Bindura University of Science Education



ANTIMICROBIAL EFFECT OF *AZANZA GARCKEANA* LEAF AND ROOT EXTRACTS ON *STAPHYLOCOCCUS AUREUS*.

By

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A research project submitted in partial fulfilment of the requirements for the Bachelor of Science Honour's Degree in Biological Sciences.

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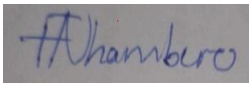
Abstract

Staphylococcus aureus is a notorious gram-positive bacterium which causes a wide range of infections. The overuse of antibiotics in treatment of infections gave rise to antibiotic resistant *S. aureus*. This highlights the need for alternative antibacterial agents. This research aimed to investigate the antimicrobial effects of *Azanza garckeana* (Malvacae family) leaves and roots against *S. aureus*. Aqueous and ethanolic extracts were obtained from *A. garckeana* leaves and roots from a local tree in Domboshava, Goromonzi district of Mashonaland East province in Zimbabwe. Maceration method was used to extract phytochemicals in the leaves and roots, using distilled water and 70% ethanol as solvents. Phytochemical tests were carried out to detect the presence of some phytochemicals in *A. garckeana* leaves and roots. The agar well diffusion method was used to determine the susceptibility of *S. aureus* to *A. garckeana* extracts in triplicates. To determine the Minimum inhibitory concentration of *A. garckeana* extracts on *S. aureus,* broth micro-dilution was done in duplicates. The detected phytochemicals in both *A. garckeana* leaf and root extracts were tannins, saponins, steroids, flavonoids, and alkaloids while glycosides were detected in root extracts only. *A. garckeana* leaf and root (aqueous and ethanolic) extracts inhibited the growth of *S. aureus* at concentrations of 100, 50, and 25mg/ml. One-way ANOVA showed there is statistically significant difference among the means of the zones of inhibition of *A. garckeana*, leaf and root (aqueous and ethanolic) extracts and the mean of the positive control $F(4,100) = 32{,}45$, ($p<0.05$). Tukey's HSD post hoc analysis showed that streptomycin had significantly larger zone if inhibition than all extracts ($p<0{,}01$) and only root ethanolic extract had significantly larger zone of inhibition than aqueous leaf extract ($p = 0{,}034$). Root ethanolic, aqueous root, leaf ethanolic and aqueous leaf extracts inhibited the growth of *S. aureus* with mean MIC values of 12,5, 25, 25 and 50mg/ml, respectively. Streptomycin (control) had the highest zone of inhibition of 29mm. Root ethanoic extract had the highest antimicrobial activity with an average mean zone of inhibition of 26mm against *S. aureus*. *A. garckeana* may be a potential alternative for treating infections caused by *S. aureus.*

Approval form

The undersigned certify that they have read the dissertation titled 'Antimicrobial effect of *Azanza garckeana* leaf and root extracts on *Staphylococcus aureus*.' and confirm that it is suitable for submission to the Biological Sciences Department, Faculty of Science and Engineering, for assessment.

Signature of student:  Date: 12/04/2026

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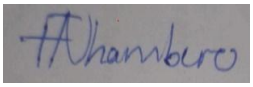
Signature:  Date: 12/04/2026

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Dedication

I dedicate this research project to my father, Mr TC Nhamburo, my brother, Mr Mhonderwa, my sisters and my late mother Mrs L Nhamburo as well as to all research biologists.

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Firstly, l would like to thank God, the author and finisher of my faith for guiding me throughout this research project. I would like to extend my gratitude to my Father, Mr TC Nhamburo and my brother, Mr Mhonderwa for their financial support throughout this academic journey. My heartfelt thanks goes to Dr Manyanga, my supervisor for his informed guidance and tireless mentorship. My special thanks goes to Central Veterinary Laboratory for providing me with resources to carry out this research and to Mr G Bonjani my laboratory supervisor.

List of abbreviations

ANOVA- Analysis of variance

AMR- Anti-microbial resistance

AST- Antimicrobial Susceptibility Test

FAO- Food and Agriculture Organisation

MBC- Minimum Bacterial Concentration

MDR- Multi-drug resistance

mg- milligrams

MIC- Minimum Inhibitory Concentration

mm- millimetres

MRSA- Methicillin-resistant *S. aureus*

SAB- *S. aureus* Bacteraemia

VRSA- Vancomycin-resistant *S. aureus*

WHO- World Health Organisation

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CHAPTER 1 INTRODUCTION

1.1 Background of study

Infectious diseases, particularly those caused by bacteria, continue to present a serious risk to worldwide public health. The emergence of antimicrobial resistance (AMR) is a pressing concern, as it renders many conventional antibiotics ineffective, leading to prolonged hospitalizations and escalated healthcare expenditure in medical care(WHO, 2018). Antimicrobial resistance was recognised among the ten most significant worldwide health risks by the World Health Organisation. There is a need for novel antimicrobial drugs which are effective to microbes. This need has driven research towards exploring natural sources, particularly medicinal plants, which have been used as traditional medicine for centuries.

Azanza garckeana (Malvaceae), often referred to as snotapple, is a deciduous tree indigenous to tropical and subtropical Africa (Mojeremane &Tshwenyane, 2004)). It is widely distributed in countries like Zimbabwe, Zambia, Tanzania, and South Africa. Multiple plant structures, which are the fruit, root, and leaves, are used for their nutritional, medicinal, and economic value (Maroyi, 2022). Traditionally, *A. garckeana* is employed in the treatment of various health conditions, which include skin diseases, respiratory infections and stomach ailments (Yusuf et al., 2020), suggesting potential antimicrobial properties.

Staphylococcus aureus is a Gram-positive bacterium which leads to various infections, which includes minor skin infections and conditions like endocarditis, pneumonia and sepsis which are life threatening (Dalia et al., 2015). The bacterium is a notorious opportunistic pathogen and has a remarkable ability to be antibiotic resistant, especially Methicillin-resistant *Staphylococcus aureus* (MRSA), a prime example of a multi-drug resistant pathogen (Mauchaza et al., 2016). The increasing occurrence of drug-resistant *S. aureus* strains underscores the urgent need for new and effective treatment options.

Given the traditional uses of *A. garckeana* to treat various infections and the increasing concern of antibiotic-resistant bacteria, investigating the antimicrobial potential of this plant is of significant scientific and public health interest.

1.2 Statement of the Problem

The increasing issue of antimicrobial resistance (AMR), particularly in pathogenic bacteria like *Staphylococcus aureus*, is a formidable challenge to modern medicine. The efficiency of conventional antibiotics is diminishing, leading to extended illnesses, treatment failures, higher mortality and morbidity rates. While synthetic drugs have been the cornerstone of antimicrobial therapy, the escalating crisis of resistance necessitates the urgent exploration of alternative sources for new antimicrobial agents. *Azanza garckeana* has been employed over many years as a traditional African medicine for treating conditions associated with microbial infections. However, there is a lack of comprehensive scientific evidence and a knowledge gap regarding the specific antimicrobial effects of its leaf and root extracts, particularly against *Staphylococcus aureus*. The bioactive compounds contained in different parts of the plant may vary, and their respective antimicrobial activities have not been thoroughly evaluated. Therefore, there is the critical need to scientifically validate the traditional use of *A. garckeana* leaves and roots as antimicrobial agents and to determine their efficacy against a clinically significant pathogen, *Staphylococcus aureus*, which contributes the most to the global burden of infectious diseases.

1.3 Aim

This study aims to evaluate the antimicrobial activity of *A. garckeana* leaf and root extract on *S. aureus.*

1.4 Objectives

The objectives of this study are:

1.4.1 To determine if *A. garckeana* leaf and root (aqueous and ethanolic) extracts inhibit the growth of *S. aureus.*

1.4.2 To qualitatively screen for the presence of major secondary metabolites, including flavonoids, tannins, saponins, alkaloids and glycosides in A. *garckeana* leaf and root (aqueous and ethanolic) extracts.

1.4.3 To determine the minimum inhibitory concentrations (MIC) of *A. garckeana l*eaf and root (aqueous and ethanolic) extracts on *S. aureus.*

1.5 Research questions

The research seeks to answer the following questions:

1.5.1 Do *A. garckeana* leaf and root (aqueous and ethanolic) extracts inhibit the growth of S. *aureus?*

1.5.2 What are the phytochemicals present in *A. garckeana* leaf and root (aqueous and ethanolic) extracts?

1.5.3 If they inhibit the growth, what are the Minimum inhibitory concentration (MIC) values of *A. garckeana* leaf and root (aqueous and ethanolic) extracts on *S. aureus?*

1.6 Hypothesis

1.6.1 (Ho) There is no significant difference in the mean of zones of inhibition of *A. garckeana* leaf aqueous, leaf ethanolic, root aqueous and root ethanolic extracts against *S. aureus*.

1.6.2 (Hi) There is a significant difference in the means of zones of inhibition of *A. garckeana* leaf aqueous, leaf ethanolic, root aqueous and root ethanolic extracts against *S. aureus*.

1.7 Significance of study

Many pathogens have developed resistance to various antimicrobial drugs which has caused a global health issue of Antimicrobial resistance (AMR). There is need to explore antimicrobial activity of *A. garckeana* root and leaf extracts as an alternative antimicrobial drug to mitigate the issue of AMR. Antimicrobial resistance is recognized as the most pressing worldwide health concern of the 21st century, each year, millions of people become infected with pathogens that have lost susceptibility to standard antibiotics, this leads to extended illnesses, higher death rates and rising costs of medical care (WHO,2021). Because antibiotic drugs are becoming less effective, there is need to develop alternative treatments. *S. aureus* is one of the pathogens that have the ability to rapidly develop resistance with MRSA being the dominant strain that is also resistant to other antibiotics apart from methicillin. This research aims to address a critical public health concern of antimicrobial resistance by evaluating a local sourced plant, *A. garckeana* as a potential antimicrobial drug against *S. aureus*. This study also contributes to the scientific validation of ethnobotanical knowledge, supporting sustainable use of indigenous flora as antimicrobial agents. This research will scientifically validate traditional medicinal practices which could give rise to the development of new plant based drugs. The

findings from this research will provide a literature for potential future studies that aim to extract and identify bioactive compounds responsible for the observed antimicrobial activity.

CHAPTER 2 LITERATURE REVIEW

2.1 The Global Crisis of Antimicrobial Resistance

Antimicrobial resistance (AMR) is a pressing worldwide health issue caused by the incorrect use of antibiotics in many sectors (Ahmed et al., 2024). The World Health Organization (WHO) and other international organisations have repeatedly emphasized the need for concerted efforts to combat this crisis (WHO, 2018). AMR occurs due to the resistance of microorganisms, such as bacteria, viruses and fungi to the drugs engineered to kill them, lowering drug efficiency and increasing the rate of disease spread, prolonged illness, and death. A key driver of this resistance is limited knowledge regarding the application of antimicrobials and misuse of antibiotics in humans and animals (O' Neil, 2014). An estimated number of 700 000 people globally die due to illnesses caused by antimicrobial resistance every year and there is an estimation that by 2050, the number will rise to 10 million (Salam et al., 2023). Development of potential antibiotics has been slow, and the pipeline of novel drugs is failing to cope up with the emergence of resistance, creating a dire need for alternative therapeutic strategies (Ventola, 2015).

2.2 *Staphylococcus aureus*: A Key Opportunistic Pathogen

Staphylococcus aureus is a bacterium that causes both hospital-acquired and community-acquired infections. The literature extensively documents its capability to cause a broad range of diseases, which include superficial skin infections like boils and abscess and conditions such as septicaemia, bacteraemia, and osteomyelitis (Wang et al., 2023). In the world, the number of people infected with *S. aureus* bacteraemia (SAB), increased from 10 to30 people per 100 000 people from 2014 to 2016 (Gnanamani et al., 2017). In the United Kingdom, from 1991 to 2006 there was a threefold increase in the number of hospitalizations for cutaneous abscess which was caused by *S. aureus*. (Wang et al., 2023). Methicillin-resistant *Staphylococcus aureus* (MRSA) has complicated the treatment protocols on *S. aureus* infections, as these strains are also resistant to various antibiotics, which includes penicillin and augmentin (Mhondoro et al., 2019). *S. aureus* has become resistant to multiple antibiotics through a wide range of pathways which include altered penicillin-binding protein (PBP2a) which decreases beta-lactam efficiency, this is how Methicillin Resistant (MRSA) developed. According to Parthasarathy and Chougale, (2021), the development of resistance in *S. aureus* began in the

1940s where *S. aureus* transitioned from being susceptible to antibiotics to developing multi-drug resistant including MRSA and vancomycin resistant *S. aureus* (VRSA). Parthasarathy and Chougale, (2021) further categorized the development of resistance into waves with the first wave from 1940s-1950s where penicillin was introduced and *S. aureus* developed resistance via the blaZ gene which produces penicillase to hydrolyse the drug and second wave was from 1960s-1970s where methicillin was introduced to overcome penicillase producing strains, however, MRSA appeared in 1961. From 1980s-1990s, MRSA became a global pandemic and this was during the third wave and there was an increase in the utilisation of vancomycin, this contributed to the development of vancomycin resistant *S. aureus* (VRSA). From the 1990s up to the present day, is the fourth wave and MRSA has escaped hospital settings causing widespread community-associated infections which are becoming difficult to treat with standard antibiotics due to multi drug resistance of the bacterial strains. Recent literature highlights the rising concerns about multi drug resistance (MDR). The 2022 Global antimicrobial Resistance and use Surveillance system (GLASS) report highlighted an increasing AMR rate of 35% for MRSA and data forecasts that S. aureus may reach 100% resistance to tetracycline and gentamicin in certain clinical settings by 2027 (WHO, 2023). The literature on *S. aureus* emphasizes the need for continuous surveillance and discovery of potential anti-staphylococcal agents to combat this persistent threat. Some researchers such as Snowden et al, (2014) and Pius et al, (2018) have focused on using plant extracts to control *S. aureus* particularly MRSA strains, focusing on evaluating the MIC values and anti-biofilm potential. *Quercus infectoria* wood extract has shown to inhibit S. aureus growth in vitro with MIC values of (0.05-0.2 mg/ml) against MRSA hospital isolates and *Garcinia mangostana* ethanoic extract of the ring has also shown effectiveness against MRSA and the MIC values ranged from (0.05-0,4mg/ml) (Snowden et al, 2014). Both ethanoic and aqueous extracts of cinnamon (*Cinnamonus* species) bark have also inhibited the growth of the bacteria with strong inhibition zones up to 23mm (Pius et al, 2018). Modern recent searches have emphasized on the anti-biofilm activities of plant extracts against *S. aureus* and there are some plants which showed to have anti-biofilm potential. Plant extracts from coffee (*Coffea arabica*) and grapes (*Vitis vinifera*) inhibited biofilm growth of *S. aureus* by up to 80-85% (Barbarossa et al., 2025). Although researchers have focused on the inhibitory effects of other plants on *S. aureus*, there is a gap in knowledge about the inhibitory effects of indigenous plants particularly *A. garckeana* against *S. aureus*.

2.3 *Azanza garckeana*

2.3.1 *Azanza garckeana* classification and botanical characteristics

According to Bioltif et al,(2020) the botanical classification of *Azanza garckeana* is as follows:

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Super division: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Magnoliopsida – Dicotyledons

Subclass: Dilleniidae

Order: Malvales

Family: Malvaceae – Mallow family

Genus: *Azanza*

Species: *Azanza garckeana*

Azanza garckeana is typically a semi-deciduous tree or shrub. Its height is variable, ranging from 3-15metres, the height depends on the habitat. (Bioltif et al 2020). This tree often exhibits multiple stems that can be either crooked or straight, occasionally branching right from the base. These stems can grow up to a diameter of 25cm when measured at breast height. The tree has a rough bark that appears in shades of greyish-black to brown and is marked with vertical fissures. (FAO, 1983). While the branches are covered in fine hairs during their early development, they tend to lose this texture and gradually become smooth as they mature. The leaves are notable for their round shape, typically measuring between 8 and 12cm while supported by elongated stalks. They are characterized as simple and alternate, featuring a roundish form with 3 to 5 lobes, which are covered in pubescence (Mojeremane and Tshwenyane, 2004). The leaves generally display a heart-shaped base with 5 to 7 prominent veins, terminating in a tip that is either rounded or bluntly pointed. The leaf edges are smooth and can reach sizes of 5 to 20cm in diameter, they are also notably rough in texture on both surfaces with raised veins. In their juvenile stage, the leaves exhibit a velvety texture on the dorsal side while green the ventral side. The plant produces large solitary flowers measuring

up to 6 cm in length situated on long pedicels in axils of uppermost leaves (FAO, 1983). These bisexual blooms feature a distinct fused cup-shaped calyx. The flowers have 5 petals and many stamens, the 5 petals have dark purple or dark red centre and are purplish in colour (FAO, 1983). The flowers have five petals and transform from a yellow colour when young to a purplish in colour when they mature, they typically appear during wet season. (FAO, 1983). Following the flowering period, woody spherical fruits develop during the dry season, specifically from April to August (Bioltif et al, 2020). Reaching diameters between 2.5cm and 4cm, the fruits are covered in fine hairs and are divided into four distinct segments. While they are characterized by a hard, woody exterior that is tardily dehiscent, the contents are edible. When chewed, the fruit palp creates a sweet glutinous, gum-like substance and they typically begin to be consumed in August. Internally, the seeds are hemispherical, measuring up to 10mm in length. (FAO, 1983). The timber of this plant is noted for its strength, durability and greyish appearance with heartwood that displays a darker, deep-grey shade.

2.3.2 Ethnobotanical and Phytochemical Studies on *Azanza garckeana*

Azanza garckeana has been the subject of several ethnobotanical and some pharmacological studies. Ethnobotanical literature documents that the plant's various parts have been used in traditional medicine across different African regions to treat illnesses such as painful mensuration, coughs and chest pains. The plant's roots were used to make a decoction and it was orally taken for treatment of earache, the infusion of the leaves and roots was also taken orally as an anti-emetic (Michael et al., 2015).This plant serves as a local resource offering various biological advantages including anti-inflammatory, analgesic, and wound-healing effects as well as aphrodisiac and utero-tonic qualities. Furthermore, it is utilized in traditional medicine in the treatment of conditions such as infertility, respiratory issues like coughs, chest pains and sexually transmitted infections. (Adam, 2019). Pharmacological researchers have reported several activities of the plant, including antibacterial, anti-fungal, anti-hyperglycaemic, antimalarial and iron absorption (Ochokwu et al., 2020).Throughout the regions where it grows, various parts of *A. garckeana* including its stems, bark, roots and fruits are documented for their diverse therapeutic qualities. These plant parts are frequently utilized in the clinical management and treatment of numerous health conditions. (Maroyi, 2022). Traditional uses such as the treatment of coughs, chest pains and stomach ailments suggest

potential antimicrobial activity. There is a gap in knowledge about the antimicrobial effects of the plant's leaves and roots on *S. aureus*.

The chemical analysis *A. garckeana* highlights its richness in bioactive compounds. In the study by Mutindi (2014) crude root extracts were analysed, leading to the isolation of specific pure compounds such as betulinic acid, stigmasterol, and various gossypol derivatives including 1, 6, 6-Dimethoxygossypol and 2, 6 Methoxygossypol 3. Furthermore, the roots contain a broad spectrum of phytochemicals which include, flavonoids, tannins, saponins and alkaloids, alongside essential nutrients like amino acids and ascorbic acids. (Mutindi, 2014). Due to the therapeutic potential of some of the above compounds, nutritionists often advocate for the consumption of these phytochemicals to promote overall wellness. (Zhang,et al, 2015). *Azanza garckeana* aqueous fruit palp extract has been found to contain a variety of bioactive compounds. Research by Zakawa et al, (2021) indicates the presence of phenols (16.54%) alkaloids (6.11%), tannins (3.85%), flavonoids (1.76%), and saponins (2.46%). According to Zhang et al. (2015), the promoting qualities of these phytochemicals are likely linked to their ability to neutralise free radicals. An overabundance of reactive nitrogen and oxygen species is a key factor in the development of numerous long term illnesses, therefore, the antioxidant properties of these compounds provides a vital protective function. In addition to secondary metabolites, the plant is a source of essential proteins. Momodu et al., (2022) identified several amino acids within the leaves and fruit of *A. garckeana*, the most prominent among these were lysine, leucine, glutamic acid and aspartic acid, which collectively account for approximately 9.67 to 12.97g per 100g of the plant material. According to Ibrahim and Idoko (2023) aqueous fruit extracts successfully isolate tannins, saponins, phenols, alkaloids, flavonoids, steroids, carbohydrates and terpenes. While some studies have focused on the phytochemical composition of the crude root and fruit extract, there is a relative scarcity of detailed phytochemical composition of the plant's leaf and root ethanol extracts.

2.4 Previous Studies on the Antimicrobial Properties of *Azanza garckeana*

There is scarcity of literature regarding the antimicrobial potential of *A. garckeana*. For example, some research has shown that crude extracts of the leaves and stem bark exhibit antimicrobial properties on *Escherichia coli* and *Enterococcus faecalis* which have been associated with phytochemicals like alkaloids, flavonoids and phenolic elements (Maroyi, et al, 2022). *A. garckeana* fruit pulp extract inhibited the *S. aureus* growth with a MIC value of 500mg/ml (Zakawa et al, 2021). The tree's fruit pulp extract also possess anti-inflammatory,

wound-healing and analgesic properties (Yusuf et al., 2020). The fruit pulp extract also showed to have inhibitory effect on *Klebsiella pneumonia* giving an MIC value of 12,5 mg/ml (Yusuf et al, 2020) However, these studies were focused on the fruit pulp extract and had a limited range of bacterial species. There is scarce literature concerning a targeted investigation into the growth inhibitory effect of both the root and leaf extracts of *A. garckeana* on a specific, clinically relevant pathogen like *Staphylococcus aureus*. This study aims to generate information about a focused and systematic evaluation of the antimicrobial effects of these plant parts on *S. aureus*. The findings will contribute valuable scientific data to validate use of *A. garckeana* and pave the way for future studies on the isolation of the active compounds.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study Area

This study was conducted at Central Veterinary Laboratory, a veterinary Laboratory located in Harare Central Business District, Harare province, Zimbabwe.

3.2 Source of research Materials

The *Azanza garckeana* fine and young leaves and roots were collected from a three-year old mature *A. garckeana* local tree in Domboshava, Goromonzi district, Mashonaland East Province, Zimbabwe.

3.3 Research design

The maceration method was used for phytochemical extraction as described by(Azwanida, 2015). For extraction, distilled water and 70% ethanol were the two solvents used. Four extractions were prepared which were Root aqueous (RD), Root ethanoic (RE), Leaf aqueous (LD) and Leaf ethanoic (LE). These four extractions were used for phytochemical analysis. For AST, serial dilutions were done for each extract to produce concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12,5mg/ml, 6,25mg/ml and 3,125mg/ml. From these concentrations, only 100, 50, 25 and 3,125mg/ml were used as treatment in Antimicrobial Susceptibility Testing (AST), with streptomycin as the positive control. Each was replicated 3 times. A total of 24 test tubes were used during the serial dilutions in each replicate. MIC detection was done in duplicates and only one micro-titre plate was used in each replicate.

3.4 Research procedure

3.4.1 Sample collection

For phytochemical extraction, fine and young *Azanza garckeana* leaves were collected and placed in a glass container. The roots were uprooted using a hole and a pick and they were placed in a separate glass container. The samples were transported to the laboratory for sample preparation.



Figure 3.1 *A. garckeana* leaves on *A. garckeana* tree.

3.4.2 Sample preparation

Distilled water was used to wash the samples. Roots were dried in the oven heater at 40℃ for 24 hours and the leaves were dried under shade for five days. A pestle and motor was then used to grind each of the samples into powder form and a sieve was used to separate lager particles and finer particles. The powder was then used in phytochemical extraction.



Figure 3.2*: A. garckeana* powdered roots.

3.4.3 Preparation of Extracts

Per each sample, for extraction, a balance was used to measure 10g of the sample and it was added to 100ml of the solvent (distilled water and 70% ethanol) in a glass beaker to make an initial concentration of 100mg/ml. The mixture was left for 72 hours at room temperature. A whatman filter paper number 1 was used to filter the extracts to remove residues. A rotary evaporator connected to a pump to was used to evaporate excess solvent in the extracts and to obtain extracts at 40℃ and at 0.38 rotations per minute (rpm).

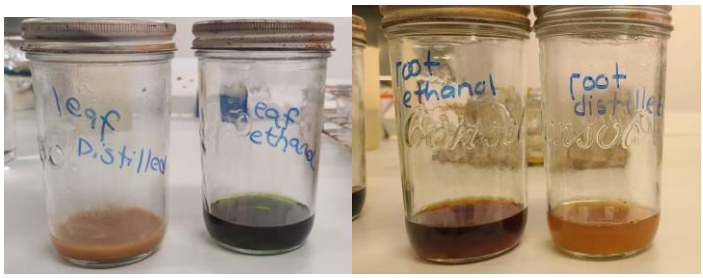

Figure 3.3: Ethanol and aqueous extracts of *A. garckeana* leaves and roots.

3.4.4 Phytochemical analysis

Qualitative phytochemical screening was conducted on aqueous and ethanolic extracts of both roots and leaves to test for the presence of some secondary metabolites. Tannins were detected by the development of a green hue upon the addition of 5% ferric chloride to 2ml of extract. Saponin presence was determined through the persistent frothing of the extract when agitated with distilled water. Glycosides were detected by heating the extract with sulphuric acid to produce a red colour, while steroids were indicated by a brown reaction following the addition of acetic and sulphuric acid. Flavonoids were confirmed using 2M sodium hydroxide to yield a yellow colour, and alkaloids were identified by a brown precipitate after treating the extract with hydrochloric acid and ammonium hydroxide.

3.4.5 Antimicrobial Susceptibility test (AST)

The agar well diffusion method was used in the antimicrobial susceptibility test as described by Balouiri et al., (2016). The bacterial inoculum was prepared up to 0,5 Mc Farland standard. 100µl of the inoculum was transferred using a micropipette onto Muller Hinton agar plates. A glass spread was used to spread the inoculum in all the plates. The plates were left for 5minutes to allow the inoculum to dry. 6mm diameter holes were punched into the agar using a sterile cork borer. In each plate, four holes were punched, each hole representing the four dilutions which were used in AST. 100µL of the extract was placed in each hole. For the positive control, a separate agar plate was punched at the centre and 100µl of streptomycin antibiotic was placed in the hole. The plates were incubated at 37℃ for 24 hours.

3.4.6 Determination of the Minimum inhibitory concentration (MIC).

To determine the Minimum inhibitory concentrations (MICs), The broth micro dilution technique was done as described by Kowalska-Krochmal & Dudek-Wicher, (2021). A 96 well micro titre plate was used. 100µl of nutrient broth was placed in each well excluding the first wells and 200µl of the extracts were placed in the first wells. Serial dilutions were done by using a multichannel pipette to suck 100µl of the extract in the first wells and placing it in the second wells thus diluting the extract with broth in the second wells and repeating until the last dilution in the last wells. In the micro titre plate, Dilutions up to 10^{-10} were obtained . 50µl bacterial inoculum was added in each well. The micro titre plate was shaken, covered and incubated at 37℃ for 24 hours.

3.5 Data collection

3.5.1 Phytochemicals

For each extract, the presence of phytochemicals in *A. garckeana* extracts were denoted by a specific colour change. The colour change observed in each test was recorded if the phytochemicals recorded as absent or present.

3.5.2 Antimicrobial Susceptibility Test

Inhibitory effect was shown by a halo clear zone around the well known as zone of inhibition. A Vernier calliper was used to measure the zones of inhibition for each of the extract concentrations (100mg/ml, 50mg/ml, 25mg/ml and 3.125mg/ml) and the control. The AST was done in triplicates so the mean zones of inhibition were calculated.

3.5.3 Minimum Inhibitory Concentration (MIC)

The broth micro-dilution technique was used to detect the Minimum Inhibitory Concentration (MIC) value of *A. garckeana* leaf and root extracts. A drop of tetrazolium dye was placed in each well to clearly show if bacteria is present or absent. If the bacteria are present (no inhibition) the dye turns red and if the bacteria are not present (inhibition) the dye remains clear. For each extract the MIC (lowest concentration that inhibits bacterial growth (lowest concentration that has no colour change) was observed and it was recorded. MIC detection was done in duplicates and the mean minimum inhibitory concentrations were calculated and recorded.

3.6 Data Analysis

SPSS (IBM 20), a statistical analysis tool was used and the data obtained in AST was subjected to a normality test and one-way analysis of variance was used to determine the levels of difference among the mean zone of inhibition of *A. garckeana* leaf and root, (aqueous and ethanolic) extracts and the positive control as antimicrobials on *S. aureus* This is done to determine if there are significant differences in the antimicrobial activity of the extracts and the positive control. Turkey's HSD post hoc was done to determine which treatments were different from each other and the positive control.

CHAPTER 4: RESULTS ANALYSIS

4.1 Antimicrobial activity of *A. garckeana*

Aqueous and ethanol extracts showed inhibitory potential at 100, 50 and 25mg/ml. The highest antimicrobial activity was detected at 100mg/ml of root ethanolic extracts and the lowest antimicrobial activity was detected at 25mg/ml of aqueous leaf extract as shown by table 4.1. All extracts at 3,125mg/ml could not inhibit the growth of *S. aureus*. Root ethanolic extracts showed high inhibitory potential than other extracts. The control showed a higher antimicrobial activity than *A. garckeana* extracts. The data was normally distributed so it passed the normality test and one- way ANOVA showed that the differences in the means of zones of inhibition of root and leaf (aqueous and ethanolic) extracts and the mean of positive control were statistically significant, $F(4,100) = 32.45$ ($p < 0.01$) as shown in appendix E. Turkey's HSD post hoc showed that the positive control had significantly larger zone of inhibition than all plant extracts, ($p < 0,001$), only root ethanolic extract had a significantly large zone of inhibition than aqueous leaf extract, ($p=0,034$) and all other pairwise comparisons among extracts were not significantly different, ($p > 0,05$).



Figure 4.1: Antimicrobial susceptibility (AST) test showing zones of inhibition.

Table 4.1 Results showing average means of zones of inhibition of the different treatments

Extract concentration (mg/ml)	**Root Ethanoic**	**Root distilled**	**Leaf Ethanoic**	**Leaf Distilled**
100	26	22	18	16
50	25	20	18	16
25	16	13	15	12
3.125	8	8	8	8
Control	29			

4.2 Phytochemical analysis

The results of phytochemical analysis of *A. garckeana* leaf and root (aqueous and ethanolic) extracts revealed the presence of saponins, tannins, steroids, and alkaloids in all extracts and glycosides were present in root extracts only as shown in table 4.2.

Table 4.2: Phytochemicals present in *A. garckeana* leaf and root extracts.

Phytochemical	Root distilled	Leaf distilled	Root ethanoic	Leaf ethanoic
Tannins	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	_	+	_
Steroids	+	+	+	+
Flavonoids	+	+	+	+
Alkaloids	+	+	+	+

KEY: - Absent, + Present.

4.3 The Minimum Inhibitory Concentration (MIC) value of *A. garckeana* leaf and root extracts

Root ethanolic extract had the lowest mean MIC value of 12,5mg/ml and leaf aqueous extracts had the highest mean MIC value of 50mg/ml.

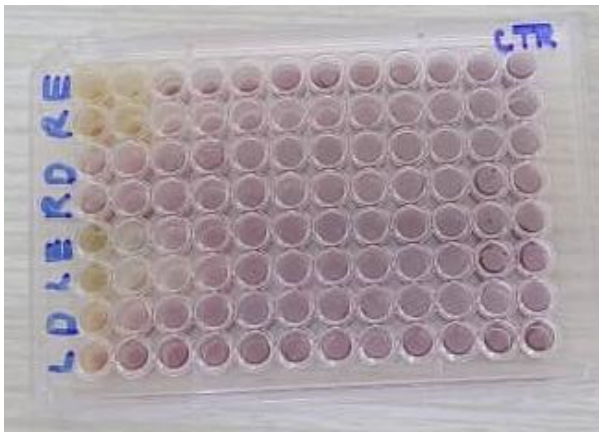

Figure 4.2: Broth micro-dilution test showing the MIC values of *A. garckeana* extracts.

Table 4.3 Mean MIC values of *A. garckeana* extracts on *S. aureus.*

Extract	MIC value
Root ethanoic	12.5mg/ml
Root distilled	25mg/ml
Leaf ethanoic	25mg/ml
Leaf distilled	50mg/ml

CHAPTER 5: DISCUSSION

5.1 Antimicrobial effect of *A. garckeana*

The purpose of this study was to determine the antimicrobial effect of *Azanza garckeana* leaf and root extracts against *S. aureus*. The results confirmed that *A. garckeana* has a significant antimicrobial effect against *S. aureus*. The findings align with the previous study by (Zakawa et al, 2021) who reported the antimicrobial effect of *A. garckeana* fruit palp extract on *S. aureus*. However, due to institutional resource constraints, testing was restricted to *S.aureus* ATCC 25923, thereby precluding the evaluation of clinical methicillin-resistant (MRSA) phenotypes.Results obtained may not be applicable to other *S. aureus* strains.

5.2 Phytochemicals in *A. garckeana*

The study revealed the presence of phytochemicals such as alkaloids, tannins, saponins, glycosides, steroids and flavonoids in *A. garckeana* extracts as similar to (Mutindi, 2014) who identified the same phytochemicals in the plant. The presence of these phytochemicals attributes to the antimicrobial properties of the plant (Zhang et al, 2015). However, further research is required to understand the mechanisms underlying these effects. These findings are limited since only specific laboratory tests were used to detect the presence of some phytochemicals and not phytochemical screening and bioassay-guided fractionation. There is need for further research to identify all the phytochemicals that are present in the plant.

5.3 Anti-microbial susceptibility of *A. garckeana*

In this study, the *A. garckeana* aqueous and ethanolic leaf and root extracts had significant antimicrobial effect against *S. aureus* whilst the aqueous leaf extract showed weak antimicrobial activity and root ethanolic extract had high antimicrobial activity. This study aligns with the study conducted by Zakawa et al, 2021 who revealed that *A. garckeana* fruit palp ethanolic extract had high potent antibacterial activity against S. *aureus*. The antimicrobial activity observed is likely due to the phytochemicals present which are known to possess distinct bactericidal mechanisms. The highest mean zone of inhibition obtained in this study was of *A. garckeana* ethanolic root extract, followed by aqueous root extract, followed by ethanolic leaf extract and the least was aqueous leaf extract. The highest yield

obtained could have been attributed by the superior extraction efficiency of ethanol which is driven by its lower dielectric constant compared to distilled water. In this study, root ethanoic extract revealed the highest antimicrobial effect of 26 ± 1.9mm mean zone of inhibition against *S. aureus* at 100mg/ml concentration. The control (streptomycin) had the highest mean zone of inhibition of 29 ± 1.9mm so streptomycin showed to have greater antimicrobial activity than *A. garckeana*. This could be due to the presence of a single isolated highly refined active pharmaceutical ingredient (API) present in the purified antibiotic. However, only one antibiotic (streptomycin) was used as a positive control. There is need for further research in finding out the Minimum, Bacterial Concentration (MBC) of *A. garckeana* against *S. aureus*.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the study has shown that *Azanza garckeana* leaf and root extracts have antimicrobial effects against *S. aureus* in vitro althoughthere was only one bacterial strain used in this study and yet literature has many bacterial strains of *S. aureus*. The ethanolic extracts of *A. garckeana* leaves and roots were found to have more inhibitory potential compared to the aqueous extracts. The root extracts were found to be more effective than the leaf extracts. Findings suggest that ethanol may be able to extract bioactive compounds from *A. garckeana* leaves and roots effectively than distilled water. However, streptomycin showed to have higher inhibitory potential than *A. garckeana* extracts. *A. garckeana* revealed its bacteriostatic effect against *S. aureus*. This can be used to develop natural remedies and reduce the reliance on antibiotics. These findings suggest that *A. garckeana* leaf and root extracts could be a potential therapeutic for the treatment of diseases caused by *S. aureus*.

6.2 Recommendations

- There is need to establish whether other plant parts like seeds and barks have the same antimicrobial effect as the *A. garckeana* leaves and roots.
- All the parts of *A. garckeana* could be combined to determine if their combined effect is the same as the individual.
- The antimicrobial activity of *A. garckeana* can be further evaluated using other solvent extraction systems for improved performance.
- The minimum bactericidal concentration (MBC) test may be used to determine the exact lowest concentration of *A. garckeana* leaf and root extracts required to kill the bacteria.

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APPENDICES

Appendix A Sample preparation

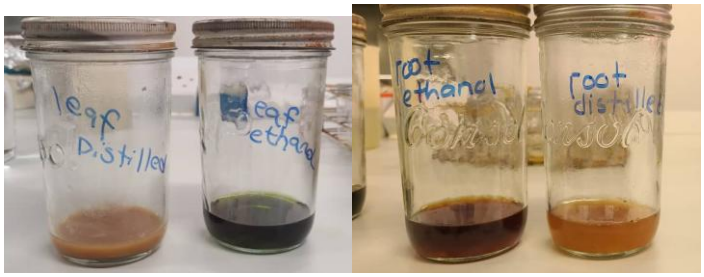


Roots being grounded into powder by pestle and mortar



Powdered plant roots and leaves being measured using a beam balance

Appendix B Solvent extraction



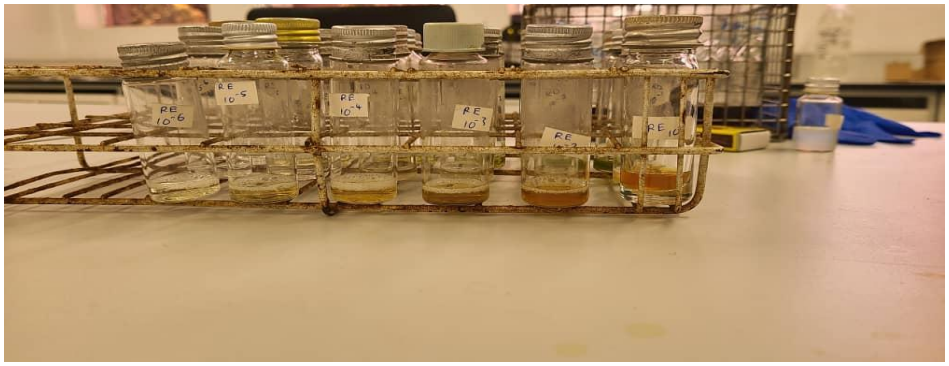
A. garckeana extracts in glass beakers



A. garckeana extracts on a rotary evaporator

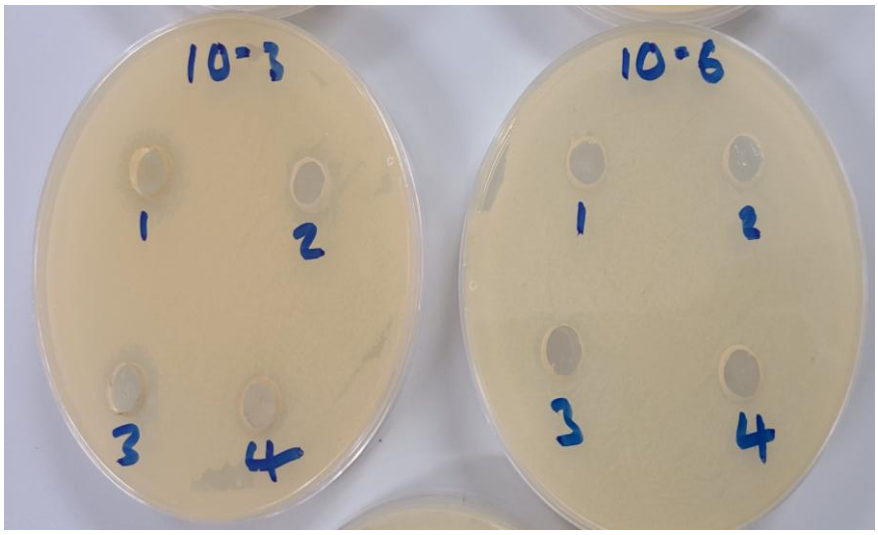
Appendix C Serial dilutions



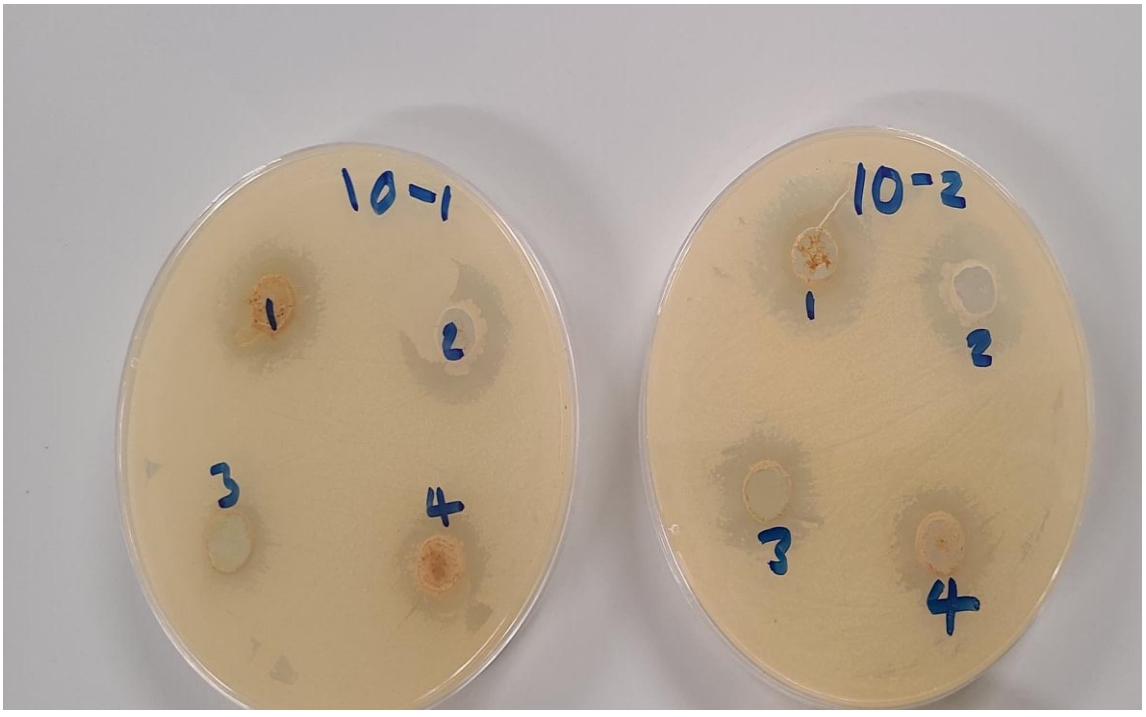




Appendix D Zones of inhibition



22mg/ml and 3.125 mg/ml concentrations



100mg/ml and 50mg/ml Concentrations

Appendix E Normality tests and Analysis of Variance

Tests of Normality							
		Kolmogorov-Sirona			Shapiro-Wilk		
	Concentration(mg/ml)	Statistic	df	Sig.	Statistic	Df	Sig.
Zone of Inhibition (mm)	100mg/ml	.153	9	.200	.931	9	.491
	50mg/ml	.149	9	.200	.909	9	.306
	25mg/ml	.236	9	.159	.806	9	.024
	12.5mg/ml	.228	9	.194	.824	9	.039
	Positive Control	.160	9	.200	.945	9	.639

One- way ANOVA

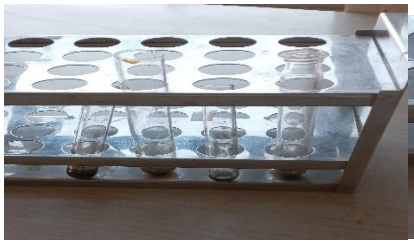
Source	Sum of squares	Df	Mean square	F	Sig.
Between groups	2772.333	4	693.08	32.450	<.001
Within groups	2136.167	100	21.362		
Total	4908.500	104			

Appendix F Turkey's HSD Post hoc

Turkey's HSD Post hoc analysis

I Group	J Group	Mean difference (I –J)	Std. Error	Sig	95% CL Lower	95% CL Upper
Root Ethanolic	Root Aqueous	2.250	1.683	.578	-2.47	6.97
Root Ethanolic	Leaf Ethanolic	3.750	1.683	1.26	-0.97	8.47
Root Ethanolic	Leaf Aqueous	5.000	1.683	**.034**	0.28	9.72
Root Ethanolic	Positive Control	-11.833	1.683	< **.001**	-16.55	-7.11
Root Aqueous	Leaf Ethanolic	1.500	1.683	.856	-3.22	6.22
Root Aqueous	Leaf Aqueous	2.750	1.683	.372	-1.97	7.47
Root aqueous	Positive control	-14.083	1.683	< **.001**	-18.81	-9.36
Leaf Ethanolic	Leaf Aqueous	1.250	1.683	.879	-3.47	5.97
Leaf Ethanolic	Positive Control	-15.583	1.683	< **.001**	-20.31	-10.86
Leaf Aqueous	Positive Control	-16.833	1.683	< **.001**	-21.55	-12.11

Appendix G phytochemicals



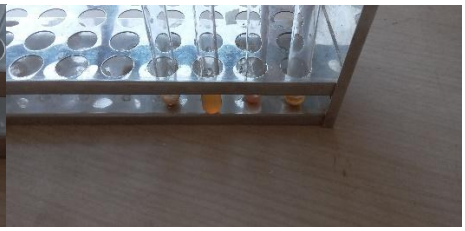
Tannins test in *A. garckeana* extracts.



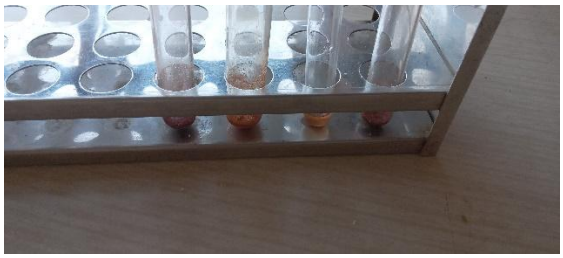
Glycosides test in *A. garckeana* extracts.



Steroids test in *A. garckeana* extracts



Flavonoids test in *A. garckeana* extracts

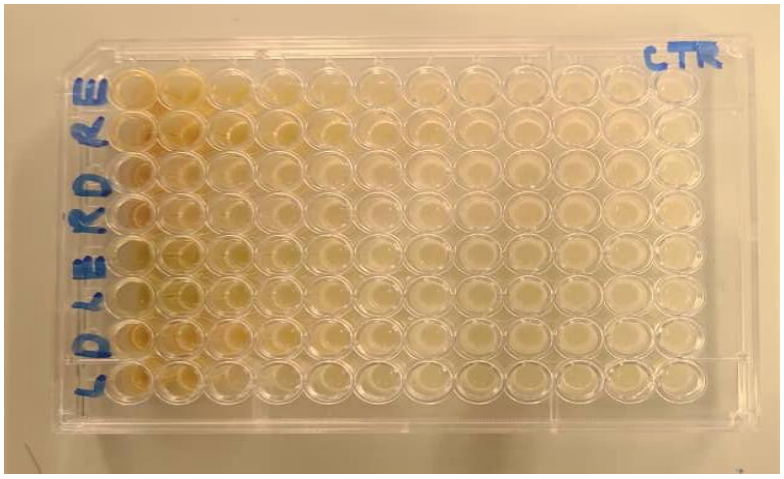


Alkaloids test in *A. garckeana* extracts.

Appendix F Broth micro-dilution



Micro-pipette, pipetting nutrient broth



Micro-titre plate with broth, extract and bacteria.