EFFICACY OF *Thunbergia alata* AND *Senna didymobotrya* EXTRACTS AGAINST SELECTED BACTERIA COMMONLY ASSOCIATED WITH SKIN INFECTIONS IN KERICHO REFERRAL HOSPITAL, KENYA

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DECLARATION AND APPROVAL

Declaration

This thesis is my original work and has not been presented for any award of a diploma or conferment of a degree in this institution or any other University.

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DEDICATION

To my dear mum and my children Hazel Jerop and Ivan Kibet

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ABSTRACT

Bacterial infections are widely distributed and cause deadly infectious diseases such as skin, soft tissue and respiratory tract infections, meningitis, and tuberculosis. Bacterial infections are very common and can be acquired easily since bacteria are ubiquitous. The infections, however, have become a challenge to modern healthcare providers, due to the myriad of side effects of the costly conventional drugs. There is a need therefore to source for alternative remedies that are easily available, affordable, and effective. This study was carried out to determine the efficacy of *Senna didymobotrya* and *Thunbergia alata* crude plant extracts against *Staphylococcus aureus,Streptococcus pyogenes* and *Pseudomonas aeruginosa* in Kericho County. Leaves of the said plants were sourced from Bomet and Kabianga, dried, milled into powder and solvent extracted using Hexane, Dichloromethane: Methanol at a ratio of 1:1an Methanol. Phytochemicals present in each plant extract were evaluated using standard laboratory procedures. Antibacterial activity and Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentration (MBC) were determined using the disc diffusion technique under sterile conditions. Discs impregnated with standard antibacterial drugs were used as a positive control. Leaves of *S. didymobotrya* and *T. alata* were found to be rich in alkaloids, flavonoids, terpenoids, glycosides and tannins irrespective of plant collection site, solvent of extraction or plant species. *S. didymobotrya* and *T. alata* plant extracts significantly inhibited the growth of the exposed microbes in the following order: *S. aureus,* ≥ *S. pyogenes* and ≥ *P. aeruginosa* bacteria in comparison with commercial antibiotics (penicillin, chloramphenicol, and erythromycin). The MIC values of the isolates ranged from 20×10-3 mg/ml to 4.8×10-3 mg/ml. However, inhibition by plant extracts showed re-growth of *S. pyogenes* after 36 hours, suggesting a bacteriostatic nature. These results suggest that *S. didymobotrya* and *T. alata* leaves contain significant amounts of alkaloids, flavonoids, terpenoids, glycosides and tannins. Hence can be used as traditional medicine to manage *S. aureus,S. pyogenes* and *P. aeruginosa* bacteria found on human skin.

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LIST OF ABBREVIATIONS AND ACRONYMS

AIDs:	Acquired Immunodeficiency syndrome
ANOVA:	Analysis of Variance
AST:	Antimicrobial Sensitivity Testing
DCM:	Dichloromethane
GDP:	Gross Domestic Product
MBC:	Minimum Bactericidal Concentration
MIC:	Minimum Inhibitory Concentration
µL:	Microliter
ml:	Milliliter
mm:	Millimeter
TSB:	Tryptic Soy Broth
MSA:	Mannitol Salt Agar
EMB:	Eosin Methylene Blue
TSI:	Tripple Sugar Iron Test
MHA:	Muller Hinton Agar
NA:	Nutrient Agar

DEFINITIONS OF TERMS

Antimicrobials: refer to a group of agents that share the common aim of reducing the possibility of infection and sepsis.

Infectious diseases: are disorders that are caused by organisms such as bacteria, viruses, fungi or parasites that can be passed, directly or indirectly from one person to another.

Inhibition zone: is an area of media where bacteria are unable to grow, due to the presence of a drug that impedes their growth.

Minimum inhibitory concentration: the lowest concentration of antimicrobial that will inhibit the visible growth of microorganism after an overnight incubation**.**

In vitro: is a process performed outside the living organism, in a tube or a Petri dish.

CHAPTER ONE

INTRODUCTION

1.1 Overview

This chapter provides the background of the study, a statement of the problem, objectives, hypothesis, justification and significance of the study and the scope of the study.

1.2 Background of the Study

Humans and animals are susceptible to diseases associated with bacterial (Verbrugghe et al., 2012). Bacterial infections are widely distributed worldwide and cause most of the deadly infectious diseases such as skin, soft tissue and respiratory tract infections, meningitis, and tuberculosis (Russo et al., 2016). These infections are very common and can be acquired easily since bacteria are ubiquitous (Doron & Gorbach, 2008). Bacterial infections exist in many forms and significantly affect human health. The sources of infectious diseases are vast, but in most cases arise from infectious microorganisms such as bacteria that can establish growth or replication in humans, harming specific systems of the human body (Hay et al., 2014). This has contributed to unsustainable socio-economic development following the emergence of antimicrobial-resistant strains of pathogens to the available conventional management regime (Kitonde et al*.,* 2014). The observed resistance is thought to be due to indiscriminate and irrational use of antibiotics that has led to the microbe's development of antibacterial resistance to available drugs making it difficult to treat.

Skin infections caused by *Staphylococcus aureus* and β-haemolytic streptococci and coryneform are the common bacteria causing skin infections (Del Giudice, 2020).They infect the epidermal layer of the skin, the follicles and penetrate to the

deeper layers of skin causing necrotic ulcers (Török & Conlon, 2005).These infections if left untreated may spread throughout the body. Examples include folliculitis, trichomycosis, erysipeloid, impetigo, cellulitis, boils, skin, staphylococcal scalded syndrome, and Lyme disease (Mahajan et al., 2022). Some secondary infections are intertrigo and athlete foot. Antibiotics are the effective treatment for bacterial skin infections (Pujalte et al., 2023).

To date, there is no known method to reverse antibiotic resistance by bacteria. Since antibiotic resistance is a natural way in which bacteria adopt to antimicrobial agents. The choice of antimicrobial agents for the treatment of various ailments has not been forthcoming since microbes change their behaviour hence making treatment with antibiotics a nightmare to healthcare providers. Antimicrobial agents are characterized according to their mechanism of action, that is, interference with cell wall synthesis, DNA and RNA synthesis, lysis of the bacterial membrane, inhibition of protein synthesis and inhibition of metabolic pathways (McManus, 1997). The choice of antibiotics for treatment targets three or all the mechanisms of action on the pathogen. On the other hand, bacteria may become resistant to antibiotic inactivation, target modification, efflux pump and plasmid efflux.

Hence making it difficult to treat leading to the emergence of more lethal strains compared to the parent strain (Dey et al*.,* 2022). The clinically available medicine is no longer effective against antibiotic-resistant strains. Hence there is a need to explore other remedies including the use of medicinal plants which are within reach of many Kenyans such as *Thunbergia alata* and *Senna didymobotrya*.*T. alata* has been evaluated for the antibacterial activity of Chloroformstem extract of *Thunbergia alata* against *Pseudomonas aeruginosa* and significant antibacterial activity of higher concentration of ethanolic leaf extract of *Thunbergia alata* against Salmonella typhi

(Romero et al., 2023). Some Gram positive and Gram-negative bacteria showed significant antibacterial activity, Methanolic extract of flower of *T. alata* showed antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteusmirabilis* and *Streptococcus pyogenes* due to the presence of phenols, alkaloids and flavonoids in the flower extract of that plant (Al-Snai, 2019).

S.didymobotrya is a potential medicinal plant and the medicinal values are explored well in many parts of the world by traditional practitioners (Jeruto et al., 2017). In Kenya, traditionally the Kipsigis community has been using these plants to control malaria as well as diarrheal (Korir et al*.,* 2012). The pastoralists of West Pokot peel the bark, dry the stem and burn it into charcoal that they use to preserve milk (Tabuti, 2007). The plant is also useful for the treatment of fungal, bacterial infections, hypertension, haemorrhoids, sickle cell anaemia, fibroids and backache, to stimulate lactation and to induce uterine contraction and abortion (Tabuti, 2007). According to Reddy et al. (2010), presence of phenolic compounds, flavonoids and carotenoids in the ethyl acetate extract of leaves are responsible for pronounced antibacterial activities *S didymobotrya* produce these chemicals to protect themselves, butrecent research demonstrates that they can protect humans and animals against diseases (Doss & Anand, 2012). A number of phytochemicals are known, some of which include alkaloids, saponins,flavonoids, tannins, glycosides, anthraquinones, steroids and terpenoids.

Many communities have been using medicinal plants to treat different ailments for many years. Plant extracts have been known since ancient times to treat various ailments and are a better choice in the search for curative medicine for ailments

caused by different pathogenic agents (Anand et al., 2022). Therefore, a need to investigate the efficacy of *Thunbergia alata* and *Senna didymobotrya* extracts for the potential treatment of bacteria that cause skin infections. In the work herein, the efficacy of crude extracts of *T.alata* and *S. didymobotrya* as antibacterial agents are evaluated against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* bacteria specimens.

1.3 Statement of the Problem

The challenge of bacterial skin infection is a major health problem globally, regionally, and locally, indeed infections with bacteria have been reported to cause severe skin infections and abscesses (Esposito et al., 2017). Indeed, every human being desires good-looking and healthy skin, but this has not been the case, this has been impeded by the expensive drugs to treat bacterial skin infection and has been worsened by the emergence of multi-drug resistance pathogens of *Staphylococcus*, *Streptococcus* and *Pseudomonads* bacterial species to available conventional drugs. These drugs are expensive and unaffordable to a wide cross-section of rural communities. Therefore, there is a need to explore other remedies including the use of medicinal plants which are within reach of many Kenyans to treat the infection. *S. didymobotrya* and *T. alata* plants grow in the wild in Kericho and are thus readily available and can be explored for their potential as a source of antimicrobial drugs against *S. aureus, S.pyogenes* and *P.aeruginosa.* Moreover, the leaf extracts of the *T.alata* and *S.didymobotrya* have not been tested for treatment of the said bacteria although other plants have been tested for treatment of diseases caused by *S. aureus* and *E. coli* which showed some significance in the treatment of the diseases caused by these pathogenic bacteria (SIDA, 2013).

1.4 General Objective

To determine the efficacy of *Thunbergia alata* and *Senna didymobotrya* extracts against selected bacteria associated with skin infections in Kericho Referral Hospital, Kenya.

1.5 Specific Objectives

i. To extract and profile the phytochemicals present in *T. alata* and *S. didymobotrya* leaves.

ii. To evaluate the antibacterial activity of crude leaf extracts of *T. alata* and *S. didymobotrya* against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* under laboratory conditions.

iii. To determine the Minimum Inhibitory Concentration of crude leaf extracts of *T. alata* and *S. didymobotrya* extracts against *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* bacteria under laboratory conditions.

1.6 Research Questions

i. What are the types of phytochemicals present in *T. alata* and *S. didymobotrya* leaf extracts?Do *S. didymobotrya* and *T. alata* crude leaf extracts inhibit the growth of *S. aureus, S. pyogenes* and *P. aeruginosa*?

ii. What is the Minimum Inhibitory Concentration (MIC)of *T. alata* and *S. didymobotrya* crude leaf extracts against *S. aureus, S. pyogenes* and *P. aeruginosa?*

1.7 Justification

Humans are subjected to bacterial infections that largely contribute to unsustainable socio-economic development. *Senna didymobotrya* and *Thunbergia alata* occur

widely in Kericho and are within reach of many Kenyans living in Kericho County. Confirmation of the potency of otherwise locally available products of *Senna didymobotrya* and *Thunbergia alata* against *S. aureus, S. pyogenes,* and *P. aeruginosa* bacterial infection will be a great relieve to many Kenyans who cannot afford the expensive antimicrobial drugs. Secondly, though *Senna didymobotrya* and *Thunbergia alata* have been used in the treatment of other ailments, information on activity against *S. aureus, S. pyogenes,* and *P. aeruginosa* is scanty. The findings of this study fill this gap in information.

1.8 Significance of the Study

This study has provided more scientific information on the potential use of *T. alata* and *S. didymobotrya* extracts as drugs for managing bacterial infections. The findings of the phytochemicals in *T. alata* and *S. didymobotrya* add to the sum of knowledge already existing on phytochemicals and the biological activity of other medicinal plants. In addition, the research findings here in will enable the promotion and conservation of both *T. alata* and *S. didymobotrya* plants and help the nation's health management team and health care providers in formulating effective policies on the treatment of bacterial infections using extracts from these plants. The study findings also serve as a guide in identifying the active biomolecules that can be used as substrates in formulating inexpensive alternative natural antimicrobial agents. This drives a strong agenda for the conservation of *T. alata* and *S. didymobotrya* plants for the generation of pharmaceutical substrates that could earn income for the locals and enhance the nation's GDP.

1.9 Scope of the Study

This study was limited to identification, extraction, isolation, and screening for antimicrobial activity of leaf extracts of *S. didymobotrya* and *T. alata* leaves, sourced from Kericho and Bomet Counties and used against *S. aureus, S. pyogenes,* and *P. aeruginosa* under laboratory conditions.

1.10 Limitations of the Study

This study was limited to mature *S. didymobotrya* and *T. alata* leaves sampled from Bomet and Kabianga, as well as access to the clinical isolates from Kericho referral hospital.

1.11 Assumption of the Study

It was assumed that mature *S. didymobotrya* and *T. alata* leaves have active phytochemical that are active against *S. aureus, S. pyogenes,* and *P. aeruginosa.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

This chapter covers infections caused by pathogenic bacteria, bacterial resistance, management of bacterial resistance, traditional management of bacterial resistance, conventional management of bacterial resistance the medicinal plants with potential effects on bacterial skin infections, botanical description, traditional uses, and chemical constituent of plant species medicinal plants in Kenya and the bacteria causing skin infections.

2.2 Infections Caused by Pathogenic Bacteria

Skin infections caused by *Staphylococcus aureus* and β-haemolytic streptococci and coryneform are the common bacteria causing skin infections (Del Giudice, 2020).They infect the epidermal layer of the skin, the follicles and penetrate to the deeper layers of skin causing necrotic ulcers (Török & Conlon, 2005).These infections if left untreated may spread throughout the body. Examples include folliculitis, trichomycosis, erysipeloid, impetigo, cellulitis, boils, skin, staphylococcal scalded syndrome, and Lyme disease (Mahajan et al ., 2022). Some secondary infections are impertrigo and athlete foot. Antibiotics are the effective treatment for bacterial skin infections (Pujalte et al., 2023). Dermatitis also known as eczema, identified by rash, itchy skin, redness and swelling. The commonest type of dermatitis is atopic dermatitis (AD). It is a common inflammatory skin disease, characterized by pruritus in infant and hyperkeratosis and lichenification (Sethi, 2021). Itching predominates in AD and *S. aureus* takes over the lesions formed by scratching (Del Giudice, 2020). Erythroderma is an inflammatory dermatitis caused due to

malfunctioning of skin metabolism. This rapidly spreading disease is accompanied with shivering, and pyrexia (Nasir, 2010).

Impetigo most common disease found in children ranging between age 2 to 5 years. The causative agents of the infection are *S. aureus*,β hemolytic streptococci or anaerobic bacteria (Pereira, 2014). A typical form, non-bullous impetigo begins with single red lesion that quickly advances to vesicle, which may further rupture to form dry honey coloured crust (Cole & Gazewood, 2007). Bullous impetigo is *Staphylococcus* induced scaled skin syndrome affecting neonate. This disease affects anogenital areas and buttocks in infants (Van Praag et al., 1997).

Pyodermathese infections are primarily impetigo and secondarily effects of other lesions caused due to invasion of a streptococci or *S. aureus* (Bessa, 2023). Scabies common eco parasitic, directly transmitted infection caused by mites, *Sarcoptesscabei*, that burrow into the skin causing scabies. It is a bacterial infection caused by streptococci. An intensely itchy papule in the webs of fingers, wrists, elbows, and buttocks is typical of scabies (Sharaf, 2024).

Erysipelas in contrast to cellulitis, is superficial infection caused by Group A β-hemolytic streptococci affecting dermis while later affects reticular dermis and subcutaneous fats (Hirschmann & Raugi, 2012). Cellulitis spreads rapidly, and gives painful and warmth feeling in the indurated area. The conditions like ulcers, trauma, eczema etc. damage the protective covering of epidermis and allow bacterial access of group A beta-hemolytic Streptococcus and *S. aureus* to the interior tissue (Bezie et al., 2005). General symptoms of infection involve chills, fever, and malaise. People suffering from systemic lupus erythematosus, hematologic cancer, alcohol abuse, diabetes mellitus, or nephrotic syndrome are more prone to Pneumococcal cellulitis (Chin-Hong, 2016) Folliculitis are the superficial inflammation of hair follicle with

papules or postules at the base. It is differentiated as bacterial and fungal. Bacterial folliculitis mostly appears in men. Pseudo folliculitis occurs when hair shaft pierce in the wall of hair follicle causing skin to appear pigmented (Durdu & Ilkit, 2013).

Rashes are red, inflamed, scaly, or itchy spots on skin are called rash (da Rosa Hoefel et al., 2023). Allergy, irritation, structural defects like malfunctioning oil glands or blocked pores and infection due to any underlying disease are some causes of rash. Examples: dermatitis, eczema, acne, psoriasis, hives, and pityriasis rosea (Lyons & Ousley, 2014). Skin abscess (boil or furuncle)are the collection of pus under skin due to localized skin infection is known as boil (Sukumaran & Senanayake, 2016). Sometimes abscesses may need draining by a doctor to get cured. Furuncles is an infection of hair follicle progressing to the adjoining subcutaneous tissue forming abscess generally caused by *S. aureus* or MRSA (Bessa, 2023). *Pseudomonas aeruginosa* has a variety of virulence factors that contribute to its pathogenicity which include production of exotoxins, proteolytic enzymes and haemolysins that destroy cells and tissue (Hoge et al., 2010). Some strains produce alginate, a polysaccharide polymer that inhibits phagocytosis.

P. aeruginosa remains an opportunistic pathogen that requires a compromised host to establish an infection. The organism either disrupts or takes advantage of loss of protection offered by intact epidermis in cases like of burns, puncture wounds, eye trauma (Das et al., 2020). Other infections include infections of the respiratory tract (hospital acquired pneumonia in patients in respirators), urinary tract, wounds, blood, central nervous system, burns, ear and cystic fibrosis (Moore et al., 2016). The organism has been incriminated as a leading cause of wound infections. A wound is a break in the skin which can either be acute or chronic. An acute wound is caused by

damage to the skin which can be accidental or surgical (Degreef, 1998). A chronic wound is one that does not heal in the normal manner and duration mostly due to underlying pathological cause for example diabetic wounds or bed sores (FrykbergRobert, 2015). In compromised patients such infections are often severe and often life-threatening. These infections have high mortality which is associated with ineffective empirical therapy (Giamarellou, 1995). Patients with infected non-healing wounds pose a huge problem for hospitals at times taking up to 50% of medical ward beds and strain already overstretched facilities. The effective treatment of these wound and skin infections is hampered in a great way by presence of multiple drug resistant bacteria (Kaiser et al., 2021).

2.3 Antimicrobial Resistance

Antimicrobial resistance happens when germs like bacteria and fungi develop the ability to defeat the drugs designed to kill them. That means the germs are not killed and continue to grow making treatment for such infections difficult and sometimes impossible (Levy, 2013). Antimicrobial resistance is an urgent global public health threat, killing at least 1.27 million people worldwide and associated with nearly 5 million deaths in 2019 (Stoneham et al., 2021). A recent study in Kenya found that 7–17% of hospitalized patients had infections caused by *S. aureus, S. pyogenes,* and *P. aeruginosa* bacteria, with 39% of isolates meeting the “difficult-to-treat resistance” definition (Sohaili et al., 2024).

Antimicrobial resistance has the potential to affect people at any stage of life, as well as the healthcare, veterinary, and agriculture industries. This makes it one of the world’s most urgent public health problems (Salam et al., 2023). Bacteria do not have to be resistant to every antibiotic to be dangerous. Resistance to even one antibiotic

can mean serious problems. For example, Antimicrobial-resistant infections that require the use of second- and third-line treatments can harm patients by causing serious side effects, such as organ failure, and prolonged care and recovery, sometimes for months (Ahmad et al., 2021).

Many medical advances are dependent on the ability to fight infections using antibiotics, including joint replacements, organ transplants, cancer therapy, and the treatment of chronic diseases like diabetes, asthma, and rheumatoid arthritis. In some cases, these infections have no treatment options other than plant remedies. If antibiotics lose their effectiveness, then we lose the ability to treat infections using conventional drugs and in such a case herbal medicine becomes a choice (Abebe & Birhanu, 2023). The search for novel antibacterial treatments has led researchers to explore a wide range of natural sources, including plants from diverse ecosystems, to uncover novel microbial resistance-fighting compounds (Muteeb, 2023). Among the ecosystems that have amassed significant attention are arid and rainforest environments, despite their extreme differences in climate and biodiversity, each offers unique potential for the discovery of antibacterial compounds.

2.3.1 Management of antibiotic resistance

Antibiotics are used against bacterial infections, for they are used to treat strep throat, which is caused by streptococcal bacteria, and skin infections caused by staphylococcal bacteria (Stoneham et al., 2021). Although antibiotics kill bacteria, they are not effective against viruses. Therefore, they will not be effective against viral infections such as colds, coughs, many types of sore throat, and influenza (flu). Using antibiotics against viral infections will not cure the infections or keep other individuals from catching the virus. Antibiotics should only be used for specific

ailments to avoid unnecessary, harmful side effects which may contribute to the development of antibiotic-resistant bacteria (Muteeb et al., 2023).

Patients and health care professionals alike can play an important role in combating antibiotic resistance. Health care professionals should prescribe antibiotics only for infections they believe to be caused by bacteria. There are ways in which bacteria become resistant to antibiotics among them is not taking the antibiotics as prescribed, medication should not be stopped when the dose is halfway just because one is feeling better (Landas, 2020). It is important to take the medication as prescribed by the doctor, even if one feels better. If treatment stops too soon, and an individual becomes sick again, the remaining bacteria may become resistant to the antibiotic that has been taken (Landas, 2020). Antibiotics are most effective when they are taken as prescribed, skipped doses can make the antibiotic-resistant to the pathogen (Khaled et al., 2024). All drugs have side effects therefore one should inform the health care professionals of unusual symptoms or side effects, in case of side effects a drug is discontinued, and an alternative antibiotic is used to complete treatment(Hamilton et al., 2020). This way antimicrobial resistance can be controlled.

2.3.2 Traditional management of bacterial resistance

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years in many parts of the world (El Sheikha, 2017). In many rural areas of developing countries, they continue to be used as the primary source of medicine. About 80% of the people in developing countries use traditional medicines for their health care (Payyappallimana, 2010). The natural products derived from medicinal plants have proven to be an abundant source of biologically active

compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals (Sen & Samanta, 2015).

With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-infective compounds. As there are approximately 500,000 plant species occurring worldwide, of which only 1% has been phytochemically investigated, there is great potential for discovering novel bioactive compounds. There have been numerous reports of the use of traditional plants and natural products for the treatment of oral diseases (Arumugam et al., 2020) as well. Many plant-derived medicines used in traditional medicinal systems have been recorded in pharmacopoeia as agents used to treat infections and a number of these have been recently investigated for their efficacy against oral microbial pathogens. The general antimicrobial activities of medicinal plants and plant products, such as essential oils, have been reviewed previously (Vaou et al., 2021).

2.3.3 Conventional management of antibiotic resistance

Antimicrobial agents are possibly the most successful drugs deployed in the 20th century. These drugs are indispensable in many medical treatments such as intensive care, chemotherapy, organ transplantation, care of preterm babies, and surgical procedures, which could not be performed effectively without the availability of effective antibiotics. Their use reduces human mortality and morbidity (Abdul-Aziz et al., 2020).

Today different classes of antibiotics with distinct modes of action are available to fight diverse micro-organisms. However, the number of resistant microorganisms, the

geographic locations affected by drug resistance, and the breadth of resistance in single organisms are increasing globally (Uddin et al., 2021). The continued evolution and spread of multiple-antibiotic resistance in human pathogens is an alarming clinical challenge (Church & McKillip, 2021). It is reported that, around 90 % –95 % of *S. aureus* strains worldwide are resistant to penicillin and, in most Asian countries 70%–80% are also methicillin-resistant (Turner et al., 2019). Also, Gram-negative bacteria such as Pseudomonas, Acinetobacter, Escherichia, and Enterobacter spp. are rapidly becoming very problematic due to their nosocomial status and expression of MDR phenotypes, which makes the treatment of the infections difficult (Di Franco et al., 2021). According to (Fletcher, 2015) the rise in the frequency of resistance among human pathogenic bacteria is a complex problem driven by many interconnected factors, in particular the extensive use of antibiotics in both human and veterinary medicine, aquaculture and agriculture. Even more worrying is the fact that bacteria can develop resistance to multiple classes of antibiotics simultaneously (Alanis, 2005). In addition, genetic resistance determinants were also detected in members of microbial communities from natural environments, raising concern about the risk that those antibiotic resistance reservoirs might pose to human and ecological health (Ahmad et al., 2021).

The increase in the frequency of MDR bacteria and the subsequent absence of access to effective antimicrobial agents represent one of the most threatening health care problems with worldwide concern (Pulingam et al., 2022). Understanding the mechanisms by which bacteria defend themselves against antimicrobial agents is essential to avert this public health threat. According to (Sultan et al., 2018), many factors contribute to the emergence of resistant phenotypes to antimicrobial agents, including the degree of the expression of resistance determinants, the capacity of the

microorganisms to maintain resistance mechanisms, the capacity of transmission, the bacterial fitness, and the potential for reversibility.

2.4 Medicinal Plant

Herbal medicine is any preparation containing one or more active herbal substances or extractives (Folashade et al., 2012). Medicinal plants are plants whose (leaves, seeds, stem, and roots) extract infusions; decoctions and powder are used in the treatment of different diseases of humans, plants, and animals (Titanji et al., 2008). About 20,000 plant species are used for medicinal purposes as reported by WHO (Fan et al., 2012).

According to WHO, traditional medicine is one of the ways to achieve total healthcare coverage for the world's population (Bodeker & Ong, 2005). Medicinal plants are used for primary health care by 70 % - 90 % of populations in developing countries (Tangjang et al., 2011). For example, Rubiacea is used to manage venereal diseases and constipation in Sierra Leone (Lifongo et al., 2014). The root infusion of *N. latifolia* is used for the treatment of gonorrhoea in Sudan and the roots are used as chewing sticks to manage toothache and dental carries (Me et al., 2016). In other countries like Ghana, the roots and leaves are used for managing stomachache and sores while in Nigeria, the fruits are used for treating piles and dysentery (Muhammad & Amusa, 2005). Medicinal plants can also cure deadly diseases such as AIDs and cancer that have resisted conventional drugs (Gurib-Fakim, 2006).

Skin diseases, diarrhoea, malaria, respiratory infections, and bacterial and fungal infections are the most common health problems in rural areas (Gracey & King, 2009). In resource-limited countries, a lot of medicinal plants are used traditionally for the treatment of these diseases (Greenwell & Rahman, 2015). Drug inventions from ethno-pharmacology and natural products are still an important exception in

uplifting the deprived livelihood of rural communities (Gupta, et al., 2014) especially those afflicted with contagious diseases like bacterial skin infections and dermatophytes.

2.4.1 Medicinal Plants in Kenya

In Kenya traditional medicine is used in the treatment of diseases caused by bacterial and fungal pathogens (Kokwaro, 2009). Not only is the usage extended to rural areas but also in the urban regions as demonstrated by an increased number of traditional practitioners all over the country (Orwa, et al., 2009). Since there is a lot of inequality in healthcare service provision, those who live in urban areas enjoy easy access to health facilities as compared to their rural counterparts, who live miles away from the medical facilities (Nyatepe, 2014). In Kenya many communities rely on traditional medicine, for instance in the Eastern province, Embu and Mbeere Districts, the residents use a diversity of herbal medicinal plants, to treat several diseases (Kareru et al., 2008).

The Kenyan pastoralists of Samburu have also kept their knowledge of the use of local plants for a diversity of purposes (Bussmann, 2006). The Samburu still depends on a diet of milk, blood from animals and soups made from natural herbs, berries, and other wild fruits. This culture has made herbal knowledge remain widespread in the community (Bussman, 2006). The Nandi community also relies on medicinal plants to treat various human and livestock diseases (Kigen, et al., 2019). Some plants extracted for medicinal use in Kenya include; *Dovyalis abyssinica* for respiratory disorders, *Warbugia ugandensis* for toothache, *Olea africana* for eye ailment and *Ximenia americana* oil for wound treatment. Medicinal plants are known to older people above 50 years old as compared to young literate people (Hu et al., 2020). The

use of plants of medicinal importance by the literates in the community can be attributed to a lack of general preparation procedures and scientific information on their efficiency as well as their toxicity levels (Mbuni et al., 2020). Additionally, the collection as well as storage methods were identified as essential considerations by the literate members of the community. This indicates that there exists a generational disconnection in the passage of traditional medicinal plant knowledge.

Most medicinal plants have a higher concentration of secondary metabolites and are readily available in all seasons of the year (Abdallah et al., 2023). Leaves are also highly utilized because they are obtained easily in large quantities in contrast to other plant parts and can regrow once plucked. Moreover, most traditional healers prefer to use leaves as they are considered to accumulate active ingredients through photosynthetic pigments such as alkaloids and tannins (Rashid et al., 2021). A myriad of methods of preparation are used in most communities in Kenya, decoction was the most widely used method of preparation mainly because of the ease of using water to prepare them (Rankoana, 2022). Such a large variety of preparation methods that have been studied has been highlighted in some parts of Kenya and other countries. It has been established that more than one method is used in preparing many of the medicinal plants studied. However, the type of plant species, condition of ailment being treated, and plant parts used determined the method of preparation (Amjad et al., 2020). Among the Marakwet, Luhya, and Pokot communities, the common way of administration of the prepared medicine was through drinking, which is in line with many other studies.

Gastrointestinal ailments were the most frequently treated using medicinal plants followed by sensory-neuron diseases. In the same way, disorders of the

gastrointestinal system and parasitic infections were the commonly treated ailments. Stomach-ache and diseases related to the digestive system could be attributed to poor sanitation because of high levels of poverty within most communities as indicated by other studies. Most communities in Kenya use some plant species to treat certain ailments due to ease of accessibility and their effectiveness in treating the diseases (Maiyo et al., 2024). The medicinal plants contain important bioactive constituents including glycosides, acids, saponins, tannins, terpenoids, and alkaloids which have medicinal value. *Carissa spinarum L*. is mainly used in treating malaria, chest pains, epilepsy, diarrhoea, coughs, breast cancer, arthritis, and gonorrhoea (Maobe, 2014). Different ecological climatic conditions have been characterized by different plant diversity, hence pointing to some of the probable reasons for similarities and differences in plants of medicinal value found in most communities (Mbuni et al., 2020). Preparation methods of herbal medicine and their use have been documented in other regions. However, 82% of medicinal applications are new and unique to different users (Nadaf et al., 2023).

2.5 *Thunbergia alata* (Black-eyed Susan Vine) Description

Thunbergia alata is a soft, perennial evergreen plant from the Acanthus family (Acanthaceae). The leaves are heart- or arrow-shaped, softly hairy and sometimes toothed. Many flowers are borne singly in leaf axils with a small calyx enclosed in 2 large, ridged bracts. The corolla is usually bright orange in wild plants. The inside of the tube is a striking dark maroon or purplish black. The fruit is like a bird's head with a spherical base and a long 'beak (Sultana, et al, 2015). The plant species of *Thunbergia alata* (Acanthaceae), is known as black-eyed Susan and in Colombia called “ojo de poeta”.

2.5.1 Distribution and Habitat

Thunbergia alata is found in tropical regions of Africa, in Kenya, mostly in Nandi County, Uasin-Gishu, South Rift and Central Kenya. It is also found in other parts of Africa notably, parts of Swaziland, South Africa, and Uganda. In Africa, it is usually found on forest margins, but it can occur in dryer, open areas. It is sometimes seen along roads near settlements; it can survive in environments with full sun or light shade. It is half-hardy, needing warm conditions but, with protection, the young plants can survive some frost in cold areas (Quijano-Abril et al., 2021).

2.5.2 Uses of *Thunbergia alata*

The plant is used mainly as an ornamental plant since it grows and flowers relatively quickly. It makes a good screen when used to cover unsightly dead trees or walls. It needs some support, as it cannot cling. It is used as a vegetable or stock feed. Medically it is used for skin problems, cellulitis, back and joint pains, eye inflammation, piles, rectal cancer, gall sickness and ear problems (Toyang & Verpoorte, 2013). Its chloroform stem extracts have been evaluated and found to possess antibacterial activity against *Pseudomonas aeruginosa.* It was also found that a higher concentration of its ethanolic leaf extract had significant antibacterial activity against *Salmonella typhi*. It is also reported that it has been evaluated for its antifungal, antiviral, and antitumor properties. In addition, Black-eyed Susan is traditionally used within Kalenjin, Luhya and Samburu communities to treat inflammation, fever, and malaria. *T. alata* is a plant species native to East Tropical Africa, widely cultivated and naturalized in tropical regions (Romero et al., 2023).

Figure 2. 1 Image of *T. alata* plant

2.6 *Senna didymobotrya*

It is a flowering plant in the legume family, belonging to the Fabaceae family, an evergreen aromatic large shrub or small tree. Stems spreading and much branched, covered with fine hairs, flowers and cut stems smell of peanut butter although some maintain the smell of mice. It invades roadsides, wastelands, urban areas, open spaces, grasslands, savannahs, woodlands and riparian vegetation. It can form large dense mono-specific stands, displacing mature plant species and inhibiting wildlife movement. Its leaves are toxic and have been reported to cause numerous livestock deaths (Weldemariam & Dejene, 2021)

2.6.1 Origin and geographical distribution

Senna didymobotrya is native to tropical Africa where it is found from Congo East to Ethiopia and South to Namibia, Zimbabwe, and Mozambique. It has been introduced as an ornamental plant in many tropical countries including the Comoros, Madagascar, Mauritius, and South Africa. It was originally introduced into tropical Asia and America as a fodder, green manure, and cover crop, but is now mainly cultivated as an ornamental (Weldemariam & Dejene, 2021).

2.6.2 Description of *Senna didymobotrya*

This is a deciduous shrub that grows up to 4.5 to 9 m tall. Its leaves are arranged spirally with 8 to 18 pairs of leaflets; stipules are broad and oval, petiole 1-8 cm long, mostly rounded at the apex, and hairy on both sides. The inflorescence is bisexual with sepals upto1.5 cm long, petals are not equal but yellow. The two lower stamens are large, ovary superior and woolly. The style is slender and bent. The fruit is flattened, the pod is 8-12cm x1.5-2.5 cm, transversely partitioned and dehiscent by 2 valves with 9-19 seeds. The seeds are compressed with a distinct areole on each face (Dirr, 1990).

2.6.3 Ecology of *Senna didymobotrya*

The wide variety of species and ecological adaptations makes at least a handful of Sennas suitable for any climate (Jeruto, et al., 2017) *S. didymobotrya* is common in deciduous bush land, along lake shores, streams, rivers and other damp localities, in grassland and woodland, from sea level up to 2500 m altitude (Igunza et al., 2019). At times it is found in old plantations and hedges near buildings. In its natural habitat, *S. didymobotrya* often grows in areas where human activities have taken place, in riparian, grassland and evergreen bush land and it tolerates light frost (Sievers, 2013). The plant is exotic in India, Indonesia, Malaysia, and Sri Lanka. *S. didymobotrya* has been naturalized in Australia and parts of America (Jeruto et al., 2017). It is common in undisturbed areas grows rapidly and is widely distributed in the tropical and subtropical regions (Upadhaya et al., 2004).

2.6.4 Uses of *Senna didymobotrya*

It is widely used as a medicinal plant, especially in East Africa, where a decoction or infusion from the leaves, stems and roots is drunk as a laxative and purgative for the

treatment of abdominal pains. It is also taken to expel intestinal worms and to treat ringworms (Webster et al., 2008). The decoction of *S. didymobotrya* is drunk for the treatment of malaria, other fevers, and jaundice. Other plant parts of *S. didymobotrya* have been used to treat different ailments, roots, stems and leaves have been used for treatment as shown in table 1 below.

Table 2. 1

Uses of S.didymobotrya

Decoction	Uses	References
Roots	Antidote for Poisoning Anti-Malaria Expelling Retained Placenta Jaundice Fever Backache Venereal Diseases	(Ogunniyi et al., 2023)
Leaf	Laxative Abdominal Pain Anti-emetic Removal of Ticks in Livestock Bacterial Diseases Fibroids Diarrhea	(Osunga et al., 2023)
Stem	Dried burned for milk preservation	(Sadia et al., 2022)

The plant *S.didymobotrya* and its features are shown in Figure 2.2 below.

Figure 2. 2 Image of S. *didymobotrya* Plant

2.7 Phytochemicals from Different Plants

Phytochemicals are bioactive molecules of plant origin. They are regarded as secondary metabolites (SMs) because the plants that manufacture them may have little need for them (Mendoza & Silva, 2018). They are naturally synthesized from all parts of the plant body bark, steam, leaves, flowers, fruit, and seeds contain bioactive components (Jain et al., 2019).

Medicinal plants are the most important component of the diet for good health. As the plant is the source of medicine, plays an important role in the health services globally (Sofowora et al., 2013). The plants are consumed both by animals and human beings as food. Therefore, the bioactive component in the plant is found in the system of human beings and animals and thus cures some diseases by default. Most people, living in rural areas depend largely on herbal remedies for the treatment of different types of ailments (Kigen et al*.,* 2016). Medicinal plants are of great interest to researchers in the field of life science especially biotechnology where most pharmaceutical industries depend on plant parts for the production of pharmaceutical drugs (Atanasov et al*.,* 2015). It has been reported that aqueous and methanolic extracts from plants used in allopathic medicines were potential sources of antiviral, antitumor and antimicrobial agents (Garg & Roy, 2020).

2.7.1 Types of phytochemicals

2.7.1.1 Flavonoids

Flavonoids are responsible for most pigments that colour flowers, fruits, and seeds (Falcone-Ferreyra et al., 2012). These secondary metabolites are widely spread in plants and are classified into subclasses: chalcones, flavones, isoflavones, flavanone, flavanols, flavandiols anthocyanins, and proanthocyanidins or condensed tannins. The

different flavonoids have different biological functions. Among them is protection against ultraviolet (UV) radiation and phytopathogens, signalling during nodulation, auxin transport, as well as the colouration of flowers as a visual signal that attracts pollinators (Salinitro et al., 2017).

Flavonoids are also responsible for the display of colour in many plants, which may protect leaf cells from photo-oxidative damage, enhancing the efficiency of nutrient retrieval during senescence (Winkel-Shirley, 2002). Flavonoids, a major class of polyphenols, widely present in the plant kingdom, represent a large group of PSMs which are either produced constitutively or induced by environmental stresses (Laoué et al., 2022). A variety of derivatives of the initial phenyl propanoid serve roles in plant structural integrity, UV photo protection, reproduction, and internal regulation of plant cell physiology and signalling. Phenylpropanoids also act as key chemical modulators of plant communication with insects and microbes. They are either attractants or repellents, as phytoalexins against pathogens and herbivores. They are attractants to pollinators through flower colour. They also induce root nodulation when excreted by symbiotic nitrogen-fixing rhizobia (Tak & Kumar, 2020).

Flavonoids play a role in protecting humans against diseases. Flavones and catechin are the most powerful flavonoids for protecting the human body against the ill-effects of oxidative stress. (Sen & Chakraborty, 2011). Quercetin, Morin, and myricetin exhibit protective effects in preventing cancer, liver, and cardiovascular diseases e.g. atherosclerosis, coronary heart disease arterial hypertension and heart failure. Therefore, flavonoids as antioxidants reduce oxidative stress and thus lead to beneficial health effects (Perez-Vizcaino & Duarte, 2010). Flavonoids also help fight pathogenic micro-organisms that enter the body system and interfere with the

functioning of the human body (Santhiravel et al., 2022). Bacterial and viral infections are prevented due to the strong antibacterial and antiviral activities of flavonoids. They also exhibit powerful anticancer activity making them a strong phytochemical to produce cell cycle inhibition in proliferating lymphoid cells and exert growth-inhibiting effects on several malignant tumour cells. Flavonoids have a wide range of health-promoting benefits that contribute to their important biological, anti-oxidative and pharmacological activities (Shen et al., 2022).

2.7.1.2 Alkaloids

These are natural products that contain heterocyclic nitrogen atoms and are always basic. The name of alkaloids derives from their alkaline nature, and it was used to describe any nitrogen-containing base (Roy, 2017). Almost all the alkaloids have a bitter taste. The alkaloid quinine for example is one of the bitter-tasting substances. Alkaloids are significant for the survival of plants because they ensure protection against micro-organisms (bacterial and fungal activities). Insects and herbivores are deterrents against other plants by means of allelopathy (Doughari, 2015). The use of alkaloids containing plants as dyes, spices drugs or poisons can be traced back to almost the beginning of civilization. Alkaloids have many pharmacological activities including anti-hypertensive anti-arrhythmic effects antimalarial and anti-cancer actions, and anti-arrhythmic effects. Some alkaloids have stimulant properties such as caffeine and nicotine, morphine is used as an analgesic and quinine as the antimalarial drug (Abdullahi & Hamza, 2020).

2.7.1.3 Tannins

Tannins are plant-based poly phenols which are astringent and are found in different parts of the herbs and plants that are consumed as food and feed. They are categorized

into two groups: condensed tannins (non-hydrolyzable) and hydrolysable tannins. Condensed tannins are the most commonly occurring ones that are generally found in stems, legumes, trees, and forages. On the other hand, hydrolyzable tannins are found in seed pods, bark, wood, leaves, and fruits (Sharma et al., 2021). Tannins have beneficial effects, the beverages containing tannins like tea, wines and beer are more popular among adults while products with tannins such as chocolates, and ice creams are common among children. Tannins in these products are beneficial in one or the other form and provide relief from various types of ailments such as reducing the risk of diabetes by enhancing glucose uptake and thus lowering blood sugar levels (Sharma et al., 2021).

Diluted tannin solution is applied over an open wound as it precipitates the protein of the wound, thereby making a protective covering and preventing bleeding to aid in faster healing (Shirmo-hammadli et al., 2018). Condensed tannins are effective against various types of allergies such as asthma, hypersensitive pneumonitis, allergic rhinitis, mite allergens, carpet dust and many more (Cipriani et al., 2017). Tannins possess some of the biological properties such as anti-inflammatory, anti-cancerous, anti-allergic (Choudhury et al., 2019), anthelmintic, and antimicrobial (A. P. Singh & Kumar, 2020). Antiviral against enteric virus, herpes simplex virus, polio virus (Behl et al*.,* 2021). Medicinally, these are employed as anti-hemorrhoid, anti-diarrheal and for treatment of hemostatic. Tannin's ability to form a protective covering and helps the tissue from getting infected and is thus used to get immediate relief during skin ulcers, dysentery, soaring of throats, diarrhoea, haemorrhaging and fatigue (Sarwar & Ahmed, 2022) Besides, tannin also acts as a precipitating agent (especially in liquor industries) and has good effects on vascular health (Sharma et al., 2021)

2.7.1.4 Terpenoids

Terpenoids are the most abundant compounds in natural products; they are a set of important secondary metabolites in plants with diverse structures. Terpenoids play important roles in plant growth and development, response to the environment, and physiological processes (Yang et al*.,* 2020). As raw materials, Terpenoids are used in pharmaceuticals, food, and cosmetics industries (Tetali, 2019). Terpenoids possess antitumor, anti-inflammatory, antibacterial, antiviral, and antimalarial effects, promote transdermal absorption, prevent, and treat cardiovascular diseases, and have hypoglycaemic activities (Yang et al*.,* 2020). In addition, previous studies have also found that Terpenoids have many potential applications, such as insect resistance, immunoregulation, anti-oxidation, antiaging, and neuroprotection (Jibira & Atawuchugi, 2021).

2.7.1.5 Saponins

These phytochemicals help to keep plants healthy, and they can do the same for human beings. These compounds give a permanent froth when shaken with water (Singh & Kumar, 2017). They also cause haemolysis of cells. Saponins are made by plants as a defence mechanism to protect themselves against infections and pests (Tiku, 2020). Traditionally, Saponins have been used as natural detergents. Saponins also have anti-fungal, antiviral, and antibacterial activity, their medicinal value is due to their expectorant effect.

2.7.1.6 Glycosides

Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical

available for use (Imohiosen et al., 2014). Many such plant glycosides are used as medications. Some glycosides contain nitrogen and sulphur water-soluble phyto-constituents found in the cell sap (Ajobiewe et al., 2020). Chemically, glycosides contain a carbohydrate (glucose) and no glucose carbohydrate part (aglycone or genin) phenols or glycerol represents aglycone (Ajobiewe et al., 2020).

2.8 Bacterial Pathogens

2.8.1 *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive coccus about 1µm in diameter. The cocci are mainly arranged in grape-like clusters, and they are pervasive, they are resistant to dry conditions and high salt concentrations (Schleifer & Bell, 2015). It is one of the main pathogens which can be acquired easily in hospitals and the environment. *Staphylococcus aureus* was first discovered in 1880 in Aberdeen, Scotland, by surgeon Alexander Ogston from patients with ulcerated sores (Ogston, 1984). *Staphylococcus aureus* belongs to the genus Staphylococcus, Vermiculites; is positive for Gram stain, an aerobic or anaerobic; and grows optimally at 37 °C and pH 7.4 (Guo et al., 2020). The colonies on the blood agar plate are thick, shiny and round with a diameter of 1-2 mm (Yuan et al., 2020). The organism is often hemolytic in blood agar due to the production of four types of hemolysins (alpha, beta, gamma, and delta). Nearly all isolates of *S. aureus* produce coagulase enzyme, a virulence factor that also helps in the identification of the organism (Balachander & Alexander, 2021) *S. aureus* does not form spores or flagella, but possesses a capsule, can produce golden yellow pigment, and decompose Mannitol (Guo et al., 2020).

Additionally, it has also been found that tests of plasma coagulase, lactose fermentation and deoxyribonuclease are positive in *S. aureus* (Kateete et al., 2010). It

has been reported that *S. aureus* can survive in an inanimate environment existing as a colonizer and may form biofilms (Stefani & Goglio, 2010). It is found in the nose of 30% of healthy individuals and may be found on the skin. It causes infection mostly at the site of lowered host resistance like the damaged skin or mucous membrane (Grice & Segre, 2011). For decades, *S. aureus* has been predominately a nosocomial pathogen and is a leading cause of mortality and morbidity in hospitals (Gnanamani et al., 2017). However, in the community, *S. aureus* infections are on the rise. The important clinical *S. aureus* infections are bacteremia, infective endocarditis, skin, and soft tissue infections, osteoarticular infections and cardiopulmonary infections (Giormezis et al., 2021). *S. aureus* possesses a battery of virulence factors. These factors enable the organism to be successful as a pathogen to cause a wide range of human and animal infections. Virulence factors help in attachment to host cells, breaking down the host immune shield, tissue invasion, causing sepsis and eliciting toxin-mediated syndrome (Gnanamani et al., 2017).

2.8.2 *Streptococcus pyogenes*

Pathogenic streptococci, commonly called *strep*, are a heterogeneous group of gram-positive bacteria, non-motile, non-spore-forming coccus that tend to grow in chains (Lemos et al., 2013). It produces a large variety of extracellular enzymes and toxins and has β-hemolytic properties. In this group, *Streptococcus pyogenes* (group A β-hemolytic *Streptococci*) is one of the most important bacterial pathogens (Hughes et al., 2009). The different serotypes of group A *Streptococci* (GAS) produce extracellular enzymes that break down host molecules. streptokinase, enzymes that activate a host blood factor that dissolves blood clots, the cytolysins streptolysin O and streptolysin. Both kill host leukocytes and capsules of M protein, which help to retard phagocytosis (Persson, 2019). *S. pyogenes* is widely distributed among humans,

some become asymptomatic carriers. *Streptococcus* species are associated with many bacterial diseases in both humans and animals. Arthritis, neonatal sepsis, meningitis, and pneumonia are some examples of diseases in humans.

In animals, they mainly cause mastitis (Fiedler et al., 2015). *S. pyogenes* is capable of infecting humans, mainly through adhesion and colonization of the host mucosal surface epithelial cells of the upper respiratory tract (Franklin et al., 2013). The pathogenicity of *S. pyogenes* is apparent in some different mild infectious diseases, such as mild pharyngitis (strep throat), scarlet fever (rash), impetigo (infection of the superficial layers of the skin) or cellulitis (infection of the deep layers of the skin) (Leung et al*.,* 2018). Invasive, toxigenic infections can result in life-threatening infections such as necrotizing fasciitis, myositis, and streptococcal toxic shock syndrome (Morgan, 2011). Patients may also develop immune-mediated post-streptococcal sequelae, such as acute rheumatic fever and acute glomerulonephritis, following acute infections caused by *Streptococcus pyogenes* as reported in Hon Kong (Drăghici & Csep, 2013).

2.8.3 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative bacillus, non-sporing, non-capsulated and usually motile by one or two polar flagella (Lim et al*.,* 2018). It is a strict aerobe but can grow anaerobically in the presence of nitrate. *P. aeruginosa* can infect almost any external site or organ. Most community infections are mild as opposed to hospital patient infections. *P. aeruginosa* rarely causes disease in healthy humans. It is usually linked with patients whose immune system is compromised by diseases or trauma (Al-Araji & Ali, 2012). It gains access to these patients' tissues through the burns, for the burn victims, or through an underlying disease, like cystic fibrosis. First, *P. aeruginosa*

adheres to tissue surfaces using its flagellum, pili and exo-S. It replicates to create infectious critical mass; and lastly, it causes tissue damage using its virulence factors (Al-Araji & Ali, 2012). Since the powerful exotoxins and endotoxins released by *P. aeruginosa* during bacteremia continue to infect the host even after *P. aeruginosa* has been killed off by antibiotics, acute diseases caused by *P. aeruginosa* tend to be chronic and life-threatening (Malhotra et al*.,* 2019).

Furthermore, except for the cystic fibrosis strain, most *P. aeruginosa* strains that attack compromised patients tend to be non-mucoid (Malhotra, 2018). Although a small number of patients infected with *P. aeruginosa* developed severe sepsis with lesions with black centres. Most patients exhibited no obvious pathological effects of the colonization*. P. aeruginosa* is known as one of the most life-threatening bacteria and is noted as a priority pathogen for the Research and Development of new antibiotics by the World Health Organization (Breijyeh et al., 2020). Common antimicrobial agents like antibiotics frequently exhibit limited efficacy due to adaptability and high intrinsic antibiotics (Gellatly & Hancock, 2013).

2.9 Biochemical Test for Identification of Bacteria

Bacteria are classified and identified to distinguish one organism from another and to group similar organisms by criteria of interest to microbiologists or other scientists. Identification is the practical use of classification criteria to distinguish certain organisms from others, to verify the authenticity or utility of a strain or a particular reaction, or to isolate and identify the organism that causes a disease. Different metabolic activities displayed by different species of bacteria are employed for biochemical identification. Several biochemical tests used for identification are discussed below.

2.9.1 Catalase test

The catalase test is used to differentiate those bacteria that produce enzyme catalase such as *staphylococci,* from non-catalase-producing bacteria such as *streptococci.* Catalase acts as a catalyst in the breakdown of hydrogen peroxide into oxygen and water (Christopher et al., 2020). To find out if a particular bacterial isolate can produce catalase enzyme, a small portion of the isolate is mixed into hydrogen peroxide solution. Bubbles of oxygen are released if the organism is a catalase producer. The lack of catalase is evident in the lack of bubble production. The morphologically similar enterococcus or streptococcus catalase negative and Staphylococcus catalase positive can be differentiated using the catalase test (Fayisa & Tuli, 2023).

2.9.2 Citrate test

Citrate agar is used to test the organism's ability to utilize citrate as a source of energy. Simmons's citrate media contain citrate as a sole source of carbon and inorganic ammonium salts as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme citrate perm-ease capable of converting citrate to pyruvate (Sivaramakrishnan & Razia, 2021). Pyruvate can then enter organisms' metabolic cycle to produce energy. When bacteria metabolize citrate, the ammonium salts are broken down to ammonia which increases alkalinity. The shift in pH turns the bromothymol blue indicator in the media from green to blue in a positive test and retains the blue colour in a negative test (Pervin et al., 2019).

2.9.3 Tripple sugar iron test (TSI)

Tripple sugar iron test is a microbiological test named for its ability to test micro-organism's ability to ferment sugars and produce hydrogen sulphide. Most of the

bacteria can ferment particular sugars (GES, 2000). The sugars that bacteria can ferment and those that they cannot ferment are the characteristics of a bacteria and thus an important criterion for its identification (Lanyi, 1988). An agar slant of a special media with multiple sugars constituting a pH-sensitive dye (phenol red), 1%sucrose, 1%lactose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulfate, is used in carrying out this test. The media is prepared and allowed to solidify at an angle result in an agar slant test tube at a slanted angle (Some et al*.,* 2021). The TSI is designed to differentiate among the micro-organisms based on the differences in carbohydrate fermentation patterns and hydrogen sulphide production. Carbohydrate fermentation is indicated by gas production and a change in the colour of the pH indicator from red to yellow (Parija, 2023).

2.9.4 Oxidase test

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme. The oxidase test detects the presence of a cytochrome oxidase system that will catalyze the transport of electrons between electron donors in bacteria and a redox dye tetramethyl-p-phenylene-diamine (Lal et al., 2019). The dye is reduced to a deep purple colour. This test is used to assist in the identification of *Pseudomonas, Neisseria, Alcaligen, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella* all of which produce the enzyme cytochrome oxidase (Mishra & Agrawal, 2012).

2.9.5 Mannitol salt agar (MSA)

Mannitol salt agar (MSA) is a selective and differential media (Shields & Tsang, 2006). The high concentration of salt (7.5%) is selected for members of genius *Staphylococcus* since they can tolerate high saline levels. Organisms from other

genera may grow but they typically grow very weakly. MSA also contain the sugar mannitol and a pH indicator phenol red. If an organism can ferment mannitol, an acidic by-product will be formed which will cause the phenol red indicator in the media to turn yellow (Petersen & McLaughlin, 2016). Most pathogenic *Staphylococcus*, such as *Staphylococcus aureus*, will ferment Mannitol. While the non-pathogenic *Staphylococcus* will not ferment Mannitol (Lu et al*.,* 2018).

2.10 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that inhibits the growth of bacterial strains (Kowalska-Krochmal & Dudek-Wicher, 2021). MIC depends on the micro-organism, the affected human being (in vivo only), and the antibiotic itself. It is usually expressed in micrograms per millilitre (μg/ml) or milligrams per litre (mg/L). The MIC is determined by preparing solutions of the chemical in vitro at increasing concentrations, incubating the solutions with separate batches of cultured bacteria, and measuring the results using agar dilution (Wiegand et al., 2008). Results have been graded into susceptible (often called sensitive), intermediate or resistant increased exposure, or resistant to a particular antimicrobial by using a breakpoint. Breakpoints are agreed-upon values, published in guidelines of a reference body, such as the U.S. Clinical and Laboratory Standards Institute (CLSI), the British Society for Antimicrobial Chemotherapy (BSAC) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Silley, 2012). MICs are often used by diagnostic laboratories mainly to confirm resistance but most often are used as a research tool to determine the *in vivo* activity of new antibiotics (Andrews, 2001)

While MIC is the lowest concentration of an antibacterial agent necessary to inhibit visible growth, Minimum Bactericidal Concentration (MBC) is the minimum concentration of an antibacterial agent that results in bacterial death. The closer the MIC is to the MBC, the more bactericidal the compound (Salada et al., 2015). The purpose of measuring the minimum inhibitory concentration is to ensure that antibiotics are chosen efficiently to increase the success of treatment and to offer the right regimen to the patient.

2.11 Antimicrobial Sensitivity Testing

Antimicrobial sensitivity testing is a measure of the susceptibility of bacteria to antibiotics, it is used because some bacteria may have resistance to antibiotics; Antimicrobial sensitivity test is a method employed to choose the antibiotic that will effectively treat a bacterial infection. The method employed in antimicrobial sensitivity testing is the Kirby-Bauer disk diffusion susceptibility test. To determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria, to various antimicrobial compounds. To assist a physician in selecting treatment options for his or her patients (Kovale et al*.,* 2021). The pathogenic organisms are grown on Mueller-Hilton Agar (MHA) in the presence of various antimicrobial-impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism (Maugeri et al., 2019).

2.12 Conceptual Framework

The antibacterial activity of the crude extracts from the selected plants against *S. aureus,S. pyogenes* and *P. aeruginosa* depends on the structural composition of the cell membrane of the microbe. The pharmacokinetics of the drug to the side of action and the polarity of the solvent used in extraction will determine the activity of the extracts. The independent variables are the extracts from *S. didymobotrya* and *T alata* while the dependent variables are the zones of inhibition of *S. aureus*, *S. pyogenes* and *P. aeruginosa.* The intervening variables are the solvents used for extraction.

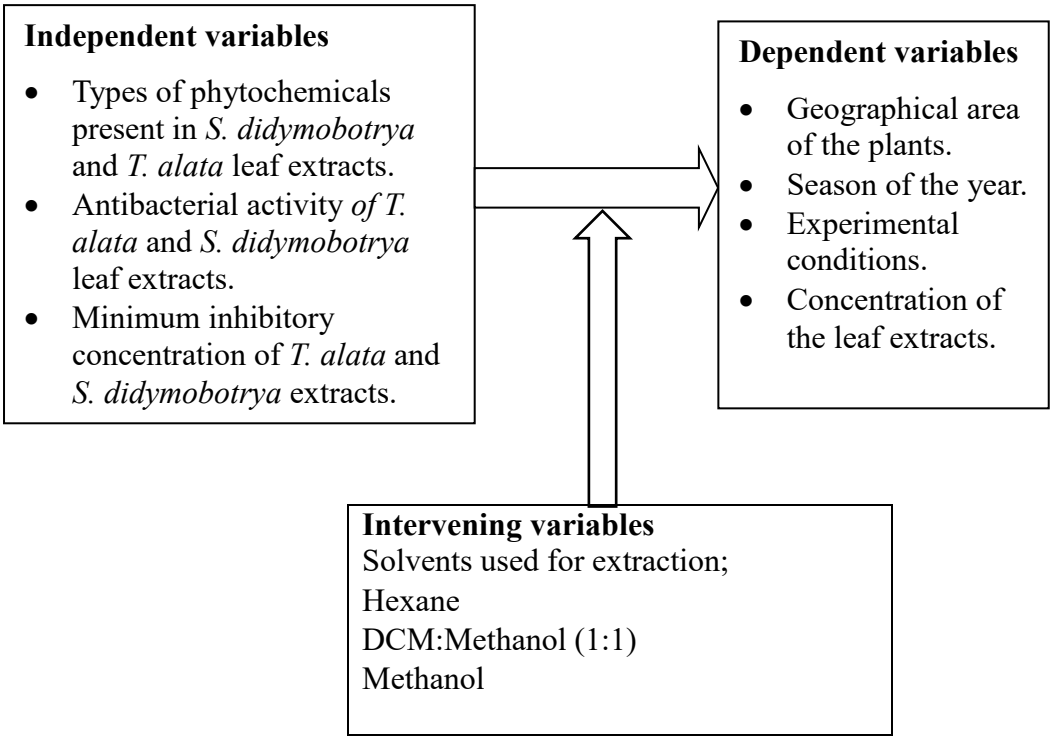


Figure 2. 3 Conceptual Framework

2.13 Identification of Knowledge Gaps

Research on antibacterial and other medicinal plant products has gained interest as an alternative source of medicine. The continued antimicrobial resistance among bacterial strains has continued to be a challenge to society and has continued to cause a lot of suffering in the resource-limited community. Plants have bioactive molecules

that have many components with many moieties. This makes them withstand the variant behaviour in microbial agents. *S. didymobotrya* and *T. alata* being plants have bioactive molecules but are they active against the identified microbes? This is a phenomenon that must be proven.

Evidence of individual plant efficacy in the treatment of bacterial infection is essential since they are widely used in the treatment of other ailments. For example, the bark, stem, and roots of *S. didymobotrya* have been used against Candida, as an anti-fungal, and as a laxative (Kigen et al., 2014). The leaves of *Thunbergia. data* have been used as a remedy for toothache, for treatment of enterobacteria and treatment of stomach pains (Gupta & Singh, 2022). Therefore, there is a need to investigate the leaves of *T. alata* and *S. didymobotrya* against *S. aureus S. pyogenes* and *P. aeruginosa,* which cause skin infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter deals with the experimental design, the study area, sampling techniques methods used in phytochemicals extraction and the antibacterial activity of the plant extracts.

3.2 Research Design and Sample Collection

The experimental design was used in this study discussed herein. Mature *S. didymobotrya* and *T. alata* were selected and collected in a simple random sampling technique from the Bomet and Kabianga regions. Leaves of the plants were plucked and placed in sampling bags and transported to the Botany Laboratory University of Kabianga. In the laboratory, the leaves were dried, and a few samples were pressed using a plant press. The pressed leaves were taken for identification at the Botany Department of the National Museums of Kenya. The plant leaves were then dried at room temperature, crushed, and milled into fine particles. The particles were then extracted separately using hexane, dichloromethane: methanol (1:1) and methanol. Extracts were then exposed to different bacterial strains to evaluate antimicrobial sensitivity testing. Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of extracts were evaluated. The extracts were also evaluated for phytochemicals present using standard laboratory procedures.

3.3 Plant Collection and identification

Mature *S. didymobotrya* and *T. alata* were sourced from two sites, Kabianga, Kenya (0.41º S, 35.16º E, 1805m above sea level), and Bomet, Kenya (0.31º S, 35.2 º E, 1981m above sea level). Kabianga has a tropical rainforest climate, with a mean

annual temperature ranging from 19.36 (66.85ºF) to 23.74℃ and a mean annual rainfall of 353.2 millimetres (13.91 inches). It receives rain about 88.42% of the time. Bomet on the other hand has a mean annual temperature of 18.65 ^{O}C. Bomet typically receives about 340.25 millimetres of precipitation and has 310.91 rainy days (85.18 % of the time) annually.

3.4 Solvent extraction of phytochemical from *S. didymobotrya* and *T alata* leaves

The grounded leaf powder was separately solvent extracted according to (Bitwell et al*.,* 2023) and as shown in (figure 3.1), 460gm of the initial powder was put into a 2.5L reagent bottle, and then 1.5L of the solvent was added, and the bottle corked. The corked reagent bottle was swirled to ensure that the whole powder was submerged. It was left to stand for 72 hours before filtration. The mixture was then filtered using Whatman filter paper no1, and the filtrate was collected using a conical flask. The filtrate was then evaporated under vacuum, and dried to a constant weight (R1). The yield of the extract is evaluated as a percentage (%) of the initial weight of the grounded leaf powder as follows:

% Yield of extracts = $\frac{R1}{460} \times 100$

Where R1 represents the weight of the dried extract (gm)

The dry extract was thereafter stored in a 10ml beaker covered with a parafilm and kept at 4 ºC awaiting use. The residue was dried for subsequent extraction using solvents of different polarities. The extracted mass appeared greenish. This concentrated greenish -brown gummy semi-solid mass of extract was then coded and labelled for identification purposes and then stored aseptically in a refrigerator awaiting use.

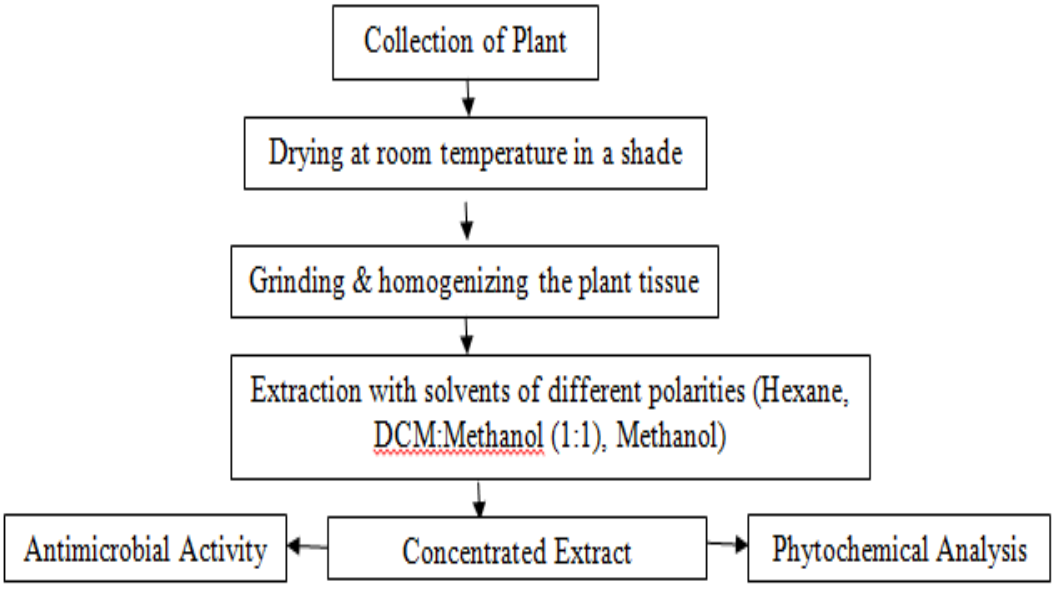


Figure 3. 1 Schematic Diagram of Solvent Extraction

3.5 Source and Reconstitution of Bacterial Species

Bacteria species (*Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa*) were obtained from Kericho Referral hospital as clinical isolates. The isolates were cultured in disposable plates on Eosin-Methylene Blue (EMB) agar and frozen in nutrient agar vials. The samples in the plates and vials were then transported to the microbiology laboratory at the University of Kabianga in a cooler box. The isolates were then subjected to favourable conditions to re-grow before sub-culturing in Nutrient agar plates for further confirmatory tests.

3.5.1. Confirmatory test for *S. aureus, S. pyogenes* and *P. aeruginosa*

The samples were handled aseptically and sub-cultured by inoculating onto the nutrient agar plates under a bio safety cabinet, the inoculated plates were then incubated at 37 ℃ for 24hour after which growth of the micro-organisms was observed. Once the growth of micro-organisms was observed, isolates were identified

using the standard morphological and culture characteristics by performing Gram staining procedures followed by biochemical tests.

3.5.1.1 *Microscopy -Gram stain test*

A loopful of bacteria suspension was picked from the culture plates using a sterile loop and spread on a clean glass slide to make a thin smear. The slide was then air dried and heat fixed by passing it through a flame. When the slide had been fixed Gram staining was carried out as follows; the smear was covered with the primary stain (crystal violet) for 60 seconds and then poured off. The smear was covered with a mordant (Lugol's iodine) solution for 30 seconds, this was to fix the primary stain. The iodine solution was then poured off and washed with water, then followed by washing with a 95 % alcohol decolourizer this was done until no more stain was coming out of the smear. The slide was then thoroughly washed using running water and counter-stained with a secondary stain (safranin) red for 30 seconds this was then followed by washing the slide with water, blotting with an absorbent paper towel and air dried.

The slide was observed using a light microscope, under an x100 power objective. Micro-organisms that retain the crystal violet stain are Gram-positive while those that lost the primary stain and retained the red colour are Gram-negative bacteria (Atlas & Synder, 2011). *S. aureus* and *S. pyogenes are* both Gram-positive. *S. aureus* is spherical and appears in clusters. *S. pyogenes* is cocci in pairs or chains *P. aeruginosa* are gram-negative rods. Further confirmatory tests were done by subjecting the micro-organism to biochemical tests. To further confirm the strains of microorganisms under this study, biochemical tests carried out are (catalase, mannitol salt agar, Simon's citrate, oxidase, and Tripple sugar iron tests).

3.5.1.2 *The biochemical tests -Catalase Test*

This test is useful in distinguishing microorganisms that can produce the enzyme Catalase from those that lack this enzyme. This test was performed from a blood-free inhibitory medium nutrient agar. Catalase is an enzyme that converts hydrogen peroxide into water and oxygen. Catalase activity tests are used to distinguish *staphylococci* from e*nterococci* and *streptococci*. In this study a colony from the cultured plates were picked aseptically using a sterilized wire loop, spread on a clean glass slide and a drop of hydrogen peroxide added. Production of gas bubbles on the smear indicated a positive test for *P. aeruginosa* and a negative test for S. *aureus*.

3.5.1.3 *Mannitol Salt Agar (MSA) test*

This is a type of media that selects organisms such as staphylococcus species which can survive in areas of high salt concentration. The differential ingredient in MSA is the sugar mannitol; therefore, organisms capable of utilizing the mannitol as a food source will produce acidic by-products of fermentation that will lower the pH indicator, phenol red to yellow. In this study, a colony of bacteria was picked from a nutrient agar plate using a sterilized loop and sub-cultured in MSA by streak method and incubated for 24 hrs at 37°C. After 24 hrs. of incubation, the media turned yellow an indication for a positive test for *S. aureus.*

3.5.1.4 *Simmons citrate agar test.*

Citrate agar is used to test the organism's ability to utilize citrate as a source of energy. Simmon's citrate media contain citrate as a sole source of carbon and inorganic ammonium salts as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme citrate permease capable of converting citrate to pyruvate. Pyruvate can then enter an organism's metabolic cycle for the production of energy. When bacteria metabolize citrate, the ammonium salts are broken down to

ammonia which increases alkalinity. The shift in pH turns the bromothymol blue indicator in the media from green to blue in a positive test and retains the blue colour in a negative test. In this study, a bacterial colony was picked from a well-isolated colony and streaked on the slant in a back-and-forth manner using a straight wire and incubated for 24 hrs. at 37℃. A change in the colour from green to blue along the slant indicates the presence of *S. aureus* and *P. aeruginosa.*

3.5.1.5 *Oxidase test*

The oxidase test is used to determine if a bacterial organism possesses the cytochrome oxidase enzyme. The oxidase test detects the presence of a cytochrome oxidase system that will catalyse the transport of electrons between electron donors in bacteria and a redox dye tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour. This test is useful in the identification of *Pseudomonas, Neisseria, Alcaligen, Aeromonas, Campylobacter, Vibrio, Brucella* and *Pasteurella* all of which are known to produce the enzyme cytochrome oxidase. In this study, an impregnated oxidase disc on a glass slide and using a sterile inoculating loop, a colony of test bacteria was picked from a fresh culture and smeared on the disc. The colour change was observed and the time taken was noted. Development of a purple to deep blue colour within 10-30 seconds, indicated a positive test for *P. aeruginosa*.

3.5.1.6 *Tripple sugar iron test*

Tripple sugar iron test is a microbiological test named for its ability to test micro-organism's ability to ferment sugars and produce hydrogen sulphide. Most of the bacteria can ferment particular sugars. The sugars that bacteria can ferment and those that they cannot ferment are the characteristics of bacteria and thus an important criterion for their identification (R. Gupta *et al.,* 2018). An agar slant of a special media with multiple sugars constituting a pH-sensitive dye (phenol red), 1%sucrose,

1% lactose, 0.1% glucose, as well as sodium thiosulfate and ferrous sulphate is used in carrying out this test.

The media is prepared and poured into a test tube and allowed to solidify at an angle that results in an agar slant. An agar slant of a special media with multiple sugars constituting a pH-sensitive dye (phenol red), 1%sucrose, 1%lactose,0.1% glucose, as well as sodium thiosulfate and ferrous sulfate, is used in carrying out this test. The media is prepared and allowed to solidify at an angle to result in an agar slant test tube at a slanted angle. The TSI is designed to differentiate among the microorganisms based on the differences in carbohydrate fermentation patterns and hydrogen sulphide production. Carbohydrate fermentation is indicated by gas production and a change in the colour of the pH indicator from red to yellow.

In this study, a well-isolated colony was picked using a straight inoculating needle and inoculated on the TSI slant by first stabbing through the centre to the bottom of the tube and then streaking the surface of the agar slant. The tube cap was left loose, before incubating at 37 ℃ for 24hrs. a change in colour from red to yellow indicated the presence of *S. pyogenes.*

3.5.1.7 *Coagulase test*

The coagulase test is used to differentiate *S. aureus* which produces the enzyme coagulase. From *S. epidermis* and *S. saprophyticus* which do not produce coagulase. Coagulase is an enzyme–like protein that causes plasma to clot by converting fibrinogen to fibrin. A drop of distilled water was placed on each end of the slide. Emulsify the colony of the organism in each of the drops to make two thick suspensions. Loopful rabbit plasma was added to one of the suspensions and mixed gently. Clumping was produced within 10 seconds due to the presence of the organism. No plasma was added to the second suspension. This was used to

differentiate any granular appearance of the organism from true Coagulase clumping. Clumping within 10 seconds was an indication of positive results for *Staphylococcus aureus.*

3.6 Media Preparation

The preparation of the media was done by following the manufacturer's directions for all mediums that were used in this study. The media was weighed using an analytical balance and dispensed into a 500mL conical flask with the intended volume of distilled water. It was allowed to dissolve by swirling to mix while heating on a hot plate set at 380 ℃. The mixture was then sterilized by autoclaving at 121 ^{O}C, for 15 minutes and then allowed to cool to 45℃. The media was then dispensed aseptically into sterile petri dishes and culture tubes under a biosafety cabinet. The media in the plates were allowed to solidify by closing the plates halfway.

3.7 Swab preparation

The cotton wool swabs to be used in spreading the inoculum on the plates were prepared from a cotton wool roll and a wooden splint broken into two. The prepared swabs were then put into the McCartney with an aluminium screw cap and sterilized in an autoclave. A sterilization tape was wrapped on the tube to indicate complete sterilization. Sterilization was done using an autoclave at 121ºc for 15 minutes, after which it was stored aseptically under a biosafety cabinet. The sterilized piece to be used for spreading the inoculum on the plates was first soaked in distilled water for ease of spreading.

3.8 Impregnation of paper discs with plant extracts

The paper discs were prepared from Whatman filter paper No.1 using a paper punch with a diameter of 6 mm. The discs were then put in a McCartney bottle with an

aluminium screw cap for autoclave sterilization at 121℃ for 15 minutes with an indicator tape to indicate complete sterilization. The pre-sterilized paper discs were soaked separately in each of the leaf extract dilutions for 1 hour before use. The plant dilutions were made by weighing 50gm of the extract in 10ml of distilled water to get the highest concentration of the extract. Then 1drop of the solution was dropped onto the disc and allowed to soak for 1hour before use.

3.9 Inoculation of bacteria into the plates

Using a wax pencil, each of the plate lids was marked for identification purposes with the plant's name. The bacterial isolates (inoculum) were introduced into replica plates containing Mueller Hinton agar media. This was done by picking a colony of the bacteria aseptically using the wet swab and spreading it on the Mueller Hinton agar plate. Finally, the swab was run around the edge of the plate to ensure that the entire plate surface was seeded. The plates were then allowed to dry for 10 minutes at room temperature before mounting the paper discs with the leaf extracts onto them. The discs were dispensed aseptically on the plate using sterilized pointed forceps. The discs were pressed to ensure there was contact between the agar and the disc. The plates were then incubated at 37 °C for 24 hours.

3.10 Empirical Procedures

The following experimental procedures were carried out.

3.10.1 Phytochemical profiling of crude extracts of *S. didymobotrya* and *T. alata* leaves

The leaf extracts of the two plants were screened for the presence of phytochemicals separately using standard procedures as follows:

3.10.2.1 Test for alkaloids

The respective plant extracts were tested for alkaloids by adding 5ml of the extract in respective extracting solvents put in a 10ml test tube and 1mL of Wagner's reagent was introduced. Then shaken for 1min and allowed to stand. The appearance of a reddish /brown precipitate signifies the presence of alkaloids (Mir et al., 2016).

3.10.1.2 Test for saponins

The extract was tested for saponins by mixing 2ml of the extract with 6mL of distilled water and shaken vigorously. Production of persistent foam for ten minutes indicates the presence of saponins (El Aziz et al., 2019).

3.10.1.3 Test for flavonoids

The extracts were screened for flavonoids by mixing 2ml of the extract with 2ml of dilute sodium hydroxide (NaOH). An intense golden yellow precipitate soluble in HCL indicated positive results for flavonoids (Dinda et al., 2010).

3.10.1.4 Test for terpenoids

Terpenoids were tested by adding 1ml of ethyl acetate to 5ml of the extract followed by the addition of 2mL chloroform to the mixture and shaking vigorously. About 3ml of concentrated Sulphuric (H_2SO_4) acid was then carefully added. A reddish-brown colouration at the interface indicated the presence of terpenoids (Ludwiczuk et al., 2017).

3.10.1.5 Test for glycosides

Glycosides were tested as follows; 0.5gm of the extract was dissolved in 2m glacial acetic acid containing two drops of 10% ferric chloride ($FeCl_3$) solution. 1ml of concentrated Sulphuric acid was then added alongside under-layering the mixture. A

brown ring at the interphase indicated the presence of glycosides (Francisco & Pinotti, 2000).

3.10.1.6 Test for tannins

Tannins were tested as follows; 0.5gm of the extract was dissolved in 2ml distilled water and four drops of ferric chloride reagent were added. A blue-black precipitate indicates the presence of tannins (Hagerman & Butler, 1978).

3.10.2 Evaluation of the antibacterial activity of *T. alata* and *S. didymobotrya* leaf extracts.

The Kirby –Bauer technique was used to determine the antibacterial activity of the plant extracts, against three bacterial strains (*S. aureus, S. pyogenes* and *P. aeruginosa*). The media used to evaluate antimicrobial activity was Mueller Hinton Agar (MHA). The bacterial growth inhibition was evaluated using the Kirby Bauer technique as the standard method for antimicrobial sensitivity testing. This test was performed by streaking bacterial inoculums to the surface of the plate (of 90 mm diameter) Mueller Hinton agar. 50 mg of each plant extract was weighed using the precision balance and dissolved in 10 ml of the extracting solvent. The paper discs were soaked in the mixture and allowed to stand for 20 minutes. The impregnated paper discs were aseptically placed on the surface of the inoculated plates. The plates were then incubated at 37°C for 24 hours. Paper discs (6 mm) containing standard antibiotics chloramphenicol 500mg, penicillin 500mg and erythromycin were used as positive control. The inhibition zones were recorded in millimetres as the diameter of growth-free zones around the discs using a clear ruler. The diameter of the inhibited zone is related to the susceptibility of the isolate to the plant extract. Each extract and standard antibiotic were tested independently in triplicate.

3.10.3 Minimum Inhibitory Concentration of Leaf Extracts of *T. alata* and *S. didymobotrya* Extracts against *S. aureus, S. pyogenes* and *P. aeruginosa* Bacteria.

Minimum inhibitory concentration was defined as the lowest concentration where no visible turbidity was observed, in this test, the broth dilution technique was employed. The plant extracts were prepared by weighing 1 mg of the extract and diluting it with 10 ml of distilled water, to get the highest concentration of 10mg ml-1in distilled water. Followed by several dilutions of concentration ranging from 2ml to 8ml. Using tryptose Soy broth, inoculation of a loopful suspension of the test micro-organism was added to the tubes and incubated for 24 hours at 37^{O}c. The control tubes did not contain the plant extracts, but the test microorganisms. After incubation, the visual turbidity was assessed and compared with the negative control. The lowest concentrations where no turbidity was observed were determined and noted as the minimum inhibitory concentration.

3.10.4 Bactericidal concentration

The minimum bactericidal concentration (MBC) was determined from the broth dilution resulting from MIC tubes by sub-culturing onto antimicrobial-free Mueller Hinton agar. The bacterial strains were picked aseptically from the tubes which showed no growth. A loop full was then picked and inoculated into the plates with sterilized Mueller Hinton Agar, pre-poured medium and incubated at 37^{O}C for 2-3 days. Bacterial growth was observed each day noting the plates with growth and concentration of the plant extract used.Absence of growth on the fourth day indicated the bactericidal nature of the extract. This was regarded as the lowest concentration of the extract that prevented growth of the bacteria that killed more than 85 % of the original inoculum.

3.11 Data Collecting Instruments

Data collecting instruments in the laboratory included observation of growth and inhibition zones and taking photographs of different plates with different bacteria species, the growth pattern of each species and the zone inhibited by the extracts of the two plants.All the instruments used in this study were calibrated.

3.12 Data Analysis and Presentation

The collected data were entered into Microsoft excel sheets for organization and later MIC and MBC determined using SPSS version 20.The antimicrobial sensitivity testing of the plant extract in different solvents against *S. pyogenes, S. aureus* and *P. aeruginosa*. The amount of plant extracts were determined by subjecting to different proportions of hypothesis testing.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter shows the results and discussions of this study. The results are presented as follows: Phytochemical extraction from the leaves of *S. didymobotrya* and *T. alata* and phytochemicals profile of crude extracts from the leaves of *S. didymobotrya* and *T. alata.* This is followed by results on the evaluation of antimicrobial activity testing of the crude plant extracts and finally Minimum inhibitory (MIC) and Minimum bactericidal concentrations (MBC) of the leaf extracts.

4.2 Extraction and Profiling of Phytochemical Present in T. alata and S. didymobotrya Leaves

Extracts from the leaves of S. didymobotrya and T. alata were separately profiled for the presence of alkaloids, flavonoids, saponins, glycosides, tannins and terpenoids using standard procedures.

4.2.1 Phytochemicals from T. alata Kabianga and Bomet

The number of extracts from the leaves of *T. alata* from Bomet and Kabianga ecological sites using solvents of different polarities were as shown Table 4.1

Table 4. 1

Amount of Extracts (%) from the Leaves of T. alata from Kabianga and Bomet

Experiment		1	2	3	Average (%)	
Solvent of extraction	Site of plant	% yield of the extracts			Bomet	Kabianga
Hexane	Bomet	2.31	2.14	1.59	2.01	
	Kabianga	2.81	2.58	2.47		2.62
Dcm:Methanol(1:1)	Bomet	3.89	3.11	2.58	3.19	
	Kabianga	4.89	4.18	3.92		4.33
Methanol	Bomet	2.83	2.27	1.53	2.21	
	Kabianga	1.39	1.08	0.88		1.12
Total yield (%)					7.41	8.07

The yield of extracts from the Leaves of *T. alata* collected from Bomet ranged from 2.01% in hexane to 3.19% in DCM: Methanol giving a total yield of 7.41 % of extracts. Similarly, the yield of extracts from the leaves of *T. alata* collected from Kabianga ranged from 1.12% in methanol to 4.33% in DCM: Methanol giving a total yield of 8.07% of extracts. The threshold of the two plants should have given a z-value of 0.293. But on testing these proportions on the yield of extracts from the two sites gave a z value of 0.190suggesting that these results are not significantly different at a 95% level of significance (Kothari et al., 2009).

4.2.2 Phytochemical extraction from the leaves of *S. didymobotrya.*

Extracts from the leaves of *S. didymobotrya* from Bomet and Kabianga ecological sites using solvents of different polarities are shown in Table 4.2

Table 4. 2

Number of Extracts (%) from the Leaves of S. didymobotrya

Experiment		1	2	3	Average %	
Extracting Solvent	Site of plant	% yield of the extracts	Bomet	Kabianga		
Hexane	Bomet	1.2	1.04	0.96	1.15	
	Kabianga	9.53	8.37	7.33		8.41
Dcm: Methanol 1:1	Bomet	8.63	8.23	7.81	8.22	
	Kabianga	8.37	8.15	7.26		7.92
Methanol	Bomet	1.29	1.10	0.70	1.03	
	Kabianga	1.00	0.87	2.28		1.38
Total Yield (%)					10.40	17.71

The yield of extracts from the leaves of *S. didymobotrya* collected from Bomet ranged from 1.03-8.22% in methanol and DCM /methanol1:1 solvent respectively giving a total yield of 10.4% of extracts. Similarly, the yield of extracts from the Leaves of *S. didymobotrya* collected from Kabianga ranged from 1.38-8.41% in methanol and hexane solvents respectively giving a total yield of 17.71% of extracts. Testing of these proportions on the yield of extracts from the two sites gave a z value of 0.0987 suggesting that these results are not significantly different at 95% level of significance. In general DCM/Methanol 1:1 gave the highest amount of phytochemical irrespective of plant collection sites and species.

4.3 Phytochemical Profile of Crude Extracts from *S. didymobotrya* and *T. alata* Leaves

The phytochemicals from the two plants were profiled using solvents of different polarities and the presence of a particular phytochemical is indicated by (+) and absence by (-) the indicators are also used for positive tests like colour, foam or formation of a ring in case of presence of a particular phytochemical. As presented in Table 4.3

Table 4. 3

Profile of phytochemicals of S. didymobotrya and T. alata leaves using Methanol, DCM: Methanol (1:1) and Hexane

Test reagent	Phytochemicals	*Methanol*				*DCM: Methanol 1:1*				*Hexane*			
		T. a		*S. d*		*T. a*		*S. d*		*T. a*		*S. d*	
		K	*B*	*K*	*B*	*K*	*B*	*K*	*B*	*K*	*B*	*K*	*B*
Wagner's reagent	Alkaloids	+	+	+	+	-	-	-	-	+	+	+	+
NaOH+ HCL	Flavonoids	-	-	+	+	-	+	+	+	+	+	+	+
Distilled water	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
Glacial acetic acid+$FeCl_3$+conc H_2OSO_4	Glycosides	-	-	-	-	-	-	-	-	+	+	+	+
FeCl	Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Acetaldehyde +conc.H_2SO_4	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+

Key:

T.a= *Thunbergia alata*

S.d =*Senna didymobotrya*

K=Kabianga

B =Bomet

It was found that both Methanol and Hexane extracted alkaloids, saponins, tannins and terpenoids from both plants (*S. didymobotrya* and *T. alata)* irrespective of the site of collection as indicated in the table above.

4.4 Evaluation of the antibacterial activity of crude leaf extracts.

The antimicrobial sensitivity testing of the crude plant extracts is presented in Table 4 showing the zone inhibited by the plant extracts against the selected bacteria. The crude plant extracts from the Kabianga region showed a greater zone of inhibition ranging from 1.7 mm to 4.8 mm in diameter. The methanolic crude extract of *S. didymobotrya* showed a greater zone of inhibition against *S. aureus* at 4.8 mm and 4 mm in *S. pyogenes.* However, after 36 hrs of incubation, the zone inhibited by the crude extract on *S. pyogenes* showed growth of the micro-organisms, meaning the bacteria were not fully cleared but were suppressed. This was observed on all the crude plant extracts from all regions and irrespective of the extracting solvent on *S. pyogenes*. The methanolic crude extract of *T. alata* from the same region showed a greater zone of inhibition against *S. aureus* at a 4 mm inhibition zone. The plant extracts from the Bomet region inhibited a zone ranging from 1.3 mm to 4.3 mm. The hexane crude plant extract from the Bomet region showed the least zone of inhibition against *P.aeruginosa.* The commercial antibiotics which were tested against the three bacteria showed a greater zone of inhibition of 4 mm in Chloramphenicol while the Erythromycin and Penicillin ranged from 00 mm to 3.5 mm respectively irrespective of the micro-organism (table 4.5). This showed that the plant crude extracts exhibited much higher activity against *S. aureus,* followed by *S. pyogenes* and lastly *P. aeruginosa.*

Table 4. 4

Zones of inhibition (mm) by Plant Extracts against test bacteria

Test bacteria	Plant extracts	Plant collection site			
		Kabianga		Bomet	
		Mean zone of inhibition in millimetres(mm)			
		S. didymobotrya	*T. alata*	*S. didymobotrya*	*T. alata*
S.pyogenes	Hexane	3.5	3.2	2.0	3.3
	DCM;Methanol (1:1)	3.2	3.0	2.8	3.0
	Methanol	4.0	3.5	4.0	3.3
P. aeruginosa	Hexane	3.1	2.5	1.3	2.8
	Dcm: methanol(1:1)	2.6	2.7	2.6	2.7
	Methanol	1.7	2.8	2.9	3.0
S.aureus	Hexane	3.0	2.8	1.7	2.9
	DCM:Methanol (1:1)	3.4	4.0	3.0	3.3
	Methanol	4.8	4.0	4.3	3.0

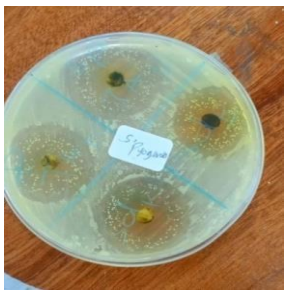
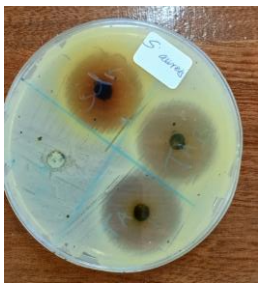
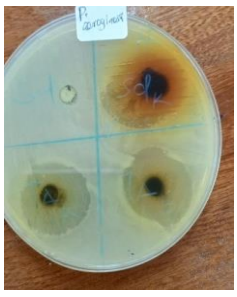
a)  b)  c) 

Figure 4.1 *Images Showing Zones of inhibition of a) S. pyogenes b) S. aureus c) P. aeruginosa*

The commercial antibiotics which were tested against the three bacteria showed zones of inhibition of 4 mm in Chloramphenicol while the Erythromycin and Penicillin ranged from 3.5mm to 00 mm respectively irrespective of the micro-organism (table

4.6). This showed that the test bacteria did not respond to penicillin, or it had developed some mechanisms to evade the action of the drug.

Table 4. 5

Mean Zones of Bacterial Inhibition (mm) against Standard Antibiotics

Antibiotic		Test bacteria Mean zone of inhibition in millimetres (mm)		
		S. pyogenes	*P. aeruginosa*	*S. aureus*
Chloromphenical	1.6	2.5	4.0	
Penicilline	0.0	0.9	0.0	
Erythromycine	2.1	2.1	3.5	

Figure 4. 2 *Image Showing Zones of inhibition by commercial antibiotics*

The commercial antibiotics were tested against each micro-organism and the results showed that penicillin is less active on all the three micro-organisms since the zone inhibited ranged from 00 mm to 0.9 mm. The gram-positive *S. aureus* and gram-negative *P. aeruginosa* showed a higher zone of inhibition on the plate with chloramphenicol as compared to those with erythromycin and penicillin. The zones of inhibition exhibited by the commercial antibiotics are smaller than those inhibited by the plant extracts.

4.5 Minimum Inhibitory Concentration

The tubes were observed for the presence of turbidity; the turbidity of the tubes indicates the amount of microbial growth with the least turbid tubes correlating with the absence of microbes. The tube with no plant extract was opaque and turbid because the microbes were able to flourish. The lowest concentrations where no turbidity was observed were determined and noted as the minimum inhibitory concentration (Parvekar et al., 2020). The table below shows the values in millilitres of the minimum inhibitory concentration.

Table 4. 6

MIC Values (ml) of Plant Extract against Test Organisms

Test bacteria	Plant extracts	Plant collection site			
		Kabianga		Bomet	
		MIC values in (mg/ml) $\times 10^{-3}$			
S. Pyogenes		*S. didymobotrya*	*T. alata*	*S. Didymobotrya*	*T. alata*
	Hexane	4.8	4.8	4.8	8.0
	DCM:Methanol	8.0	4.8	4.8	8.0
	(1:1 Methanol	8.0	8.0	8.0	8.0
P. aeruginosa	Hexane	8.0	8.0	8.0	8.0
	DCM:Methanol	20	20	20	20
	1:1 Methanol	20	20	20	20
S. Aureus	Hexane	20	20	8.0	8.0
	DCM:Methanol	20	8.0	8.0	8.0
	1:1 Methanol	20	20	20	20

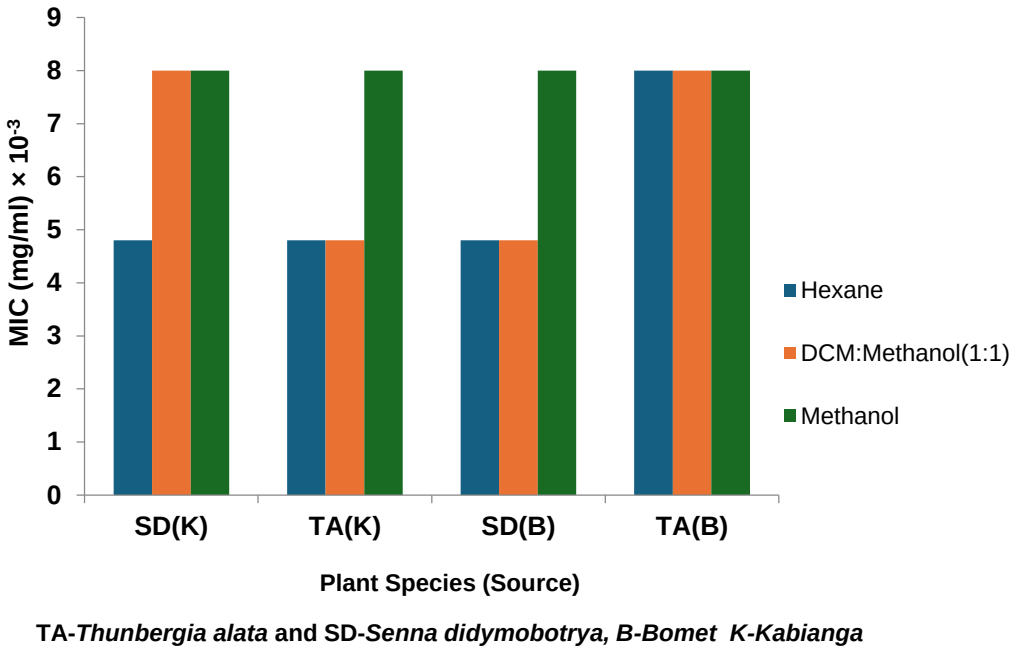


Figure 4. 3 *MIC of T. alata and S. didymobotrya* plant extracts against *S. pyogenes*

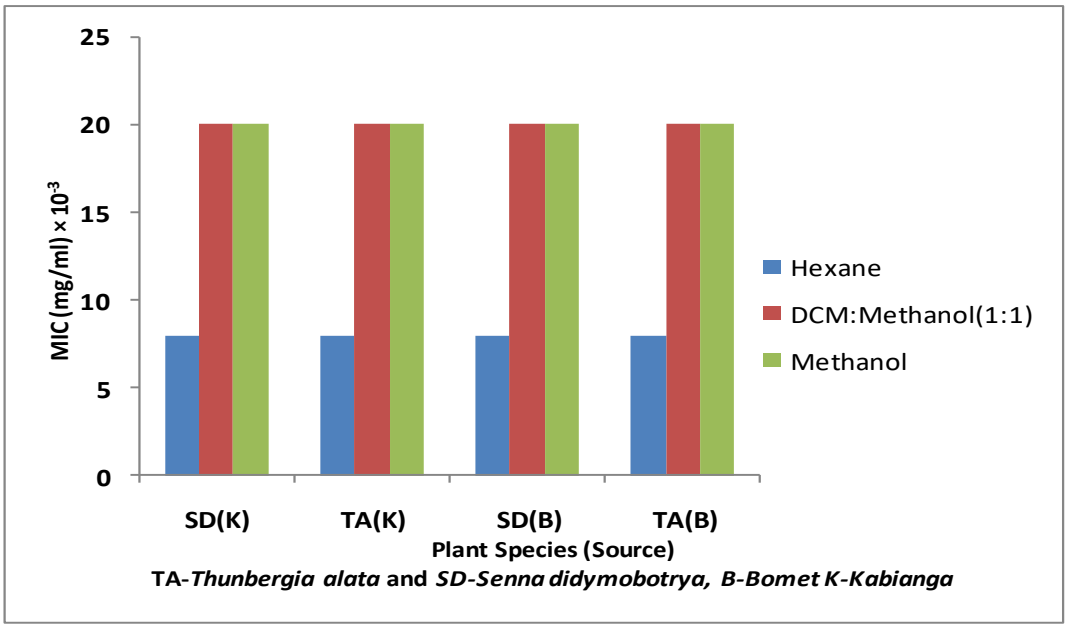


Figure 4. 4 *MIC of T. alata and S. didymobotrya* plant extracts against *P. aeruginosa*

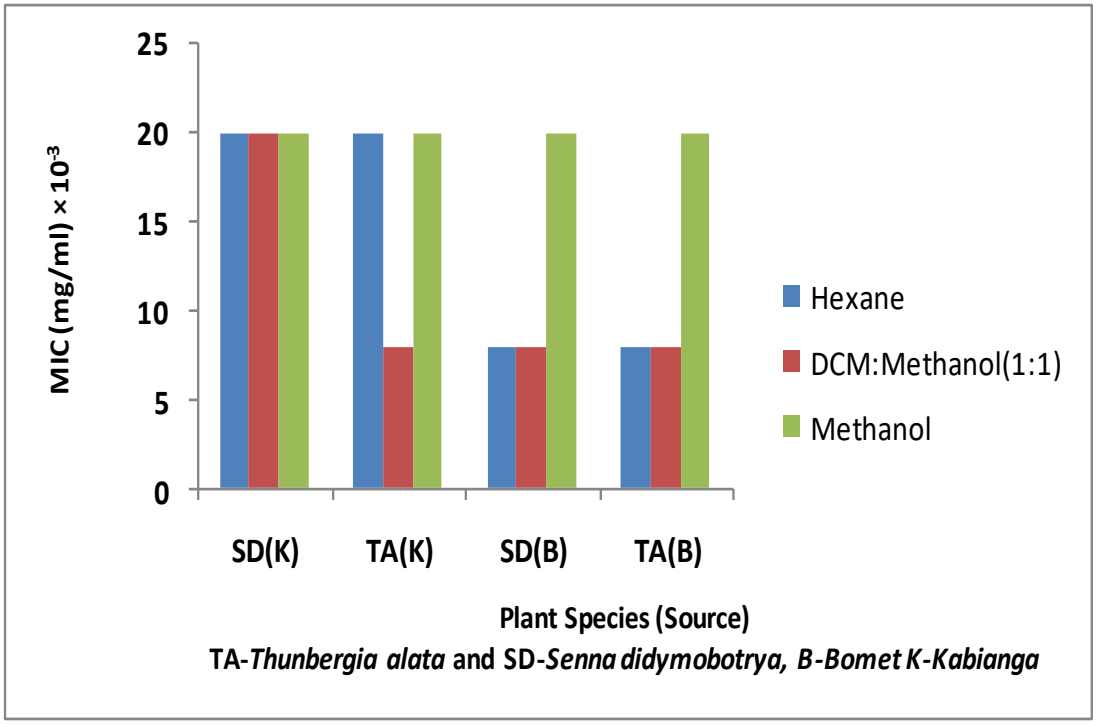


Figure 4. 5 *MIC of T. alata and S. didymobotrya* plant extracts against *S. aureus*

Table 4.6 and Figures 4.3, 4.4 and 4.5show the MIC values (mg/ml) ×10 $^{-3}$of *S. didymobotrya* and *T. alata* plant extracts against *S. pyogenes*, *S. aureus* and *P. aeruginosa.* Irrespective of plant species, methanolic extracts inhibited *S. aureus* and *P. aeruginosa* at 20 ×10^{-3} (mg/ml), however lower MIC values ranging from 4.8- 8.0 ×10^{-3} (mg/ml) were observed in *S. pyogenes*. Such results indicate that these plant extracts could be bacteria static necessitating the evaluation of MBC. The most interesting activity was obtained from *S. didymobotrya* from the two regions.

4.6 Minimum Bactericidal Concentration (MBC)

The Minimum bactericidal was determined from the broth dilution test resulting from the MIC tubes by subculturing a loopful of the bacterial suspension from the MIC tubes on Mueller Hinton agar. The lowest concentration of the extract at 8.0 x 10^3 (mg/ml) which showed no growth was recorded as the MBC. But after 48 hours there

was growth on the plates which were seeded with *S. pyogenes.* This meant that the plant extracts were bacteriostatic at some point not allowing it to grow but the bacteria were kept at their stationary phase, and when cultured on the plates with the MHA it was able to form colonies.

4.7 Discussions

Antimicrobial resistance is a problem that continues to challenge the healthcare sector in many parts of the world both in developed and developing countries. The emergence and spread of multidrug-resistant pathogens have threatened the current antibiotic therapy. This has demanded a search for a new source of antimicrobial activity of different medicinal plant extracts against human pathogens that cause skin infections. Although the plant extracts exhibited good antimicrobial activity towards the tested bacterial isolates, *S. pyogenes* was only suppressed and not completely eradicated by the plant extracts as was exhibited by the antimicrobial sensitivity tests after 36 hours.

Literature studies show that *T. alata* chloroform stem extract has some antibacterial activity against *Pseudomonas aeruginosa* while a significant antibacterial activity of higher concentration of ethanolic leaf extract has been observed against *Salmonella typhi* (Gado et al*.,* 2021). Ethanolic crude extract of *Thunbergia alata* leaves have significant antifungal, anti-tumour and antiviral activity Methanolic crude extract of *Thunbergia grandiflora* leaves have significant microbial activity against some Gram-positive and Gram-negative bacteria. Methanolic extract of the flower of *Thunbergia grandiflora* showed antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes* due to the presence of phenols, alkaloids and flavonoids

(Sultana et al., 2015).*S. didymobotrya* leaves and pods have been evaluated and found to contain several anthraquinone derivatives e.g., emodin, chrysophanol, physcione and knipholone. Other compounds isolated from leaves are aloe-emodin, rhin and small quantities of dianthrone, emodin, catechinic, tannins, flavonoids and aloe-emodin B-glucoside (Nzuki, 2016).

Anthraquinones exhibit other biological effects including diuresis, vaso-relaxation and induction of muscular contractions, antioxidant properties as well as antibacterial and antifungal activities. Emodin is known to be a feeding deterrent against a wide range of organisms (Izhaki, 2002). The roots of *S. didymobotrya* have been used in the treatment of venereal diseases, jaundice, fever, and backache as reported by (Nyamwamu et al., 2015), while the leaf infusion /decoction has been used in the treatment of bacterial diseases, fibroids, and diarrhoea (Singh & Singh, 2010). The stem has also been evaluated for its biological use and found to have a preservative effect on milk (Kawanga, 2017). The current study shows some significant variations in the phytochemical contents, like Alkaloids, flavonoids, Saponins, Terpenoids and glycosides. The variation is due to several environmental factors such as climate, and altitude as mentioned by Pant et al., (2021).

The phytochemical screening of crude plant extracts of *S. didymobotrya* and *T. alata* revealed the presence of alkaloids, flavonoids, saponin, tannin and terpenoids as shown in tables number 4.3. These classes of phytochemicals are known to show curative activity against several pathogenic micro-organisms, and this could explain the reason why it has been used majorly by traditional herbalists to treat a wide range of ailments (Saxena et al., 2013).

The in-vitro antimicrobial susceptibility testing presented in Table 4.4 showed the zones inhibited by the plant extract in mm against *S. pyogenes, S. aureus* and *P. aeruginosa.* The plant extract exhibited a considerable zone of inhibition against the test micro-organisms, the highest being *S. aureus* 4.8 mm in methanolic plant extract of *S. didymobotrya* from Kabianga region. Its level surpassed the degree of commercial antibiotics. The lowest is 0.8mm on *P. aeruginosa* hexane extract from *S. didymobotrya* from the Bomet region. Although these plant extracts were sensitive to *S. pyogenes* with an inhibited zone of 4.00mm, the plant extract seemed to be bacteriostatic and not bactericidal. This is because after 36 hours there was growth of the micro-organism on the Mueller Hinton agar plate seeded with *S. pyogenes* and with all the plant extract in the test. *S. pyogenes* displayed some level of resistance.

However, in comparison with the reference antibiotics especially Chloramphenicol showed that the plant extract exhibited a much higher antimicrobial activity against *S. aureus.* The zones of inhibition inhibited by most antibiotics against some of the reference bacteria were found to be equal or close to those of plant extract. However, in a study carried out by (Musau & Wanjiru, 2020) their methanolic crude extract showed a big zone of inhibition on both *S. aureus* and *P. aeruginosa* at 24.0 mm and 25.5 mm respectively, contrary to what was found in this study. This variation in zones of inhibition could be attributed to the fact that the plant extract from the two regions could have had different concentrations of the phytochemicals.

Due to different ecological zones and different environmental factors, this was categorically stated by (Pant et al*.,* 2021) in their literature that plants of the same species grown in different environments have different concentrations of a particular secondary metabolite. This research agrees with his findings because the plants from

the Bomet region showed a large layer of foam when tested for Saponins compared to those from the Kabianga region. This is because plants must produce a specified amount of phytochemicals to overcome environmental stress (Yeshi et al., 2022).

From the results of MIC presented in Table 4.6, it was observed that the greatest activity of extracts against *S. aureus* and *P. aeruginosa* was 4.8 x 10^3 (mg/ml) as its MIC while MBC of 8.0 x10^3 (mg/ml) was noted similarly. The results on inhibition of bacterial growth have shown that the extracts are active at high concentrations and inactive at low concentrations. Thus, the study findings suggest that the inhibition of bacterial growth activity is dependent on the concentration. The activity of the extracts against the test micro-organisms may indicate a greater zone of inhibition at a higher concentration. This is an important observation to be considered especially when formulating a therapeutic substance against multi-drug–resistant organisms. The victory of traditional medicine may be attributed to the administration of concentrated doses over a long period. This study agrees with the study done by (Ojo et al., 2022) on the concentration of the drug administered to the Swiss albino rat infected with *P. aeruginosa* and *S. aureus* over 10 days.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

This chapter presents the summary of results, conclusions, and recommendations.

5.2 Summary of Results

The yield of extracts from the Leaves of *T. alata* collected from Bomet ranged from 2.01% in hexane to 3.19% in DCM: Methanol (1:1) giving a total yield of 7.41% of extracts. Similarly, the yield of extracts from the Leaves of *T. alata* collected from Kabianga ranged from 1.12% in methanol to 4.33% in DCM: Methanol (1:1) giving a total yield of 8.07% of extracts. Testing of these proportions on the yield of extracts from the two sites gave a z-value of 0.19 suggesting that these results are not significantly different at 95% level of significance.

The yield of extracts from the leaves of *S. didymobotrya* collected from Bomet ranged from 1.03-8.22% in methanol and DCM /methanol (1:1) solvent respectively giving a total yield of 10.4% of extracts. Similarly, the yield of extracts from the Leaves of *S. didymobotrya* collected from Kabianga ranged from 1.38-8.41% in methanol and hexane solvents respectively giving a total yield of 17.71% of extracts. Testing of these proportions on the yield of extracts from the two sites gave a z value of 0.0987 suggesting that these results are not significantly different at 95% level of significance. The yield of plant extracts is not affected by site conditions but by plant species. Phytochemicals analysis and profile of Methanolic, DCM/Methanol (1:1) and hexane leaf extracts of *S. didymobotrya* and *T. alata* indicated the presence (+) of alkaloids, flavonoids, terpenoids, glycosides and tannins irrespective of plant collection sites, extracting solvent and plant species.

The ability of *S. didymobotrya* and *T. alata* plant extracts to inhibit the growth of *S. aureus, S.pyogenes* and *P. aeruginosa* bacteria was evaluated as the diameter of growth-free zones around discs in a growth media. In general *S. didymobotrya* crude plant extracts from the Kabianga region showed a greater zone of bacterial growth inhibition ranging from 2.7 to 3.8mm in diameter. The methanolic crude extract of *S. didymobotrya* showed a greater zone of inhibition against *S. aureus* at 4.8mm and 4mm in *S. pyogenes.* However, after 36 hours of incubation, the zone inhibited by the crude extract on *S. pyogenes* showed re-growth of the micro-organisms, meaning the bacteria were not fully cleared but were suppressed. This was observed on all the crude plant extracts from all regions and irrespective of the extracting solvent on *S. pyogenes.*

The methanolic crude extract of *T. alata* from the Kabianga region showed a greater zone of inhibition against *S. aureus* at a 4mm inhibition zone. The plant extracts from the Bomet region exhibited a zone of inhibition ranging from 1.3 mm to 4.3mm. The hexane crude plant extract from the Bomet region exhibited a zone of inhibition ranging from 1.6 to 4.8 mm against the test micro-organisms. The commercial antibiotics which were tested against the three bacteria showed a greater zone of inhibition of 4mm in chloramphenicol while the erythromycin and penicillin ranged from 0.9mm to 3.5mm respectively irrespective of the micro-organism. This showed that the plant crude extracts exhibited much higher activity against *S. aureus*, followed by S. *pyogenes* and lastly *P. aeruginosa.*

The commercial antibiotics were tested against each micro-organism and the results showed that penicillin is less active on all the three micro-organisms since the zone inhibited ranged from 0.0mm to 0.9mm. The gram-positive *S. aureus* and gram-

negative *P. aeruginosa* showed a higher zone of inhibition on the plate with chloramphenicol as compared to those with erythromycin and penicillin. The zones of inhibition exhibited by the commercial antibiotics are smaller than those inhibited by the plant extracts. The MIC of plant extracts was 4.8 x 10^3 (mg/ml) this was obtained from the tubes that showed turbidity, the turbidity of the tubes indicates the amount of microbial growth with the least turbid tubes correlating with the absence of microbes. The tube with no plant extract was opaque and turbid because the microbes were able to flourish. The lowest concentrations where no turbidity was observed were determined and noted as the minimum inhibitory concentration (Parvekar et al., 2020).

The Minimum bactericidal was determined from the broth dilution test resulting from the MIC tubes the tubes that were less tubid at 8.0 x10^3 (mg/ml) showed no growth of all the microorganisms and was recorded as MBC. But after 48 hours there was growth on of the plates which were seeded with *S. pyogenes,* this meant that the plant extracts were bacteriostatic at some point not allowing it to grow but the bacteria were kept at their stationary phase, and when cultured on the plates with the MHAs it was able to form colonies.

5.3 Conclusions

The leaves of the *S. didymobotrya* and *T. alata* are rich in phytochemicals in alkaloids, flavonoids, terpenoids, glycosides and tannins irrespective of plant collection site and extracting solvents. The crude plant extracts were able to inhibit a large zone of 4.8mm compared to commercial antibiotics at 4mm. Plant crude extracts were bactericidal on *S. aureus* and *P. aeruginosa* while for *S. pyogenes* it was bacteriostatic.

5.4 Recommendations

i. Measures to closely monitor dosage completion and post –treatment follow-up for serious infections should be initiated where possible to prevent emergence of antimicrobial resistance.

ii. It is common for people to purchase antimicrobials without prescription. Most people use antimicrobials and do not complete the recommended doses. Relevant bodies should ensure rational use of antimicrobials by restricting over the counter sales of antimicrobial agents with prescription. There should be enhanced antimicrobial stewardship, by adopting measures that will improve antibiotic prescribing practices and stop the excessive indiscriminate use of antimicrobials.

iii. Different extracting solvents can also be used including aqua to extract maximum phytochemicals from the medicinal plants. This can be advantageous for the pharmaceutical industry to explore effective drugs from different species of *Thunbergia* plant.

5.5 Suggestions for Future Prospects

i. Purification, identification, and characterization of unexplored bioactive compounds in these plants that possess pharmacological activities is an area of future work.

ii. Further investigations are necessary to evaluate the antibacterial, antifungal antiviral and antiparasitic activity of the two plants.

iii. Other parts of the plants need to be studied especially the different species of Thunbergia alata to evaluate their potential therapeutic agents.

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APPENDICES

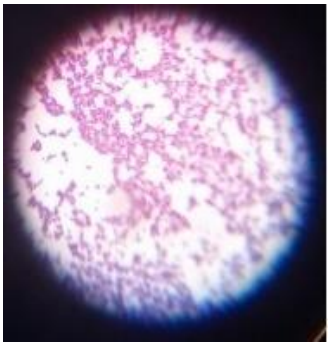
Appendix I: Confirmatory Tests

S. aureus

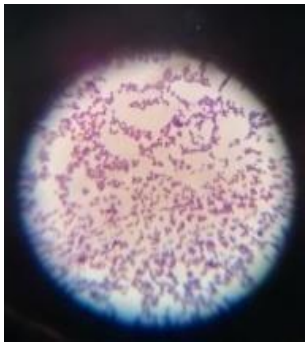
S. pyogenes

P. aeruginosa

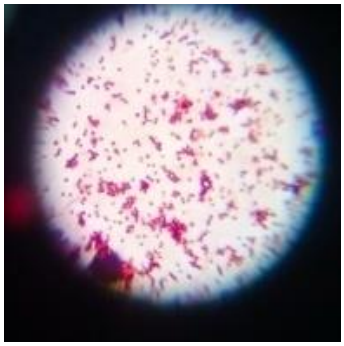
Microscopic examination of a) *S.aureus*and b)*S.pyogenes* and c)*P.aeruginosa*



a)



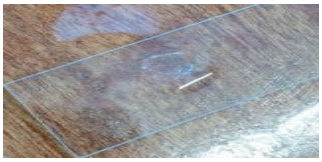
b)



c)

Catalase test bubbles of hydrogen peroxide

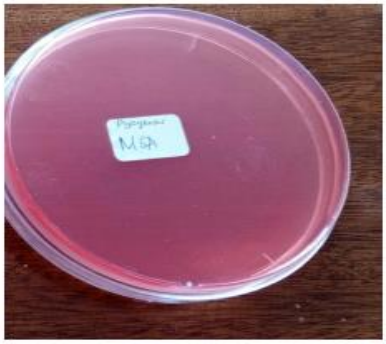
a) *S. aureus* slide positive test b) negatve tests *S.pyogenes*and c) *P.aeruginosa slides*



Simmons citrate test

Simmons citrate test negative for a) *S. pyogenes* and b) positive test for *P. aeruginosa*

Mannitol salt agar



a)Positive test for *S. aureus* and b)negative test for *S. pyogenes*

Tripple sugar iron test


McCateney tube slants positive test for *S.pyogenes* and negative test for *P.aeruginosa*

Eosin methylene blue test

Growth of *P. aeruginosa* on EMB agar plate.

Appendix II: NMK Plant Identification Confirmation

NATIONAL MUSEUMS OF KENYA

REF: NMK/BOT/CTX

21st November 2021

Selina Kotut
Kabianga University
P.O. Box 20200 – 20200
Tel. 0734 -903710
KERICHO

Dear Madam,

PLANT IDENTIFICATION

The plant specimens you brought to us for identification has been determined as follows:

1. *Thunbergia alata* Sims **(Family: Acanthaceae)**
2. *Senna didymobotrya* (Fresen.) Irwin & Barneby **(Family: Fabaceae)**

Thank you for consulting the EAH for plant identification confirmation.

Yours Sincerely

Mathias Mbale
For: Head, Botany Department

Appendix III: Approval Letter from BGS

UNIVERSITY OF KABIANGA
ISO 9001:2015 CERTIFIED
OFFICE OF THE DIRECTOR, BOARD OF GRADUATE STUDIES

REF: PGC/MIC/007/16 **Date: 26TH MARCH, 2021**

Kotut J. Selina,
Biological Sciences,
University of Kabianga,
P.O Box 2030- 20200,
KERICHO.

Dear Ms. Kotut,

RE: CLEARANCE TO COMMENCE FIELD WORK

I am glad to inform you that the Board of Graduate Studies during its meeting on 8th May 2019 approved your research proposal entitled **"Efficacy of Thunbergia Alata and Senna Didymobotrya Extracts against Selected Dermatophytes in Kericho County, Kenya".**

I am also acknowledging receipt of your corrected proposal via email and hard copy. You are now free to commence your field work on condition that you obtain a research permit from NACOSTI.

Please note that, you are expected to publish at least one (1) paper in a peer reviewed journal before final examination (oral defense) of your Masters thesis.

Thank you.

Yours Sincerely,

UNIVERSITY OF KABIANGA
26 MAR 2021
BOARD OF GRADUATE STUDIES

Prof. J. K. Kibett
DIRECTOR, BOARD OF GRADUATE STUDIES.

cc 1. Dean, SST
2. HOD, Biological Sciences
3. Supervisors

Appendix IV: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 359389

Date of Issue: 11/April/2023

RESEARCH LICENSE

This is to Certify that Ms.. SELINA JEMUTAI KOTUT of University of Kabianga, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Kericho on the topic: EFFICACY OF THUNBERGIA ALATA AND SENNA DIDYMOBOTRYA EXTRACTS AGAINST SELECTED BACTERIA THAT CAUSE SKIN INFECTION IN KERICHO COUNTY, KENYA for the period ending : 11/April/2024.

License No: NACOSTI/P/23/24051

359389

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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