chopra, R. (2018). A comprehensive review on *Hibiscus sabdariffa* L.: Morphological characteristics, phytochemistry, and therapeutic potential. *Phytotherapy Research*, 35(8): 4222-4241.

Rodríguez-Melcón, C., Alonso-Calleja, C., García-Fernández, C., Carballo, J. and Capita, R. (2021). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for twelve antimicrobials (biocides and antibiotics) in eight strains of Listeria monocytogenes. *Biology*, 11(1): 46-58.

Sanou, A., Konate, K., Dakuyo, R., Kabore, K., Sama, H. and Dicko, M. H. (2022). *Hibiscus sabdariffa*: Genetic variability, seasonality and their impact on nutritional and antioxidant properties. *PloS One*, 17(3): e0261924.

Sapian, S., Ibrahim Mze, A. A., Jubaidi, F. F., Mohd Nor, N. A., Taib, I. S., Abd Hamid, Z., Zainalabidin, S., Mohamad Anuar, N. N., Katas, H., Latip, J., Jalil, J., Abu Bakar, N. F. and Budin, S. B. (2023). Therapeutic potential of *Hibiscus sabdariffa* Linn. in attenuating cardiovascular risk factors. *Pharmaceuticals*, 16(6): 807-820.

Schwalbe, R., Steele-Moore, L. and Goodwin, A. C. (2007). Antimi*crobial susceptibility testing protocols*. CRC Press.

Stracy, M., Snitser, O., Amer, Y., Parizade, M., Katz, R., Rimler, G., Wolf, T., Herzel, E., Koren, G., Kuint, J., Foxman, B., Chodick, G., Shalev, V. and Kishony, R. (2022). Minimizing treatment-induced emergence of antibiotic resistance in bacterial infections. *Science*, 375(6583): 889–894.

Sujith, S., Solomon, A. P. and Rayappan, J. B. B. (2024). Comprehensive insights into UTIs: from pathophysiology to precision diagnosis and management. *Frontiers in Cellular and Infection Microbiology*, 14: 1402941.

Tao, A., Li, Y., Chen, J., Li, J., Xu, J., Lin, L., Zhang, L. and Fang, P. (2024). Development of Roselle (*Hibiscus sabdariffa* L.) transcriptome-based simple sequence repeat markers and their application in Roselle. *Plants*, 13(23) : 3517-3530.

Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C. and Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, 9(10): 2041-2058.

Whelan, S., Lucey, B. and Finn, K. (2023). Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: The molecular basis for challenges to effective treatment. *Microorganisms*, 11(9): 2225-2239.

Woo, S., Marquez, L., Crandall, W. J., Risener, C. J., & Quave, C. L. (2023). Recent advances in the discovery of plant-derived antimicrobial natural products to combat antimicrobial resistant pathogens: insights from 2018-2022. *Natural Product Reports*, 40(7): 1271–1290

Zhou, Y., Zhou, Z., Zheng, L., Gong, Z., Li, Y., Jin, Y., Huang, Y. and Chi, M. (2023). Urinary tract infections caused by uropathogenic *Escherichia coli*: Mechanisms of infection and treatment options. *International Journal of Molecular Sciences,* 24(13): 10537-10552.

Zouine, N., Ghachtouli, N. E., Abed, S. E. and Koraichi, S. I. (2024). A comprehensive review on medicinal plant extracts as antibacterial agents: Factors, mechanism insights and future prospects. *Scientific African*, 26: e02395.

APPENDIX

Preparation of Media

Nutrient Agar Preparation

Twenty-eight (28g) grams of the nutrient agar powder was weighed and suspended in 1 litre of distilled water. The solution was mixed properly and allowed to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes. The molten liquid was poured into the petri dish and allowed to solidify. The plates were stored in a refrigerator.

Muller Hinton Agar Preparation

Thirty-eight (38g) grams of the powdered medium was weighed and suspended in one litre of distilled water. It was heated with frequent agitation and boiled for one minute to completely dissolve the medium.The mixture was autoclaved at 121ºC for 15minutes. It was allowed to cool to 45ºC and poured into petri dishes. The poured plates were allowed to solidify and were used to culture the samples after drying.

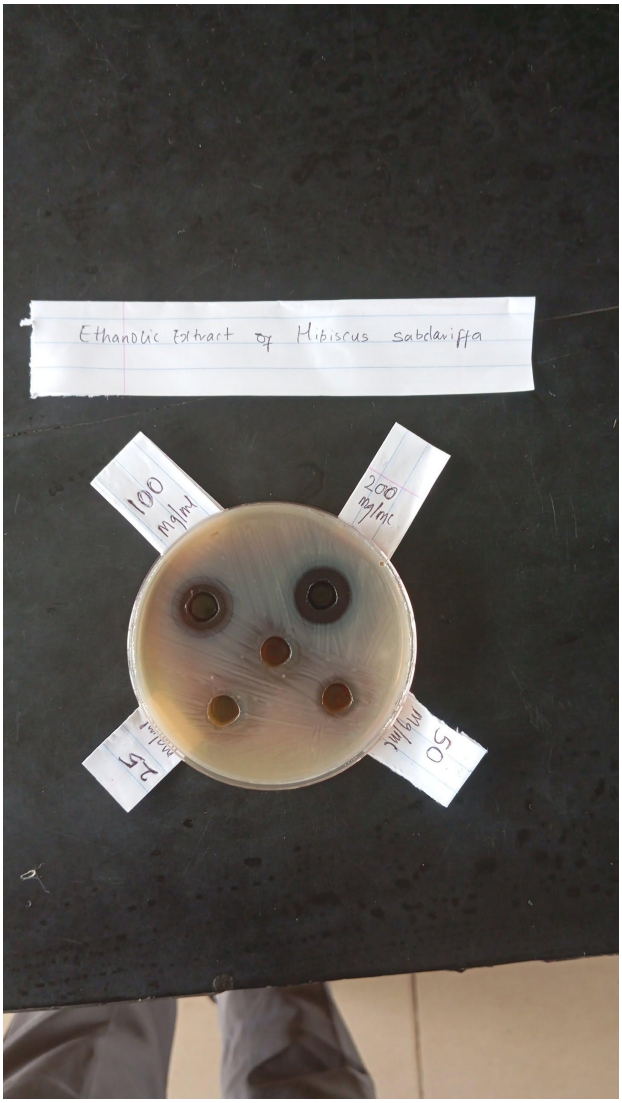

Figure 10: MIC of ethanolic extracts of *Hibiscus sabdariffa* flower against *Escherichia coli*