PHYTOCHEMICAL ANALYSIS, *IN VITRO* TESTING OF ANTIBACTERIAL PROPERTIES, ANTI-INFLAMMATORY ACTIVITY, AND CYTOTOXICITY OF AQUEOUS AND DICHLOROMETHANE LEAF EXTRACTS OF *Solanum incanum* AND *Tamarindus indica*

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirement for the Award of the Degree of Master of Science in Biochemistry of Chuka University.

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DECLARATION AND RECOMMENDATION

Declaration

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

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Recommendation

This thesis has been examined, passed and submitted with our approval as University supervisors.

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DEDICATION

I dedicate this research Heavenly Father, my immediate wife Esther Wanjiku, daughter Shirleen Nduta, and friends who continuously supported me through spiritual guidance, financial support, and encouragement.

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ABSTRACT

The prevalence of bacterial infections and inflammatory-related diseases is increasing. As an alternative, the pharmaceutical sector is currently focusing on studying medicinal plants to generate alternative therapies for these health concerns. *Solanum incanum* and *Tamarindus indica* are among the plant species utilized in traditional medicine to address these issues. Most conventional drugs used to manage inflammation and bacterial infections have side effects, while some are expensive, hence the need to have alternative plant-based therapies. Additionally, there is limited information on the potency of *Solanum incanum* and *Tamarindus indica*, which is vital in process of commercializing its active metabolites to improve disease management. Therefore, this study analyzed phytochemicals present in aqueous and dichloromethane leaf extracts of the two plants and tested their antibacterial, anti-inflammatory, and cytotoxic properties. The phytochemical analysis was done using standard chemical tests followed by Gas Chromatography-Mass Spectroscopy of dichloromethane extracts. The antibacterial activity of the extracts was tested against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* using disc diffusion method, minimum inhibitory Concentration, and minimum bactericidal concentrations assays. The anti-inflammatory potency of the extracts was tested using an Erythrocyte stabilization assay, while the toxicity of the extract was tested using brine shrimp lethality tests. Phytochemical analysis indicated presence of flavonoids, tannins, saponins, phenols, and alkaloids in all the plant extracts. Additionally, the *T. indica* dichloromethane and *S. incanum* water extracts had no glycosides, while anthraquinones were absent in all the extracts. The antibacterial assay revealed significant difference in antibacterial activity between the plant extracts at different concentrations. The Minimum Inhibitory Concentrations of *T. indica* extracts ranged between 62.5 µg/mL and 125 µg/mL, while those of *S. incanum* ranged between 62.5 µg/mL and 250 µg/mL. The Minimum Bactericidal Concentration of both plants ranged between 125 µg/mL and 500 µg/mL. The *S. incanum* dichloromethane extracts had the highest percentage erythrocyte stabilization at 1000 µg/ml, 57.64 ±13.90%, while *S. incanum* water extract had the lowest stabilization at 2000 µg/ml, 19.06 ±14.43%. *T. indica* dichloromethane extract has the highest toxicity (LD_{50} of 113.57 µg/mL) while *S. incanum* DCM extract was the least toxic (LD_{50} of 2341 µg/mL). The plant extracts have demonstrated the potential of being used for therapeutic purposes after further analysis for the identification of the active compounds. Therefore, this research provides preliminary data on their antibacterial and anti-inflammatory activity and cytotoxicity of the extracts, which is foundational for further research.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
CDC-	Center for Disease Control and Prevention
CI	Confidence Intervals
CV	Coefficient of Variation
COVID-19	Corona Virus Disease-19
COX	Cyclooxygenase
DCM	dichloromethane
EDTA	Ethylenediaminetetraacetic Acid
Erk	Extracellular Receptor Kinase
GC-MS	Gas Chromatography–Mass Spectrometry
GIT	Gastrointestinal Tract
HCl	Hydrochloric Acid
IkB	Inhibitor of NF-κB
JAK-STAT	Janus Kinase/Signal Transduction and Activator of Transcription
JNK	Jun N-Terminal Kinase
LD_{50}	Lethal Concentration 50%
LOX	Lipoxygenase
LSD	Least Significant Difference
MAPK	Mitogen-Activated Protein Kinase
MAPKK	Mitogen-Activated Protein Kinase Kinase
MAPKKK	Mitogen-Activated Protein Kinase Kinase Kinase
MBC	Minimum Bactericidal Concentration
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
NACOSTI	National Commission for Science, Technology and Innovation
NaH_2PO_4	Monosodium phosphate
Na_2HPO_4	Disodium hydrogen phosphate
NF-kB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
Nrf_2	Nuclear Factor Erythroid– Related Factor 2
NSAIDs	Non-Steroidal Anti-inflammatory Drugs
PPARs	Peroxisome Proliferator-Activated Receptors

RBCs- Red Blood Cells

Rcl A, p65, p50, c-Rel- Transcription factors

r-RNA Ribosomal Ribonucleic Acid

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Most communities in Kenya, including the Samburu, Kikuyu, Embu, and Meru have been using various plants to prevent, manage and treat diseases (Gakuya *et al*., 2020; Allegra *et al*., 2019; Mariita *et al*., 2011). The successful use of medicinal plants is attributed to their phytochemical composition, justifying the necessity of phytochemical analysis in herbal plants research (Yoo *et al*., 2018). Phytochemical analysis elucidates they chemical compound in plant extracts and their respective concentrations. The preliminary analysis is fundamental for subsequent elucidation of chemical structures and determination of pharmaceutically relevant secondary metabolites (Yoo *et al*., 2018). Currently, there is limited information on the phytochemical composition of *S. incanum* and *T. indica* leaf extracts from Tharaka Nithi County. Therefore, it is important to analyze the phytochemical contained in the two plant and link them to their health benefits.

Traditional practitioners have been using *S. incanum* and *T. indica* to cure bacterial infections and diseases caused uncontrolled inflammation (Akanmu *et al*., 2018; Enoc *et al*., 2018). The practitioners justify their efficacy by their wound healing capabilities, and potency in managing gastrointestinal tract illnesses (Taye *et al.*, 2011). Chronic wounds are infected with various bacterial strains, including *S. aureus* and therefore, for a chronic wound to heal, the extracts used should have antiseptic properties. The two plants are also used locally by Ameru, Aembu, and Abaluhya traditional practitioners to manage bacterial infections (Otieno and Analo, 2012). Researchers have reported that both ethanolic and aqueous leaf extracts of *S. incanum* were effective against *S. aureus, S. typhi*, and *E. coli* (Sbhatu and Abraha, 2020). Similar results were reported when against *Streptococcus pyogenes* and *Pseudomonas aerugninosa* when methanol was used as an extraction solvent (Akanmu *et al.*, 2018). However, *S. incanum* is less effective against *Klebsiella pneumonia* (Akanmu *et al*., 2018). Other studies concluded that ethanolic and methanolic leaf and fruit extracts of *T. indica* were effective against several bacterial species, including *Bacillus subtilis, E. coli,* and *P. aeruginosa* (Hassan *et al*., 2019; Abdallah and Ali, 2018). Based on the solvents used in all these studies, it is evident that the researchers focused on polar phytochemicals,

leaving a knowledge gap on the efficacy of less polar phytochemicals present in the extracts. Less polar solvents such as Dichloromethane (DCM) effectively extract non-polar phytochemicals as a prerequisite for analysis of the target compounds.

S. incanum and *T. indica* from other world regions have been assessed for their anti-inflammatory potency. Flavonoid-rich root extracts of *S. incanum* effectively managed acute inflammation at dosages above 6.5 mg/kg (Enoc *et al*., 2018). Similary, a study using *S. incanum* ethanolic leaf extracts from Brazil, reported that the plant effectively controlled inflammation at dosages above 75 mg/kg (da Costa *et al*., 2015). Further, researchers confirmed that the ethanolic extracts of the *T. indica* roots and back have ability to control inflamation, with the roots being superior to the back (Borquaye *et al*., 2020). These reports on the potency of the plants in managing bacterial diseases and inflammation explain why traditional healers have successfully managed bacterial and inflammatory diseases over the years. However, no publications available on the anti-inflammatory potency of *S. incanum* and *T. indica* used in eastern Kenya despite their regular use in Ameru traditional medicine.

Several researchers assessed the toxicity of various plant extracts. For instance, researchers reported that dichloromethane extracts of *Solanum lycocarpum* leaves had lower toxicity than ethyl acetate and hexane extracts, demonstrating the impact of solvent choice in phytochemical extraction (da Costa *et al*., 2015). Also, using brine shrimp lethality assay, researchers found that several plant species used by South African communities were cytotoxic at concentrations above 1mg/ml (Ghuman *et al*., 2016). Additionally, four plant species used to treat skin diseases and cosmetic purposes were found toxic against fibroblasts and keratinocytes at high concentrations (Ziemlewska *et al*., 2021). A study using *T. indica* seeds demonstrated that vincristine sulfate extracts of the seeds were less toxic than ethanolic and methanolic extracts (Hassan *et al*., 2019). These studies show variations in toxicity levels in plants extracted using different solvents. Due to this, it was also important to evaluate the toxicity levels of various concentrations of water and DCM extracts of *S. incanum* and *T. indica*. The toxicity analysis informed on the toxicity of the selected plants used in folk medicine.

Although several researchers have assessed the potency of the *S. incanum* and *T. indica* from different ecological locations, there is a need to evaluate the efficacy of the plants used by traditional practitioner in Tharaka Nithi county in Eastern Kenya. Similar plant species from different ecological locations have different phytochemical constituents and varying medical benefits (Ghasemzadeh *et al.*, 2018). The differences are attributed to molecular diversity caused by climatic, topographic divergence in different world zones, soil properties, inter-species interactions and seasonal variations (Defossez *et al*., 2021; Kumar *et al.*, 2017). Besides this, studies on the two plants used polar solvents, and there is limited information on the toxicity profile of their leaf extracts. Therefore, this research qualitatively analyzed phytochemicals in aqueous and dichloromethane extracts of the two plants and investigated their antibacterial potency against *S. aureus, S. typhi,* and *E. coli*. In addition, the study provided information on the anti-inflammatory potency and cytotoxicity of the water and DCM extracts of the two plants.

1.2 Statement of the Problem

The advent of multidrug-resistant microbes and toxicity caused by most anti-inflammatory medications pose a significant health problem globally. Besides, most antibiotics have devastating side effects. For example, antibiotics such as azithromycin and ciprofloxacin increase heart rate, while others like amoxicillin increase the risk of arrhythmias. Aminoglycosides (such as gentamicin) and β- lactam antibiotics can cause acute kidney injury. Additionally, antibiotics like kanamycin and streptomycin are teratogenic and therefore, cannot be used by pregnant women. Some anti-inflammatory drugs cause gastrointestinal mucosa, which occurs in more than 71% of chronic NSAID users, and liver toxicity, which occurs in approximately 5 cases per 100 000 diclofenac prescriptions and 14 per 100 000 piroxicam prescriptions. Traditional practitioners in Eastern Kenya use *S. incanum* and *T. indica* to manage bacterial-related diseases. However, the two plants (from Tharaka Nithi) has not been scientifically evaluated for their antibacterial and anti-inflammatory potency. In addition, prescribing the two plants without scientific evidence of their toxicity threshold may cause harm to the users. For example, 1453 intoxication cases were reported by researchers in 2018, demonstrating the risk of uncontrolled used of medicinal plants. The information on plant toxicity is especially important because of the uneven doses of extracts used to

manage various illnesses in traditional settings. Therefore, evaluating the potency and toxicity of *S. incanum* and *T. indica* plant parts used in traditional settings will improve user safety.

1.3 General Objective

i. To determine the phytochemical composition, antibacterial properties, anti-inflammatory activities, and cytotoxicity of aqueous and dichloromethane leaf extracts of *Solanum incanum* and *Tamarindus indica.*

ii. To determine the phytochemical composition and bioassaying of aqueous and dichloromethane leaf extracts of *Solanum incanum* and *Tamarindus indica.*

1.4 Specific Objectives

i. To determine the phytochemical composition of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica*.

ii. To determine the antibacterial activity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* using diffusion and dilution methods.

iii. To determine the anti-inflammatory activity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* in erythrocytes.

iv. To determine the cytotoxicity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica* using the brine shrimp lethality test.

1.5 Hypotheses

$H0_1$ There is no difference in phytochemical composition in aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica*.

$H0_2$ There is no significant difference in the antibacterial activity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica,* and ciprofloxacin.

$H0_3$ There is no significant difference in the anti-inflammatory activity of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica,* and diclofenac sodium.

$H0_4$ There is no significant difference in cytotoxicity between different concentrations of aqueous and dichloromethane leaf extracts of *S. incanum* and *T. indica*.

1.6 Justification

Bacterial cause mild and severe diseases in animals and humans (Ventola, 2015). Currently, more than 700 000 deaths caused by drug-resistant bacteria are reported annually globally, and the number might rise to more than ten million incidenced by 2050 (Serra-Burriel *et al*., 2020). Increased bacterial-related diseases will, in turn, increase the healthcare burden. Currently, antibacterial resistance causes the global healthcare system more than $ 300 billion, with the current analysis predicting an increase of the cost to more than one trillion dollars by 2050 (Dadgostar, 2019). Diseases caused by inflammation, such as Inflammatory Bowels Disease (IBD) and rheumatoid arthritis, have affected a significant percentage of the global population (Mody, 2017). A study on IBD prevalence in Sub-Saharan Africa ranked Kenya in the third position after Nigeria and Ghana (Watermeyer *et al*., 2020). The new diagnosis Rheumatoid arthritis is also increasing in Kenya, with the most affected being senior citizens. With these challenges, it is important to find alternative plant-based solutions through scientific assessment of their bioactivity.

Communities in Tharaka Nithi have been using different medicinal plants, including *S. incanum* and *T. indica* to manage various medical concerns (Kathambi *et al*., 2020). *S. incanum* leaves are prepared in traditional practice to manage sore throats, pneumonia, rheumatoid arthritis, and asthma, while *T. indica* leaves help manage gastrointestinal illnesses, treat gum inflammation, and promote wound healing (Sbhatu and Abraha, 2020; Dakone and Guadie, 2016). The effectiveness of plant extracts depends on their phytochemical compositions. Phytochemicals have varying polarities, which influence the mode of extraction and bioactivity. The regular use of *S. incanum* and *T. indica* to manage bacterial and inflammatory-related diseases rationalize the need for scientific studies to assess their potency and safety. Therefore, this research provided preliminary information on the phytochemical composition of the two plants, which will enable further studies to elucidate the chemical structures of the compounds and subsequent large-scale production of active metabolites to manage bacterial infections and manage inflammation (Yoo *et al*., 2018). Also, the research generated toxicity profiles of the *S. incanum* and *T. indica*, which is vital in consequent formulation of safe dose for human use.

CHAPTER TWO
LITERATURE REVIEW

2.1 Use of Plants in Traditional Medicine

In the past few decades, researchers have been studying different types of plants and their medicinal relevance. The studies premiered the manufacture of numerous plant-derived compounds that are currently used to manage various diseases (Abubakar and Haque, 2020). More phytochemical studies are underway to solve the menace of bacterial and inflammatory-related diseases (Dakone and Guadie, 2016). Despite having several types of antibiotics and having done numerous studies on major bacteria strains, the prevalence of bacterial-related infections is still high (Ventola, 2015). As a result, it is important to focus on alternative methods, such as traditional medicine to managing the diseases Herbals plants were prepared using different extraction modes, with the most common approaches being soaking specific plant parts in hot or cold water, or direct application of plant powder on a wound (Abubakar and Haque, 2020; Nankaya *et al*., 2019). Other practices include chewing fresh leaves or direct use of sap from different parts of a plant (Abubakar and Haque, 2020).

2.1.1 Modes of Phytochemical Extraction and Their Efficacy

Extraction processes generate active compounds from inert plant material (Altemimi *et al*., 2017). As such, medicinal plants must undergo this process before being used for medication or in experimental processes. For experimental purposes, factors such as the time when the plant part is harvested, harvesting method, plant authentication and proper drying and milling are important (Abubakar and Haque, 2020). These activities precede phytochemical extraction and analysis procedures, and thus they are likely to determine the yield. Several factors determine the efficiency of an extraction process. The first factor is the solvent to be used, which in turn determines the most compatible extraction method (Altemimi *et al*., 2017). The target phytochemicals is the second factor that determines the extraction method. For example, when targeting non-polar phytochemicals, a researcher should use less polar solvents like DCM, ethyl acetate, and hexane (Altemimi *et al*., 2017). Currently, several solvents are commercially available and can be used in phytochemical extraction (Abubakar and Haque, 2020). Table 1 below summarizes the most commonly used solvents and their polarity index.

Table 1: A summary of the most utilized solvents in phytochemical extraction. The higher the polarity index, the more polar the solvent is.

Solvents	Polarity Index
Water	1.000
Methanol	0.762
Ethanol	0.654
n-Butanol	0.586
Acetone	0.355
Dichloromethane	0.309
Chloroform	0.259
Ethyl acetate	0.228
Diethyl ether	0.117
Petroleum ether	0.117
n-Hexane	0.009

From Table 1, water is the most polar solvent while n-hexane is the least polar. Due to this water is the best solvent when targeting polar phytochemicals, while n-hexane is more applicable when targeting non-polar secondary metabolites (Altemimi *et al*., 2017). In addition to polarity considerations, it is also important to consider their miscibility (when two solvents are used), and their safety (Abubakar and Haque, 2020; Zhang *et al*., 2018). Other essential factors when selecting the solvent include cost, recovery method, boiling temperature, viscosity, and reactivity (Abubakar and Haque, 2020).

Maceration is among the common phytochemical extraction methods. The process involves mixing milled plant material with a menstruum (extracting solvent) in a container (Abubakar and Haque, 2020). The mixture is soaked for several days. After the soaking duration, the menstruum with the target compound is separated from the micelle by decantation or filtration (Zhang *et al*., 2018). The extraction solvent is evaporated at the most suitable temperature to avoid degradation of the target compounds (Altemimi *et al*., 2017). Infusion extraction method is similar to maceration. However, infusion can use hot or cold solvent and the mixture is incubated for shorter periods (a few hours). Besides, unlike maceration, infusion permits the use of fresh plant extracts. Digestion is another alternative phytochemical extraction method, which uses heat to extract active metabolites. In this process, a powdered part of a plant is mixed with the most suitable solvent, and the mixture heated at 50 ºC for a specified duration (Abubakar and Haque, 2020). Decoction extraction mode also

involves heating the powder-menstruum mixture, using water only as an extraction solvent.

Other extraction methods include percolation, Soxhlet extraction, microwave-assisted extraction, and ultrasound extractions. Percolation is done using a percolator to boost the efficiency of the extraction (Zhang *et al*., 2018). Soxhlet extraction uses a Soxhlet extractor where the extracting solvent is heated and allowed to pass through the plant material (Mahire and Patel, 2020). The extracted material then flows to the extraction flask. The method reduces the volume of extraction solvent used. Microwave-assisted extraction uses electromagnetic radiations (frequencies from 300 MHz - 300 GHz) to facilitate solvent movement through the plant material (Abubakar and Haque, 2020; Zhang *et al*., 2018). Although microwave assisted extraction is that it minimizes the extraction time, it can only be used to extract relatively stable phytochemicals such as flavonoids and phenolics. In ultrasound extraction, sound waves disrupt plant cell walls and thus improving phytochemicals-menstruum interaction (Altemimi *et al*., 2017). Despite its poor reproducibility, it is effective when only a small sample of plant material is available.

2.1.2 Traditional Use of *Solanum incanum* and *Tamarindus indica*

S. incanum is a herb classified under the *Solanaceae* family. The plant, also known as Sodom apple (English), *mtunguja mwitu* (Kiswahili), *mutongu* (Kikuyu), and *ochok* (Luo), grows in most regions in Kenya. *S. incanum* is believed to have originated in Africa, but is also found in the Middle East and India. The plant is common in dry, overgrazed areas and grows along roads and in woodlands. Its yellow and purple flowers (which can exist in clusters or solitary form), and broad and thorny leaves, are some of the characteristics that differentiate it from other plants of the same family. *S. incanum* also has spikes on its base, stem, and branches, mainly for protection against herbivores. *S. incanum* grows to a height of about 1.8 meters and is known for managing snakebites (Kathambi *et al*., 2020). Additionally, *S. incanum* is routinely used to manage human diseases such as inflammation, diabetes, malaria, and bacterial infection (Sbhatu *et al*., 2020). Its parts have varying medical benefits. For example, the roots and stem are used to manage toothache and headaches, the fruits for wound healing, and the leaves to treat bacterial infections (Feyera *et al*., 2017). Recent studies have

also proven that methanolic and aqueous extracts of *S. incanum* are effective against Multiple Drug-resistant pathogens like *P. aeruginosa, K. pneumoniae, S. aureus,* and *S. pyogenes* (Akanmu *et al*., 2019). Figure 1 on the is an image of *S. incanum* used in traditional medicine.

Figure 1: The image of *S. incanum.* The plant has broad green leaves, thorns on its stem and leaves and purple and yellow flowers.

Tamarindus indica is a tree that can attain 30 m at maturity (Ebifa-Othieno *et al*., 2017). The plant is common within the tropics and has small-sized leaves with smooth margins. Naturally, the plant grows in lowlands, up to 1500 meters above sea level, and regions with temperatures between 20 ºC and 35 ºC, and annual rainfall of between 800 mm to 3000 mm (Agroforestry database, 2009). It parts have different therapeutic purposes and some serve as ingredients. For instance, the plant's pulp is used to prepare Chutneys and Curries, which are common in India (Agroforestry database, 2009). The fruits of the plants, which are consumed raw, have high ascorbic and β-carotene content and also contain calcium, potassium, phosphorus, magnesium, and sodium (Okello *et al*., 2018; Okello *et al*., 2017). Its leaves and back are applied to wounds to eliminate infections and promote healing, while the leaves help manage inflammation and bacterial diseases (Kamakech *et al*., 2019). Studies also show that the seeds of the plant can be used to manage diabetes and heart diseases (Bhadoriya *et al*., 2018). The back is burned, and its ash is used to improve human digestion and minimize discomfort, while fresh backs are used as a tonic. The plant’s extracts are also added to lotions to manage rashes, and treat boils and ulcers (Agroforestry database, 2009). In traditional settings, the plant is also used in managing amenorrhea and asthma, while its fresh

leaves are used to manage rheumatoid arthritis, treat wounds, and as a pain reliever (Komakesh *et al*., 2019). Besides, hot water extracts are used in treating conjunctivitis, while its powdered fruits are effective in treating diarrhea and managing dysentery (Agroforestry database, 2009). Currently, *T. indica* seeds are being studied for efficacy in COVID-19 treatment, especially among people with chronic diseases such as obesity (Morais *et al*., 2021). Figure 2 is a picture of *Tamarindus indica* used in traditional medicine.

Figure 2: Picture of *Tamarindus indica.* The tree had small leaves with a smooth lining.

The efficiency of plant extracts hinge on the mode of extraction, the solvents used, and the part of the plant selected. More research should be done to confirm the its potency in treating various diseases. Focusing on the non-polar phytochemicals will help build a complete scientific profile for the two plants, and hence the need to use less polar solvents (Rokosz *et al*., 2018).

2.2 Phytochemical Composition of Plant Extracts

Phytochemicals metabolically produced to buffer them against biotic and abiotic stress, attract pollinators, and enhance symbiosis (Seca and Pinto, 2019; Guerriero *et al*., 2018). Several researchers have assessed and published phytochemical profiles of *S. incanum* and *T. indica* from different ecological zones and revealed high molecular

diversity of the compounds. However, some studies reported contradicting findings. For instance, some researchers demonstrated that *S. incanum* and *T. indica* do not have anthraquinones, while others reported a significant amount of anthraquinones in *T. indica* (Belayneh *et al*., 2021; Abdalla and Muhammad, 2018). Other compounds present in high concentration in *T. indica* include pthalic acid, Butyl Octyl Ester and 2, 3-betanediol (Yetayih and Ravichandran, 2020; Sharma *et al*., 2021). Apart from the contradiction on the presence or absence of anthraquinones, previous studies concur on the existence of phenols, glycosides, terpenoids, alkaloids and glycosides in *S. incanum* and *T. indica* (Belayneh *et al*., 2021; Abdalla and Muhammad, 2018).

2.2.1 Phytochemicals Classes and their Medical Benefits

Phytochemical profiling is essential when evaluating the medical benefits of a plant extracts. Currently, researchers have identified several classes and subclasses of phytochemical compounds and elucidated their medical importance (Guerriero *et al*., 2018). The compounds include alkaloids, flavonoids, saponins, tannins, phenolics, and terpenoids.

2.2.1.1 Alkaloids and their Medicinal Importance

Alkaloids have a nitrogen atom either within or outside the cyclic structure, which makes them basic. However, the nitrogen can also be weakly acidic or neutral in some cases. Generally, alkaloids are low-molecular structures with a bitter taste (Eguchi *et al*., 2019). It is estimated that alkaloids make up 20% of all secondary metabolites in a plant (Dey *et al*., 2020). Currently, researchers have isolated and characterized more than 12000 alkaloids (Dey *et al*., 2020). The classification of alkaloids is dependent on the starting compound in the biosynthetic pathway, ring structure and biogenesis (Eguchi *et al*., 2019). Most alkaloids are synthesized from specific amino acids (Heinrich *et al*., 2021). Based on the biogenesis, alkaloids are classified into pseudoakaloids, protoalkaloids, and true alkaloids (Dey *et al*., 2020). True alkaloids have nitrogen atom within its structure, are derived directly from amino acids, and forms water-soluble salts. Most true alkaloids in this category are solid, with nicotine being the only liquid true alkaloid (Dey *et al*., 2020). Table 2 summarizes major true alkaloids and their amino acid precursors.

Protoalkaloids are also synthesized from amino acids. The major difference between protoalkaloids and true alkaloids is that for protoalkaloids, the nitrogen from the amino acid precusors is not included in the main cyclic structure (Bhambhani *et al*., 2020). These alkaloids are synthesized from L-tryptophan and L-tyrosine. Examples of alkaloids in this category include hordenine, mescaline, and yohimbine. On the other hand, pseudoalkaloids are not synthesized from amino acids but by linking other metabolic pathways to amino acid pathways. Examples of such alkaloids include caffeine, ephedrine, and capsaicin (Dey *et al*., 2020). Based on structure, alkaloids are grouped into tropane, pyrrolizidine, piperidine, quinolines, isoquinoline, indole, steroidal, imidazole, purine, and Pyrrolidine alkaloids. These alkaloids also have varying chemical properties.

Table 2: A summary of major true alkaloids and their amino acid sources

Amino Acid Precursors	Groups of Alkaloids
L- serine	Egort alkaloids
L- Tryptophan	Indole alkaloids
L- Proline	Quinoline alkaloids
L- Threonine	Aspidosperma alkaloids
L- Asparagine L- Alanine L- ornithine L- Aspartate L- arginine	Marine alkaloids
L- ornithine	Tropane alkaloids Pyrrolizidine alkaloids Pyrrolidine alkaloids
L- Histidine	Imidazole alkaloids Manzamine alkaloids
L- Lysine L- Isoleucine L- Leucine	Piperidine alkaloids Indolizidine alkaloids Quinolizidine alkaloids

Alkaloids are proven effective in managing bacterial, viral, and fungal-related infections (Thawabteh *et al*., 2019). Besides, alkaloids have antihelminth, antioxidant and anticoagulatory properties, and can effectively manage neurodegenerative diseases (Dey *et al*. 2020; Hussain *et al*. Their diversity is the basis for their variations in

biological activity of alkaloids varies (Heinrich *et al*., 2021). Although alkaloids are medically important, some are not safe for human use (Dey *et al*., 2020).

2.2.1.2 Terpenoids and their Medicinal Benefits

Terpenoids are among the most common class of phytochemicals. They are synthesized from isoprenoid units, which are chemically modified to produce different types of these compounds (Cox-Georgian *et al*., 2019). Terpenoids are classified depending on the carbons present in their structure (Guerriero *et al*., 2018). The simplest terpenoid class has five carbons (hemiterpenes) while the most complex has more than 40 carbons (Proshkina *et al*., 2020). An example of 40-carbon terpene is carotenoids. Other classes of terpenoids are monoterpenes (C= 10), sesquiterpenes (C=15), diterpenes (C=20), and triterpenes (C=30) and polyterpenes with more than 40 carbons (Cox-Georgian *et al*., 2019). Currently, more than 55 000 terpenoids have been identified through chemical identification methods, and most of them assessed for their pharmacological benefits (Prado-Audelo *et al*., 2021).

Each class of terpenoids has various medical benefits. For instance, several monoterpenes are insect repellants, while several sesquiterpenes are used to treat bacterial infections, manage migraines, and treat malaria (Cox-Georgian *et al*., 2019). Other terpenoids are geroprotectors, which means that they can help reverse the aging process (Proshkina *et al*., 2020). Diterpenes from species such as *Euphorbia* and *Salvia miltiorrhiza* have cardioprotective properties (Cox-Georgian *et al*., 2019). Most terpenoids reduce inflammation by interfering with Mitogen-Activated Protein Kinases (MAPKs), reducing pro-inflammatory mediator's expression and interfering with microphage function (Cho *et al*., 2017).

2.2.1.3 Phenolic Compounds and their Medicinal Importance

Most plant-based phenolics are produced through the pentose phosphate and shikimate pathways. These compounds contain a hydroxyl group attached to benzene rings (Lin *et al*., 2016). More than 8000 phenolics have been identified and characterized (Dzialo *et al*., 2016). Phenolics can exist in simple forms such as phenolic acids or more complex forms such as flavonoids. Phenolic acids contain at least one carboxyl group attached to the benzene ring (Kumar and Goel, 2019). Most phenolics have antiviral,

anti-inflammatory, hepatoprotective, and antiallergic (Albuquerque *et al*., 2021; Lin *et al*., 2016). Some flavonoids have nutritional benefits and can be used to manage chronic conditions such as diabetes. Plant phenolics can also be used to manage skin conditions and conditions like acne and wrinkles (Dzialo *et al*., 2016). Besides, polyphenolics have neuroprotective properties and have antioxidant activities, hence reducing aging and risks of cardiovascular diseases (Nardini, 2022).

2.2.1.4 Flavonoids and their Medicinal Benefits

Flavonoids are polyphenolic structures synthesized by almost all plants and are commonly presents in vegetables and fruits (Panche *et al*., 2016). More than 10 000 flavonoids with varying medical benefits have been identified (Ullah *et al*., 2020). Most flavonoids have anticancer, cardioprotective, antibacterial, and antioxidant effects (Tungmunnithum *et al*., 2018). Besides, some leaf and fruit flavonoids have antiviral activities, while others have antimutagenic and anticarcinogenic properties (Ullah *et al*., 2020). Their activity is based on their enzyme inhibitory activities against acetylcholinesterase, cyclooxygenase, and butyrylcholinesterase (Panche *et al*., 2016). Besides flavonoids can manage mental health issues such as Alzheimer's disease by promoting neurogenesis, reducing oxidative stress on neurons, and elevating the synthesis of Brain-Derived Neurotrophic Factor (BDNF), which promotes neural function (Hole and Williams, 2021).

2.2.1.5 Saponins and their Medicinal Importance

Chemically, saponins combine triterpenes or steroids with sugar residues. The steroids and triterpene backbones are naturally synthesized through the mevalonic acid pathway in plants (Broker *et al*., 2018). Like other phytochemicals, saponins are chemically diverse and have varying medicinal benefits, which include managing viral and bacterial infections (Sharma *et al*., 2021). For example, saponins from *Albizia adianthifolia* have high potency aganst bacteria (mostly Gram-negative). Due to their antiviral properties, researchers are assessing their efficacy in managing COVID-19, HIV, and rotaviruses (Sharma *et al*., 2021). Besides, saponins have cardioprotective properties and can prevent stroke, myocardial infarction, coronary atherosclerosis and hyperlipidemia (Sign and Chaughuri, 2018; Marrelli *et al*., 2016). Triterpene saponins regulate inflammatory by inhibiting hyaluronidase, which control inflammatory

pathways, and interfering with AMP-activated Protein Kinase (AMPK) pathway (Liu *et al*., 2021; Grabowska *et al*., 2020).

2.2.1.6 Tannins and their Pharmaceutical Relevance

Tannins are complex polyphenols that occur in different plant species. Tannins have antibacterial activity against selected bacterial strains. Their efficacy depends on their chemical structure, flexibility, and size exposure time, temperature, and pH (Puljula *et al*., 2020; Kaczmarek, 2020). In plants, tannins eliminate bacterial wilt and other similar diseases (Vu *et al*., 2017). Tannins inhibit inflammation by inhibiting prostaglandin E production and synthesis of pro-inflammatory cytokines (Kitabatake *et al*., 2021). Medical benefits of plant extracts are attributed to their phytochemical compositions, and thus the need for characterization of metabolites in medicinal plants (Yoo *et al*., 2018).

2.3 Bacterial Infections, Antibiotic Use, and Herbal Therapies

2.3.1 Prevalence of Bacterial Infections

Antibacterial resistance has significantly affected the global healthcare system. It is projected that the diseases caused by resistant bacterial strains may cause approximately ten million mortalities by 2050 (de Kraker *et al*., 2016). The infection cases will demand an economic input of approximately 100 trillion US dollars (Varma *et al*., 2018). The US, being one of the strongest healthcare systems, is also a victim. The CDC reported that more than 2.8m American citizens are infected with resistant pathogens annually, among which, 35 000 succumb to the infections (CDC, 2021). In Africa, antibiotic resistance has been reported among several bacterial strains including *Streptococcus pneumonia, Haemophilus influenza, Escherichia coli, Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* (Tadesse *et al*., 2017). Similarly, in Kenya, a 15-20% decrease in antibiotic susceptibility has been linked with increase in enteric infections (Kariuki *et al*., 2021).

2.3.2 Antibiotics production and Use

The efforts to produce effective antibiotics were boosted by a detailed understanding of the morphology, genetic makeup, and metabolism of bacterial cells. Antibiotic is a general term given to all drugs that act against bacterial cells (Nemeth *et al.*, 2015).

Advanced technology has simplified the production of drugs, with the pharmaceutical industry producing numerous antibiotics with varying mechanisms of action. In clinical settings, drug selection is based on their efficiency, availability, cost, and various contraindications (Bush and Bradford, 2016). Antibiotics can be either bactericidal or bacteriostatic (Nemeth *et al.*, 2015).

2.3.2.1 Classes of Antibiotics

Bacteriostatic antibiotics are a class of antibiotics that inhibit bacterial growth (Nemeth *et al.*, 2015). *In vivo*, this class of antibiotics reduce bacterial multiplication and thus allowing the immune system to clear the infection. Due to this, this class of antibiotics is considered less effective without the intervention of the immune system. The basic mechanisms of action of bacteriostatic antibiotics include interference with protein biosynthesis, inhibiting DNA replication, and interference with cell metabolism (Barrenechea *et al*., 2021). In each reproduction cycle, a bacterial cell has to replicate its genome. The replication process is dependent on the proteins such as DNA polymerases, single-strand binding proteins, helicases and topoisomerases (Fisk *et al*., 2013). Interference with any of these proteins affects the DNA replication process, and thus, inhibiting bacterial replication.

The common classes of bacteriostatic antibiotics include macrolides (e.g., azithromycin), glycylcyclines (e.g., tigecycline), tetracyclines (for example, doxycycline), sulphonamides (sulfamethoxazole) and lincosamides (clindamycin). Tetracycline and glycylcyclines have similar modes of action. The antibiotics bind ribosomal subunit, which hinders the recruitment of aminoacyl-trna, inhibiting elongation of polypeptide chains (Kim *et al*., 2018). Although the drugs effectively complement the immune system, they might not always be effective for immunocompromised people such as those living with HIV/AIDs (Seddon and Bhagani, 2018). When growth conditions are changed, the inhibited bacteria species are more likely to regain their replicative potential and virulence.

Bactericidal antibiotics are chemical agents designed to kill specific bacterial strains. Interference with the growth conditions can affect the potency of a bactericidal drug, making it bacteriostatic. The main mechanisms of action associated with bactericidal

drugs are inhibition of cell wall biosynthesis, interference with DNA replication pathways, and interference with the membrane and protein metabolism (Baquero and Levin, 2021). Examples of bactericidal antibiotics include β-lactam antibiotics.

2.3.2.2 Mode of Action of β- lactam antibiotics

β-lactam antibiotics are the most prescribed antibacterial drugs globally, accounting for more than 65% of the prescriptions (Bush and Bradford, 2016). The main drug classes under this classification include penicillins, monobactams, carbapenems, and cephalosporins (Pandey and Cascella, 2019). Cephalosporins are highly prescribed subclass of *β*-lactam antibiotics (Bush and Bradford, 2016). All these antibiotics have a β-lactam cyclic structure, which deter the cell wall biosynthetic pathway. Once the cell wall is compromised, the bacterial cell succumbs to osmotic pressure, hence causing cell death (Bush, 2018). Figure 1 demonstrates the general structure of β-lactam antibiotics.

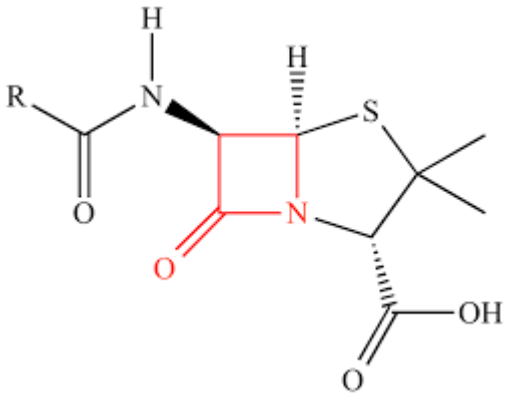


Figure 3: General structure of β-lactam antibiotics. The β-lactam ring binds covalently with penicillin-binding proteins and thus inhibiting the cell wall biosynthesis pathway. The situation causes the death of bacterial cells, mostly due to osmotic pressure.
Source: https://www.chem.ucla.edu/~harding/IGOC/B/beta_lactam.html

2.1.2.3 Cell Wall Biosynthesis and Drug Target

The β-lactam antibiotics pose their activity by inhibiting cell-wall biosynthetic pathway. The cell wall protects cell organelles, and stabilizes bacteria under high osmotic pressure. It is made up of peptidoglycan, which is synthesized from basic sugar components, N-acetylglucosamine and N-acetylmuramic acid, in alternating patterns (Scheffers and Pinho, 2005). Peptidoglycan biosynthesis occurs in three compartments,

which are the periplasmic space, the cytoplasmic membrane, and the cytoplasm. The preliminary biosynthetic process start in the cytoplasm where fructose-6-phosphate is converted to UDP- N-acetylglucosamine, under the Glm enzyme catalysis. UDP- N-acetylglucosamine is also metabolized to UDP-N-acetylmuramyl-pentapeptide (UDP-Mpp), by Mur enzymes (Liu and Breukink, 2016). In the second phase, the synthesized compounds are attached to the membrane lipid receptor known as bactoprenol, forming MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol. The N-acetylglucosamine that had been initially synthesized is added to the new compound, forming GlcNAc-β-(1,4)-MurNAc-(pentapeptide)-pyrophosphoryl-undecaprenol (Scheffers and Pinho, 2005). The lipid-based intermediates facilitate the translocation of the basic components to the periplasmic space.

The final phase of the pathway is polymerization of the building blocks. The major reactions, transpeptidation, and transglycosylations occur with the help of penicillin-binding proteins (Scheffers and Pinho, 2005). The transglycosylation reaction links the MurNAc to the fourth carbon of glucosamine residues, producing undecaprenyl-pyrophosphate as a by-product. The subsequent reaction involves dephosphorylation of the byproduct to yield bactoprenol, which is recycled for the next biosynthetic process. In the transpeptidation phase, the D-Ala-D-Ala bond is cleaved, to provide the required energy for the transpeptidation reaction. The reaction terminates with forming a new bond between the amino group in the cross-bridge and the penultimate D-alanine (Scheffers and Pinho, 2005). The newly formed peptidoglycan is added to the existing layers. The transpeptidase enzyme, that catalyzes the transpeptidation reaction, is the main target for β- lactam antibiotics (Bush and Bradford, 2016).

2.1.2.4 Resistance to β-lactam Antibiotics and Available Solutions

The increasing global cases of β-lactam resistance is worrying. Most bacterial species have devised effective approaches to evade the action of basic β-lactam antibiotics, through β-lactamases expression which degrade penicillins and other related antibiotics, hence reducing their efficacy (Zeng and Lin, 2013). The researchers noted that murein fragments induce production of β-lactamases during the cell wall biosynthesis, to minimize risk of antibiotic interference (Zeng and Lin, 2013). Therefore, although β-lactamases production is generally low, its increased expression

during cell wall biosynthesis justifies the ability of bacterial cells to evade the action of β-lactam antibiotics. Currently, more than 2770 β-lactamases have been identified and characterized. Contrary to the ancient classification that grouped beta-lactamases into cephalosporinases or penicillinases only, the recent classification of the enzymes is based on functional characteristics rather than the general action against antibiotics (Bush, 2018). The classification led to the grouping of beta-lactamases into several classes.

Class A β-lactamase enzymes are coded by chromosomal and plasmid genes and are susceptible to inhibitors like avibactam, tazobactam, clavulanate and sulbactam. The class A β-lactamases cause carbapenems resistance (Bomono, 2017). Class B of the enzymes are zinc metalloenzymes that degrade β-lactam antibiotics. The presence on zinc ions in their structure make it possible to inhibit them with chemical agents such as EDTA. An example of a bacteria with class B β-lactamase is *Stenotrophomonas maltophilia* (Bomono, 2017). Class C β-lactamases are more potent against cephalosporins and penicillins. However, the enzymes are susceptible to tazobactam and clavulanate. Class D β-lactamases are more potent against oxacillinase and carbapenemes and are generally more effective than class A and C enzymes (Bush, 2018). The classification of the β-lactamases was boosted by molecular innovations (Bomono, 2017). Although gram-negative bacteria are the common producers of the enzymes, gram-positive bacterial cells also produce the enzymes in significant amounts.

Compounds such as clavulanate, sulbactam, and tazobactam are used to inhibit β-lactamases *in vivo*. The β-lactam backbone in these compounds bind into the enzyme's, blocking the binding of the antibiotic (Bomono, 2017). Avibactam is also a potent inhibitor but is 50% less effective than tazobactam, sulbactam, and clavulanic acid (Drawz *et al.*, 2014). Figure three shows the three commonly used β-lactam inhibitors. Boronic acid inhibitors, such as glycylboronates also inhibit β-lactamases through competitive inhibition.

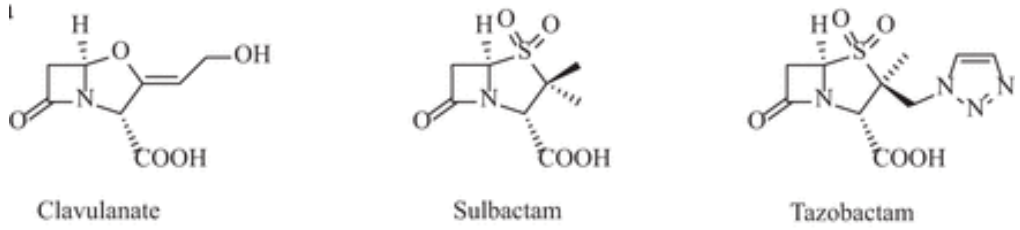


Figure 4: The molecular structure of the three common β -lactamase inhibitors currently in use (Drawz *et al*., 2014). The three compounds have β-lactam backbones, similar to those in β-lactam antibiotics. Clavulanate and Sulbactam are the most common β-lactamase inhibitors in the commercially available antibiotics.

2.3.3 Using Plants extracts as Antibiotics

Herbal presciptions manage bacterial illnesses in folk medicine. The need to validate their application stimulated scientific studies in different settings. Through these studies, the researchers have confirmed that several plant species are effective when used as antibiotics. An example of such studies was done in Kisumu, Kenya, where the researchers noted that 44 plant species used in Luo traditional medicine were effective in managing respiratory infections (Mailu *et al*., 2020). Similarly, researchers have confirmed several herbal extract were effective against *Salmonella typhi, P. aeruginosa, E. coli,* and *S. aureus* (Kariuki *et al.*, 2014). The researchers also noted that the extraction method influenced the potency of the extracts from the five plants, with the aqueous extracts being more effective than extracts obtained using organic solvents (Kariuki *et al.*, 2014). Besides treating normal bacterial infections, some extracts are also potent against multidrug resistant strain. For instance, *Calpurnia aurea* from Ethiopia is highly effective against multidrug-resistant *S. aureus* while *Croton macrostachyus* was effective against several multidrug-resistant bacterial species, including *E. coli* and *P. aeruginosa* (Romha *et al.*, 2018). A similar trend was reported using several plants from Pakistan (Khan *et al*., 2018). The high efficacy against multidrug resistant bacteria was based on their capacity to inhibit biofilm formation.

Studies on different *S. incanum* extracts have shown that its leaves are effective against several bacteria. For instance, using aqueous and ethanolic extraction, researchers noted that stem, leaf, and fruit were effective agains several bacterial like *Bacillus subtilis,* producing zones of inhibition of up to 16.06 mm (Sbhatu and Abraha, 2020). Besides, *S. incanum* methanolic leaf extracts were effective against multiple drug-resistant

pathogens, for example, *P. aeruginosa, K. pneumoniae, S. aureus,* and *S. pyogenes* (Akanmu *et al*., 2019). These studies have shown that polar phytochemicals from the plant have antimicrobial activity.

Studies have also used clinical isolates to assess the potency of various extracts. *T. indica* methanolic extracts were more effective that aqueous extracts against selected clinical isolates (Abdalla, 2018). Besides, the two extracts demonstrated high antibiotic activity against *E. coli* clinical isolates than *Shigella* sp, with the largest zones being up to 14.62 mm in diameter (Abdalla, 2018). *T. indica* ethanolic seed extracts produced similar results, but with smaller zones or approximately 13.5 mm (Hassan *et al*., 2019). These studies justify the efficacy of selected plant species in managing bacterial infections. However, more analysis using similar plant species from different world zones is vital to identify a lead compound that can be used in antibiotic production.

2.4 Inflammation pathways and Anti-inflammatory Drugs

Inflammation is an immunological process initiated by the innate immune cells to counter harmful stimuli such as exposure to toxic compounds, irradiation, physical injury, and infections (Chen *et al*., 2018; Bennett *et al*., 2017). Although the main aim of initiating the cascade is to counter the effects of the negative stimuli, in some cases, inflammation is a threat to human health (Chen *et al*., 2018). As such, researchers have developed several research models to assess the potency of anti-inflammatory drugs. One of the models is the RBC stabilization assay.

The RBC stabilization assay is among the most preferred *in vitro* model to study the ability of selected extacts to control inflamation. RBCs are used in this model, due to their membrane similarity to the lysosomal membrane, and their sensitivity to increase in temperature, which triggers hemolysis, releasing hemoglobin, which can be detected using UV-Vis technique. Lysosomes are actively involved in inflammatory pathways (Kardile *et al*., 2016). During inflammation, lysosomes rapture releasing pro-inflammatory mediators, which mobilize immune cells to protect the human body against pathogens. Additionally, lysosomal enzymes instigate lipid peroxidation, causing loss of tissues function. Erythrocytes are prone to hemolysis when exposed to hypotonic solutions or heat. The heat-induced hemolysis *in vitro* is comparable to *in*

vivo lysosomal lysis due to elevated body temperatures at the inflammation site (Bonam *et al*., 2019). Temperature increase at the inflammation site aims to create an environment that is not suitable for multiplication of infectious pathogens (Blomqvist and Engblom, 2018). In addition to inhibiting cyclooxygenase enzymes, Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have also been shown to stabilize lysosomes, thus reducing the release of inflammatory mediators (Anosike *et al*., 2012). The finding explains the high stabilization effects of the NSAIDs used in clinical practice.

2.2.1 Inflammatory Pathways and their Roles in Inflamation

Several pathways that facilitate the inflammatory process have been identified. These pathways include the NF-κB pathway, MAPK pathway, and JAK-STAT pathway (Chen *et al*., 2018).

2.2.1.1 NF-κB pathway and Inflammation

Nuclear Factor-kB (NF-κB) is a transcription factor that facilitates the inflammatory process. NF-κB activation is dependent on several compounds, including Tumor Necrosis Factors, bacterial lipopolysaccharides and interleukins (Shalapour and Karin, 2018). The NF-kB has been well elucidated in macrophages, which justifies the roles of these cells in initiating inflammatory responses (Dorrington and Fraser, 2019). NF-κB is a combination of five transcript factors. These factors are RelB, P50, RelA (p65), p52 and c-Rel (Chen *et al*., 2018). NF-kB activity is inhibited by IκB proteins found in the cytoplasm, reducing transcription of inflammatory genes. Phosphorylation of IκB proteins reduces its inhibitory activity against NF-κB. This triggers transfer expression of inflammatory genes, producing inflammatory cytokines, as demonstrated in Figure 4.

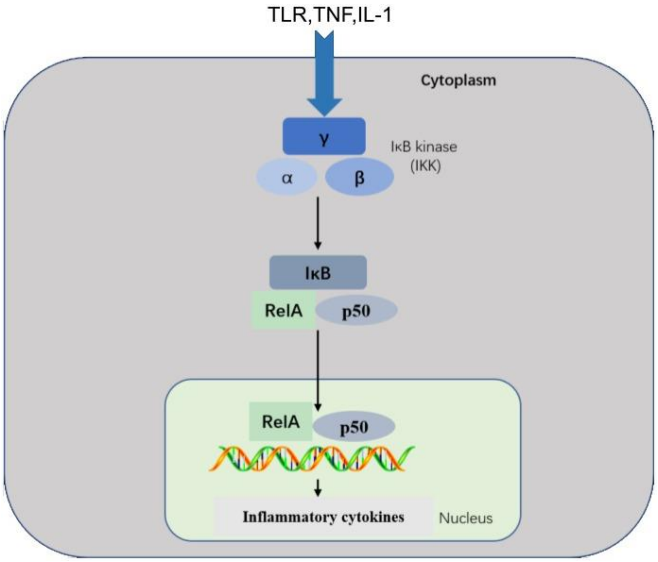


Figure 5: The NF-kB pathway. The binding of TLR, tumor necrosis factor and interleukin-1 leads to the activation of IkB kinases in the cytoplasm. The kinases phosphorylate IkB and thus inactivate it. The inactivation leads to the release of NF-kB (for example, RelA and p50), which translocate to the nucleus, initiating the transcription of inflammatory cytokines (Chen *et al*., 2018).

2.2.1.2 MAPK pathway and Inflammation

Mitogen-activated Protein Kinase (MAPK) pathway is a process that leads to the phosphorylation of three proteins; MAPKK kinase, MAPK kinase, and MAPK. Phosphorylation of these proteins leads to activation of other cytoplasmic proteins, through phosphorylation of their serine and threonine residues (Moens *et al.*, 2013). The pathway controls apoptosis, metabolism, cell proliferation, differentiation, and inflammation (Huang *et al.*, 2010). The MAPK pathway is initiated by stress such as heat shock and inflammatory mediators. The shock activates MAPKKKs (e.g., Raf, Mlk 3, TAK), which activates MAPKKs (e.g., MKK1/2). The MAPKK subsequently activates MAPKs (e.g., Erk1/2, JNK, and p38) (Chen *et al.*, 2018). The activated MAPKs move to the nucleus, where they initiate the transcription of inflammatory cytokines.

2.2.1.3 JAK-STAT pathway and Inflammation

The Janus Kinase/Signal Transduction and Activator of Transcription (JAK-STAT) signaling pathway aid inflammatory responses (Banarjee *et al.*, 2017). Molecules such as interleukin-6 activate the cascade, which terminates with the transcription of inflammatory cytokines under the influence of STATs (Chen *et al.*, 2018). JAKs are

tyrosine kinases, which are among the initial molecules in the cascade (Seif *et al.*, 2017). The JAK-STAT pathway is a drugs target for managing inflammatory psoriasis, bowel disease, and rheumatoid arthritis (Banarjee *et al.*, 2017).

Uncontrolled inflammation causes several illnesses such as cancer, cardiovascular diseases, non-fatty alcoholic liver disease, chronic kidney disease, rheumatoid arthritis, asthma, Crohn's disease, and lung inflammation (Furman *et al*., 2019; Schett and Neurath, 2018). Besides this, inflammation has major contribution in tumorigenesis and metastasis (Shalapour and Karin, 2015). Due to this, it is important to evaluate available options to reduce inflammation-related mortality and mobidity.

2.4.2 Convectional Management of Inflammation

Anti-inflammatory, for example, corticosteroids, NSAIDs, and JAK inhibitors are currently used to manage inflammation-related diseases (Luo *et al*., 2020; Heinemeier *et al.*, 2017; Cruz-Topete and Cidlowski, 2015). The potency of corticosteroids is based on their *in vivo* immunomodulatory properties (Cruz-Topete and Cidlowski, 2015). Glucocorticoids, a class of corticosteroids, binds to glucocorticoid receptors inhibiting transcription of several genes, including those involved in inflammation (Ramamoorthy and Cidlowski, 2017). Prednisolone is an example of a corticosteroid used in clinical settings (Williams, 2018). More than 96% of prescritpions are NSAIDs such as ibuprofen and diclofenac sodium (Wongrakpanich *et al.*, 2018; van Walsem *et al*., 2015). The drugs inhibit cyclooxygenase, which catalyzes prostaglandin synthesis and thus intercepting pain and inflammatory pathways (Heinemeier *et al*., 2017). JAK inhibitors such as Ruxolitinib and tofacitinib target the JAK/STAT signaling cascade, inhibiting inflammatory cytokine production (Luo *et al.*, 2020; Fragoulis *et al.*, 2019).

2.4.3 Traditional Management of Inflammation

Scientific studies have helped assess the effectiveness of various plants used to treat inflammation-related diseases. Plants such as *Sedum sarmentosum* significantly reduce chronic inflammation and hence their use to treat rheumatoid arthritis, asthma, and ulcerative colitis (Kim *et al.*, 2018). Also, phenolic compounds from *Magnolia officinalis* and *Artemisia herba alba* are effective in addressing similar health issues (Ambriz-Perez *et al.*, 2016). Although several plant species are used in traditional

practice to address these health issues, they have different effective dosages due to variations in the chemical structures of their metabolites (Enoc *et al*., 2018; da Costa *et al*., 2015).

Analysis of several African plant species have revealed their potency to manage inflammation. These plants include *Phyllostachys nigrar* from Egypt, effective at 500 mg/kg in rats, and *Croton macrostachyus, Oxygonum sinuatum,* and *Ajuga remota* from Kenya (Oguntibeju, 2018). Their anti-inflammatory potency is based on the activity of plant metabolites including alkaloids, glycosides, terpenoids, polysaccharides, resins, steroids, cannabinoids, and phenolics (Awuchi, 2019; Ambriz-Perez *et al*., 2015).

Several researchers have evaluated the of *S. incanum* and *T. indica* in controlling inflammation. For example, *S. incanum* ethyl acetate extract effectively controls inflammation at 75 mg/kg, while its flavonoid rich extract achieves the same outcome at 6.5 mg/kg dose (Enoc *et al*., 2018; da Costa *et al*., 2015). Besides, researchers have noted that different parts of a plant may have varying anti-inflammatory potency. For instance, *T. indica* ethanolic back extract has an effective dose of 154.5 ± 2.6 mg/kg, while the root extract manages inflammation at 118.1 ± 1.9 mg/kg (Borquaye *et al*., 2020).

2.5 Cytotoxicity of Plant Extracts

Although traditional practitioners are using various medicinal herbs to treat illnesses, in most cases, the concentration of the extracts is not known (Falya *et al*., 2020). Some extracts might be toxic at high concentrations (Alelign *et al*., 2020). Plant extracts can be tested for their acute cellular toxicity, teratogenicity, mutagenic and carcinogenic effects (Falya *et al*., 2020). The three toxicity levels assessed in phytochemical toxicity studies are acute toxicity, sub-acute toxicity, and chronic toxicity (Alelign *et al*., 2020). Acute toxicity is tested by administering an increasing dose of an extract, and its effects assessed within a short period (Subha and Geetha, 2017). In mice models, the animals are exposed to varying concentrations of an extract for 14 days (Alelign *et al*., 2020; Subha and Geetha, 2017). Unlike these *in vivo* tests, brine shrimp lethality test is easier to observe the impact of the treatment calculate the lethal dose.

Studies using *S. incanum* and *T. indica* from other regions of the world have shown that some extracts were toxic at higher doses. Unripe *S. incanum* fruits are toxic to sheep, while *T. indica* seeds were toxic at concentrations lower than 100 µg/ml (Hasan *et al*., 2019; Thaiyah *et al*., 2011). On the othr hand, *T. indica* aqueous pulp extract does not have significant toxicity after long-term use (Iskandar *et al*., 2017). Due to the effects of various solvents on toxicity thresholds of a plant extracts, there is a need to confirm the *in vitro* toxicity of aqueous and DCM extracts of *S. incanum* and *T. indica*.

This research qualitatively analyzed phytochemicals present in water and DCM extracts of *S. incanum* and *T. indica* and evaluated their efficacy in treating bacterial infections and managing inflammation. The antibacterial efficacy of the plants were tested using *S. aureus, E. coli,* and *S. typhi* standard strains, while the anti-inflammatory activity was evaluated using the RBC stabilization method. In addition, the research evaluated the toxicity of their leaf extracts using the brine shrimp lethality test.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Site

The leaves of *S. incanum* and *T. indica* were obtained from their natural habitat in Karingani ward, Tharaka Nithi County (0° 17' 60.00" N Longitude: 38° 00' 0.00" E) in Eastern region of Kenya, with an area or 2609 km^2 as demonstrated in figure 6 (Kathambi *et al.,* 2020). The altitude of the region is between 600 M and 5200 M above sea levels, with bimodal rainfall pattern producing precipitations of 500 mm - 2200 mm. The temperature can be as low as 14 °C in highlands and as high as 36 °C in lowlands (Ogolla *et al*., 2019). Deciduous montane forest covers more area in highlands, which the most common vegetation in the lowlands are shrubs and dry-forest vegetation.

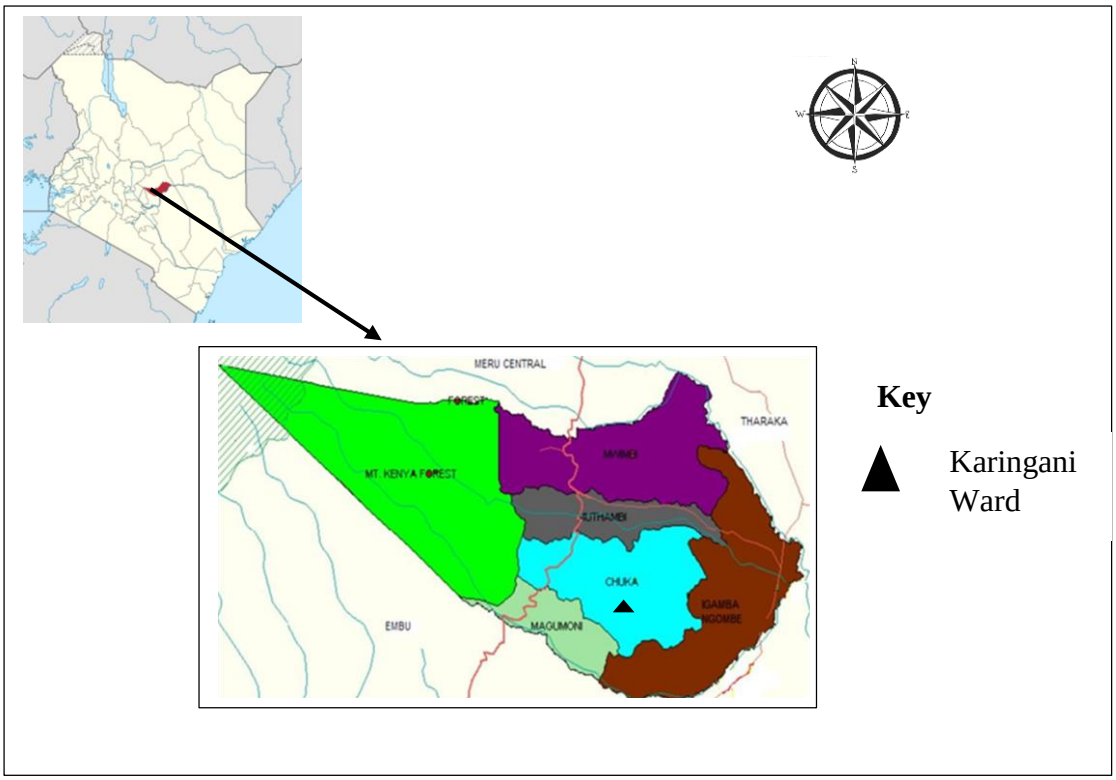


Figure 6: Map of Tharaka Nithi County and Its Location on the Kenyan Map.

Based on the 2019 census, the county had a population of 393 177 people, most of with only less than 7% living in urban areas. Traditionally, a larger proportion of people living in rural areas used traditional herb to treat various diseases, with the knowledge of the medically useful herbs transferred from one generation to the next. The community has a record of about 200 plant species used to treat human and animal diseases, with *S. incunum* and *T. indica* being among the most utilized plants. Leaves

are the second most common used plant part (34% of all plants) after roots (36%) (Kathambi *et al.,* 2020). Additionally, use of medicinal herbs for children under 5 years of age is high, with more than 89.4% of parents acknowledging using various plants to manage childhood related diseases. 70.2% use the users in the County believe that the herbal medications are safer than conventional drugs (Nzuki, 2016).

3.2 Research Design

The antibacterial assay was performed using a 3×3×2×2 factorial design laid out in Complete Randomized Design (CRD). The four factors used were three bacterial stains, three concentrations, two plant species, and two extraction modes. The anti-inflammatory assay was done using a 4×2×2 factorial design laid out in CRD. Three factors used were four concentrations, two plant species, and two modes of extraction. Three replicates were used in the two research layouts. The toxicity test was conducted using a 3×2×2 factorial design laid in CRD. The three factors used for the study were three concentrations, two modes of extractions, and two plant species. Each concentration in the toxicity assay was replicated five times.

3.3 Data Collection

The data for the study was collected from the chemical tests of the phytochemicals, GC-MS analysis, disc diffusion methods, MIC and MBC, anti-inflammatory tests and cytotoxicity analysis as described in the procedures below.

3.3.1 Sample Preparation and Extraction of Phytochemicals

The plants used for this study were confirmed and approved by a local herbalist. The sampling of *S. incanum* was based on their age (plants that have not produced any flowers), while only fresh *T. indica* leaves were picked for the study. The leaves were collected while green, washed with running water at room temperature and dried away from direct sunlight until they were completely dry. The duration taken to complete the drying process depended on the succulence of the leaves. Each dried sample was milled independently using a blender to obtain a leaf powder. The powdered samples were stored in waterproof bags to avoid contamination and moisture contact. The extraction adhered to Jimoh *et al*. (2019) protocol with minor modifications. Three hundred grams of each powdered sample were soaked in four different reagent bottles containing 1000

mL of the respective solvents (Distilled water and DCM) for 48 hours. The mixtures were vigorously shaken by hand every 12 hours to facilitate extraction. The mixtures were filtered using a cotton cloth and Whatman No. 1 filter papers into four separate beakers, by placing the cotton cloth on a filter paper. A rotary facilitates the concentration of the DCM crude extract at 40 ^{o}C and 100 mbar. The water filtrates were subjected to a freeze-drying process in a freeze-dryer. To maximize the extraction process, each sample was divided into approximately 200 mL portions, which were poured into different plates of the freeze-drier. The freeze-drying process was done for seven days. The yield of the extract was calculated using the formula below.

$$\text{Percentage Yield} = \frac{\text{Weight of the extracts after concentration}}{\text{Weight of the powdered leaves used}} \times 100$$

3.3.2 Determination of Phytochemical Composition

3.3.2.1 Test for Phenols

The test was performed as described by Mumtaz *et al*. (2014). One milliliter of each extract was added to test tubes containing one milliliter of distilled water. Two drops of Iron III chloride ($FeCl_3$) were added, and the mixture was shaken several times. A bluish-green colour indicated the presence of phenols. The data on presence or absence of the phenols was tabulated.

3.3.2.2 Test for Saponins

The test was performed as described by Mumtaz *et al*. (2014). Two grams of powdered samples were added to 20 mL of distilled water and boiled in a water bath for five minutes. The mixture was filtered using filter paper. To 10 mL of the filtrate, 5 mL of distilled water was added, and the mixture was shaken vigorously. A stable foam indicated the presence of saponins. The data on presence or absence of saponins was tabulated.

3.3.2.3 Test for Flavonoids

The test was performed as described by Mumtaz *et a*l. (2014). One gram of the powdered extracts was heated in 10 mL ethyl acetate for five minutes. The mixture was filtered, and one milliliter of dilute ammonia was added to the samples. The yellow colour indicated the presence of flavonoids. The data on presence or absence of the flavonoids was tabulated.

3.3.2.4 Test for Alkaloids

The test was conducted as described by Rao *et al*. (2016). Two milliliters of each extract were mixed with 0.2 mL of 2 N HCl. One milliliter of Meyer's reagent was added, and the mixture was shaken. The yellow colour indicated the presence of alkaloids. The data on presence or absence of the phytochemicals was tabulated.

3.3.2.5 Test for Terpenoids

Two milliliters of chloroform were mixed with 0.5 mL of each extract, and three milliliters of concentrated sulfuric acid were added. The mixture was gently shaken. Red or brown colour at the interphase between sulfuric acid and chloroform indicated the presence of terpenoids (Rao *et al*., 2016). The data on presence or absence of the terpenoids was tabulated.

3.3.2.6 Test for Glycosides

In one milliliter of distilled water, 0.5 g of each extract was added. Three drops of aqueous sodium hydroxide were added, and the mixture was shaken. The yellow colour indicated that glycosides are present (Rao *et al*., 2016). The data on presence or absence of the glycosides was tabulated.

3.3.2.7 Test for Anthraquinones

Twenty milliliters of chloroform were added to a test tube containing one gram of powdered leaf. The mixture was heated in a water bath for five minutes. The extract was filtered and allowed to cool before 10% ammonia was added (Auwal *et al*., 2014). The mixture was vigorously shaken, and the upper layer of the mixture was observed for pink coloration. The data on presence or absence of the anthraquinones was tabulated.

The described tests were used to determine the presence or absence of the selected phytochemicals, which are important in understanding the basis of the bioactivity of the plant extracts.

3.3.2.7 Gas Chromatography-Mass Spectrometer (GC-MS)

The plant extracts were subjected to GC-MS analysis to obtain a spectrum that was used to identify the main compounds present in in the extracts. Solid extracts obtained during the extraction phase were dissolved in DCM. To 1 mL of DCM, 0.2 g of the extract was added. The mixtures were hand-shaken until the extracts are dissolved. The samples were filtered using a 45 µm membrane filter paper to obtain an injectable sample (Komappa *et al*., 2020). The carrier gas for analysis was helium, which was set at a flow rate of 1.0 mL/min. Other conditions set on the GC-MS machine include 16.2 psi, 1 µL injector, a split ratio of 1:50, interphase temperature at 300 ºC and ion-source temperature at 250 ºC. The results were generated using a GC-MS software by comparing the phytochemicals present to those in the library database.

3.3.3 Determination of Antibacterial Activity

Three bacterial strains (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *S. typhi* ATCC 6539) were used to test the antibacterial activity the *S. incunum* and *T. indica* aqueous and DCM extracts. The bacteria strains were obtained from the biological sciences laboratory at Chuka University.

3.3.3.1 Disc Diffusion Assay

The disc diffusion method was done using the modified Kirby-Bauer assay as described by Hudzicki (2019). Muller Hinton Agar (MHA) was prepared by dissolving 19 g of the agar powder in 500 mL of distilled water. The mixture was autoclaved at 121 ºC for 15 minutes. Thirty-Six Petri dishes were labeled and subdivided into four quadrants using a marker pen. The label contained the name of the bacteria, the plant species, the extraction solvent used, and the concentration of the extract. Fifteen milliliters of the media were poured into each of the thirty-six sterile Petri dishes and allowed to solidify. The microorganisms described in section 3.3.3 were serial diluted and plated on MHA plates, and a concentration of 47×10^3 CFU/ ml was obtained by multiplying the number of colonies per plate with the dilution factor. Using a sterile wire loop, the microorganisms were uniformly spread on the Petri dishes containing MHA. Discs (6 mm diameter) were made from a Whatman filter paper No.1 using a paper punch. The discs were covered with an aluminum foil and sterilized by autoclaving at 121ºC for 15 minutes. Three concentrations (3000 µg/mL, 1500 µg/mL, and 750 µg/mL) of each

extract were prepared as described by Burman *et al*. (2018). Ten microliters of each sample were applied to the 6 mm discs so that each disc was had a different concentration. The discs were dried at room temperature, covered in sterile Petri dishes for thirty minutes. The dried discs were placed on MHA plates with the bacterial strains, one at a time, using a pair of sterilized forceps. Each disk was placed at the center of a quadrant. A positive control disk (ciprofloxacin commercial disk) was placed on the fourth quadrant. Each Petri dish thus contained three discs with the extract at the same concentration and one positive control placed in independent quadrants. The forceps were sterilized after each transfer stage. In three separate MHA plates negative controls (6 mm discs soaked in water and DCM) were added, such that, each microorganism had one water and one DCM negative control disc. All the plates were incubated for 24 hours at 37 ºC after which the zones of inhibition produced by the extracts and control were measured in millimeters using a ruler. The data was recorded and used to compare the potency the prepared samples to the positive and negative controls using statistical analysis software.

3.3.3.2 Determination of Minimum Inhibition Concentration

Determination of Minimum Inhibition Concentration (MIC) was conducted according to Bussmann *et al*. (2011) with minor modifications. The process was done only for the extract that showed a strong antibacterial activity (zone of inhibition ≥ 10 mm) (Mariita *et al*., 2011). All the plant extracts were diluted to attain 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL concentrations. Five hundred milliliters of nutrient broth were prepared by dissolving 6.5 g of nutrient broth powder into 500 mL of distilled water in a conical flask. The mixture was gently mixed and heated to ensure all the powder had dissolved. The conical flask was tightly covered with aluminum foil and the mixture was autoclaved at 121 ºC for 15 minutes. The broth was allowed to cool at room temperature for subsequent procedures.

For the first extract, a setup for MIC contained two sets of four test tubes each, with each tube containing 2 mL of nutrient broth. To the first tubes in each set, 500 µg/mL (1 mL) of the first extracts was added and mixed by pipetting the extract up and down. From the first tubes, one milliliter of the mixture was obtained and transferred to the second tubes. The same procedure was used for the third and fourth tubes. The next

stage involved the addition of 100 µL of the individual bacterial strains (prepared as described in section 3.3.3.1) into each tube containing plant extracts, such that set one contained *S. aureus* while set two contained *E. coli*. The same procedure was repeated for the other three extracts. For the positive control, ciprofloxacin was diluted to attain the same concentration as the extracts. The control setup was similar to that for the extracts, but the extracts were replaced with the standard drug. Two tubes, one inoculated with *S. aureus* and another inoculated with *E. coli*, were used as negative controls. All the tubes were incubated at 37 ^{o}C for 24 hours, after which visible turbidity in the tubes was assessed. The minimum concentration without any visible turbidity was used for the MBC assay.

3.3.3.3 Determination of Minimum Bactericidal Concentration

The assay was conducted as described by Mostafa *et al*. (2018) with minor modifications. Twenty-four Petri dishes were labeled, and MHA was prepared as described in section 3.3.3.1. The sub-culturing was done by taking 100 µL samples from the two lowest concentration tubes (from section 3.3.3.2) without any visible bacterial growth. The samples were inoculated in independent MHA plates. A sterile wire loop was used to streak the samples on the media. All the Petri dishes were incubated for 24 hours at 37 ^{o}C, after which the MBC values were obtained. The lowest concentration without visible growth on MHA after the specified duration was recorded as MBC. The subculturing was done in duplicates for each concentration. The data from this assay helped determine whether the extracts have bacteriostatic or bactericidal effects on the three strains.

3.3.4 Determination of anti-Inflammatory Activity

An anti-inflammatory assay was performed using the membrane stabilization method as described by Yesmin *et al*. (2020) with minor modifications. Twelve milliliters of blood samples were collected from healthy sheep and transferred to EDTA tubes and gently shaken five times. This helped prevent hemolysis and ensure that the blood samples were well mixed with the anticoagulant. The blood-containing tubes were transported in cool boxes. In preparation for the assay, the blood was removed from the cool boxes and kept at room temperature for 20 minutes. The samples were centrifuged at 2500 rpm for 5 minutes, and the packed cells were washed three times

with isosaline (0.9% w/v NaCl, pH 7.2). A 10 % v/v suspension of the erythrocytes was prepared by adding 4 mL of the cells to 36 mL of Isosaline. A phosphate buffer (0.15 M, pH 7.4) was prepared by mixing 121.5 mL of 0.15 M $NaH_2PO_4.2H_2O$ and 28.5 mL of 0.15 M Na_2HPO_4 solutions (Kezia *et al*., 2020). Positive control was prepared by dissolving 0.1g of diclofenac sodium in 100 mL isosaline to form the mother liquor. The solution was diluted using isosaline to attain a 100 µg/mL concentration.

S. incanum water extract was diluted into four concentrations (500 µg/mL, 1000 µg/mL, 2000 µg/mL, and 4000 µg/Ml), and 1 ml of each concentration added into four different test tubes. To the test tube, 0.25% w/v hyposaline (2 mL), 0.15 M sodium phosphate buffer (1 mL), 10% erythrocytes (0.5 mL) was added, creating a 4.5 mL mixture. The same procedure was repeated for all the four extracts (Kezia *et al*., 2020). This mixture formed the test setup for the subsequent hemolyzing conditions. In the negative control, the extracts were replaced with 1 mL isosaline, while in the positive control, the extracts were replaced with 1 mL diclofenac sodium solution. The tubes were incubated in a water bath at 50 ^oC for 20 minutes and then centrifuged at 3000 rpm for five minutes. Two milliliters of the supernatant were gently pipetted into cuvettes. The absorbance of the supernatant was read at 560 nm. The experiment was performed in three replicates for all the test samples and the controls. The percentage of hemolysis was estimated by assuming the hemolysis produced by the negative control was 100%. The percentage of erythrocyte membrane stabilization or protection was calculated using the following formula described by Yesmin *et al*. (2020).

$$\text{Percentage protection} = 100 - (\text{OD sample} / \text{OD control}) \times 100$$

The data obtained from this assay were analyzed to compare the effectiveness of the extracts and the positive control (diclofenac sodium). The data also demonstrated the difference in efficacy between the plant extracts.

3.3.5 Determination of Cytotoxicity of Plant Extracts

3.3.5.1 Hatching the Brine Shrimp

Twenty-seven grams of table salt was dissolved in 3 ml of distilled water in a rectangular jar. An airline was placed on one side of the jar to enhance air circulation. Fifteen grams of brine shrimp eggs were placed to the top of the water jar, and mixed

with the salt solution (Sarah *et al*., 2017). A 75-watt bulb was placed a few inches from the jar and was switched on. The eggs hatched after 24 hours, after which nauplii were obtained for toxicity testing.

3.3.5.2 Toxicity Testing

All the extracts prepared in procedure 3.3.1 were diluted to attain three different concentrations (10 µg/mL, 100 µg/mL and 1000 µg/mL) in 10 mL seawater containing 1% dimethyl sulfoxide (DMSO). Five replicates were prepared for each concentration. Nauplii were picked by sterilized forceps and exposed to the three concentrations such that each tube with the extracts contained ten live nauplii (Sarah *et al*., 2017). The positive control setup was prepared using vincristine sulphate, while the hatching solution containing 1% DMSO was used as the negative control. The number of live nauplii were counted after 24 hours. A nauplius was considered dead after 30 seconds of no forward motion. The percentage of death of nauplii was calculated as follows.

$$\text{Percentage Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii + Number of live nauplii}} \times 100$$

The LD_{50} was calculated using the probit analysis using statistical software. An extract was considered toxic if 50% of the nauplii died at a lower concentration compared to other extracts.

3.4 Statistical Analysis

The values of average zones of inhibition produced by the four plant extracts and the percentage RBC stabilization assay results were analyzed using one-way Analysis of Variance (ANOVA). The significance level for the differences was set at a 5% probability level, after which Least Significant Difference (LSD) mean separation procedure was applied. The two data sets revealed the differences in antibacterial activities and anti-inflammatory activities of the extracts respectively. The percentage death data obtained from the brine shrimp lethality test was analyzed using probit analysis, and LD_{50} was obtained using regression at a 5% probability level. The data compared toxicity levels of the four extracts. All the analysis was done using STATA software.

3.5 Ethical Consideration

Ethical permission was sought from the ethics committee at Chuka University, and a copy sent to National Commission for Science, Technology and Innovation (NACOSTI) for a research permit. The minimum possible number of animals was used according to the guidelines by Ferdowsian and Beck (2011). The process followed safety procedure to avoid harming the animals. Academic integrity was maintained by ensuring that no work was copy-pasted and all information used was well cited. All the practices adhered to laboratory safety guidelines.

CHAPTER FOUR

RESULTS

4.1 Yields of the Extracts

Each plant had a different yield, which varied based on the mode of extraction used. *S. incunum* water extract had the highest yield (4.7% w/w) while *T. indica* had the lowest yield (1.07% w/w). For both plants, water extracts had a higher yield than DCM extracts. The percentage yields are tabulates in Table 3.

Table 3: A summary of the percentage yield of the extracts obtained after the final concentration of the extracts. Aqueous extraction had higher yield than DCM extraction.

Extract	Percentage Yield (w/w)
S. incanum (DCM)	2.54
T. indica (DCM)	1.07
S. incanum (Water)	4.7
T. indica (Water)	2.53

4.2 Qualitative Phytochemical Analysis

4.2.1 Chemical Identification of Phytochemicals

The chemical analysis demonstrated that all the four extracts contained phenols, saponins, flavonoids, alkaloids and terpenoids. However, glycosides were absent in *T. indica* water extract and *S. incunum* DCM extract. None of the extracts had anthraquinones. Table 4 summarizes the qualitative phytochemical analysis results.

Table 4: Chemical qualitative phytochemical analysis results.

Extract Phytochemicals	*S. incanum* (Water)	*T. indica* (Water)	*S. incanum* (DCM)	*T. indica* (DCM)
Phenols	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Glycosides	+	-	-	+
Anthraquinones	-	-	-	-

A Positive (+) indicates presence, while a negative (-) indicates an absence of selected phytochemicals.

4.2.1 Estimation of phytochemical composition using GC-MS

The GC-MS analysis revealed compounds that are unique to selected extracts. The phytochemicals are of different classes and are summarized in table 5. The main compound identified in GC-MS are flavonoids, phenols, and alkaloids. The percentage abundance also varied between the phytochemical classes, with phthalic acid being the main compound identified in *T. indica* extract.

Table 5: A Summary of GC-MS Results of *T. indica* and *S. incanum* DCM extracts

Plant Extract	Phytochemical	Molecular Formulae	% Abundance	Phytochemical Class
T. indica	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{22}O_4$	21.96	Flavonoid
	Diisooctyl phthalate	$C_{24}H_{38}O_4$	20.16	Flavonoid
	Phthalic acid, butyl tetradecyl ester	$C_{26}H_{42}O_4$	14.73	Flavonoid
	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	13.47	Alkaloid
	Phthalic acid (2-cyclohexylethyl isobutyl ester)	$C_{16}H_{22}O_5$	12.22	Flavonoid
	Phthalic acid, butyl isohexyl ester	$C_{18}H_{26}O_4$	9.57	Flavonoid
	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O$	7.89	Phenols
S. incanum	2-chloro-2-methyl-Butane	$C_5H_{11}Cl$	42.63	Alkane
	1,2-Benzisothiazol-3-amine	$C_7H_6N_2S$	5.34	Alkaloid
	N-(4-methoxyphenyl)-2,2-dimethyl	$C_{12}H_{17}NO_2$	52.04	Phenols

4.3 Determination of antibacterial Activity

4.3.1 Disc Diffusion Assay

All the had significant differences in potency against the three bacterial strains. The control, ciprofloxacin, had the largest zones of inhibition against the three bacterial strains while *T. indica* water extract was the least effective against *S. aureus* 6.37 ±0.39 mm. The overall analysis is attached ass appendix I.

4.3.1.1 Activity of the Extracts against *E. coli*

Table 6 summarizes the differences in activity of the four extracts against *E. coli* at 3000 µg/ml. There was no significant difference between *S. incanum* DCM extract (22.03 ±0.31 mm) and *T. indica* water extract (21.67 ±0.35 mm). Ciproflaxacin (Positive Control) had the biggest zones at 29.27 ±0.40.

Table 6: Differences in zones of inhibition of the four extracts against *E. coli* at 3000 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	29.27 ±0.40[a]
S. incanum	DCM	22.03 ±0.31[b]
T. indica	Water	21.67 ±0.35[b]
S. incanum	Water	20.01 ±0.92[c]
T. indica	DCM	17.87 ±0.39[d]
LSD		0.3633

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD = Least Significant Difference.

A different trend as observed at 1500 µg/ml. *T. indica* water extract had the second zones after the positive control. There were no significant differences of the inhibition zones of *S. incunum* DCM, *T. indica* DCM and *S. incunum* water extract as demonstrated in Table 7.

Table 7: Differences in zones of inhibition of the four extracts against *E. coli* at 1500 µg/ml.

Plant/ Control	**Extraction**	**Zones of Inhibition (mm ± SEM)**
Control	Water	29.27 ±0.40[a]
T. indica	Water	18.07 ±0.61[bc]
S. incanum	DCM	17.73 ±0.23[bcd]
T. indica	DCM	17.37 ±0.47[bcde]
S. incanum	Water	17.33 ±0.67[cde]
LSD		0.056

[a] Means represented by the same letter are not significantly different at α= 0.05.

All the extracts had the smallest zones at the lowest concentration, 750 µg/ml. There was no significant difference between *S. incanum* DCM and *T. indica* DCM extracts. *T. indica* water sanple had the smallest zones *E. coli* at 750 µg/ml as demonstrated in table 8.

Table 8: Variations in zones of inhibition of the four extracts against *E. coli* at 1500 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	29.27 ±0.40[a]
S. incanum	DCM	16.93 ±0.06[b]
T. indica	DCM	16.93 ±0.40[b]
S. incanum	Water	14.53 ±0.80[c]
T. indica	Water	13.07 ±0.67[d]
LSD		0.460

[a] Means represented by the same letter are not significantly different at α= 0.05.

4.3.1.2 Activity of the Extracts against *S. typhi*

At 3000 µg/ml, all the four extracts were significantly different from each other. The control had the largest zone of inhibition, 27.23 ± 0.40 mm, while *T. indica* water extract had the smallest zones of inhibition 17.77 ±0.15 mm. For each extract, the inhibitions zones diminished with reduction in concentrations. The results of the analysis are summarized in Table 9.

Table 9: Variations in zones of inhibition of the four extracts against *S. typhi* at 3000 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	27.23 ± 0.40[a]
S. incanum	Water	21.67 ±0.42[b]
T. indica	DCM	19.93 ±0.15[c]
T. indica	Water	18.77 ±0.60[d]
T. indica	Water	17.77 ±0.15[e]
LSD		1.000

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD; least significant difference. Significant difference was noted across all extracts.

At 1500 µg/ml, there were significant difference between the control and all the four extracts. However, there was no significant difference between *S. incanum* water and DCM extracts. Among the extracts, *T. indica* water extract had the largest zone of inhibition, 18.20 ±0.82 mm, while, *S. incunum* DCM extract had the smallest zone of inhibition 16.60 ±0.36 mm. The data is displayed in Table 10.

Table 10: Variations in zones of inhibition of the four extracts against *S. typhi* at 1500 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	27.23 ± 0.40[a]
T. indica	Water	18.20 ±0.82[b]
T. indica	DCM	17.78 ±0.25[c]
S. incanum	Water	16.77 ±0.16[d]
S. incanum	DCM	16.60 ±0.36[d]
LSD		0.770

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD; least significant difference. No significant differences between *S. incunum* water and DCM extracts.

T. indica had wide inhibition zones against *S. typhi* at 750 µg/ml, while *S. incanum* DCM had the smallest zone. There was no significant difference between *S. incunum* and *T. indica* water extracts. Table 11 presents details on the analysis.

Table 11:Differences in zones of inhibition of the four extracts against *S. typhi* at 750 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	27.23 ± 0.40[a]
T. indica	DCM	16.27 ±0.35[b]
S. incanum	Water	15.93 ±0.21[bc]
T. indica	Water	15.17 ±0.25[cd]
S. incanum	DCM	14.77 ±0.50[d]
LSD		0.761

[a] Means represented by the same letter are not significantly different at α= 0.05

4.3.1.3 Activity of the Extracts against *S. aureus*

There were significant differences in activity of the extracts across all the extracts against *S. aureus* at 3000 µg/ml. The positive control, had the largest zone, 24.83 ±0.42 mm, while *S. incanum* water extract had the smallest zone, 9.00 ±0.1 mm as demonstrated in Table 12.

Table 12: Differences in zones of inhibition of the four extracts against *S. aureus* at 3000 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	24.83 ±0.42[a]
S. incanum	DCM	14.80 ±0.69[b]
T. indica	DCM	13.90 ±0.62[c]
T. indica	Water	12.00 ±0.72[d]
S. incanum	Water	9.00 ±0.1[e]
LSD		0.901

[a] Means represented by the same letter are not significantly different at α= 0.05

All the four extracts followed a similar trend at 1500 µg/ml. After the positive control, *S. incanum* DCM extract had the largest zone, 13.70 ±0.2 mm, while *S. incanum* water extract had the smallest zone, 7.80 ±1.25 mm as demonstrated in Table 13.

Table 13: Differences in zones of inhibition of the four extracts against *S. aureus* at 1500 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	24.83 ±0.42[a]
S. incanum	DCM	13.70 ±0.2[b]
T. indica	DCM	11.90 ±0.82[c]
T. indica	Water	10.83 ±0.25[d]
S. incanum	Water	7.80 ±1.25[e]
LSD		1.07

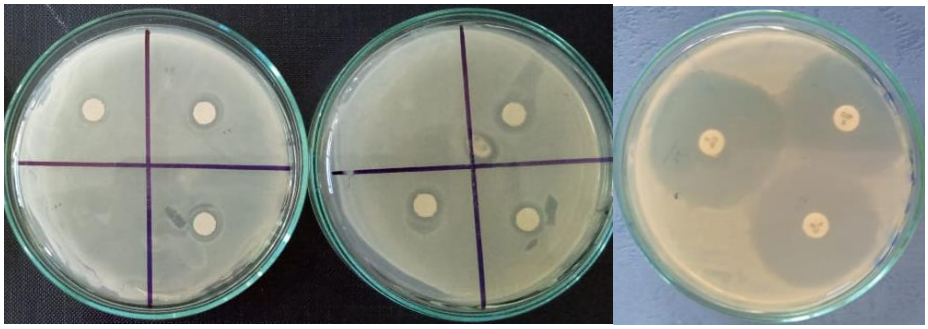
[a] Means represented by the same letter are not significantly different at α= 0.05

At 750 µg/ml, all the four extracts followed a different trend. There were no significant differences between three plant extracts *S. incanum* water and DCM extracts, and *T. indica* water extracts. Table 14 is a detailed representation of the differences in activity of the four extracts against *S. aureus*. Figure 6 is the images of *T. indica* against *S. aureus*.

Table 14: Differences in zones of inhibition of the four extracts against *S. aureus* at 750 µg/ml.

Plant/ Control	Extraction	Zones of Inhibition (mm ± SEM)
Control	Water	24.83 ±0.42[a]
T. indica	DCM	9.23 ±0.82[b]
S. incanum	Water	6.57 ±0.32[c]
S. incanum	DCM	6.47 ±0.39[c]
T. indica	Water	6.37 ±0.39[c]
LSD		0.10

[a] Means represented by the same letter are not significantly different at α= 0.05



1 2 3

Figure 7: Images of *T. indica* extracts against *S. aureus* at 750 µg/ml.

The bacteria was grown in MHA at 37 ^{o}C for 24 hours. Plate 1 has *T. indica* water extract, plate 2 *T. indica* DCM extracts and plate 3 was the positive control. From the image, the DCM extract had larger zones than water extracts.

There were significant differences in the four extracts applied against different bacterial strains at $\alpha = 0.05$. All the controls had the highest inhibition zones, with the largest highest zone being that of ciprofloxacin against *E. coli* (29.27 ±0.40 mm). *T. indica* water extract was the least active, against *S. aureus* at 750 µg/ml, with inhibition zones of 6.37 ±0.39 mm. On the other hand, the *S. incanum* DCM extract at 3000 µg/ml had the largest zone of inhibition (18.90 ±2.38 mm). The mean inhibition of the four extracts at different concentrations is detailed in Table 15.

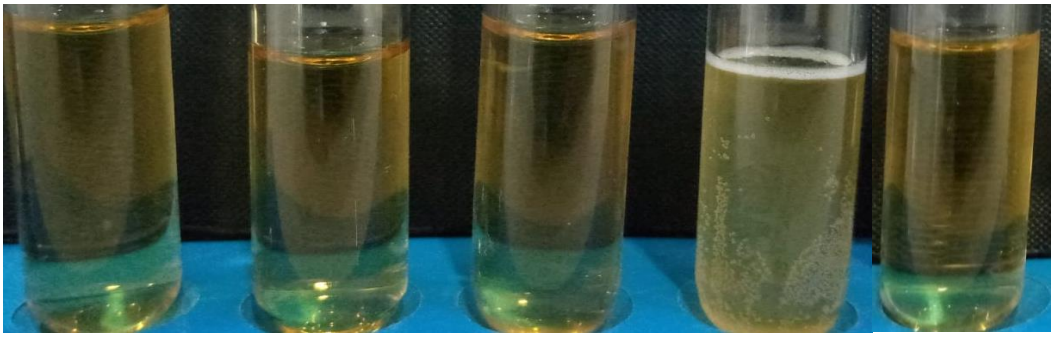
Table 15: Detailed representation of the differences in zones of inhibition for the four extracts at three concentrations against three bacteria strains.

Plant/ Controls	Extraction Method	Bacteria	Concentration (µg/mL)	Zone of inhibition (mm ± SEM)
Control	Water	*E. coli*	5	29.27 ±0.40[a]
Control	Water	*S. typhi*	5	27.23 ± 0.40[b]
control	Water	*S. aureus*	5	24.83 ±0.42[c]
S. incanum	DCM	*E. coli*	3000	22.03 ±0.31[d]
T. indica	Water	*E. coli*	3000	21.67 ±0.35[d]
S. incanum	Water	*S. typhi*	3000	21.67 ±0.42[d]
S. incanum	Water	*E. coli*	3000	20.01 ±0.92[e]
T. indica	DCM	*S. typhi*	3000	19.93 ±0.15[e]
T. indica	Water	*S. typhi*	3000	18.77 ±0.60[f]
T. indica	Water	*S. typhi*	1500	18.20 ±0.82[fg]
T. indica	Water	*E. coli*	1500	18.07 ±0.61[fgh]
T. indica	DCM	*E. coli*	3000	17.87 ±0.39[fh]
T. indica	DCM	*S. typhi*	1500	17.77 ±0.25[ghi]
S. incanum	DCM	*S. typhi*	3000	17.77 ±0.15[ghi]
S. incanum	DCM	*E. coli*	1500	17.73 ±0.23[ghi]
T. indica	DCM	E. coli	1500	17.37 ±0.47[ghij]
S. incanum	Water	*E. coli*	1500	17.33 ±0.67[hij]
S. incanum	DCM	*E. coli*	750	16.93 ±0.06[ijk]
T. indica	DCM	*E. coli*	750	16.93 ±0.40[ijk]
S. incanum	Water	*S. typhi*	1500	16.77 ±0.16[jkl]
S. incanum	DCM	*S. typhi*	1500	16.60 ±0.36j[kl]
T. indica	DCM	S. typhi	750	16.27 ±0.35[kl]
S. incanum	Water	*S. typhi*	750	15.93 ±0.21[lm]
T. indica	Water	*S. typhi*	750	15.17 ±0.25[mn]
S. incanum	DCM	*S. aureus*	3000	14.80 ±0.69[n]
S. incanum	DCM	*S. typhi*	750	14.77 ±0.50[n]
S. incanum	Water	*E. coli*	750	14.53 ±0.80[no]
T. indica	DCM	*S. aureus*	3000	13.90 ±0.62[op]
S. incanum	DCM	*S. aureus*	1500	13.70 ±0.2[op]
T. indica	Water	*E. coli*	750	13.07 ±0.67[p]
T. indica	Water	*S. aureus*	3000	12.00 ±0.72[q]
T. indica	DCM	*S. aureus*	1500	11.90 ±0.82[q]
T. indica	Water	*S. aureus*	1500	10.83 ±0.25[r]
T. indica	DCM	*S. aureus*	750	9.23 ±0.82[s]
S. incanum	Water	*S. aureus*	3000	9.00 ±0.1[s]
S. incanum	Water	*S. aureus*	1500	7.80 ±1.25[t]
S. incanum	Water	*S. aureus*	750	6.57 ±0.32[u]
S. incanum	DCM	*S. aureus*	750	6.47 ±0.39[u]
T. indica	Water	*S. aureus*	750	6.37 ±0.39[u]
LSD				0.8639
R- Square				0.993
CV				3.30%

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference, R-square= demonstrates the accuracy of the model and CV= coefficient of variation.

4.3.2 Minimum Inhibitory concentration

The MIC of *T. indica* aqueous extract was 125 µg/mL against *E. coli* and 125 µg/mL against *S. aureus*, while *S. incanum* water extract's MIC was 250 µg/mL against *E. coli* and 250 µg/mL and against *S. aureus*. The *T. indica* DCM extract MIC was 125 µg/mL against *E. coli* and 125 µg/mL against *S. aureus*, while *S. incanum* DCM extract had an MIC of 62.5 µg/mL against *E. coli* and 125 µg/mL against *S. aureus*. Figure 7 represents MIC dilution tubes for *T. indica* DCM extract against *S. aureus*. From the setup, there was bacterial growth in tube 4 alone while tube 1, 2, 3 and 5 had no visible turbidity.



1 2 3 4 5

Figure 8: MIC dilution tubes for *T. indica* DCM extract against *S. aureus.*

The bacterial was grown in MH- broth at 37 ^{o}C for 24 hours. The concentrations of the extracts were 500 µg/ml (tube 1), 250 µg/ml (Tube 2), 125 µg/ml (Tube 3) and 62.5 µg/ml (tube 4). Tube 5 was the positive control. Turbidity is visible on in tubes tube 4 alone.

4.3.3 Minimum Bactericidal Concentration

The *T. indica* aqueous extract had an MBC value of 250 µg/mL against *E. coli* and 250 µg/mL against *S. aureus*, while *S. incanum* water extract had an MBC of 250 µg/mL against *E. coli* and 500 µg/mL against *S. aureus*. The MBC setup for *S. incanum* against *S. aureus* is demonstrated in figure 9. The MBC values of *T. indica* DCM extract were 125 µg/mL against *E. coli* and 500 µg/mL against *S. aureus*. *S. incanum* DCM extract had an MBC of 125 µg/mL against *E. coli* and 250 µg/mL against *S. aureus*.

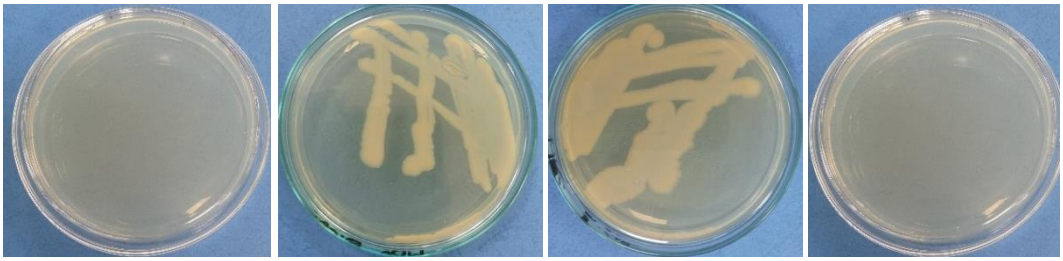


Plate 1 Plate 2 Plate 3 Plate 4

Figure 9: Images of MBC plates of *S. incanum* water extract against *S. aureus.* The bacteria was cultured nutrient agar at 37 ^{o}C for 24 hours. The dilutions were, 500 µg/Ml (Plate 1), 250 µg/mL (Plate 2) and 125 µg/Ml (Plate 3). Plate 4 was the positive control.

4.4 Determination of Anti-Inflammatory Activity of Plant Extracts

The four extracts demonstrated significant differences in RBC stabilization at different concentrations. All the differences were tested at α= 0.05. At 4000 µg/ml, *S. incanum* and *T. indica* DCM extracts were more effective than the control (diclofenac sodium). *T. indica* water extract was the least effective with a percentage stabilization of 31.99 ±0.06% as demonstrated in Table 16. The percentage stabilization of all the four extracts and the control were significantly different from each other. The overall analysis is attached ass Appendix II.

Table 16: Percentage stabilization of the four extracts at 4000 µg/ml.

Plant/ Control	Extraction	Percentage Stabilization (mm ± SEM)
S. incanum	DCM	59.41 ±0.06[a]
T. indica	DCM	56.94 ±0.20[b]
Control	Water	55.33 ±0.08[c]
S. incanum	Water	40.91 ±0.04[d]
T. indica	Water	31.99 ±0.06[e]
LSD		1.610

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference

At 2000 µg/ml, all the extract had different percentage stabilizations. However, at this concentration, *T. indica* DCM extract had a higher stabilization, 77.70 ±0.09% than *S. incanum* DCM extract, 59.60 ±0.16%. The two extracts were more effective than the control. Water extracts were less effective, with *S. incanum* water extract being the least effective, as demonstated in Table 17.

Table 17: Percentage stabilization of the four extracts at 2000 µg/ml.

Plant/ Control	Extraction	Percentage Stabilization (mm ± SEM)
T. indica	DCM	77.70 ±0.09[a]
S. incanum	DCM	59.60 ±0.16[b]
Control	Water	55.33 ±0.08[c]
T. indica	Water	30.95 ±0.06[d]
S. incanum	Water	2.59 ±0.39[e]
LSD		4.270

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference

The largest difference between the extracts was reported at 1000 µg/ml. *S. incanum* DCM extract had the highest percentage stabilization, 83.87 ±0.10% while *S. incanum* water extract had the lowest percentage stabilization, 16.58 ±0.06%. The DCM extracts were also more effective than the control, while the water extracts were the least effective.

Table 18: Percentage stabilization of the four extracts at 1000 µg/ml.

Plant/ Control	Extraction	Percentage Stabilization (mm ± SEM)
S. incanum	DCM	83.87 ±0.10[a]
T. indica	DCM	55.61 ±0.47[b]
Control	Water	55.33 ±0.08[c]
T. indica	Water	36.12 ±2.03[d]
S. incanum	Water	16.58 ±0.06[e]
LSD		0.280

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference

A different trend was noted at 500 µg/ml. The control was more effective that all the four extracts, with a stabilization of 55.33 ±0.08%. Also, both *T. indica* extracts (water and DCM) had higher RBC stabilization compared to the *S. incanum* extracts. For each of the plant species, DCM extracts had higher percentage stability compared to water extracts as demonstrated in Table 19.

Table 19: Percentage stabilization of the four extracts at 500 µg/ml.

Plant/ Control	Extraction	Percentage Stabilization (mm ± SEM)
Control	Water	55.33 ±0.08[a]
T. indica	DCM	40.29 ±0.22[b]
T. indica	Water	35.08 ±0.38[c]
S. incanum	DCM	17.81 ±0.18[d]
S. incanum	Water	16.15 ±0.06[e]
LSD		1.66

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference

Significant differences in percentage stabilization were noted across all samples at different concentrations. *S. incanum* DCM extract at 1000 µg/mL concentration had the highest stabilization (83.87 ±0.10%), followed by *T. indica* DCM extract at 2000 µg/mL concentration (77.70 ±0.09%), while the *S. incanum* water extract at 2000 µg/mL concentrations had the lowest stabilization (2.59 ±0.39%). However, there were no significant differences in percentage stabilization between several treatments. For example, no statistical differences between *S. incanum* DCM extract at 2000 µg/mL concentrations (59.60 ±0.16%) and *S. incanum* DCM extract at 4000 µg/mL concentration (59.41 ±0.06%). Detailed information on the RBC stabilization assay is provided in table 20.

Table 20: A comprehensive overview of the differences in percentage stabilization of the extracts at different concentrations.

Plant/ Control	Extraction	Concentration (µg/mL)	Percentage Stabilization ± SEM
S. incanum	DCM	1000	83.87 ±0.10[a]
T. indica	DCM	2000	77.70 ±0.09[b]
S. incanum	DCM	2000	59.60 ±0.16[c]
S. incanum	DCM	4000	59.41 ±0.06[c]
T. indica	DCM	4000	56.94 ±0.20[d]
T. indica	DCM	1000	55.61 ±0.47[e]
Control	Water	100	55.33 ±0.08[e]
S. incanum	Water	4000	40.91 ±0.04[f]
T. indica	DCM	500	40.29 ±0.22[f]
T. indica	Water	1000	36.12 ±2.03[g]
T. indica	Water	500	35.08 ±0.38[h]
T. indica	Water	4000	31.99 ±0.06[i]
T. indica	Water	2000	30.95 ±0.06[j]
S. incanum	DCM	500	17.81 ±0.18[k]
S. incanum	Water	1000	16.58 ±0.06[l]
S. incanum	Water	500	16.15 ±0.06[l]
S. incanum	Water	2000	2.59 ±0.39[m]
LSD			0.8474
R-Square			0.999654
CV %			1.208101

[a] Means represented by the same letter are not significantly different at α= 0.05. LSD= Least Significant Difference, R-square= demonstrates the accuracy of the model and CV= coefficient of variation

4.5 Determination of Cytotoxicity of the Plant Extracts

The extracts had different toxicity thresholds, which were higher than the positive control (Vincristine Sulfate). *S. incanum* DCM extract had a LD_{50} of 2341 µg/mL. as demonstrated in table 21. The summary of the models used to analyze the toxicity of the extracts is represented in Appendix III.

Table 21: The LD values of *S. incanum* DCM extract. The LD_{50} of the extracts was obtained at the 0.50 probability level. Analysis done at $\alpha = 0.05$.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	2.056	0.000523	14.437
0.20	27.620	0.637	93.637
0.30	155.193	32.695	712.077
0.40	638.825	196.348	15769.00
0.50	2341.00	560.532	491144.00
0.60	8575.00	1379.00	17754360.00
0.70	35298.00	3470.00	938025872.00
0.80	198331.00	10347.00	1.22645×10^{11}
0.90	2662237.00	51949.00	1.93134×10^{14}

The *S. incanum* water extract had a lower LD_{50} value, 1000 µg/Ml, compared to *S. incanum* DCM extract. The summary of the regression analysis is represented in Table 22.

Table 22: The LD values of *S. incanum* water extract. The LD_{50} of the extracts was obtained at the 0.50 probability level. Analysis done at $\alpha = 0.05$.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	833.690	796.296	872.841
0.20	891.578	851.587	933.448
0.30	932.261	890.445	976.041
0.40	966.991	923.617	1012.402
0.50	1000.000	955.145	1046.960
0.60	1034.135	987.749	1082.699
0.70	1072.660	1024.546	1123.033
0.80	1121.605	1071.296	1174.277
0.90	1199.485	1145.682	1255.814

T. indica water extract had an LD_{50} of 1104.53 µg/mL. The toxicity threshold of the *T. indica* water extract was higher than *S. incanum* water extract. Therefore, the *T. indica* water extract is less toxic than *S. incanum* water extract. The findings are represented in Table 23.

Table 23: The LD values of *T. indica* water extract at 0.50 probability. Analysis done at $\alpha = 0.05$.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	913.844	864.093	966.459
0.20	980.051	926.696	1036.478
0.30	1026.690	970.796	1085.802
0.40	1066.571	1008.506	1127.980
0.50	1104.532	1044.400	1168.126
0.60	1143.844	1081.572	1209.701
0.70	1188.276	1123.585	1256.692
0.80	1244.823	1177.054	1316.495
0.90	1335.010	1262.331	1411.874

T. indica DCM extracts had the lowest LD_{50} values of 113.58 µg/mL. The results show that the *T. indica* DCM extract was more toxic than the *T. indica* water extract and *S. incanum* extracts. The finding of LD_{50} values is summarized in Table 24.

Table 24: The LD values of *T. indica* DCM extract. The LD_{50} of the extract was obtained at the 0.50 probability level. Analysis done at $\alpha = 0.05$.

Probability	Concentration (µg/mL)	95% Fiducial Limits	
0.10	95.948	90.541	101.679
0.20	102.108	96.353	108.207
0.30	106.419	100.421	112.775
0.40	110.089	103.884	116.664
0.50	113.568	107.167	120.351
0.60	117.156	110.553	124.154
0.70	121.196	114.365	128.435
0.80	126.313	119.194	133.858
0.90	134.422	126.846	142.451

The positive Control had the lowest toxicity threshold with an LD_{50} of 11.92 µg/mL. Therefore, the positive control is more toxic than the four as demonstrated in Table 25.

Table 25: The LD values of positive control (vincristine sulfate) at 0.50 probability level. Analysis done at $\alpha = 0.05$.

Probability	Concentration	95% Fiducial Limits	
0.10	3.620	1.138	5.711
0.20	5.620	2.549	7.971
0.30	7.528	4.253	10.185
0.40	9.567	6.266	12.858
0.50	11.920	8.548	16.661
0.60	14.853	11.056	22.767
0.70	18.875	13.940	33.586
0.80	25.285	17.798	56.086
0.90	39.254	24.828	125.593

CHAPTER FIVE

DISCUSSION

5.1 Percentage Yield of the Extracts

The percentage yield varied across the four extracts. *S. incunum* water extract had the highest yield (4.7% w/w), followed by *S. incanum* DCM extract at 2.54% and *T. indica* water extract at 2.53%, while *T. indica* DCM extract had the lowest yield (1.07% w/w). Other extraction modes used by other authors had a higher yield. For example, hydromethanol leaf extract of *S. incanum* yield was at 28.45% w/w as reported by Belayneh *et al*. (2021), while methanolic leaf extracts of the same plant had a 16.5% w/w yield Andargie *et al.* (2022). Similarly, other studies also had a higher yield of *T. indica* extracts using water (15.35 % w/w), acetone (20.54 % w/w) and methanol (31.37% w/w) (Sandesh *et al*., 2014). However, Nwodo *et al.,* 2011 achieved a lower yield using cold water (5.76% w/w), hot water (5.21% w/w) and ethanol (4.38% w/w).

Unlike the current study where a rotary vaporator was used, the studies reporting high yield use hot oven at 39 ^{o}C (Andargie *et al.,* 2022; Belayneh *et al.*, 2021). For the *T. indica* extracts, the researchers used Soxhlet extraction method, which is more efficient (Sandesh *et al*., 2014). For the analysis, it is evident that the mode of extraction and the devices used affects the percentage yield. Therefore, although rotary evaporators reduce extraction time, reduce decomposition of the phytochemicals and promote solvent penetration, it yields is lower compared to using a temperature-controlled oven (Lezoul *et al*., 2020). The second factor that caused variation in extraction is solvent choice. Regardless of the method of extraction and devices used, it is evident that the yield reduced with a reduction in solvent polarity. In the present study, water and DCM were the solvent of choice. Water is highly polar with a polarity index of 10.2, while DCM is less polar with a polarity index of 3.1 (Altemimi *et al*., 2017). The aqueous extraction protocol had a higher extract yield than the DCM methods. The higher yield obtained in water extraction demonstrates that most phytochemicals in the leaves were polar. Simillary, in other studies, water extraction had higher yield, justifying the conclusion on the plants having high concentrations of polar solvents. Extraction temperature could have also been a factor contributing to the differences. When extraction temperature is increased, the extraction time reduces. For example, increasing the extraction temperature to 60 ^{0}C reduces extraction time to 120 minutes

(Che Sulaiman *et al*., 2017). At room temperature, researchers recommend extraction for 48 hours (Sieberi *et al*., 2020). Although a 48-hour extraction was used in this study to increase yield, the production was lower than when a higher temperature was used by other researhers.

Phytochemical distribution in plants of the same species also affects the yield. The variation occurs due to internal and external factors. Internal factors that affect phytochemical generation include genetic profiles, phenological stage, and plant organs. The two plants, *S. incanum* and *T. indica*, are from different families, hence the differences in genetic profiles. Plant growth phase determines the quantity of phytochemicals present (Adegbaju *et al*., 2020). Studies have shown that harvesting plants before flowering increases the yield of various phytochemicals and thus, harvesting *S. incanum* and *T. indica* before flowering guaranteed high phytochemical yield (Jimoh *et al*., 2018). External factors that affect phytochemical yield include light, pathogens, herbivores attack, temperature and soil parameters such as pH (Adegbaju *et al*., 2020). The internal and external factors contributed to the variations in the types of phytochemicals present in *S. incanum* and *T. indica*. Also, the variation in growth conditions in different regions could have contributed to the variation in phytochemical yield.

5.2 Qualitative Phytochemical Analysis

The chemical qualitative analysis of phytochemicals revealed that *S. incanum* and *T. indica* water and DCM extracts contained phenols, saponins, flavonoids, alkaloids and terpenoids. However, *S. incanum* DCM extract and *T. indica* water extract tested negative for glycosides while anthraquinones tested negative in all extracts. The GC-MS results concurred on the availability of flavonoids, phenols and alkaloids. The analysis demonstrated some similarities and differences in the metabolites present to those published in the literature. For example, similar to the results in this study, other researchers have shown that *S. incanum* leaf extracts do not have anthraquinones (Belayneh *et al*., 2021). The chemical test results from the *T. indica* water extracts were similar to results published in other studies (Abdalla and Muhammad, 2018). However, the researchers noted that the *T. indica* leaf extracts had significant amounts of anthraquinones, which were not identified in this analysis. The findings from

qualitative analysis of *S. incanum* extracts were similar to those reported by Sbhatu and Abraha (2020). The researchers also reported that *S. incanum* ethanolic leaf extracts had significant amounts of glycosides, which were absent in *S. incanum* DCM extract used in thus study (Sbhatu and Abraha, 2020).

The analysis revealed several compounds that had similar chemical structure (with minor variations) to those identified by other researchers. For example, the *S. incanum* extract used in this study had 2-chloro-2-methyl-butane, while previous studies found significant amounts of 2, 3-butanediol (Yetayih and Ravichandran, 2020). The minor differences between the two compounds are attributed to the molecular diversity of the same species growing in different regions of the world (Adegbaju *et al*., 2020). A similar trend was noted in *T. indica* GC-MS results. Although the extract has several unique phytochemicals such as 1, 2-Benzisothiazol-3-amine, majority of the compounds identified in this study are similar to those published by other authors. For example, the *T. indica* extract had Phthalic acid, which was also confirmed in other extracts (Sharma *et al.*, 2021; Mehdi *et al*., 2020). In addition, the plant had several esters with structural similarities to those identified by other researchers. Butyl tetradecyl ester from *T. indica* used in this study had a similar chemical structure to Butyl Octyl Ester, as identified by Sharma *et al*. (2021). Despite the high variations among the extracts, the analysis shows that phthalic acid is a common compound in *T. indica* from different world zones.

The differences in phytochemicals present in the plants of the same species are influenced by climatic variation, interspecies interactions, soil properties, and solvent differences (Defossez *et al*., 2021; Wakeel *et al*., 2019; Silva *et al.*, 2018; Kumar *et al*., 2017). The solvents used have different polarities, with water being more polar than DCM (Abubakar and Haque, 2020). Due to this, phytochemicals present in the water extracts are more polar than those in the DCM extracts. The difference in solvents could have also contributed to the variation in phytochemicals identified using GC-MS. In most studies, the researchers used more polar solvents such as methanol and ethanol, while in this case, phytochemicals were extracted with DCM, a less polar solvent. The differences in compounds identified in this study to those reported by other researchers

is also attributed to various ecological variations such as differences in altitude (Kumar *et al.*, 2017).

5.3 Determination of Antibacterial Activities of the Plant Extracts

The disc diffusion method revealed that the zones of inhibition against all the bacteria varied with concentration, with 3000 µg/ml concentration being the most effective while the 750 µg/ml being the least effective. Gram negative bacteria were more prone, with *S. incanum* DCM extract being more effective against *E. coli* (22.03 ±0.31 mm). *T. indica* water extract at 750 µg/ml was the least effective against *S. aureus* with zones of 6.37 ±0.39 mm. All the extracts had different MIC and MBC values. For instance, *T. indica* aqueous extract had an MIC of 125 µg/ml and MBC of 250 µg/mL against *E. coli*, while *S. incanum* had an MIC of 125 µg/mL and an MBC of 250 µg/mL against *S. aureus*. The MIC and MBC values were higher against *S. aureus* that *E. coli*.

The findings align with those reported by other researchers using the same plant species or plants of the same genus. For example, Sbhatu *et al.* (2020) noted that the ethanolic leaf extracts of *S. incanum* had zones of 16.06 mm against *E. coli*, 11. 34 mm against *S. typhi* and 16.04 mm against *S. aureus*. The smaller zones of inhibition also aligned with their MIC and MBC values. The researchers reported higher MIC and MBC values of 1.56 mg/ml in *E. coli* and 1.56 mg/ml against *S. aureus*. Contrary to the findings in this research, Taye *et al*. (2011) reported no zones of inhibition when methanolic leaf extracts were used against E. coli. On the other hand, Lima *et al*. (2017) reported that *T. indica* extracts were effective at 500 µg/ml, a value within the ranges used in thus study. The differences in activities of *S. incanum* against the same bacterial strains is attributed to differences in solvents used, which affects the class of phytochemicals extracted.

The minor differences in the antibacterial efficacy of the two plants used in this study are as a resule of the similarities in their phytochemical composition. From the preliminary chemical tests for the phytochemicals, all the four extracts have similar metabolites, apart from *S. incanum* DCM and *T. indica* water extract, which tested negative for glycosides. Several phytochemicals tested in the preliminary chemical analysis are known to possess antibacterial activities, and some are also effective

against MRSA (Okwu *et al*., 2019). All four extracts tested positive for phenols, which are have significant actibacterial potency. The compound inhibits bacterial growth by interfering with biofilm formation and inhibiting β lactamases (Mandal *et al*., 2017). Due to this, phenolics can be used to increase drug sensitivity among drug-resistant bacterial such as *S. aureus* (Miklasińska-Majdanik *et al*., 2018).

Saponins also have antibacterial activities against several bacterial strains. Saponins extracted from *Albizia adianthifolia* were effective against Gram-negative bacteria, with MIC's varying from as low as 16 µg/mL to 128 µg/ml (Sanfack *et al*., 2019). The four extracts had high saponins levels (due to the large volume of stable foam in the test tubes), which justifies the high efficacy of *S. incanum* and *T. indica* against the two Gram-negative bacteria compared to *S. aureus*. Studies on specific flavonoid classes have also shown that this phytochemical class significantly affects bacterial growth, with some flavonoids being more effective than standard drugs (Farhadi *et al*., 2019). For example, a purified chalcone extract of various plants was found to be six times more effective than the antibiotics used in hospitals. Some flavonoids are more effective against Gram-negative bacteria than the Gram-positive strains. For instance, the MIC values of flavone against *S. aureus* was found to be 1000 µg/mL, while its MIC against *E. coli* and *P. aeruginosa* are 500 µg/ml (Adamczak *et al*., 2019). Similar trends were noted with the four extracts used in this study. All the extracts used in this study were less effective against *S. aureus* than against Gram-negatives.

The *S. incanum* and *T. indica* extracts had a high alkaloid composition, which are proven effective aganst numerous bacteria. For instance, researchers reported that alkaloid extracts of *Berberis microphylla* had MIC values of 83-333 µg/mL and MBC values of 167- 717 µg/ml (Manosalva *et al*., 2016). However, some alkaloids are less effective, with MIC values exceeding 1.67 mg/mL (Mabhiza *et al*., 2016). *S. incanum* and *T. indica* have different alkaloid compositions, which could have affected their efficacy against the three bacterial strains.

Furthermore, the extracts had terpenoids, which exhibit their antibacterial activity through the hydroxyl groups present in their chemical structure (Guimarães *et al*., 2019). Electron microscopy on bacteria strains subjected to different concentrations of

terpenoids revealed significant disruption on bacterial cell membrane (Guimarães *et al*., 2019). Having an extracellular membrane could have increased the susceptibility of *E. coli* and *S. typhi* used in this study, denoted by larger zones of inhibition compared to those of *S. aureus*. Comparing the findings in this study to those published by other authors, it is evident that the phytochemicals present were the basis for the antibacterial effeciacy of the extracts. The diversity of phytochemicals of the same class contributed to the differences in antibacterial activities of *S. incanum* and *T. indica*.

Plant phytochemicals have varying modes of action. The most common include DNA damage, inhibition of synthesis of bacterial proteins, losing ATP, disruption of cell walls and cell membranes which causes leakage in cell constitutes, and direct interference with synthesis of cell walls and cell membrane components (Khameneh *et al*., 2021). Also, some studies have shown that the phytochemicals often affect bacterial communication, biofilm formation, and the efficiency of efflux pumps, whose role is to reduce the intracellular concentration of toxic compounds and metabolites (Khare *et al*., 2021). In addition, some phytochemicals, such as phenols, inhibit β- lactamases produced by various bacterial trains (Mandal *et al*., 2017). The ability to compromise the efficiency of efflux pumps and inhibit β- lactamases justify the efficacy of the extracts when used alongside conventional medications (Manosalva *et al*., 2016). Therefore, the phytochemicals present in the *S. incanum* and *T. indica* extracts could have imposed their antibacterial activity using one or more of these mechanisms of action.

Phthalic Acid Esters (PAE) produced by *T. indica* is effective against *S. aureus, E. faecalis, P. aeruginosa* and *E. coli* (Huang *et al*., 2021). The *T. indica* extracts in this study had significant amounts of diisooctyl phthalate which has antifungal and antibacterial activities (Habib and Karim, 2009). Researchers have also shown that diisooctyl phthalate is effective against several bacterial species, including *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and *Streptococcus pyogenes* (Zellagui *et al*., 2012). Although 1,2-Benzenedicarboxylic acid concentration was the lowest among all compounds extracted in this study, the phytochemical has high antibacterial activity (Shoge *et al*., 2016). The compound generated zones of inhibition between 27-32 mm when tested against *E. coli, S. aureus, S. typhi, Shigella dysentriae*, and *Streptococcus*

faecalis (Shoge *et al*., 2016). 1,2-Benzisothiazol-3-amine identified in *S. incanum* and *T. indica* has significant antibacterial effects. Other researcher had extracted the same compound and other structurally similar alkaloids and reported high activity against several bacterial strains (Priyanka *et al*., 2015). These specific phytochemicals identified through GC-MS analysis provides more insight on the basis for the high antibacterial activity of *S. incanum* and *T. indica*. These compounds can be extracted from the two plants used in this study and analyzed independently for their ability to treat bacterial infections.

5.4 Determination of Anti-inflammatory Activity of Plant Extracts

The anti-inflammatory analysis revealed that all the extracts were effective in stabilizing RBCs at different concentrations. However, the stabilization was not dose-dependent, in that S. incanum DCM extract has high stability at 1000 µg/ml (83.87 ±0.10%), while T. indica DCM extract had the highest stability at 2000 µg/ml (77.70 ±0.09%). Across all the concentrations, the DCM extracts were more effective that water extracts. Additionally, the first three concentrations, (4000 µg/ml, 2000 µg/ml and 1000 µg/ml) had a higher stability than the control (diclofenac sodium).

The findings from this assay support other studies conducted on the anti-inflammatory activities of plants of the same genus as *S. incanum* and *T. indica*. *Solanum aethiopicum*, extracts inhibited hemolysis of RBCs at all doses between 800 µg/mL to 100 µg/ml (Anosike *et al*., 2012). These researchers reported percentage membrane stabilization of between 86.67 ± 3.06% and 46.53 ± 2.52%, with the highest stabilization noted at 800 µg/ml, while the lowest stabilization was reported at 400 µg/mL. Stabilization at 100 µg/mL was higher than at 400 µg/ml (a non-dose-dependent manner). Similarly, in this study, the concentrations used ranged from 4000 µg/ml to 500 µg/ml, with the highest stability reported at 1000 µg/ml. Other researchers reported that *Solanum diploconos* hydroalcoholic extracts had anti-inflammatory activity at low concentrations (Benvenutti *et al*., 2021). Further, *in vivo* studies have also shown that plants of *Solanum* genus such as *Solanum lycocarpum* and *Solanum xanthocarpum* has high anti-inflammatory activity at varying concentrations (da costa *et al*., 2015; More *et al*., 2013). Plants of the same genus are likely to have several

similar phytochemicals, which justify, the anti-inflammatory ability of *S. incanum* used in this study.

The RBC stabilization model has been used in assessing the efficacy of other plant extracts. For instance, researchers noted that *Piper chaba* ethanolic root extract had 52.667% RBC stabilization efficacy at 500 µg/ml, while *Aloe vera* had a 20.86 ± 0.77% stabilization efficiency at 1000 µg/ml (Paul *et al*., 2021; Yesmin *et al*., 2020). Besides, the model is also applicable when comparing plants of different species (Chowdhury *et al*., 2017). Although with different efficacy levels, all the extracts used in this study had significant protection on the erythrocyte membrane. The plant extracts could pose anti-inflammatory properties by acting on one or more enzymes involved in inflammation, or by interfering with inflammatory related signaling pathways.

While assessing the analgesic and anti-inflammatory efficacy of *T. indica* roots, bark, seeds stem and leaves, Komakech *et al*. (2019) noted that the extracts from the parts effectively managed inflammation at a low concentration. The researchers recommended that more research should be done on other plant parts, hence the basis of this research. Similar to their findings, this study also noted that the leaf extracts had high anti-inflammatory activity. Researchers using *in vivo* models also reported that ethanolic stem extracts of *T. indica* is effective at 154.5 ± 2.6 mg/kg, while the root extracts of the plant effectively managed oedema at 118.1 ± 1.9 mg/kg (Borquaye *et al*., 2020). Compiling the available evidence with the findings in this study, all parts of *T. indica* are effective in managing inflammation. The similarity is attributed to the similarities in phytochemical composition of the plant parts. Due to this, *T. indica* seeds and other parts can be used to manage conditions such as arthritis (Sundaram *et al*., 2015).

The potency of the extracts depends on the phytochemicals present and the concentration of individual phytochemical classes. Having high concentration of a bioactive compound guarantees high anti-inflammatory activity. The differences in phytochemical composition explain why *T. indica* DCM extract was more effective than *S. incanum* extracts. Besides, the *T. indica* at 4000 µg/ml to 1000 µg/ml concentrations had a better RBC stabilization percentage than the standard drug. The

quantity of anti-inflammatory phytochemicals varied in the four plant extracts used in this study. The phytochemicals with the highest RBC stabilization are non-polar. This is because the DCM extracts of the two plants had better stabilization compared to the water extracts. Several phytochemicals present in the extracts have anti-inflammatory potency. The chemical tests reveal that all the extracts had significant amounts of terpenoids. Terpenes control inflammation by activating AMP-Activated Protein Kinase (AMPK), which stimulates various energy metabolic pathways, such as lipid oxidative pathways (Liu *et al*., 2021; Miki *et al*., 2019). Studies have shown that decreased activity of AMPK caused low-grade inflammation, which is associated with diseases such as atherosclerosis and type 2 diabetes (Miki *et al*., 2019). Terpenes also control inflammation by inhibiting pathways such as the NF-Kβ that lead to the production of inflammatory mediators such as IL-1 and TNF-α, and also inhibits production of LPS-induced inflammatory mediators (Prado-Audelo *et al*., 2021; Liu *et al*., 2021). The presence of terpenes in *S. incanum* and *T. indica* suggests that the two plants could manage inflammation-related diseases by interfering with these pathways.

The DCM extracts of *S. incanum* and *T. indica* also had high concentrations of alkaloids and flavonoids, as demonstrated in the GC-MS results. Besides this, the chemical tests revealed that the water and DCM extracts of the two plants had high amounts of saponins. These three phytochemicals are effective in manageing diseaases caused by unregulated inflammation. For instance, Hashmi *et al*. (2018) demonstrated that phytochemicals of this class often inhibit the COX-2 enzyme. The enzyme is involved in the synthesis of PGE, which stimulates inflammatory pathways, leading to production of pro-inflammatory mediators. The ability of alkaloids to inhibit COX-2 has also triggered research interest to assess how various alkaloid derivatives can be used to inhibit tumor growth. In addition to the inhibition of COX-2, alkaloids also inhibit LOX-5, an enzyme involved in thromboxane production (Wang *et al*., 2021). Other publications have also revelaed that alkaloids from various plant extracts can be used to manage Inflammatory Bowel Disease (IBD) due to their ability to inhibit MAPK and NF-κB signalling pathways (Yang *et al*., 2021; de Almeida *et al*., 2017). The major alkaloid identified in the *T. indica* DCM extract was 1,2-Benzisothiazol-3-amine, which, alongside other isothiazole derivatives, have been proven effective in managing inflammation (Alam *et al*., 2021). *S. incanum* used in this study also had a

significant amount of isothiazole derivatives, which justify its high percentage RBC stabilization. *S. incanum* also had a high concentration of propenamide derivatives, which are proven to have anti-inflammatory and anti-oxidant activity (Gökçe *et al*., 2009). The two compounds could be the reason why the extracts were effective stabilizing the RBCs.

Flavonoids and saponins also inhibit enzymes and signaling pathways involved in inflammation. In addition to their efficacy in inhibiting COX-2 and LOX-5, flavonoids also inhibit nitric oxide synthase, autophagy and inflammasome activation, and also interfere with several inflammation-related signaling pathways such as Nrf2, PPAR, and AP-1 (Lim *et al*., 2019; Chen *et al*., 2018). The three pathways have major contributions to developing Alzheimer's disease and other related inflammatory diseases (Liu *et al*., 2019). These pathways are used as new drug targets for anti-inflammatory drugs (Lim *et al*., 2019). For example, the Nrf2 is a promising therapeutic target due to its ability to regulate heme oxygenase-1 (HO-1), which plays a crucial role in modulating the vascular phases of inflammation (Saha *et al*., 2020). Phthalic acid reported in this study had high anti-inflammatory activities, anti-diabetic, antitumor, and antiviral properties (Huang *et al*., 2021). The high concentration of phthalic acid derivatives explains the high anti-inflammatory activity of the *T. indica* extracts. Although the phytochemical was not identified in *S. incanum* extracts, it is common among other plant species within the *Solanaceae* family. The Glycosides present in *S. incanum* (water extract) and *T. indica* (DCM extract) could also help manage inflammation. Glycosides achieve this by regulating the MAPK, NF-κB and JNK pathways (Dong *et al*., 2018). The presence of diverse phytochemical classes in *S. incanum* and *T. indica* could be the reason for high anti-inflammatory activity.

5.5 Determination of the Cytotoxicity of the Plant Extracts

The toxicity analysis demonstrated varying ranges of toxicity thresholds, with S. incanum DCM extract having the highest threshold at 2341 µg/mL. On the other hand, *S. incanum* water extract had an LD_{50} of 1000 µg/ml, while *T. indica* water extract had an LD_{50} of 1104.53 µg/mL. Among the four extracts, *T. indica* DCM extracts had the lowest LD_{50} values of 113.58 µg/mL. Studies on different parts of *S. incanum* revealed that the plant contains cytotoxic phytochemicals. For example, an *in-vitro* toxicity

analysis of *S. incanum* hydromethanol root and leaf extracts demonstrated that the extracts were toxic at 2 g/kg (Belayneh *et al*., 2021). Furthermore, *S. incanum* fruit extracts induced sub-acute toxicity at 400 mg/kg dosages (Feyera *et al*., 2017). *S. incanum* fruits were toxic to sheep, causing brain congestion, damage to hepatocytes and kidney cells, lung emphysema, neural necrosis and damage of Purkinje cells (Thaiyah *et al.,* 2011). Although no *in vivo* tests on *S. incanum* were identified, other studies have shown that plants of the genus *Solanum* have varying toxicity levels (Niño *et al*., 2006). *Solanum ovalifolium* had LD_{50} values of 1.00 mg/mL, *Solanum leucocarpun* (LD_{50} 0.44 mg/mL), *Solanum deflexiflorum* (0.68 mg/mL) and *Solanum lepidotum* (1.00 mg/mL) (Niño *et al*., 2006). The study linked the high toxicity of the extracts against brine shrimps to the high steroids, triterpenes and saponins present in the extracts. In this study, *S. incanum* DCM extract had LD_{50} of 1000 µg/mL, close to the value reported in other plants within the *Solanum* genus. The results suggest that *S. incanum* is less toxic when used at high concentration.

Available literature have shown varying toxicity of *T. indica* extracts. Researchers demonstrated that *T. indica* ethanol extracts had LD_{50} levels of between 832 µg/mL and 5 019 µg/ml (Nwondo *et al*., 2011). The LD_{50} values reported in this study are within the range reported by Nwondo *et al*. (2011). *T. indica* ethanol and methanol seed extracts have lower toxicity thresholds, ranging between 30 µg/mL and 100 µg/mL. The higher toxicity values could be due to the difference in the phytochemical present in the fruits and leaves of the extract. *T. indica* DCM bark extracts could help manage cancer, due to its ability to induce apoptosis in PA-1 cells and HeLa cells (Shirisha and Varalakshmi, 2017). Besides this, the toxicity of various extracts of *T. indica* justifies its application in root canal treatment in endodontic procedures (Wulandari, 2008).

The brine shrimp lethality test demonstrated different toxicity levels for *the S. incanum* and *T. indica* extracts. Additionally, the DCM extracts had lower toxicity compared to the water extracts. The difference in toxicity is dependent on the differences in phytochemicals present in extracts. Several phytochemicals compounds could have contributed to the differences in toxicity levels. For example, researchers have demonstrated that pyrrolizidine alkaloids have high cytotoxicity levels in animal models (Seremet *et al*., 2018). Similarly, the chemical tests of plant extracts used in this

study revealed that the four extracts contained alkaloids, which was confirmed using the GC-MS analysis. The high alkaloid composition could have influenced the toxicity levels of the plant extracts used in this study.

Flavonoids and sesquiterpenes also determine the toxicity levels of plant extracts (Butala *et al.*, 2021). Other studies have also proven that crude saponin extracts are highly toxic to animal cells and can be used in cancer treatment (Sobolewska *et al*., 2020; Alam *et al*., 2017). The chemical tests demonstrated high amounts of saponins. Although the GC-MS analysis could not reveal the actual saponins present, this class of phytochemicals could have contributed to the toxicity of the plant extracts. Presence of glycosides in plat extracts may also increase their toxicity levels (Vu *et al*., 2019). *T. indica* DCM extracts and *S. incanum* water extracts contained glycosides that could have also contributed to the toxicity of the two plant extracts.

The toxicity of the plant extracts is linked with the presence of toxic primary and secondary metabolites. Plants produce different proteins that protect them against fungi, bacteria, insects and animals (Dang and Van Damme, 2015). Knowledge on toxic proteins in plants facilitated genetic engineering, to introduce the toxic products to protect other plants from diseases and pathogens. Intoxication cases due to uncontrolled use of medicinal plants has increased in the recent times. For example, 1453 intoxication cases have been reported after analyzing 127 articles addressing toxic metabolites in medicinal plants (Ghorani-Azam *et al*., 2018). Researchers have identified several modes of actions linked with plant toxicity. For example, researchers reported that presence of ribosome inactivating metabolites interfere with rRNA N-glycosidase activity (Tumer, 2015). Inactivation of the enzyme affects translation process, which in turn causes cell death. Additionally, toxic metabolites interact with nucleic acid and biomembranes affecting their stability, while most terpenoids and phenolics interact with receptors of various neurotransmitters, affecting their function (Wink, 2015). The metabolites also interact with various cellular elements through ionic, hydrophobic and hydrogen bond. Besides, the toxicity of some plants is linked with presence of elements such as lead, arsenic, Cadmium and Aluminum (Brima, 2017). Also, due to the toxicity of some herbal extracts, the WHO formulated guidelines

to ensure that the population is not exposed to higher concentration of the toxic elements per dose.

The toxicity tests of the plants reveal that all four extracts had varying toxicity thresholds. Although cytotoxicity of plant extracts is associated with negative effects in the human body, such as damage to neurons, kidney, and liver cells, the toxic compound can also be effective in managing cancer by inducing apoptosis of cancer cells (Yu *et al*., 2017). Besides, toxic extracts minimize cancer metastasis. Apoptotic effects on several cells lines suggests that *T. indica* seeds induced cell death can be analyzed further for anticancer potency (Hussein *et al*., 2017). In this study, *T. indica* leaf extracts had lower toxicity thresholds, which suggest that the extract could also affect various cell lines. As such, the toxic phytochemicals in the extract can generate lead compounds for cancer management.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The assays generated data that confirmed the efficacy of the *S. incanum* and *T. indica* DCM as used in traditional medicine. Therefore, the analysis led to the following conclusions.

i. *S. incanum* water extract had the highest phytochemical yield (4.7% w/w), while *T. indica* DCM extract had the lowest yield (1.07% w/w). *S. incanum* DCM extract and *T. indica* water extracts had yield of 2.54% w/w and 2.53% w/w respectively. The chemical tests for the phytochemicals revealed that *S. incanum* and *T. indica* contained several phytochemical classes. However, glycosides were absent in *T. indica* water extract and *S. incanum* DCM extract. Anthraquinones were absent in all four extracts. The GC-MS analysis revealed availability of flavonoids, phenols and alkaloids.

ii. The antibacterial assay revealed that *S. icnanum* and *T. indica* water and DCM extraxts were active against *E. coli, S. typhi* and *S. aureaus*. However, the extracts were more effective against Gram-negative bacteria (*E. coli* and *S. typhi*) than Gram-positive bacteria (*S. aureus*). The MIC of *T. indica* extracts ranged between 62.5 µg/mL and 125 µg/mL, while those of *S. incanum* ranged between 62.5 µg/mL and 250 µg/mL, while the MBC values of both plants ranged between 125 µg/mL and 500 µg/mL. The MIC and MBC values against *S. aureus* were higher than those of *E. coli*.

iii. *S. incanum* and *T. indica* were effective in stabilizing RBCs, with *T. indica* DCM extract being the most effective, while *S. incanum* water extract was the least effective. The RBC stabilization efficiency for both plants was not dose-dependent.

iv. *S. incanum* and *T. indica* extracts had varying toxicity levels. *S. incanum* water extract was more toxic than DCM extract, while *T. indica* DCM extract were more toxic than *T. indica* water extract.

6.2 Recommendations

The study has shown that the two plants are effective in managing bacterial infections caused by *E. coli, S. typhi* and *S. aureus* and in controlling inflammation. The efficacy

of the plants justifies their application in traditional medicine. In addition, the study revealed that different modes of extraction might affect the efficacy of various plant extracts. The brine shrimp lethality test effectively demonstrated the toxicity levels of the plant extracts. To improve scientific knowledge on these plants there is a need for;

i. Fractionation of various phytochemical classes to identify the actual class of phytochemicals with antibacterial, anti-inflammatory and cytotoxic effects.
ii. Testing the plant extracts against multi-drug resistant bacteria such as MRSA and drug resistant *E. coli*.
iii. Using animal models for *in-vitro* testing of anti-inflammatory and cytotoxicity of the two plants.

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APPENDICES

Appendix I: ANOVA table for the model used in the Anti-bacteria assay of the plant extracts

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	40	3200.542051	80.013551	283.51	<.0001
Error	76	21.448718	0.282220		
Corrected Total	116	3221.990769			

R-Square	CV	Root MSE	Zone of inhibition
0.993343	3.303860	0.531244	16.07949

Appendix II: ANOVA table representing the Mean stabilization for the plant extracts used.

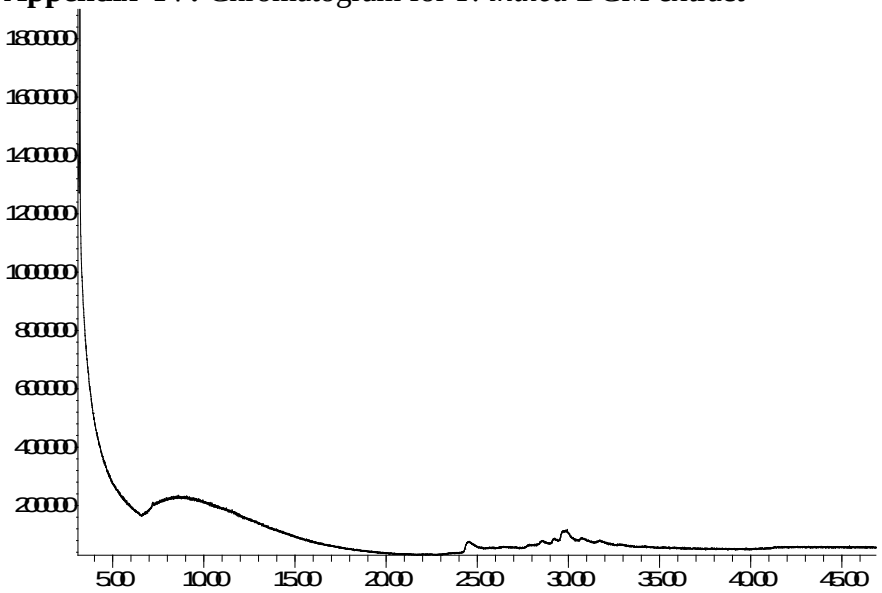
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	23967.64020	1331.53557	5129.01	<.0001
Error	32	8.30747	0.25961		
Corrected Total	50	23975.94767			

R-Square	CV	Root MSE	Stabilization Mean
0.999654	1.208101	0.509518	42.17510

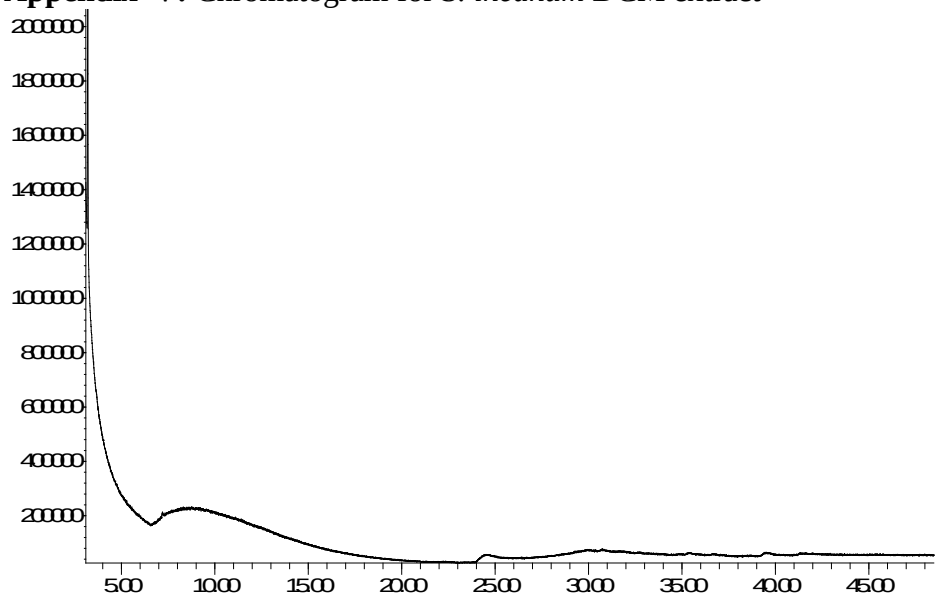
Appendix III: A summary of the regression model used for the toxicity assay

Sample	Parameter	Estimate	Standard Error	95% CI Limits		Chi-Square	Pr > ChiSq
S. incanum DCM	Intercept	-2.4225	0.5563	-3.5129	-1.3321	18.96	<.0001
	Log10(Conc)	0.7190	0.2381	0.2523	1.1857	9.12	0.0025
S. incanum Water	Intercept	-83.4442	0.2828	-83.99	-82.889	87036.8	<.0001
	Log10(Conc)	27.8147	0.0000	27.8147	27.8147		
T. indica Water	Intercept	-81.2398	0.3311	-81.888	-80.591	60191.1	<.0001
	Log10(Conc)	26.6957	0.0000	26.6957	26.6957		
T. indica DCM	Intercept	-61.6781	0.3858	-62.434	-60.922	25564.1	<.0001
	Log10(Conc)	30.0099	0.0000	30.0099	30.0099		
Positive Control	Intercept	-4.5691	1.1443	-6.8120	-2.3262	15.94	<.0001
	Log10(Conc)	4.2451	1.0335	2.2196	6.2707	16.87	<.0001
Negative Control	Intercept	0.0000	0.0000	0.0000	0.0000	0.0000	
	Log10(Conc)	0.0000	0.0000	0.0000	0.0000	0.0000	

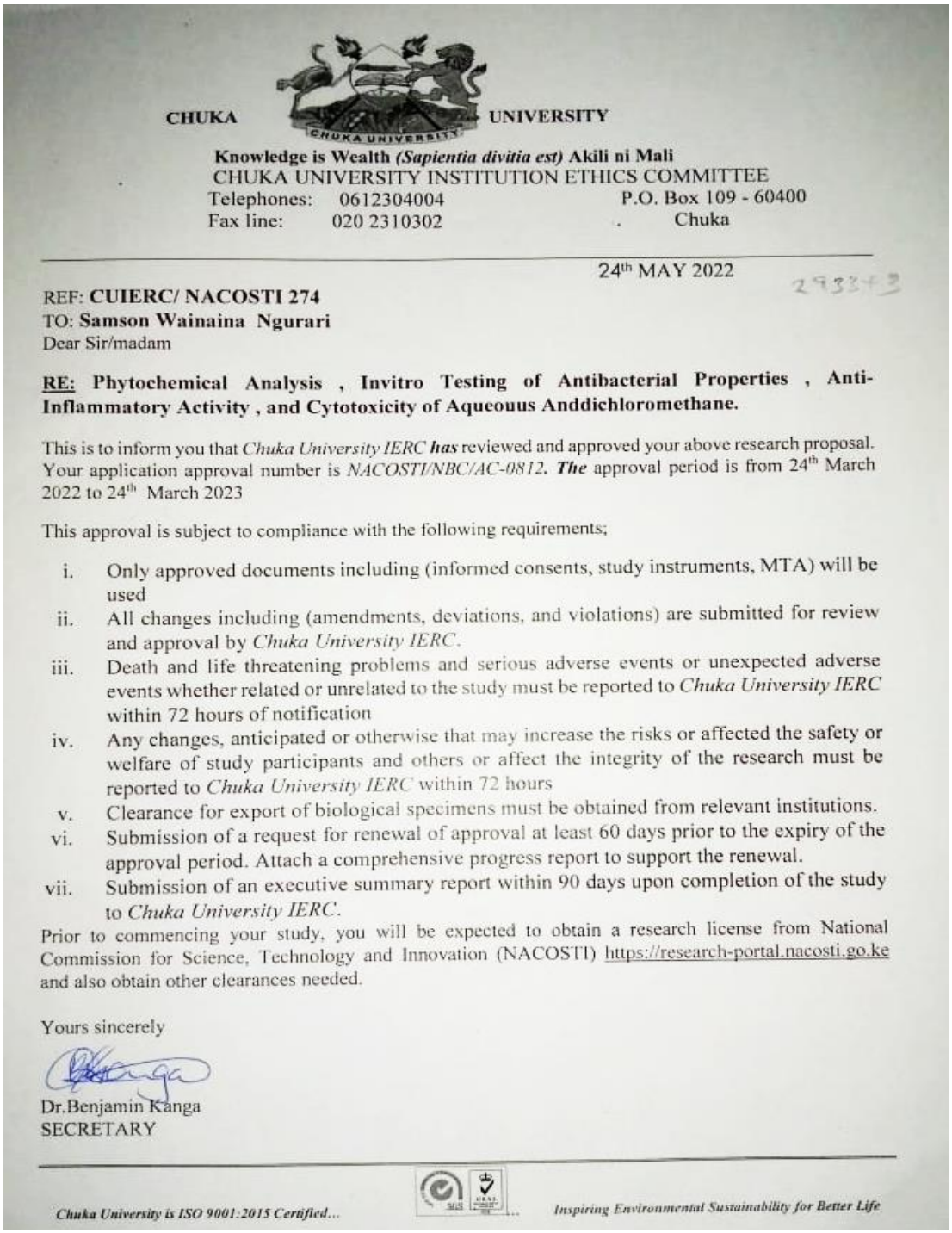
Appendix IV: Chromatogram for *T. indica* DCM extract



Appendix V: Chromatogram for *S. incanum* DCM extract



Appendix VI: Approval letter from ethical committee



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CHUKA UNIVERSITY INSTITUTION ETHICS COMMITTEE

Telephones: 0612304004 P.O. Box 109 - 60400

Fax line: 020 2310302 Chuka

24th MAY 2022

293343

REF: **CUIERC/ NACOSTI 274**

TO: **Samson Wainaina Ngurari**

Dear Sir/madam

RE: Phytochemical Analysis , Invitro Testing of Antibacterial Properties , Anti-Inflammatory Activity , and Cytotoxicity of Aqueouus Anddichloromethane.

This is to inform you that *Chuka University IERC* ***has*** reviewed and approved your above research proposal. Your application approval number is *NACOSTI/NBC/AC-0812.* ***The*** approval period is from 24th March 2022 to 24th March 2023

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by *Chuka University IERC.*
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to *Chuka University IERC* within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected the safety or welfare of study participants and others or affect the integrity of the research must be reported to *Chuka University IERC* within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to the expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to *Chuka University IERC.*

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr.Benjamin Kanga
SECRETARY

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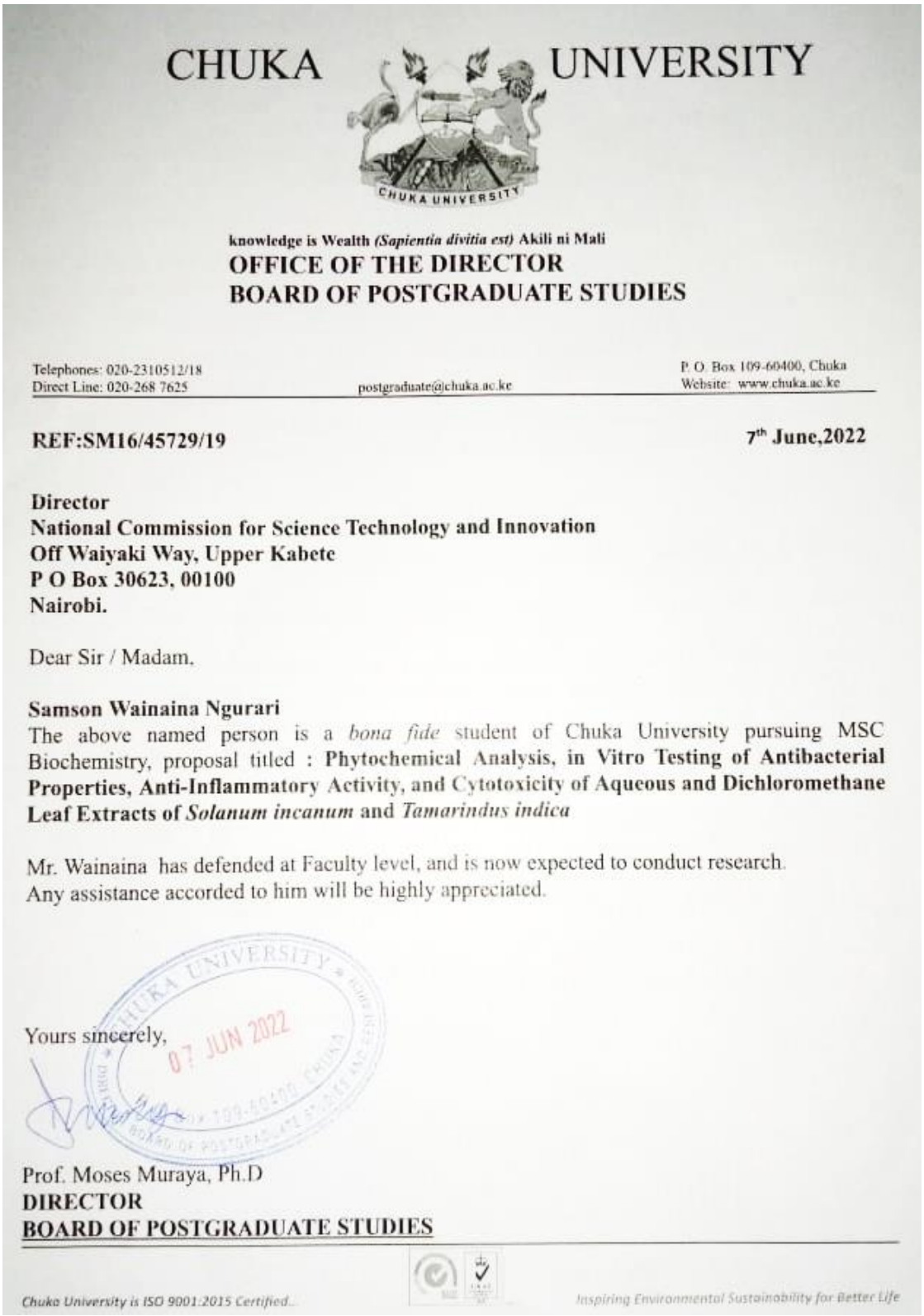
Appendix VII: Approval letter from board of post graduate studies



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REF:SM16/45729/19 **7th June,2022**

Director
National Commission for Science Technology and Innovation
Off Waiyaki Way, Upper Kabete
P O Box 30623, 00100
Nairobi.

Dear Sir / Madam,

Samson Wainaina Ngurari
The above named person is a *bona fide* student of Chuka University pursuing MSC Biochemistry, proposal titled : **Phytochemical Analysis, in Vitro Testing of Antibacterial Properties, Anti-Inflammatory Activity, and Cytotoxicity of Aqueous and Dichloromethane Leaf Extracts of *Solanum incanum* and *Tamarindus indica***

Mr. Wainaina has defended at Faculty level, and is now expected to conduct research.
Any assistance accorded to him will be highly appreciated.

CHUKA UNIVERSITY 07 JUN 2022 BOARD OF POSTGRADUATE STUDIES

Yours sincerely,

Prof. Moses Muraya, Ph.D
DIRECTOR
BOARD OF POSTGRADUATE STUDIES

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Appendix VIII: Research License from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 293343

Date of Issue: 25/August/2022

RESEARCH LICENSE

This is to Certify that Mr.. Samson Wainaina Ngurari of Chuka University, has been licensed to conduct research in Tharaka-Nithi on the topic: PHYTOCHEMICAL ANALYSIS, IN VITRO TESTING OF ANTIBACTERIAL PROPERTIES, ANTI-INFLAMMATORY ACTIVITY, AND CYTOTOXICITY OF AQUEOUS AND DICHLOROMETHANE LEAF EXTRACTS OF Solanum incanum AND Tamarindus indica for the period ending : 25/August/2023.

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