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DEPARTMENT OF BIOLOGICAL SCIENCES

Phytochemical Screening And Antimicrobial Activity Assessment Of Trichilia Emetica, Azadirachta Indica, And Chamaemelum Nobile Against Staphylococcus Aureus.

BY

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APPROVAL FORM

TITLE OF DISSERTATION 'PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY ASSESSMENT OF TRICHILIA EMETICA, AZADIRACHTA INDICA, AND CHAMAEMELUM NOBILE AGAINST STAPHYLOCOCCUS AUREUS'.

The undersigned certify that they have read the dissertation and it is suitable for submission to the Faculty of Science and checked for conformity with the Faculty

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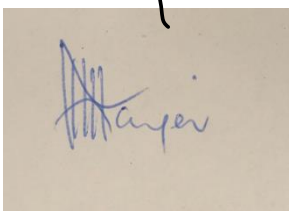
Supervisor:  **Date: 06/06/24**

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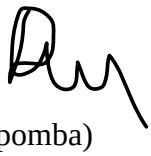
Signature  Date 06/06/24

(Lincy Kapomba)

DEDICATION

I would like to dedicate this piece of work to my parents who have shown me great, profound and benevolent concern for my education and life. This work would have never been a success without them.

ACKNOWLEDGEMENTS

One of the joys of completion is to look over the journey and remember all the persons who have helped and supported me along the ebbs and tides of this project study. It is my pleasure and privilege to appreciate the people whose constant efforts, motivation and well wishes paved a path for me to complete this project study. First and foremost, I would like to thank the Almighty God for his wisdom and guidance that he rendered me throughout the project study period and for the gift of life. I would like to express my heartfelt gratitude to my immediate supervisor Mr Munosiyei who nurtured me to the best of his knowledge and experience during this research project. My special appreciation also goes to the senior management for giving students an opportunity to explore the working environment practically. I would also like to express my heartfelt gratitude to my parents and fellow colleagues for their unwavering support.

LIST OF ACRONYMS

ANOVA - Analysis of Variance

CVL - Central Veterinary Laboratory

MHA - Mueller Hinton Agar

MIC - Minimum Inhibitory Concentration

ml - Millimeter

MRSA - Methicillin Resistant *S. aureus*

SPSS - Statistical Package for the Social Sciences

ABSTRACT

The prevalence of skin infections caused by *S. aureus*, coupled with the development of antibiotic-resistant strains, poses considerable challenges in terms of effective treatment, prevention, and control. The rise of antimicrobial resistance further exacerbates the challenges associated with skin infections, emphasizing the need for alternative effective treatments. The study aimed to determine the phytochemical composition of *Trichilia emetica* (bark), *Azadirachta indica* (leaves), and *Chamaemelum nobile* (flower) extracts and their antimicrobial properties against *S. aureus*. The experiment was conducted at the Central Veterinary Laboratories in Harare. Plants were collected and identified by specialist botanists. Dried and pulverised plant parts were soaked for 72 hours in extraction solvents that were water and methanol. A stock concentration for each herb was prepared by diluting it with dimethyl sulphoxide. Phytochemical analysis was done using gas chromatography and mass spectrometry. Phytochemical tests for alkaloids, flavonoids, phenols, saponins, glycosides, steroids, proteins, and carbohydrates were conducted. The Kirby-Bauer disk diffusion test was done by inoculating the surface of the Muller-Hinton agar plates with the identified bacteria using the spread plate technique. From the phytochemical tests, all the plant extracts contained alkaloids, saponins, and tannins for both methanol and distilled water solvent extracts. Tannins, glycosides, phenols, steroids, and flavonoids were only present in *T. emetica*. The ANOVA mean square for *T. emetica* 0.611 under methanolic extracts represented greater potential inhibition of the herbal extract against the suppression of *S. aureus* compared to *A. indica* and *C. nobile* paper disks containing the herbal extract. The lowest concentration or Minimum Inhibitory Concentration was lowest for *T. emetica* 12.5g/ml followed by *A. indica* 25g/ml and finally *C. nobile* 50mg/ml. It is concluded that all the plants that were under study exhibited therapeutic effects against strains of *S. aureus.*

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CHAPTER 1

1.0 INTRODUCTION

The skin, being a habitat for various microbiota, hosts microorganisms such as fungi and yeast in skin folds and non-pathogenic mycobacteria in areas rich in sebaceous secretions. However, certain bacterial agents, notably *S. aureus*, can cause skin infections, posing a significant health concern. *S. aureus* is of particular importance due to its increasing antibiotic resistance and its role as a major cause of nosocomial infections globally (Bisignan, 2005). Infections associated with this bacterium range from sepsis to pneumonia, osteomyelitis and skin infections, can become life-threatening if the bacteria penetrate deeper into the body, affecting the bloodstream, joints, bones, lungs or heart (ASM, 2016).

The use of synthetic skin medicines containing harsh chemicals has raised concerns, as these substances may lead to skin irritation, redness, dryness, or allergic reactions (Liang, 2020). Moreover, the production and disposal of synthetic skin medicines often involve harmful chemicals, impacting the environment negatively (Chaturvedi et al., 2021). The emergence of antimicrobial resistance (AMR) has further complicated the management of skin infections (Aljeldah, 2022).

In response to these challenges, there has been increased interest in the utilization of medicinal plants for the treatment of skin diseases. Medicinal plants have been traditionally employed for their antimicrobial and anti-inflammatory properties, providing an alternative to synthetic skin medicines. Extracts from various plant parts such as leaves, stems, roots, and fruits have been utilized in the treatment of injuries and diseases and to promote overall health (Kareru *et al*., 2010). Ethnomedically, the topical application of plant extracts particularly from *A. indica, C. nobile,* and *T. emetica* has been observed for its antimicrobial and anti-inflammatory effects in treating skin diseases like eczema, ringworm, and pruritus (Sharma *et al*., 2014).

This study focused on the analysis of crude extracts from *A. indica* (leaves), *C. Nobile* (flower), and *T. emetica* (bark) for their antibacterial and anti-inflammatory activities, with a particular emphasis on their potential use against *S. aureus* infections. *A. indica, C. nobile* and *T. emetica* were chosen for their well-documented traditional uses and the presence of bioactive compounds that may contribute to their therapeutic properties.

1.1 Problem statement

The prevalence of skin infections caused by *S. aureus*, including antibiotic-resistant strains, poses a considerable challenge in terms of effective treatment, prevention and control. Synthetic skin medicines containing harsh chemicals can lead to adverse reactions such as skin irritation, allergic reactions, and even systemic toxicity (Guo et al., 2020). Additionally, the production and disposal of these medicines often result in environmental risks, such as water pollution from pharmaceutical waste and the release of hazardous chemicals into the atmosphere (Chaturvedi et al., 2021). The rise of antimicrobial resistance further exacerbates the challenges associated with skin infections, emphasizing the need for alternative effective treatments.

1.2 Justification

Herbal medications have gained popularity especially in developing countries for their perceived efficacy in primary skin care. *A. indica*, *C. nobile*, and *T. emetica* are believed to possess antimicrobial, antioxidant and anti-inflammatory properties, making them potentially effective against *S. aureus* infections (Sharma et al., 2023). The bioactive compounds in these plants can be extracted and standardised, offering a locally accessible and cost-effective alternative to antibiotics and creams (Afreen et al., 2021). This study aims to contribute valuable insights into the potential use of these plant extracts for combating skin infections caused by *S. aureu*s.

1.3 Significance of the study

The study of phytochemical properties of *T. emetica, C. nobile* and *A. indica* against *S. aureus* infections holds several significant importance. With the rise of antibiotic resistant *Staphylococcus* strains there is an urgent need for alternative treatment options and this offers the potential for developing alternative antimicrobial agents that can be used individually or in combination with other existing antibiotics so as to overcome resistance and enhance treatment efficacy. Phytochemical analysis can lead to the discovery of new bioactive compounds with antimicrobial properties and the compounds can serve as leads for the development of novel drugs or as inspiration for synthetic modifications to enhance their effectiveness and reduce toxicity (Baron, 2006). The use of these plants may enhance the efficacy of antibiotics and reduce the required dosage or mitigate adverse effects of drugs and this approach may improve treatment outcomes or cases of antibiotic resistance (Álvarez-Martínez et al., 2021). The other significance of *T. emetica*, *C. nobile* and *A. indica* is that they have a very long history of

traditional use due to their medicinal properties and conducting phytochemical analysis on these plants helps in validating their traditional use as well as preserving traditional knowledge related to their traditional use and preserve traditional knowledge related to therapeutic potential (Crottaeau *et al*., 2006).

1.4 Aim of the study

The aim of this study was to determine the phytochemical composition and the antimicrobial effectiveness of crude extracts of *indica* (leaves), *C. Nobile* (flower), and *T. emetica* (bark) against *S. aureus.*

1.5 Objectives of the study

The specific objectives of this study were to:

i. Determine the phytochemical composition of the extracts.
ii. Determine the antimicrobial properties of the extracts against *S. aureus.*

1.6 Research questions

i. What are the phytochemicals present in *A. indica*, *C. nobile,* and *T. emetica*?
ii. How do the antimicrobial properties of water and methanolic extracts of A. indica, C. nobile, and T. emetica compare to Staphylococcus aureus?

1.7 Hypothesis

H_0: *A. indica, C. nobile,* and *T. emetica* extracts have no antimicrobial properties against *S. aureus*.

H_1: *A. indica*, *C. nobile* and *T. emetica* extracts have antimicrobial properties against *S. aureus*.

1.8 Limitations of the study

The study focused only on phytochemical analysis of *T. emetica*, *C. nobile* and *A. indica* so other species with potential antimicrobial properties may not be used. Variability in the plant material as the phytochemical chemical composition of plants can vary depending on factors like geographical location, climate, soil or plant maturity thus studies conducted with plants from different sources may yield inconsistent results making it a challenge in establishing standardized profiles of active compounds. Choice of solvent extraction method and extraction conditions can impact the phytochemical composition of the extracts because different

extraction protocols may extract different compounds leading to a variation in the observed antimicrobial activity. Phytochemical analysis and antimicrobial testing usually done in vitro that is in the laboratory strains of *S. aureus* and these studies may provide initial insights into the potential antimicrobial activity and may not fully reflect the complex interactions that occur in vivo on human skin. As many plants contain multiple active compounds that may work synergistically to enhance their antimicrobial activity, identifying and optimising the interactions between compounds can be complex (Baron, 2006).

CHAPTER 2

2.0 LITERATURE REVIEW

Infectious diseases are caused by different types of pathogenic microbes, for example bacteria, viruses, parasites or fungi (WHO, 2007). These infectious diseases have resulted in more than 17 million deaths per year worldwide and represent 43% of deaths in developing countries (WHO, 2007). These infections are of major concern because they represent a major public health problem in the world and especially in developing countries where they are endemic.

Bacterial infections are responsible for 70% of these deaths (Gangoue, 2003). The discovery of antibiotics significantly reduced the spread of these pathologies but the remarkable effectiveness of these antibiotics was accompanied by their massive and abusive use leading to the emergence of bacterial resistance (Ben *et al*., 2005). This resistance contributed to making pathologies linked to microbes the leading cause of death in the world, killing more than 50,000 people per day (Ahmad *et al*., 2001) and contributing to the need to search for new molecules. Medicinal plants remain potential sources of new active molecules. According to WHO (2002), an estimated 80% of the African population engaged in this practice for their own health.

2.1 *S. aureus* infections

Staphylococcal infections also known as staph infections are caused by a genus of bacteria called Staphylococcus and it has more than 30 strains but the most human pathogen is *S. aureus*. A few common skin infections caused by staph *S. aureus* are boils. Boils are the most commontype of staph infection, they are pockets of white pus that start where a hair follicle or oil glandis. The boil is tender and red where the infection is located on the skin.

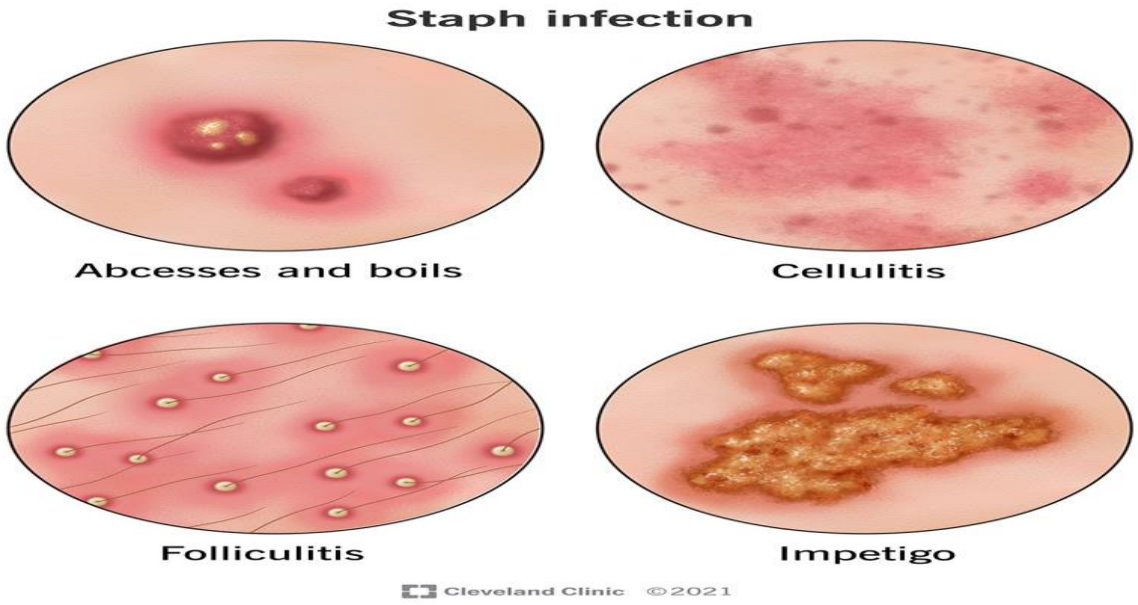

Figure 1: Illustration of skin infection types caused by *S. aureus*

S. aureus is a gram-positive and glucose-positive cocci of the Staphylococcus family and an opportunistic infection agent in human beings. Skin infection occurs upon contact with the Staphylococcus bacteria as the bacteria is contagious and usually enters the skin through a cut then pus may form (DeLeo, Diep and Otto, 2009). This strain is responsible for causing skin infection and this can produce boils, blisters, or redness on the skin. The unfortunate part is that infections can be anywhere on the body including the face. Staphylococcus bacteria can cause infections on the breasts or chest as people can develop mastitis which causes inflammation or swelling as well as abscesses in the breasts. Symptoms of staphylococcus infections usually depend on the area where the infection has occurred and it causes pimples that may look red and filled with puss. *S. aureus* infections can be either primary or secondary and these infections include impetigo, folliculitis, furuncles, and primary abscesses. Abscesses and boils are painful sores that form under the skin causing redness and pain. Cellulitis is a type of infection that causes swollen red, painful tissue under the skin whereas folliculitis is a

small pimple that forms under the hair follicle and causes massive pain. Staphylococcal scalded skin syndrome is a serious infection that causes the skin to peel off all over the body affecting infants and small children (Tong *et al.*, 2015).

2.2 Problems associated with antibiotics in treating staphylococcus infections

Antibiotics are highly used to treat Staphylococcus infections usually in the skin but these then pose some problems that are associated with their use. The problem to note is the antibiotic resistance of *S. aureus* including methicillin-resistant *S. aureus* which is developing to multiple antibiotics and this is due to overuse as well as misuse of antibiotics. It becomes challenging to effectively treat Staphylococcus infections as the bacteria become more resistant leading to treatment failure. As issues of antibiotic resistance arise, the range of treating Staphylococcus infections is becoming increasingly limited. In some notable cases, infections may become difficult to treat leading to an increased risk of adverse effects and complications and they may range from mild to severe (Taconelli *et al*., 2008). Some side effects may include gastrointestinal disturbances, allergic reactions as well as skin rashes, or may go as far as kidney or liver toxicity. The use of antibiotics in treating staphylococcus infections contributes to the disruption of the microbiome. That is, antibiotics can disrupt the natural balance of microbial communities leading to severe life-threatening infections. The discovery and development of new antibiotics have significantly slowed down in recent years and have limited the options for treating antibiotic-resistant staphylococcus infections and underscores the urgent need for alternative strategies example combination therapies or the use of plant extracts as most of the traditional plant possess antimicrobial, antioxidant as well as anti-inflammatory properties that may combat antibiotic resistance (Tacconelli *et al*., 2008).

2.3 *T. emetica*

The powdered bark of *T. emetica* is a popular remedy for stomach and intestinal ailments. The bark is also used to produce a pinkish decoction used for the treatment of fever and also as a purgative.

Figure 2: Illustration of *Trichilia emetica* tree bark, showcasing its characteristic rough texture and dark brown colouration.

A study by Adeyemi *et al* (2017) investigated the phytochemical composition and medicinal properties of *T. emetica*. The research highlighted the presence of bioactive compounds such as alkaloids, flavonoids and tannins which contribute to its antioxidant and antimicrobial properties. This plant has also been used as a traditional medicine to treat abdominal pains, dermatitis, hemorrhoids, jaundice as well as chest pains. In another study Njau *et al* (2019) evaluated the traditional uses of *T. emetica* in East Africa. They found that indigenous communities utilize different parts of the plant for various medicinal purposes including treating malaria, gastrointestinal disorders, and skin infections. An evaluation of the antimicrobial activity of this plant against *S. aureus* and the results showed that the methanol extract had moderate antimicrobial activity against *S. aureus*. A study that was published in the journal of Ethnopharmacology evaluated the antiflammatory effects of a methanol extract of *T. emetica* in animal models and this exhibited a significant inhibition of inflammation in the tested models thus making it a potential an antiinflammatory agent (Baatile *et al*., 2019).

2.4 *A. indica*

The leaves of *A. indica* are rich in antioxidants such as flavonoids, phenolic compounds and carotenoids and these help in neutralising harmful free radicals in the body which cause oxidative stress and damage to cells.

Figure 3: Illustration of *A. indica* showcasing the plant leaves.

A comprehensive review by Naqvi *et al*., (2019) discussed the pharmacological activities of *A. indica*. The study highlighted its antidiabetic, anticancer, anti-inflammatory and immunomodulatory properties. Additionally, it emphasized the potential of *A. indica* extracts in pest control and agricultural applications. Another study by Biswas *et al*., (2019) explored the therapeutic potential of its extracts against microbial infections. The research demonstrated the antimicrobial activity of *A. indica* compounds against various bacteria, fungi and parasites. The extracts were found to inhibit the growth and adhesion of pathogens. *A. indica* leaves have been reported to also possess anti-inflammatory properties as the active compounds example nimbin, nimbidin and quercetin have been shown to inhibit pro-inflammatory enzymes and cytokines that are involved in the inflammatory response. The anti-inflammatory properties of *A. indica* leaves may have potential benefits in conditions that are associated which chronic inflammation example arthritis, dermatitis and gastrointestinal disorders (Lavanya *et al.*, 2016). In a study that was carried out by Sharma *et al.,(* 2019) their main objective was to analyse the

phytochemicals in various extracts of the *A. indica* leaves and a comparative evaluation of the antibacterial activity of extracts against *E. coli* and *S. aureus* as well as comparative evaluation of antioxidant activity using 1.1 diphenyl-2-picrylhydrazyl radical scavenging assay. Results obtained indicated that there was presence of alkaloids, flavonoids and terpenoids but tannins suggested that bioactive compounds that are found in leaves of *A. indica* contribute to its pharmacological activities (Gupta *et al.*, 2013).

2.5 *C. nobile*

C. nobile is a flowering plant that has been utilized for its medicinal properties for decades as it contains various bioactive compounds that then contribute to its pharmacological properties. A study by Srivastava et al (2010) reviewed the pharmacological properties of *C. nobile*.

Figure 4: Illustration of *Chamaemelum nobile*, displaying the characteristics of white petals and yellow disc florets.

The research highlighted its anti-inflammatory, antioxidant, antimicrobial, and sedative effects. For the anti-inflammatory properties, the flowers contain compounds like chamazulene, alpha-bisabolol, and flavonoids which have been shown to inhibit inflammatory mediators thus reducing inflammation and then this can be useful in conditions such as skin irritations, gastrointestinal disorders, and respiratory conditions. The presence of flavonoids and other phenolic compounds contribute to the antioxidant properties and the effects have potential benefits in reducing oxidative stress and preventing chronic diseases. According to (Srivastava,

et al., 2010), *C. nobile* flowers possess sedative and anxiolytic properties that have calming and relaxing effects have been traditionally used to promote sleep and relief anxiety. The anti-microbial properties of this flower is due to the presence of terpenoids that exhibit antimicrobial effects. The study also discussed the use of *C. nobile* in traditional medicine for alleviating digestive disorders, anxiety and skin conditions. Koulivand *et al* (2013) investigated the anxiolytic effects of *C. nobile*. The research indicated that *C. nobile* extracts have potential anxiolytic properties, reducing anxiety symptoms in animal models.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Study site

The experiments were conducted at the Central Veterinary Laboratories in Harare Zimbabwe in the molecular biology and analytical chemistry laboratories.

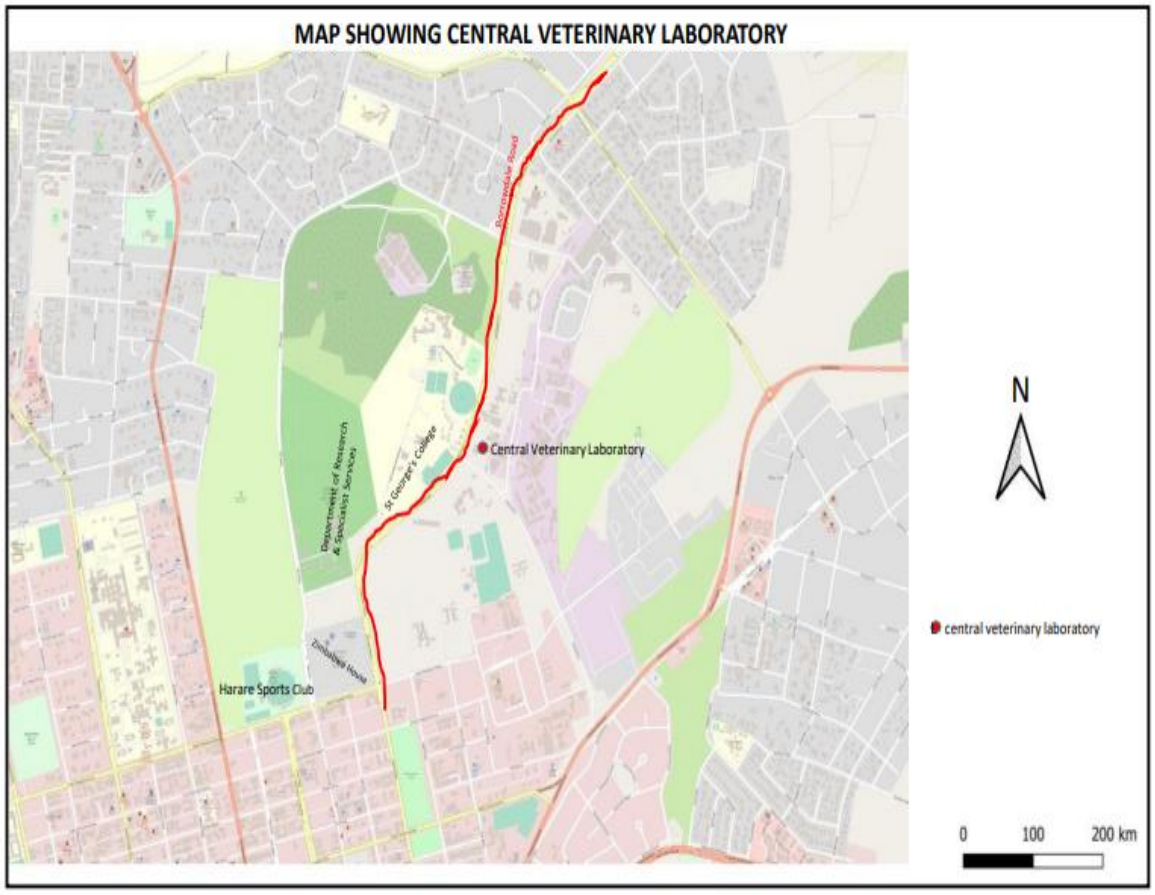

Figure 5: Illustration of the location of Central Veterinary Laboratories in Harare

3.2 Plant collection and identification

The plants were collected from areas around Harare, Zimbabwe and the plants were identified at Botanical gardens by specialist botanists. Samples of fresh bark of *T. emetica*, leaves of *A. indica* and flowers of *C. nobile* plant species were taken for crude herbal solvent extraction for the inhibition trials of bacterial experiments.

3.3 Plant preparation and extraction

The plant material was washed thoroughly with distilled water and air dried under the shade. Plant material was then grounded using pestle and mortar to make a fine powder. The powder was sieved and weighed. The powder was then stored in airtight containers.

Figure 6: Illustration of aqueous and methanolic extracts of *Azadirachta indica* leaves obtained by maceration, arranged by concentration

Water and methanol were used for the solvent extraction process in which the plant powder was soaked in the solvents for 72 hours in a dark room with gentle shaking (Karthikeyan *et al.*, 2020). After 72 hours, the mixture was filtered and the filtrate was concentrated to make sure all the solvent had been lost. The concentrated extract was reconstituted by mixing with dimethyl sulphoxide (DMSO) to give a stock solution which was be used for further testing at varying concentrations. The crude extracts were stored in airtight bags for future use. Gas chromatography and mass spectrometry was conducted to determine the chemical composition of the phytochemicals from the herbs.

The quality and quantity of the antioxidant extracts from plants are defined by the nature of the employed solvent because various antioxidants have various chemical properties and polarity.

Figure 7: Illustration of test tubes with aqueous and methanolic extracts of *Azadirachta indica*, Chamaemelum nobile, and Trichilia emetica, labeled with the plant species and extraction solvent.

3.4 Sterility test

An aliquot of 0.1 milliliters of all extracts was poured on a nutrient agar plate and left for two minutes. Incubation was done overnight at 37℃ to observe the antibacterial activity.

3.5 Phytochemical screening

The different extracts of the herbs were screened for phytochemicals such as alkaloids, cardiac glycosides, carbohydrates, flavonoids, phenols, proteins, steroids, saponins, and tannins.

3.6 Qualitative analysis of phytochemicals in plant extracts

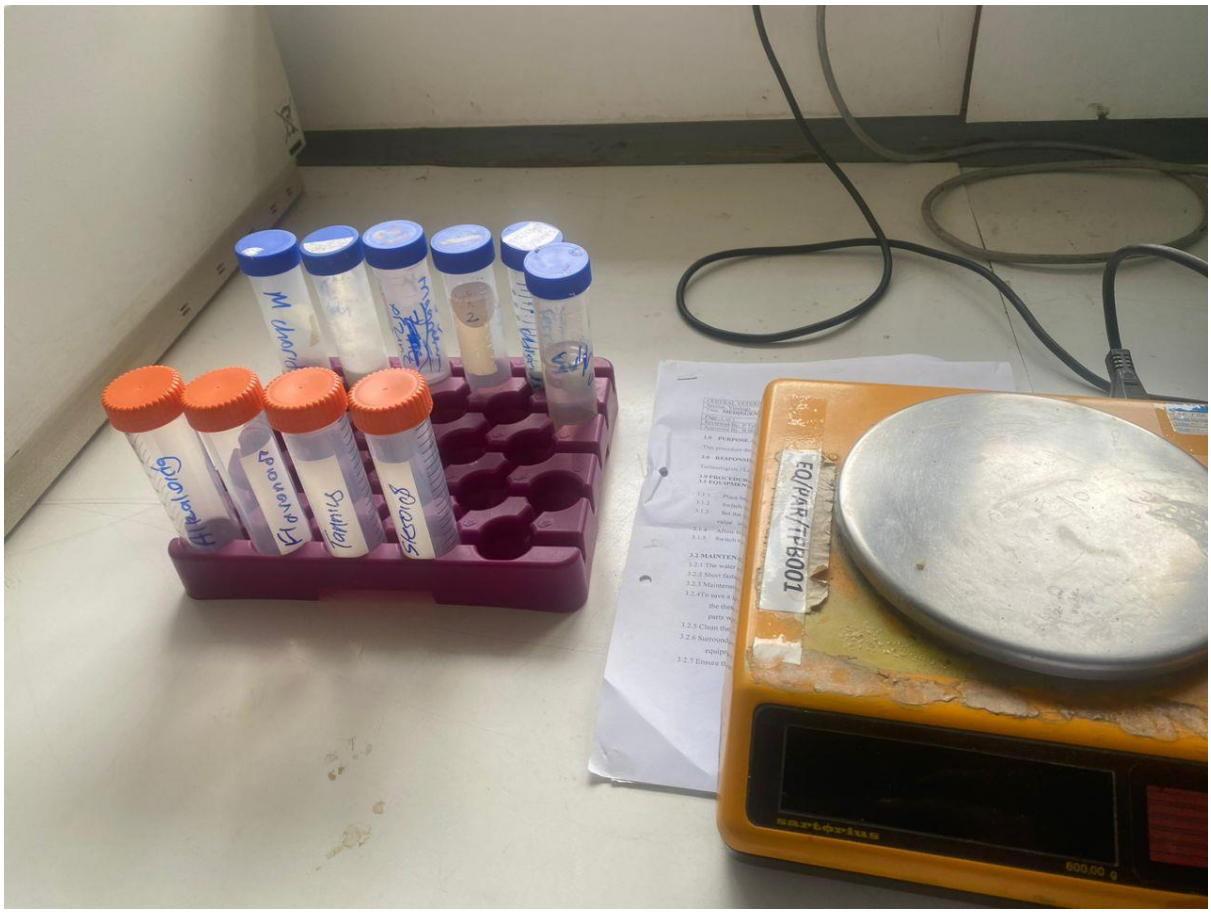

Figure 7: Illustration of phytochemical analysis laboratory setup, featuring labeled test tubes with plant extracts, a test tube rack, and an analytical balance.

3.6.1 Alkaloids Mayer's Test (Potassium Mercuric Iodide)

For the alkaloids Mayer's test, a solution was prepared by dissolving 1.36 gm of mercuric chloride in 60 ml of distilled water, adding 5 gm of potassium iodide, and diluting the mixture to 100 ml with distilled water. To 1.0 ml of the acidic aqueous solution of the samples, a few drops of the reagent were added. The presence of alkaloids was indicated by the formation of a white or pale precipitate.

3.6.2 Flavonoids

In a test tube containing 0.5 ml of alcoholic and aqueous extracts of the samples, 5-10 drops of dilute hydrochloric acid and a small piece of zinc or magnesium were added. The solution was then boiled for a few minutes. The presence of flavonoids was confirmed by the development of a reddish-pink or dirty brown color.

3.6.3 Phenols (Ferric Chloride Test)

To 1.0 ml of the alcoholic solution of samples, 2.0 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution were added. The formation of a blue or green color indicated the presence of phenols.

3.6.4 Tannins

Half a gram of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 10% ferric chloride solution (light yellow) were added, and the formation of a brownish-green or blue-black coloration confirmed the presence of tannins.

3.6.5 Saponins

For the saponins test, 1.0 ml of the extract was mixed with 4 ml of distilled water and agitated in a graduated cylinder for 3 minutes. The presence of foam indicated the presence of saponins.

3.6.6 Glycosides

A small amount of the sample was dissolved in 1 ml of water, and an aqueous solution of sodium hydroxide was added. The formation of a yellow color indicated the presence of glycosides.

3.6.7 Steroids

To 2.0 ml of the sample, 1.0 ml of concentrated sulfuric acid was added carefully along the sides of the test tube. The presence of steroids was confirmed by the formation of a red color chloroform layer.

3.6.8 Proteins

Millon’s Test for the proteins Millon’s test, a solution was prepared by digesting one part of mercury with two parts of nitric acid, and the resulting solution was diluted with two volumes of water. To a small quantity of decoction, 5-6 drops of Millon’s reagent were added. The formation of a precipitate turning red upon heating indicated the presence of proteins.

3.6.9 Carbohydrates

Molish Test was used. In a test tube containing 2.0 ml of the plant sample, 2 drops of freshly prepared 20% alcoholic solution of α-naphthol were added and mixed. To this solution, 2.0 ml of concentrated sulfuric acid was added to form a layer below the mixture. The presence of carbohydrates was confirmed by the formation of a red-violet ring at the junction of the solution, disappearing upon the addition of an excess of alkali solution.

3.7 Antimicrobial susceptibility patterns of the crude extracts of *T. emetica*, *A. indica*, and *C. nobile* against *S. aureus*

The Bauer-Kirby (1996) disc diffusion method, a culture-based microbiology assay commonly used in diagnostic and drug discovery laboratories was utilized for this susceptibility test. The agar used for the media was Muller-Hinton agar. The media plates that were used were labeled for the bacterial species *S. aureus*. The test was performed by inoculating the surface of 10 Muller-Hinton agar plates with the identified bacteria, and subcultures from the bacteria bank using the spread plate technique using a cotton swab. Four Muller-Hinton agar plates were labeled for the herbal extracts alongside herbal dilutions of 6.5mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml, 75mg/ml, and 100mg/ml.

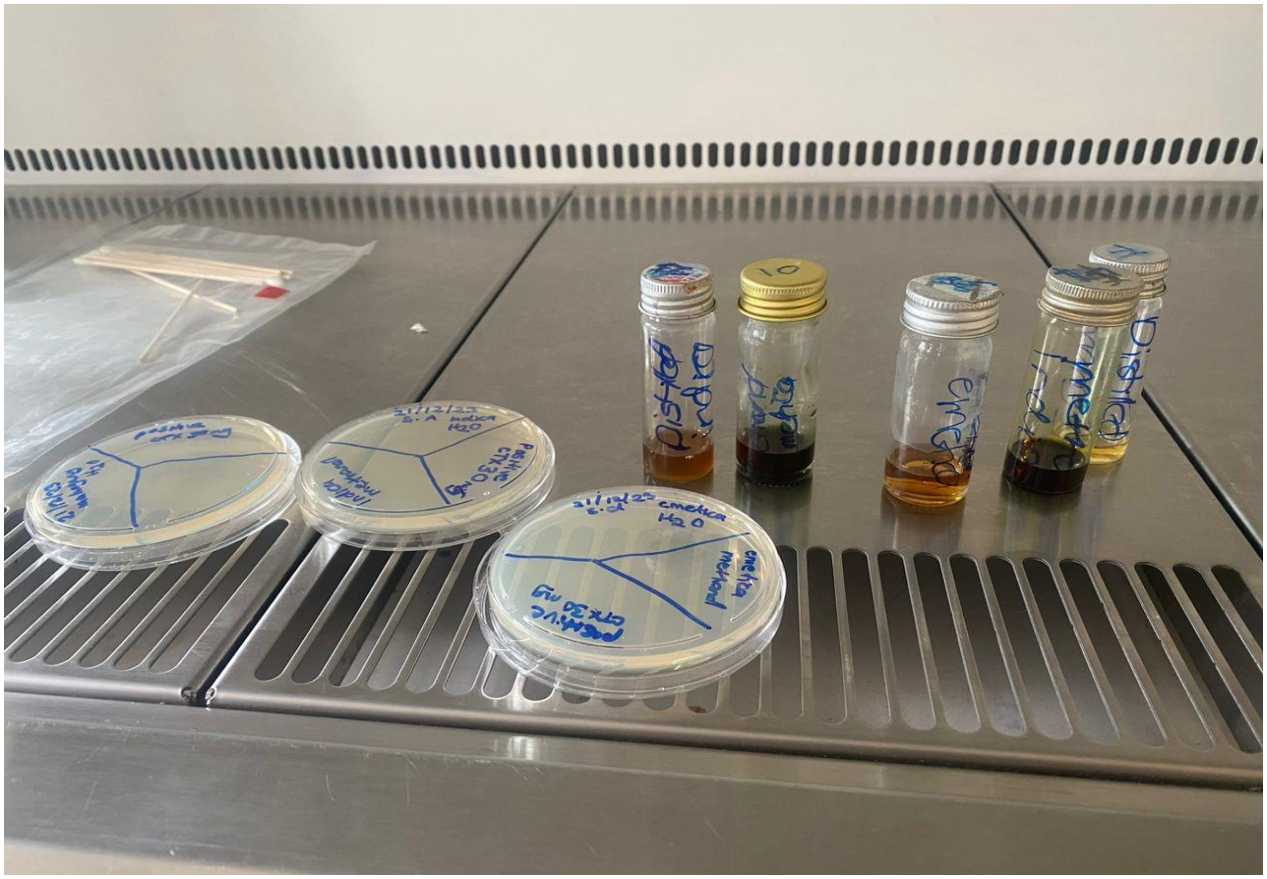

Figure 8: Kirby-Bauer disc diffusion test of methanolic and aqueous extracts of *Azadirachta indica, Chamaemelum nobile,* and *Trichilia emetica* against *Staphylococcus aureus*.

3.8 Disk diffusion assay

Distilled water was used as a negative control and oxytetracycline was used as a positive control. Six-millimetre (6mm) paper disks were added to a sterile petri dish. Five grams (5g) of each of the three selected plants extracts was added to each disk and the disks was left to dry for at least 2 hours. The agar plates were labelled and incubated at 37^0C for 24 hours.

The paper disks containing the herbal extracts and the antibiotic was applied to the agar plates at a distance to prevent the overlapping of inhibition zones. One Muller-Hinton agar plate of bacterial isolate was inoculated with either the antibiotic or the herbs treated as a standard reference to validate the test and the environment (control plate). The control plates of the strain were prepared to reveal high bacterial counts since there was no suppression effect. Incubation was done for 24 hours at 37°C. The measurements of the length of the zones of inhibition were recorded. The susceptibility of the bacterial isolate to each plant extract and the antibiotic oxytetracycline was compared based on the length of the zone of inhibition. Zones of inhibition were measured in millimeters (mm) using a vernier caliper.

3.10 Minimum Inhibitory Concentration MIC

Ringer solution was used to dilute the stock solution using a two-fold dilution series. The selected plant extracts were diluted into various concentrations in sterile nutrient broth in a test tube. One ml of the bacteria sample was inoculated into each test tube containing nutrient broth and one ml of different plant extracts and incubated at 37°C for 24 hours.

mic was calculated using the equation below:

mic (mg/ml) = Dilution Factor X Final concentration

3.11 Data analysis

Bluesksky spss version 10.2.1 was used for ANOVA on the suppression of *S. aureus*. IBM SPSS version 23 was used for correlation analysis. MS excel was used to produce regression trend models for crude extract inhibition.

CHAPTER 4

4.0 RESULTS

4.1 Comparative analysis of the phytochemical constituents of *T. emetica, A. indica* and *C. nobile*.

T. emetica, A. indica, and *C. nobile* were found to possess most of the tested phytochemicals as shown in Table 1 below.

Table 1: Phytochemical screening of methanolic and aqueous extracts of Azadirachta indica, Trichilia emetica, and Chamaemelum nobile, highlighting the presence of alkaloids, flavonoids, saponins, tannins, proteins, glycosides, phenols, and steroids

	A. indica	*T. emetica*	*C. Nobile*
Alkaloids	+	+	+
Flavonoids	+	+	-
Sapponins	+	+	+
Tannins	+	+	+
Proteins	+	+	+
Glycosides	+	+	+
Phenols	+	+	+
Steroids	+	+	+

Key

+ present

-absent

4.2 Crude herbal suppression of *Staphylococcus aureus* by methanolic extracts of *T. emetica, A. indica,* and *C. nobile*

T. emetica, A. indica and *C. nobile* methanolic extracts were found to possess the ability to suppress *S. aureus* and *T. emetica* exhibited the greatest potential in inhibiting the growth of *S. aureus* as shown in Figure 10 below.

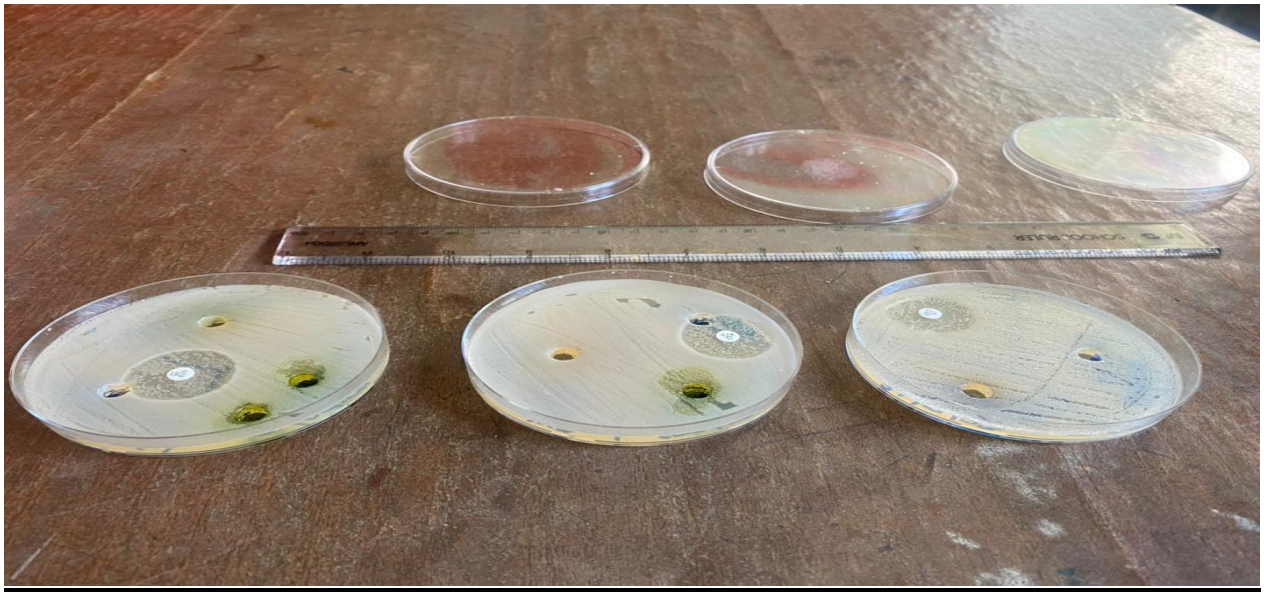

Figure 9: Results of the inhibition of *S. aureus* by methanolic crude extracts of herbal plant extracts under study.

Table 2 below shows that as the concentration is increased the magnitude of inhibition also increased. It shows that there was a greater contribution of solvent used and the plant species. *T. emetica* methanolic crude extracts had greater potential to inhibit *S. aureus* growth.

Table 2: Minimum inhibitory concentration (MIC) of methanolic crude extracts of *Azadirachta indica, Trichilia emetica,* and *Chamaemelum nobile* against *Staphylococcus aureus*

Dilution mg/ml	6.5	12.5	25	50	75	100
A. Indica	0	0.12	0.4	0.9	1.2	1.5
T. emetica	0.4	0.9	1.2	1.7	2.1	2.5

C. nobile	0	0	0.11	0.34	0.81	1.3
CTX	2.5	2.5	2.5	2.5	2.5	2.5

Table 3 below shows that as the concentration increased, the magnitude of inhibition also increased. Compared to methanolic extracts, it shows distilled water extracts had a lower potential to inhibit *S. aureus* growth.

Table 3: Minimum inhibitory concentration (MIC) of distilled water crude extracts of *Azadirachta indica*, *Trichilia emetica*, and *Chamaemelum nobile* against *Staphylococcus aureus*.

Dilution	6.5	12.5	25	50	75	100
A. *Indica*	-	-	0.1	0.22	0.53	0.7
T. emetica	-	0.11	0.22	0.31	0.72	1.3
C. nobile	-	-	-	0.10	0.22	0.43
CTX	2.00	2.00	2.00	2.00	2.00	2.00

4.3. Statistical Analysis results of Antimicrobial Activity.

A one-way analysis of variance (ANOVA) was used to determine whether the antimicrobial activity of the methanolic and aqueous extracts of Azadirachta indica, Trichilia emetica, and Chamaemelum nobile against Staphylococcus aureus differed significantly. The MIC values obtained from each plant species and extraction method were compared to determine statistical significance.

4.3.1. ANOVA results of the Antimicrobial activity of methanolic crude extracts

The table below shows the results of an ANOVA analysis used to determine the minimum inhibitory concentration (MIC) values of methanolic crude extracts from three plant species: *Azadirachta indica*, *Trichilia emetica*, and *Chamaemelum nobile*, against Staphylococcus aureus. The analysis shows that for Azadirachta indica, the between-groups sum of squares is

1.845, with 5 degrees of freedom, yielding a mean square of 0.369. The calculated F-ratio is 0.00, with a significance value (Sig.) of 0.00, indicating that there is no variation in the extract's MIC values.

Similarly, *Trichilia emetica* has a between-groups sum of squares of 3.053 while keeping the same degrees of freedom, resulting in a mean square of 0.611, an F-ratio of 0.00, and a significance value of 0.00. Finally, *Chamaemelum nobile* has a between-groups sum of squares of 1.307, a mean square of 0.261, and an F-ratio of 0.00, with a significance level of 0.00.

Notably, the within-groups sums of squares for all three extracts are zero, indicating a lack of variability within each group. Overall, the results show that the differences in MIC values between the three plant extracts are statistically significant, despite the lack of within-group variability, indicating their potential effectiveness against *Staphylococcus aureus*.

Table 4: ANOVA analysis results of the minimum inhibitory concentration (MIC) values for methanolic crude extracts of *Azadirachta indica*, *Trichilia emetica*, and *Chamaemelum nobile* against *Staphylococcus aureus*

		Sum of Squares	df	Mean Square	F	Sig.
A. indica	Between Groups	1.845	5	0.369	0.00	0.00
	Within Groups	.000	0	.		
	Total	1.845	5			
T.emetica	Between Groups	3.053	5	0.611	0.00	0.00
	Within Groups	.000	0			
	Total	3.053	5			
C. nobile	Between Groups	1.307	5	0.261	.0.00	0.00
	Within Groups	.000	0			

	Total	1.307	5

The output above indicates that *C. nobile* (mean square (0.261) had little suppression on *S. aureus*.

4.3.2. ANOVA results of the Antimicrobial activity of Aqueous Crude Extracts

The results in the table show an ANOVA analysis of the minimum inhibitory concentration (MIC) values for aqueous crude extracts of three plant species: *Chamaemelum nobile, Trichilia emetica*, and *Azadirachta indica*, against a specific test organism. For *Chamaemelum nobile*, the sum of squares for between-group variation is 0.075, with one degree of freedom, resulting in a mean square of 0.075. The calculated F-ratio is 0.563, with a significance level (Sig.) of 0.482, indicating that there is no statistically significant difference in MIC values between the groups. Within groups, the sum of squares is 0.800 with six degrees of freedom, yielding a mean square of 0.133.

In the case of *Trichilia emetica*, the between-groups sum of squares is 0.075 with one degree of freedom, resulting in a 0.075 mean square. The F-ratio is 0.094, with a significance level of 0.770, indicating a lack of significant differences in MIC values. This extract has a within-groups sum of squares of 4.800, with a mean square of 0.800 and 6 degrees of freedom. Finally, for *Azadirachta indica*, the between-groups sum of squares is 0.133, yielding an F-ratio of 0.429 and a significance level of 0.537. The within-group sum of squares is 1.867, which yields a mean square of 0.311. Collectively, these findings show that there are no statistically significant variations in MIC values between the aqueous extracts of the three plant species, implying a consistent efficacy profile against the tested organism.

Table 5: ANOVA analysis results of the minimum inhibitory concentration (MIC) values for aqueous crude extracts of *Azadirachta indica, Trichilia emetica*, and *Chamaemelum nobile* against *Staphylococcus aureus*

		Sum of Squares	df	Mean Square	F	Sig.
C.nobile	Between Groups	.075	1	.075	.563	.482
	Within Groups	.800	6	.133		
	Total	.875	7			

T. emetica	Between Groups	.075	1	.075	.094	.770
	Within Groups	4.800	6	.800		
	Total	4.875	7			
A. indica	Between Groups	.133	1	.133	.429	.537
	Within Groups	1.867	6	.311		
	Total	2.000	7			

	Mean	Std. Deviation	N
Control	44.833	37.1169	6
A.indica	.2583	.29288	6
T.emetica	.4433	.48698	6
C.nobile	.1250	.17295	6

Model		*C.nobile*	*A.indica*	*T.emetica*
Correlations	*C.nobile*	1.000	-.246	-.774
	A.indica	-.246	1.000	-.407
	T.emetica	-.774	-.407	1.000
Covariances	*C.nobile*	21649.368	-2174.883	-6319.486
	A.indica	-2174.883	3621.519	-1358.732
	T.emetica	-6319.486	-1358.732	3075.555

Coefficient Correlations output indicated that *T. emetica* (0.4433) initiated a greater inhibition of *Staphylococcus aureus* growth, followed by A. *indica* (0.2583) and *C. nobile* (0.1250) had the lowest mean inhibition recorded during the study. The model correlation between *T. emetica* and C .*nobile* (-0.774) had the least correlation of coefficient diagnostics.

CHAPTER 5

5.0 DISCUSSION

Plant extracts demonstrated varying antibiotic capabilities owing to their distinct phytochemical compositions, which play a pivotal role in eradicating pathogenic microorganisms. The effectiveness of inhibiting bacterial growth was significantly influenced by the solvents utilised during the extraction process. In the examination of *T. emetica*, *A. indica*, and *C. nobile*, *T. emetica* displayed the most antibiotic properties against S. *aureus*. Notably, extracts from different solvents exhibited diverse antibacterial activities, manifesting in distinct antibacterial spectra for various bacteria and varying levels of antibacterial potency against the same bacterium. This variability arises from the presence of different active ingredients in the solvents of extraction.

Commonly employed solvents for extraction encompass hexane, ethyl acetate, methanol, chloroform, ethanol, water and various solvent combinations in appropriate proportions. The solvent extraction method stands as the predominant approach for extracting active compounds, with methanol and distilled water being the chosen solvents in this study. Adebayo (2012), suggests that the antibacterial mechanisms of plant extracts primarily target the cell membrane, cell wall, nucleic acids, or bacterial metabolic pathways. However, further research is required to comprehensively understand these mechanisms.

S. aureus infections are prevalent in both community and hospital settings, posing a substantial risk due to the bacterium's production of diverse virulence factors. The emergence of multidisciplinary strains, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus*, constitutes a severe threat to human health (Lee *et al*., 2017).

Qualitative tests conducted on methanol extracts of *T. emetica*, *C. nobile* and *A. indica* have revealed the presence of secondary metabolites such as alkaloids, terpenoids, saponins, phenols, tannins, and steroids. These compounds are believed to have contributed to the observed antibacterial activity except flavonoids, which were absent in *C. nobile*. *T. emetica* and *A. indica* exhibited more potent secondary metabolites compared to *C. nobile*. The ANOVA output summary indicates that the differences in the phytochemical constituents of each crude extract are less significant.

A. indica, recognised as a highly useful medicinal plant, has been traditionally used for its antiseptic, antiviral, antipyretic, anti-inflammatory, anti-ulcer and antifungal properties.

Phytochemical constituents like flavonoids and saponins are believed to contribute to the plant's anti-inflammatory, antimicrobial, antioxidant, and antiviral activities (Galeane *et al*., 2017).

The antibacterial activity observed in each extract tested in this study underscores the significance of ethno pharmacological data as a foundation for discovering bioactive compounds from plants. According to the World Health Organization (WHO, 1997; Martinez *et al*., 2015), a medicinal plant is one whose organs contain substances suitable for therapeutic purposes or serve as precursors in drug synthesis.

The different antibacterial activities exhibited by the extracts can be attributed to variations in their components, resulting in distinct antibacterial effects (Akihisa *et al*., 1996). This emphasizes the importance of understanding the specific bioactive compounds present in plant extracts for their potential therapeutic applications.

The antibacterial properties of the herbal methanolic crude extracts from *T. emetica, A. indica* and *C. nobile* against *S. aureus* was almost different across the herbal plant species. *Trichilia emetica* had more powerful antibacterial properties against *S. aureus* compared to *A. indica* and *C. nobile,* this may be due to the presence of phytochemicals found in greater concentration in *T. emetica* compared to others. The findings of this study demonstrate the three plant species' antimicrobial potential against Staphylococcus aureus. Among the methanolic crude extracts, *Trichilia emetica* had the highest antibacterial activity, with a minimum inhibitory concentration (MIC) of 6.5 mg/mL. This value was significantly lower than those found in *Azadirachta indica* (12.5 mg/mL) and *Chamaemelum nobile* (25 mg/mL), indicating that T. emetica contains compounds with higher antimicrobial effectiveness.

Distilled water as a solvent for the extraction of phytochemicals responsible against microbial growth have got some significant impact.The lowest concentration or Minimum Inhibitory Concentration was low for *T. emetica* 12.5g/ml followed by *A. indica* 25g/ml and finally *C. nobile* 50mg/ml. The presence of phytochemical constituents such as flavonoids, terpenoids, steroids, steroids and alkaloids in high concentration may have contributed to the possible suppression observed on the pathogenic *S. aureus* though distilled water was a poor solvent compared to methanol. From the ANOVA analysis output higher mean square value of *T. emetica* compared to other plants under study indicates that this selected tree species might have greater potential above all on the inhibition/ suppression of *S. aureus.* The strain produces several virulence factors, such as staphyloxanthin, hemolysins and lipase therefore the ability

of the solvent extraction method to yield potential antibacterial phytochemicals may inhibit the growth of *S. aureus* and its infections if this crude herbal extracts are included in the formulation of herbal skin creams or vaselines (Dinge *et al.,* 2000).

In the comparative analysis of methanolic extracts and distilled water extracts concerning the suppression of *S. aureus*, the choice of solvent for phytochemical extraction plays a pivotal role in enhancing the inhibition of bacterial growth (Chen *et al*., 2011; Lin *et al*., 2013; Mitani *et al*., 2018). Currently, methanol and distilled water are commonly utilized as solvents for extracting phytochemicals from plant species (Galeane *et al*., 2017). The analysis revealed that methanolic extracts of *T. emetica* exhibited a greater potential for inhibiting the growth of *S. aureus* compared to *A. indica* and *C. nobile*, using both methanol and distilled water as solvents (Chen *et al*., 2011; Lin *et al*., 2013; Mitani *et al*., 2018). The variance in antibacterial efficacy among plant species suggests that the choice of solvent influences the extraction process and subsequent antibacterial activity.

The biological functions of medicinal plants are intricately linked to their antibacterial constituents, such as tannins, steroids, saponins, phenolic compounds, flavonoids, terpenes, alkaloids, glycosides and peptides (Nikaido, 1994; Hu and Kitts, 2005; Jassim *et al*., 2012). These compounds operate through various mechanisms, with saponins managing inflammation (Adebayo and Issah, 2012), tannins reacting with proteins to treat inflammation, alkaloids interacting with DNA and phenolic compounds forming complexes with dissolved proteins or cell membranes to eradicate bacteria.

Reports indicate that alpha-tocopherols can disrupt the efflux pumps of *S. aureus*, reducing resistance (Tintino *et al*., 2016), while organic acids create a more acidic bacterial growth environment, inhibiting the formation of capsular polysaccharides and exerting antibacterial effects. It was noted that distilled water may not extract certain plant components like flavonoids as effectively as methanol, leading to a lower overall extraction efficiency (Chen *et al*., 2011; Lin *et al*., 2013; Mitani *et al*., 2018). Methanol, known for its polarity and volatility, serves as an effective solvent capable of extracting both lipophilic and hydrophilic molecules, facilitating their removal at room temperature (Galeane *et al*., 2017).

In summary, the choice of solvent for phytochemical extraction significantly influences the antibacterial activity of plant extracts against *S. aureus*, and a thorough comparative analysis sheds light on the differential efficacy of methanolic and distilled water extracts from various plant species (Chen *et al*., 2011; Lin *et al*., 2013; Mitani *et al*., 2018).

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study provided a baseline assessment for the possible use of crude herbal tree extracts as substitutes for the treatment and eradication of diseases and also to evaluate the potential therapeutic effects against the problematic pathogenic strains like *S. aureus*. This is due to the phytochemicals and antimicrobial properties of the plants under study. Antimicrobial resistance prominent in the today world has affected the growth of agricultural productivity due to resistant infections induced by resistant genes.

6.2 Recommendations

Future studies should prioritise the inclusion of various plant parts, given the potential variance in phytochemical components found in different parts like leaves and stems. There is a pressing need for increased attention and research to delve into the antibacterial mechanisms inherent in these natural compounds. This exploration is crucial as it lays the foundation for identifying effective natural drugs or food additives. To accomplish this, it is imperative to focus on purifying the active substances to assess antibacterial activity and elucidate the corresponding mechanisms. Ongoing efforts should also be directed towards exploring the abundant antibacterial properties of diverse plants, elucidating the structures and properties of natural products and most significantly, providing a clear understanding of the underlying antibacterial mechanisms**.**

REFERENCES

Afreen, S., Syed, W. and Banerjee, S. (2021). Herbal based drug discovery for skin and surgical infectious diseases. Biopharmacological activities of medicinal plants and bioactive compounds, 101.

Akihisa, T., Yasukawa, K., Kimura, Y., Takase, S., Yamanouchi, S. & Tamura, T. (1996). Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. *Phytochemistry, 43*, 1255–1260.

Aljeldah, M.M. (2022). Antimicrobial resistance and its spread is a global threat. Antibiotics, 11(8), p.1082.

Álvarez-Martínez, F.J., Barrajón-Catalán, E. and Micol, V. (2020). Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. Biomedicines, 8(10), p.405.

Astafieva, A. A., Rogozhin, E. A., Odintsova, T. I., Khadeeva, N. V., Grishin, E. V. & Egorov, T. A. (2012). Discovery of novel antimicrobial peptides with unusual cysteine motifs in dandelion *Taraxacum officinale* Wigg. flowers. *Peptide*, 266–271.

Bandyopadhyay, U., Biswas, K., Sengupta, A., Moitra, P., Dutta, P., Sarkar, D., Debnath, P., Ganguly, C. K. & Banerjee, R. K. (2004). Clinical studies on the effect of Neem (*Azadirachta indica*) bark extract on gastric secretion and gastroduodenal ulcer. *Life Sciences, 75*(24), 2867-2878.

Ben, F., Todd, R. & Brightly, P. (2005). Les bactéries multi résistantes isolées chez les malades hospitalisés dans un service de maladies infectieuses. *Tin Infectiol*, 12–15.

Biswas, K., Chattopadhyay, I., Banerjee, R. K. & Bandyopadhyay, U. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 1336–1345.

Chaturvedi, P., Shukla, P., Giri, B.S., Chowdhary, P., Chandra, R., Gupta, P. and Pandey, A. (2021). Prevalence and hazardous impact of pharmaceutical and personal care products and antibiotics in environment: A review on emerging contaminants. Environmental research, 194, p.110664.

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antibiotics in environment: A review on emerging contaminants. Environmental research, 194, p.110664.

Chen, Y., Wong, R. W., Seneviratne, C. J., Hägg, U., McGrath, C., Samaranayake, L. P. & Kao, R. (2011). The antimicrobial efficacy of *Fructus mume* extract on orthodontic bracket: a monospecies-biofilm model study in vitro. *Archives of Oral Biology, 56*(1), 16-21.

Crotteau, C. A. & Wright, S. T. (2006). What is the best treatment for infants with colic? *Journal of Family Practice, 55*(7), 634-636.

DeLeo, F. R., Diep, B. & Otto, M. (2009). Host defense and pathogenesis in *Staphylococcus aureus* infections. *Infectious Disease Clinics of North America*, 17–34.

Elgamouz, A. & Tijani, N. (2018). Dataset in the production of composite clay-zeolite membranes made from naturally occurring clay minerals. *Data in Brief*, 345-421.

Forster, H. B., Niklas, H. & Lutz, S. (1980). Antispasmodic effects of some medicinal plants. *Planta Medica*, 309–319.

Fupi, V. W. K. & Mork, P. C. (1982). Mafura nut oil and meal: processing and purification. *Journal of the American Oil Chemists' Society*, 94–98.

Germanò, M. P., D'Angelo, V., Sanogo, R., Catania, S., Alma, R., De Pasquale, R. & Bisignano, G. (2005). Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl. (Meliaceae). *Journal of Ethnopharmacology, 96*(1-2), 227-232.

Griesbach, R. J. (2005). Biochemistry and genetics of flower color. *Plant Breeding Reviews*, 89–114.

Guo, Y., Song, G., Sun, M., Wang, J. and Wang, Y. (2020). Prevalence and therapies of antibiotic-resistance in Staphylococcus aureus. Frontiers in cellular and infection microbiology, 10, p.107.

Gupta, A. K., Ahirwar, N. K., Shinde, N., Choudhary, M., Rajput, Y. S. & Singh, A. (2013). Phytochemical Screening and Antimicrobial Assessment of Leaves of *Adhatoda vasica, Azadirachta indica* and *Datura stramonium*. *Environmental Science*, 36-69.

Kareru, P. G., Keriko, J. M., Kenji, G. M., Thiong'o, G. T., Gachanja, A. N. & Mukiira, H. N. (2010). Antimicrobial activities of skincare preparations from plant extracts. *African Journal of Traditional, Complementary and Alternative Medicines, 7*(3), 214-218.

Komane, B. M., Olivier, E. I. & Viljoen, A. M. (2011). *Trichilia emetica* (Meliaceae)–A review of traditional uses, biological activities and phytochemistry. *Phytochemistry Letters*, 1–9.

Kumar, A., Poe, K. & Joe, S. (2009). Phytochemicals investigation on a tropical plant, *Syzygium cumini* from Kattuppalayam, Erode district, Tamil Nadu, South India. *Pakistan Journal of Nutrition*, 83–85.

Kumar, S., Pandey, A. K. & John, S. (2013). Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal*, 2009.

Lavanya, J., Odette, E. & Selvam, S. (2016). Antioxidant and antimicrobial activity of selected medicinal plants against human oral pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*, 71–78.

Lee, J. H., Kim, Y. G., Park, J. G. & Lee, J. (2017). Supercritical fluid extracts of *Moringa oleifera* and their unsaturated fatty acid components inhibit biofilm formation by *Staphylococcus aureus*. *Food Control*, 74–82.

Liang, W. (2020). Toxicity and effect of chemicals in skin care products on human health. In IOP Conference Series: Earth and Environmental Science (Vol. 512, No. 1, p. 012081). IOP Publishing.

Mahady, G. B. (2005). Medicinal plants for the prevention and treatment of bacterial infections. *Current Pharmaceutical Design*, 2405–2427.

Mariita, R. M., Ogol, C. K. P. O., Oguge, N. O. & Okemo, P. O. (2011). Methanol extract of three medicinal plants from Samburu in Northern Kenya show significant antimycobacterial, antibacterial and antifungal properties. *Research Journal of Medicinal Plant*, 54–64.

Martin, M., Paul, D., Orwin, M. & Schlievert, P. (2000). Exotoxins of *Staphylococcus aureus*. *Clinical Microbiology Reviews*, 4441-4502.

Martinez, M., Poirrier, P., Chamy, R., Prüfer, D., Schulze-Gronover, C., Jorquera, L. & Ruiz, G. (2015). *Taraxacum officinale* and related species—An ethnopharmacological review and its potential as a commercial medicinal plant. *Journal of Ethnopharmacology*, 244-262.

Munir, A. A. (1996). A taxonomic review of *Lantana camara* L. and *L. montevidensis* (Spreng.) Briq. (Verbenaceae) in Australia. *Journal of the Adelaide Botanic Garden*, 1–27.

Nascimento, G. F., Locatelli, J., Freitas, P. C. & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 247–256.

Nikaido, H. (1994). Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science*, 382–388.

Oseni, L. A. & Yussif, I. (2012). Screening ethanolic and aqueous leaf extracts of *Taraxacum officinale* for in vitro bacteria growth inhibition, 205-224.

Sakai, H. & Misawa, M. (2005). Effect of sodium azulene sulfonate on capsaicin-induced pharyngitis in rats. *Basic & Clinical Pharmacology & Toxicology*, 54–59.

Sama, F. L., Mouokeu, R. S., Tume, C., Njateng, G. S., Kamchueng, M. O., Ndonkou, N. J. & Kuitate, J. R. (2015). Anti-*Staphylococcus aureus* activity of methanol extracts of 12 plants used in Cameroonian folk medicine. *BMC Research Notes*, 1–6.

Savithramma, N., Rao, M. L. & Suhrulatha, D. (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research*, 579–584.

Sharma, A., Sundaram, S., Malviya, R., Verma, S., Fuloria, N.K., Fuloria, S., Sekar, M., Mishra, N. and Meenakshi, D.U. (2023). Patient care and treatment strategies for skin diseases in sub-Saharan Africa: Role of traditional and western medicines. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 23(3), pp.69-85.

Sharma, G. A., Thakur, A. N., Lal, S. O. & Nadda, R. K. (2019). Comparative analysis of phytochemical, antibacterial, and antioxidant activity of different extracts of *Azadirachta indica* leaves. *Asian Journal of Pharmaceutical and Clinical Research*, 151–154.

Sharma, J., Gairola, S., Sharma, Y. P. & Gaur, R. D. (2014). Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. *Journal of Ethnopharmacology*, 140–206.

Srivastava, J. K., Shankar, E. & Gupta, S. (2010). Chamomile: A herbal medicine of the past with a bright future. *Molecular Medicine Reports*, 895–901.

Stanley, J. R. & Amagai, M. (2006). Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. *New England Journal of Medicine*, 1800–1810.

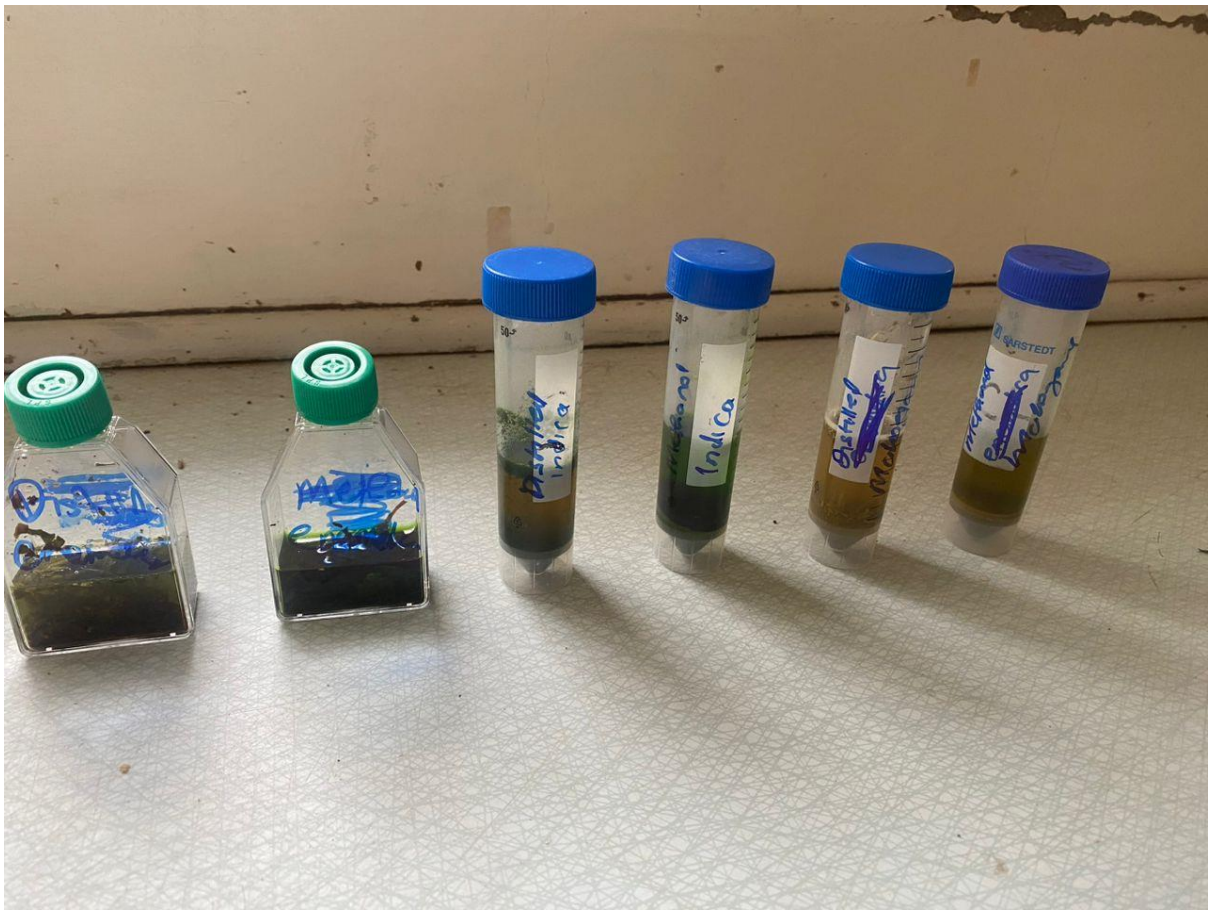
Tacconelli, E., De Angelis, G., Cataldo, M. A., Pozzi, E. & Cauda, R. (2008). Does antibiotic exposure increase the risk of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation? A systematic review and meta-analysis. *The Journal of Antimicrobial Chemotherapy*, *61*(1), 26-38.

Tong, S. Y., Davis, J. S., Eichenberger, E., Holland, T. L. & Fowler, V. G. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, *28*(3), 603–661.

World Health Organization (WHO). (2007). WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues, 2340.

APPENDICES

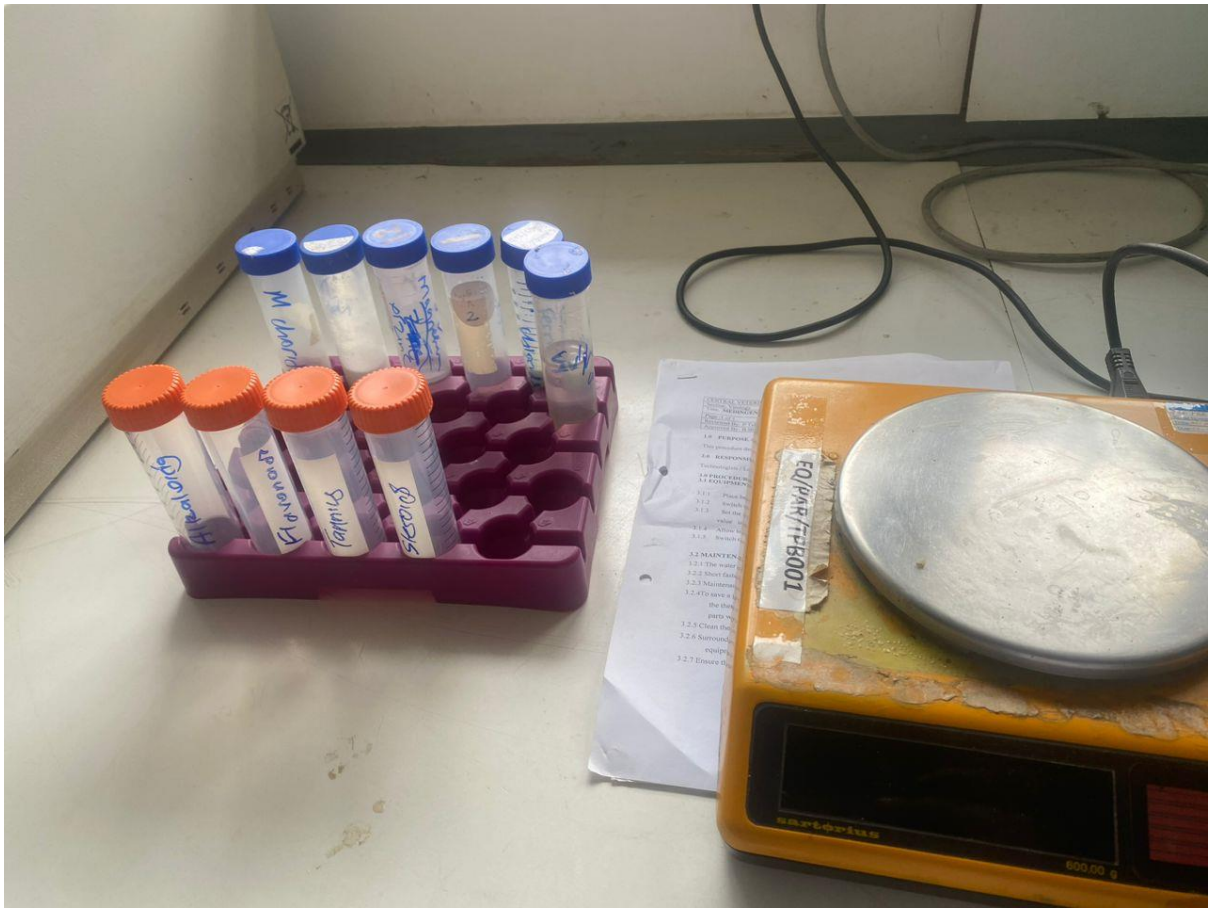
APPENDIX 1: Plant collection and extraction in distilled water and methanol



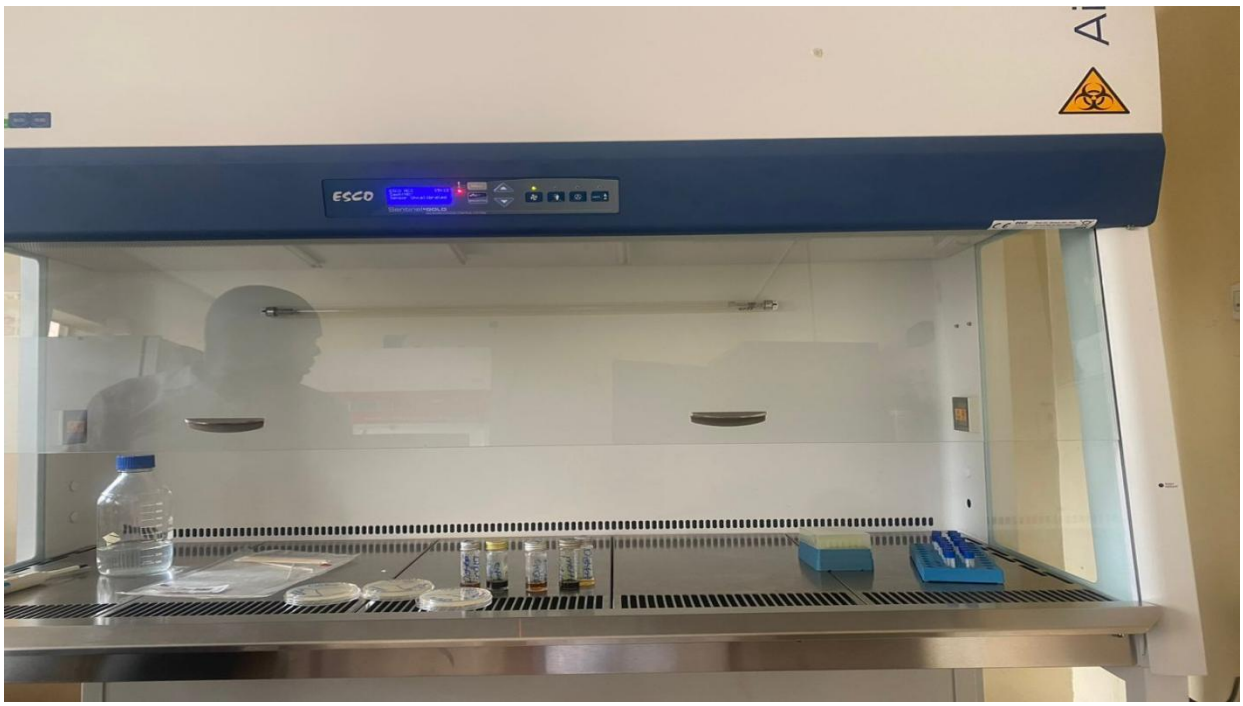
APPENDIX 2: Plant extracts in Distilled water and methanol

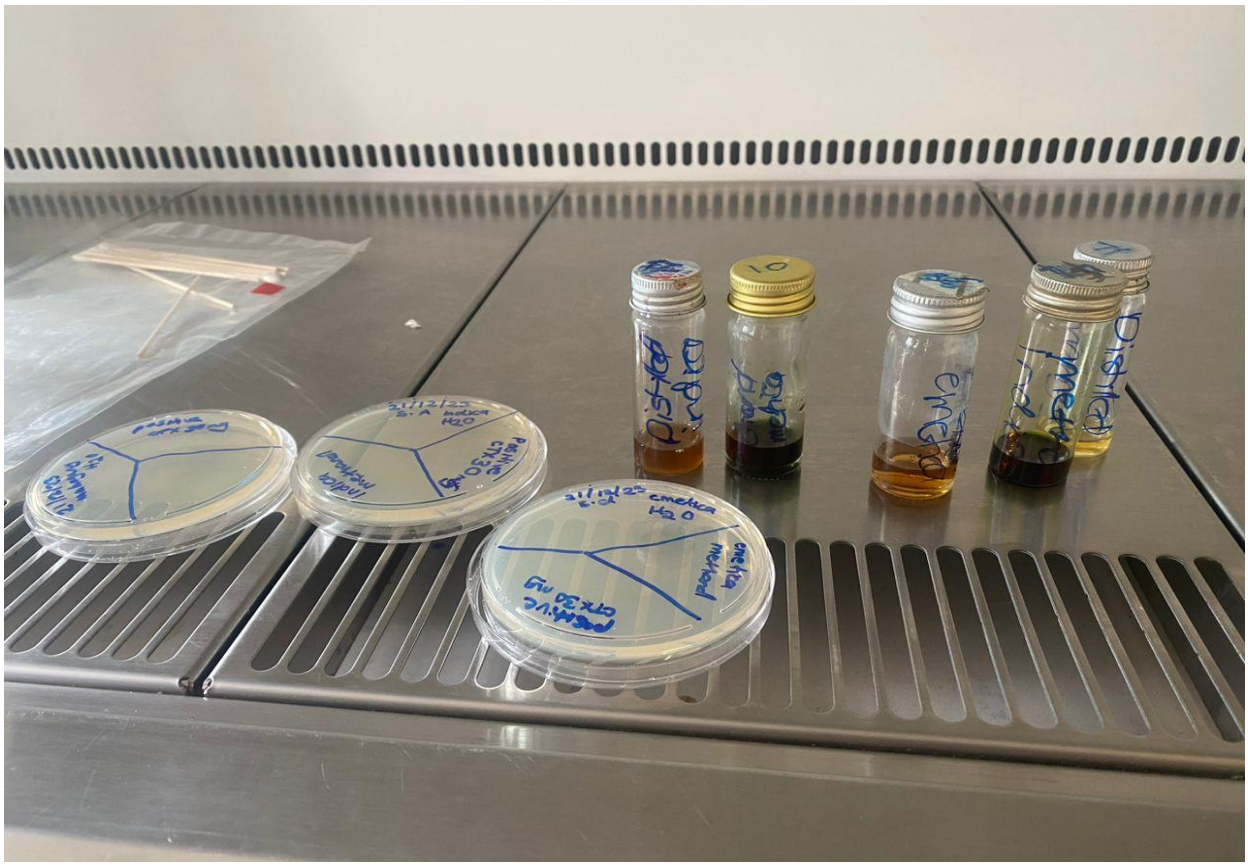
APPENDIX 3: Incubator at CVL and plant extracts in distilled water

APPENDIX 4: Phytochemical tests on the plant extracts



APPENDIX 5: Antimicrobial tests using the Kirby-Bauer test





APPENDIX 6: Student carrying the tests at the CVL