IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF ETHYL ACETATE EXTRACTS OF *Xerophyta spekei* (Baker), *Senna singueana* (Delile) and *Grewia tembensis* (Fresen)

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October, 2022

DECLARATION

I, Paul Ochieng Nyalo, declare that the work presented in this thesis is my original work and has not been submitted for a degree or any other award in any other university or any other institution.

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DEDICATION

My heartwarming gratitude goes to my dear wife Jacqueline Ogutu Nyalo, our sons Aron Paul Nyalo and Magnus Paul Nyalo, our daughter Malgorzata Paul Nyalo, my sister Penina Akinyi Nyalo and my parents Mr. Samwel Nyalo and the late Magret Samwel Nyalo (Nyaugenya) for their prayers, support as well as encouragement towards my studies.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BHA	Butylated Hydroxyanisole
BHT	Butylated Hydroxytoluene
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric Reducing Antioxidant Power
HIV	Human Immunodeficiency Virus
MBC	Minimum bactericidal concentration
MDR	Multi drug resistant
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
Mm	Millimeter
MZI	Mean Zones of Inhibition
NACOSTI	National Commission for Science, Technology and Innovation
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
OS	Oxidative Stress
PG	Propyl Gallate
RNA	Ribonucleic Acid
RNS	Reactive nitrogen species
ROS	Reactive Oxygen Species
SEM	Standard Error of Mean
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

Bacterial infections are a leading cause of mortality and morbidity worldwide. During bacterial diseases, the elevation of host immune response occurs, which involves production of free radicals in response to the bacterial infection. Overproduction of free radicals in excess of the antioxidants leads to oxidative stress. To combat the bacterial diseases, conventional antibiotics are normally used. Conventional antibiotics are associated with side-effects such as hypersensitivity reactions, toxicity, and bacterial pathogens developing resistance against them. Artificial antioxidants are said to be carcinogenic. This study sought to confirm folklore use and validate the antibacterial and antioxidant activities of *Grewia tembensis*, *Senna singueana,* and *Xerophyta spekei*, which have been widely used in the Mbeere community in Kenya. The *in vitro* antibacterial activities of the plant extracts were investigated on Gram positive bacteria (*Bacillus subtilis* ATCC 21332 and *Staphylococcus aureus* ATCC 25923) and Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 1408). Ciprofloxacin (100 µg/ml) drug was used as a standard reference, whereas 5% DMSO was used as a negative reference. The antibacterial tests included disc diffusion, minimum inhibitory and bactericidal concentrations. Antioxidant properties of the extracts were determined through scavenging effects of DPPH, H_2O_2 (hydrogen peroxide), and hydroxyl radicals and iron chelating effects, as well as FRAP assay. *S. singueana* extract showed broad-spectrum activity against tested bacterial microbes producing mean zones of inhibition (MZI) from 07.67±0.33 to 17.67±0.33 mm. Ethyl acetate stem bark extract of *G. tembensis* showed a notable antibacterial effect on *S. aureus* only with MZI ranging from 07.07±0.07 to 12.33±0.33 mm. Likewise, ethyl acetate leaf extract of *G. tembensis* also exhibited an antibacterial effect against only *S. aureus* with MZI ranges of 08.33±0.33 to 11.67±0.33 mm, while the ethyl acetate extract of *X. spekei* revealed notable antibacterial effects on studied Gram positive bacteria only with inhibition zones ranging from 07.67±0.33 to 14.67±0.33 mm. *S. singueana* extract exhibited significantly higher antibacterial effects than all the other plant extracts ($p<0.05$). *X. spekei* extract had significantly higher *in vitro* antioxidant activity than all the other extracts ($p<0.05$). The quantitative phytocompounds screening demonstrated the availability of compounds with known antibacterial and antioxidant effects. Therefore, the current study recommends ethnomedicinal and therapeutic use of *S. singueana* as antibacterial agent against *S. aureus, B. subtilis, S. typhi* and *E. coli* while *X. spekei* as antibacterial agent against *S. aureus, B. subtilis* and finally *G. tembensis* as antibacterial agent against *S. aureus.* They can also be used as antioxidant agents after a comprehensive study of their toxicity and safety profiles.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Microbial infections, especially from bacteria, account for about 38% of human diseases and about 30% of emerging human infectious diseases (Lindahl and Grace, 2015). Most bacterial diseases re-emerge due to human factors and the emergence of antibiotic resistance among bacteria (Lindahl and Grace, 2015). Some bacteria are normal flora that live and survive in the human body without causing any pathological effects (Faust *et al*., 2012). During bacterial infections, the immune system of the body is activated and recognizes pathogens through pathogen-associated molecular patterns and fights the invading bacterial pathogens to eradicate them from the body (Hayashi *et al*., 2001).

Due to lack of resources, infectious diseases reports from developing countries are not well documented (Mutuli *et al*., 2016). In Kenya, microbial pathogens causing a majority of human diseases are generally those with high antibiotic resistance. The top five killers in Kenya are infectious diseases, although the data on bacterial infections are not well documented because a majority of illnesses and deaths occur outside the hospitals (GARP-KWG, 2011). The burden of

infectious diseases is highly prevalent among Kenyans from poor communities (Feikin *et al*., 2011).

In 2001, it was estimated that microbial infections affected about 320 million children aged 0 and 14 years in Africa (Peltola, 2001). In 2006, microbial infections per one hundred thousand children aged above 2 years were 2440, whereas those aged above 5 years were 1192, with an estimated 4.4 million deaths annually in children below 5 years in sub-Saharan Africa (Brent *et al*., 2006).

Infectious bacterial diseases are a great danger to both human and animal health and are the main source of morbidity as well as mortality worldwide (Kitonde *et al*., 2013). The spread of infectious diseases has reportedly been facilitated by increased human population in major cities, environmental degradation, and lack of proper health care services (Noah and Fidas, 2000). Some conditions that lead to the increased number of individuals with compromised immunity have also led to a large number of people being subjected to bacterial infections (Ford *et al*., 2015).

During energy generation in body cells, free radicals are generated, which, at high concentrations, results in oxidative stress, but at low or moderate levels, they are effective in cellular and immune responses (Pham-Huy *et al*., 2008).

The development of chronic infections like arthritis, cancer, cardiovascular and other neurodegenerative diseases are known to be mediated by oxidative stress (Pham-Huy *et al*., 2008). During bacterial infections, the elevation of host immune response occurs, which involves reactive oxygen as well as nitrogen species (RNS/ROS) production to counter the bacterial infection.

Overproduction of free radicals above available antioxidant concentrations in the body results in oxidative stress (OS), which can potentiate apoptosis, tumorigenesis, and immune response (Spooner and Yilmaz, 2011), thereby causing harm to the body (Pizzino *et al*., 2017). Free radicals play dual roles during bacterial infections, which include shielding against invading microorganisms. However, if in excess, free radicals can lead to tissue damage during the ensuing inflammation as a result of immune response (Pohanka, 2013).

Synthetic antioxidants such as tertiary butyl hydroquinone, butylated hydroxytoluene as well as propyl gallate, which are broadly used in the preservation of food for human consumption, have been thought to cause serious harmful health effects like DNA damage on the sensitive organs of humans like the liver and the brain (Kulisic *et al*., 2004) and are also proved to be carcinogenic (Kanwal *et al.*, 2015). In addition, these synthetic antioxidants are

not readily available to patients due to factors such as cost and inaccessibility (Asif, 2015).

There has been a rise in the use of counterfeit antibacterial agents due to the increased expense of medication, especially in underdeveloped countries, some of which are of sub-standard quality. Their use has led to loss of lives and resulted in antibacterial drug-resistant pathogens (Kelesidis *et al*., 2007). Antibacterial resistance to commonly used antibiotics is currently increasing at an alarming rate, which has complicated the management of bacterial infections (Ayukekbong *et al*., 2017). Some of the elements that cause this phenomenon include misuse of over-the-counter antibiotics, noncompliance to antibiotic dosage due to their adverse side effects, and use of under-dose due to the high expense of medication (Ayukekbong *et al*., 2017). Therefore, there is an urgent need for alternative and complementary antibacterial therapies which are affordable and have fewer side effects.

There is a greater need for the use of plant-derived antibiotics due to the challenges of conventional antibacterial therapies. Since time immemorial, medicinal plants have been relied upon as therapeutic agents to prevent or heal diseases as they are associated with fewer side effects and are affordable (Gupta *et al.*, 2017). Plants are important sources of molecules, which can be utilized in developing new antibacterial and antioxidant agents, and it is estimated that 44%

of new drugs being developed are of plant origin (Burgos *et al*., 2015). Some plants like *Eclipta prostrata (L)* have previously exhibited both antibacterial and antioxidant properties (Karthikumar *et al*., 2007). Essential oil of *Artemisia annua* was also shown to possess both antibacterial and antioxidant activities (Juteau *et al*., 2002). Plant antioxidants like polyphenols, carotenoids, glutathione have exhibited antioxidant effects by interrupting free radicals' chain reactions (Aziz *et al*., 2019). Due to these properties, plant antioxidants are being considered as a source of alternative and complementary therapy against oxidative stress.

Natural antioxidants and antibacterial components have been discovered in medicinal plants (Altemimi *et al*., 2017). However, only a few have been scientifically evaluated and validated (Imanirampa and Alele, 2016). The three plants used in the current study; *Xerophyta spekei*, *Senna singueana* and *Grewia tembensis* are used by herbalists as medicinal herbs in the Mbeere community in Embu County (Kareru *et al*., 2007). However, their antibacterial and antioxidant activities are yet to be confirmed scientifically. Therefore, this experiment was executed to explore, establish and confirm the three medicinal plant extracts' antibacterial and antioxidant capabilities *in vitro*.

1.2 Statement of the problem

Conventional antibacterial drugs which have been relied on for management and treatment of bacterial infections have drawbacks including severe side-effects

such as severe fatal adverse drug reactions, hypersensitivity reactions, effects on normal flora, drug interactions, and toxicity (Djieyep *et al*., 2015; Stanković *et al*., 2016), high cost of medication, poor patient adherence (Lopes *et al*., 2013; Biasi-Garbin *et al*., 2016) and bacterial pathogens developing resistance mechanisms against them (Venkatesan *et al*., 2009). Additionally, conventional drugs used to manage and treat illnesses caused by oxidative stress and bacterial infections are costly, unaffordable, and inaccessible, especially in rural and remote regions.

It is estimated that about 80% of the world's population still depend on herbal medicine as their main healthcare source (Bacha *et al*., 2016), particularly in developing nations (Gurnani *et al*., 2014). Traditional healers have indigenously used medicinal plants to cure several diseases, including bacterial infections (Nankaya *et al.*, 2020). The Mbeere community uses the three medicinal plants utilized in this study as a remedy to cure a variety of diseases. However, their indigenous uses lack scientific validation.

1.3 Justification of the study

The challenges associated with conventional management and treatment of both bacterial infections and oxidative stress have led to scientists shifting their attention to finding alternative therapies such as using medicinal plants as they are valued not only for their dietary use but also for their medicinal use (Anushia

et al., 2009). Therefore, new therapeutic agents with both antibacterial and antioxidant activities are more desirable.

The present study sought to confirm the folklore use of *G. tembensis*, *S. singueana*, and *X. spekei* in the treatment of bacterial infections by the Mbeere community in Embu County and validate their antibacterial as well as their antioxidant activities. The current study also determined the phytochemical compositions of ethyl acetate extracts of *G. tembensis*, *S. singueana*, and *X. spekei* to determine the basis of their medicinal activities.

1.4 Null Hypotheses

i. The ethyl acetate extracts of *X. spekei*, *S. singueana*, and *G. tembensis*, have no *in vitro* antibacterial and antioxidant activities.

ii. The ethyl acetate extracts of *X. spekei*, *S. singueana*, and *G. tembensis* do not contain phytochemicals associated with antibacterial and antioxidant activities.

1.5 Objectives of the study

1.5.1 Main objective

To explore the *in vitro* antibacterial and antioxidant effects of *X. spekei*, *S. singueana*, and *G. tembensis* ethyl acetate extracts.

1.5.2 Specific objectives

i. To determine the *in vitro* antibacterial properties of *X. spekei*, *S. singueana*, and *G. tembensis* ethyl acetate extracts on selected bacterial species.

ii. To determine *in vitro* antioxidant effects of ethyl acetate extracts of *X. spekei*, *S. singueana*, and *G. tembensis.*

iii. To determine quantitative phytochemical constituents of ethyl acetate extracts of *X. spekei*, *S. singueana*, and *G. tembensis*.

1.6 Significance of the study

The main aim of this study was to determine if the ethyl acetate plant extracts of *X. spekei*, *S. singueana*, and *G. tembensis* have antibacterial activities against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus*. The study also determined the antioxidant effects of the studied plant extracts. The determination of phytochemicals present in the extracts also helped in proposing the use of these herbal plants as the primary material in the development of antibacterial as well as antioxidant agents. This also provides an alternative to the conventionally available antibacterial and antioxidant drugs.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bacterial diseases

Pathogenic bacterial illnesses are linked with the increase in morbidity and mortality rates, especially amongst hospitalized patients undergoing transplantation, cancer treatment, and immune-compromised patients (Santos *et al.*, 2009). Effective and efficient antibacterials are critical in reducing this morbidity and mortality rates worldwide (Ndihokubwayo *et al*., 2013). Conventional antibacterial agents have had a decrease in efficacy due to an increase in multidrug-resistant microbes. There is, therefore, a critical need to evaluate natural products from plants with unique structure and potency against these pathogens (Ameya *et al*., 2017) and with a beneficial health impact (Balakrishnan *et al*., 2014; Romha *et al*., 2018).

Bacterial infections have been on the rise globally due to the increase in immunosuppression as a result of chronic infections like HIV/AIDs, the increase in human population, especially in urban areas leading to overcrowding, poor hygiene, and lack of adherence to conventional antibacterial use leading to antibacterial resistance to conventional antibiotics (Cyrus *et al*., 2008). With this global increase of bacterial infections, some of the bacterial infections like diarrhea have become a life-threatening global concern (Samie *et al*., 2005).

Bacterial infections kill an estimated 14.7 million people worldwide each year, out of which 14.2 million cases are from developing countries. Additionally, sub-Saharan African countries lead in mortality rates with about 6.8 million global deaths due to bacterial infections, while South Asia follows with 4.4 million annual bacterial infections related deaths (Michaud, 2009). However, some East African countries like Kenya lack accurate data on bacterial infections related deaths because most of the deaths occur in households out of hospital facilities, making it difficult to gain such data (Feikin *et al*., 2011).

2.2 Bacterial pathogens

S. aureus is a Gram-positive human microbe that clinically manifests as a skin infection (Miller and Kaplan, 2009). *S. aureus* has the ability to adjust and progress because of its resistance traits and virulence factors (Tang and Stratton, 2010). *S. aureus* infections can lead to various clinical illnesses and is the most common cause of infective endocarditis and bacteremia. It causes osteoarticular infections, skin and soft tissue infections, pleuropulmonary infections like pneumonia (Tong *et al*., 2015). It can as well cause food-borne related diseases (Shan *et al*., 2007). Plants like *Ocimum gratissimum* have demonstrated *in vitro* antibacterial effects against *S. aureus* (Omodamiro and Jimoh, 2015).

S. typhi is a Gram-negative bacillus categorized under the Enterobacteriaceae family (Parry *et al*., 2002). It's a worldwide prime causative agent of gastroenteritis, with an estimate of 2.8 billion diarrheal cases each year (Bula-

Rudas *et al*., 2015). Globally, typhoid fever which is caused by *S. typhi* (Dougan and Baker, 2014), is the major cause of high mortality rates, with an estimate of around 26.9 million *S. typhi* illnesses and 269,000 *S. typhi* related mortalities (Buckle *et al*., 2012). Most countries have reported the outbreak of typhoid fever, whose spread is majorly associated with poor sanitation, especially in high population areas, poor hygiene practices, and unclean food and water (Nyamusore *et al*., 2018). Due to the emergence of *S. typhi* resistance strains, there has been a challenge in antibiotic treatment of typhoid fever (Bula-Rudas *et al*., 2015). *Ocimum gratissimum* has been shown to be effective against *S. typhi* (Omodamiro and Jimoh, 2015).

E. coli is a Gram-negative bacterium of the *Escherichia* genus (Tenaillon *et al*., 2010) and causes most urinary tract infections (Kibret and Abera, 2014). *E. coli* is a common human gut inhabitant as a member of the normal flora (Sarba *et al*., 2019) and also an important pathogen causing worldwide morbidity and mortality (Clements *et al*., 2012). *E. coli* pathotypes include Enteropathogenic *E. coli* causing infant diarrhea, Enterohemorrhagic *E. coli* causing food poisoning, Enteroinvasive *E. coli* causing shigellosis like diarrhea, Enteroaggregative *E. coli* causes travelers' and infant diarrhea, Enterotoxigenic *E. coli* majorly causes acute diarrhea in children below 5 years and travelers' diarrhea and Diffusely Adherent *E. coli* (DAEC) causing acute diarrhea in

children below 5 years (Clements *et al*., 2012). *Euphorbia hirta* has been demonstrated to be effective on *E. coli* (Ogbulie *et al*., 2007).

B. subtilis, an opportunistic Gram-positive aerobic pathogen, is associated with frequent food contamination (Yassin and Ahmad, 2012). Traditionally *B. subtilis* has been revealed to cause food-borne illnesses in humans whose symptoms are commonly accompanied by diarrhea (Gopal *et al*., 2015). *B. subtilis* is a rod-shaped bacterium and is occasionally found in soil, water, and the air. It is also a model bacterial organism for protein secretion and cell division studies (Kovács, 2019). It is regarded as nonpathogenic and sometimes is found on the mucous membranes of healthy human beings (Molnár *et al*., 2011). However, in immune-suppressed patients, it can lead to opportunistic infections (Jeon *et al*., 2012). *Alpinia galanga* and *Zingiber officinale* crude extracts have been demonstrated to have *in vitro* antibacterial effects against *B. subtilis* (Hamad *et al*., 2016).

2.3 Bacterial infections and oxidative stress

Formation of ROS/RNS may lead to OS. These ROS/RNS are detrimental to life when there is disequilibrium between them and antioxidants, whereas under normal physiological conditions are essential in life. The ROS/RNS are also released in enzymatic reactions (Zamudio-Cuevas *et al*., 2015). Free radicals like reactive oxygen species can be produced endogenously by aerobic organisms

during their normal aerobic process as well as exogenously from chemicals and other environmental factors (Katoch and Begum, 2003; Ifeanyi, 2018).

In most cases, during bacterial infections, the host's immune system may successfully eliminate the invading bacterial pathogen, but in some cases, the bacteria may persist in the host body. During this period, free radicals like ROS are produced as an immediate defense against bacterial attack (Hartog *et al.*, 2016). The produced ROS act by signaling immune response in addition to killing the invasive bacterial microbes (Spooner and Yilmaz, 2011). ROS involved in this process include superoxide, which in the presence of NADPH oxidase enzyme, causes further creation of other bactericidal ROS leading to the effective killing of the bacterial organism (Kobayashi *et al.*, 2018). The bacterial pathogen's continued survival in the host body results in the creation of free radicals either endogenously through the immune cells (Boncompain *et al.*, 2010) or exogenously through the conventional antibacterial agents used (Grant and Hung, 2013; Carini *et al.*, 2017) which ultimately leads to oxidative stress as the amount of oxidants exceeds the amount of antioxidants (Vaishampayan and Grohmann, 2022) leading to other chronic diseases like cancer, diabetes, and arthritis. Additionally, when these ROS are produced in excess, this leads to cellular damage of the host cells, which eventually subjects the host cell to more bacterial infection susceptibility (Rahal *et al.*, 2014).

2.4 Conventional antibacterial and antioxidant chemotherapy

Bacterial diseases are conventionally managed using antibiotics which are classified as either bactericidal if they kill or destroy the bacteria or bacteriostatic if they only slow down the bacterial growth (Kohanski *et al*., 2010; Nemeth *et al*., 2015). Some antibiotics are natural by-products of microorganisms (Fymat, 2017). The current conventional antibiotics used in treating and managing bacterial infections are classified into the following major classes; Aminoglycosides, Cephalosporin, Macrolides, Penicillin, Sulphonamide drugs, and Tetracycline (Fymat, 2017).

2.4.1 Penicillins

Penicillins are antibiotics belonging to the β-lactam family and are widely used in primary healthcare for the management of bacterial infections as they have bactericidal effects. They are well distributed in the body and are highly effective against the most susceptible bacteria. They work by inhibiting bacterial cell wall biosynthesis (Barker *et al*., 2017). The major antibacterial agents in this class include; Amoxicillin, Ampicillin, Carbenicillin, Methicillin, Penicillin G, Penicillin K, Spectinomycin, and Ticarcillin. They are utilized in the treatment of tonsillitis, ear, and skin diseases, upper respiratory tract, as well as sore throat infections due to *S. aureus* bacterial pathogen (Fymat, 2017). Despite their broad use, there has been an emergence of penicillin resistance bacterial strains, increased reported cases of penicillin-related allergies, and severe adverse side

effects of penicillin which have so far limited the widespread use of this antibiotic (Barker *et al*., 2017).

2.4.2 Aminoglycosides

Aminoglycosides are broad-spectrum antibiotics derived from Actinomycetes. They are particularly active against Enterobacteriaceae bacterial family and were amongst the early first-line antibacterial agents in the early days of antibacterial therapy (Krause *et al*., 2016). Aminoglycosides act by interfering with the DNA or RNA synthesis of the bacteria and include Amikacin, Aureomycin, Gentamicin, Kanamycin, Streptomycin, Tetramycin, Tobramycin, and Vancomycin (Fymat, 2017). Despite their wide use as a broad spectrum of conventional antibacterial agents, aminoglycosides have adverse effects like depletion of normal bacterial flora in the gut, epiglottitis, and tonsillitis (Fymat, 2017), nephrotoxicity, and ototoxicity (Durante-Mangoni *et al*., 2009).

2.4.3 Cephalosporins

Cephalosporins belong to the broad-spectrum beta (β)-lactam group of antibiotics. They are bactericidal and are closely related chemically to penicillins (Vardanyan and Hruby, 2016). They interfere with bacterial cell wall synthesis and protein synthesis. They are commonly used to treat respiratory tract diseases (Vardanyan and Hruby, 2016), mixed aerobic and anaerobic soft tissue and skin infections, gynecological infections and can also be used for surgical prophylaxis (Zaffiri *et al.*, 2012). Cephalosporins are generally reported to have adverse effects like skin eruptions, anaphylaxis, urticaria, serum sickness reactions,

fever, eosinophilia, acute interstitial nephritis, and drug-induced cytopenias (Harrison and Bratcher, 2008).

2.4.4 Tetracyclines

Tetracyclines are broad-spectrum bacteriostatic antibiotics that work by inhibiting protein synthesis (Vardanyan and Hruby, 2016). Some of the drugs in this group include Doxycycline, Methacycline, Minocycline (Fymat, 2017). During the administration of tetracyclines, they get incorporated into the tissues that get calcified, which can lead to enamel hypoplasia (Vennila *et al*., 2014). In addition, tetracyclines are suspected to promote the emergence of antibiotic resistant microorganisms in the environment (Daghrir and Drogui, 2013).

2.4.5 Macrolides

Macrolides are generally bacteriostatic antibiotics generated from various strains of *Streptomyces* though some may be bactericidal when administered at high doses. Macrolides include erythromycin, azithromycin, clarithromycin, roxithromycin, and dirithromycin (Vardanyan and Hruby, 2016). They act by inhibiting protein synthesis during bacterial cell translation (Kannan *et al.*, 2014). Macrolides have been considered the drug of choice in the management of streptococcal tonsillopharyngitis, acute otitis infections, and oral cavity infections (Steel *et al*., 2012). Despite these uses, bacterial resistance to macrolides is increasingly becoming a major concern (Vardanyan and Hruby, 2016). They are reported to cause adverse side effects like gastrointestinal upset

and cardiac arrhythmias, as well as increased resistance among respiratory infection pathogens (Altenburg *et al*., 2011).

2.4.6 Sulfonamides

Sulfonamides belong to a group of broad spectrum synthetic antibiotics that contain sulfonamide chemical groups. They act by interfering with the folic acid synthesis in the bacteria (Fymat, 2017; Tačić *et al*., 2017). Sulfonamides drugs include Sulfanilamide, Sulfamethoxazole, and Sulfisoxazole. They are used in the management of urethritis, intestinal infections, and the prevention of pneumocystis. However, this group of antibiotics has several adverse side effects, including hypersensitivity reactions (Schnyder and Pichler, 2013), nausea, vomiting, diarrhea, persistent headache, seizures, and slow/irregular heartbeat (Fymat, 2017).

The body's disparity between free radicals and antioxidants can lead to oxidative stress, which causes cell damage and can lead to degenerative conditions and chronic diseases, including diabetes, formation of plaques within the artery leading to hypertension, and aging (Jayasri *et al*., 2009). The effects of OS are preventable by the use of antioxidants which protect cells and tissues from the destructive effects of these unstable oxidants (Mattson and Cheng, 2006).

During oxidative stress, the body produces antioxidants which include enzymes and vitamins, to counteract the effects caused by the oxidants. However, these

antioxidants can get overwhelmed by some factors like environmental factors that prevent the antioxidants from detoxifying the oxidants hence making the oxidants remain in the body as they cannot be detoxified and eliminated from the body (Halliwell and Gutteridge, 2015). Accumulation and increase of OS in the body leads to the destruction of the base pairs of the DNA molecule, causing mutations and other chronic conditions like cancer (Wei *et al*., 2001).

The unstable oxidants in the body attach to negatively charged ions around them to gain stability, a process that causes cellular damage leading to chronic conditions like autoimmune disorders, diabetes, cardiovascular-related conditions, among others. As a result, the antioxidants donate ions to these unstable free radicals to stabilize them, thus counteracting their effects (Uddin *et al*., 2008). In addition to naturally produced antioxidants, synthetic antioxidants are also used as counteraction measures against oxidative stress effects in the body. These synthetic antioxidants include propyl gallate, nordihydro-guaretic acid, butylated hydroxyanisole, butylated hydroxytoluene, metal chelating agent, and tertiary butyl hydroquinone (Hamid *et al*., 2010).

2.4.7 Butylated hydroxytoluene (BHT)

Butylated hydroxytoluene (BHT) is the most commonly used synthetic antioxidant and is considered safe to use in food and pharmaceutical products. It is a hydrophobic molecule derived from phenol and acts as an antioxidant by preventing autoxidation when oxygen molecules in the body attack the

unsaturated organic molecules in the body. It is also considered an artificial equivalent of tocopherol antioxidants. BHT counteracts the consequences of oxidative stress by converting peroxy free radicals generated in the body to stable hydroperoxides through the donation of hydrogen atoms to the unstable peroxy free radicals (Yehye *et al*., 2015). However, BHT has been found to increase the weight of the liver and reduce the activities of some liver enzymes, as well as having effects on the kidneys and the skin. In addition, it has been shown to have tumor-promoting effects (Lanigan and Yamarik, 2002).

2.4.8 Butylated hydroxyanisole (BHA)

The other commonly used synthetic antioxidant is Butylated hydroxyanisole (BHA) which has been used as both antioxidant and a food preservative as well as used in rubber and petroleum industries. BHA was initially used in petroleum industries to prevent petroleum products from oxidative gumming but has been used in food as an antioxidant since 1954. However, this synthetic antioxidant has been known to be a tumor promoter when used at high concentrations (Atta *et al*., 2017).

2.4.9 TBHQ (tert-butylhydroquinone)

TBHQ (tert-butylhydroquinone) is another synthetic antioxidant with a benzene ring structure which is commonly used in the feed industry (Atta *et al*., 2017) as a food preservative predominantly employed to stabilize and preserve fats (Ooi *et al*., 2013). However, despite its wide antioxidant use, a number of studies have

shown that tBHQ can induce carcinogenicity at high dosages (Gharavi *et al*., 2007).

2.4.10 Propyl gallate

Propyl gallate is majorly used in food, cosmetics, and pharmaceutical companies (Garrido *et al.,* 2012). Propyl Gallate is generally used to protect oils and fat containing foods from formation of peroxides. However, Propyl gallate also irritates the skin, eyes and is also a dermal sensitizer. Inhalation of Propyl gallate is considered hazardous (FEEDAP *et al*., 2020).

2.5 Plant-based treatment and management of bacterial infections and oxidative stress-related diseases

Several experiments have revealed that plants have both antioxidant and antibacterial activities. Phytochemicals found in plants can inhibit bacterial pathogenesis with less toxicity effects on human bodies thus have been utilized as alternative human medicine replacing the conventional synthetic modern medicines (Ceylan *et al*., 2019). Antioxidants protect living organisms from cellular damage due to oxidative stress. Antioxidants can quench free radicals hence preventing oxidation of unstable molecules, which causes cellular damage hence promoting positive health benefits to humans. Plants have phytochemicals with antioxidant activities, which can scavenge and inhibit the production of free radicals at the same time modifying intracellular redox reactions in the body (Kaneria *et al*., 2009). Some of the major phytochemicals produced by plants

with antioxidant and antibacterial activities include phenolics, tannins, alkaloids, terpenoids, steroids, saponins, and flavonoids.

2.5.1 Phenolic compounds

Phenols represent the largest portion of plant phytochemicals and are composed of hydroxycinnamic and hydroxybenzoic acids (Altemimi *et al*., 2017). Phenols act as antibacterial agents by denaturing the bacterial proteins and binding to the peptidoglycan wall of the bacteria hence breaking its cell wall (Khlif *et al*., 2015). Generally, most phenols have a superior antibacterial effect on Gram-positive over Gram-negative pathogens (Patra, 2012). They protect against free radicals (Koche *et al*., 2016) by chelating metal cations, donating hydrogen atoms or electrons in addition to scavenging free radicals (Minatel *et al*., 2017).

2.5.2 Flavonoids

These are phytocompounds with low molecular weight. They are found as aglycones and methylated derivatives. They have been shown to have both antibacterial and antioxidant effects (Koche *et al*., 2016). Flavonoids are believed to exert antibacterial activities by inhibiting the nucleic acid synthesis in bacteria, interfering with cytoplasmic membrane functionality, and inhibiting energy metabolism by the bacteria (Cushnie and Lamb, 2005; Xie *et al*., 2015). They are plant phytochemicals with the largest antioxidant properties (Tungmunnithum *et al*., 2018) and offer protection against OS through scavenging free radicals, acting as reducing agents, hydrogen donors as well as chelate irons (Takaidza *et al*., 2018).

2.5.3 Tannins

Tannins are phytochemicals in plants with several phenolic rings. They are classified as either hydrolyzable tannins, which have a mixture of phenols and ester linkages in their structures, or condensed tannins, which contain condensed flavonoid units (Altemimi *et al*., 2017). They have both antioxidant and antibacterial activities (Koche *et al*., 2016). The antibacterial effects of tannins are reported to be as a result of their ability to bind to bacterial proteins, complex with the bacterial cell wall, inhibit bacterial enzymes as well as disrupt bacterial plasma membrane (Dimech *et al*., 2013). Traditionally, they have been utilized to cure bacterial diarrhea (Mbaebie *et al*., 2012). Additionally, they are good free radical scavengers hence defending the body from oxidative harm (Mbaebie *et al*., 2012). They also interfere with the Fenton reaction process by chelating metal ions hence retarding oxidation and inhibiting lipid peroxidation (Amarowicz, 2007).

2.5.4 Alkaloids

These are plant chemicals with a bitter taste containing heterocyclic nitrogen atoms. They are known to have antibacterial and antioxidant effects, amongst other health benefits (Koche *et al*., 2016). Alkaloids' antibacterial properties owe to their ability to disrupt bacterial cell division, inhibit respiratory and enzyme functions, disrupt plasma membrane function, and affect bacterial gene virulence (Othman *et al*., 2019). Alkaloids exert their antioxidant activity by capturing and scavenging free radicals by donating electrons to unstable free radicals, thus

inhibiting their oxidation process (Chen *et al*., 2016; Thawabteh *et al*., 2019). They work against superoxide anions free radicals (Rau *et al*., 2014) by converting the superoxide radical to hydroperoxyl radical ($HO_2^{\bullet}$), which is then converted to hydrogen peroxide (H_2O_2) (Kasote *et al*., 2015). The H_2O_2 is finally converted to O_2 and H_2O (Nimse and Pal, 2015).

2.5.5 Terpenoids

Terpenoids are plant compounds considered the most common type of plant secondary metabolites. Their known medicinal functions include antibacterial and antioxidant activities (Koche *et al*., 2016). Terpenoids act as antioxidants through the donation of hydrogen to free radicals to stabilize them, thus diminishing the lipid oxidation progression (Takaidza *et al*., 2018). In addition to being effective antioxidants, terpenoids protect the body against bacterial infections by disrupting the bacterial membrane, thus killing or hindering bacterial growth (Jasmine *et al*., 2011). They can also interfere with the bacterial membrane permeability altering the protein topology of the bacteria, thus causing disturbances across the bacterial respiration chain (Khameneh *et al*., 2019).

2.5.6 Saponins

The name saponin comes from the fact that these groups of compounds form stable foam while in aqueous solutions like soap. Saponins have several biological effects like anticarcinogenic, antiprotozoal, antibacterial as well as antioxidant effects (Koche *et al*., 2016). The detergent-like properties of saponins increase the bacterial cell membrane permeability increasing

antibacterial agents' influx, thus leading to their antibacterial activity (Arabski *et al*., 2012). However, most saponins exhibit superior antibacterial effects on Gram-positive pathogens than Gram-negative pathogens (Patra, 2012). Saponins reduce the effects of cardiovascular diseases by scavenging low-density lipoproteins in the body (Moyo *et al*., 2013). They also have proton donating capacity to free radicals hence stabilizing them as well as scavenging nitric oxide radicals hence reducing their harmful effects in the body (Nafiu and Ashafa, 2017).

2.5.7 Steroids

Steroids are phytochemicals that play important roles in the growth of plant cells. Steroids have both vinyl and peroxide bonds in their structures, giving them a resemblance to sterols which are found in the bacterial cells; thus, the steroids replace the normal sterols in the bacterial cell membrane, thus disrupting the bacterial cell membrane hence killing the bacteria (Dogan *et al*., 2017). Steroids prevent the effects of unstable free radicals by donating electrons to make them stable (Mansoori *et al*., 2020).

2.5.8 Fatty acids

Fatty acids are phytochemicals that have carboxylic acids with either straight or branched aliphatic chains and can be saturated or unsaturated (Casillas-Vargas *et al*., 2021). They have various biological properties, including antibacterial and antioxidant properties (Karimi *et al*., 2015). Fatty acids confer their antibacterial properties by intensifying the pathogen's membrane permeability, instigating

bacterial cell lysis, and by inhibiting the activity of several bacterial enzymes (Yoon *et al*., 2018). Fatty acids are known to scavenge free radicals and are directly involved in scavenging superoxide free radicals, thus having antioxidant effects (Richard *et al*., 2008).

2.6 Extraction solvent

Effective extraction of phytochemicals and yields is reliant on the solvent type used for the extraction process as various solvents and phytochemicals have different polarities and solubilities (Asghar *et al.*, 2016). Solvents used in extraction include n-butanol, methanol, ethyl acetate, n-hexane, dichloromethane, water, ethanol, among others. In the current experiment, ethyl acetate solvent was used. Ethyl acetate is a polar solvent having a 4.4 polarity index in addition to 77.1^{o}C boiling point (BP) (Sadek, 2002), thus the ease of extraction of polar compounds and subsequent concentration using a rotary evaporator. In a study done by Praveena and Pradeep (2012), the antibacterial properties of ethyl acetate extracts were higher compared to methanol and hydroalcoholic extracts. Another study by Arora and Sood (2017) showed that ethyl acetate is the best organic extractant with high inhibition zones compared to aqueous extracts. A different previous study demonstrated that ethyl acetate extracts had greater antibacterial activities relative to chloroform and methanol extracts (Bajpai *et al*., 2009).

2.7 Studied plants

2.7.1 *Xerophyta spekei* (Baker)

X. spekei, also called *Vellozia spekei*, is a member of the Velloziaceae family. It's common in Kenya, Zambia, Tanzania, Zimbabwe, and Ethiopia. It is a shrub of about 2 - 5 meters tall with 6 - 12 cm thick branched stems. The leaves are congregated on one edge of the branch (Wanga *et al*., 2020). The leaves are covered by short glandular trichomes, which are denser in margins with non-secretory hairs (Péli and Nagy-Déri, 2018). The flowers appear 1 - 4 in a leaf axial at the stem apex (Wanga *et al*., 2020).

Figure 2.1: *X. spekei*

The Kamba call it Kiandui, and its stem bark is used by herbalists in Makueni to treat burns (Kisangau and Herrmann, 2007). In the Embu community, it is

known as Kianduri and is used to treat dog bites and diabetes (Kareru *et al.*, 2007). It is also used as an antivenom where the ashes of the whole plant are applied at the site of the snake bite (Omara, 2020). The Mbeere community uses it to treat dog bites and diabetes (Kareru *et al*., 2007). Traditional herbalists in South Africa use it as an analgesic and an anti-inflammatory (Kellermann, 2010). The Taita who live along Mount Kasigau call *X. spekei* Kidue and use its stem to make strong brooms or paint brushes (Medley and Kalibo, 2007).

2.7.2 *Senna singueana* (Delile)

S. singueana, which is also known as scrambled egg, is an African traditional medicinal plant with many medicinal uses throughout the African continent and is categorized in the Fabaceae family (Shawa *et al*., 2016; Sobeh *et al*., 2017). It is a deciduous shrub with a light, open crown; it can grow to 1 - 15 meters tall. The bole can be 15 cm in diameter, it has a spectacular flowering display which often takes place in the dry seasons (Hishe *et al*., 2018).

Figure 2.2: *S. singueana*

It's used as a therapy for diarrhea, conjunctivitis, bilharziasis, and coughs in different communities (Kidane *et al*., 2014; Hiben *et al*., 2016; Shawa *et al*., 2016). The Mbeere community calls it Mukengeta, and they use it to treat anthrax and elephantiasis (Kareru *et al*., 2007). It has been shown to have anticancer and antimalarial effects (Gerezgher *et al*., 2018). Its leaves are used in treating stomach pains, whereas the fruits are considered lactogenic, and the root bark has been used to cure helminthic infections (Shawa *et al*., 2016). Additionally, Nigerian communities use it as traditional therapy for diabetes mellitus (Ibrahim and Islam, 2014).

2.7.3 *Grewia tembensis* (Fresen)

G. tembensis is a small multi-stemmed perennial shrub about 4m high with long, narrow, smooth, gray stems belonging to the family Tiliaceae. The leaves are green, thinly hairy, and slightly rough above with jagged margins. Flowers are white to pink, and the fruits are usually 2 - 4 lobed, green when young but turn orange when mature. It grows in moderately dry areas in Eastern Africa (Gebreyohannes, 2013).

Figure 2.3: *G. tembensis*

The Kipsigis call it Chesarebut, the Mbeere call it Muruba (Kokwaro, 2009), while the Maasai in Kajiado county calls it Eirri (Gebreyohannes, 2013). *G. tembensis* is used in Djibouti to treat microbial infections like abscesses and furuncle (Hassan-Abdallah *et al*., 2013), while the fruits are eaten by the Kamba from the Ithanga division (Njoroge *et al*., 2017). The Maasai in Kajiado county in Kenya uses *G. tembensis* in combination with sheep dung to close gaps in

skull fissures (Kiringe, 2006), while the Maasai from Sekenani Valley, Maasai Mara, Kenya use it as fodder for animals, for dental hygiene, and fencing of their homestead (Bussmann *et al*., 2006).

The Turkana community uses *G. tembensis* to treat coughs and eat its fruits as food (Mutie *et al*., 2020). The Kamba community calls it Muvindaviti or Mutuva and uses its roots to treat typhoid (Kokwaro, 2009), heartburn, hypochondriasis (Wanzala *et al*., 2016), reduced appetite, and swollen diaphragm (Mutie *et al*., 2020). In Tanzania, the Pare community calls it Mkokoro and uses it to treat breast cancer (Matata *et al*., 2018). Some African communities have been reported to use *G. tembensis* as human feed (Tefera *et al*., 2007), while pastoralists in Southern rangelands in Ethiopia use it as livestock feed (Abebe *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant materials collection and preparation

Fresh *X. spekei* (whole plant without roots), *S. singueana* (leaves), and *G. tembensis* (leaves and stem barks) parts were gathered from Gikuyari village, Thura Sub Location, Thura Location, Siakago Division, Mbeere North Sub County in Embu County, Kenya (Figure 3.1) in May 2021 with the assistance of a local practicing traditional herb doctor. GPS coordinates for *S. singueana* were 0°35'39" N, 37°38'12" E, while for *X. spekei* were 0°36'34" N, 37°37'15" E, and for *G. tembensis* were 0°35'38.55552" S, 37°38'12.3252" E. The plants were transported to Kenyatta University, where they were identified by a recognized taxonomist (Mr. Lukas Karimi of Department of Pharmacognosy Kenyatta University), and a voucher specimen for each plant was preserved at Kenya National Museum's herbarium for future reference. Voucher numbers for the specimens were allocated as PN/001/27698/2018, PN/002/27698/2018, and PN/003/27698/2018 for *S. singueana*, *X. spekei*, and *G. tembensis*, respectively. The plants were properly washed using running tap water, rinsed using distilled water (dH_2O), and chopped into small pieces. They were then shade dried for 28 days, finely ground into powder prior to storage at room temperature in airtight vessels ready for the extraction process.

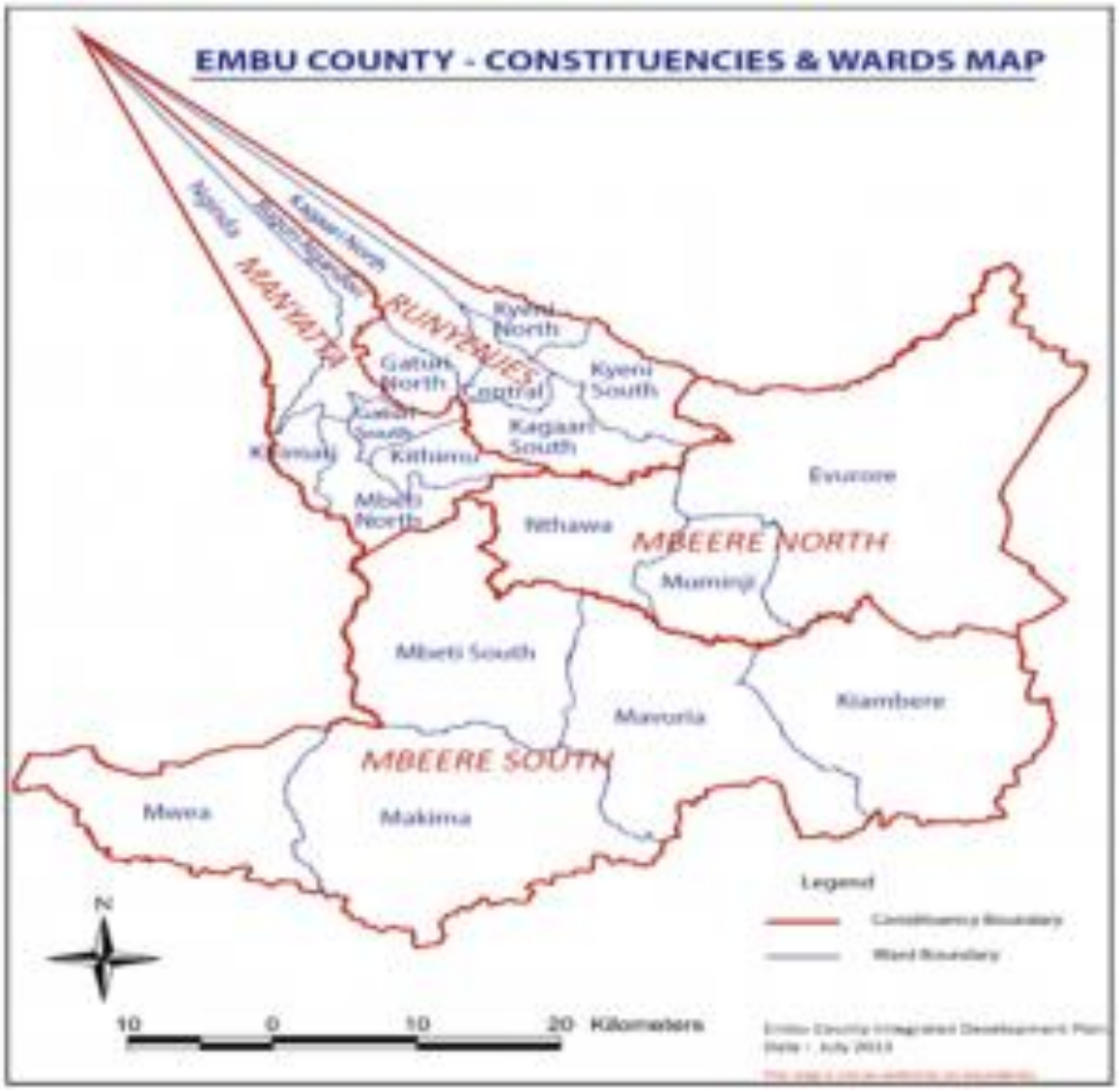

Figure 3.1: Map showing Mbeere North Sub-County, Embu County

3.2 Extraction procedure

Four hundred grams (400 grams) of dry powder of *S. singueana* leaves and *G. tembensis* stem bark were separately soaked in 1.2 L of ethyl acetate for 72 hours. In addition, 800 grams of *X. spekei* whole plant dry powder and 300 grams of *G. tembensis* dry leaves powder were separately soaked in 2.4 L and 0.9 L of ethyl

acetate, respectively, for 72 hours. The solutions were occasionally swirled to achieve complete dissolution.

After 72 hours, the solutions were decanted and vacuum filtered with the help of a Buchner funnel and Whatman's filter paper No. 1. Thereafter, using a rotary evaporator, the filtrates were separately concentrated to evaporate the solvent at 90 rpm at 60°C under vacuum. The extract yields of the plants were determined according to the following equation:

$$Percentage\ Yield\ = \left(\frac{K1}{K2}\right) \times 100\ \%$$

Where K1-mass of concentrated plant extracts, K2-dry mass of the powdered plant before extraction (Felhi *et al*., 2017).

The resultant extracts were stored at 4°C in clean, sterile glasses awaiting bioassay studies.

3.3 Antibacterial assays

3.3.1 Test microorganisms and controls

Bacterial isolates used for antibacterial assays were sourced from Kenyatta University's Microbiology Laboratory, Biochemistry, Microbiology, and Biotechnology (BMB) Department. They consisted of Gram positive (*B. subtilis* ATCC 21332 and *S. aureus* ATCC 25923) and Gram negative (*E. coli* ATCC 25922 and *S. typhi* ATCC 1408). Ciprofloxacin was utilized as a positive reference (reference antibiotic) because it is a broad spectrum antibiotic commonly used to

treat bacterial diseases caused by the studied bacteria in this study, whereas DMSO (5 %) was utilized as a negative reference.

3.3.2 Media preparation

Mueller Hinton Agar and Mueller Hinton Broth media were both prepared and sterilized using an autoclave at 121^{o}C, following the manufacturer's instructions. All the media used were obtained from HIMEDIA Laboratories manufacturers. Mueller Hinton Agar (MHA) was prepared by weighing 38 grams of the MHA powder in a clean flat bottomed conical flask, 1 L of distilled water was then added, rigorously mixed before heating in a water bath for complete powder dissolution. This was sterilized for 15 mins at 121^{o}C, 15 psi pressure. The media was left to cool to 55^{o}C. Thereafter, approximately 20 ml were dispensed into sterile Petri dishes in a Biosafety Cabinet No. 2. Culture plates were allowed to set at room temperature, then packed upside down in sterile plastic bags and stored at 2 – 8^{o}C until use (Sieberi *et al*., 2020).

In a clean conical flask, 21 grams of Mueller Hinton Broth powder were liquefied with 1 L distilled water (DH_2O) to make Mueller Hinton Broth (MHB). The blend was heated in a water bath for complete dissolution. Nine (9) ml of the mixture were then transferred to bijou bottles for sterilization by autoclaving at 121^{o}C, 15 psi pressure for 15 minutes.

3.3.3 Sterile paper discs' preparation

Whatman's filter papers No 1 were punched with the aid of a paper punch to prepare 6mm diameter paper discs. Prior to sterilization, they were placed in bijou bottles and autoclaved at 121°C for 15 minutes. After which, they were stored in a dry clean place until use.

3.3.4 McFarland turbidity standard preparation

McFarland turbidity standard (std) was made according to a protocol discussed by Hudzicki (2009) with slight modifications by combining 0.5 ml of 1.175% Barium chloride ($BaCl_2$) solution with 99.5 ml of 1% Sulphuric acid (H_2SO_4). The Barium sulfate mixture was then aliquoted into screw-capped tubes and kept in a dark place at room temperature awaiting use.

3.3.5 Inocula suspension preparation

Inocula suspension was prepared according to the protocol described by Hudzicki (2009), where 4 – 5 pure colonies of each sub-cultured bacterial strain were picked using a sterile inoculating wire loop and then suspended in sterile normal saline (10 ml) in a universal bottle in a Biosafety cabinet. To obtain a standardized suspension for all bacteria, the prepared inocula were adjusted to 0.5 McFarland standard.

3.3.6 Preparation of extracts concentrations and disc impregnation

One hundred (100) mg of *X. spekei, S. singueana,* and *G. tembensis* (stem bark and leaf) extracts were weighed then placed in sterile 2ml micro-centrifuge tubes. One (1) ml of 5 % DMSO was added, the blend properly vortexed thereafter sonicated

to ensure complete dissolution to achieve 100 mg/ml stock solution concentration (Adefuye *et al*., 2011).

Two-fold serially diluted concentrations were prepared by taking 500µl of extracts stock solution and mixing with 500 µl of 5 % DMSO to attain concentrations beginning from 50mg/ml to 3.125mg/ml. Fifteen (15) µl of serially diluted extracts were used to impregnate sterile discs made as illustrated in section 3.3.3 above. The discs were left in the Biosafety cabinet to air dry for about 20 minutes before being placed on the surface of inoculated media. Ciprofloxacin powder (100 µg) dissolved in 1000 µl of sterile normal saline (Bhalodia and Shukla, 2011) was applied as a positive reference while DMSO (5 %) was applied as a negative reference.

3.3.7 Antibacterial sensitivity tests

Antibacterial sensitivity assays were conducted using the disc diffusion technique in triplicates as explained by Hudzicki (2009). Sterile cotton swabs were dipped in the bacterial inocula prepared as outlined in section 3.3.5 and spun on the tube's sides to eliminate surplus fluid. After which, they were streaked all over the already prepared Mueller Hinton Agar media. To guarantee the inocula's even distribution, the plates were rotated approximately 60 degrees each time. The inoculated plates were then left to dry for about 5 minutes in a Biosafety cabinet before placing the discs on the surface. Using sterile forceps, the 6mm paper discs impregnated with various dilutions of the plant extracts, 5 % DMSO (negative control) or

Ciprofloxacin (positive control) were then placed on the agar surface, one at a time. The plates were placed in sterile condition at normal room temperature (RTP) for around 15 mins to allow for infiltration of the extracts, 5 % DMSO, and Ciprofloxacin into the Mueller Hinton Agar media (Manandhar *et al*., 2019) then incubated at 37 degrees Celsius (^{o}C) for 24 hours (Mwitari *et al*., 2013), after which the clear zones around the discs were determined in millimeters using a ruler and recorded in spreadsheets. Based on criteria detailed by Mwitari *et al*. (2013), the antibacterial activity of the studied extracts and the positive control was determined as follows;

i. Zones of inhibition < 7 mm were considered not to have any activity,
ii. Zones between 8 – 11 mm were considered active and
iii. Zones > 11 mm were considered very active.

3.3.8 Minimum inhibitory concentrations (MICs)

To determine the minimal inhibitory concentration, a broth dilution experiment was done in triplicates following the protocols as performed by Nikolic *et al.* (2014) and Sarker *et al.* (2007). Extracts were double diluted to concentrations from 100mg/ml to 1.5625mg/ml in different sterile 96-well plates containing MHB. This was done by adding equal volumes (100 µl) of the extracts to MHB. After dilution, 20 µl of each test bacterial suspension, adjusted to standard turbidity (0.5 McFarland), was pipetted to the wells prior to 24 hour 37 °C incubation. Finally, 1% of resazurin solution (50 µl) was added to every well to serve as an indicator. Thereafter, the plates were re-incubated at 37°C for 30 mins (Elshikh *et al*., 2016).

The minimum concentration that inhibited visible blue to pink resazurin color change was considered minimum inhibitory concentration (Elshikh *et al*., 2016). Ciprofloxacin powder (100 µg/ml) was diluted the same way the extracts were diluted, while 5 % DMSO was used as a negative reference.

3.3.9 Minimum bactericidal concentrations (MBCs)

Using a sterile cotton swab, 10 µl of the materials from every well having concentrations at and above the MICs of studied antibacterial agents were spread all over the surface of the MHA plate (Sieberi *et al*., 2020), followed by 37 °C incubation for 24 hours. Minimum bactericidal concentration was documented as the minimal concentration with no visible bacterial growth on MHA (Kang *et al*., 2011). Bacterial growth on the Mueller Hinton Agar plates was recorded as bacteriostatic effects of the extracts, whereas lack of bacterial growth on the Mueller Hinton Agar plates was considered as bactericidal effects of the investigated extracts. This was done in triplicates for each experiment.

3.4 Determination of *in vitro* antioxidant activities

3.4.1 *In vitro* DPPH radical scavenging activity

The plant extracts' ability to mop 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals was done in triplicates as conducted by Oyedemi *et al.* (2010), with minor modifications (plant extracts' and the standard's concentrations used in the current study were different from the ones used in the previous study). Plant extracts and ascorbic acid (reference) were prepared at various concentrations beginning from

15.625µg/ml to 500µg/ml. DPPH (1mM) solution was prepared in methanol. One ml of each dilution of the test extract and the standard were separately placed in clean test tubes, after which DPPH (0.5ml) and methanol (3ml) solutions were added. The blend was thoroughly vortexed for 5 minutes after which; it was set aside in a dark cupboard for 30 mins at ambient temperature. A blank solution containing 3 ml methanol and 0.5 ml DPPH solutions was also prepared. Using a spectrophotometer, the solutions' absorbances were measured at 517 nm against blank. The plant extracts' % DPPH free radical quenching properties were computed as;

$\%\ DPPH\ radical\ scavenging\ capability = \frac{Abs\ Blank - Abs\ Sample}{Abs\ Blank} \times 100$ (Zahin *et al*., 2009)

Half Maximal Inhibitory Concentration (IC_{50}), representing the concentration at which 50 % of the DPPH radicals were mopped, was analyzed using linear regression analysis.

3.4.2 Determination of ferric reducing antioxidant effect

Plant extracts' ferric reducing activity was done following the protocol used by Gangwar *et al.* (2014), with little modifications (plant extracts' and the standard's concentrations used in the current study were different from the ones used in the previous study). Approximately 2.5ml solution of test extracts and reference (Ascorbic acid) at varying concentrations starting from 7.8125µg/ml to 500µg/ml were separately blended with 2.5ml phosphate buffer (pH6.6, 0.2 M) along with 2.5ml potassium ferricyanide (1%) followed by 20 minutes incubation at 50^{o}C.

Thereafter, 2.5ml of 10% trichloroacetic acid was added into the blend and vortexed before centrifugation for 10 minutes at 3000 rotations per min (rpm). The supernate (5ml), DH_2O (5ml), and 0.1% ferric chloride (1ml) were mixed, incubated at normal room temperature for 10 minutes, after which a spectrophotometer set at 700 nm was used to read the absorbance against blank (Rahman *et al*., 2015). The blank solution comprised all the reagents other than the plant extracts and ascorbic acid. This was done in triplicates.

3.4.3 Determination of *in vitro* hydroxyl radical scavenging effects

The extracts' ability to quench hydroxyl radicals was done based on protocols described by Bajpai and Agrawal (2015) and AsokKumar *et al.* (2009). A blend solution of extracts/control (Gallic Acid) at varied concentrations (15.625 µg/ml to 500 µg/ml) 500 µl, 28.0 mM 2-deoxy-2-ribose dissolved in $KH_2\ PO_4$ –KOH buffered solution (20.0mM, pH 7.4) 100µl, EDTA (1.04mmol L^{-1}) 100µl, ascorbic acid (1.0µM) 100µl, $FeCl_3$ (200mM) 100µl and hydrogen peroxide (1.0mM) 100 µl. The blend was incubated at 37 ^oC in a water bath for 1 hour then 1 % cold Thiobarbituric Acid (TBA) 1000 µl along with 2.8% Trichloroacetic Acid (TCA) 1000 µl solutions were added before heating the blend at 100^oC for 15 minutes where a noticeable pink color developed thereafter, the mixture was cooled in cold water. Absorbance was recorded against blank using a spectrophotometer set at 532nm. Assays were run in triplicates. The % radical quenching capability was computed as;

$Percentage\ hydroxyl\ radical\ scavenging\ capacity = \frac{AB-AT}{AB} \times 100$ (Arika *et al*., 2019)

Where; AB= Blank absorbance, AT= Extract/control absorbance.

3.4.4 Iron (Fe^{2+}) chelating activity assay

Iron (Fe^{2+}) Chelating assay was demonstrated following protocols of Arika *et al.* (2019), with slight adjustments (plant extracts' and the standard's concentrations used in the current study were different from the ones used in the previous study). Various dilutions (15.625µg/ml to 500µg/ml) of test extracts and EDTA (control) at a volume of 1ml of each concentration were separately blended with 1ml of ferrous sulfate (0.125 mM) thereafter, ferrozine (0.3125 mM) 1000 µl was added to commence the reaction. The blend was vortexed, incubated at ambient room temperature for 10 mins before reading the absorbance of the plant extracts/reference at 562 nm against blank, which had all the reagents except the extracts and the standard. All the experiments were conducted in triplicates.

The iron chelating properties of the plant extracts and the standard were calculated as follows;

$\%\ Iron\ chelating\ activity = \frac{AB-AS}{AB} \times 100$ (Indracanti and Sivakumar, 2019)

Where; AB= Blank Absorbance, AS= Sample/standard Absorbance.

3.4.5 Determination of hydrogen peroxide radical scavenging effect

Hydrogen peroxide (H_2O_2) free radical mopping capacity of studied plant extracts was done using the protocol of Arika *et al.* (2019). Six hundred (600) µl of H_2O_2 (40mM) prepared using buffered phosphate solution (1.0 M, pH 7.4) was separately

blended with 400 µl of various dilutions (15.625µg/m to 500µg/ml) of the extracts and ascorbic acid (reference). The blend was then allowed to stand for 10 mins at standard room temperature. Optical density was read at 230 nm against a blank that only had phosphate buffer solution (Al-Amiery *et al.*, 2015). All the experiments were performed in triplicates. H_2O_2 free radical mopping effect was calculated as follows;

$\%\ Hydrogen\ peroxide\ scavenging\ activity = \frac{B0-B1}{B0} \times 100$ (Arika *et al.*, 2019)

Where; B0= blank absorbance, B1= sample/control absorbance.

3.5 Quantitative phytochemical screening

Phytochemical screening in the current study was done using GC-MS to determine and quantify phytochemicals available in the ethyl acetate extracts of *X. spekei*, *S. singueana*, and *G. tembensis.*

3.5.1 Preparation of extracts for Gas Chromatography-Mass Spectrometry (GC-MS)

Four clean microcentrifuge tubes (2.0 ml) were labeled as XS, SS, GTL, and GTB for *X. spekei*, *S. singueana*, *G. tembensis* leaf, and *G. tembensis* stem bark extracts, respectively. To each labeled tube, one milligram of the respective test extracts was added followed by 1000 µl of ethyl acetate to liquefy the samples. The samples were vortexed for 1 min then sonicated for 15 minutes, after which they were all centrifuged at 1,400 rotations per min for 5 mins. The resultant supernate (1

mg/ml), dried over anhydrous Na_2SO_4, was used to prepare experimental solutions in triplicates at a concentration of 100 ng/µL (Arika *et al*., 2019).

3.5.2 Gas Chromatography-Mass Spectrometry analysis

This was done by GC-MS on 7890A Gas-Chromatograph joined to a 5975C mass selective detector (Agilent Technologies), which consists of an HP5 MS low bleed capillary column (30 m long, 0.25 mm wide, as well as 0.25 µm film thick). Operating parameters of the mass spectrometer included; relative detector gain mode, 70eV of ionization energy, 3.3 mins of filament delay time, 1666µ/sec of scan speed, 40 - 550m/z of scan range, 230 °C ion source temperature, and 180 °C quadrupole temperature.

Helium gas (99.9%) was applied as a carrier gas at a steady flow speed rate of 1.25ml per minute. Mass transfer temperature was programmed at 200 °C while injector line transfer temperature at 250 °C, with 1 µl injection volume. The oven temperature was programmed at 35 °C for 5 mins followed by a 10 °C/min increase to 280 °C for 24.5 mins and then raised at a rate of 50 °C per min to 285 °C for 20.5mins and a total run period of 50 mins (Arika *et al*., 2019). To identify the phytocompounds found in each extract, a comparison of the obtained data was matched with mass-spectral library search reports from the National Institute of Standards and Technology (NIST) 08 and 11, where each unique peak represented a particular chemical substance (Arika *et al*., 2019).

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3.6 Data management and statistical analysis

The data were tabulated in a Microsoft excel spreadsheet and organized before being imported into Minitab software version 17.00 (Pennsylvania State University), where descriptive statistics values were conveyed as mean ± STD (standard) error of the mean (SEM). One-way Analysis of Variance (ANOVA) for inferential statistics and Tukey's post hoc test for pairwise comparison as well as separation of means were used. A statistically significant p value of <0.05 was used. Graphs and tables were used to present the findings.

For GC-MS data, the various compounds were recognized primarily on their retention time (RT) and fragmentation pattern in comparison with the NIST 08, 11 library search report. For identification of the compounds, an identity match of above 60% with the library phytocompounds was required (Mworia *et al*., 2021). The compound names, molecular weights, and structures were established. The components' concentrations were expressed in µg/g. Descriptive statistics were also done for the three replicates as above.

CHAPTER FOUR

RESULTS

4.1 Yields of the plant extracts

Among the tested extracts, *S. singueana* had the highest yield (4.99%), producing a dark green solid extract, followed by *G. tembensis* leaf (2.99%), which was a dark green semi-solid extract, *X. spekei* (2.01%), a dark brown sticky extract, and *G. tembensis* stem bark (1.87%), which produced a brown solid extract (Table 4.1).

Table 4.1: Plant extracts' percentage yield and description

Extract	% yield (w/w)	Description
S. singueana	4.99	Dark green solid extract
G. tembensis stem bark	1.87	Brown solid extract
X. spekei	2.01	Dark brown sticky extract
G. tembensis leaf	2.99	Dark green semi solid extract

4.2 Antibacterial activities of ethyl acetate leaf extract of *S. singueana* (Delile)

S. singueana extract exhibited notable antibacterial effects against all the tested bacteria in this study. This was manifested by the visible inhibitory zones surrounding the discs impregnated with various dilutions of the extract (Plate 4.1).

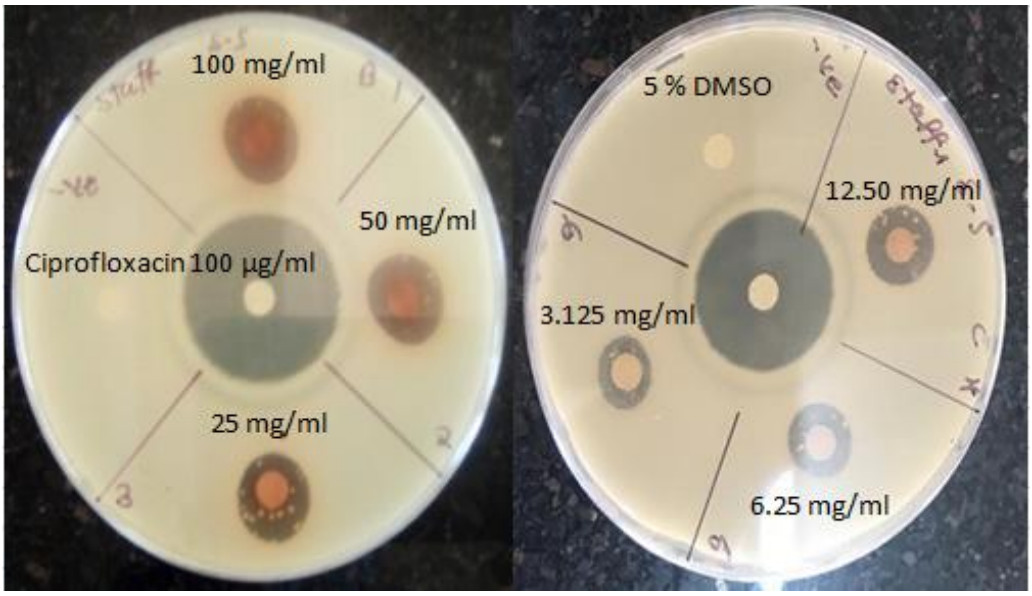

Plate 4.1: Zones of inhibition caused by *S. singueana* extract against *S. aureus*

Generally, *S. singueana* extract demonstrated a greater effect against tested Gram-positive (+ve) pathogens (*B. subtilis* and *S. aureus*), recording greater than (>) 12 mm of mean zones of inhibition (MZI) at extract concentration ranges from 25.00mg/ml to 100.00mg/ml. At 12.5mg/ml concentration, it had a MZI > 12 mm against *S. aureus* (Table 4.2). The extract showed activity at all tested concentrations against the tested Gram-positive pathogens. However, *S. singueana* extract displayed no effect against tested Gram-negative (-ve) pathogens at concentration ranges from 3.125mg/ml to 12.5mg/ml (Table 4.2).

At 100 mg/ml concentration, *S. singueana* extract displayed antibacterial activities against both *S. aureus* and *B. subtilis* that were significantly different from the activities recorded by Ciprofloxacin, DMSO, and extract concentrations ranging

from 3.125mg/ml to 50.00mg/ml ($p<0.05$; Table 4.2). The extract's concentration at 100 mg/ml recorded MZI on *E. coli* and *S. typhi* that were not statistically significant with 50 mg/ml concentration ($p>0.05$; Table 4.2) but significantly distinct with concentration ranges from 3.125mg/ml to 25 mg/ml ($p<0.05$; Table 4.2). *S. singueana* extract at 50 mg/ml concentration showed activities against all pathogens tested, although the activities were not significantly different from the activities of extract concentration 25mg/ml on *E. coli* and *S. aureus* ($p>0.05$; Table 4.2). Similarly, *S. singueana* extract effect at concentration 25 mg/ml was significantly different from activities of extract concentrations ranges of 3.125mg/ml to 12.5mg/ml against all pathogens except against *S. aureus* whose activity was statistically insignificant with concentration of 12.5 mg/ml ($p>0.05$; Table 4.2). At concentration 12.5 mg/ml, *S. singueana* extract had antibacterial effects against *S. aureus* and *B. subtilis* pathogens only even though the effects were not significantly different from activities of concentration 6.25 mg/ml ($p>0.05$; Table 4.2). Similar activities were observed with concentration of 3.125 mg/ml, whose effect was statistically insignificant to that of 6.25 mg/ml ($p >0.05$; Table 4.2).

In vitro antibacterial activity of *S. singueana* extract was dose-dependent with recorded MZI against all the tested bacterial pathogens, increasing with an increase in extract concentrations (Table 4.2).

Table 4.2: Antibacterial activities of ethyl acetate leaf extracts of *S. singueana*

Treatment	Mean zones of inhibition (mm)			
	S. aureus	***B. subtilis***	***E. coli***	***S. typhi***
5% DMSO	06.00 ± 0.00^{g}	06.00 ± 0.00^{g}	06.00 ± 0.00^{d}	06.00 ± 0.00^{d}
Ciprofloxacin (100 µg/ml)	26.33 ± 0.33^{aC}	29.67 ± 0.33^{aB}	32.67 ± 0.33^{aA}	28.33 ± 0.33^{aB}
S. singueana extract (mg/ml)				
100	17.67 ± 0.33^{bA}	17.67 ± 0.33^{bA}	09.67 ± 0.33^{bB}	10.67 ± 0.33^{bB}
50	15.33 ± 0.33^{cA}	14.33 ± 0.33^{cA}	08.67 ± 0.33^{bcB}	09.67 ± 0.33^{bB}
25	13.67 ± 0.33^{cdA}	12.33 ± 0.33^{dA}	07.67 ± 0.33^{cB}	07.67 ± 0.33^{cB}
12.5	12.67 ± 0.33^{deA}	10.67 ± 0.33^{eB}	06.00 ± 0.00^{dC}	06.00 ± 0.00^{dC}
6.25	10.67 ± 0.67^{efA}	09.67 ± 0.33^{efA}	06.00 ± 0.00^{dB}	06.00 ± 0.00^{dB}
3.125	09.33 ± 0.88^{fA}	08.33 ± 0.33^{fA}	06.00 ± 0.00^{dB}	06.00 ± 0.00^{dB}

Values of Mean Zones of Inhibition are expressed as Mean ± SEM. Values with the same lowercase superscript letter within the same column are not significantly different ($p>0.05$) while values with different uppercase superscript letter across the rows are significantly different ($p<0.05$) after one-way ANOVA followed by Tukey's post hoc test.

4.3 Antibacterial activities of ethyl acetate stem bark extracts of *G. tembensis* (Fresen)

Antibacterial activities of *G. tembensis* ethyl acetate stem bark extract were tested at different concentrations against bacterial pathogens *S. aureus*, *B. subtilis*, *E. coli*, as well as *S. typhi* in comparison with the standard antibiotic, Ciprofloxacin, and the diluent, DMSO. The extract exhibited antibacterial activities on *S. aureus* only with MZI ranging from 07.07 mm to 12.33 mm in diameter (Plate 4.2; Table 4.3).

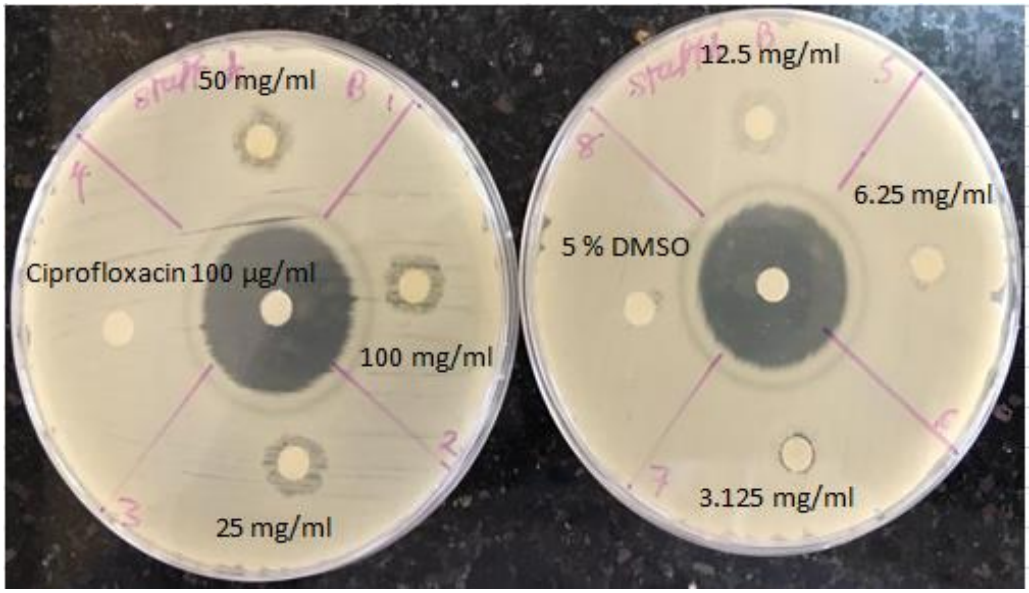

Plate 4.2: Zones of inhibition caused by *G. tembensis* stem bark extract against *S. aureus*

There was no notable effect against *B. subtilis* (Gram-positive) and Gram-negative (*S. typhi*, and *E. coli*) on all the extract concentrations (Table 4.3). The ethyl acetate stem bark extract of *G. tembensis* at concentration 100 mg/ml, recorded an MZI >12 mm against *S. aureus* which was significantly different from concentrations ranging from 50mg/ml to 3.125mg/ml ($p<0.05$; Table 4.3). There was statistical

insignificance in the antibacterial effects of extract concentrations 50mg/ml and 25 mg/ml on *S. aureus* ($p>0.05$; Table 4.3). Likewise, there was statistical insignificance in the effects of extract concentrations 6.25mg/ml and 12.5mg/ml and extract concentrations 6.25mg/ml and 3.125mg/ml ($p>0.05$; Table 4.3), although there was no activity at concentration 3.125 mg/ml.

The inhibition zones increased with an increase in extract concentrations against *S. aureus* (Table 4.3; Plate 4.2).

Table 4.3: Antibacterial activities of ethyl acetate stem bark extracts of *G. tembensis*

Treatment	Mean zones of inhibition (mm)			
	S. aureus	***B. subtilis***	***E. coli***	***S. typhi***
5% DMSO	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}
Ciprofloxacin (100 µg/ml)	26.33 ± 0.33^{aC}	29.67 ± 0.33^{aB}	32.67 ± 0.33^{aA}	28.33 ± 0.33^{aB}
G. tembensis stem bark extract (mg/ml)				
100	12.33 ± 0.33^{bA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
50	10.33 ± 0.33^{cA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
25	09.33 ± 0.33^{cA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
12.5	07.67 ± 0.33^{dA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
6.25	07.07 ± 0.07^{deA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
3.125	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}

Values of Mean Zones of Inhibition are expressed as Mean ± SEM. Values with the same lowercase superscript letter within the same column are not significantly different ($p>0.05$) while values with different uppercase superscript letters across the rows are significantly different ($p<0.05$) after one-way ANOVA followed by Tukey's post hoc test.

4.4 Antibacterial activities of ethyl acetate leaf extracts of *G. tembensis* (Fresen)

G. tembensis leaf extract antibacterial activity was tested at different concentrations against selected bacterial pathogens *S. aureus*, *B. subtilis*, *E. coli*, and *S. typhi* in comparison with the standard antibiotic, Ciprofloxacin, and the diluent, DMSO. The extract exhibited antibacterial activities on *S. aureus* only with MZI ranging from 08.33 mm to 11.67 mm in diameter (Plate 4.3; Table 4.4).

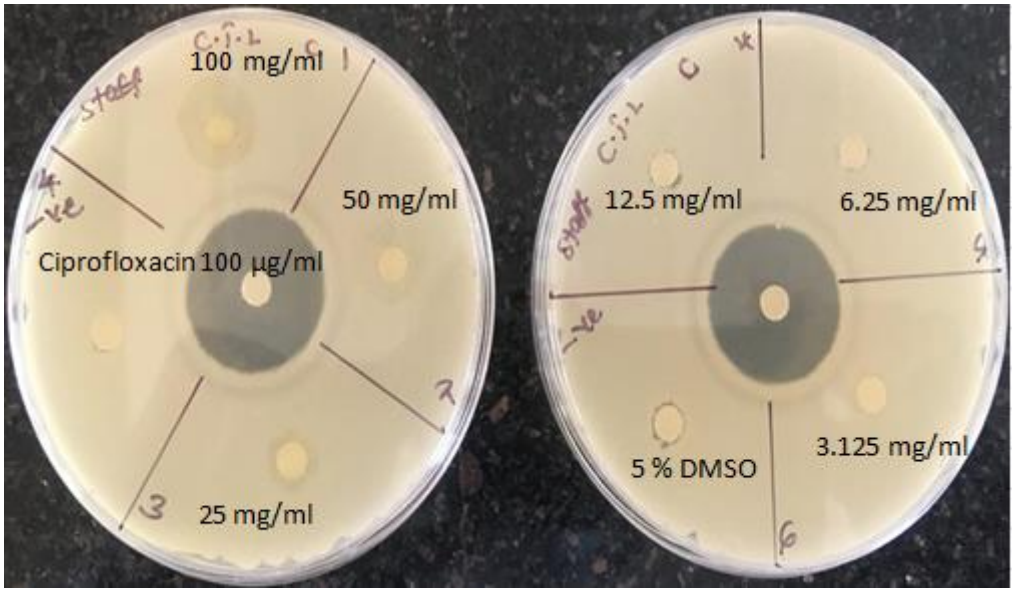

Plate 4.3: Zones of inhibition caused by *G. tembensis* leaf extract against *S. aureus*

The extract and DMSO were inactive on *E. coli*, *B. subtilis*, and *S. typhi* in all the extract concentrations (Table 4.4). Similarly, the extract also showed no activity on *S. aureus* at concentrations ranging from 3.125mg/ml to 12.5mg/ml (Table 4.4). There was no statistical significance in the effect of the extract concentration 100

mg/ml and 50 mg/ml ($p>0.05$; Table 4.4). Same statistical insignificance was noted in the extract concentration 25mg/ml and 50 mg/ml ($p>0.05$). *G. tembensis* leaf extract's antibacterial activity increased with an increase in concentration (Table 4.4), with Ciprofloxacin producing significantly larger zones of inhibition than all the extract concentrations and DMSO ($p<0.05$; Table 4.4).

Table 4.4: Antibacterial activities of ethyl acetate leaf extracts of *Grewia tembensis*

Treatment	Mean zones of inhibition (mm)			
	S. aureus	***B. subtilis***	***E. coli***	***S. typhi***
5% DMSO	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}
Ciprofloxacin (100 µg/ml)	26.33 ± 0.33^{aC}	29.67 ± 0.33^{aB}	32.67 ± 0.33^{aA}	28.33 ± 0.33^{aB}
G. tembensis leaf extract (mg/ml)				
100	11.67 ± 0.33^{bA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
50	10.00 ± 0.58^{bcA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
25	08.33 ± 0.33^{cdA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
12.5	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}
6.25	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}
3.125	06.00 ± 0.00^{e}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}

Values of Mean Zones of Inhibition are expressed as Mean ± SEM. Values with the same lowercase superscript letter within the same column are not significantly different ($p>0.05$) while values with different uppercase superscript letters across the rows are significantly different ($p<0.05$) after one-way ANOVA followed by Tukey's post hoc test.

4.5 Antibacterial activities of ethyl acetate extracts of *X. spekei*

Ethyl acetate extract of *X. spekei* showed notable antibacterial activities against tested Gram-positive bacteria, whereas there was no notable effect against tested Gram-negative bacteria (Table 4.5).

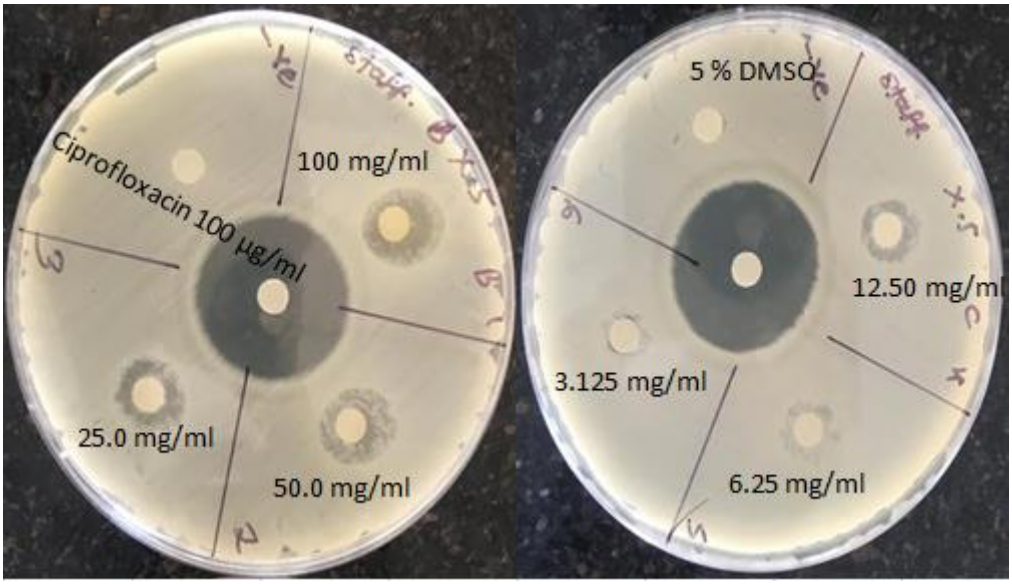

Plate 4.4: Zones of inhibition caused by *X. spekei* extract against *S. aureus*

X. spekei extract showed high effects on *B. subtilis* and *S. aureus* with recorded MZI of > 12 mm at extract concentrations between 50mg/ml and 100mg/ml (Table 4.5), although there was no statistical significance in the effects of both extract concentrations ($p>0.05$). There was no activity against *B. subtilis* at concentrations 3.125mg/ml and 6.25mg/ml. However, *X. spekei* extract showed activity against *S. aureus* in all the tested concentrations (Table 4.5). Additionally, at 12.5mg/ml and 25 mg/ml concentrations, there was statistical insignificance in antibacterial effects on *B. subtilis* and *S. aureus* ($p>0.05$; Table 4.5). Concentrations 6.25mg/ml and

12.5mg/ml and concentrations 3.125mg/ml and 6.25mg/ml also showed no statistical significance in their antibacterial effects against *S. aureus* ($p > 0.05$; Table 4.5). The antibacterial activity of *X. spekei* extract on the tested Gram-positive bacteria was concentration-dependent (as concentration increased, extract's activity also increased) (Table 4.5).

Table 4.5: Antibacterial activities of ethyl acetate extracts of *X. spekei*

Treatment	Mean zones of inhibition (mm)			
	S. aureus	***B. subtilis***	***E. coli***	***S. typhi***
5% DMSO	06.00 ± 0.00^{f}	06.00 ± 0.00^{d}	06.00 ± 0.00^{b}	06.00 ± 0.00^{b}
Ciprofloxacin (100 µg/ml)	26.33 ± 0.33^{aC}	29.67 ± 0.33^{aB}	32.67 ± 0.33^{aA}	28.33 ± 0.33^{aB}
X. spekei extract (mg/ml)				
100	14.67 ± 0.33^{bA}	14.33 ± 0.33^{bA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
50	13.33 ± 0.33^{bA}	13.33 ± 0.33^{bA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
25	11.33 ± 0.33^{cA}	11.00 ± 0.58^{cA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
12.5	09.67 ± 0.33^{cdA}	09.67 ± 0.33^{cA}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
6.25	08.33 ± 0.67^{deA}	06.00 ± 0.00^{dB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}
3.125	07.67 ± 0.33^{efA}	06.00 ± 0.00^{dB}	06.00 ± 0.00^{bB}	06.00 ± 0.00^{bB}

Values of Mean Zones of Inhibition are expressed as Mean ± SEM. Values with the same lowercase superscript letter within the same column are not significantly different ($p>0.05$) while values with different uppercase superscript letters across the rows are significantly different ($p<0.05$) after one-way ANOVA followed by Tukey's post hoc test.

4.6 Comparison of antibacterial activities of ethyl acetate extracts of *S. singueana*, *X. spekei,* and *G. tembensis* against *S. aureus*

Antibacterial effects of *S. singueana*, *X. spekei*, and *G. tembensis* extracts were tested against *S. aureus* and compared using one-way ANOVA. At concentrations beginning from 6.25mg/ml to 100mg/ml, *S. singueana* extract demonstrated significantly greater antibacterial effects against *S. aureus* than the other extracts ($p<0.05$; Figure 4.1). However, at concentration 3.125 mg/ml, *S. singueana* and *X. spekei* extracts demonstrated no statistical significance in their antibacterial effects ($p>0.05$; Figure 4.1). *G. tembensis* leaf and stem bark extracts demonstrated statistically insignificant antibacterial effects from 25 mg/ml to 100 mg/ml concentrations against *S. aureus* ($p>0.05$; Figure 4.1).

The ethyl acetate extracts of *X. spekei* demonstrated significantly different antibacterial effects from the other extracts against *S. aureus*, at 12.5mg/ml to 100 mg/ml concentration ranges ($p<0.05$; Figure 4.1). At concentration 6.25mg/ml, *X. spekei* extract exhibited statistically insignificant effects with *G. tembensis* stem bark extract against *S. aureus* ($p>0.05$; Figure 4.1). Comparative activities of *G. tembensis* leaf extract at concentrations range of 3.125mg/ml to 12.5mg/ml and *G. tembensis* stem bark extract at concentration 3.125 mg/ml were not done as there were no extract activities at these concentrations (Figure 4.1).

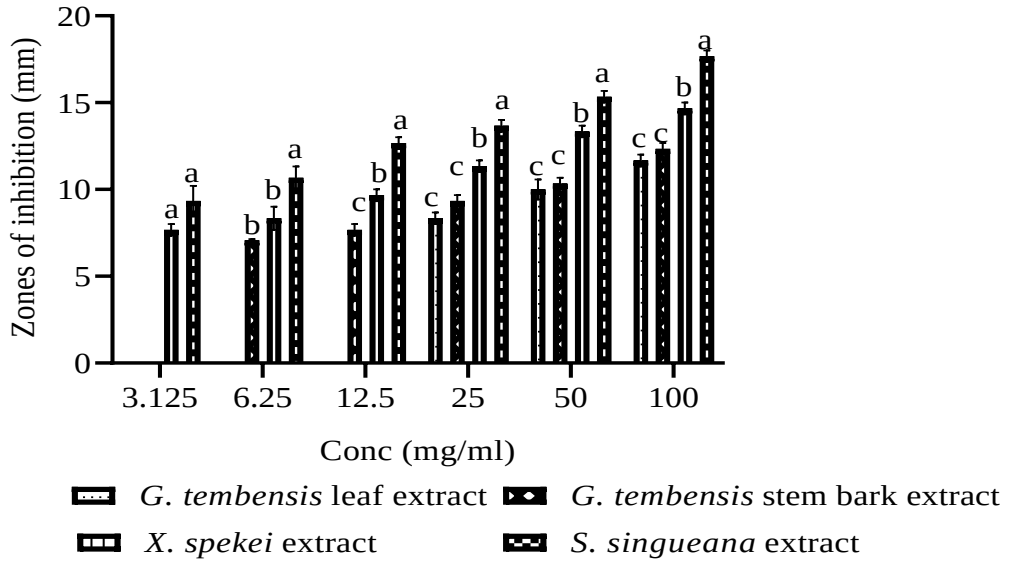

Figure 4.1: Comparison of antibacterial activities of ethyl acetate extracts of *S. singueana, X. spekei,* and *G. tembensis* against *S. aureus*. Bar graphs with distinct letters within a given concentration are significantly insignificant (p<0.05).

4.7 Comparison of antibacterial effects of the studied ethyl acetate extracts against *B. subtilis*

The ethyl acetate extracts of *S. singueana* and *X. spekei*, demonstrated statistical insignificance in their activities against *B. subtilis* at concentrations beginning from 12.5mg/ml to 50mg/ml (p>0.05; Figure 4.2). Although, at a concentration 100 mg/ml, *S. singueana* extract demonstrated significantly higher antibacterial effects against *B. subtilis* than *X. spekei* extract (p<0.05; Figure 4.2). Both leaf and stem bark extracts of *G. tembensis* had no activity against *B. subtilis* at all tested concentrations thus, they were not subjected to comparative analysis (Figure 4.2). Similarly, *X. spekei* extract concentration ranges of 3.125mg/ml to 6.25mg/ml had no activity against *B. subtilis*; thus, the two concentrations were also not subjected

to comparative analysis as only *S. singueana* extract had activity at these two concentrations against *B. subtilis* (Figure 4.2).

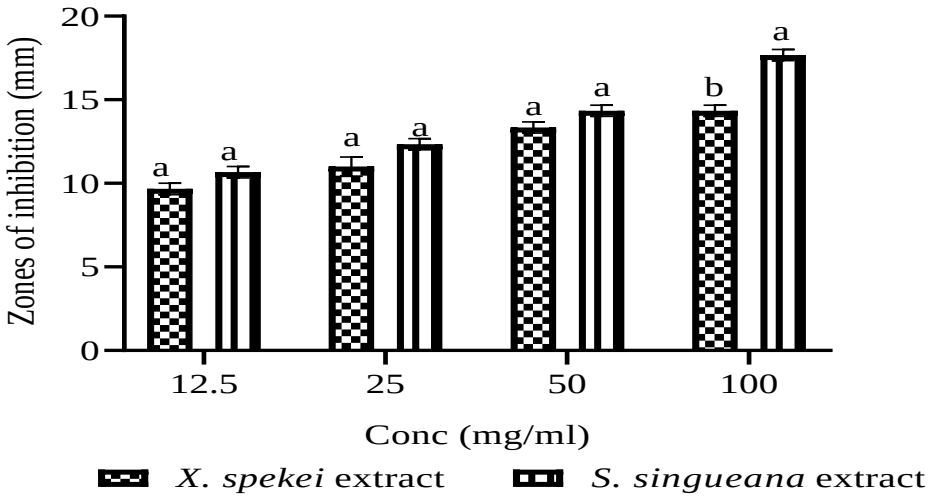

Figure 4.2: Comparison of antibacterial effects of ethyl acetate extracts of *S. singueana* and *X. spekei* against *B. subtilis*. Bar graphs with similar letters within a particular concentration are statistically insignificant (p>0.05).

4.8 Minimum inhibitory concentrations

Selected test extracts showed varied bacterial growth inhibitions and were thus subjected to minimum inhibitory and bactericidal concentrations depending on the pathogens they had effects on.

The MIC means ranged from 1.30±0.26 to 20.83±4.17 mg/ml, 5.21±1.04 to 25.00±0.00 mg/ml, 25.00±0.00mg/ml, and 33.33±8.33mg/ml for ethyl acetate leaf extracts of *S. singueana*, *X. spekei*, stem bark, and leaf extracts of *G. tembensis*, respectively (Table 4.6). *S. singueana* extract showed statistically insignificant inhibition effects on *S. aureus*, *E. coli*, and *B. subtilis* (p>0.05). Similar activity was

observed in the inhibitory effects of *S. singueana* extract on *E. coli* and *S. typhi* (Table 4.6). *X. spekei* extract showed significantly different inhibition effects on *B. subtilis* as well as *S. aureus* ($p<0.05$; Table 4.6), while the reference drug, Ciprofloxacin demonstrated statistically insignificant inhibitory effects against all the tested pathogens ($p>0.05$; Table 4.6).

Table 4.6: Minimum inhibitory concentrations of *S. singueana*, *X. spekei*, and *G. tembensis* extracts

	Concentration (mg/ml)				
Bacterial strain	***S. singueana* extract**	***X. spekei* extract**	***G. tembensis* stem bark extract**	***G. tembensis* leaf extract**	**Ciprofloxacin (µg/ml)**
S. aureus	1.30 ± 0.26^{b}	5.21 ± 1.04^{b}	25.00 ± 0.00^{a}	33.33 ± 8.33^{a}	0.16 ± 0.03^{a}
B. subtilis	3.13 ± 0.00^{b}	25.00 ± 0.00^{a}	NA	NA	0.16 ± 0.03^{a}
S. typhi	41.67 ± 8.33^{a}	NA	NA	NA	0.13 ± 0.03^{a}
E. coli	20.83 ± 4.17^{ab}	NA	NA	NA	0.05 ± 0.00^{a}

Values were conveyed as mean±std error of mean. Values with similar superscript letters within a particular column are insignificantly distinct after one-way Analysis of Variance and Tukey's post hoc ($p>0.05$) NA stands for not active.

In comparison, *S. singueana* extract exhibited inhibitory effects at lower concentrations against all the tested bacterial pathogens than the other extracts (Table 4.7), although its mean inhibitory concentrations were not statistically significant to those of *X. spekei* and Ciprofloxacin against *S. aureus* ($p>0.05$; Table 4.7). Similarly, the inhibitory activities of both stem bark and leaf extracts of *G. tembensis* on *S. aureus* were not statistically significant ($p>0.05$; Table 4.7). *S. singueana* extract's mean inhibitory concentration values were significantly greater

than those of Ciprofloxacin on *B. subtilis*, *S. typhi*, and *E. coli* ($p<0.05$), although the inhibitory effects of *S. singueana* extract were at significantly lower concentrations than those of *X. spekei* extract against *B. subtilis* ($p<0.05$; Table 4.7).

Table 4.7: Comparison of MICs of the studied ethyl acetate extracts and Ciprofloxacin

	Minimum Inhibition Concentration (mg/ml)			
Treatment	***S. aureus***	***B. subtilis***	***S. typhi***	***E. coli***
Ciprofloxacin (µg/ml)	0.16 ± 0.03^{b}	0.16 ± 0.03^{c}	0.13 ± 0.03^{b}	0.05 ± 0.00^{b}
S. singueana extract	1.30 ± 0.26^{b}	3.13 ± 0.00^{b}	41.67 ± 8.33^{a}	20.83 ± 4.17^{a}
X. spekei extract	5.21 ± 1.04^{b}	25.00 ± 0.00^{a}	NA	NA
G. tembensis stem bark extract	25.00 ± 0.00^{a}	NA	NA	NA
G. tembensis leaf extract	33.33 ± 8.33^{a}	NA	NA	NA

Values expressed as mean±std error of mean. Values with identical superscript letters along the column are insignificantly distinct (one-way Analysis of Variance and Tukey's post hoc) ($p>0.05$). NA stands for not active.

4.9 Minimum bactericidal concentrations

Generally, the extracts had higher MBC values than MIC values against each of the test bacterial pathogens (Tables 4.6 and 4.8). Mean MBC were from 12.50 ±0.00 to 100.00± 0.00 mg/ml, 33.33±8.33 to 100.00±0.00mg/ml, 100.00±0.00mg/ml and 100.00±0.00mg/ml for *S. singueana*, *X. spekei* and *G. tembensis* (stem bark and leaf) extracts respectively (Table 4.8).

Both *S. singueana* and *X. spekei* extracts showed significantly higher bactericidal effects against *S. aureus* than *B. subtilis* ($p < 0.05$; Table 4.8). Bactericidal activities of *S. singueana* extract against *E. coli* and *S. typhi* were not statistically significant ($p > 0.05$; Table 4.8). Additionally, *S. singueana* extract's bactericidal effect against *S. aureus* was at lower concentrations than the other extracts (Table 4.8).

Table 4.8: Minimum bactericidal concentrations of *S. singueana, X. spekei,* and *G. tembensis* extracts

	Concentration (mg/ml)				
Bacterial strains	***S. singueana* extract**	***X. spekei* extract**	***G. tembensis* stem bark extract**	***G. tembensis* leaf extract**	**Ciprofloxacin (µg/ml)**
S. aureus	12.50 ± 0.00^{c}	33.33 ± 8.33^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	1.30 ± 0.26^{a}
B. subtilis	41.67 ± 8.33^{b}	100.00 ± 0.00^{a}	NA	NA	0.65 ± 0.13^{ab}
S. typhi	100.00 ± 0.00^{a}	NA	NA	NA	0.78 ± 0.00^{ab}
E. coli	100.00 ± 0.00^{a}	NA	NA	NA	0.26 ± 0.06^{b}

Values expressed as mean±std error of mean. Values with identical superscript letters within a given column are insignificantly distinct (one-way Analysis of Variance and Tukey's post hoc) ($p > 0.05$).NA stands for not active.

In comparison to Ciprofloxacin, the bactericidal effect of *S. singueana* extract against *S. aureus*, was not at a statistically significant concentration with Ciprofloxacin ($p > 0.05$) but at a significantly lower concentration than other extracts ($p < 0.05$; Table 4.9). Both leaf and stem bark extracts of *G. tembensis* demonstrated statistically insignificant bactericidal effects against *S. aureus* ($p > 0.05$). However, *S. singueana* extract demonstrated bactericidal effects at significantly lower

bactericidal concentrations than *X. spekei* extract but at significantly higher concentrations than Ciprofloxacin against *B. subtilis* ($p<0.05$).

Table 4.9: Comparison of MBCs of the studied ethyl acetate extracts and Ciprofloxacin

	Minimum Bactericidal Concentration (mg/ml)			
Treatment	***S. aureus***	***B. subtilis***	***S. typhi***	***E. coli***
Ciprofloxacin (µg/ml)	1.30 ± 0.26^{c}	0.65 ± 0.13^{c}	0.78 ± 0.00^{b}	0.26 ± 0.06^{b}
S. singueana extract	12.50 ± 0.00^{c}	41.67 ± 8.33^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}
X. spekei extract	33.33 ± 8.33^{b}	100.00 ± 0.00^{a}	NA	NA
G. tembensis stem bark extract	100.00 ± 0.00^{a}	NA	NA	NA
G. tembensis leaf extract	100.00 ± 0.00^{a}	NA	NA	NA

Values expressed as mean±std error of mean. Values with identical superscript letters along the column are insignificantly distinct (one-way Analysis of Variance and Tukey's post hoc) ($p>0.05$). NA stands for not active.

4.10 *In Vitro* DPPH radical scavenging activities of *G. tembensis*, *S. singueana*, and *X. spekei*

The test plant extracts displayed DPPH radical scavenging effect across all concentrations in a dose-dependent trend. As plant extracts' concentration decreased, DPPH radical scavenging capacity also decreased. Both stem bark and leaf extracts of *G. tembensis* demonstrated statistically significant DPPH activities at all the dilutions ($p<0.05$; Table 4.10). *S. singueana* extract and ascorbic acid showed significantly different DPPH radical quenching effects at all the dilutions ($p<0.05$; Table 4.10) except at concentrations between 250.00µg/ml and 500.00µg/ml where their DPPH free radical scavenging effects were statistically

insignificant ($p>0.05$; Table 4.10). Additionally, *X. spekei* extract demonstrated significantly different DPPH radical scavenging activity in all dilutions ($p<0.05$; Table 4.10) except at 125.00µg/ml and 250.00µg/ml concentrations where the extract showed no statistical significance in DPPH radical scavenging activity ($p>0.05$; Table 4.10).

The ethyl acetate extract of *X. spekei* demonstrated a significantly greater scavenging activity of DPPH radicals with an IC_{50} value of 33.00±1.47 µg/ml in comparison to the other plant extracts, which had IC_{50} values of 47.97±0.69µg/ml, 69.66±1.01µg/ml and 86.88±2.64µg/ml for *S. singueana*, *G. tembensis* leaves and *G. tembensis* stem bark extracts, respectively ($p<0.05$; Table 4.10).

Table 4.10: *In Vitro* DPPH radical scavenging activities of *G. tembensis, S. singueana,* and *X. spekei* extracts

Concentrations (µg/ml)	*X. spekei* extract	*S. singueana* extract	*G. tembensis* leaf extract	*G. tembensis* stem bark extract	Ascorbic acid
			Percentage (%)		
15.625	34.80±1.56^{e}	29.67±1.09^{e}	23.00±1.22^{f}	17.13±0.98^{f}	43.13±1.14^{e}
31.250	55.13±0.71^{d}	45.60±1.10^{d}	36.60±1.29^{e}	35.27±1.33^{e}	60.53±1.22^{d}
62.500	64.87±1.34^{c}	54.87±1.16^{c}	48.53±1.34^{d}	43.20±0.95^{d}	71.27±1.23^{c}
125.000	76.80±1.25^{b}	72.27±1.05^{b}	60.53±1.20^{c}	54.47±1.28^{c}	80.07±0.81^{b}
250.000	82.40±1.33^{b}	80.27±1.79^{a}	71.27±1.30^{b}	65.00±1.01^{b}	87.33±1.39^{a}
500.000	88.47±1.22^{a}	84.47±0.98^{a}	78.47±1.27^{a}	74.80±1.33^{a}	92.53±0.87^{a}
IC_{50} value (µg/ml)	33.00±1.47$_{D}$	47.97±0.69$_{C}$	69.66±1.01$_{B}$	86.88±2.64$_{A}$	20.54±2.24$_{E}$

Values expressed as mean ±SEM of the triplicates. Means that do not share the same superscript letter along the column are significantly different ($p<0.05$). For IC_{50} values, means that do not share the same subscript letter across the row are significantly different ($p<0.05$). (One-way ANOVA and Tukey's Post hoc tests).

At 125.00µg/ml to 500.00µg/ml concentration ranges, ascorbic acid and *X. spekei* extract had no statistically significant DPPH radical scavenging activities. The same observation was made for the activities of *S. singueana* and *X. spekei* extracts at the same concentrations ($p>0.05$; Figure 4.3). At concentration 15.625 µg/ml, there was a statistical insignificance in DPPH radical scavenging activities between *X. spekei* and *S. singueana* extracts ($p>0.05$; Figure 4.3). Between 31.250µg/ml and 62.500µg/ml concentrations, *X. spekei* extract showed significantly greater DPPH free radical scavenging capacity than the remaining extracts ($p<0.05$; Figure 4.3).

Both stem bark and leaf extracts of *G. tembensis* demonstrated statistically insignificant DPPH radical scavenging activities in all the concentrations ($p>0.05$; Figure 4.3) except at 15.625µg/ml and 125.00µg/ml concentrations, where the leaf extract showed significantly higher DPPH radical scavenging effect than the stem bark extract ($p<0.05$; Figure 4.3).

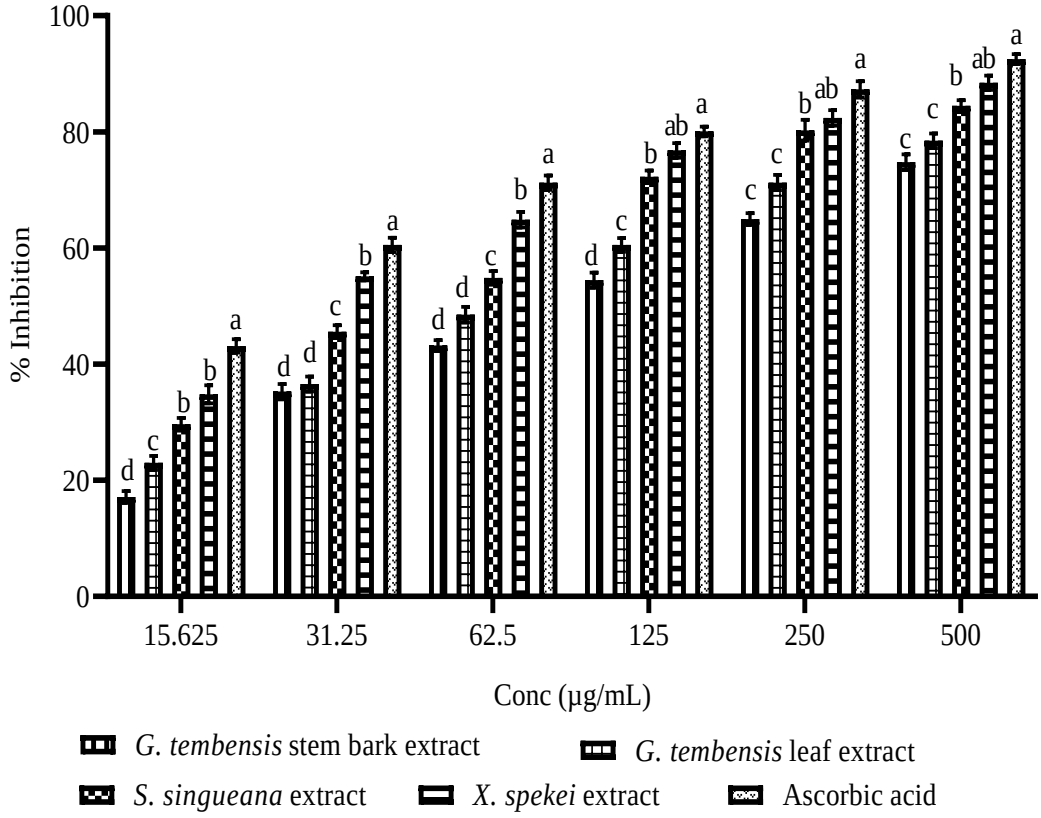

Figure 4.3: Comparison of DPPH radical scavenging activities of *S. singueana*, *X. spekei*, and *G. tembensis* extracts. Bar graphs with distinct letters within a given concentration are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's Post hoc tests).

4.11 Ferric reducing antioxidant activity of ethyl acetate extracts of *G. tembensis*, *S. singueana*, and *X. spekei*

The seven tested concentrations of ethyl acetate plant extracts exhibited a dilution dependent ferric reducing effect. As the concentrations increased amongst all the tested extracts, the ferric reducing antioxidant power also increased. Ascorbic acid showed no statistical significance in all the dilutions ($p<0.05$; Table 4.11) while *G. tembensis* leaf extract showed statistically insignificant ferric reducing activities between concentrations 7.8125 µg/ml and 15.625µg/ml, 31.250µg/ml and 15.625µg/ml and between concentrations 62.500µg/ml and 31.250µg/ml ($p>0.05$; Table 4.11). Similarly, *G. tembensis* stem bark extract demonstrated statistically insignificant ferric reducing activities between concentrations 125.00µg/ml and 250.00µg/ml, concentrations starting from 15.625µg/ml to 62.500µg/ml and concentrations beginning from 7.8125µg/ml to 31.250µg/ml ($p>0.05$; Table 4.11). *S. singueana* extract showed no statistical significance in its ferric reducing activities at concentrations ranges of 7.8125µg/ml to 31.250µg/ml and concentrations between 250.00µg/ml and 125.00µg/ml ($p>0.05$; Table 4.11). Additionally, *X. spekei* extract showed statistically insignificant ferric reducing activities at concentrations between 15.625µg/ml and 7.8125µg/ml ($p>0.05$; Table 4.11).

Table 4.11: Ferric reducing antioxidant activity of ethyl acetate extracts of *G. tembensis*, *S. singueana*, and *X. spekei*

Concentrations (µg/ml)	*X. spekei* extract	*S. singueana* extract	*G. tembensis* leaf extract	*G. tembensis* stem bark extract	Ascorbic Acid
		Optical Density (700nm)			
7.8125	0.104±0.008^{f}	0.096±0.006^{d}	0.059±0.009^{e}	0.053±0.008^{d}	0.119±0.006^{g}
15.625	0.163±0.007^{f}	0.136±0.008^{d}	0.101±0.007^{de}	0.090±0.011^{cd}	0.286±0.006^{f}
31.250	0.365±0.036^{e}	0.236±0.032^{d}	0.120±0.006^{de}	0.117±0.007^{cd}	0.467±0.035^{e}
62.500	0.784±0.035^{d}	0.520±0.033^{c}	0.183±0.007^{d}	0.159±0.010^{c}	0.877±0.027^{d}
125.000	1.109±0.031^{c}	0.705±0.057^{b}	0.434±0.023^{c}	0.356±0.034^{b}	1.316±0.031^{c}
250.000	1.327±0.074^{b}	0.878±0.041^{b}	0.560±0.027^{b}	0.413±0.017^{b}	1.570±0.065^{b}
500.000	1.944±0.031^{a}	1.096±0.052^{a}	0.676±0.034^{a}	0.539±0.028^{a}	2.129±0.021^{a}

Triplicates' mean values expressed as mean±Std Error of Mean. Means with distinct superscript letters along the column are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's post hoc tests).

In comparison, the stem bark and leaf extracts of *G. tembensis,* recorded statistically insignificant ferric reducing activities in all tested concentrations ($p>0.05$; Figure 4.4). Among the extracts, *X. spekei* extract exhibited significantly stronger ferric reducing activity than the other extracts ($p<0.05$; Figure 4.4). Ascorbic acid and *X. spekei* extract, at 31.250µg/ml and 62.500µg/ml concentrations, showed no statistical significance in their ferric reducing activities ($p>0.05$; Figure 4.4), although, at the same concentrations, *X. spekei* extract demonstrated significantly higher ferric reducing effect than the other extracts ($p<0.05$; Figure 4.4).

At concentration 31.250 µg/ml, *S. singueana* extract demonstrated statistically insignificant ferric reducing power with both stem bark and leaf extracts of *G. tembensis* ($p>0.05$; Figure 4.4). Similarly, at 15.625 µg/ml concentration, *S. singueana* and *X. spekei* extracts recorded statistically insignificant ferric reducing power and the same applied to *S. singueana* and *G. tembensis* leaf extracts ($p>0.05$; Figure 4.4). At concentration 7.8125 µg/ml, there was no statistical significance in ferric reducing activities among ascorbic acid, *X. spekei,* and *S. singueana* extracts ($p>0.05$; Figure 4.4).

These findings showed that *X. spekei* extract had a higher reducing power amongst the tested plant extracts followed by *S. singueana* extract followed by *G. tembensis* leaf extract, and finally, *G. tembensis* stem bark extract which had the lowest reducing power (Table 4.11; Figure 4.4).

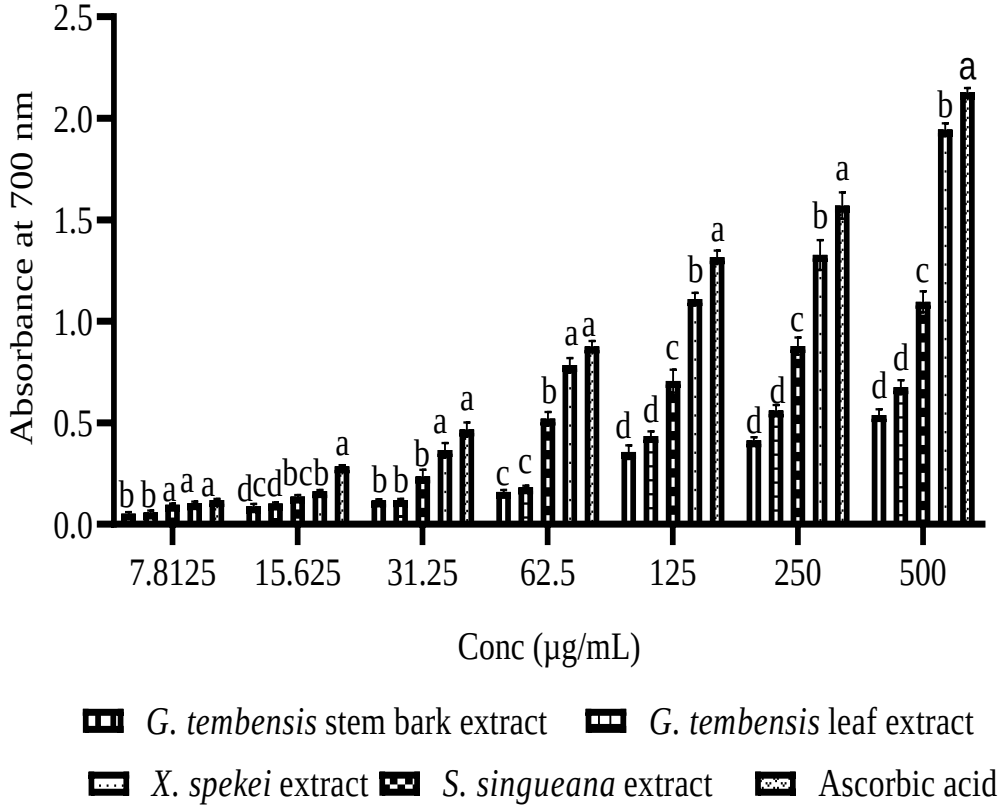

Figure 4.4: Comparison of ferric reducing antioxidant activity of *S. singueana*, *X. spekei*, and *G. tembensis* extracts. Bar graphs with distinct letters within a particular concentration are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's Post hoc tests).

4.12 Hydroxyl radical scavenging activity of ethyl acetate extracts of *G. tembensis*, *S. singueana*, and *X. spekei*

The test plant extracts displayed an efficient hydroxyl free radical scavenging capability in all the plant extracts' dilutions which occurred in a dilution-dependent trend. As the extracts' concentrations decreased, their ability to scavenge hydroxyl (˙OH) radicals also decreased and vice versa. There were significant differences in

hydroxyl radical scavenging effects amongst all the dilutions in each of the tested plant extracts, including the standard gallic acid ($p<0.05$; Table 4.12).

The findings of this study also showed that the IC_{50} value of *X. spekei* extract was not statistically significant to that of *S. singueana* extract. Additionally, both leaf and stem bark extracts of *G. tembensis* demonstrated no statistical significance in their IC_{50} values ($p>0.05$; Table 4.12).

Table 4.12: Hydroxyl (˙OH) radical scavenging activity of ethyl acetate extracts of *S. singueana*, *G. tembensis*, and *X. spekei*

Concentration (µg/ml)	*X. spekei* extract	*S. singueana* extract	*G. tembensis* leaf extract	*G. tembensis* stem bark extract	Gallic acid
	Percentage (%)				
15.625	26.91±0.76^{f}	22.96±1.12^{f}	19.69±1.25^{f}	14.69±0.79^{f}	35.56±1.18^{f}
31.250	35.93±0.98^{e}	30.62±1.42^{e}	25.00±1.23^{e}	21.17±1.31^{e}	47.41±1.21^{e}
62.500	48.95±0.80^{d}	47.53±1.17^{d}	39.44±0.98^{d}	37.96±1.26^{d}	67.53±1.35^{d}
125.000	70.93±0.98^{c}	67.84±0.92^{c}	63.83±0.97^{c}	60.43±1.23^{c}	79.88±0.80^{c}
250.000	83.95±0.99^{b}	80.86±1.18^{b}	76.48±1.13^{b}	74.69±1.03^{b}	86.61±0.89^{b}
500.000	88.83±0.91^{a}	88.09±1.03^{a}	82.59±0.98^{a}	80.00±0.96^{a}	93.40±0.76^{a}
IC_{50} value (µg/ml)	61.23±0.21 $_{B}$	67.84±1.34 $_{B}$	84.50±0.70 $_{A}$	91.78±4.09 $_{A}$	35.33±0.88 $_{C}$

Values expressed as mean ±SEM of the triplicates. Means that do not share the same superscript letter along the column are significantly different ($p<0.05$). For IC_{50} values, means that do not share the same subscript letter across the row are significantly different ($p<0.05$). (One-way ANOVA and Tukey's Post hoc tests).

In comparison, gallic acid had significantly greater hydroxyl (•OH) radical scavenging activity than all the extracts at all the concentrations ($p<0.05$; Figure 4.5), except at concentration 250.00 µg/ml, where its ability to scavenge hydroxyl radicals was statistically insignificant to that of *X. spekei* extract ($p>0.05$; Figure 4.5). *X. spekei* and *S. singueana* extracts demonstrated no statistical significance in their •OH radical scavenging capabilities at all the tested dilutions ($p>0.05$; Figure 4.5). *S. singueana* extract demonstrated statistical insignificance in its •OH radical scavenging effect with *G. tembensis* leaf extract in all concentrations ($p>0.05$; Figure 4.5), except at 62.500 µg/ml and 500.00 µg/ml concentrations, where *S. singueana* extract showed significantly greater hydroxyl radical scavenging effect than *G. tembensis* leaf extract ($p<0.05$; Figure 4.5). Similarly, in all the concentrations tested, both *G. tembensis* stem bark and leaf extracts demonstrated no statistical significance in their hydroxyl radical scavenging activities ($p>0.05$; Figure 4.5) except at concentration 15.625 µg/ml where *G. tembensis* leaf extract exhibited significantly higher hydroxyl radical quenching capability than the stem bark extract of *G. tembensis* ($p< 0.05$; Figure 4.5).

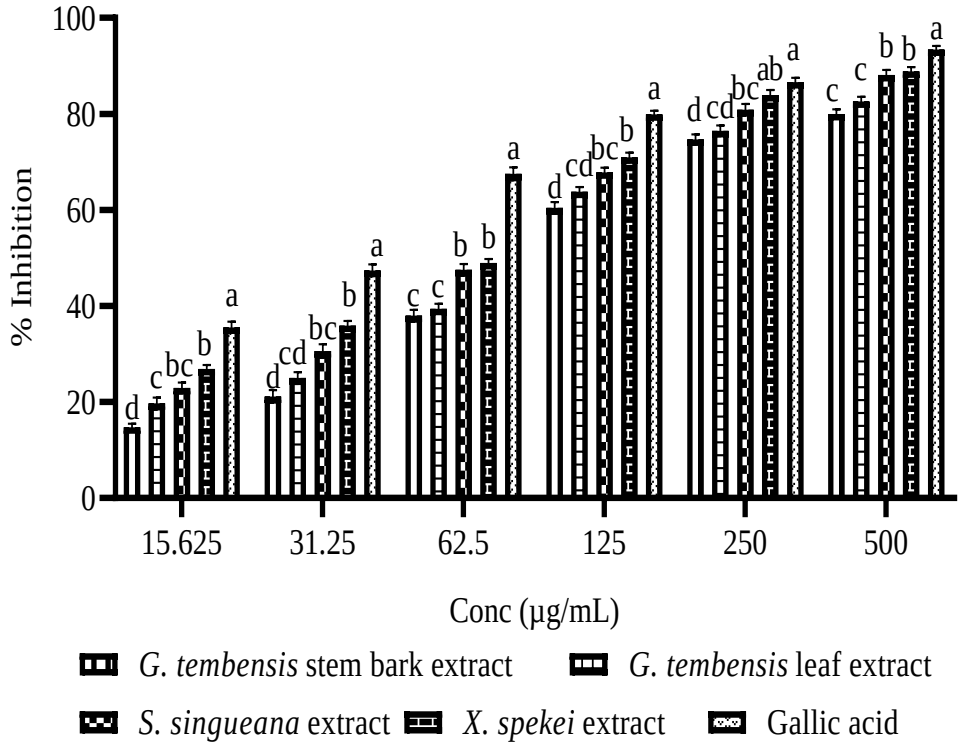

Figure 4.5: Comparison of ˙OH radical quenching activity of *S. singueana, X. spekei,* and *G. tembensis* extracts. Bar graphs with distinct letters within a given concentration are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's Post hoc tests).

4.13 Iron chelating activities of ethyl acetate extracts of *G. tembensis, S. singueana,* and *X. spekei*

The tested plant extracts demonstrated a strong efficacy of iron chelating activity across all the plant extracts concentrations, which occurred in a dilution reliant manner. Plant extracts' ability to chelate irons decreased with a decrease in extracts' concentration. There was no statistical significance in iron chelating activities of plant extracts and the standard EDTA across all tested dilutions ($p<0.05$; Table 4.13).

X. spekei had a significantly lower IC_{50} value of 43.56±0.46µg/ml as opposed to *S. singueana*, *G. tembensis* leaf, and *G. tembensis* stem bark extracts which had IC_{50} values of 59.40±0.90µg/ml, 89.78±0.55µg/ml, and 120.70±0.71µg/ml respectively ($p<0.05$; Table 4.13).

Table 4.13: *In vitro* iron chelating activity of ethyl acetate extracts of *X. spekei, S. singueana,* and *G. tembensis*

Concentrations (µg/ml)	*X. spekei* extract	*S. singueana* extract	*G. tembensis* leaf extract	*G. tembensis* stem bark extract	EDTA
			Percentage (%)		
15.625	29.84±0.63^{f}	26.60±0.35^{f}	21.68±0.76^{f}	19.27±0.53^{f}	37.16±0.72^{f}
31.250	48.85±0.86^{e}	40.40±0.66^{e}	31.49±0.82^{e}	28.19±0.69^{e}	60.40±0.85^{e}
62.500	57.82±0.69^{d}	51.12±0.50^{d}	41.02±0.72^{d}	37.03±0.55^{d}	76.77±0.63^{d}
125.000	73.63±0.72^{c}	64.85±0.70^{c}	57.16±0.56^{c}	49.67±0.60^{c}	83.14±1.04^{c}
250.000	81.62±0.64^{b}	73.76±0.77^{b}	67.03±0.67^{b}	66.14±0.49^{b}	87.86±0.61^{b}
500.000	88.55±0.54^{a}	83.56±0.58^{a}	80.59±0.52^{a}	79.14±0.55^{a}	94.79±0.61^{a}
IC_{50} value (µg/ml)	43.56±0.46 $_{D}$	59.40±0.90 $_{C}$	89.78±0.55 $_{B}$	120.70±0.71 $_{A}$	25.05±0.79 $_{E}$

Values expressed as mean ±SEM of the triplicates. Means that do not share the same superscript letter along the column are significantly different ($p<0.05$). For IC_{50} values, means that do not share the same subscript letter across the row are significantly different ($p<0.05$). (One-way ANOVA and Tukey's Post hoc tests).

In comparison, *X. spekei* extract had significantly higher iron chelating activity than the other extracts across all the dilutions ($p<0.05$; Figure 4.6). Similarly, *S. singueana* extract had significantly higher iron chelating activity than both stem bark and leaf extracts of *G. tembensis* at all the concentrations ($p<0.05$; Figure 4.6). Both leaf and stem bark extracts of *G. tembensis* demonstrated statistically insignificant iron chelating activities across all the concentrations ($p>0.05$; Figure 4.6), apart from 62.50µg/ml and 125.00µg/ml concentrations, where *G. tembensis* leaf extract had significantly higher iron chelating activity than *G. tembensis* stem bark extract ($p <0.05$; Figure 4.6).

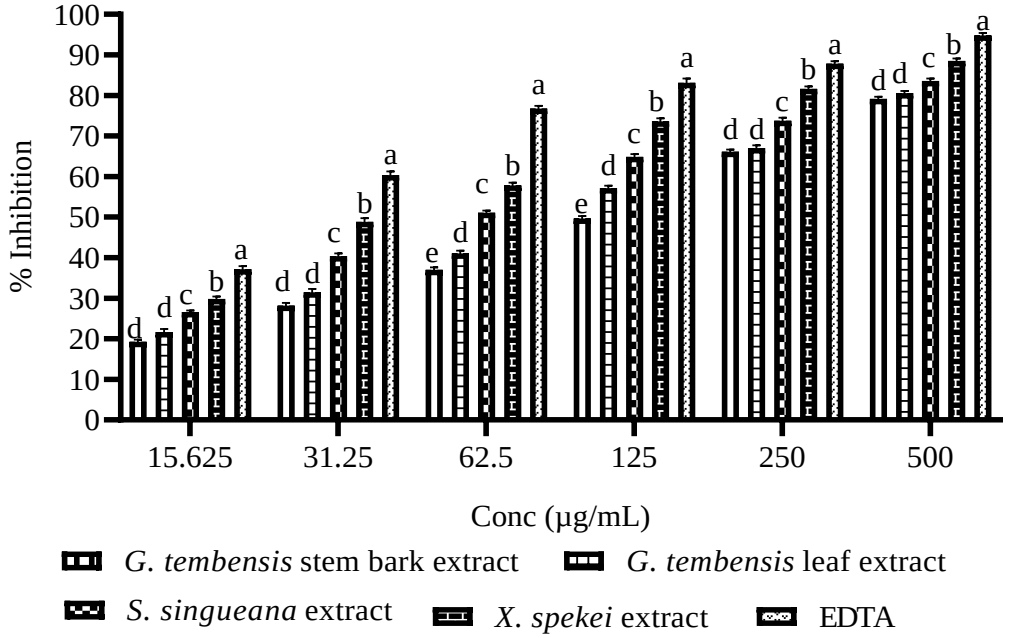

Figure 4.6: Comparison of iron chelating activity of *S. singueana, X. spekei,* and *G. tembensis* extracts. Bar graphs with distinct letters within a particular concentration are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's Post hoc tests).

4.14 *In vitro* H_2O_2 radical quenching activity of *X. spekei, S. singueana,* and *G. tembensis* ethyl acetate extracts

All the tested extracts displayed a concentration dependent efficacy of hydrogen peroxide (H_2O_2) radical scavenging ability. As the concentrations increased amongst all the tested extracts, the H_2O_2 radical scavenging capacity also increased. *S. singueana* extract showed statistically significant hydrogen peroxide scavenging abilities across all concentrations ($p<0.05$; Table 4.14) except at concentrations 125.00µg/ml and 500.00µg/ml where it demonstrated no statistical significance in its hydrogen peroxide scavenging activities ($p>0.05$; Table 4.14). All

concentrations for *G. tembensis* stem bark and leaf extracts exhibited significantly different H_2O_2 scavenging effects from one another ($p<0.05$; Table 4.14) except at concentrations 250.00µg/ml and 500.00µg/ml where they both demonstrated no statistical significance in their hydrogen peroxide scavenging activities ($p>005$; Table 4.14). *X. spekei* extract demonstrated statistical insignificance in its hydrogen peroxide scavenging activity between concentrations 500.00µg/ml and 250.00µg/ml and concentrations 125.00µg/ml and 250.00µg/ml ($p>0.05$; Table 4.14).

Ethyl acetate extract of *X. spekei* had an IC_{50} value of 29.65±0.69µg/ml which was significantly lower compared to *S. singueana*, *G. tembensis* leaf and *G. tembensis* stem bark extracts which had IC_{50} values 35.18±0.50µg/ml, 42.92±0.50µg/ml, and 55.78±1.91 µg/ml respectively ($p<0.05$; Table 4.14).

Table 4.14: *In vitro* H_2O_2 radical quenching activities of *X. spekei, S. singueana,* and *G. tembensis* ethyl acetate extracts

Concentrations (µg/ml)	*X. spekei* extract	*S. singueana* extract	*G. tembensis* leaf extract	*G. tembensis* stem bark extract	Ascorbic acid
			Percentage (%)		
15.625	35.62±1.02^{e}	30.52±0.69^{e}	27.97±1.25^{e}	20.33±0.87^{e}	43.92±0.79^{e}
31.250	55.62±1.18^{d}	54.31±0.79^{d}	47.97±0.82^{d}	40.26±1.30^{d}	61.57±1.11^{d}
62.500	71.70±0.96^{c}	65.62±0.96^{c}	60.72±1.36^{c}	53.86±1.31^{c}	73.01±1.25^{c}
125.000	80.85±1.13^{b}	79.28±1.25^{b}	73.66±1.25^{b}	69.54±0.64^{b}	81.31±1.09^{b}
250.000	84.90±1.20^{ab}	82.29±0.66^{b}	79.61±0.97^{a}	78.82±0.97^{a}	85.03±0.75^{b}
500.000	89.41±1.71^{a}	88.95±1.48^{a}	83.01±1.03^{a}	82.75±0.97^{a}	91.96±1.02^{a}
IC_{50} value (µg/ml)	29.65±0.69 $_{D}$	35.18±0.50 $_{C}$	42.92±0.50 $_{B}$	55.78±1.91 $_{A}$	19.14±0.61 $_{E}$

Values expressed as mean ±SEM of the triplicates. Means that do not share the same superscript letter along the column are significantly different ($p<0.05$). For IC_{50} values, means that do not share the same subscript letter across the row are significantly different ($p<0.05$). (One-way ANOVA and Tukey's Post hoc tests).

In comparison, *X. spekei* and *S. singueana* extracts demonstrated no statistical significance in their hydrogen peroxide radical scavenging activities at concentration ranges of 125.00µg/ml to 500.00µg/ml with ascorbic acid ($p>0.05$; Figure 4.7). At 62.500 µg/ml concentration, *X. spekei* extract demonstrated statistically insignificant hydrogen peroxide scavenging activity with ascorbic acid ($p>0.05$; Figure 4.7).

X. spekei extract demonstrated a significantly higher hydrogen peroxide radical scavenging effect than *S. singueana* extract at 15.625µg/ml and 62.500µg/ml concentrations ($p<0.05$; Figure 4.7). *S. singueana* extract showed significantly higher hydrogen peroxide radical scavenging ability than *G. tembensis* leaf extract at 500.00µg/ml, 125.00µg/ml, as well as 31.25µg/ml concentrations ($p<0.05$; Figure 4.7). Similarly, *G. tembensis* leaf extract demonstrated significantly higher hydrogen peroxide radical scavenging activity than *G. tembensis* stem bark extract at concentration ranges of 15.625µg/ml to 62.500µg/ml ($p<0.05$; Figure 4.7).

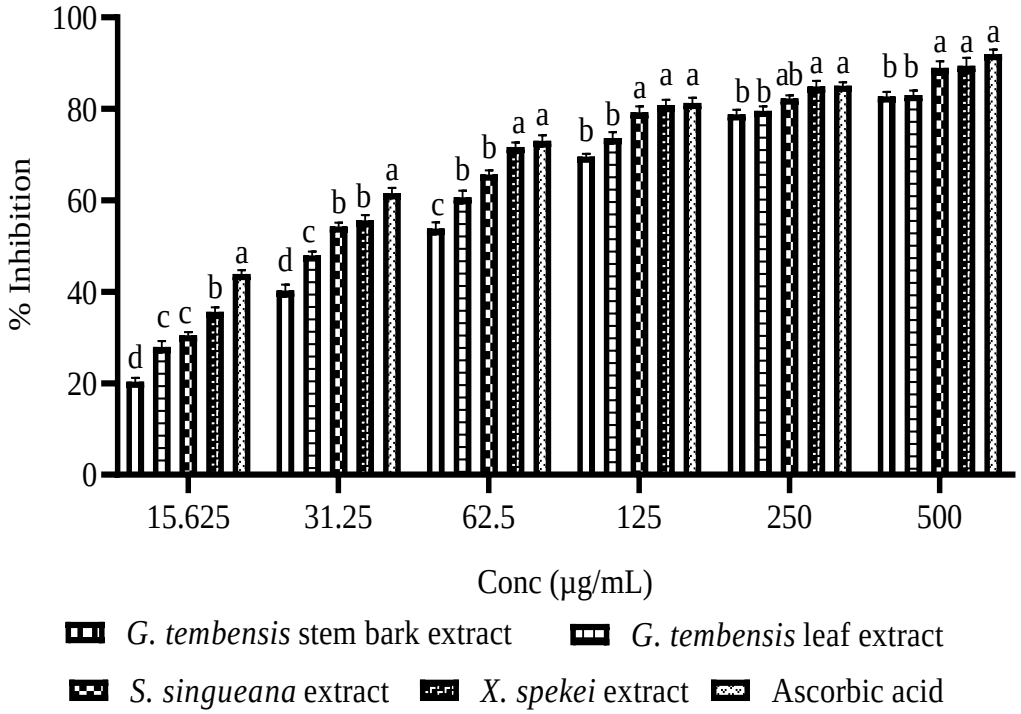

Figure 4.7: Comparison of *in vitro* H_2O_2 radical quenching activity of *S. singueana, X. spekei,* and *G. tembensis* extracts. Bar graphs with distinct letters within a given concentration are significantly different ($p<0.05$). (One-way Analysis of Variance and Tukey's Post hoc tests).

4.15 Quantitative phytochemical composition of ethyl acetate extracts of *S. singueana, G. tembensis* stem bark, *G. tembensis* leaf, and *X. spekei*

The *S. singueana* leaf extract displayed the presence of a total of 51 compounds, out of which 33 compounds have known biological activities. Based on the obtained results, Oxazolidine, 2-ethyl-2-methyl- an Oxazoline compound had the least concentration of 0.02±0.00µg/g, whereas Squalene, a triterpenoid, had the highest concentration of 5.24±0.07 µg/g (Table 4.15). The findings revealed composition of 51.84% hydrocarbons, 24.3% terpenoids, 15.18% fatty acids, 3.26% tocopherols, 3.33% phenolic compounds, 1.03% iodo compounds, 0.28% steroids, 0.28% benzene derivatives, 0.09% heteroaromatic molecules, 0.09% volatile organic compounds, 0.07% cyclic secondary amines, 0.15% aminopyridine, 0.05% alkaloids and 0.03% oxazoline compounds (Table 4.15).

Table 4.15: Quantitative phytochemical compounds analysis in ethyl acetate extract of *S. singueana*

RT(mins)	Compound	%abundance	MF	Conc (µg/g)	Chemical Class
38.78	Beta.-Sitosterol	0.28	$C_{29}H_{50}O$	0.17±0.00	Phytosterol
44.42	Gamma.-Cyano-3-methyl-5,10-dihydrobenzo[f]indolizine	0.09	$C_{14}H_{12}N_2$	0.06±0.00	Heteroaromatic molecule
47.31	1(3H)- Isobenzofuranone, 6,7– dimethoxy-3- [2-(2-methoxyphenyl) -2- Oxoethyl]-	0.09	$C_{19}H_{18}O_6$	0.05±0.00	Volatile organic substances
46.71	1,2,3- Propatriol, 1-indol-4- yl (ether)	0.24	$C_{11}H_{13}NO_3$	0.14±0.00	Hydrocarbon
27.34	1,4- Dioxaspiro [4.5]decane-6 -carboxylic acid, dimethylamide	2.53	$C_{11}H_{19}NO_3$	1.51±0.02	Cyclohexane
21.79	1-Cyclopentyleicosane	0.57	$C_{25}H_{50}$	0.34±0.00	Hydrocarbon
27.06	1-Hexadecanol, 3,7,11,15-tetramethyl-	0.95	$C_{20}H_{42}O$	0.57±0.01	Terpene alcohol
44.18	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	0.72	$C_{17}H_{14}O_4$	0.43±0.01	Hydrocarbon
13.72	2-Cyclohexylpiperidine	0.05	$C_{11}H_{21}N$	0.03±0.00	Alkaloid
46.38	2-Pyridinamine, N-(4, 5-dihydro-5-methyl-2-thiazolyl) -3-methyl-	0.15	$C_{10}H_{13}N_3S$	0.09±0.00	Aminopyridine
29.69	3,7,11,15- Tetramethyl -2- hexadecen-1 -ol	5.57	$C_{20}H_{40}O$	3.32±0.04	Terpene alcohol
21.51	4-Nonanol, 2,6,8-trimethyl-	0.56	$C_{12}H_{26}O$	0.33±0.00	Fatty alcohol
49.10	5-(2-Oxo-6-phenyl-1,2-dihydropyrimidinyl-4)uracil	0.02	not found	0.01±0.00	
25.51	7-Octenal, 3,7-dimethyl-	1.78	$C_{10}H_{18}O$	1.06±0.01	Monoterpenoid
25.06	9- Octadecenoic acid, methyl ester, (E)-	2.05	$C_{19}H_{36}O_2$	1.22±0.02	Fatty acid derivative
28.18	9-Tricosene, (Z)-	4.53	$C_{23}H_{46}$	2.70±0.04	Hydrocarbon
44.35	Acetamide, N- methyl-N-(2-phenylethyl)-	0.28	$C_{11}H_{15}NO$	0.17±0.00	Benzene derivative
03.39	Butane, 2-chloro-2-methyl-	0.33	$C_5H_{11}Cl$	0.20±0.00	Chlorinated hydrocarbon

16.95	Cyclohexadecane, 1,2-diethyl-	0.52	$C_{20}H_{40}$	0.31±0.00	Hydrocarbon
23.37	Decane, 3,8-dimethyl-	0.41	$C_{12}H_{26}$	0.24±0.00	Hydrocarbon
25.88	Docosane	3.51	$C_{22}H_{46}$	2.09±0.03	Hydrocarbon
19.40	Dodecane, 2,6,11-trimethyl-	0.68	$C_{15}H_{32}$	0.40±0.01	Aliphatic alkane
20.16	Dodecanoic acid	4.17	$C_{12}H_{24}O_2$	2.48±0.03	Fatty acid
19.65	Dodecanoic acid, methyl ester	0.72	$C_{13}H_{26}O_2$	0.43±0.01	Fatty acid derivative
21.64	Eicosane (C20)	0.95	$C_{20}H_{42}$	0.57±0.01	Hydrocarbon
30.38	Fumaric acid,4- methyl pent-2-yl tridecyl ester	1.28	$C_{23}H_{42}O_4$	0.76±0.01	Fatty acid derivative
29.14	Hexacosane	3.44	$C_{26}H_{54}$	2.05±0.03	Hydrocarbon
29.89	Hexadecane (C16)	4.50	$C_{16}H_{34}$	2.68±0.04	Hydrocarbon
34.24	Hexadecane, 8-hexyl-8-pentyl-	1.82	$C_{27}H_{56}$	1.08±0.01	Hydrocarbon
24.07	Hexadecanoic acid, ethyl ester	2.31	$C_{18}H_{36}O_2$	1.38±0.02	Fatty acid derivative
24.33	Isopropyl hexadecanoate	1.01	$C_{19}H_{38}O_2$	0.60±0.01	Fatty acid derivative
40.55	Lupan-3-ol	0.33	$C_{30}H_{52}O$	0.20±0.00	Triterpenoid
23.46	Methyl hexadecanoate	1.60	$C_{17}H_{34}O_2$	0.95±0.01	Fatty acid derivative
18.88	Methyleugenol	0.52	$C_{11}H_{14}O_2$	0.31±0.00	Phenylpropanoid
23.84	n-Hexadecanoic acid	1.48	$C_{16}H_{32}O_2$	0.88±0.01	Fatty acid derivative
26.73	Nonadecane (C19)	2.61	$C_{19}H_{40}$	1.50±0.02	Hydrocarbon
30.72	Octacosane	3.41	$C_{28}H_{58}$	2.03±0.03	Hydrocarbon
06.68	Oxazolidine, 2-ethyl-2-methyl-	0.03	$C_6H_{13}NO$	0.02±0.00	Oxazoline compound
28.37	Pentacosane	3.63	$C_{25}H_{52}$	2.16±0.03	Hydrocarbon
28.65	Phenol, 2,4-bis (1-methyl-1- phenylethyl)-	2.81	$C_{24}H_{26}O$	1.67±0.02	Phenolic compound
25.21	Phytol	3.54	$C_{20}H_{40}O$	2.11±0.03	Diterpenoid

26.08	Phytol acetate<E->	3.34	$C_{22}H_{42}O_2$	1.99±0.03	Diterpenoid
10.55	Pyrrolidine, 2-decyl-1-methyl-	0.07	$C_{17}H_{35}N$	0.04±0.00	cyclic secondary amine
31.07	Squalene	8.79	$C_{30}H_{50}$	5.24±0.07	Triterpenoid
32.85	Tetracosane	4.30	$C_{24}H_{50}$	2.56±0.03	Hydrocarbon
27.57	Tetracosane	4.18	$C_{24}H_{50}$	2.49±0.03	Hydrocarbon
25.72	Tetratetracontane	2.40	$C_{44}H_{90}$	1.43±0.02	Hydrocarbon
31.70	Tricosane	5.72	$C_{23}H_{48}$	3.41±0.05	Hydrocarbon
21.19	Tridecane, 1-iodo-	1.03	$C_{13}H_{27}I$	0.61±0.01	Iodo compound
24.21	Undecane, 5,5-dimethyl-	0.83	$C_{13}H_{28}$	0.50±0.01	Hydrocarbon
35.06	Vitamin E	3.26	$C_{29}H_{50}O_2$	1.94±0.03	Tocopherol

Key: Conc= Concentration**, Mins=**Minutes**, MF=**Molecular Formula, **RT=**Retention Time

Phytochemical screening results for stem bark extract of *G. tembensis* as presented in table 4.16, showed n-Hexadecanoic acid, a fatty acid derivative, had the highest concentration at 88.06±3.04 µg/g while the compound 2-Pentanone a ketone had the lowest concentration at 0.40±0.00 µg/g. As table 4.16 shows, the extract comprised 38.1% fatty acids, 30.6% terpenoids, 11.6% hydrocarbons, 10.2% steroids, 4.0% phenolic compounds, 2.3% aldehyde compounds, 1.4% ketones, 0.6% tocopherol, 0.6% benzene derivatives, 0.4% methoxybenzoic acid compounds and 0.2% aromatic amine compounds.

Table 4.16: Quantitative phytochemical Compounds analysis in ethyl acetate stem bark extract of *G. tembensis*

RT(mins)	Compound	% abundance	MF	Conc (µg/g)	Chemical Class
03.36	2-Pentanone	0.1	$C_5H_{10}O$	0.40±0.00	Ketone
18.64	AR- Curcumene	0.3	$C_{15}H_{22}$	1.83±0.07	Sesquiterpenoid
19.30	2(4H)- Benzofuranone, 5,6,7,7a- tetrahydro- 4,4,7a-trimethyl-	0.4	$C_{11}H_{16}O_2$	2.03±0.07	Diterpenoid
19.55	Dodecanoic acid	0.7	$C_{12}H_{24}O_2$	3.667±0.13	Fatty acid
20.10	3- Hydroxy-4-methoxybenzoic acid	0.4	$C_8H_8O_4$	2.43±0.07	Methoxybenzoic acid
20.58	Benzenamine, 4-cyclohexyl-	0.2	$C_{12}H_{17}N$	1.17±0.03	Aromatic amine
21.09	Heptadecane	0.2	$C_{17}H_{36}$	1.37±0.03	Hydrocarbon
21.18	Nonahexacontanoic acid	0.2	$C_{69}H_{138}O_2$	1.07±0.03	Fatty acid
21.42	Benzoic acid, 2,4- dihydroxy- 3,6- dimethyl-, methyl ester	0.6	$C_{10}H_{12}O_4$	3.40±0.12	Benzene derivative
21.56	Octacosane	0.3	$C_{28}H_{58}$	1.53±0.05	Hydrocarbon
21.67	Tetradecanoic acid	1.0	$C_{14}H_{28}O_2$	5.36±0.19	Fatty acid
22.31	Pentadecanoic acid, methyl ester	0.4	$C_{16}H_{32}O_2$	2.25±0.08	Fatty acid derivative
22.45	Phytol acetate<E->	0.6	$C_{22}H_{42}O_2$	3.02±0.11	Diterpenoid
22.53	2- Pentadecanone,6,10,14- trimethyl-	0.8	$C_{18}H_{36}O$	4.21±0.15	Diterpenoid
22.70	Pentadecanoic acid	0.6	$C_{15}H_{30}O_2$	3.21±0.11	Fatty acid
22.89	Phytol, acetate	0.5	$C_{22}H_{42}O_2$	2.60±0.09	Diterpenoid
23.04	Nonadecane	0.3	$C_{19}H_{40}$	1.79±0.06	Hydrocarbon
23.32	Methyl hexadecanoate	2.0	$C_{17}H_{34}O_2$	10.78±0.37	Fatty acid derivative
23.52	Palmitoleic acid	0.8	$C_{16}H_{30}O_2$	4.16±0.14	Fatty acid
23.77	n-Hexadecanoic acid	16.2	$C_{16}H_{32}O_2$	88.06±3.04	Fatty acid
23.98	Hexadecanoic acid,ethyl ester	2.6	$C_{18}H_{36}O_2$	13.95±0.48	Fatty acid derivative
24.49	cis-10-Heptadecenoic acid	1.1	$C_{17}H_{32}O_2$	5.84±0.20	Fatty acid

24.95	Methyl linoleate	2.2	$C_{19}H_{34}O_2$	12.22±0.42	Fatty acid derivative
25.01	7,10,13-Hexadecatrienoic acid, methyl ester	1.4	$C_{17}H_{28}O_2$	7.51±0.26	Fatty acid derivative
25.14	Phytol	1.3	$C_{20}H_{40}O$	6.874±0.24	Diterpenoid
25.22	Methyl octadecanoate	0.7	$C_{19}H_{38}O_2$	3.93±0.14	Fatty acid derivative
26.05	Tridecanedial	1.4	$C_{13}H_{24}O_2$	7.85±0.27	Aldehyde
26.45	Adipic acid, 2-ethylhexyl isobutyl ester	2.5	$C_{18}H_{34}O_4$	13.76±0.48	Fatty acid derivative
26.71	Tricosane	1.8	$C_{23}H_{48}$	9.91±0.34	Hydrocarbon
26.97	Methyl 18-methylnonadecanoate	1.2	$C_{21}H_{42}O_2$	6.67±0.23	Fatty acid derivative
27.25	4,8,12,16-Tetramethylheptadecan-4-olide	2.6	$C_{21}H_{40}O_2$	14.05±0.49	Terpenoid
27.55	Tetracosane	1.8	$C_{24}H_{50}$	9.93±0.34	Hydrocarbon
27.81	Octadecanal	0.9	$C_{18}H_{36}O$	4.71±0.16	Long-chain aldehyde
28.34	Pentacosane	1.4	$C_{25}H_{52}$	7.56±0.26	Hydrocarbon
28.41	Phenol, 2,5-dimethyl-, acetate	2.1	$C_{10}H_{12}O_2$	11.27±0.39	Phenolic compound
28.59	Phenol, 2,4- bis (1- methyl-1- phenylethyl)-	1.9	$C_{24}H_{26}O$	10.3±0.36	Phenolic compound
29.11	Hexacosane	1.3	$C_{26}H_{54}$	7.26±0.25	Hydrocarbon
29.86	Tritriacontane	2.7	$C_{33}H_{68}$	14.61±0.50	Hydrocarbon
30.13	Tetracosanoic acid, methyl ester	1.2	$C_{25}H_{50}O_2$	6.56±0.23	Fatty acid derivative
30.69	Octacosane	1.8	$C_{28}H_{58}$	9.63±0.33	Hydrocarbon
30.82	Heneicosyl acetate	1.4	$C_{23}H_{46}O_2$	7.42±0.26	Fatty alcohol
31.04	Squalene	2.1	$C_{30}H_{50}$	11.31±0.39	Triterpenoid
31.27	trans-Geranylgeraniol	0.8	$C_{20}H_{34}O$	4.13±0.14	Diterpenoid
32.14	2,6,10, 14- Hexadecatetraen-1- ol,3,7,11,15- tetramethyl-, acetate, (E,E,E)-	0.7	$C_{22}H_{36}O_2$	3.79±0.13	Diterpenoid
33.00	Hexacosyl acetate	1.0	$C_{28}H_{56}O_2$	5.32±0.18	Fatty alcohol
34.99	Vitamin E	0.6	$C_{29}H_{50}O_2$	3.09±0.11	Tocopherol
35.90	5-Decanone	1.3	$C_{10}H_{20}O$	6.93±0.24	Ketone

36.19	Tetracosyl acetate	0.9	$C_{26}H_{52}O_2$	5.00±0.17	Fatty alcohol
36.77	Campesterol	3.2	$C_{28}H_{48}O$	17.39±0.60	Phytosterol
37.41	Stigmasterol	3.8	$C_{29}H_{48}O$	20.54±0.71	Phytosterol
37.61	Ursa- 9(11),12- dien-3-ol	2.4	$C_{30}H_{48}O$	12.97±0.45	Triterpenoid
38.68	.gamma.-Sitosterol	1.3	$C_{29}H_{50}O$	7.14±0.25	Phytosterol
39.30	.alpha.-Amyrin	1.4	$C_{30}H_{50}O$	7.81±0.27	Triterpenoid
39.46	.beta.-Amyrin	0.9	$C_{30}H_{50}O$	4.78±0.17	Triterpenoid
40.01	Lup-20(29)-en-3-one	3.6	$C_{30}H_{48}O$	19.67±0.68	Triterpenoid
40.58	Taraxasterol	3.2	$C_{30}H_{50}O$	17.59±0.61	Triterpenoid
41.82	Stigmast-4-en-3-one	1.9	$C_{29}H_{48}O$	10.58±0.37	Phytosterol
42.49	2,2,4a,6a,8a,9,12b,14a- Octamethyl- 1,2,3,4,4a,5,6,6a, 6b,7,8,8a, 9,12,12a,12b,13,14,14a, 14b- eicosahydropicene	1.8	$C_{30}H_{50}$	9.77±0.34	Triterpenoid
43.01	Lupan-3-ol, acetate	3.0	$C_{32}H_{54}O_2$	16.36±0.57	Triterpenoid
43.83	Friedelan-3-one	4.3	$C_{30}H_{50}O$	23.17±0.80	Triterpenoid

Key: Conc= Concentration**, Mins=**Minutes**, MF=**Molecular formula, **RT=**Retention time

The major class of secondary metabolites identified in the leaf extract of *G. tembensis* were 59.33% of terpenoids, 16.17% fatty acids, 15.08% hydrocarbons, 6.58% phenolic compounds, 1.79% ester compounds, 0.79% steroids, 0.19% naphthalene compounds, 0.04% aldehyde, 0.03% toloudine derivative (Table 4.17). Further, phytochemical investigations of this plant extract as presented in table 4.17, showed the presence of 40 compounds with trans-3-Penten-2-ol, an alkenol compound, having the lowest concentration at 0.01±0.00 µg/g while Squalene, a triterpenoid, having the highest concentration at 14.17±0.24 µg/g.

Table 4.17: Quantitative phytochemical compounds' analysis of ethyl acetate leaf extract of *G. tembensis*

RT (mins)	Compound	%abundance	MF	Conc(µg/g)	Chemical Class
03.76	trans-3-Penten-2-ol	0.02	$C_5H_{10}O$	0.01±0.00	Alkenol
11.25	Pivalaldehyde, semicarbazone	0.04	$C_6H_{13}N_3O$	0.03±0.00	Aldehyde
11.40	Prilocaine	0.03	$C_{13}H_{20}N_2O$	0.02±0.00	Toluidine derivative
13.50	Limonene	0.22	$C_{10}H_{16}$	0.16±0.00	Monoterpenoid
19.10	1, 4-Naphthalenedione,5, 8-dihydroxy-2,7- dimethoxy-	0.19	$C_{12}H_{10}O_6$	0.14±0.00	Naphthalene compound
19.47	2 (4H)- Benzofuranone,5,6,7,7a- tetrahydro- 4,4, 7a-trimethyl-	1.34	$C_{11}H_{16}O_2$	1.01±0.02	Diterpenoid
21.74	Cyclopentane, 1,2-dibutyl-	0.62	$C_{13}H_{26}$	0.46±0.01	Cyclic alkane
22.49	Bicyclo [3.1.1] heptane, 2,6, 6-trimethyl-, (1.α., 2.β, 5.α.)-	3.92	$C_{10}H_{18}$	2.95±0.05	Terpenoid
22.60	2-Pentadecanone, 6,10, 14-trimethyl-	1.14	$C_{18}H_{36}O$	0.86±0.01	Diterpenoid
22.74	3,7,11, 15-Tetramethyl-2- hexadecen-1-ol	1.22	$C_{20}H_{40}O$	0.92±0.02	Terpene alcohol
22.94	Phytol, acetate	2.06	$C_{22}H_{42}O_2$	1.55±0.03	Diterpenoid
23.39	5,9,13- Pentadecatrien-2-one, 6,10,14-trimethyl -,(E,E)-	2.88	$C_{18}H_{30}O$	2.17±0.04	Terpenoid
23.75	n-Hexadecanoic acid	3.49	$C_{16}H_{32}O_2$	2.63±0.04	Fatty acid
24.01	Ethyl hexadecanoate	3.95	$C_{18}H_{36}O_2$	2.98±0.05	Fatty acid
24.13	trans-Geranylgeraniol	2.14	$C_{20}H_{34}O$	1.62±0.03	Diterpenoid
24.96	Heneicosane	1.84	$C_{21}H_{44}$	1.39±0.02	Hydrocarbon
25.02	Trans-13- Octadecenoic acid, methyl ester	1.39	$C_{19}H_{36}O_2$	1.05±0.02	Fatty acid derivative
25.16	Phytol	4.60	$C_{20}H_{40}O$	3.47±0.06	Diterpenoid
25.65	9,12,15- Octadecatrienoic acid, ethyl ester,(Z,Z, Z)-	5.52	$C_{20}H_{34}O_2$	4.17±0.07	Fatty acid
25.96	Carbonic acid, pentadecyl 2,2,2-trichloroethyl ester	1.14	$C_{18}H_{33}Cl_3O_3$	0.86±0.01	Fatty acid derivative
26.05	Phytol acetate<E->	2.92	$C_{22}H_{42}O_2$	2.20±0.04	Diterpenoid
26.72	Tricosane	3.10	$C_{23}H_{48}$	2.34±0.04	Hydrocarbon
27.03	Eicosane	1.52	$C_{20}H_{42}$	1.14±0.02	Hydrocarbon

27.55	Tetracosane	3.77	$C_{24}H_{50}$	2.85±0.05	Hydrocarbon
28.54	Phenol, 3,5-dimethyl-	1.58	$C_8H_{10}O$	1.19±0.02	Phenolic compound
28.62	Phenol, 2,4- bis (1-methyl-1- phenylethyl)-	3.34	$C_{24}H_{26}O$	2.52±0.04	Phenolic compound
30.14	Octadecane	2.50	$C_{18}H_{38}$	1.89±0.03	Hydrocarbon
31.04	Squalene	18.78	$C_{30}H_{50}$	14.17±0.2	Triterpenoid
31.30	Nerolidol 1	1.40	$C_{15}H_{26}O$	1.05±0.02	Sesquiterpenoid
32.28	Adipic acid, .beta.-citronellyl tetradecyl ester	0.68	$C_{28}H_{52}O_4$	0.51±0.01	Fatty acid derivative
32.44	2,6-Octadienal, 3,7-dimethyl-, (Z)-	1.30	$C_{10}H_{16}O$	0.98±0.02	Monoterpenoid
32.82	Hexacosane	1.70	$C_{26}H_{54}$	1.28±0.02	Hydrocarbon
33.01	13-Tetradecen-1-ol acetate	1.79	$C_{16}H_{30}O_2$	1.35±0.02	Ester compound
35.00	dl-.alpha.-Tocopherol	1.66	$C_{29}H_{50}O_2$	1.25±0.02	Phenolic compound
37.43	Stigmasterol	0.16	$C_{29}H_{48}O$	0.12±0.00	Phytosterol
38.67	.gamma.-Sitosterol	0.63	$C_{29}H_{50}O$	0.47±0.01	Phytosterol
39.45	.beta.-Amyrin	9.03	$C_{30}H_{50}O$	6.81±0.12	Triterpenoid
39.98	Lup-20(29)-en-3-one	3.65	$C_{30}H_{48}O$	2.75±0.05	Triterpenoid
40.50	Taraxasterol	2.31	$C_{30}H_{50}O$	1.75±0.03	Triterpenoid
43.84	Friedelan-3-one	0.42	$C_{30}H_{50}O$	0.32±0.01	Triterpenoid

Key: Conc= Concentration**, Mins=**Minutes**, MF=**Molecular formula, **RT=**Retention time

Phytochemical compounds of *X. spekei* extract, showed several secondary metabolites in different concentrations with Ursa-9(11),12-dien-3-one, a triterpenoid, having the highest concentration at 38.39±2.40 µg/g whereas 1,16-Cyclocorynan-17-oic acid, 19, 20- didehydro-, methyl ester, (16S, 19E)-, an alkaloid, having the lowest concentration at 0.04±0.00 µg/g (Table 4.18). Additionally, phytochemical screening of ethyl acetate of *X. spekei* extract, revealed 33.34% terpenoids, 27.90% fatty acids, 23.93% steroids, 9.76% hydrocarbons, 1.92% phenolic compounds, 1.02% tocopherols, 0.83% tetracarboxylic acids, 0.97% benzene derivatives, 0.20% ketones, 0.11% dialkylaminodiphenylbutanol ester and 0.02% alkaloid (Table 4.18).

Table 4.18: Quantitative analysis of phytochemical compounds in ethyl acetate *X. spekei* extract

RT (mins)	Compound	%abundance	MF	Conc(µg/g)	Chemical Class
21.68	1-tert-Butyl-3-nitrobenzene	0.37	$C_{10}H_{13}NO_2$	1.01±0.06	Benzene derivative
27.01	Heneicosane, 10-methyl-	1.09	$C_{22}H_{46}$	2.96±0.19	Hydrocarbon
41.46	9,19- Cyclo-25, 26-epoxyergostan-3-ol, 4,4, 14- trimethyl-, acetate	1.21	$C_{33}H_{54}O_3$	3.28±0.21	Phytosterol
8.63	Pyrroliphene	0.11	$C_{23}H_{29}NO_2$	0.29±0.02	Dialkylaminodiphenylbutanol ester
21.81	Tetradecanoic acid	0.44	$C_{14}H_{28}O_2$	1.19±0.07	Fatty acid
23.52	Z-11-Pentadecenol	0.20	$C_{15}H_{30}O$	0.55±0.03	Ketone
23.40	Benzenethiol, 2,4,6-tris(1-methylethyl)-	0.24	$C_{15}H_{24}S$	0.64±0.04	Phenolic compound
25.22	Methyl stearate	0.49	$C_{19}H_{38}O_2$	1.33±0.08	Fatty acid derivative
25.15	Phytol	0.58	$C_{20}H_{40}O$	1.58±0.10	Diterpenoid
27.82	9-Undecen-2-one, 6,10-dimethyl-	0.95	$C_{13}H_{24}O$	2.59±0.16	Monoterpenoid
25.81	1-Docosene	2.53	$C_{22}H_{44}$	6.88±0.43	Hydrocarbon
41.83	Stigmast-4-en-3-one	5.96	$C_{29}H_{48}O$	16.19±1.01	Phytosterol
06.65	1,16- Cyclocorynan -17-oic acid, 19, 20- didehydro-, methyl ester, (16S, 19E)-	0.02	$C_{20}H_{22}N_2O_2$	0.04±0.00	Alkaloid
25.56	Linoleic acid ethyl ester	4.68	$C_{20}H_{36}O_2$	12.7±0.79	Fatty acid derivative
32.81	Octacosane	0.44	$C_{28}H58$	1.19±0.07	Hydrocarbon
25.00	9-Octadecenoic acid, methyl ester, (E)-	0.69	$C_{19}H_{36}O_2$	1.87±0.12	Fatty acid derivative
18.93	Phenol, 3,5-bis(1, 1-dimethylethyl)-	0.21	$C_{14}H_{22}O$	0.56±0.03	Phenolic compound
38.91	4,4, 6a,6b,8a,11,12, 14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a, 14b-octadecahydro- 2H-picen-3-one	6.63	$C_{30}H_{48}O$	18.01±1.13	Terpenoid

21.99	Pentadecanol<n->	0.57	$C_{15}H_{32}O$	1.56±0.10	Fatty acid derivative
23.98	Ethyl hexadecanoate	1.29	$C_{18}H_{36}O_2$	3.51±0.22	Fatty acid derivative
24.95	Hexadecane	0.96	C16H34	2.59±0.16	Hydrocarbon
26.04	Phytol, acetate	3.52	$C_{22}H_{42}O_2$	9.57±0.60	Diterpenoid
26.45	Tributyl acetylcitrate	0.83	$C_{20}H_{34}O_8$	2.25±0.14	Tetracarboxylic acid
29.86	50.47 Heneicosane	2.27	$C_{21}H_{44}$	6.18±0.39	Hydrocarbon
37.00	Ursa-9(11),12-dien-3-one	14.14	$C_{30}H_{46}O$	38.39±2.40	Triterpenoid
27.26	4,8,12,16-Tetramethylheptadecan-4-olide	2.96	$C_{21}H_{40}O_2$	8.04±0.50	Triterpenoid
40.03	Lup-20(29)-en-3-one	1.48	$C_{30}H_{48}O$	4.03±0.25	Triterpenoid
40.43	4,22-Stigmastadiene-3-one	1.02	$C_{29}H_{46}O$	2.78±0.17	Phytosterol
19.90	Hexadecene<1->	0.53	$C_{16}H_{32}$	1.43±0.09	Hydrocarbon
23.35	Methyl hexadecanoate	0.27	$C_{17}H_{34}O_2$	0.73±0.05	Fatty acid derivative
28.610	Phenol, 2,4-bis(1-methyl-1- phenylethyl)-	1.47	$C_{24}H_{26}O$	3.99±0.25	Phenolic compound
29.10	Hexacosane	1.71	$C_{26}H_{54}$	4.65±0.29	Hydrocarbon
33.74	Widdrol	0.49	$C_{15}H_{26}O$	1.34±0.08	Sesquiterpenoid
38.45	Lanost-8-en-3-one	0.82	$C_{30}H_{50}O$	2.21±0.14	Triterpenoid
32.38	2H-1-Benzopyran-6-ol, 3,4-dihydro- 2,8-dimethyl-2-(4,8,12 -trimethyltridecyl)-, [2R-[2R*(4R*,8R*)]]-	1.02	$C_{27}H_{46}O_2$	2.76±0.17	Tocopherol
34.20	Heneicosane	0.23	$C_{21}H_{44}$	0.63±0.04	Hydrocarbon
21.56	Benzoic acid, 2, 4-dihydroxy-3, 6-dimethyl-, methyl ester	0.60	$C_{10}H_{12}O_4$	1.64±0.10	Benzene derivative
22.57	2-Pentadecanone, 6,10,14-trimethyl-	0.31	$C_{18}H_{36}O$	0.84±0.05	Diterpenoid
23.59	Palmitoleic acid	0.37	$C_{16}H_{30}O_2$	1.00±0.06	Fatty acid
25.38	9,12-Octadecadienoic acid (Z,Z)-	10.11	$C_{18}H_{32}O_2$	27.46±1.72	Fatty acid derivative
34.86	17- (1,5-Dimethylhexyl)-10,13- dimethyl-2,3,4,7,8,9,10,11, 12,13,14,15,16, 17-tetradecahydro-1h-cyclopenta[a]Phenanthren-3-ol	2.18	$C_{27}H_{48}O$	5.93±0.37	Phytosterol

23.70	n-Hexadecanoic acid	8.99	$C_{16}H_{32}O_2$	24.41±1.53	Fatty acid derivative
31.04	Squalene	1.46	$C_{30}H_{50}$	3.97±0.25	Triterpenoid
37.41	Stigmasterol	0.87	$C_{29}H_{48}O$	2.36±0.15	Phytosterol
38.67	.gamma.-Sitosterol	12.69	$C_{29}H_{50}O$	34.46±2.15	Phytosterol

Key: Conc= Concentration**, Mins=**Minutes**, MF=**Molecular formula, **RT=**Retention time, **MW=**Molecular weight

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

The current study evaluated the *in vitro* antibacterial activities of ethyl acetate extracts of *X. spekei, S. singueana,* and *G. tembensis* stem bark and leaves on *B. subtilis*, *S. typhi*, *E. coli*, as well as *S. aureus*.

5.1.1 *In vitro* antibacterial activity of ethyl acetate extracts of *S. singueana, X. spekei,* and *G. tembensis*

There is no literature on *in vitro* antibacterial effects of ethyl acetate leaf extracts of *S. singueana*. Previous studies on *S. singueana* extract reported several biological activities such as, anti-malarial and antioxidant effects of ethanol leaf extracts (Hiben *et al.*, 2016; Gerezgher *et al.*, 2018), anti-nociceptive effects for methanol leaf extracts (Hishe *et al*., 2018), antioxidant activities of methanol bark and leaf extracts (Gebrelibanos *et al*., 2008), hypoglycemic capability of aqueous leaf extracts (Mukhtar *et al*., 2020), and antibacterial activities of aqueous leaf extracts (Kareru *et al*., 2008; Aguoru and Ogaba, 2010). Ethanol leaf extracts of *Senna occidentalis*, *Senna hirsuta, Senna siamea, Senna obtusifolia, Senna polyphylla* and *Senna alata*, which also belong to the same genus, were found to have antibacterial activities (MA *et al*., 2019). Previous experiments have also demonstrated that both aqueous and methanol extracts of *S. singueana* are relatively non-toxic, thus, safe for use (Shawa *et al*., 2015; Umar, 2019).

In the current experiment, *S. singueana* extract inhibited the bacterial growth of the test pathogens, producing MZI ranging from 07.67±0.33 to 17.67±0.33 mm with higher effects against Gram-positive bacterial pathogens than on Gram-negative bacteria. This is in consensus with a past report which illustrated that the methanol, acetone, and chloroform root extracts of *S. singueana* had greater activities on Gram-positive (+ve) bacteria (*Streptococcus pyogenes*, *S. aureus*, and *Streptococcus pneumonia*) than Gram-negative (-ve) pathogens (*Pseudomonas aeruginosa*, *S. typhi*, *E. coli*, and *Klebsiella pneumonia*) (Gebremariam *et al*., 2014). This also concurs with a report by Kareru *et al.* (2008), which indicated that the aqueous leaf extract of *S. singueana* had higher effects on *S. aureus* and *B. subtilis* than it had on *E. coli*. However, our current findings are partly contrary to the reports of Shawa *et al.* (2016), which suggested that aqueous leaf and root extracts of *S. singueana* were inactive on *S. aureus* and *Pseudomonas aeruginosa*.

These findings could be as a result in the difference in cell wall structure of both Gram positive and Gram negative bacteria. Gram positive bacteria have a thick porous peptidoglycan wall which allows for easy penetration of antibacterial agents unlike Gram negative bacteria which have a thin rigid peptidoglycan wall making it hard for antibacterial agents to penetrate the bacteria (Zia-Ul-Haq *et al*., 2013).

This is considerably the initial report about the antibacterial activities of ethyl acetate stem bark extracts of *G. tembensis*. However, previous studies have shown

the biological capabilities of stem bark extracts of other plants of the genus *Grewia*. *Grewia tiliaefolia* methanol stem bark extract demonstrated antidiabetic effects (Kumar *et al*., 2012), ethanol bark extracts of *Grewia asiatica* exhibited antidiabetic, anti-hyperlipidemic as well as antioxidant activities (Khatune *et al*., 2016). Ethanol stem bark extracts of *G. mollis* have exhibited antibacterial activities on *E. coli, S. aureus* as well as *Streptococcus* sp (Shagal *et al*., 2012). *G. asiatica* ethanol stem bark extracts have been found to be safe when tested against Brine shrimp (Parveen *et al*., 2013). Chloroform leaf extract of *G. asiatica* has antihyperglycemic activity (Priyanka *et al*., 2010), whereas the methanol leaf extracts showed antimalarial activities (Paul, 2015).

The ethyl acetate stem bark extracts of *G. tembensis* exhibited notable antibacterial effects against *S. aureus* only with MZI ranges of 07.07±0.07 to 12.33±0.33 mm. This concurs with a report by Akwu *et al.* (2020), which confirmed that lupeol compounds from the stem bark of *Grewia lasiocarpa* were inactive on *S. typhi* and *E. coli*. Chloroform stem bark extract of *G. lasiocarpa* demonstrated antibacterial effects on tested Gram-positive microbes but had no activity on all the tested Gram negative pathogens (Akwu *et al*., 2019). Additionally, this also agrees with a study which indicated that the ethyl acetate leaf extracts of *G. plagiophylla* displayed no antibacterial activity against *E. coli* and *S. typhi* but had activity against *S. aureus* (Douglas and Gitonga, 2016). On the contrary, the findings of Iftikhar *et al.* (2020)

showed that *G. optiva* had a wide antibacterial spectrum of activity on *E. coli, S. aureus, S. typhi* as well as *Streptococcus pneumoniae.*

Similarly, this is considerably the initial study about the antibacterial activity of ethyl acetate leaf extracts of *G. tembensis*. However, previous experiments have demonstrated that the leaves of other *Grewia* genus plants have pharmacological activities. The methanol, n-hexane, and ethyl acetate leaf extracts of *G. pubescens* have been shown to possess antioxidant and antibacterial effects (Hamid *et al*., 2016), whereas *G. optiva* aqueous leaf extract exhibited analgesic, anti-cholinesterase as well as antibacterial capabilities (Iftikhar *et al*., 2020). Both aqueous and methanol *G. asiatica* leaf extracts have also been demonstrated to have anticancer and antimicrobial activities (Zia-Ul-Haq *et al*., 2013).

Ethyl acetate leaf extracts of *G. tembensis* exhibited antibacterial capability against only *S. aureus* in a dilution-dependent trend with MZI ranging from 08.33±0.33 to 11.67±0.33 mm. These findings relate to those of Mostafa *et al.* (2018), who determined that ethanol seed extracts of *Cuminum cyminum* were effective against only *S. aureus* but were inactive on Gram-positive (*B. cereus*) and Gram-negative (*E. coli*, *P. aeruginosa* and *S. typhi*) bacterial strains. Additionally, our findings also agree with a report that found both ethanol and aqueous *G. mollis* extracts to have demonstrated no impact against *S. typhi* (Shagal *et al*., 2012).

The current study noted that there is lack of published reports available on antibacterial capabilities of ethyl acetate extracts of *X. spekei*. Stem barks of *X. spekei* are used by South African traditional healers to relieve asthma, for general aches, as an anti-inflammatory, and for postpartum hemorrhage (Seaman, 2005). *Xerophyta retinervis* which belongs to the same genus as *X. spekei* has illustrated antimicrobial, antiulcer as well as anti-inflammatory activities. Crude extracts of *X. retinervis* and *X. villosa* also demonstrated antiplasmodial activities (Wiesner, 2008). In South Africa, *X. retinervis* is used by traditional herbalists to cure rhinitis and headache (Semenya and Maroyi, 2018). The whole plant of *X. retinervis* is traditionally used in the Pretoria region of South Africa as a therapy for asthma and nose bleeding (Motlhatlego, 2014).

The ethyl acetate extract of *X. spekei* produced notable inhibition zones starting from 07.67±0.33 to 14.67±0.33mm against *S. aureus* and inhibition zones starting from 09.67±0.33 to 14.33±0.33mm against *B. subtilis* but had no antibacterial activities on the tested Gram-negative microbes. This was in consensus with the findings of a study by Seaman (2005) that illustrated that ethyl acetate extracts of *X. retinervis* had antibacterial activity on *S. aureus*. This also agrees with an experiment that showed no antibacterial effect on Gram-negative *K. pneumoniae* and *P. aeruginosa* (Motlhatlego, 2014).

Generally, the studied plant extracts had a significantly better antibacterial activity on Gram-positive than on Gram-negative microbes. This agrees with Abebaw *et al.* (2018), who demonstrated that most Gram-positive microbes are more prone to antibacterial agents, unlike Gram-negative microbes. This is because the latter has a three-layered envelope that consists of three layers, with the outer layer distinguishing the Gram-negative from Gram-positive as it is the protective layer consisting of lipopolysaccharides. The second layer is a rigid peptidoglycan layer that determines the cell shape. The third layer is a phospholipid bilayer useful in bacterial transport, structure, and biosynthetic activities (Abebaw *et al.,* 2018). Gram-negative's drug resistance is due to the existence of the outer membrane. On the contrary, the absence of this layer makes Gram-positive microbes more susceptible to antibacterial agents (Breijyeh *et al*., 2020). This has made Gram-positive pathogens more sensitive to crude plant extracts and bioactive constituents because of their cell wall structure (Zia-Ul-Haq *et al*., 2013). This could be the possible explanation as to why the studied extracts had higher activities on Gram-positive bacteria.

However, our findings generally were in contrast with Sieberi *et al*. (2020), who showed that the methanol-dichloromethane blend of *Polygala sphenoptera, Fuerstia africana,* and *Centella asiatica* had a better impact on the Gram-negative than the Gram-positive microbes tested. The antibacterial activity by these extracts could be attributed to the presence of a lipophilic outer membrane consisting of

lipopolysaccharide molecules with affinity for lipophilic molecules (Sieberi *et al*., 2020).

The antibacterial activities of the studied plant extracts in the current experiment are ascribable to the presence of various phytocompounds. Phytochemicals facilitate their antibacterial actions by preventing bacterial growth, interfering with some bacterial metabolic processes, and modulate signal transduction (Godstime *et al*., 2014).

Compounds like terpenoids have known antibacterial activities (Nami *et al.*, 2016), although their antibacterial mechanisms of action remain largely unknown. However, it is speculated that they can cause bacterial membrane disruption due to their lipophilic nature (Awa *et al*., 2012), as well as interfere with bacterial oxygen uptake and oxidative phosphorylation, which are two important essential processes in bacteria (Mahizan *et al*., 2019). Phytol, a diterpenoid which was found in *S. singueana* extract at a concentration of 2.11±0.03 µg/g, has known antibacterial activity (Rukshana *et al*., 2017). Phytol acetate<E-> a diterpenoid present in stem bark extract of *G. tembensis* at 3.02±0.11 µg/g concentration has been known to have antibacterial effects (Jasna *et al*., 2018). 2-Pentadecanone, 6,10,14-trimethyl- a diterpenoid present in *X. spekei* extract at 0.84±0.05 µg/g concentration, have also exhibited antibacterial activities (Arora and Meena, 2017) respectively. Additionally, Lup-20(29)-en-3-one, a triterpenoid, which was present in *G.*

tembensis leaf extract at 2.75±0.05 µg/g concentration, has also been known to have antibacterial effects (Nimbeshaho *et al*., 2020).

Hydrocarbon phytochemicals can either be saturated or unsaturated (Brielmann *et al*., 2006). The majority of the hydrocarbons were alkanes. Alkanes are an important class of hydrocarbons that are synthesized by many organisms naturally for protection against environmental threats. Alkanes act by interfering with bacterial cell membrane integrity and function, leading to bacterial cell death (Kang and Nielsen, 2017). Alkanes have good antibacterial effects on *S. aureus* and *E. coli* (He, 2009; Rouis-Soussi *et al*., 2014). Octacosane, a straight-chain alkane, which was found in *G. tembensis* stem bark extracts at 1.53±0.05 µg/g concentration, has antibacterial effects (Karthikeyan *et al.*, 2016), whereas other straight-chain alkanes, Tetracosane and Tricosane, which were both present in *S. singueana* and *G. tembensis* leaf extracts at 6.13±0.03 µg/g and 2.34±0.04 µg/g concentrations respectively, have also demonstrated antibacterial effects (Konovalova *et al*., 2013; Vijay *et al*., 2018; Mini *et al.,* 2019). Another hydrocarbon, Heneicosane, which was found in *X. spekei* extract at 0.63±0.04 µg/g concentration, has previously shown antibacterial activities (Biswas *et al*., 2017).

Many plants utilize fatty acids in defense against pathogenic bacteria. While fatty acids' exact antibacterial mechanism of action is poorly known (Zheng *et al*., 2005), their prime target is disrupting the electron transport chain of bacterial cell membranes. Fatty acids can also act by inhibiting bacterial enzyme activity,

impairing nutrient uptake, and direct bacterial cell lysis (Desbois and Smith, 2010). Fatty acid like n-Hexadecanoic acid (palmitic acid), found *G. tembensis* leaf extracts at 2.63±0.04 µg/g concentration, has been shown to possess antimicrobial effects (Awa *et al.,* 2012). Another fatty acid, Hexadecanoic acid ethyl ester, which was present in both *G. tembensis* stem bark and *S. singueana* extracts at varied concentrations of 13.95±0.48 µg/g and 1.38±0.02 µg/g, has also been found to possess antimicrobial capabilities (Musa *et al*., 2015; Nami *et al*., 2016). Palmitoleic acid, found in *X. spekei* extract at 1.00±0.06 µg/g concentration has known antibacterial effects (Ali *et al.,* 2017; Teh *et al.,* 2017).

Alkaloids, a structurally diverse group of plant secondary metabolites, exert their antibacterial activity by inhibiting bacterial enzyme activity, thus killing the bacteria (Othman *et al.,* 2019). An alkaloid, 2-Cyclohexylpiperidine, which was present in *S. singueana* leaf extract at 0.03±0.00 µg/g concentration has been known to have antimicrobial activities (Omar *et al*., 2018).

Phenolic compounds, large heterogeneous secondary plant metabolites, are broadly spread in plants and are important constituents of human diet (Pourreza, 2013). In addition, phenolic compounds are known for their cell lysis in addition to membrane-disturbing capabilities as their mode of antibacterial activity (Reichling *et al*., 2006). Phenol, 3,5-bis (1, 1-dimethylethyl)-, which was present in *X. spekei* extract at 0.56±0.03 µg/g concentration has been shown to have antibacterial

activities (Rai *et al*., 2016). Phenol, 2,5-dimethyl-, acetate which was found in *G. tembensis* stem bark extract at 11.27±0.39 µg/g concentration has displayed antimicrobial activities (Mukhtar *et al*., 2016).

Phytosterols are important structural components in plant membranes. They stabilize plant cell phospholipid bilayers just like cholesterols in animal cell membranes (Sharma *et al*., 2017). Phytosterols have demonstrated good antibacterial activities (Fahmi *et al*., 2014). Stigmasterol, which was present in *G. tembensis* stem bark, and leaf extracts at varied concentrations of 20.54±0.71 µg/g and 0.12±0.00 µg/g respectively, has been previously shown to have antibacterial activities (Mailafiya *et al*., 2018). Stigmast-4-en-3-one, a phytosterol, present in *X. spekei* extract at 16.19±1.01 µg/g concentration, has been shown to have antibacterial effects (Ragasa *et al*., 2009). β-Sitosterol, which was present in *S. singueana* extract at 0.17±0.00 µg/g concentration, has been found to exhibit antimicrobial activities (Sen *et al*., 2012).

Vitamin E (α tocopherol), a fat-soluble carotenoid, is useful in deactivating photosynthesis-derived reactive oxygen species in plants (Brewer, 2011). Vitamin E, which was present in both *G. tembensis* stem bark and *S. singueana* extracts at varied concentrations of 3.09±0.11 µg/g and 1.94±0.03 µg/g, has antimicrobial activities (Al-Salih *et al.*, 2013; Nguyen *et al.*, 2020). Other compounds like fatty alcohols demonstrated antibacterial activities (Belkacemi *et al*., 2020).

Pentadecanol, which was present in *X. spekei* extract, at a concentration of 1.56±0.10 µg/g, has been shown to have antibacterial activities (Chatterjee *et al*., 2018). A previous experiment by Malarvizhi *et al.* (2019) confirmed that 3,7,11, 15-tetramethyl-2- hexadecen-1-ol, a terpene alcohol that was present in both *S. singueana* and *G. tembensis* leaf extracts at varied concentrations of 3.32±0.04 µg/g and 0.92±0.02 µg/g, has been demonstrated to have antibacterial effects.

5.1.2 *In vitro* antioxidant activity of the test plant extracts

In the current study, antioxidant activity of the test plant extracts in all the assays was dose dependent. As the extracts' concentration decreased, the antioxidant capacity also decreased. These findings concur with previous studies that demonstrated dose-dependent antioxidant activity of studied extracts (Rahman *et al.*, 2015; Zou *et al*., 2015). In a past study, Shon *et al.* (2004) also found that as the concentration of ethyl acetate *Allium cepa* extracts increased, the DPPH free radical scavenging activity also increased. Similar observations were also described by Sharma and Vig (2013), who showed that *Parkinsonia aculeata* L. both aqueous and methanol leaf extracts' *in vitro* antioxidant effects increased with an increase in concentration.

Ferric reducing power results in this study demonstrated a dose dependent trend. Our findings concur with Sharma *et al.* (2013), who confirmed that, the reducing capacity of aqueous and methanol leaf extracts of *Parkinsonia aculeata* L. decreased with a decrease in extract concentration. Similarly,

Narendhirakannan *et al.* (2010) demonstrated that *Allium Sativum* L. extracts' reducing power increased with an increase in extract concentration.

The findings of the current study confirm that the plant extracts demonstrated significant hydroxyl radical scavenging activities. These findings are similar to those of Amudha *et al.* (2016) who demonstrated that the ethyl acetate (EtOAc) and ethanol extracts of *Cordia retusa* had hydroxyl radical scavenging activities. Our findings also concurred with the findings of Sharma *et al.* (2013), who found that the *in vitro* hydroxyl radical mopping capacity of *Parkinsonia aculeata* L. aqueous and methanol leaf extracts were significantly lower than the reference.

X. spekei extract exhibited significantly the greatest iron chelating impact amongst the studied extracts trailed by *S. singueana* and *G. tembensis* leaf and stem bark extracts, respectively. These findings agree Wong *et al.* (2014) who demonstrated that *Vernonia amygdalina, Pereskia bleo, Clinacanthus nutans, Hedyotis diffusa, Callicarpa formosana,* and *Leonurus cardiaca* showed strong chelating activities but lower than the standard. Our observations also concur with Amudha *et al.* (2016), who observed that the standard EDTA had a higher iron chelating activity than the ethanol and ethyl acetate extracts of *Cordia retusa*.

In the current study, each studied extract demonstrated good hydrogen peroxide radical scavenging activity. This was consistent with a previous report by Bhatti *et al.* (2015) that confirmed that *Ranunculus arvensis* L aqueous, chloroform, methanol together with acetone extracts displayed potent hydrogen peroxide radical scavenging activity in a similar trend as the studied extracts in the current experiment. Our current observations also agree with those of Narendhirakannan and Rajeswari (2010), who found that *Allium sativum* L. extracts had potent hydrogen peroxide scavenging activity though their activities were significantly lesser than those of the reference. Consistently, Shon *et al.* (2004) demonstrated that *Allium cepa* ethyl acetate extract exhibited lower hydrogen peroxide radical scavenging activity than those of the reference.

Antioxidant activities exhibited by ethyl acetate extracts of *S. singueana*, *G. tembensis* leaf, *G. tembensis* stem bark, and *X. spekei* can be due to the various phytocompounds that work synergistically to overcome free radicals (Ishino *et al*., 2010). Several biological compounds, including phenolic compounds, lipids like fatty acids, phytosterols, and fatty acids esters, terpenoids like monoterpene, diterpenes, and triterpenes, alkaloids, and hydrocarbons like alkanes and alkenes were detected. They have been known to exert their antioxidant capability through multi-step processes that involve initiating, propagating and eventually terminating free radicals (Ishino *et al*., 2010).

Terpenoids, the most diverse and largest class of plant phytochemicals (Yadav *et al.*, 2015), are known to have cancer-protective, cardio-protective effects (Wang *et al.*, 2019) anti-malarial and insecticidal activities (Yang *et al.*, 2020). Terpenoids have also been known to have antioxidant activities. They act as free radical scavengers (Torres-Martínez *et al*., 2017) and as chelating agents (Mohandas and Kumaraswamy, 2018). Alpha.-Amyrin, a triterpenoid, present in *G. tembensis* stem bark extract at a concentration of 7.81±0.27 µg/g, has known antioxidant activities (Obafemi *et al.*, 2017), whereas 7-Octenal, 3,7-dimethyl-, a monoterpenoid present in *S. singueana* extract at a concentration of 1.06±0.01 µg/g has also been known to have antioxidant effects (Salem *et al*., 2018). Limonene, a monoterpenoid, which was present in *G. tembensis* leaf extract at 0.16±0.00 µg/g concentration, exhibited antioxidant activities (Bacanli *et al.*, 2015; Baschieri *et al*., 2017). Additionally, Ursa-9(11), 12-dien-3-one, a triterpenoid, present in *X. spekei* extract at 38.39±2.40 µg/g concentration, has also been found to have antioxidant effects (Sushma *et al*., 2017).

Hexadecane, a hydrocarbon present in *X. spekei* extract has been shown to have antioxidant effects (Padma *et al*., 2019). Octadecane, another hydrocarbon, present in *G. tembensis* leaf extract at a concentration of 1.89±0.03 µg/g, has antioxidant effects (Adeyemi *et al*., 2017). Similarly, Tritriacontane, another hydrocarbon present in *G. tembensis* stem bark extract at 14.61±0.50 µg/g, has been established to have good antioxidant effects (Akpuaka and Ahmed, 2013), whereas

Tetratetracontane, present in *S. singueana* extract at 1.43±0.02 µg/g concentration, has also been shown to have antioxidant activities (Kumari and Menghani, 2021).

Fatty acids exert antioxidant activity by scavenging free radicals (El-Agbar *et al.*, 2018). Ethyl hexadecanoate acid ethyl ester, a fatty acid, which was present in *S. singueana* at 1.38±0.02 µg/g concentration, has been shown in previous studies to have antioxidant activities (Moon and Cha, 2020). Linoleic acid, another fatty acid found in *X. spekei* extract having a concentration of 12.7±0.79 µg/g, is reported to exhibit antioxidant effects (Kim *et al*., 2020). Alsor fatty acid, 9,12, 15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-, present in *G. tembensis* leaf extract at 4.17±0.07 µg/g concentration, has been proved to possess antioxidant capabilities (Falowo *et al*., 2017). Lauric acid, a fatty acid found in *G. tembensis* stem bark extract at 0.04±0.00 µg/g concentration, possesses good antioxidant activities (Gani *et al*., 2020).

Phenolic substances exhibit antioxidant effects by scavenging free radicals (Sei *et al*., 2014). Methyleugenol, a phenolic compound, was present in *S. singueana* leaf extract at 0.31±0.00 µg/g concentration and is known to have antioxidant activities (Nascimento *et al*., 2020). Phenol, 3,5-bis (1,1-dimethylethyl)-, another phenolic phytocompound, present in *X. spekei* extract at 0.56±0.03 µg/g concentration has good antioxidant activities (Rai *et al*., 2016).

Phytosterols exert antioxidant activities by directly scavenging free radicals (Vezza *et al*., 2020). Their biological effects include; antioxidant and anti-inflammatory activities (Salehi *et al*., 2020). Campesterol, a phytosterol present in *G. tembensis* stem bark extract, with a concentration of 17.39±0.60 µg/g is a known antioxidant agent (Pandey *et al.,* 2014; Bhuyar *et al.,* 2020). Additionally, 17-(1, 5-dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1h-cyclopenta [a]phenanthren -3-ol, a phytosterol present in *X. spekei* extract at 5.93±0.37 µg/g concentration, has also been shown to have antioxidant effects (AlAmery and AlGaraawi, 2020). β-Sitosterol, another phytosterol, which was present in *S. singueana* extract at 0.17±0.00 µg/g concentration has been found to have antioxidant effects (Babu and Jayaraman, 2020).

Tocopherol's antioxidant mode of action is basically through the donation of phenolic hydrogen to lipid-free radicals thus preventing oxidation (Luzia and Jorge, 2011). Vitamin E, present in both *G. tembensis* stem bark and *S. singueana* extracts at varied concentrations of 3.09±0.11 µg/g and 1.94±0.03 µg/g respectively has been confirmed to possess antioxidant activities (Al-Salih *et al.,* 2013; Nguyen *et al*., 2020).

5.2 Conclusions

The current experiment aimed at confirming the traditional use of *S. singueana*, *X. spekei,* and *G. tembensis* medicinal plants against frequent bacteria, causing various

human diseases including *E. coli, B. subtilis, S. typhi,* and *S. aureus* by investigating their *in vitro* antibacterial activity, antioxidant and the presence of phytochemicals with such activities. The ethyl acetate extracts of the studied plant extracts demonstrated varied antibacterial activities with *S. singueana* extract having activity on all the tested pathogens (*S. aureus, B. subtilis, E. coli*, and *S. typhi*) whereas, *X. spekei* extract, had activity on *S. aureus* and *B. subtilis* only, and finally, both the stem bark and leaf extracts of *G. tembensis* had activity on *S. aureus* alone. However, all the studied extracts demonstrated *in vitro* DPPH, H_2O_2, and hydroxyl radical scavenging activities, in addition to ferric reducing antioxidant capacity and iron chelating activities. The test extracts also contained phytochemicals with antibacterial and antioxidant activities. Overall, our current findings indicate that the studied extracts can be activity candidates to extract antibacterial and antioxidant agents for managing infectious diseases and oxidative stress-related illnesses.

5.3 Recommendations

5.3.1 Recommendations from the study

i. The ethyl acetate extracts of *S. singueana* can be developed further as antibacterial agents against *S. aureus, B. subtilis, S. typhi* and *E. coli* while *X. spekei* extract can be used to develop antibacterial agents against *S. aureus, B. subtilis* and finally *G. tembensis* extracts may be applied in the development of *S. aureus* antibacterial agent.

ii. The ethyl acetate extracts of *S. singueana, X. spekei*, and *G. tembensis* may be utilized as alternative antioxidant agents.

5.3.2 Suggestions for future studies

i. Bioassay guided fractionation and isolation of antibacterial and antioxidant phytocompounds need to be done.

ii. *In vivo* investigation of the extracts' antioxidant and antibacterial activities needs to be conducted.

iii. The extracts' *in vitro* modes of action for antioxidant and antibacterial effects needs to be investigated.

iv. Determination of safety of the tested plant extracts needs to be done.

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APPENDICES

Appendix I: Compounds shared amongst the extracts

SN	MW	MF	CLASS	COMPOUND NAME	CONCENTRATION (g/mol) PER EXTRACT			
					GTL	**GTB**	**SS**	**XS**
1.	410.70	$C_{30}H_{50}$	Triterpenoid	Squalene	14.17±0.24	11.31±0.39	5.24±0.07	3.97±0.25
2.	338.70	$C_{24}H_{50}$	Hydrocarbon	Tetracosane	2.85±0.05	9.93±0.34	2.56±0.03	**NP**
3.	324.60	$C_{23}H_{48}$	Hydrocarbon	Tricosane	2.34±0.04	9.91±0.34	3.41±0.05	**NP**
4.	352.70	$C_{25}H_{52}$	Hydrocarbon	Pentacosane	**NP**	7.56±0.26	2.16±0.03	**NP**
5.	254.41	$C_{16}H_{30}O_2$	Fatty Acid	Palmitoleic acid	**NP**	4.16±0.14	**NP**	1.00±0.06
6.	324.50	$C_{21}H_{40}O_2$	Terpenoid	4,8,12, 16tetramethylheptadecan-4-olide	**NP**	14.05±0.49	**NP**	8.04±0.50
7.	268.50	$C_{19}H_{40}$	Hydrocarbon	Nonadecane	**NP**	1.79±0.06	1.50±0.02	**NP**
8.	296.60	$C_{21}H_{44}$	Hydrocarbon	Heneicosane	1.39±0.02	**NP**	**NP**	0.63±0.04
9.	270.50	$C_{17}H_{34}O_2$	Fatty Acid methyl ester	Methyl hexadecanoate/ Hexadecanoic acid, methyl ester	**NP**	10.78±0.37	0.95±0.01	0.73±0.05
10.	290.50	$C_{20}H_{34}O$	Diterpenoid	trans-Geranylgeraniol	1.62±0.03	4.13±0.14	**NP**	**NP**
11.	284.50	$C_{18}H_{36}O_2$	Fatty Acid esters	Hexadecanoic acid, ethyl ester	**NP**	13.95±0.48	1.38±0.02	**NP**
12.	366.70	$C_{26}H_{54}$	Hydrocarbon	Hexacosane	1.28±0.02	7.26±0.25	2.05±0.03	4.65±0.29

13.	284.50	$C_{18}H_{36}O_2$	Fatty Acid esters	Ethyl hexadecanoate	2.98±0.05	**NP**	**NP**	3.51±0.22
14.	256.42	$C_{16}H_{32}O_2$	Fatty Acid derivative	n-Hexadecanoic acid/palmitic acid	2.63±0.04	88.06±3.04	0.88±0.01	24.41±1.53
15.	282.50	$C_{20}H_{42}$	Hydrocarbon	Eicosane	1.14±0.02	**NP**	0.57±0.01	**NP**
16.	296.50	$C_{19}H_{36}O_2$	Fatty Acid methyl ester	9-Octadecenoic acid, methyl ester, (E)-	**NP**	**NP**	1.22±0.02	1.87±0.12
17.	430.70	$C_{29}H_{50}O_2$	Tocopherol	Vitamin E	**NP**	3.09±0.11	1.94±0.03	**NP**
18.	412.70	$C_{29}H_{48}O$	Phytosterols	Stigmasterol	0.12±0.00	20.54±0.71	**NP**	2.36±0.15
19.	414.70	$C_{29}H_{50}O$	Phytosterols	gamma.-Sitosterol	0.47±0.01	7.14±0.25	**NP**	34.46±2.15
20.	426.70	$C_{30}H_{50}O$	Triterpenoid	beta.-Amyrin	6.81±0.12	4.78±0.17	**NP**	**NP**
21.	424.70	$C_{30}H_{48}O$	Triterpenoid	Lupenone/ Lup-20(29)-en-3-one	2.75±0.05	19.67±0.68	**NP**	4.03±0.25
22.	426.70	$C_{30}H_{50}O$	Triterpenoid	Taraxasterol	1.75±0.03	17.59±0.61	**NP**	**NP**
23.	412.70	$C_{29}H_{48}O$	Steroid	Stigmast-4-en-3-one	**NP**	10.58±0.37	**NP**	16.19±1.01
24.	426.70	$C_{30}H_{50}O$	Triterpenoid	Friedelan-3-one	0.32±0.01	23.17±0.80	**NP**	**NP**
25.	268.50	$C_{18}H_{36}O$	Diterpenoid	2-Pentadecanone, 6,10,14-trimethyl-	0.86±0.01	4.21±0.15	**NP**	0.84±0.05
26.	338.60	$C_{22}H_{42}O_2$	Diterpenoid	Phytol, acetate	1.55±0.03	2.60±0.09	**NP**	9.57±0.60
27.	296.50	$C_{20}H_{40}O$	Diterpenoid	Phytol	3.47±0.06	6.874±0.24	2.11±0.03	1.58±0.10

28.	338.60	$C_{22}H_{42}O_2$	Diterpenoid	Phytol acetate<E->	2.20±0.04	3.02±0.11	1.99±0.03	**NP**
29.	228.37	$C_{14}H_{28}O_2$	Fatty acid	Tetradecanoic acid/myristic acid	**NP**	5.36±0.19	**NP**	1.19±0.07
30.	394.80	$C_{28}H_{58}$	Hydrocarbon	Octacosane	**NP**	1.53±0.05	2.03±0.03	1.19±0.07
31.	196.19	$C_{10}H_{12}O_4$	Benzene derivative	Benzoic acid, 2, 4-dihydroxy-3, 6-dimethyl-, methyl ester	**NP**	3.40±0.12	**NP**	1.64±0.10
32.	200.32	$C_{12}H_{24}O_2$	Fatty acid	Dodecanoic acid/lauric acid	**NP**	3.667±0.13	2.48±0.03	**NP**
33.	296.50	$C_{20}H_{40}O$	Terpene alcohol	3,7,11, 15-tetramethyl-2-hexadecen-1-ol	0.92±0.02	**NP**	3.32±0.04	**NP**
34.	180.24	$C_{11}H_{16}O_2$	Diterpenoid	2 (4H)-benzofuranone, 5,6,7, 7a-tetrahydro-4,4, 7a-trimethyl-	1.01±0.02	2.03±0.07	**NP**	**NP**
35.	330.50	$C_{24}H_{26}O$	Phenolic compound	Phenol, 2,4-bis(1-methyl-1-phenylethyl)-	2.52±0.04	10.3±0.36	1.67±0.02	3.99±0.25

Key: NP-Not Present, GTL-*Grewia tembensis* leaf extract, GTB-*Grewia tembensis* stem bark extract, SS-*Senna singueana* extract, XS-*Xerophyta spekei* extract

Appendix II: Research proposal approval

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Dean, Graduate School DATE: 9th April, 2021

TO: Mr. Paul Ochieng Nyalo REF: I56/27698/2018
C/o Department of Biochemistry, Microbiology
& Biotechnology

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting on 31st March, 2021, approved your Research Proposal for the M.Sc. Degree entitled, "*In Vitro* Antimicrobial and Antioxidant Activities of Ethyl Acetate Extracts of *Xerophyta spekei* (Baker), *Senna singueana* (Delile) and *Grewia tembensis* (Fresen)."

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Thank you.

JULIA GITU
FOR: DEAN, GRADUATE SCHOOL

CC. Chairman, Department of Biochemistry, Microbiology & Biotechnology

Supervisors:

1. Dr. Mathew Piero Ngugi
C/o Biochemistry, Microbiology & Biotechnology Dept.
Kenyatta University

2. Dr. George Omwenga
C/o Biochemistry, Microbiology & Biotechnology Dept.
Kenyatta University

Appendix III: Research license issued by NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 834666

Date of Issue: 29/April/2021

RESEARCH LICENSE

This is to Certify that Mr.. Paul Ochieng Nyalo of Kenyatta University, has been licensed to conduct research in Embu on the topic: IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF ETHYL ACETATE EXTRACTS OF Xerophyta spekei (Baker), Senna singueana (Delile) and Grewia tembensis (Fresen) for the period ending : 29/April/2022.

License No: NACOSTI/P/21/10159

834666

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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Appendix IV: Graduate school letter to NACOSTI

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Our Ref: I56/27698/2018

DATE: 9th April, 2021

Director General,
National Commission for Science, Technology
and Innovation
P.O. Box 30623-00100
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR. PAUL OCHIENG NYALO – REG. NO. I56/27698/18

I write to introduce Mr. Paul Ochieng Nyalo who is a Postgraduate Student of this University. She is registered for M.Sc. degree programme in the **Department of Biochemistry, Microbiology & Biotechnology.**

Mr. Nyalo intends to conduct research for a M.Sc. thesis Proposal entitled, **"*In Vitro* Antimicrobial and Antioxidant Activities of Ethyl Acetate Extracts of *Xerophyta spekei* (Baker), *Senna singueana* (Delile) and *Grewia tembensis* (Fresen)."**

Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL