



**BUSITEMA
UNIVERSITY**
Pursuing Excellence

P.O. Box 236, Tororo, Uganda
Gen: +256 - 45 444 8838
Fax: +256 - 45 4436517
Email: info@adm.busitema.ac.ug
www.busitema.ac.ug

FACULTY OF SCIENCE AND EDUCATION

DEPARTMENT OF CHEMISTRY

**PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY
OF TYLOSEMA ESCULENTUM LEAF EXTRACTS AGAINST
COMMON SKIN PATHOGENS**

BY

KANWAGI CHARLES

BU/UG/2023/2327

**A RESEARCH PROJECT REPORT SUBMITTED TO THE
DEPARTMENT OF CHEMISTRY IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE AWARD OF A BACHELOR'S
DEGREE IN SCIENCE EDUCATION OF BUSITEMA UNIVERSITY**

JUNE, 2026

DECLARATION

I **KANWAGI CHARLES**, declare that this research project is my original work and has not been submitted to any other institution for academic credit.

Signature: .....

Date: 25th/08/2026.....

APPROVAL

This research project has been submitted with the approval of the supervisor.

DR. OWOR RICHARD ORIKO

Signature: .....

Date: 25/08/2026.....

DEDICATION

I dedicate my research work to my mother Ms. Nakeeya Aisha and my uncle Mr. Ssekanjako Deo for their endless efforts and support towards my academics and Ms. Naggayi Aisha who has been there for me emotionally and spiritually.

ACKNOWLEDGEMENT

I thank my supervisor Dr. Owor Richard Oriko who always guided me and provided necessary support towards the successful completion of my research work. I appreciate the members of the Department of Chemistry in particular Ms. Alowo Florence, Ms. Chebijira Mercy, Mr. Mwesigye John Paskari, Dr Andima Moses and the laboratory technicians Ms. Amado Mary and Mr. Chebet Collins for the tireless commitment and time offered in support of the research work.

I am thankful to my fellow classmates, especially Kajubi Paul, Malunda Sowedi, Mbusa Edson and Kijejere Mubarak for their consistent help and encouragement during my research.

Above all, I express my deepest gratitude to Almighty God for granting me life, wisdom, knowledge, good health and constant protection both inside and outside the academic environment.

ABSTRACT

Skin infections present a significant public health challenge in East Africa and Africa at large. This issue is worsened by rising antimicrobial resistance among common cutaneous pathogens. *Tylosema esculentum* known as marama bean is a resilient perennial legume that is currently valued for its nutritional and medicinal properties while the chemical profile some of its parts such as leaves remains unexplored. The therapeutic effectiveness of plant-based preparations is fundamentally dependent on the concentration and diversity of secondary metabolites such as phenols, alkaloids, flavonoids among others which serve as the primary bioactive agents against pathogenic organisms. The main objective of this research was to determine the qualitative secondary metabolite composition and quantitative concentrations of the selected phytochemicals within the leaf tissue matrix of *Tylosema esculentum*. The leaves of *Tylosema esculentum* were subjected to ultrasonic-assisted extraction using pure acetone as the extraction solvent at a temperature of 45°C, the crude extract was qualitatively analyzed for the presence of flavonoids, alkaloids, tannins, phenols, saponins, steroids, terpenoids, glycosides, quinones, anthraquinones and phlobatannins. The extraction process yielded 3.492 g of crude extract from an initial sample size of 40g of leaf powder giving a total percentage extraction yield of 8.73% which demonstrated a viable extraction efficiency. The qualitative phytochemical analysis confirmed the presence of flavonoids, alkaloids, tannins, phenols, saponins, steroids, glycosides, and quinones, while terpenoids, anthraquinones and phlobatannins were absent. Quantification demonstrated that tannins were the most abundant phytochemicals at a concentration of 629.6 mg/g, followed by alkaloids at 112.2 mg/g, flavonoids at 24.9 mg/g and steroids at 24.8 mg/g. Antimicrobial assays against skin pathogens were not carried out because the required microbiology laboratory chemicals, microbial cultures and biosafety equipment were not readily available. The findings of the study suggest that *Tylosema esculentum* leaves may be a potential source of antimicrobial agents due to the exceptionally high presence of bioactive alkaloids, flavonoids, tannins and steroids and also provide basis for further antimicrobial investigations.

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	x
CHAPTER ONE: INTRODUCTION	1
1.1 Background	1
1.2 Problem statement	4
1.3 Objectives of the study	4
1.3.1 General objective	4
1.3.2 Specific objectives	4
1.4 Research questions	5
1.5 Scope of the study	5
1.6 Justification	5
1.7 Significance	5
CHAPTER TWO: LITERATURE REVIEW	7
2.1 Overview of the literature review	7
2.2 Distribution and Local Occurrence	7
2.3 Description of the plant	7
2.4 Classification of <i>Tylosema esculentum</i>	7
2.5 Ethnobotanical Uses and Traditional Knowledge	8
2.6 Biochemical constituents of <i>Tylosema esculentum</i>	8
2.7 Phytochemicals	8
2.7.1 Flavonoids	9
2.7.2 Alkaloids	9
2.7.3 Phenols	10
2.7.4 Terpenoids	10
2.7.5 Tannins	11
2.7.6 Saponins	11
2.7.7 Steroids	12
2.7.8 Quinones	13

2.7.9 Anthraquinones	13
2.7.10 Glycosides	13
2.7.11 Phlobatannins	14
CHAPTER THREE: METHODOLOGY	15
3.1 Study area	15
3.2 Research design	15
3.3 Plant material collection and preparation	15
3.3.1 Collection and authentication	15
3.3.2 Processing and drying	15
3.3.3 Extraction	15
3.4 Phytochemical screening tests	16
3.4.1 Test for alkaloids	16
3.4.2 Test for flavonoids	16
3.4.3 Test for tannins	16
3.4.4 Test for phenols	17
3.4.5 Test for saponins	17
3.4.6 Test for terpenoids	17
3.4.7 Test for steroids	17
3.4.8 Test for glycosides	17
3.4.9 Test for quinones	17
3.4.10 Test for anthraquinones	18
3.4.11 Test for phlobatannins	18
3.5 Quantification of phytochemicals	18
3.5.1 Total Flavonoid Content	18
3.5.2 Total Alkaloid Content	18
3.5.3 Total Tannins Content	19
3.5.4 Total Steroidal Content	19
3.6 Data analysis methods	19
3.7 Ethical considerations	19
CHAPTER FOUR: RESULTS AND DISCUSSION	21
4.1 Introduction	21
4.2 Presentatyon of results	21
4.2.1 Qualitative phytochemical analysis	21
4.2.2 Total phytochemical content	22
4.2.2.1 Total Flavonoids Content	22

4.2.2.2 Total Alkaloid Content.....	23
4.2.2.3 Total Tannins Content.....	24
4.2.2.4 Total Steroidal Content.....	25
4.3 Analysis of results.....	26
4.3.1 Total flavonoids content.....	27
4.3.2 Total alkaloids content.....	27
4.3.3 Total tannins content.....	27
4.3.4 Total steroidal content.....	27
4.4 Discussion of results.....	28
CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS	30
5.1 Conclusion.....	30
5.2 Recommendations.....	30
5.3 Areas for Further Research.....	31
REFERENCES	32

LIST OF TABLES

Table 1: Phytochemical screening results	21
Table 2: Total Flavonoids Content	22
Table 3: Total Alkaloid Content	23
Table 4: Total Tannins Content	24
Table 5: Total Steroidal Content	25
Table 6: Total phytochemical content	27

LIST OF FIGURES

Figure 1: Acne vulgaris	2
Figure 2: Skin infections caused by staphylococcus aureus	3
Figure 3: Common fungal infections	3
Figure 7: Calibration curve for flavonoids	23
Figure 8: Calibration curve for alkaloids	24
Figure 9: Calibration curve for tannins	25
Figure 10: Calibration curve for steroids	26
Figure 11: A bar graph showing the phytochemical content of Tylosema leaf extract	28

LIST OF ABBREVIATIONS

SDGs - Sustainable Development Goals

WHO - World Health Organisation

DMSO - Dimethyl sulfoxide

DNA - Deoxyribonucleic acid

RNA - Ribonucleic acid

UV – Ultraviolet

SD - Indicates Standard

TE - Indicates Tylosema Esculentum sample

mL - Millilitres

mg - Milligrams

μL - Microlitres

CHAPTER ONE: INTRODUCTION

1.1 Background

Skin infections caused by bacterial and fungal pathogens are among the most common health problems that affect people of all ages especially in developing countries like Uganda (Lwigale et al., 2024). They arise when pathogenic microorganisms breach the skin's protective barriers and proliferate within cutaneous tissues. The skin normally serves as a primary defense mechanism through its physical structure (keratinized epidermis), chemical environment and immunological surveillance (Madigan et al., 1997). Disruption of this barrier through cuts, abrasions, insect bites, burns, surgical wounds or underlying dermatological conditions creates an entry point for pathogens (S. Y. C. Tong et al., 2015).

Bacterial pathogens are among the most common causes of skin infections. *Staphylococcus aureus*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Escherichia coli* or *Streptococcus pyogenes* frequently colonize the skin surface and can invade deeper tissues when the barrier is compromised (Dréno et al., 2018). These organisms produce several factors such as adhesins, toxins and enzymes such as hyaluronidase, coagulase among others that facilitate tissue invasion, immune evasion and inflammation (Willey et al., 2023). This can lead to conditions such as impetigo, acne, cellulitis and abscess formation.

Fungal infections typically occur when dermatophytes such as trichophyton species, yeasts like *Candida albicans* or molds exploit moist warm environments on the skin (Hay et al., 2014). These organisms secrete keratinases that degrade keratin enabling colonization of the stratum corneum, hair or nails (Gupta et al., 2017). Such infections are generally superficial but can become invasive in immunocompromised individuals.

A number of antibiotics are widely used to treat common bacterial skin infections. Drugs such as ampiclox, penicillin, doxycycline, cephalosporins, clindamycin and trimethoprim-sulfamethoxazole are frequently prescribed. Ketoconazole, terbinafine for fungal skin infections while mupirocin ointment is often used for mild localized infections like impetigo. However, the widespread availability of these medicines without proper medical supervision together with incomplete treatment courses has contributed to rising antibiotic resistance. As a result, some skin infections are becoming more difficult and costly to manage. This growing challenge has renewed interest in alternative and complementary approaches, particularly the use of medicinal plants.

Medicinal plants have long served as an important source of therapeutic compounds for managing various human diseases including microbial infections (Newman et al., 2016) . According to the World Health Organisation's report on traditional and complementary medicine (2019), a substantial share of the global population relies on plant-based remedies as a primary source of healthcare especially in regions where modern pharmaceutical services are limited. Medicinal plants have been known to produce compounds with therapeutic properties such as antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic and gastroprotective effect (Mazimba et al., 2011). In recent years, increasing scientific focus has been directed toward indigenous and underutilized plants with the potential to yield novel pharmacologically active substances.

Tylosema esculentum commonly known as marama bean, a perennial herb is traditionally valued for its nutritional and medicinal properties (Chingwaru et al., 2015) . Like other medicinal plants, *T. esculentum* contains naturally occurring chemical compounds known as phytochemicals that help protect them from diseases and pests many of which have been found to possess antimicrobial, antioxidant and anti-inflammatory properties that are beneficial to humans (Cowan, 1999). Studies have reported that extracts from different parts of the plant exhibit antibacterial and antifungal activity. However, most studies have focused on seeds and roots with limited attention given to leaf extracts. The leaves of *T. esculentum* are readily available making them suitable for sustainable medicinal use, investigating their phytochemical composition and antimicrobial activity may provide scientific validation for their traditional use and contribute to the development of affordable antimicrobial agents.



Figure 1: Acne vulgaris



Figure 2: Skin infections caused by *staphylococcus aureus*



Figure 3: Common fungal infections

1.2 Problem statement

Antimicrobial resistance has become a growing public health concern worldwide by diminishing the effectiveness of commonly used antibiotics and antifungal drugs. Infections that were once easily treated are increasingly persistent leading to prolonged illness, higher medical costs and greater risk of complications (O'Neill, 2016). Skin infections in particular remain common in many communities and are frequently associated with pathogens such as *Staphylococcus aureus* and *Candida albicans* (Costa et al., 2024). The emergence of resistant strains of these organisms has reduced treatment options thereby encouraging the search for new and locally available plant-based antimicrobial alternatives. Although medicinal plants have long been recognized for their potential to provide natural antimicrobial compounds, many indigenous species remain scientifically underexplored (Wyk, 2017). Despite the fact that it is readily available and has been used for numerous purposes, there is limited scientific research on the antimicrobial activity of *Tylosema esculentum* leaf extracts particularly against skin-infecting microorganisms. The absence of adequate scientific data on the effectiveness of its leaves in inhibiting skin pathogens creates a knowledge gap that limits their possible therapeutic use. Without such evidence, the plant's potential contribution to the search for safe, affordable and effective alternative treatments for skin infections remains unclear. This study therefore sought to fill this gap by identifying the phytochemical constituents of *T. esculentum* leaves and assessing their antimicrobial effects against common skin pathogens.

1.3 Objectives of the study

1.3.1 General objective

To determine the phytochemical composition and assess the antimicrobial activity of *Tylosema esculentum* leaf extracts against common skin pathogens.

1.3.2 Specific objectives

- i. To determine the percentage yield of extract from *Tylosema esculentum* leaves using acetone as a solvent
- ii. To investigate the presence of major phytochemical classes in the leaf extracts
- iii. To determine the total phytochemical content within the crude leaf extracts.
- iv. To evaluate the antimicrobial efficacy of the leaf extracts against common skin pathogens

1.4 Research questions

- i. What is the total percentage yield of dry extract recovered from *Tylosema esculentum* leaves utilizing a sequential ultrasonic extraction method with pure acetone?
- ii. What are the major phytochemical compounds present in *Tylosema esculentum* leaf extracts and how much is present?
- iii. Can *Tylosema esculentum* be considered a potential natural source of antimicrobial agents?

1.5 Scope of the study

This study was aimed investigating the phytochemical composition and antimicrobial activity of *Tylosema esculentum* leaf extracts against common skin pathogens. Leaves were collected from Nagongera town council, Tororo district in Uganda and extracts were prepared by ultrasonic-assisted extraction using pure acetone. Phytochemical screening and quantification of key compounds such as flavonoids, alkaloids among others were performed using laboratory chemical tests. The study was conducted in the laboratories of Busitema university at Nagongera campus. It was carried out in three months' time, from March to June 2026

1.6 Justification

The emergence and spread of drug-resistant microbes has threatened the activity of available drugs and remains the major cause of treatment failure. The burden of morbidity and mortality has been inclined towards developing countries due to the increased prevalence of risk factors associated with economic transition (Prestinaci et al., 2015). Antibiotics that were once thought to be miracle cures are now unable to treat resistant microorganisms. Numerous multidrug-resistant microbes have been identified as severely dangerous (Tong et al., 2015). Conducting a phytochemical screening and antimicrobial evaluation of *Tylosema esculentum* leaf extracts generated scientific evidence that may support the use of the plant part as a natural antimicrobial agent. The findings can provide foundational data for future drug development, contribute to efforts aimed at combating antimicrobial resistance and promote the utilization of locally available plant resources.

1.7 Significance

The findings of this study help to scientifically validate the traditional use of *Tylosema esculentum* leaves in treating skin infections. The results may provide a basis for developing affordable plant-based antimicrobial products suitable for local communities. The research

empowers local communities by leveraging indigenous knowledge and locally available plant resources.

For Busitema University, the research serves as a valuable academic contribution and a reference for future studies in natural product chemistry and microbiology.

The results of this study have the potential to inform national and regional policies on traditional medicine, antimicrobial stewardship and community health. The integration of validated plant-based remedies into primary healthcare can support the goals of universal health coverage and the Sustainable Development Goals (SDGs) particularly SDG 3 which focuses on good health and well-being).

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of the literature review

This chapter presents existing knowledge related to *Tylosema esculentum*, its distribution, botanical characteristics, ethnobotanical uses, biochemical composition and phytochemical profile. It also mentions scientific theories explaining the antimicrobial activity of plant secondary metabolites and identifies the knowledge gaps

2.2 Distribution and Local Occurrence

Tylosema esculentum is a member of the Fabaceae (Leguminosae) family and subfamily Cercidoideae with synonyms such as *Bauhinia esculenta* and *Bauhinia bainesii*. It has documented occurrences in Botswana, Namibia and South Africa as well as localized areas of East Africa including Uganda (Uzabakirho, 2016). The plant exhibits irregular growth throughout the warm season and thrives in soils that are low in organic content, nitrogen and phosphorus. It grows best at temperatures ranging from 28–37 °C and requires approximately three hours of sunlight before and after midday, with a light intensity of around 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Cullis et al., 2019). It requires low rainfall, between 100 and 900 mm and long periods of drought. It is found on sandy and limestone soils and in grassland vegetation. The plant's adaptation to arid and semi-arid environments is largely attributed to its large underground tuber which stores water and nutrients and its ability to thrive in sandy nutrient-poor soils (Germishuizen & Meyer, 2003).

2.3 Description of the plant

Tylosema esculentum commonly known as marama bean and as gemsbok bean is a perennial herb with tuberous roots and a creeper with prostrate, trailing stems up to 3 m long arising from a large tuber with forked tendrils which facilitate climbing opposite the leaves (Castro et al., 2005). The leaves are bifoliolate and leathery and has small yellow flowers that give way to hard, woody pods enclosing dark, rounded seeds rich in structural oils and complex proteins. Beneath the soil, it forms a massive, water-storing fibrous tuber that acts as an energy reservoir during prolonged dry spells (Nepolo & Chimwamurombe, 2012).

2.4 Classification of *Tylosema esculentum*

Domain Eukaryota

Kingdom Plantae

Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Genus	Tylosema
Species	Tylosema esculentum (Burch.) A.Schreib

2.5 Ethnobotanical Uses and Traditional Knowledge

Across various indigenous African communities, different parts of *Tylosema esculentum* serve crucial roles in traditional medicine and survival diets (Omotayo A.O & Aremu O, 2021). Young tubers are consumed either baked, boiled or roasted while those older than 2 years become fibrous and bitter and usually not eaten. The beans rich in protein and oil are eaten boiled or roasted (Chingwaru et al., 2015). The seed oil is used for cooking and making butter in Botswana. Crushed leaf paste and expressed leaf sap are applied topically by traditional healers to soothe scalds, accelerate wound closure and suppress superficial skin rashes or boils. This historic use points to the presence of active chemical compounds capable of inhibiting opportunistic surface pathogens (Gwamba et al., 2025).

2.6 Biochemical constituents of *Tylosema esculentum*

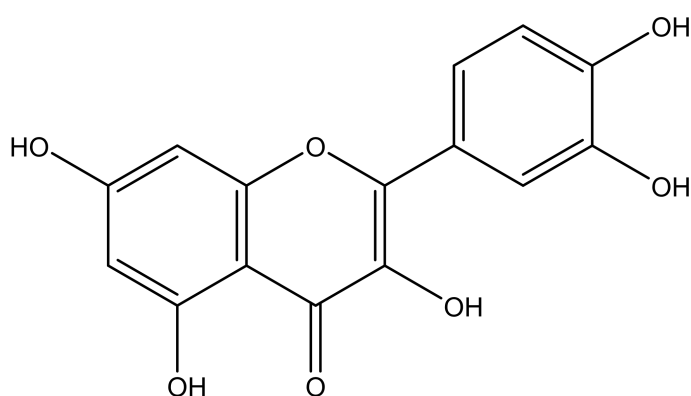
The seeds of *T. esculentum* are highly nutritious, boasting a substantial protein content, ranging from 29% to 39% and significant oil content 24% to 42% (Vilakazi et al., 2025). The golden yellow seed oil is particularly rich in mono- and di-unsaturated fatty acids including oleic and linoleic acids. Furthermore, marama beans are a good source of macronutrients such as calcium, iron, zinc, phosphate, magnesium, potassium, phosphorus, sulphur and B-vitamins including folate. The protein composition is notable for its high levels of amino acids like tyrosine, aspartic acid and glutamic acid (Nyembwe et al., 2014).

2.7 Phytochemicals

Phytochemicals are bioactive compounds produced by plants as part of their defense mechanisms. They are also known as plant secondary metabolites which have been widely studied for their antimicrobial, antioxidant and therapeutic properties.

2.7.1 Flavonoids

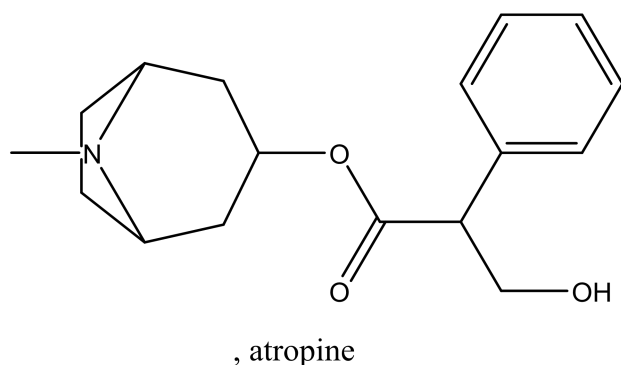
Flavonoids belong to the large class of polyphenolic secondary metabolites. They are structured around a 15-carbon skeleton consisting of two benzene rings linked by a heterocyclic pyran ring. Major structural categories include flavones, flavonols, flavanones, and isoflavones with examples like quercetin, catechin, rutin, kaempferol among others. Their antimicrobial property relies on complexing with bacterial cell walls. Their antimicrobial activity involves membrane disruption, inhibition of nucleic acid synthesis, enzyme inhibition and complex formation with microbial cell walls and proteins (Cushnie , 2011).



quercetin

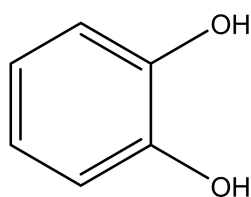
2.7.2 Alkaloids

Alkaloids are a diverse class of organic compounds containing basic nitrogen atoms. They are categorized based on their heterocyclic rings into types like quinoline, isoquinoline, indole, and piperidine alkaloids, with examples including quinine, nicotine, atropine, codeine, strychnine, berberine and ephedrine. These compounds exhibit potent antimicrobial activity. Some alkaloids exert antimicrobial effects through DNA intercalation, inhibition of topoisomerases, membrane disruption, and interference with metabolic pathways (Cushnie, 2014).



2.7.3 Phenols

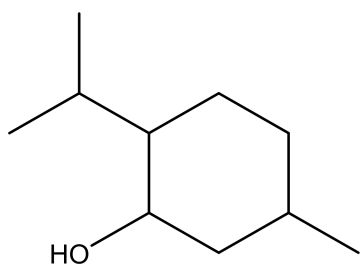
Phenols are simple aromatic compounds possessing one or more hydroxyl groups attached directly to a benzene ring. This class includes phenolic acids, coumarins, and simple phenols like catechol and resorcinol. Their antimicrobial action is highly effective. They can denature proteins, disrupt membranes and inhibit essential microbial enzymes, ultimately interfering with microbial growth and survival (Chibane et al., 2019).



catechol

2.7.4 Terpenoids

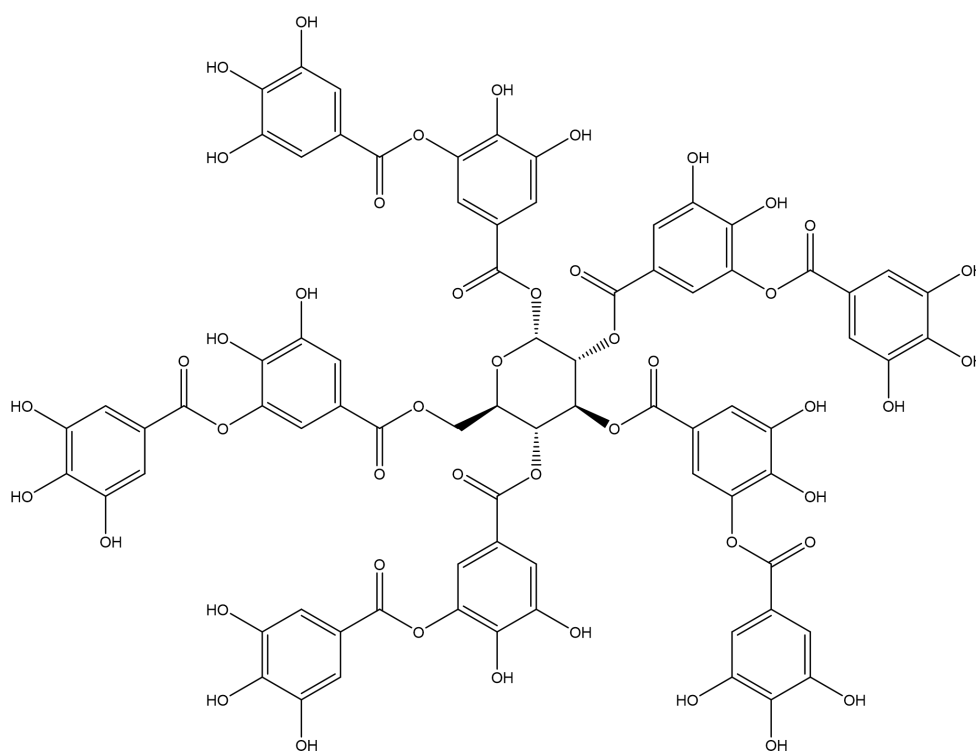
Terpenoids are fat-soluble molecules built from repeating combinations of five-carbon isoprene blocks. Depending on the size of their carbon frame, they are classified into monoterpenes, sesquiterpenes, or diterpenes, with menthol and camphor being among the examples. They easily penetrate bacterial and fungal defenses. Because they dissolve well in lipids, they interact with microbial membranes, increasing permeability and impairing cellular function (Nazzaro et al., 2017).



menthol

2.7.5 Tannins

Tannins are large, complex polyphenolic compounds famous for their ability to bind and precipitate proteins. They are generally categorized as either hydrolyzable types such as gallotannins or condensed structures known as proanthocyanidins. Their germ-fighting power stems from this binding talent. They exert antimicrobial effects through protein precipitation, enzyme inhibition, membrane disruption and metal ion chelation (Tong et al., 2022).

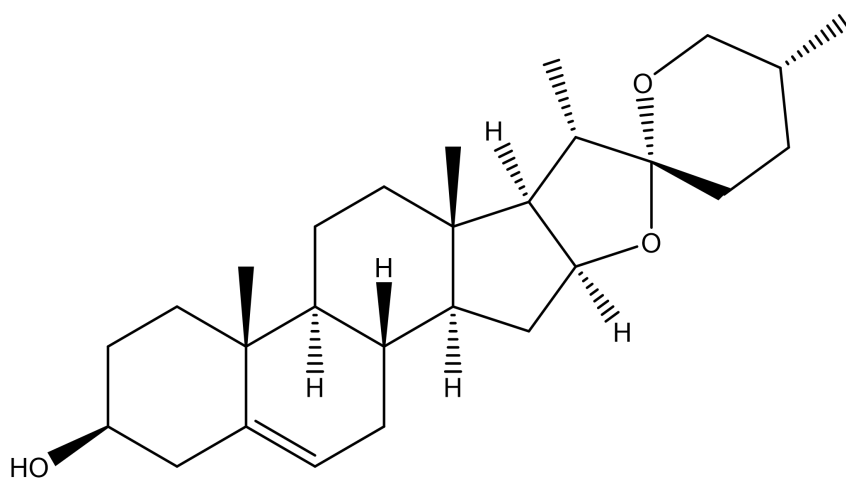


Tannic acid

2.7.6 Saponins

Saponins are natural glycosides possessing distinct surfactant properties that cause them to foam like soap in water. Chemically, they fall into two major baskets: steroidal structures and triterpenoid frameworks with diosgenin being a prominent example. Their main defensive

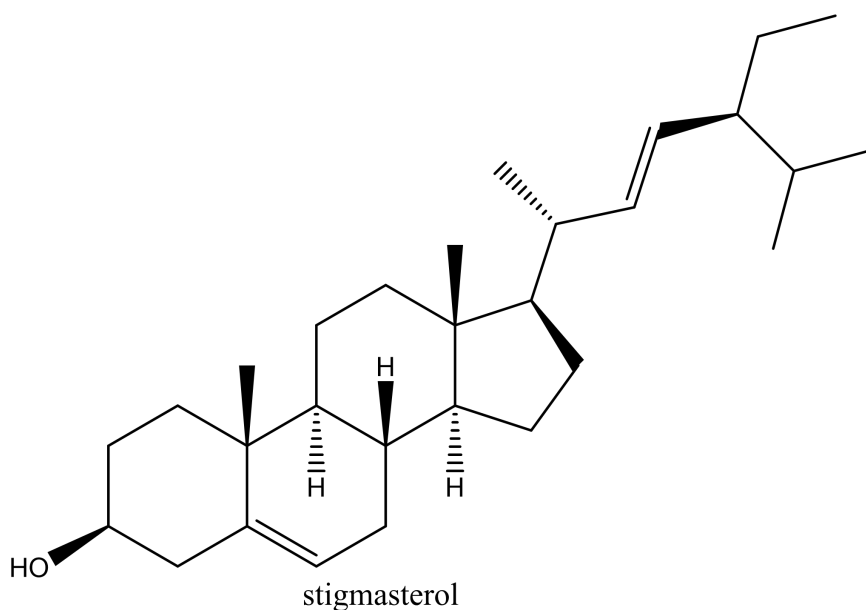
strategy involves puncturing cell boundaries. They anchor onto the sterols inside microbial membranes increasing membrane permeability and causing leakage of intracellular contents (Moses et al., 2014).



diosgenin

2.7.7 Steroids

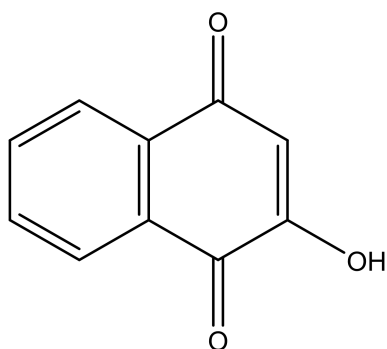
Steroids represent a specialized line of modified triterpenoids centered around a shared, four-ring fused carbon core. Plant versions, frequently called phytosterols with β -sitosterol and stigmasterol being widespread examples (Moreau et al., 2018). They fight off pathogens by embedding into the lipid bilayer of the microbe. This alters the normal fluidity of the membrane leading to leaks and structural failure (Salehi et al., 2021).



stigmasterol

2.7.8 Quinones

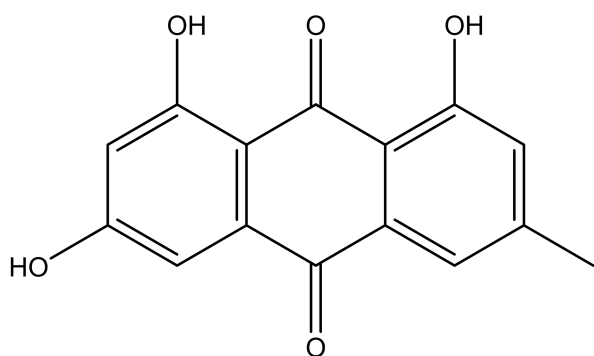
Quinones are colored aromatic compounds containing a pair of carbonyl groups situated within an unsaturated ring framework. They are sorted into groups like benzoquinones and anthraquinones, with lawsone and juglone acting as clear examples. They deal with pathogens through chemical warfare. They can form covalent interactions with microbial proteins and generate reactive oxygen species that damage cellular components (Schieber, 2018).



Lawsone

2.7.9 Anthraquinones

Anthraquinones are a dedicated branch of the quinone family featuring three fused aromatic rings at their core. Well-known examples from this group include emodin, aloin and chrysophanol. They display sharp antiviral and antibacterial traits. Their primary mode of attack involves halting the synthesis of microbial RNA and DNA, disrupt membranes and induce oxidative stress in microbial cells (Dong et al., 2016).

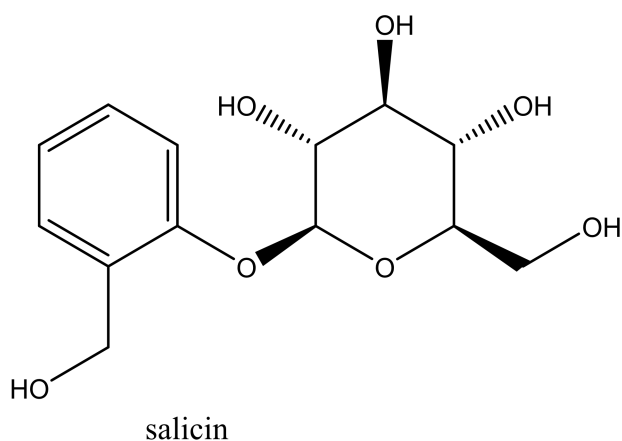


emodin

2.7.10 Glycosides

Glycosides are two-part molecules where a sugar portion, the glycone is chemically linked to a non-sugar counterpart, the aglycone. They are sorted based on the identity of that non-sugar

piece, yielding categories like cardiac glycosides and cyanogenic glycoside with amygdalin, digoxin and salicin as a common example. Glycosides exhibit antimicrobial activity through diverse mechanisms depending on the aglycone component, including membrane disruption, enzyme inhibition and interference with microbial metabolism (Wilke et al., 2021).



2.7.11 Phlobatannins

Phlobatannins are condensed tannin derivatives that may exhibit antimicrobial activity through protein binding and interference with microbial cell surfaces (Gonelimali et al., 2018).

Phytochemical screening protocols (qualitative analysis) allow rapid detection of these classes using laboratory reagents such as Mayer's reagents for alkaloids, ferric chloride for phenols, gelatin test for tannins, foam test for saponins, Shinoda's test for flavonoids, Liebermann–Burchard test for steroids and others.

CHAPTER THREE: METHODOLOGY

3.1 Study area

Plant materials were collected from Nagongera town council, Tororo district in Uganda where *Tylosema esculentum* is known to grow naturally. Laboratory analyses including extraction, phytochemical screening and antimicrobial assays were carried out in the laboratories of Busitema university at Nagongera campus.

3.2 Research design

The study employed an experimental laboratory-based methods to investigate the phytochemical composition and antimicrobial efficacy of *Tylosema esculentum* leaf extracts. The research involved the collection and preparation of plant material, extraction, qualitative and quantitative phytochemical screening

3.3 Plant material collection and preparation

3.3.1 Collection and authentication

Fresh leaves of *Tylosema esculentum* were collected from the identified location in Nagongera. The plant material was then authenticated by a qualified botanist and voucher specimens were prepared and deposited in a recognized herbarium for future reference.

3.3.2 Processing and drying

The collected leaves were thoroughly washed under running tap water followed by distilled water to remove dust and debris. The plant material was then solar dried until all the water evaporated. Once completely dried, the leaves were ground into fine powder using a grinding mill and stored in airtight containers protected from light and moisture until extraction.

3.3.3 Extraction

40gm of dried powdered plant material were weighed and mixed with 250 mL of pure acetone in a clean 500 mL beaker. The beaker was placed into a digital laboratory sonicator set to an acoustic frequency of 40 kHz at a temperature of 45°C for 45 minutes. The warm mixture was filtered using a filter paper into a conical flask to separate the liquid extract from the solid plant residue. The remaining solid plant residue was mixed with another 250 mL of pure acetone, sonication and filtration steps were repeated under identical conditions. Fresh acetone was repeatedly added to the residue and the extraction process continued several times to maximize yield. All collected liquid filtrates were combined and concentrated by

allowing solvent to evaporate in the fume hood leaving behind a pure dry crude extract. The dried crude extract obtained was weighed and kept for further analysis.

3.4 Phytochemical screening tests

The phytochemical screening test were conducted to detect the presence of major bioactive constituent classes using standard qualitative methods.

Each phytochemical test was conducted with a corresponding negative control consisting of the extract solvent without the plant extract treated with the same reagents under identical conditions. The limitation was that positive control standards were not readily available for the qualitative phytochemical tests. Consequently, the phytochemical tests relied on standard colour reactions described in the literature

3.4.1 Test for alkaloids

Mayer's test: Added 1 mL of Mayer's reagent to 1 mL of an acidic solution of crude extract in a test tube. Formation of a cream coloured precipitate indicated presence of alkaloids

Wagner's test: Added 1 mL of Wagner's reagent to the 1 mL of an acidic solution of crude extract in a test tube. Formation of a reddish-brown precipitate indicated presence of alkaloids

3.4.2 Test for flavonoids

Ferric chloride test: 5 drops of neutral ferric chloride solution were added to 1 mL of an alcoholic solution of a crude extract using a test tube. Formation of a blackish red colour indicated presence of flavonoids

Lead-acetate test: Added 5 drops of aqueous basic lead acetate solution to 1 mL of alcoholic extract using in a test tube. Formation of a white precipitate indicated presence of flavonoids

Shinoda's test: To 1 mL of an alcoholic solution of the crude extract in a test tube, a small piece of magnesium ribbon and few drops of concentrated HCl were added. Formation of a pink colour indicated

3.4.3 Test for tannins

Ferric chloride test: 5 drops of ferric chloride solution were added to 1 mL of aqueous solution of crude extract in a test tube. Formation of a blackish red precipitate indicated the presence of tannins

Gelatin test: Added gelatin solution to 1 mL of aqueous solution of crude extract in a test tube. Formation of a white precipitate indicated the presence of tannins

3.4.4 Test for phenols

Ferric chloride test: 1 mL of ferric chloride solution were added to 5 mL of an aqueous solution of crude extract in a test tube. Formation of an intense colour indicated presence of phenols

3.4.5 Test for saponins

Foam test: Mixed a little part of the crude extract with 20 mL of distilled water and then agitated in a test tube for 10 min. Form formation indicated presence of saponins

3.4.6 Test for terpenoids

Fresh plant material was treated with 5 mL of 1% aqueous hydrochloric acid for about 4 hours. The extract thus obtained was treated with 1 mL of Trim-Hill reagent in a test tube followed by heating in a water bath. There was no observable change and this indicated that terpenoids were absent

3.4.7 Test for steroids

Salkowski test: Added 1 mL of concentrated sulphuric acid to 5 mL of a chloroform solution of crude extract in a test tube allowed to stand for 5 min. Lower layer turned into red in colour which indicated presence of steroids

Liebermann-Burchard test: Added 5 drops of acetic anhydride to 5 mL of a chloroform solution of crude extract followed by adding 1 mL of concentrated sulphuric acid through the wall of the test tube and allowed the mixture to stand for 5 minutes. A reddish-brown ring at the junction of the two layers and a green colour in upper layer was observed indicating presence of steroids.

3.4.8 Test for glycosides

Molisch's test: A mixture of Molisch's reagent and concentrated sulphuric acid in a ratio of 1:1 was added to 1 mL of aqueous solution of crude extract. A reddish-violet ring at the junction of two liquids was formed which indicated presence of glycosides.

3.4.9 Test for quinones

Added 5 mL of an aqueous solution of crude extract to 5 mL of alcoholic potassium hydroxide solution. Appearance of a red colour indicating presence of quinones

3.4.10 Test for anthraquinones

Borntrager's test: 3 mL of aqueous extract was shaken with 3 mL of benzene, filtered and 5 mL of 10% ammonia solution were added to the filtrate and the mixture was then shaken. There was no observable change which indicated that anthraquinones were absent

3.4.11 Test for phlobatannins

2 mL of aqueous extract was added to 2 mL of 1% HCl and the mixture boiled. There was no observable change which indicated that phlobatannins were absent

3.5 Quantification of phytochemicals

The quantification of phytochemicals was performed using spectrophotometric methods and selected phytochemicals were calibrated against standards.

3.5.1 Total Flavonoid Content

The total flavonoid content was measured by an aluminum chloride colorimetric assay using quercetin as a standard flavonoid. A 500 μ L volume of the extract was placed in a test tube, 1 mL of 5% sodium nitrite solution was added and the mixture was incubated at room temperature for 5 minutes. After incubation, the mixture was treated with 1 mL of 10% aluminium chloride solution. After 1 minute, 1 mL of 1M sodium hydroxide solution was added and 6.5 mL of distilled water was added to the whole solution. The solution was mixed well and the absorbance was measured at 420 nm against a blank after scanning for the maximum absorbance. The same procedure was repeated for the standard solution of quercetin, based on the 5-point standard calibration curve of quercetin (31.25, 15.625, 7.813, 3.906, and 1.953 μ g/mL) in 80% methanol.

3.5.2 Total Alkaloid Content

A solution of 1 mg/mL of plant extract was prepared using dimethyl sulfoxide (DMSO). 1 mL of 2M hydrochloric acid was added to 1 mL of the DMSO-dissolved extracts and the resulting mixture was filtered using filter paper. A 500 μ L portion of the filtrate was transferred to a 250 mL separating funnel, and 5 mL of 0.1% Bromocresol green dissolved in methanol was added to this solution followed by 5 mL of phosphate buffer (pH 6.6). 5 mL of chloroform were also added into the separating funnel and the mixture was vigorously shaken, after which the funnel was left to stand and the mixture separated into different layers. The lower layer was collected in a 10 mL volumetric flask. The process was repeated with 6, 7, and 8 mL of chloroform. Caffeine was used to construct a standard curve using a

concentration range of 1.0–0.2 mg/mL. The absorbance of the sample and standard solutions was recorded at a wavelength of 470 nm against a reagent blank in a 607 UV-JENWAY spectrophotometer.

3.5.3 Total Tannins Content

A 500 µL volume of plant extract of concentration 1 mg/mL in 80% methanol was placed in a test tube and 2 mL of 0.1M ferric chloride solution in 0.1N hydrochloric acid and 0.008M potassium ferricyanide were added to the extract. The resulting mixture was mixed and the absorbance was measured at 650 nm within 10 minutes against the blank in a 607 UV-JENWAY spectrophotometer. The same procedure was repeated for the standard solution of tannic acid based on the 5-point standard calibration curve of tannic acid (1.0, 0.8, 0.6, 0.4 and 0.2 mg/mL) in 80% methanol.

3.5.4 Total Steroidal Content

A 500 µL portion of plant extract of concentration 5 mg/mL was placed in a test tube. 2 mL of 2M sulphuric acid and 2 mL of 0.5% w/v ferric chloride solution were added followed by 500 µL of 0.5% w/v potassium ferricyanide solution. The mixture was heated in a water bath at 70 °C for 30 minutes and then diluted to make up 10 mL with distilled water. The same procedure was repeated for the standard solution of Diosgenin. The absorbance was measured at 780 nm against a blank in a 607 UV-JENWAY spectrophotometer. Diosgenin was used as a standard and was serially diluted in two-fold steps (1.0, 0.5, 0.25, 0.125 and 0.0625 mg/mL) to create a standard curve.

3.6 Data analysis methods

Data analysis involved interpreting qualitative phytochemical screening outcomes by recording observable reagent-based indicators such as colour changes or precipitate formation, while quantitative assays were evaluated using UV-Vis spectrophotometric absorbance values plotted against standard calibration curves to generate regression equations used for calculating concentrations of the phytochemicals all measurements were presented in tables, calibration graphs and a comparative bar chart was drawn to clearly illustrate the relative abundance of phytochemicals in the leaf extract.

3.7 Ethical considerations

Clearance was sought from the faculty of science and education of Busitema University before collecting the data. Informed consent was obtained from the individuals where the

plant material, *Tylosema esculentum* was located. The researcher strictly followed environmental ethics, plant samples were harvested without disrupting local biodiversity. Laboratory operations followed strict safety standards and in guidance of the laboratory technician.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Introduction

This section outlines the phytochemical profile of *Tylosema esculentum* leaf extracts, showing the phytochemicals that were detected and the quantities of selected phytochemicals. It also explains what the findings mean in the context of plant chemistry and potential antimicrobial relevance. The section also connects the observed results to existing scientific knowledge and highlights how the chemical composition of the leaf extracts shows potential traditional use supports their traditional use of the extracts

4.2 Presentatyion of results

The extraction of 40g of *Tylosema esculentum* with acetone yielded 3.492 g of dried extract.

4.2.1 Qualitative phytochemical analysis

The preliminary chemical analysis revealed a diverse profile of secondary metabolites preserved within the acetone extract. The full results are summarized in the table below

Table 1: Phytochemical screening results

Phytochemical group	Test	Result
Flavonoids	Ferric chloride test	+
	Shinoda's test	
	Lead acetate test	
Alkaloids	Mayer's test	+
	Wagner's test	
Phenol	Ferric chloride test	+
Saponins	Foam test	+
Tannins	Ferric chloride test	+
	Gelatin test	
Terpenoids	Trim-Hill test	-
Steroids	Salkowski test	+

	Liebermann-Burchard test	
Glycosides	Molisch's test	+
Quinones	Alkaline test	+
Anthraquinones	Borntrager's test	-
Phlobatannins		-

Key: + Indicates Present

- Indicates Absent

4.2.2 Total phytochemical content

4.2.2.1 Total Flavonoids Content

Table 2: Total Flavonoids Content

Analyte	Concentration (µg/mL)	Absorbance
SD1	1.953	0.157
SD2	3.906	0.381
SD3	7.813	0.539
SD4	15.625	0.862
SD5	31.250	1.744
TE1		1.392
TE2		1.413
TE3		1.396

SD - Indicates Standard

TE - Indicates Tylosema Esculentum sample

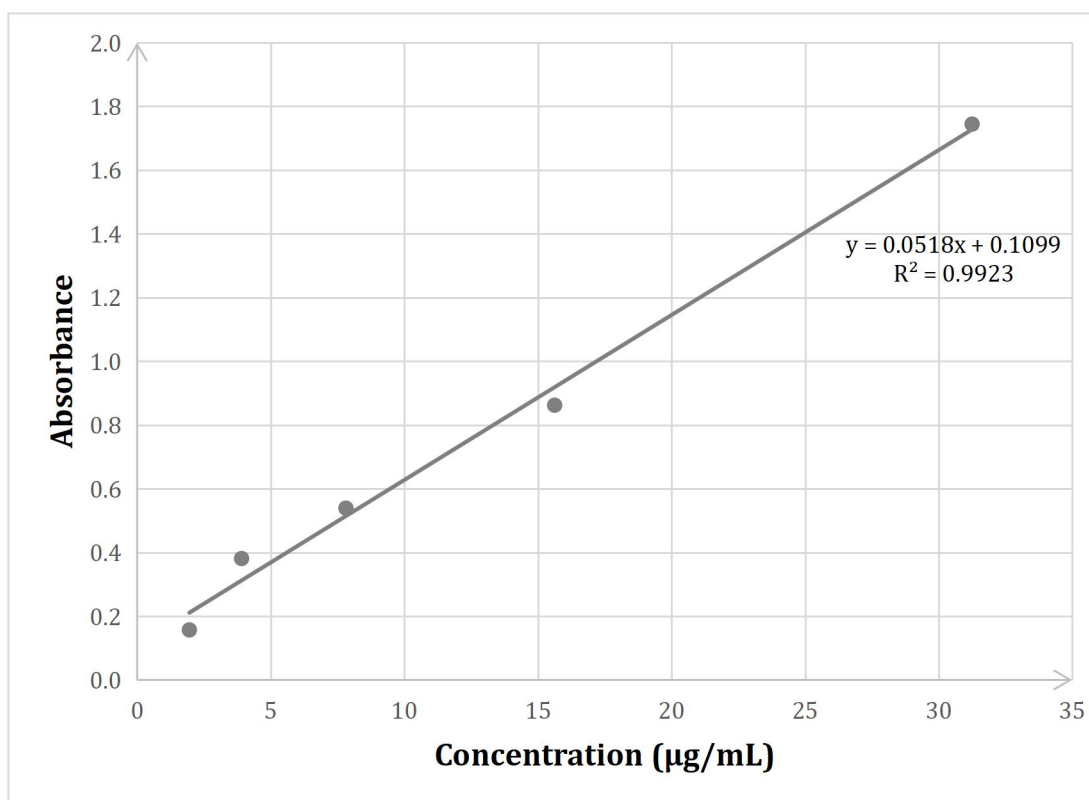


Figure 7: Calibration curve for flavonoids

4.2.2.2 Total Alkaloid Content

Table 3: Total Alkaloid Content

Analyte	Concentration (mg/mL)	Absorbance
SD1	0.2	1.431
SD2	0.4	1.662
SD3	0.6	1.784
SD4	0.8	1.913
SD5	1.0	2.126
TE1		1.386
TE2		1.391
TE3		1.374

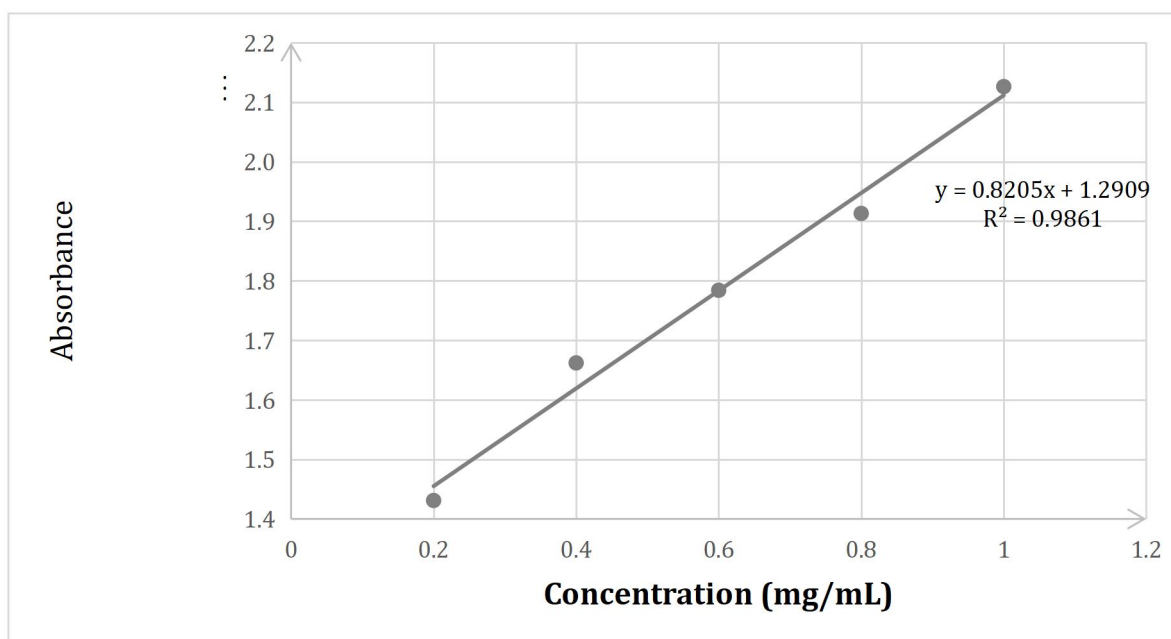


Figure 8: Calibration curve for alkaloids

4.2.2.3 Total Tannins Content

Table 4: Total Tannins Content

Analyte	Concentration (mg/mL)	Absorbance
SD1	0.2	2.098
SD2	0.4	2.15
SD3	0.6	2.389
SD4	0.8	2.489
SD5	1.0	2.543
TE1		2.335
TE2		2.340
TE3		2.380

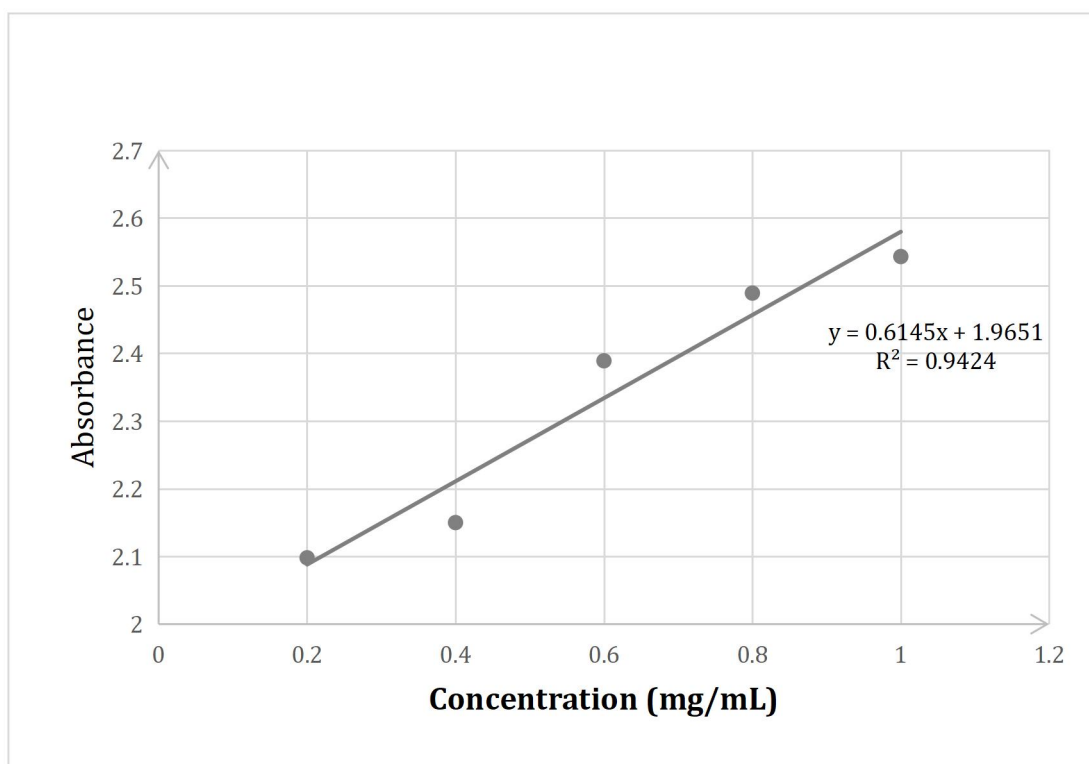


Figure 9: Calibration curve for tannins

4.2.2.4 Total Steroidal Content

Table 5: Total Steroidal Content

Analyte	Concentration (mg/mL)	Absorbance
SD1	0.0625	0.151
SD2	0.1250	0.282
SD3	0.2500	0.454
SD4	0.5000	0.581
SD5	1.0000	1.326
TE1		0.244
TE2		0.241
TE3		0.251

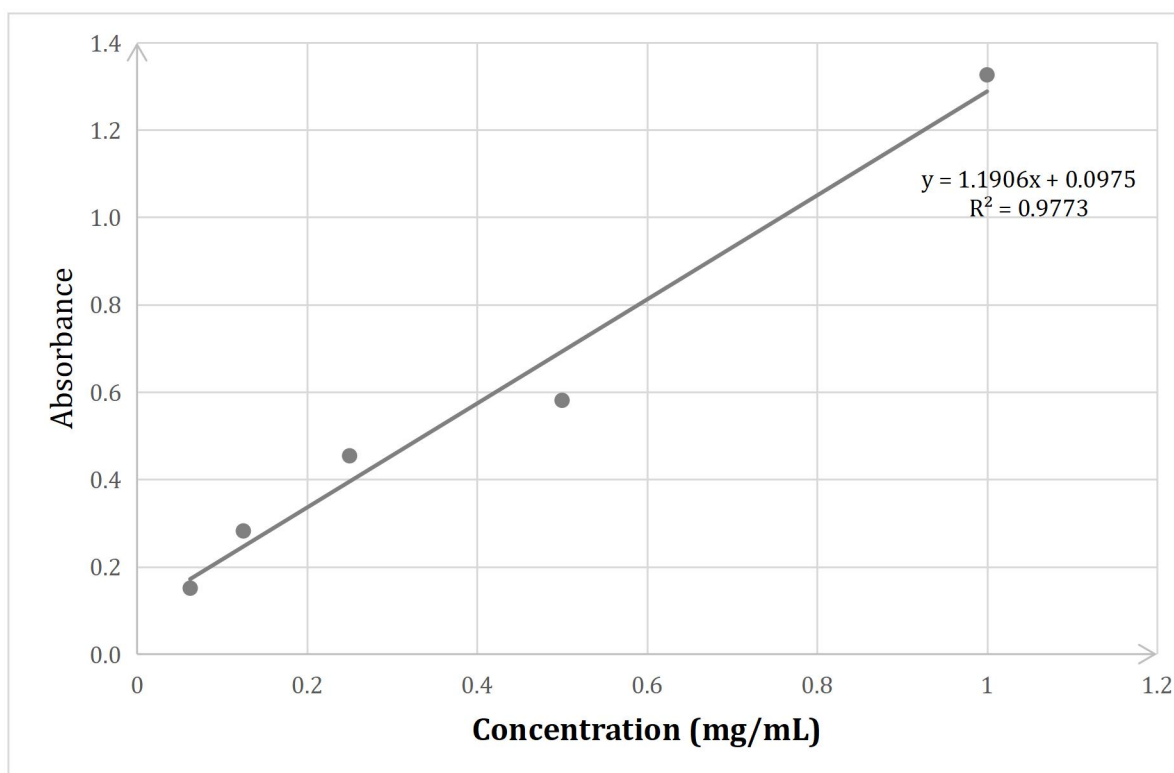


Figure 10: Calibration curve for steroids

4.3 Analysis of results

Total phytochemical content was calculated from the formulae below;

$$C = \frac{x \cdot V \cdot DF}{m}$$

C = Total flavonoid content expressed as mg of standard equivalent per gram of plant extract (mg/g).

x = Concentration of the phytochemical established from the calibration curve in mg/mL

V = Volume of the extract solution prepared in mL

M = Mass of the plant extract used during the procedure in grams.

DF = Dilution factor

$$x = \frac{y - c}{m}$$

y = Mean Absorbance

c = intercept

m = slope

4.3.1 Total flavonoids content

Mean Absorbance = 1.400

Concentration= $24.9\mu\text{g/mL} = 0.0249\text{mg/mL}$

Total flavonoids content = 24.9 mg QE/g

4.3.2 Total alkaloids content

Mean Absorbance = 1.383

Concentration= 0.1122 mg/mL

Total alkaloids content= 112.2 mg CE/g

4.3.3 Total tannins content

Mean Absorbance = 2.352

Concentration= 0.6296 mg/mL

Total tannins content= 629.6 mg TAE/g

4.3.4 Total steroidal content

Mean Absorbance = 0.245

Concentration= 0.1239 mg/mL

Total steroidal content = 24.8 mg DE/g

Table 6: Total phytochemical content

Phytochemical	Concentration (mg/g)
Alkaloids	112.2
Flavonoids	24.9
Tannins	629.6

Steroids	24.8
----------	------

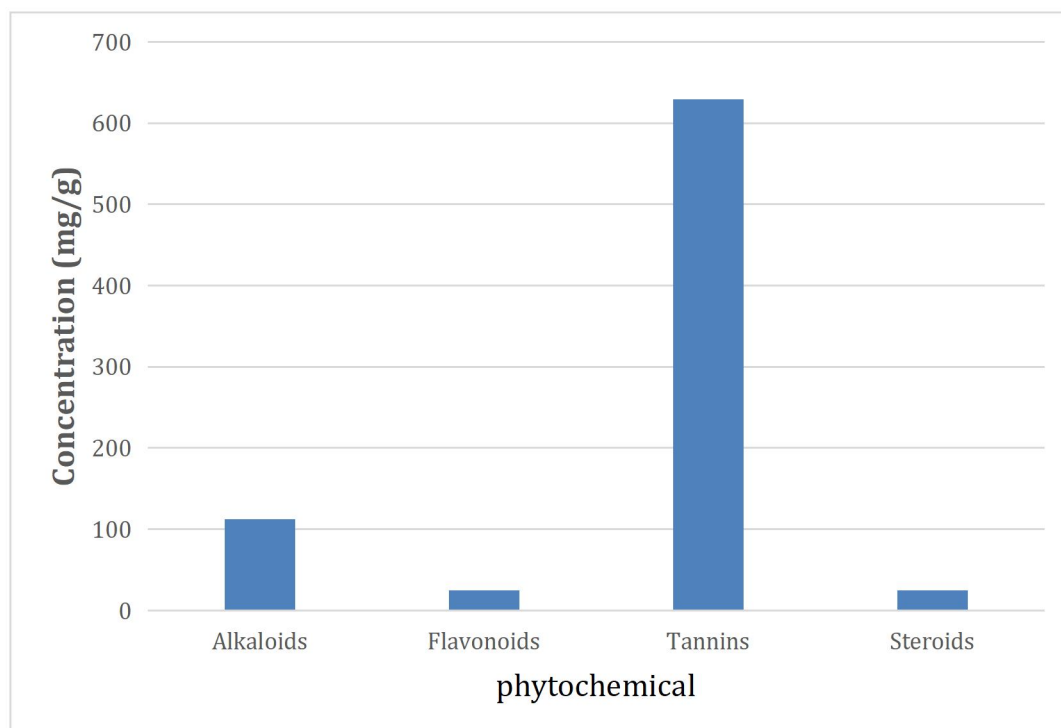


Figure 11: A bar graph showing the phytochemical content of *Tylosema* leaf extract

4.4 Discussion of results

Starting with 40.00 g of dry *Tylosema esculentum* leaf powder and using a systematic acetone-sonication extraction method, the process recovered 3.492 g of pure concentrated dark-green crude extract paste representing an extraction yield of 8.73%. This yield demonstrates that acetone combined with ultrasonic energy is highly effective at breaking down plant structures to extract a substantial amount of secondary metabolites.

The qualitative screening revealed the presence of flavonoids, alkaloids, phenols, saponins, tannins, steroids, glycosides and quinones in the acetone leaf extract of *Tylosema esculentum*. However, terpenoids, anthraquinones and phlobatannins were not detected.

The presence of multiple phytochemical groups suggests that the leaves contain a diverse range of bioactive compounds that may contribute to the medicinal properties of the plant. The positive results obtained for flavonoids, tannins, alkaloids and phenols are particularly important because these compounds have been widely reported to possess antimicrobial, antioxidant, anti-inflammatory and wound-healing properties.

The absence of terpenoids, anthraquinones and phlobatannins could be due to environmental factors, plant maturity, geographical location, climatic conditions or the extraction solvent used.

The chemical profiling of *Tylosema esculentum* leaf acetone extracts unveils a unique distribution of secondary metabolites that strongly supports its therapeutic potential. The quantification showed that tannins have the highest concentration of 629.6 mg/g. This high concentration of tannins indicates that the leaves have a high potential to precipitate proteins, inhibit microbial enzymes and interfere with microbial growth. The substantial concentration of alkaloids at 112.2 mg/g further strengthens the chemical defense profile of the leaf extracts, they work by intercalating with microbial DNA, disrupting cell division or inhibiting crucial cytoplasmic enzymes. Flavonoids (24.9 mg/g) and steroids (24.8 mg/g) were detected at lower concentrations but may contribute synergistically to the overall biological activity of the extract

Antimicrobial assays were not conducted in the study because the required microbiology laboratory chemicals, microbial cultures and biosafety equipment were not readily available. Consequently, biological activity testing could not be performed and the work was limited to extraction and phytochemical characterization only.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study demonstrates that the leaves of *Tylosema esculentum* harvested from Nagongera Town Council contain a rich matrix of medicinal compounds with an extraction efficiency yield of 8.73% using pure acetone via ultrasonic-assisted extraction, validating their effectiveness in managing skin infections and promoting wound healing. The presence of flavonoids, alkaloids, tannins, saponins, steroids, glycosides, quinones shows a strong potential of *Tylosema esculentum* leaves in treating skin conditions. The coexistence of multiple phytochemical classes with complementary mechanisms of action suggests potential synergistic effects that could enhance antimicrobial efficacy and reduce resistance development. Quantitative analysis showed that tannins were the dominant phytochemical followed by alkaloids while flavonoids and steroids occurred at lower concentrations. These findings suggest potential antimicrobial activity of the leaf extract because the detected phytochemical have previous been reported to possess antimicrobial properties. Validating the antimicrobial activity requires experimental confirmation through performing antimicrobial assays which were not carried out because the required microbiology laboratory chemicals, microbial cultures and biosafety equipment were not readily available.

5.2 Recommendations

Based on the findings and conclusions of this study, the following recommendations are made:

Evaluating the antimicrobial activity of *Tylosema esculentum* leaf extracts against common bacterial and fungal pathogens using standard microbiological techniques such as agar well diffusion, disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays

Performing detailed toxicity testing on human skin cell lines to confirm safety followed by formulation into topical creams or ointments for practical clinical use

Evaluating potential synergistic interactions between different phytochemicals and between the extract and conventional antibiotics.

5.3 Areas for Further Research

The study provides a foundation for future investigations on *Tylosema esculentum*.

Future researchers can investigate seasonal, geographical and environmental influences on the phytochemical composition of *Tylosema esculentum* to determine how these factors affect the concentration of bioactive compounds.

Isolation, purification and structural characterization of the major phytochemical constituents can be undertaken to identify the compounds responsible for the observed phytochemical profile. This can contribute to the development of novel natural products for pharmaceutical and other health-related applications.

REFERENCES

- Bouarab, L., Forquet, V., Lantéri, P., Clément, Y., Léonard-Akkari, L., Oulahal, N., Degraeve, P., & Bordes, C. (2019). Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure–activity relationship) models. *Frontiers in Microbiology*, 10, 829.
- Castro, S., Silveira, P., Coutinho, A. P., & Figueiredo, E. (2005). Systematic studies in *Tylosema* (Leguminosae). *Botanical Journal of the Linnean Society*, 147(1), 99–115.
- Chingwaru, W., Vidmar, J., Kapewangolo, P. T., Mazimba, O., & Jackson, J. (2015). Therapeutic and Prophylactic Potential of Morama (*Tylosema esculentum*): A Review. *Phytotherapy Research*, 29(10), 1423–1438.
- Costa, F. G., Mills, K. B., Crosby, H. A., & Horswill, A. R. (2024). The *Staphylococcus aureus* regulatory program in a human skin-like environment. *Nature Communications*, 15(5).
- Cowan, M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564–582.
- Cullis, C., Lawlor, D. W., Chimwamurombe, P., Bbebe, N., Kunert, K., & Vorster, J. (2019). Development of marama bean, an orphan legume, as a crop. *Food and Energy Security*, 8(3).
- Cushnie, T. (2011). Recent advances in understanding the antibacterial properties of flavonoids. *International Journal of Antimicrobial Agents*, 38(2), 99–10.
- Cushnie, T. (2014). Alkaloids: An overview of their antibacterial activities. *International Journal of Antimicrobial Agents*.
- Dong, X., Fu, J., Yin, X., Cao, S., Li, X., Lin, L., & Ni, J. (2016). Emodin: a review of its pharmacology, toxicity and pharmacokinetics. *Phytotherapy Research*, 34(6), 1207–1218.
- Dréno, B., Pécastaings, S., Corvec, S., Veraldi, S., Khammari, A., & Roques, C. (2018). *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: a brief look at the latest updates. *Journal of the European Academy of Dermatology and Venereology*, 32, 5–14.
- Germishuizen, G., & Meyer, N. (2003). *Plants of southern Africa: an annotated checklist*. National Botanical Institute, Pretoria.
- Gonelimali, F. D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., & Hatab, S. R. (2018). Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. *Frontiers in Microbiology*, 9, 1639.

- Gupta, A. K., Foley, K. A., & Versteeg, S. G. (2017). New antifungal agents and new formulations against dermatophytes. *Mycopathologia*, 182(1–2), 127–141.
- Hay, R., Johns, N., & Williams, H. (2014). The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. *Journal of Investigative Dermatology*, 134(6), 1527–1534.
- O'Neill. (2016). Tackling drug-resistant infections globally: final report and recommendations. *Review on Antimicrobial Resistance*.
- Wilke, E., & Polkowska, Ż. (2021). Amygdalin: toxicity, anticancer activity and analytical procedures for its determination in plant seeds. *International Journal of Molecular Sciences*, 9(11), 102–111.
- Lwigale, F., Kibombo, D., Kasango, S. D., Tabajjwa, D., Atuheire, C., Kungu, J., Kalule, J. B., Otita, M., Kakooza, F., Nabukenya, I., Mayito, J., & Rwego, I. B. (2024). Prevalence, resistance profiles and factors associated with skin and soft-tissue infections at Jinja regional referral hospital: a retrospective study. *PLOS Global Public Health*, 4(8).
- Madigan, M., Martinko, J., & Parker, J. (1997). *Brock biology of microorganisms*.
- Mazimba, O., Majinda, R. R. T., Modibedi, C., Masesane, I. B., Cencič, A., & Chingwaru, W. (2011). Tylosema esculentum extractives and their bioactivity. *Bioorganic & Medicinal Chemistry*, 19(17), 5225–5230.
- Moreau, R., Whitaker, B., & Research, K. H. (2018). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*, 70, 42–61.
- Moses, T., Papadopoulou, K. K., & Osbourn, A. (2014). Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology*, 49(6), 439–462.
- Nazzaro, F., Fratianni, F., & Martino. (2017). Effect of essential oils on pathogenic bacteria. *Pharmaceutical*, 6(12), 1451–1474.
- Nepolo, E., & Chimwamurombe, P. M. (2012). Assessment of genetic variations within and between populations of Marama Bean (*Tylosema Esculentum* (Burchell) Schreiber) based on Microsatellites (SSRS. *South African Journal of Botany*, 78, 31–38.
- Newman, D., Products, G. C.-J. of natural, & 2016, U. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629–661.

- Nyembwe, P., Minnaar, A., Duodu, K., Chemistry, H. de K.-F., & 2015, U. (2014). Sensory and physicochemical analyses of roasted marama beans with specific focus on compounds that may influence flavor and acceptability. *Food Chemistry*, 178, 45–51.
- Omotayo A.O, & Aremu O. (2021). Marama bean : An indigenous plant with potential for food, nutrition, and economic sustainability. *Food & Function*, 12(6), 2389–2403.
- Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health*, 109(7), 309–318.
- Salehi, B., Quispe, C., Sharifi-Rad, J., Cruz-Martins, N., Nigam, M., Mishra, A. P., Kononov, D. A., Orobinskaya, V., Abu-Reidah, I. M., Zam, W., Sharopov, F., Venneri, T., Capasso, R., Kukula-Koch, W., Wawruszak, A., & Koch, W. (2021). Phytosterols: from preclinical evidence to potential clinical applications. *Frontiers in Pharmacology*, 11.
- Schieber, A. (2018). Reactions of quinones—Mechanisms, structures, and prospects for food research. *Journal of Agricultural and Food Chemistry*, 66(50), 13051–13055.
- Tong, S. Y. C., 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.
- Tong, Z., He, W., Fan, X., & Guo, A. (2022). Biological function of plant tannin and its application in animal health. *Frontiers in Veterinary Science*, 8.
- Uzabakiriho, J. D. (2016). *Diversity and distribution of Tylosema esculentum (Marama bean) endophytic bacteria communities in Omitara, Harnas and Otjinene, eastern Namibia*. University of Namibia.
- Vilakazi, B., Mafongoya, P., & Odindo, A. (2025). The role of neglected grain legumes in food and nutrition security and human health. *Sustainability*, 17(1), 350.
- Willey, J., M.Prescott, M., L., Sandma, & Woolverton, C. J. (2023). *Microbiology* (10th editi). McGraw-Hill Education.
- Wyk, V. (2017). A Review of African Medicinal and Aromatic Plants. *Journal of Ethnopharmacology*.