2021-06

Antimicrobial investigation of Tephrosia Vogelii hook.f from Hai District in Tanzania towards development of antifungal agents for topical application

Hanolo, Stephano

NM-AIST

https://doi.org/10.58694/20.500.12479/1366

ANTIMICROBIAL INVESTIGATION OF *TEPHROSIA VOGELII* HOOK.F FROM HAI DISTRICT IN TANZANIA TOWARDS DEVELOPMENT OF ANTIFUNGAL AGENTS FOR TOPICAL APPLICATION

Stephano Hanolo

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

June, 2021

ABSTRACT

Infectious diseases such as fungal diseases are the global health problem. Moreover, antimicrobial resistance to modern fungicides and antibiotics is even perplexing in the health settings. Such situation needs serious attention to search for alternative antimicrobial agents. Thus, this study aimed to investigate antimicrobial activity of *Tephrosia vogelii* Hook.f found in Tanzania and develop antifungal agents therefrom. The *n*-hexane, dichloromethane and methanolic extracts from leaves and roots of *Tephrosia vogelii* were evaluated for antimicrobial activity against *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (clinical isolate), *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC29953), *Klebsiella pneumoniae* (ATCC 700603) and *Salmonella typhi* (NCTC 8385). A serial dilution method using the sterilized 96 wells of polystyrene microliter plates were used to determine the minimum inhibitory concentrations (MIC) of extracts. The *in vivo* toxicity of methanolic leaf and root extracts was evaluated using albino rats. Extracts doses of 600, 1200, 2000 and 5000 mg/kg body weight were evaluated for lethality test while doses of 600, 1200 and 2000 mg/kg body weight subjected for sub-acute toxicity test. Gas Chromatography-Mass Spectrometry (GC-MS) technique was employed to appraise phytochemical compounds present. Antifungal agent for topical application was formulated and evaluated through *in vitro* and *in vivo* test to establish its effectiveness. The study results revealed that *n*-hexane and dichloromethane extracts exhibited the lowest activity. Equally, methanolic root and leaf extracts exhibited high activity at MICs ranging from 0.625 - 5.0 mg/mL against assayed pathogens. Phytochemical screening of both methanolic leaf and root extracts revealed the presence of tannins, flavonoids, terpenes, fatty acids and saponins. The GC-MS analysis unveiled the presence of ten phytocompounds. Five compounds; demethylmunduserone sumatrol, munduserone, hexadecanoic acid and hexadecanoic acid, methyl ester are new and this is the first time to be reported from *Tephrosia vogelii*. Furthermore, toxicity evaluation revealed that methanolic leaf and root extracts of *Tephrosia vogelii* were not deleterious at highest dose of 5000 mg/kg body weight. Though there was no mortality recorded, histopathological examination revealed toxicity indices due to vacuolation and necrosis in hepatocytes under treatment of methanolic root extracts of dose 2000 mg/kg body weight. The herbal cream prototype ($CBTV_3$) was formulated, and it exhibited antimicrobial activity through *in vitro* and *in vivo* tests. The development of herbal cream prototype from *Tephrosia vogelii* Hook.f is hereby reported for the first time.

DECLARATION

I, Stephano Hanolo do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Stephano Hanolo **9th June, 2021**

Name and signature of candidate Date

The above declaration is confirmed by

Dr. Musa Chacha **9th June, 2021**

Name and signature of Supervisor I Date

Dr. Juma A. Mmongoyo **9th June, 2021**

Name and signature of Supervisor II Date

COPYRIGHT

CERTIFICATION

The undersigned certify that they have read and hereby recommend the dissertation entitled "Antimicrobial investigation of *Tephrosia vogelii* Hook.f from Hai district in Tanzania towards development of antifungal agents for topical application" as a fulfillment of the requirements for the Degree of Doctor of Philosophy in Life Sciences at Nelson Mandela African Institution of Science and Technology.

Dr. Musa Chacha **9th June, 2021**

Name and signature of Supervisor I Date

Dr. Juma A. Mmongoyo **9th June, 2021**

Name and signature of Supervisor II Date

ACKNOWLEDGEMENTS

First of all, I would like to acknowledge the Almighty God for his enormous grace, blessing and care in my life. All the glory is to the Almighty God who enabled me to accomplish this work. To him, I am forever grateful and indebted to.

I would like to express my special gratitude to my supervisors, Dr. Musa Chacha and Dr. Juma Mmongoyo for their diligent guidance, assistance, encouragement and invaluable advice throughout the period of this study. Yes, God will pay them.

I would like to extend my sincere thanks to the sponsor and provider of study leave, Mkwawa University College of Education, a constituent college of University of Dar es Salaam (my employer).

Special thanks to School of Pharmacy, Muhimbili University of Health and allied Sciences (MUHAS) for allowing me to use their laboratories for biological assays. Special thanks to laboratory technicians, Mr. Abdul Kidukuli and Ezekiel Marando for their assistance when I was performing biological assays. Special appreciation to College of Veterinary Medicine, Sokoine University of Agriculture (SUA) for allowing me to use its laboratories for toxicity assays. Special thanks to, Mr. Zemu Mbilu and Dr. Barnabas Mngumi for their technical assistance. More appreciation should also go to Tanzania Standard Bureau for allowing me to access their GC-MS facility to perform my phytochemical analyses.

I would like to thank all staff members at Nelson Mandela African Institution of Science and Technology (NM-AIST) for creating conducive learning environment during my study right from the beginning to the end. I extend my sincere thanks to all my fellow postgraduate students at NM-AIST for their cooperation and constructive support during my study.

Lastly but not least, Special gratitude should be to my father, Hanolo F. Mlozi, my mother Sabina Mwashilindi and the whole family for their encouragement throughout the program. I would be remiss if I did not express my heart-felt appreciations to my beloved wife, Esnath Mwankenja, together with our kids, Monica, Stephen, Loveness and Blessed for their relentless patience, prayers and moral support throughout this program. Had it not their support, realization of my doctorate degree would be impossible.

DEDICATION

This dissertation is dedicated to my parents Hanolo F. Mlozi and Sabina Mwashilindi, my loved wife Esnath Mwankenja, as well as my loved children; Monica, Stephen, Loveness and Blessed for their encouragement, prayer, patience, advice and moral support throughout my study.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

˚C	Degree Celsius
CB	Cream Base
CBTV	Formulated Herbal Cream
CDC	Centre of Disease Control and Prevention
DCM	Dichloromethane
DMSO	Dimethylsulphoxide
GC	Gas Chromatography
m/*z*	Mass per Charge Ratio
MeOH	Methanol
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MS	Mass Spectroscopy (Mass Spectrum, or Mass Spectra)
MUCE	Mkwawa University College of Education
MUHAS	Muhimbili University of Healthy and Allied Sciences
NM-AIST	Nelson Mandela African Institution of Science and Technology
OECD	Organization for Economic Co-operation Development
SUA	Sokoine University of Agriculture
TV	*Tephrosia vogelii*
TV-L	Leaf Extract of *Tephrosia vogelii*
TV-L_{DE}	Dichloromethane Leaf Extract of *Tephrosia vogelii*
TV-L_{HE}	*n*-Hexane Leaf Extract of *Tephrosia vogelii*
TV-L_{ME}	Methanolic Leaf Extract of *Tephrosia vogelii*
TV-R	Root extract of *Tephrosia vogelii*
TV-R_{DE}	Dichloromethane Root Extract of *Tephrosia vogelii*
TV-R_{HE}	*n*-Hexane Root Extract of *Tephrosia vogelii*
TV-R_{ME}	Methanolic Root Extract of *Tephrosia vogelii*
UDSM	University of Dar es Salaam
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Although they are quite often neglected, fungal diseases are challenging global health care settings, and creating the problem that has extensively conveyed a health burden to humans (Fausto *et al.*, 2019). The available estimates clearly indicate that the fungal diseases are deadly; they have been reported to affect over a billion people worldwide whereby more than 1.5 million people die annually (Bongomin *et al*., 2017). It is unfortunate that *Candida albicans* and *Cryptococcus neoformans* are responsible for the infections and are known to significantly contribute to such deaths to humans (Bongomin *et al*., 2017; Rodrigues & Albuquerque, 2018). Typically, the *C. albicans* and *C. neoformans* are opportunistic fungi, which cause fungal life-threatening diseases such as skin infections, oral infections, lung infections and central nervous system (CDC, 2019b, 2019a). Because of their relatively weaker immunity, the immune-suppressed people living with cancer, diabetes and HIV/AIDS are even more tremendously vulnerable to opportunistic *C. albicans* and *C. neoformans* (Faini *et al*., 2015; Limper *et al*., 2017; Society, 2016). Global warming contributed to the body and genetic changes of the microbes as a result of adaptation against harsh environment (Casadevall, 2018). In the same way, the climate change exacerbates the prevalence of fungal diseases and emergence of such pathogenic fungi as well as antifungal resistance worldwide (Casadevall, 2018; Garcia-Solache & Casadevall, 2010)

Nevertheless, fungal resistance to commercially available antifungal agents poses a critical global health challenge which affects the efforts to fight against fungal diseases. It has been reported that the synthetic fungal drugs such as fluconazole are increasingly becoming less effective due to resistance developed by the pathogenic fungi (Arendrup, 2014; Badiee, 2017; Kontoyiannis, 2017; Redfield, 2019; Rodrigues & Albuquerque, 2018; Sanguinetti *et al*., 2015; Vandeputte *et al*., 2012; Wiederhold, 2017). Fungal resistance to synthetic drugs is a serious and significant threat to humans and biodiversity at large. Despite such life-threatening effects, unfortunately fungal diseases are often neglected and therefore little attention is paid to them in terms of searching for alternative antifungal, which could offset the fungal resistance (Bongomin *et al*., 2017; Rodrigues & Albuquerque, 2018). Consequently, the magnitude of the problem has become even more severe particularly now

when the global statistics of the vulnerable immuno-suppressed people have considerably continued to rise (Faini *et al*., 2015; Rajasingham *et al*., 2017). Therefore, a search for alternative effective antifungal agents against *C. albicans* and *C. neoformans* is obligatory and urgently needed (Rodrigues & Albuquerque, 2018).

On the other hand, bacterial infections and antibiotic resistance are also concern to the public health. Typically, *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi*, are bacterial pathogens, which have developed resistance against the first line antibacterial agents (Labar *et al*., 2012; Okeke *et al*., 2007). For instance, *Staphylococcus aureus* developed resistance known as methicillin-resistant *Staphylococcus aureus* (MRSA), against the antibacterial drugs and hence known to pose a global problem (Labar *et al*., 2012; Okeke *et al*., 2007). Such resistance is even more dangerous because it complicates the control of the bacterial infections and treatment, thereby leading to increased morbidity and mortality (Okeke *et al*., 2007; Redfield, 2019). Low income countries especially sub-Saharan countries are more affected with a burden of such resistance to antibiotics because it is prohibitively expensive to deal with bacterial pathogens resisting antibiotics (Okeke *et al*., 2007; Redfield, 2019). This burden becomes even more challenging when there are dual bacteria-fungi interactions especially among *candida-staphylococcus* species which are often responsible pathogens for causing human diseases (Carolus *et al*., 2019). The negative effects and resistance issue of these pathogenic microbes necessitate an urgent search for new antibacterial agents capable of containing the pathogens and offsetting the bacterial resistances to safeguard the public health.

Nature through the medicinal plants resources has a promising solution to problems associated with infectious and non-infectious diseases. Since medieval times, medicinal plants have been a major source of medicines for the management and treatment of human diseases including fungal and bacterial diseases (Maregesi *et al*., 2007; Masevhe *et al*., 2015; Tabassum & Hamdani, 2014). To date, over 80% of human population still depend on medicinal plants to meet their primary healthcare to treat human ailments worldwide particularly in the poor communities which cannot afford the modern medications (Gurib-Fakim, 2006). Medicinal plants used for treating bacterial and fungal diseases have been scientifically recognized as promising natural resource from which searching of antimicrobial agents can be done (Fatima *et al*., 2017; Gupta & Birdi, 2017; Gurib-Fakim, 2006; WHO, 2018; Yarnell, 2005). For instance, in Tanzania, thousands of medicinal plant species are

traditionally used for treatment of diseases inflicting animals and human including fungal and bacterial ailments (Maregesi *et al*., 2007). The present study aims to investigate and search for potential antimicrobial agents from *Tephrosia vogelii* Hook.f. which is traditionally used for treatment of infectious human diseases.

Of particular interest from ethnomedical viewpoint, the *Tephrosia vogelii* has attracted our attention to explore its potentiality as a medicinal plant to serve as a resource of antifungal agents. Previously, studies have shown that *Tephrosia vogelii* has been traditionally used not only as abortifacient and purgative agents but also as a source of active agents for the management of ecto-parasitism, pests and schistosomiasis (Dzenda *et al*., 2008; Orwa *et al*., 2009). Moreover, *Tephrosia vogelii* has been used for treatment of ringworms and skin diseases; and its decoctions have been reported to treat scabies, yaws and constipation (Orwa *et al*., 2009). Evidences from pharmacological studies have demonstrated that extracts of *Tephrosia vogelii* leaves have substantial potencies against lice, mites, fleas, ticks and mosquito larvicidal (Anjarwalla *et al*., 2015; Kidukuli *et al*., 2015; Stevenson & Belmain, 2017). These ethnopharmacological reports appear to be adequate evidences that *Tephrosia vogelii* might be a promising resource from which novel antifungal and antibacterial agents could be discovered. Such agents could pave a way to replace the synthetic antifungal and antibacterial drugs which are challenged by the problem of fungal and bacterial resistances. Therefore, such agents could be used to offset the fungal and bacterial resistance-to-synthetic-drugs problem.

In spite of ethnopharmacological evidences on the resourcefulness of the plant, there is little or limited scientific information on the antimicrobial pharmacological activities of the leaves and roots of *Tephrosia vogelii* against opportunistic *C. albicans* and *C. neoformans* pathogens. To the best of our knowledge, reports on antibacterial potential of leaves and roots of *Tephrosia vogelii* against pathogenic bacteria such as *S. aureus, E. coli, K. pneumoniae* and *S. typhi* are scanty. Thus, this study aimed to extensively investigate the antimicrobial potential towards developing antimicrobial agents from the medicinal plant *Tephrosia vogelii* leaves and roots for topical application. This is a new strategy and trajectory to fight fungal diseases and to offset the aforementioned microbial resistances to synthetic antimicrobial drugs.

1.2 Statement of the Problem

Pathogenic microbes causing infectious diseases and microbial resistances to antimicrobial agents are the global concerns that need urgent solutions. For instance, *Candida species* are among notorious causative agents of skin and deep fungal infections, and they have increasingly developed resistance to the most used modern fungicides such as fluconazole and voriconazole (Arendrup, 2014; Lockhart *et al*., 2017; Rodrigues & Albuquerque, 2018; Sanguinetti *et al*., 2015; Vandeputte *et al*., 2012). In addition, the problem of fungal resistance to the first line fungicides is a burden to millions of individuals particularly in the developing world because they are unable to afford the second line fungicides as they are prohibitively expensive. This explains as to why fungal infections and fungal resistance issues have implicated morbidity, mortality and health care in the community particularly in the developing countries (Arendrup, 2014; Bongomin *et al*., 2017; Lockhart *et al*., 2017; Rodrigues & Albuquerque, 2018; Vandeputte *et al*., 2012).

Notably, fungal infections are a public health problem that is more pronounced in sub-Saharan African countries than any other part of the world, and Tanzania is not an exception (Cole *et al*., 2017; Faini *et al*., 2015; Limper *et al*., 2017; Okeke *et al*., 2007; Rajasingham *et al*., 2017; Silverberg *et al*., 2017; Society, 2016). The fungal diseases and emerging fungal resistance have led to many deaths and attrition of economic production including in agriculture industry in low-income countries. The fungal diseases affect human healthy, crop production and livestock. For instance, it has reported that fungal infections accounts for 18% out of 80% deaths in Tanzania (Faini *et al*., 2015). These predominant fungal infections and emerging fungal resistances to second line fungicides have caused serious economic losses. For instance, recently The United States reported to spend about USD 7 billion yearly basis (Benedict *et al*., 2019). Equally, the antimicrobial resistance estimated to effect expenditure of about USD 200 billion yearly basis and expected to sprout up to more than USD 1 trillion by 2030 worldwide (Bank, 2017). This necessitates the search for new and more powerful antifungal agents to combat such serious fungal infections and reduce the economic losses associated with fungal infections (Fausto *et al*., 2019; Rodrigues & Albuquerque, 2018).

As a result, searching for antifungal agents from medicinal plants that could help to offset the prevailing fungal infections and resistance to the available synthetic antifungal drugs and the associated economic problem is crucial. Fortunately, medicinal plants are a great source of antimicrobial agents because of their diverse phytochemical compounds capable of exerting

inhibitory effects against disease-causing microorganisms (Gurib-Fakim, 2006). Explicitly, *Tephrosia vogelii* is among potential medicinal plants with evidence as indicated that it has been used traditionally to treat human ailments since olden times (Orwa *et al*., 2009). Unfortunately, there is no scientific study to date that has been reported to explore the versatility of *Tephrosia vogelii* to address fungal infections regardless of its traditional use to cure fungal and bacterial diseases. Moreover, the medicinal features of *Tephrosia vogelii* against infectious diseases and being used as anti-ectoparasite and pesticide agents, there are no scientific information on the drug development or formulation from this species. Therefore, this study aimed at investigating the antimicrobial potential of *Tephrosia vogelii* toward developing the antifungal agents.

1.3 Rationale of the Study

The increasing of fungal infections and emerging fungal resistance to available, affordable and recommended fungicides is a global problem. The situation overburdens humans in health settings and economics. Thus, it necessitates search for new and more powerful antifungal agents capable of combating such serious fungal infections and overcoming the fungal resistances (Fausto *et al*., 2019; Rodrigues & Albuquerque, 2018; WHO, 2015). The nature is replete with medicinal plants that have successfully and traditionally been used to treat fungal diseases afflicting humans, and this fact provides potential promise solution for the problem. Interestingly, medicinal plants are less expensive and readily available in the sub-Saharan region to search for antimicrobial agents for development of antifungal drugs topical application.

1.4 Research Objectives

1.4.1 General Objective

The general objective of the study was to investigate antimicrobial activity of *Tephrosia vogelii* found in Tanzania towards development of antifungal agents for topical application.

1.4.2 Specific Objectives

The specific objectives implemented in this study were the following:

(i) To evaluate the antimicrobial activities of leaf and root extracts of *Tephrosia vogelii* against *C. albicans, C. neoformans, S. aureus, E. coli, K. pneumoniae* and *S. typhi*.

(ii) To identify phytochemicals from leaf and root extracts of *Tephrosia vogelii* that exhibited high biological activity in the first specific objective.

(iii) To evaluate toxicity of the bioactive from extracts of *Tephrosia vogelii* that exhibited high activities and considerably suitable for drug formulation.

(iv) To formulate antifungal agents for topical application from *Tephrosia vogelii* bioactive extracts that exhibited high antifungal activity with low toxicity.

1.5 Research questions

(i) Is there any antimicrobial activity of leaf and root extracts of *Tephrosia vogelii* against *C. albicans, C. neoformans, S. aureus, E. coli, K. pneumoniae* and *S. typhi*?

(ii) What are the phytochemicals found leaf and root extracts of *Tephrosia vogelii* that exhibited high biological activity in the first specific objective?

(iii) Is there any toxicity of the bioactives from extracts of *Tephrosia vogelii* that exhibited high activities and considerably suitable for drug formulation?

(iv) Is it possible to formulate antifungal agents for topical application from *Tephrosia vogelii* bioactive extracts? If yes, what are the criteria to be used in selecting the extract(s) for drug formulation?

1.6 Significance of the Study

The opportunistic pathogens such as *C. albicans* are found around many parts of human body and they are more brutal as they affect mostly people with immuno-deficiency. Discovery of new bioactive secondary metabolites for drug development remains an urgent requirement into antifungal therapeutics. Bioactive compounds from this study entail the phytocomponents of the formulated antimicrobial agent prototype (product) for topical application against fungal infections such as *C. albicans* and *C. neoformans.* Of course, the

product needs further chemical optimization and clinical trials in human. Additionally, the study contributed to fill existed gap for the lacked scientific knowledge on the antifungal potentiality and toxicity profile of medicinal *Tephrosia vogelii*. Therefore, this study has contributed to establish available bioactives against fungal and bacterial infections and herbal cream prototype which could be used to promote humans health. Of course, the study output (herbal cream product) concurs with the WHO global action plan on antimicrobial resistance that was adopted by the World Health Assembly in May 2015. Moreover, toxicity studies revealed that roots of *Tephrosia vogelii* contain compounds which could be extended to anticancer studies in future.

1.7 Delineation of the Study

Herbal drug formulations are crucial for this era where antimicrobial resistants is increasing. In this study, antifungal agent (herbal cream) for topical therapy has been formulated from methanolic leaf extract of *Tephrosia vogelii*. Such methanolic leaf extracts exhibited worthy antimicrobial activity, it also contained phytochemicals with moiety active that explains the medicinal features of the plant. Moreover, toxicity evaluation of the extract used in herbal cream formulation attested safe. Thus, a clinical trial in human is needed as for further study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fungal Diseases

The fungal diseases are among the infectious diseases caused by pathogenic fungi found in the human body and environment (Beardsley *et al*., 2018). There are many pathogens such as *C. albicans* and *C. neoformans* that are known in causing fungal infections. Certainly, fungal diseases pose a global therapeutic problem due to fact that the fungi have continued to manifest resistance to synthetic antifungal drugs. For instance, candidiasis, and fungal skin infections have become intractable and even more critical fungal problems known to affect healthcare setting globally because of the ability of the fungi to resist the synthetic drugs (Arendrup, 2014; Bongomin *et al*., 2017). Of course, it has been reported that candidiasis is the leading fungal diseases worldwide and resistance to recommended drugs is the main factor contributing to the prevalence of the problem (Bongomin *et al*., 2017). Furthermore, the study findings indicated that *C. albicans* is the most dangerous opportunistic fungal species (Fig. 1) causing candidiasis which affect several areas of the human body: mouth, skin, vagina, and blood streams (CDC, 2019a). Women are even reported to be serious affected with vulvovaginal candidiasis (David W Denning *et al*., 2018). The immune-compromised persons such as those living with HIV are even more susceptible to the candidiasis.

In the same way, *C. neoformans* causing cryptococcosis is another dangerous opportunistic fungal pathogen causing deadly fetal meningitis diseases. Like candidiasis, cryptococcosis is also dangerous to immuno-compromised individuals such as those HIV-AIDS victims and prevalent due to fact that they also manifest fungal resistance to the synthetic drugs (Fig. 2) (Rajasingham *et al*., 2017). Unfortunately, these candidiasis and cryptococcosis problems have continued to cause very serious negative health effects to humans and consequently huge negative economic impacts in the sub-Saharan countries (Society, 2016).

More shockingly, it has recently been reported that skin fungal infections and oral candidiasis are among of fungal infections that are most problematic and deadly globally (Bongomin *et al*., 2017). The WHO and global health studies have pointed out that skin diseases are amongst the global burden diseases (GBD) because they are leading in causing disability and many deaths worldwide (Dermatology, 2017; Karimkhani *et al*., 2017; WHO,

2020). Being communicable in nature, skin fungal infections have become even extra problematic amongst poor people in the developing countries (Faini *et al*., 2015). The *C. albicans* and dermatophytes are some of the top pathogenic fungi causing skin infections amongst rural people particularly schoolchildren. The skin infections caused by *C. albicans* are also made complicated because they are interactively associated with pathogenic bacteria especially *S. aurea* (Carolus *et al*., 2019; Schlecht *et al*., 2015). The schoolchildren in rural areas are more vulnerable to such skin fungal infections due to interactions with dusts either in classrooms or outside in which the spores of the fungi reside (Chikoi *et al*., 2018; Gibbs, 1996; Satimia *et al*., 1998). For instance, studies conducted in Tanzania by Chikoi and colleagues (2018) revealed that 12% - 55% schoolchildren are affected by skin infections annually, and this has contributed to morbidity and socio-economic impacts (Chikoi *et al*., 2018).

The negligence has resulted into inadequate reliable data/ reports on how many persons die of fungal diseases on a yearly basis in many countries (Fig. 3). The availability of adequate and reliable data is key to developing strategic efforts to combat these diseases (Bongomin *et al*., 2017; Denning, 2017; Rodrigues & Albuquerque, 2018; Society, 2016). It is in the same trend that treatment of such opportunistic fungal pathogens especially *C. albicans* and *C. neoformans* has been an overrun challenge and burden in the community due to fungal resistance to drugs (Bongomin *et al*., 2017; Faini *et al*., 2015; Perlin *et al*., 2017). The solution to the problem of fungal resistance and toxicity (side effects) of the petrochemicals has not sufficiently been pursued (McManus, 2017).

For the reason that the immuno-suppressed persons especially cancer patients, diabetes victims and HIV/AIDS patients are even more vulnerable to fungal diseases, it suggests that fungal diseases need no more negligence. They are life-threatening and their deaths are expected to be in shocking numbers in sub-Saharan region because these patients are predominant in the region (Bongomin *et al*., 2017; Faini *et al*., 2015; Limper *et al*., 2017; Rajasingham *et al*., 2017). Thus, searching for non-petrochemical antifungal agents against the pathogenic fungi *Candida* and *Cryptococcus* species; and resistant microbial pathogens causing infections is needed. This is crucial in order to mitigate the fungal infections and achieve Sustainable Development Goals for 2030 agenda (Rodrigues & Albuquerque, 2018; UNDP, 2015). The solution will be not only greatly important to the poor communities due to overwhelmed economically but also to the global health care settings. The present study

therefore aims to investigate secondary metabolites for antifungal agents that will particularly serve against fungal skin infections from *Tephrosia vogelii* in favor of its potentiality as ethnomedically claimed for treatment against skin infections.

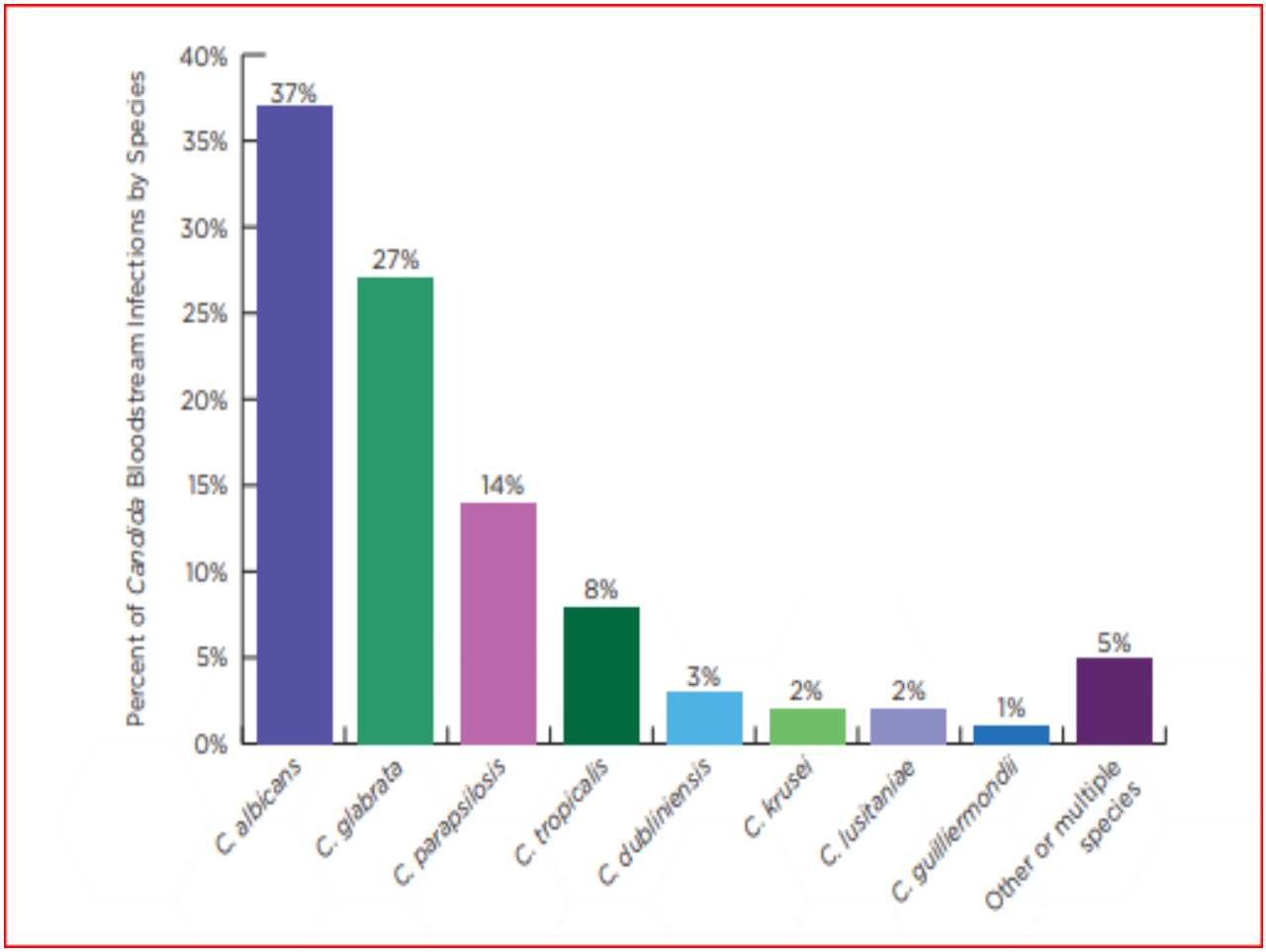


Figure 1: Percentage effects of *Candida* species (CDC, 2019a)

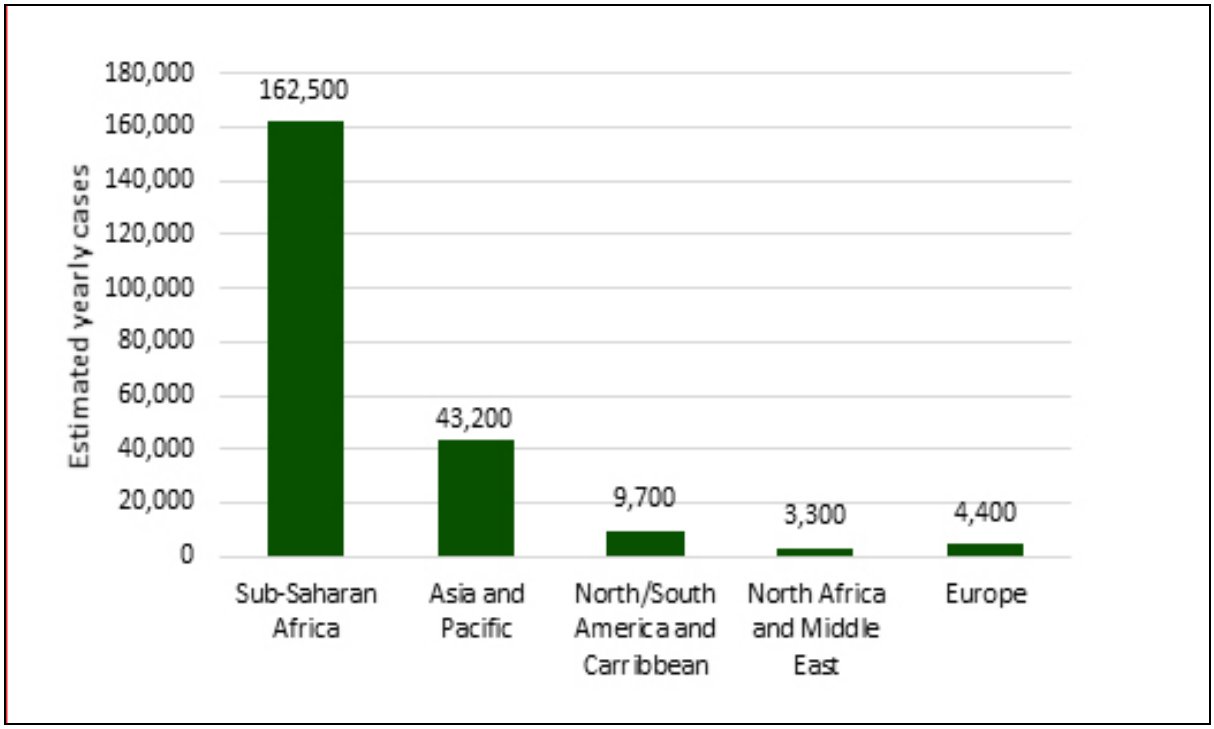


Figure 2: Global burden of HIV-related cryptococcal meningitis (Rajasingham *et al*., 2017)

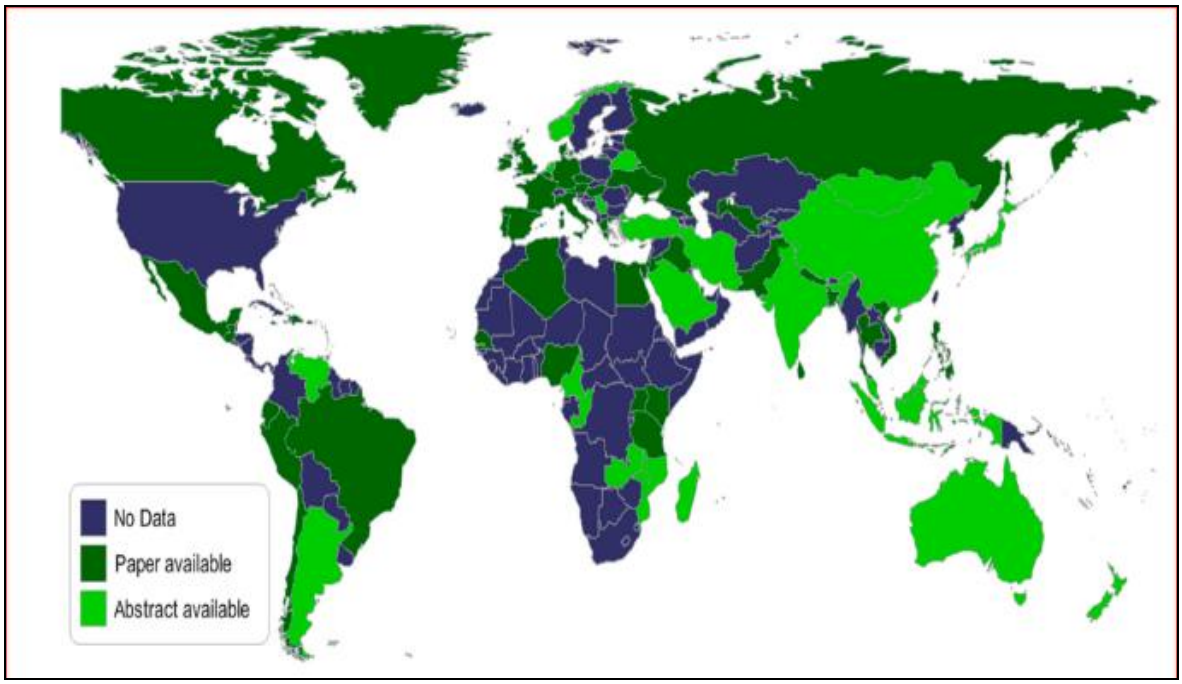


Figure 3: The global data of fungal diseases in year 2017 (Denning, 2017)

2.2 Tephrosia vogelii

By classification, the plant belongs to phylum Tracheophyta, order Fabales, class Magnoliopsida, genus *Tephrosia* and species *Tephrosia vogelii*. Of course, *Tephrosia vogelii* (Fig. 4 *vide infra*) belongs to family Fabaceae (Mwaura *et al*., 2013; Stevenson & Belmain, 2017). The *Tephrosia vogelii* has various trivial English names such as vogel's tephrosia, fish-poison-tree, fish-poison bean, and fish bean; and trivial Swahili names such as *utupa*, *mtupa*, *mibaazi* and *kibazi*. The family Fabaceae to which *Tephrosia vogelii* belongs consists of more than 700 genera, and about 20,000 species of trees, shrubs, vines, herbs and distributed worldwide (Ahmad *et al*., 2016; Rahman & Parvin, 2014). Members of Fabaceae family are popularly known to possess potential medicinal qualities traditionally used to treat a range of human ailments that include microbial infections (Ahmad *et al*., 2016; Rahman & Parvin, 2014).

The plants in this Family are endowed with diverse biologically-active secondary metabolites such as terpenoids, saponins, flavonoids, alkaloids and coumarins, which have been reported to exhibit anticancer qualities and antimicrobial activities against pathogens (Majinda *et al*., 2001; Makoshi & Arowolo, 2011). Specifically, the flavonoids have been reported to have antimicrobial, anticancer activities and neural-brain-activating abilities (Atanasov *et al*., 2015; Dewick, 2009; Maheswari *et al*., 2016; Marinova *et al*., 2005; Pietta, 2000; Prabha *et al*., 2014; Slimestad & Verheul, 2009; Yao *et al*., 2004). Similarly, the tannins have been reported to exhibit anticancer, and, antimicrobial activities, which have been found to be the inherent properties for treatment of constipation (Hussain *et al*., 2019; Singh & Kumar, 2019; Hwang, 2018). Additionally, the saponins have been reported to exhibit hepatopathy activities that assist body cells to recover from injuries (Patel *et al*., 2013). The fact that *Tephrosia vogelii* belongs to the family Fabaceae makes it an ideal ethnomedical candidate for investigating medicinal agents and drug formulations for treatment and management of several diseases such as bacterial diseases, ecto-parasitic infections, schistosomiasis, ringworm and skin diseases (Anjarwalla *et al*., 2015; Dzenda *et al*., 2008; Mwaura *et al*., 2013; Orwa *et al*., 2009). However, information on medicinal agents and drug formulations are limited. Certainly, lack of extensive pharmacological information *in vivo* toxicity studies of *Tephrosia vogelii* against fungal and bacterial pathogens limits the information on the potential of the plant against fungal and bacterial pathogens. This information could be used in the chemical industry to develop drugs for fighting the skin fungal infections. Thus, the

present study intends to use this plant extracts to carry out the antifungal studies against *C. albicans* and *C. neoformans* as well as *S. aureus*, *E. coli*, *K. pneumoniae*, and *S. typhi* focusing on drug formulations for topical applications.

Figure 4: Photo of matured *Tephrosia vogelii* as taken in the field

2.2.1 Distribution and Uses of *Tephrosia vogelii*

Tephrosia vogelii was discovered at first place in Western Africa and still found in various tropical regions of Africa (Orwa *et al*., 2009). The species is exotic in China and various regions worldwide that include nearly all habitats such as grassland, savannah-like vegetation, wasteland, forest margins and shrub land as well as fallow fields (Orwa *et al*., 2009). Moreover, the species grows well in acidic soils. The *Tephrosia vogelii* has been used as poison to stupefy fish for easy fish catching during fishing activities (Orwa *et al*., 2009). This latter use of the species caused the emanation of its popular names: "bean fish-poison-tree", "fish-poison bean" and "the fish bean". As a result, many people capitalized to grow *Tephrosia vogelii* for fishing purposes. However, authorities have banned its use as a fishing plant because use of *Tephrosia vogelii* is regarded as an illegal fishing method, probably because the stupefying chemicals are unable to select specific sizes of the fishes such that they kill even baby-fishes thereby increasing the risk of fish disappearance (Hisham *et al*., 2006; Makoshi & Arowolo, 2011). Consequently, because of the fish bean's ability to stupefy

fish non-selectively, its use a fishing agent has been banned and as a result its cultivation in many communities has also been drastically decelerating in the recent years.

Apart from being used as a fishing-plant, *Tephrosia vogelii* has been reported to form root nodules capable of fixing atmospheric nitrogen, hence the plant contributes to the improvement of soil fertility (Orwa *et al*., 2009). Interestingly, another utility of this plant has been demonstrated by some communities in East and West Africa that have cultivated this plant for managing insects like weevils, ecto-parasites in animals and other uses such as human ailments (Anjarwalla *et al*., 2015). This suggests that the plant has the potential of being used to develop pesticides for promoting agricultural productivity via integrated pest management. However, it is unfortunate to learn that over the recent years due to industrialization advancements, the local uses of *Tephrosia vogelii* as pesticides and acaricides have been neglected and instead replaced by the petrochemical-derived industrial pharmaceuticals. Although conventional drugs have replaced the local ones, undesirably the petrochemical-derived drugs have nowadays been reported to be increasingly ineffective due to the fact that the pathogens are increasingly and constantly exerting drug resistance. Moreover, these petrochemical-derived drugs are not environmentally friendly. Therefore, these challenges demonstrated by conventional drugs could be overcomed by going back to the natural resources. Evidently, nature through medicinal plants such *Tephrosia vogelii* that have been traditionally used for management of pests, ecto-parasites and human ailments are significantly in this matter.

2.2.2 Ethnomedical Features of *Tephrosia vogelii*

Ethnomedically, this species is used as an abortifacient, emetic, bactericide, purgative and cure for skin diseases, schistosomiasis, ringworm and parasitic infections in various area including Tanzania, Uganda, Gabon, Zimbabwe, Angola, Nigeria and Kenya (Anjarwalla *et al*., 2015; Dzenda *et al*., 2008). The dry, crushed leaves are used as insecticide for management of lice, fleas and ticks (Anjarwalla *et al*., 2015). The leaf and root decoctions are used in the treatment of scabies and yaws, typhoid, constipation, tuberculosis as well as anthelmintic agents (Anjarwalla *et al*., 2015; Dzenda *et al*., 2008; Orwa *et al*., 2009).

2.2.3 Pharmacological Features of *Tephrosia vogelii*

The pharmacological studies of water, methanolic and ethanolic extracts have substantiated the ethnomedical use of *Tephrosia vogelii* as acaricides, pesticides and dermatophyticides

(Anjarwalla *et al*., 2015; Li *et al*., 2015; Makoshi & Arowolo, 2011; Marango *et al*., 2017; Orwa *et al*., 2009; Stevenson & Belmain, 2017). The bioactive compounds from ethanolic and methanolic extracts reported to exhibit larvicidal effects on the subjected growth stages of mosquitoes and insects (Kalume *et al*., 2012; Kidukuli *et al*., 2015; Li *et al*., 2015; Stevenson & Belmain, 2017). The pharmacological studies warranted the application of *Tephrosia vogelii* extracts in management of ticks in domestic animals especially to small scale animal farming (Anjarwalla *et al*., 2015). The countries such as Uganda, Tanzania, Kenya and Zimbabwe are amongst the countries whose societies use leaf extracts to control ticks (Gadzirayi *et al*., 2009; Kalume *et al*., 2012; Makoshi & Arowolo, 2011; Mwaura *et al*., 2013). Since the *Tephrosia vogelii* has showed insecticidal and acaricidal features, the species reveals its potentiality of contained bioactive compounds for drug formulations and pharmaceutical development in future (Stevenson & Belmain, 2017).

Furthermore, pharmacological studies of leaf extracts of *Tephrosia vogelii* conducted by Makoshi *et al*. (2011) and other studies validate its potentiality to treat animal skin infection (Dzenda *et al*., 2008; Makoshi & Arowolo, 2011). In addition, the ethanolic-aqua (70:30) barks extracts of *Tephrosia vogelii* reported to exhibit antibacterial activity of the bacterial strains (Swamy *et al*., 2015). On the other hand, pharmacological studies of Swamy and colleagues (2015) pointed-out the usefulness of *Tephrosia vogelii* as a medicinal plant for management of bacterial diseases (Inalegwu & Sodipo, 2015; Swamy *et al*., 2015). The biological assay of ether-ethanolic extracts of *Tephrosia vogelii* seeds showed that the extracts exhibit anti-oxidants and free scavenging properties, and hence indicated the presence of flavonoids which have been reported to be responsible for such antioxidant and free radical scavenging properties (Samuel *et al*., 2019). Other studies reported that the methanolic extracts of *Tephrosia vogelii* also exhibited purgative activities after being subjected to the rabbit (Samuel *et al*., 2019). Moreover, extracts of *Tephrosia vogelii* stem barks containing flavonoids were reported to exhibit anti-cancer activities (Gbadamosi & Erinoso, 2016).

The afore-reported pharmacological studies clearly advocate that *Tephrosia vogelii* as a potential source of the future pharmaceuticals drugs. Its usefulness ranges diversely not only as a potential source of pesticidal and acaricidal agents but also antifungal, antibacterial and anticancer agents for treating human ailments. However, it seems little attention has been paid to investigate and establish the profile of the plant in terms of its antimicrobial activities

and toxicities. The present study intends to carry out the extensive investigation of *Tephrosia vogelii* bioactive compounds regarding antimicrobial activity to address the existing gap of limited information and discover the bioactive compounds (secondary metabolites) leading to formulation of the antifungal drugs for topical applications.

2.2.4 Secondary Metabolites of *Tephrosia vogelii*

The phytochemical studies from *Tephrosia vogelii* have spotlighted several isolated secondary metabolites (Fig. 5) such as terpenoids, steroids, tannins, flavonoids and rotenoids which substantiate ethnopharmacological prominence of this medicinal plant (Inalegwu & Sodipo, 2015; Makoshi & Arowolo, 2011; Tole & Neme, 2019). For instance, the terpenoids have been reported to come from predominantly aerial parts of the plant in the ethanol extracts, these include sesquiterpenes, (1β,6α,10α)-guai-4(15)-ene-6,7,10-triol1) (**A**) and lignans, (+)-lariciresinol 9'-stearate (**B**) (Wei *et al*., 2009). The flavonoids also have been reported to come from the methanolic root extracts; they include obovatin-3-methylether (**C**) and Z-tephrostachin (**D**) (Dagne *et al*., 1989). The rotenoids have been reported to come from the roots, stem barks and leaves; such phytocompounds include deguelin (**E**), tephrosin (**F**), α-toxicarol (**G**), rotenone (**H**), 12α-hydroxyrotenone (**I**) and sarcolobine (**J**) (Stevenson *et al*., 2012; Stevenson & Belmain, 2017). This information is sufficient evidence that *Tephrosia vogelii* is highly rich in compounds, which are most likely of medicinal value.

Notably, the studies of pharmacological activities of compounds **A-J** revealed different levels of *Tephrosia vogelii* biological and chemical activities against pests (Dagne *et al*., 1989; Stevenson *et al*., 2012; Stevenson & Belmain, 2017; Wei *et al*., 2009). Bioactivity tests of the pure isolated secondary metabolites especially rotenoids and extracts justified that the plant species is a potential resource for finding non-petrochemical insecticides, pesticides and acaricides (Anjarwalla *et al*., 2015; Dagne *et al*., 1989; Gadzirayi *et al*., 2009; Kalume *et al*., 2012; Li *et al*., 2015; Makoshi & Arowolo, 2011; Marango *et al*., 2017; Morris, 1999; Mwaura *et al*., 2013; Russell *et al*., 2017; Stevenson *et al*., 2012; Stevenson & Belmain, 2017). The rotenoids have been the most active chemical components that influence its applicability as a natural source of local pesticides, acaricides and fish-stupefying agents (Caboni *et al*., 2004; Ling, 2003). Interestingly, studies have indicated that the rotenones and other rotenoids are biodegradable because they decay easily and rapidly, mostly within fourteen days (Caboni *et al*., 2004; Ling, 2003). Therefore, unlike the petrochemicals, the rotenoids are user-friendly to the environment. Indeed, the pharmacological studies do not

only provide evidence for the potentiality of *Tephrosia vogelii* as a source of compounds of medicinal value but also suitable candidate for the present study to investigate alternative biodegradable and environmentally user-friendly antimicrobials.

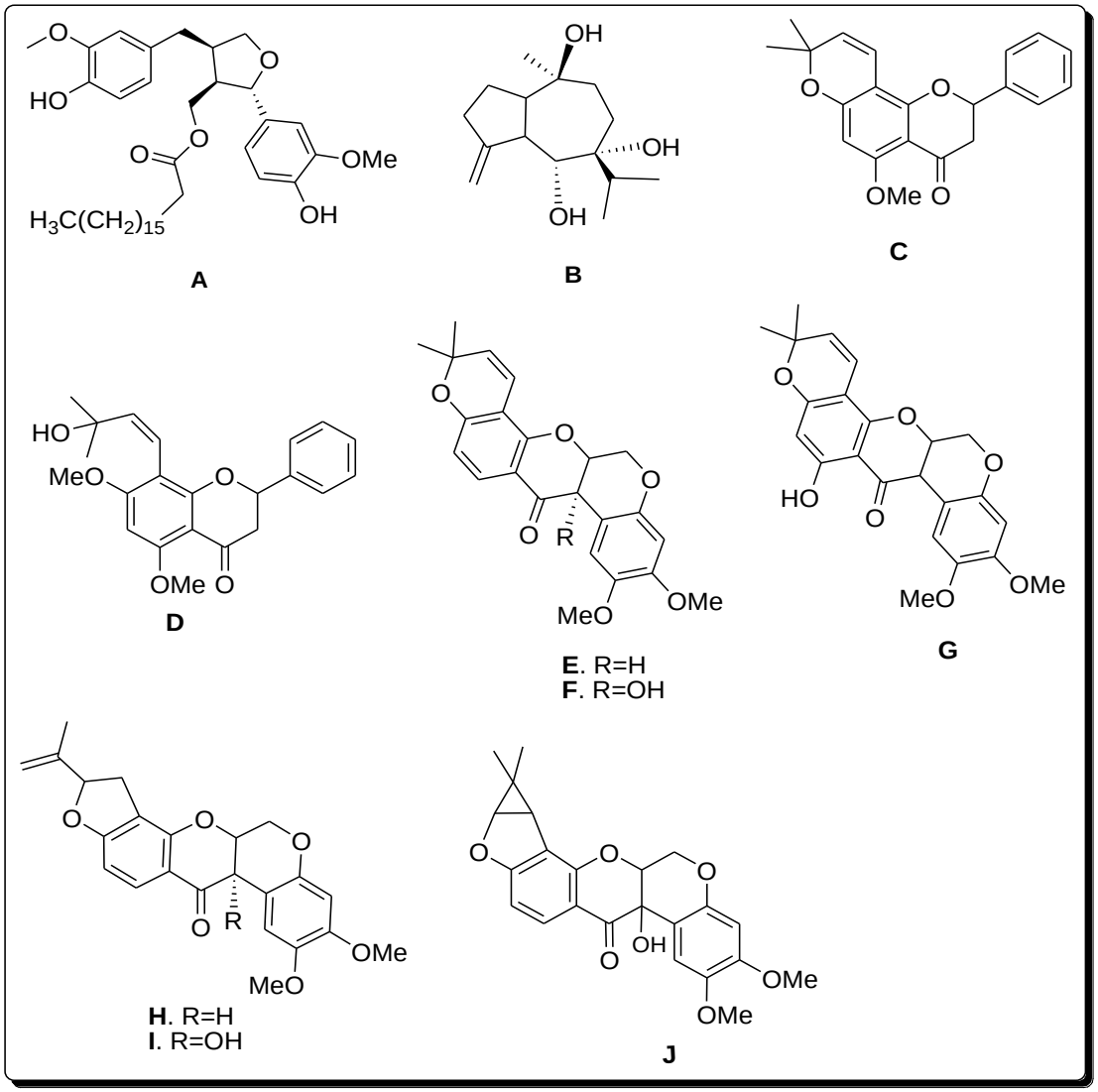


Figure 5: Reported secondary metabolites from *Tephrosia vogelii* (Stevenson & Belmain, 2017; Wei *et al*., 2009)

Consequently, the ethnomedical use and pharmacological studies of the *Tephrosia vogelii* demonstrate that this species is an ideal potential source of bioactive secondary metabolites which could be used to develop pharmaceuticals of a wide range of applications from promoting agriculture productivity to protecting the public health. For instance, its extracts could be used for formulation of pesticides and antimicrobial agents. Despite its medicinal potential against pathogenic microbes and the abundant pharmacological knowledge of this precious plant on its potential to serve as a source of anti-ectoparasite and pesticide agents,

there are no reports on the drug formulation from the plant. Also, both phytochemical and biological assays on investigating the antimicrobial activities of stems, roots, fruits, flowers and leaves of *Tephrosia vogelii;* and toxicity studies have not yet been adequately pursued and reported. Thus, this review critically argues that extensive study on the toxicity, phytochemical investigations and antimicrobial analysis of *Tephrosia vogelii* should be carried out because such a study would provide useful information towards the development or herbal formulations of antifungal and antibacterial agents for treatment of both fungal and bacterial infections.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Chemicals and Standard Drugs

All laboratory chemicals and solvents used in this study were purchased from Sigma-Aldrich and used as they are. These were: methanol, dichloromethane, *n*-hexane, dimethyl sulfoxide (DMSO), white soft paraffin, Cetostearyl alcohol, stearic acid, propyl glycol, methyl Paraben, Mueller Hinton broth (MHB), Mueller Hinton agar (MHA), Sabouraud dextrose agar (SDA) and Iodonitrotetrazolium (INT). The polystyrene microliter plates, fluconazole, clotrimazole cream and ciprofloxacin were purchased from pharmaceuticals shop.

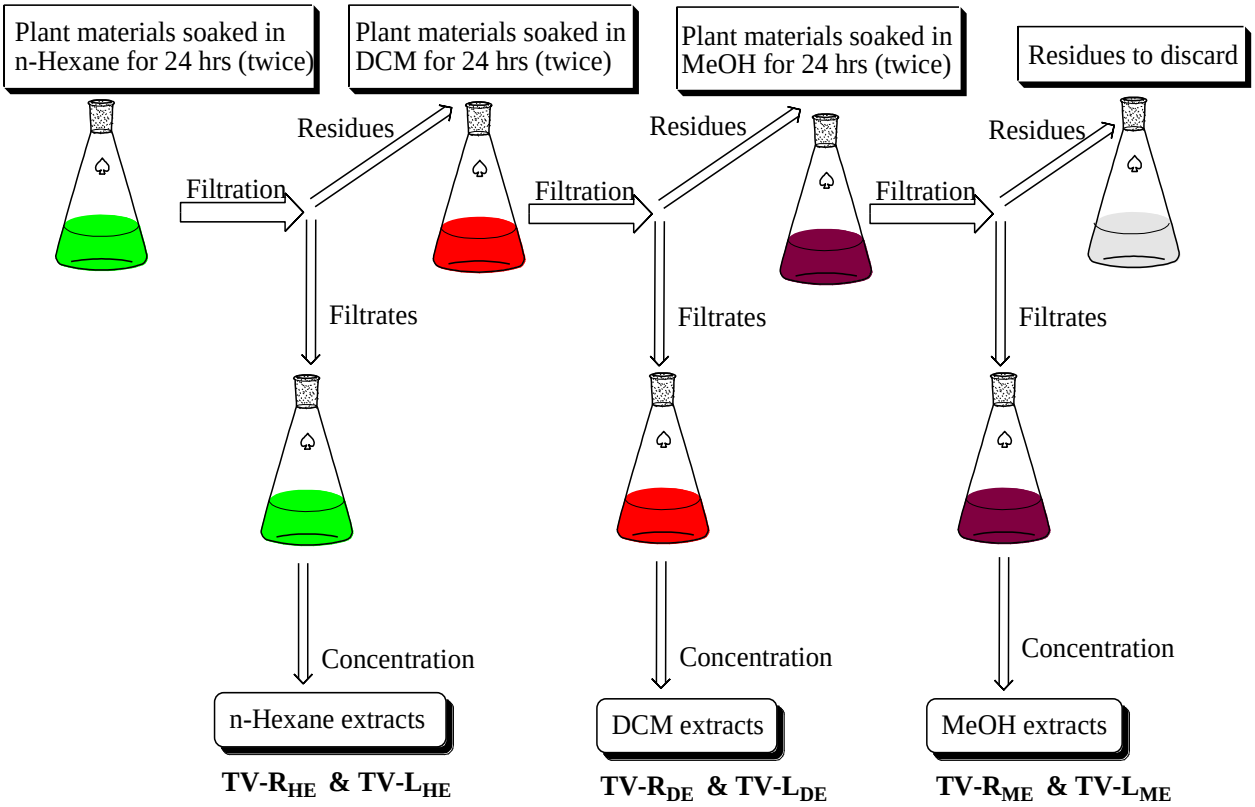
3.2 Plant Materials: Identification and Collection

The leaves and roots of *Tephrosia vogelii* were collected in January, 2019 from Hai district, Moshi region in Tanzania (Latitude S 03° 15' 6.4" and Longitude E 37° 14' 3.8"). In the course of collection of plant materials was a rainy season. The plant species was identified by Mr. Ezekiel John, the plant taxonomist and botanist from Tanzania Pesticides Research Institute (TPRI), and the voucher specimen (SH-NM102) was deposited at the Nelson Mandela African Institution of Science and Technology (NM-AIST). The collected plant materials were air-dried under shade for four weeks then pulverized to powder form for subsequent extraction.

3.3 Extractions

The pulverized 1.0 kg leaves and 0.6 kg roots were extracted separately and sequentially soaked with n-hexane, dichloromethane, and methanol for 48 hours in each solvent (Fig. 6). All solvents were completely evaporated under low pressure using a rotary evaporator at temperature below 40^{o}C to avoid thermal decomposition of volatile compounds. After evaporation, 4.8 and 3.4 g of *n*-hexane leaf and root extracts, respectively, were obtained. Also, 5.9 and 4.1 g of dichloromethane leaf and root extracts, respectively, were obtained. In the same way, 24.2 and 10.5 g of methanolic leaf and root extracts, respectively, were obtained. Then, leaf and root extracts of *Tephrosia vogelii* were stored at 4^{o}C for subsequent bioassays, phytochemical analyses and herbal formulations.

Chart Flow of Total Extraction



Note:
TV = *Tephrosia vogelii*; H = Hexane; D = Dichloromethane; M = Methanol; R = Root; L = Leaves; E = Extracts

Figure 6: Schematic of extraction processes

3.4 Antimicrobial Activities

3.4.1 Fungi Strains, Bacteria Strains and Sub-culturing

All strains used in this study were generously provided by the School of Pharmacy at Muhimbili University of Health and Allied Sciences (MUHAS). Antimicrobial bioassays were performed in the Microbiology Laboratory at MUHAS. Two fungal strains used were *C. albicans* (ATCC 90028) and *C. neoformans* (clinical isolate). Four bacteria strains used were of two categories: the Gram positive bacteria, *S.s aureus* (ATCC25923) and the Gram negative bacteria; *E. coli* (ATCC29953), *K. pneumoniae* (ATCC 700603) and *S. typhi* (NCTC 8385). All microbes, fungi and bacteria were sub-cultured onto Mueller Hinton agar (MHA). The MHA (8.0 g) was suspended in 230 mL of distilled water in 500 mL scotch bottle forming the mixture that was heated at 60 ^{o}C to dissolve the agar completely. Then the suspension was autoclaved at 121 ^{o}C for 15 minutes. The mixture was left to cool at room temperature; inoculation was conducted onto the cooled growth media and left for five days. The fungi strains inoculums were incubated at 37^{o} ^{o}C for 48 hours while bacteria inoculums were incubated at 37 ^{o}C for 24 hours.

3.4.2 Procedures for Antimicrobial Activities

The antifungal and antibacterial bioassays were conducted against *C. albicans, C. neoformans, S. aureus, E. coli, K. pneumoniae* and *S. typhi* using methanolic, dichloromethane and *n*-hexane leaf extracts and root extracts of *Tephrosia vogelii*. The inoculation of strains was carried out into Mueller Hinton broth (MHB) mixed with extracts then followed by incubation. The MHB was used as sub-culture media from MHA in this stage of experimental study. Two-fold serial dilution method (Fig. 7) was used to determine the Minimal Inhibitory Concentration (MIC) of the extracts. This method was performed using the sterilized 96 wells of polystyrene microliter plates as previously described by Eloff (1998) with minor modifications (Eloff, 1998).

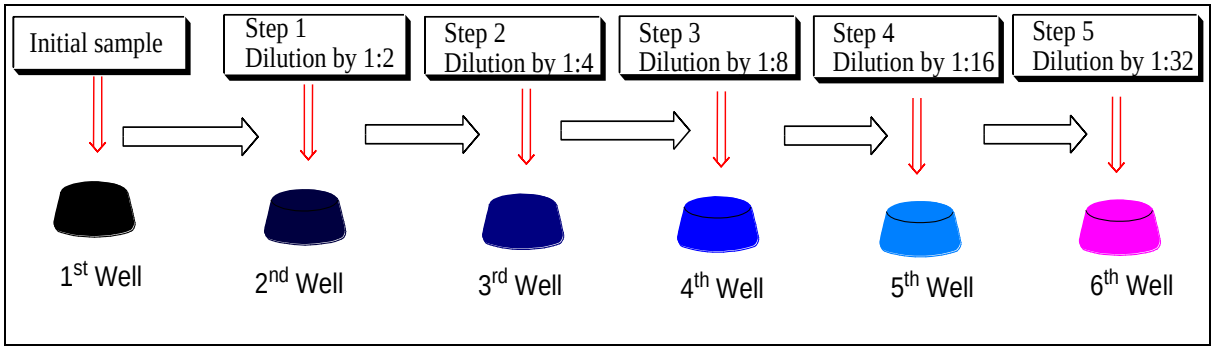


Figure 7: The concept of serial dilution

3.4.3 Determination of Minimum Inhibitory Concentration

The Minimal Inhibitory Concentration (MIC) values were determined by the two-fold serial dilution method. Forty milligrams of each crude extract were used to prepare stock solutions (40 mg/mL) by dissolving 40 mg of crude extracts in 1mL of 10% DMSO and 90% sterile tryptone broth in eppendorf tube. The turbidity of inoculated microbes (fungi and bacteria) grown into MHB cultures for screening were adjusted to standard solution which is equivalent to the 0.5 McFarland units (approximately 1.2 x 10^8 CFU/mL). The inoculums were then subjected to biological assays.

Determination of MIC was conducted in duplicates using microliter plates (Fig. 8). The MHB (50 µL) were added to each microliter plate well. Then, to each well of the first row, 50 µL of the extract (40 mg/mL) were added and well mixed to reduce the extract concentration to 20 mg/mL. The serial dilution proceeded by transferring 50 µL of the mixture from each well of the first row to each well in the second row. This dilution was carried on to the subsequent rows until the last row of the wells. The mixture (50 µL) transferred from each well of the last row were discarded. Then, 50 µL of the cultured strains (fungi and bacteria) were added to each well containing growth media poisoned with the extract completing the double dilution producing eight serial concentrations: 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.1562, and 0.07825 mg/mL. The microliter plate wells having inoculums without extracts were used as growth control (negative control). The microliter plate wells with inoculums treated with fluconazole and ciprofloxacin were used as positive control for fungi and bacteria respectively. Then, all inoculated microliter plate wells were incubated for 48 and 24 hours for fungi and bacteria strains, respectively. After incubation, 30 µL of Iodonitrotetrazolium (INT) chloride salt was added to each well and then the plates were incubated for one hour, the duration after which the results were recorded. Whereas the pink colouration developed in microplate wells after

addition of INT indicated the presence of microbes, clearance or disappearance of pink colouration indicated the growth inhibition of the fungi or bacteria. The MIC values of all extracts were determined at the lowest concentration of the extract at which a marked reduction in colour formation was noted. The negative control was used as reference for the growth of the strains under study.

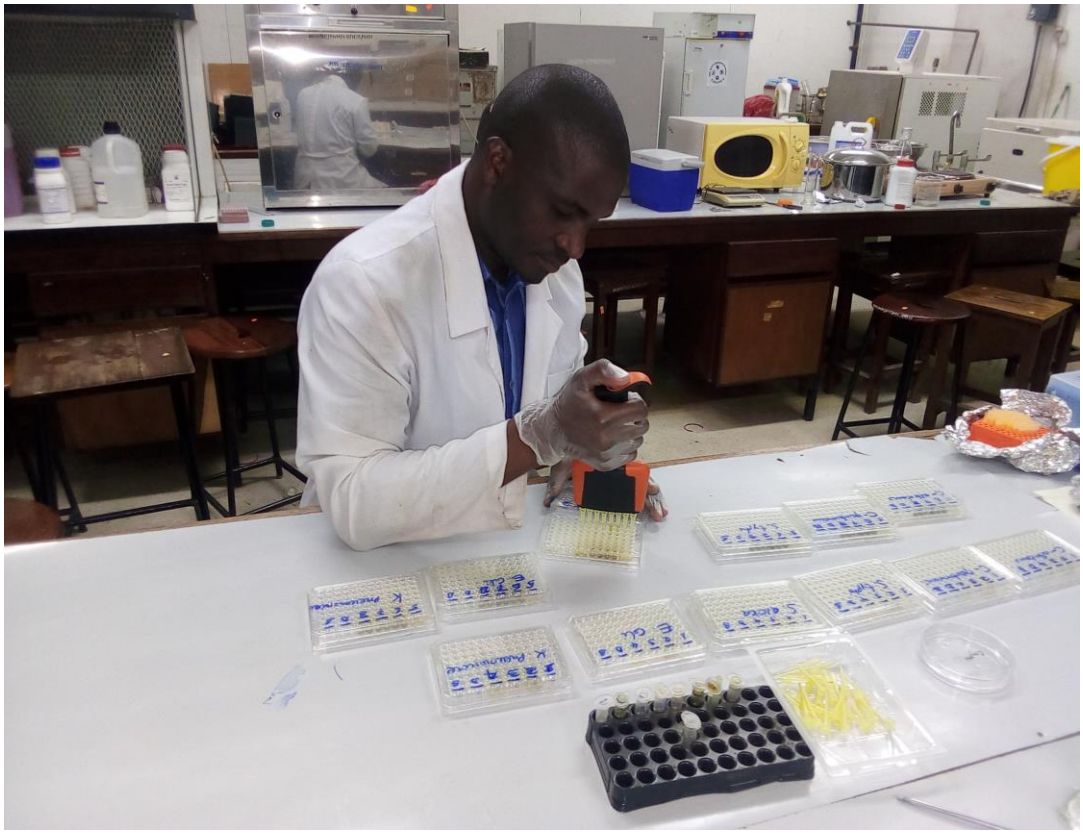


Figure 8: Laboratory work during inoculation to determine MIC of extracts

3.5 Phytochemical Investigation

3.5.1 Phytochemical Screening

Phytochemical screening of secondary metabolites from the methanolic root and leaf extracts was conducted according to standard procedures as described in previously studies (Kujur *et al*., 2010; Kumar & Thampi, 2015; Onwukaeme *et al*., 2007; Ukwubile *et al*., 2019; Zahra *et al*., 2019). All chemical tests were conducted in duplicates for experimental replication and consistency of results.

Test for tannins

Distilled water of 1 mL was added to 0.5 g of extracts. Then the mixture was stirred, filtered and few drops of ferric chloride were added to the filtrate. The formation of blue-black precipitates indicated the presence of tannins.

Test for steroids

About 0.1 g of the extract was dissolved in 1 mL of chloroform. Then 1 mL of acetic anhydride was added to the mixture. Finally, two drops of concentrated sulfuric acid were added gently to the mixture alongside the test tube. Changes of colour from violet to blue/green indicate the presence of steroids.

Test for terpenes

About 0.1 g of the extract was dissolved in 1 mL of chloroform. Then 1 mL of acetic anhydride was added to the mixture. Finally, two drops of concentrated sulfuric acid were added gently to the mixture alongside the test tube. Changes of colour from violet to pink-red indicated the presence of terpenoids.

Test for saponins

About 0.5 g of extract was added to 5 mL of distilled water and shaken well. Then the mixture was gently warmed. Persistent frothing even after warming indicated the presence of saponins.

Test for flavonoids

About 15 mL of distilled water was added to 0.25 g of plant extracts, and then mixture was filtered. About 10 mL of the filtrate collected which then divided into two test tubes each containing 5 mL. Then 5 mL of 20 % sodium hydroxide was added to 5 mL of the filtrate. In another tube of 5 mL of the filtrate 5 mL of lead acetic solution was added. Formations of yellow colour with either reagents added to the filtrate confirmed the presence of flavonoids.

Test for alkaloids

About 0.5 g of the extract was added to 3 mL of 1 % aqueous hydrochloric acid and stirred in a steam bath. The mixture was then filtered, and 1 mL of the filtrate was poured into two test

tubes, each containing 0.5 mL. Finally, 3 drops of Mayer's reagent were added to one of the test tubes, and 3 drops of 1 % picric acid were added to another test tube. The non-formation of precipitates with any of the final added two reagents confirmed the absence of alkaloids.

Test for glycosides

About 0.1 g of extract was dissolved in 1 mL of glacial acetic acid containing one drop of ferric chloride solution. Then 1 mL of concentrated sulphuric acid was added to the mixture by pouring alongside the test tube. The brown ring formation indicated the presence of glycosides.

Anthraquinones

About 50 mg of extract was heated with 1 mL 10 % ferric chloride solution and 1 mL of concentrated hydrochloric acid. Then the extract cooled and filtered, and then filtrate was shaken with equal amount of diethyl ether. The extract of the ether extract with strong ammonia did not form pink-red colour of aqueous layer indicated absence of anthraquinones.

3.5.2 GC-MS Analysis

Phytochemical analysis of methanolic leaf and root extracts was performed using Gas Chromatography-Mass Spectrometry (GC-MS) as described in the previous study by Irawan *et al.* (2018) with minor modifications (Irawan *et al*., 2018). The 7010 GC-MS Triple Quad Agilent Technologies made in Germany was used. The experiment was carried out at the institute of Tanzania Bureau Standard (TBS). In this experiment, the GC-MS Agilent Technology consisting 7890B Gas Chromatography coupled with 7010 Triple Quadruple Mass Spectrometer was employed. About 50 mg samples were dissolved in 5 mL of methanol-dichloromethane (2:3) and filtered using Whatman's no. 1 (filter paper). The splitless mode was used to inject 2 µL of each extract sample. The HP-5 column (30 m long, 0.250 mm internal diameter and 0.25 µm film thickness, Agilent Technology) was employed to separate phytochemical compounds. The flow rate of the mobile phase (carrier gas: Helium) was set at constant of 0.2 mL/min., and electron ionization (EI) mode for mass spectrometer was set at 70 eV with flame ionization detector (FID) at the scan range between *m/z* 40 and *m/z* 600 so as to produce mass spectra. The oven temperature was held at 40 ºC for 5 minutes then increased to 250 ºC that maintained for 9 minutes, then changed to 280 ºC which maintained for 14 minutes. The total running time was 30 minutes. Each sample was

run in duplicates. The structure and names of phytochemicals were ascertained through transacted molecular weight (m/z) from GC-MS chromatograms. The experimental data analysis was done through comparing obtained spectral peaks in GC-MS chromatograms with Wiley and National Institute Standard and Technique (NIST) libraries as well as known phytocomponents in the literature. The proof of identity of compounds were assumed mass spectra and retention time (RT) matched.

3.6 Toxicity Evaluation

3.6.1 Experimental Animals and Doses

Animal model experiments were performed in accordance with OECD guidelines 423 and 407 for toxicity assessment (OECD, 2001, 2008) and the previous methods with minor modifications (Chinedu *et al*., 2013; Nanthini *et al*., 2014; Olaniyan *et al*., 2016; Yadav *et al*., 2019). Fifty-five laboratory albino rats aged eight weeks were collected from Sokoine University of Agriculture (SUA). All rats were housed in plastic cages and left for acclimatization for seven days before starting treatments. During acclimatization period, rats were freely fed food and water three times a day; in the morning, afternoon and evening to make sure that they did not starve and die. During dose regime, the extracts doses (drugs) were administered to rats through oral gavaging. As a consequence, for convenient administration of extracts to rats, water was not provided in the morning (0800 hours) to noon (1200 hours) in order to induce thirst and enhance dose up take while avoiding vomiting. Therefore, water was given in the afternoon immediately after administration of the drugs and during the evening.

The extracts doses of 600, 1200, 2000 and 5000 mg/kg body weight were employed regarding previous studies with minor modifications (Porwal *et al*., 2017; Yadav *et al*., 2019). The body weights of the animals were measured using analytical balance before the drug administration regime in order to prepare appropriate doses. Weights of animals were 150 ± 5 g of which the doses were calculated on the average weight of 150 g. The amount of extracts used to prepare concentrations (doses) was established based on the calculation of the "ratio of expected dose multiplied by the weight of rats". Then, 90, 180, 300 and 750 mg of methanolic leaf and root extracts of *Tephrosia vogelii* were measured regarding rats weights and expected doses. Extracts were dissolved in 1 mL of distilled water to obtain 90, 180, 300

and 750 mg/mL solutions, which were equivalent to doses 600, 1200, 2000 and 5000 mg/kg, respectively, for dosage in rats.

3.6.2 Animal Welfare and Safety

The Rats were humanely treated to observe ethics of using animals in experimental study. The Tanzania animal welfare Act (*Animal Welfare Act: Enacted by Parliamentary of the United Republic of Tanzania*, 2008) as well as other ethical guidelines for consideration of animal rights such as the use of few numbers of animals, treatment of animals with minimal or without pain as well as age of animals to be used in experiments were observed in this study (Balls *et al*., 1995; Prescott & Lidster, 2017). During the experimental work, all rats were safely caged to protect the rats to ensure that the rats neither suffocated nor died because of unconducive environments. The sacrificed bodies and organs were sterilized and incinerated after the experiments as per institution guidelines.

3.6.3 Lethal Dose (LD_{50}) Test: Groups of Animals and Treatment

Twenty-seven rats were grouped into nine groups (Fig. 9); each group consisted of three rats (Chinedu *et al*., 2013). Animal groups were housed in separate plastic cages for ease sign observation and treatments. The LD_{50} test was an *in vivo* experiment whereby a single dose of the extract(s) administered to laboratory albino rats so as to evaluate doses causing deaths. Both methanolic root and leaf extracts of *Tephrosia vogelii* of doses 600, 1200, 2000, and 5000 mg/kg were administered to rats to determine deaths due to LD_{50} extracts within 72 hours. Except for the control group (group 9) which was not treated with extracts, the same dose was administered to all three animals of each respective group. The methanolic root extracts (TV-R, ME) of doses 600, 1200, 2000 and 5000 mg/kg were administered to four groups of rats (groups 1, 2, 3 and 4), respectively. The methanolic leaf extracts (TV-L, ME) of doses 600, 1200, 2000 and 5000 mg/kg were administered to four groups of rats (groups 5, 6, 7 and 8), respectively. The dose administrations were done once followed by observations of toxicity (clinical) signs recorded at the interval of 10 minutes, 30 minutes, 1 hour, 4 hours, 8 hours and 24 hours and 72 hours. The clinical signs of toxicity such as water intake, mortality, food intake, sedation, convulsion and deaths were recorded for 3 days.

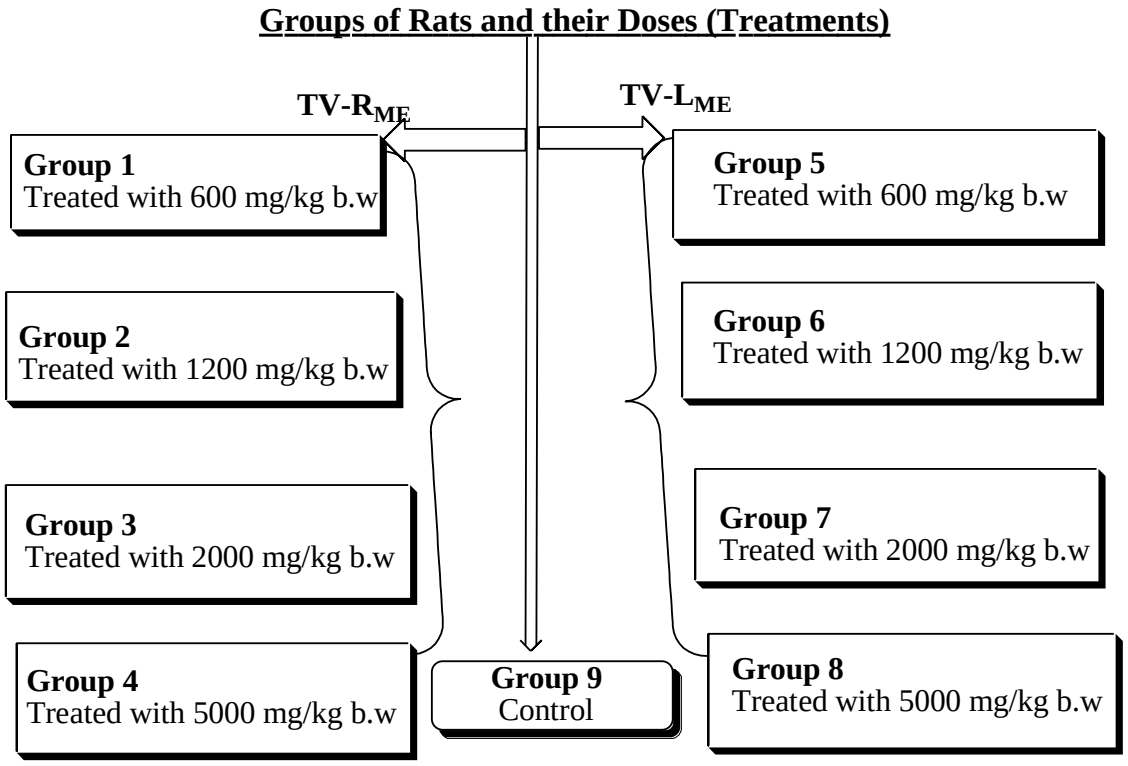


Figure 9: Sketch of animal groups with the respective doses for lethal test

3.6.4 Sub-Acute Toxicity Assay: Groups of Animals and Treatment

The sub-acute toxicity assays were performed through an animal model using laboratory albino rats carried out at SUA. Twenty-eight albino rats were divided into seven experimental groups, and each group had four rats (Fig. 10). Each group was housed in an independent plastic cage for conducting treatments. The TV-R, ME doses of 600, 1200 and 2000 mg/kg were used to treat groups 1, 2, and 3, respectively. The TV-L, ME doses of 600, 1200 and 2000 mg/kg were used to treat groups 4, 5, and 6, respectively. Group 7 was not treated with extracts as it served as a control group. The dosage regime of the subjected animals with extracts doses was conducted once per day for consecutively fourteen days. The dose administrations were done at noon and made through oral gavaging followed by clinical signs observations. Toxicity (clinical) signs were recorded at the interval of 10 minutes, 30 minutes, 60 hour, 240 hours, 480 hours and 1440 hours immediately after oral dose administration. The clinical signs of toxicity such as water intake, mortality, food intake, sedation, convulsion and general behaviour to the treated animals were recorded for 28 days. For duplicates data collection, two animals in each group were anaesthetised and sacrificed at the 15th and 29th days, respectively. At first session, two animals were sacrificed in order to see the effects of prolonged administered extracts in fourteen days (day 1-14). At second

session, two animals were sacrificed at 29th day that is 14 days later after animals stopped receiving doses from day 15-28. Animals were anaesthetised with ketamine/xylazine, 20:1 mg/kg intraperitoneal (20:1 mg/kg IP) injection to avoid pain during dissection. After the anaesthesia reached depth, the animals were sacrificed then kidney and liver organs were removed for histopathological examination. These organs were preferred because they are the target organs involved in the detoxification and excretions of the ingested harmful substances, which are more likely to manifest negative effects in the cells of the organs (Bello *et al.*, 2016). Both positive and negative effects were studied and collected as histopathological data.

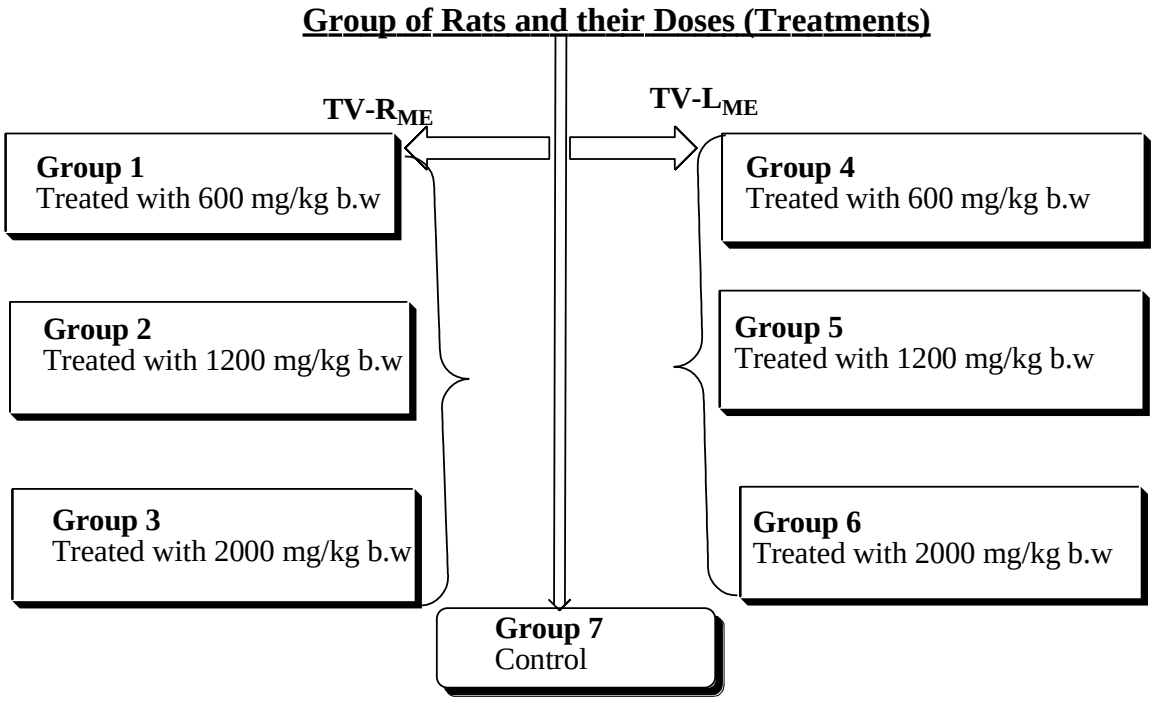


Figure 10: Sketch of animal groups with the respective doses for sub-acute toxicity test

3.6.5 Histopathology

Histopathology involves macro and microscopic examination of tissues and cells to determine the effect of administered extracts (drugs) in animals (Fig. 11). The experiment was conducted at College of Veterinary Medicine, SUA. The liver and kidney were collected from the sacrificed and dissected rats then washed with 10 % neutral buffered formalin (NBF) solution. The macroscopic examination of organs (kidney and liver) was carried out using normal eyes before fixation and staining for microscopic examination. The organs were trimmed and processed for paraffin embedding. Then fixation was carried out whereby about 4 μm thick sections of the embedded were prepared. The tissues were stained with

hematoxylin (H) and eosin (E) to ensure clear visual observation under microscope (Evans & Robinson, 2011). The stained tissues in slides were then analysed with the aid of an optical microscope to observe, if any, signs of cell degenerative changes such as inflammation and necrosis evidence. The optical microscopic analyses captured the images with the microscope Motic B1 series and scanned the images through micro-camera Moticam 480 using the Motic Images Plus 2.0 Mult-Language Application Suite Software. The histopathological analyses of hepatic-renal toxicity effects in animals' organs treated with extracts were established and compared with the control organs of the untreated animals.

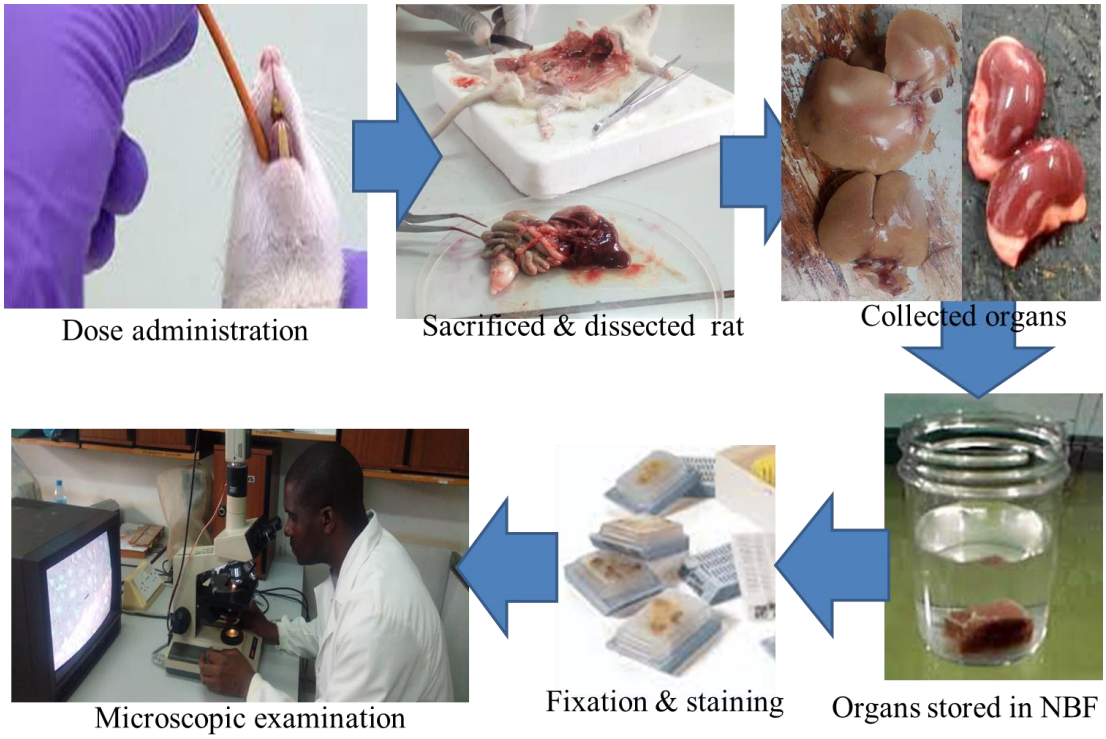


Figure 11: Sketch of steps from dose administrations to the microscopic tissue examination

3.7 Formulation and Evaluation of Herbal Cream for Topical Application

3.7.1 Formulation Procedures

The formulation of herbal cream involved uniform mixing up of the methanolic leaf extracts of *Tephrosia vogelii* with the cream base. The standard techniques with minor modifications were employed and furnished herbal cream formulations that composed of two phases (oil phase and aqueous phase) which were uniformly blended (Ali *et al*., 2013; Banerjee *et al*., 2019; Chen *et al*., 2016; Premkumar *et al*., 2014; Shankar *et al*., 2016). The oil phase with its

soluble components and aqueous phase with its soluble components were mixed (Table 1). Each phase with its components was be mixed and heated independently at temperature of about 70 ^{o}C before mixing up so as to obtain stable mixtures suitable for the storage. The mixture obtained was stirred while hot until cooled to ensure homogeneously composed of the final formulated herbal cream. Three herbal creams ($CBTV_1$, $CBTV_{2,}$ and $CBTV_3$) of ingredient concentrations, 0.05, 0.10 and 0.25 %, respectively were formulated. The MIC of extracts (Table 4.1) used as guide to prepare herbal concentrations. The extracts were used as active ingredients and cream base used as vehicle in this study aspect (Danby *et al*., 2020).

3.7.2 Physicochemical Evaluation

The physicochemical parameters such as colour, pH, homogeneity, washability, solubility and stability were pre-meditated to ensure satisfactory results for the formulated herbal cream. The physicochemical properties were evaluated accordingly using existing techniques as per previous studies (Abdellatif *et al*., 2020; Ajala *et al*., 2016). The stability of formulated drug was assessed at temperature conditions of 4 ^{o}C, 25 ^{o}C and 37 ^{o}C within 6 weeks.

Table 1: Composition for formulation of herbal cream

Oil phase		**Aqueous phase**	
S/N	**components (% w/w)**	**S/N**	**components (% w/w)**
1.	White soft Paraffin – 15.0 %	1.	Glycerine - 15.0 %
2.	Cetostearyl alcohol – 5.0 %	2.	Methyl Paraben – 0.5 %
3.	Stearic acid – 10.0 %	3.	Plant leaf extracts - 0.05, 0.1 & 0.25 % ($CBTV_1$, $CBTV_{2,}$ & $CBTV_3$), respectively
4.	Organic coconut oil – 15.0 %		
5.	Propyl glycol – 5 %	4.	Distilled water - q.s to 100 %

3.7.3 Fungi Strains, Bacteria Strains and Sub-culturing

The bioassay was performed in the Microbiology Laboratory at MUHAS. Three pathogenic strains; *C. albicans* (ATCC 90028), *S. aureus* (ATCC25923), and *E. coli* (ATCC29953) were

subjected in this study. All strain species were generously provided by the School of Pharmacy at Muhimbili University of Health and Allied Sciences. The microbes, fungi and bacteria were sub-cultured onto Mueller Hinton Agar. The MHA (8.0 g) was suspended in 230 mL of distilled water in 500 mL scotch bottle forming the mixture that was heated at 60 ^{o}C to dissolve the agar completely. To ensure sterilization then suspension was autoclaved at 121 ^{o}C for 15 minutes. Then the mixture was left to cool at room temperature followed by inoculation which was conducted onto the cooled growth media. Inoculation of strains was carried out then followed by incubation. The *C. albicans* inoculums were incubated at 37 ^{o}C for 48 hours while bacteria inoculums were incubated at 37 ^{o}C for 24 hours.

3.7.4 *In vitro* Antimicrobial Activity Evaluation of Herbal Cream

The Disc diffusion method (Disc diffusion technique) as described by Bauer and Kirby, (1966) then Herman (2009) and demonstrated by Hudzicki (2009) was employed for the antimicrobial bioassay (Bauer *et al*., 1966; Herman *et al*., 2020; Hudzicki, 2016). Antimicrobial assay was conducted to investigate the effectiveness of methanolic leaf extracts of *Tephrosia vogelii* contained in the cream formulations. Three cream formulations (25 mg $CBTV_1$, 50 mg $CBTV_2$ and 125 mg $CBTV_3$) and cream base (CB) contained in separate vials were dissolved in 5 mL of distilled water containing 5% DMSO, hence constituting concentrations of 5 mg/mL $CBTV_1$, 10 mg/mL $CBTV_2$, and 25 mg/mL $CBTV_3$. Discs of 6 mm in diameter were made from the Whatman's no. 1 (filter paper) using a paper puncher. A set of 80 discs placed into storage petri-dish plates, a Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were autoclaved at 121°C for 15 minutes. Then the sterilized MHA) and SDA base plates were seeded with the bacterial and fungal inoculum, respectively with inoculum size 1×10^{8} CFU/mL for bacteria and 1×10^{7} cell/ mL for yeast. Then 20 µL of each tested cream sample added to sterilized discs, after absorption discs were placed on the seeded agar plates. Each sample was tested in duplicate to minimize results errors. The preparation was incubated at 37 °C for 24 hours to see zones of inhibitions. The zones indicated inhibitory effects on tested samples against pathogens on the cultured plates. The zones of inhibitions were measured with a ruler and compared with the control (commercial pharmaceutical standards) to ascertain the antimicrobial viability. Ciprofloxacin and fluconazole were used as standard drugs against bacteria and fungi, respectively.

3.7.5 *In vivo* Evaluation of Formulated Herbal Cream using Mice

The herbal cream ($CBTV_3$) that indicated best effectiveness in the *in vitro* test was subjected for *in vivo* evaluation using animal model experiments. Twenty mice generously provided from University of Dar es Salaam, Mkwawa University College of Education, Department of Biological Sciences were employed in this *in vivo* evaluation of herbal cream. All mice were housed in five cages (Fig. 12) and left for acclimatization for seven days before starting treatments. During acclimatization period, mice were freely fed food and water every day. After acclimatization portion of the skin of all twenty mice were infected with *Candida albicans*. For convenient, dose regime started three days later from infection day. Animals were humanely treated to observe animal rights and ethics.

During dose regime, food and water were given to all animals while doses (drugs) were administered to mice through skin twice (morning and evening) a day for seven day. The drug was applied topically on the infected part of the skin. Any behaviour changes such as skin irritation immediately after dose administration were also recorded. Group 1 was not treated with any drug and was used as control group. Group 2 was used to evaluate the effect of cream base (drug vehicle) to the skin. Clotrimazole cream as positive control was administered to group 3 while formulated herbal cream ($CBTV_3$) was administered to group 4. The group 5 mice were treated with methanolic leaf extracts for the reason of checking their effectiveness in comparison to formulated herbal cream. After topical application dose regime animals were left for ten days in their respective cages for observation of any infections continuity particularly those treated with herbal cream, methanolic extracts and clotrimazole cream.

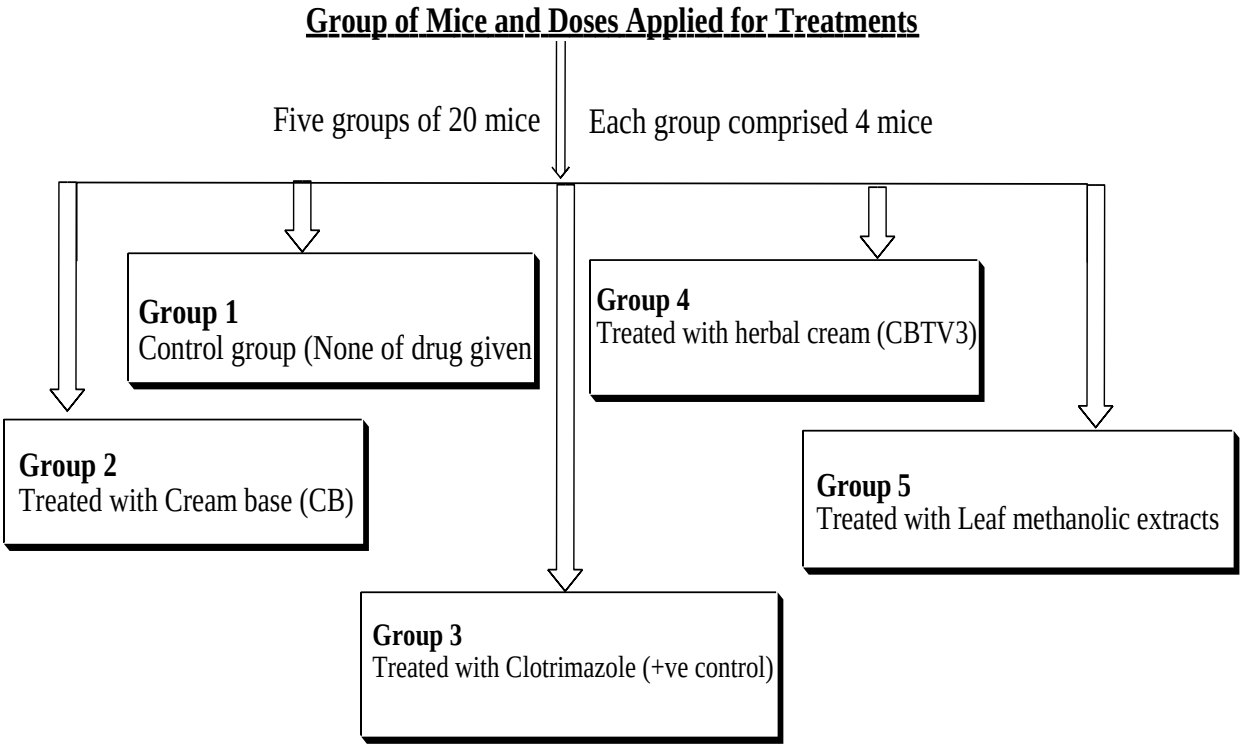


Figure 12: Sketch of animal groups with respective doses for *in vivo* herbal cream evaluation

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Antimicrobial Activities

The biological assays were performed to assess the presence of bioactive compounds in the root and leaf extracts against selected fungi and bacteria strains. The Minimum Inhibitory Concentrations (MICs), which is the key parameter determined through serial dilution method unveiled the extent to which antimicrobial activities of the extracts exerted inhibitory effects on the tested microorganisms. The MICs varied from low to high against tested strains (Table 2 and Fig. 13). The smaller MIC value indicates higher growth inhibitory activity of a given extract against the strains and vice versa (Eloff, 1998). The *n*-hexane and dichloromethane extracts of *Tephrosia vogelii* leaves and roots exhibited low activities against fungal strains because their MICs were higher than 10 mg/mL. These low activities may be attributed to non-polar and less polar compounds present in *n*-hexane and dichloromethane extracts. The activities signpost that the compounds contained in the *n*-hexane and dichloromethane extracts inhibited the growth of the fungi strains more weakly than methanolic extracts.

Notably, methanolic root extracts of *Tephrosia vogelii* exhibited moderate activities at MIC of 5 mg/mL against both *C. albicans* and *C. neoformans*. Methanolic leaf extracts of *Tephrosia vogelii* exhibited a high potency due to its relatively low MIC of 1.25 mg/mL against assayed fungi, *C. albicans* and *C. neoformans.* Henceforth, both assayed fungal strains indicated that they were equally and highly susceptible to methanolic leaf extracts of *Tephrosia vogelii*. In principle, the methanolic extracts contain polar compounds such as phenolics, flavonoids and saponins which explain why the methanolic leaf extracts demonstrated relatively higher bioactivities than other solvent extracts (Mwaura *et al*., 2013; Stevenson *et al*., 2012; Stevenson & Belmain, 2017; Swamy *et al*., 2015). Moreover, it was interesting to note that the MIC of methanolic leaf extracts was twice that of the positive control (fluconazole) suggesting superior inhibitory performance of the methanolic leaf extracts over other extracts on the fungi. The proximity of the MIC indicates that the compound(s) contained in the methanolic leaf extracts have strong inhibitory effects on the fungi strains compared to the rest of the evaluated extracts.

Table 2: Antifungal and antibacterial activities of leaf and root extracts of *Tephrosia vogelii*

Extracts and Positive Control	Minimum Inhibitory Concentration (MIC) in mg/mL for tested species					
	S. aureus	*K. pneumoniae*	*S. typhi*	*E. coli*	*C. albicans*	*C. neoformans*
TV-R, HE	10	>10	>10	>10	>10	>10
TV-L, HE	10	>10	>10	>10	>10	>10
TV-R, DE	5	5	2.5	>10	>10	>10
TV-L, DE	5	5	2.5	>10	>10	>10
TV-R, ME	1.25	2.5	2.5	>10	5	5
TV-L, ME	1.25	5	1.25	2.5	1.25	1.25
TV-R, DE+ME	10	10	5	>10	10	10
TV-L, DE+ME	1.25	5	1.25	5	5	5
Ciprofloxacin / Fluconazole	0.15625	0.3125	0.3125	0.625	0.625	0.625

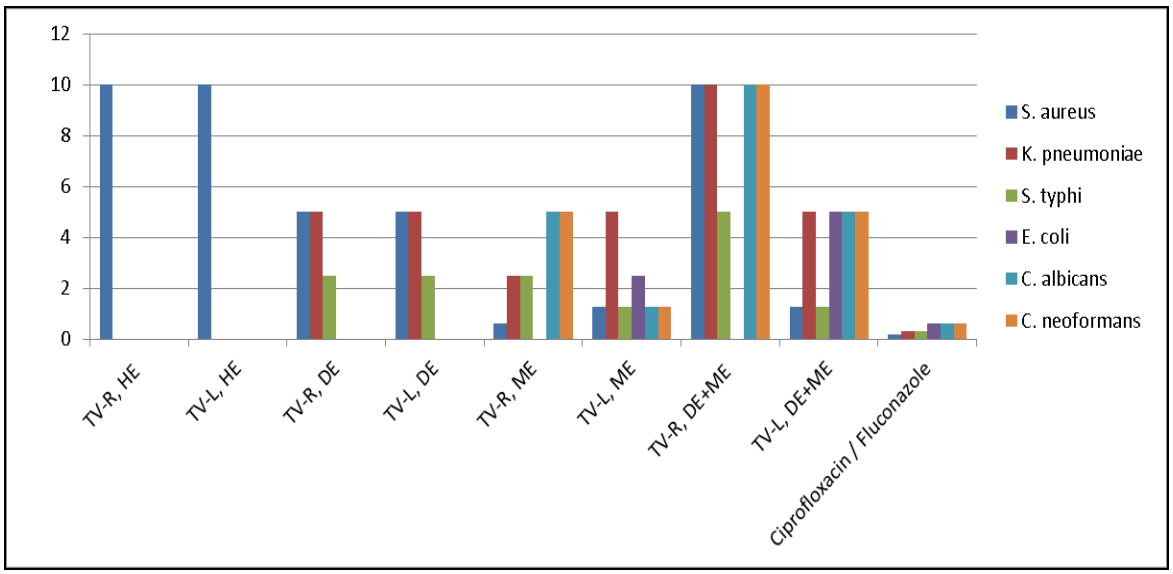


Figure 13: Graph showing MICs (Y-axis) against extracts and positive control (X-axis).

MICs of extracts greater than 10 mg/mL neglected by software programme to draw.

Key: TV-R, HE = *Tephrosia vogelii*, hexane root extract; TV-L, HE = *Tephrosia vogelii*, hexane leaf extract; TV-R, DE = *Tephrosia vogelii*, dichloromethane root extract; TV-R, ME = *Tephrosia vogelii*, methanolic root extract; TV-R, DE+ME = *Tephrosia vogelii* roots, mixed dichloromethane and methanol extracts; TV-L, DE = *Tephrosia vogelii*, dichloromethane leaf extract; TV-L, ME = *Tephrosia vogelii*, methanolic leaf extract; TV-L, DE+ME = *Tephrosia vogelii* leaves, mixed dichloromethane and methanol extracts

Moreover, it was anticipated the blends; dichloromethane with methanolic leaf extracts (TV-L, DE+ME), and root dichloromethane with methanolic extracts (TV-L, DE+ME) in 1:1 ratio could exhibit positive synergistic effects against the tested fungi. Unfortunately, all extracts blends exhibited antagonistic effects. For instance, antagonism was noted when MICs (Table 4.1) of sole methanolic extracts for both *Tephrosia vogelii* roots and leaves because the MIC values of the blends had considerably changed from small values (indicating high antifungal activity) to high values (indicating less antifungal activity). Although the blends showed more antagonistic bioactivity effects on both strains than individual extracts, the potencies/inhibitory effects of the blend extracts from the leaves on these fungal strains were still superior to those from the roots. Apparently, the interactions of compounds in the mixed extracts reduced the inhibition power of the methanolic extracts for virtually more than half against the fungi strains.

Based on study findings, the antifungal activities of methanolic leaf and root extracts of *Tephrosia vogelii* against *C. albicans* and *C. neoformans* concur with other studies of the same plant that exhibited similar activities against bovine dermatophytosis (Makoshi & Arowolo, 2011). Similarly, antifungal activities of methanolic extracts were in good alignment with other antifungal studies against pathogens causing human, animal and plant disease (Inalegwu & Sodipo, 2015; Li *et al*., 2015; Mahomoodally *et al*., 2005; Nneka & Jude, 2012). Thus, this study reports the methanolic leaf extracts and methanolic root extracts of *Tephrosia vogelii* that they are potential sources of antifungal agents against the opportunistic *Candida albicans* and *Cryptococcus neoformans* fungal strains. Nevertheless, these findings shed light to conduct toxicity, clinical trials, and structure characterization of the potential antifungal compounds from the methanolic extracts of *Tephrosia vogelii.*

As for bioassays against the bacteria strains, the leaf and root hexane extracts of *Tephrosia vogelii* exhibited the lowest activities on *Klebsiella pneumoniae, Salmonella typhi* and *Escherichia coli* because of high MICs (>10 mg/mL). Inhibitory effects of root hexane extracts on *Staphylococcus aureus* (Gram +ve bacteria) were evident at MIC of 10 mg/mL compared to Gram –ve bacteria. Presumably, unlike the Gram negative bacteria, lack of protective cell walls in *Staphylococcus aureus* might have allowed easy penetration of the bioactive agents of the extract into the bacterial cells, thereby increasing their susceptibility to root hexane extracts compared to their counterparts (Mahomoodally *et al*., 2005). The antibacterial activities of both root and leaf dichloromethane extracts of *Tephrosia vogelii*

were moderate ranging from MIC 2.5 mg/mL against *S. typhi* to MIC 5 mg/mL against both *S. aureus* and *K. pneumoniae*. However, the same extracts exhibited the lowest antibacterial activities (MIC > 10 mg/mL) against *E. coli.* Antibacterial activity with respect to *E. coli* suggested that the stains were resistant to both dichloromethane root and leaf extracts. Perhaps, the compounds contained in the extracts are not chemically capable of disrupting the cell wall and affects inner cell organelles such as nucleus of *E. coli*. Of course, this might assist to explain why the same compounds in the extracts could express considerable activities against *K. pneumoniae* and *S. typhi*.

Of particular interest, to find out the synergistic effects of the blends against bacteria strains, the bioassays using mixtures of extracts (TV-L, DE+ME and TV-R, DE+ME) were performed. As a result, the mixture of root dichloromethane and methanolic extracts exhibited low antibacterial activities as compared to individual extracts. This could mean that the chemical interactions of compounds in the mixed extracts reduced efficacy against the bacterial strains. The activities of the mixed leaf dichloromethane and methanolic extracts demonstrated the performance in much the same way as individual methanolic extracts. Therefore, the blends of leaf extracts did not exhibit any positive synergistic effects on the bacterial growth instead antagonistic effects were more pronounced. Thus, it suggests that the interacting compounds in the mixed extracts could be suppressing antibacterial activities against the tested bacteria strains.

Besides, methanolic root extracts of *Tephrosia vogelii* exhibited highest activity with MIC 1.25 mg/mL against Gram-positive bacteria, *S. aureus* followed with MIC 2.5 mg/mL for both *S. typhi* and *K. pneumoniae*. The methanolic root extracts exhibited low activity against *Escherichia coli* at MIC greater than 10 mg/mL. The methanolic leaf extracts of *Tephrosia vogelii* exhibited high antibacterial activities at MIC 1.25 mg/mL against *S. aureus* and *S. typhi*. It exhibited moderate at MIC of 2.5 mg/mL and 5 mg/mL against *E. coli* and *K. pneumoniae*, respectively. This observation is indicative of the presence of compound(s) in leaf extracts which inhibited *S. aureus, S. typhi* and *E. coli* but had less inhibitory effects on *K. pneumoniae.* Superbly, *S. aureus* and *S. typhi* were equally vulnerable to the methanolic leaf extracts of *Tephrosia vogelii*. The *E. coli* showed resistance to all extracts as they had high MIC greater than 10 mg/mL except methanolic leaf extracts to which it was susceptible at MIC of 2.5 mg/mL. Higher antibacterial activities of methanolic leaf and root extracts of *Tephrosia vogelii* are attributed to polar compounds capable of suppressing the bacterial

growth (Inalegwu & Sodipo, 2015; Li *et al*., 2015; Mahomoodally *et al*., 2005; Nneka & Jude, 2012). The antibacterial activities of ethanol-aqua extracts of *Tephrosia vogelii* barks reported by Swamy and co-workers are in good agreement with our findings that they are attributable to prospectively polar compounds as in methanolic leaf and root extracts (Swamy *et al*., 2015). Thus, the antibacterial activities obtained suggest that methanolic leaf and root extracts of *Tephrosia vogelii* are potential sources of antibacterial agents against the pathogens: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Salmonella typhi*. Conversely, antimicrobial activities of methanolic extracts prompted further studies of toxicity evaluation and phytochemical exploration.

4.2 Phytochemical Investigation

The versality of the medicinal plants against natural enemies is dependent on the secondary metabolites they synthesize. Of course, medicinal plants synthesize secondary metabolites of different functional groups that spur several chemical properties they use to their own advantage against natural enemies in their surroundings in order to ensure their survival. The enemies could range from the herbivores, and insects that feed on the medicinal plants to the microorganisms which cause diseases to the plants. Recently, researchers have a growing interest in investigating and characterizing potential secondary metabolites from medicinal plants that could be used to develop natural chemistry products to treat human ailments. The primary advantage of these phytocompounds is mainly the fight against diseases such as cancer, diabetes, bacterial, and fungal infections inflicting human and animals (Dewick, 2009; Gurib-Fakim, 2006). Mechanisms of action of such phytochemicals from medicinal plants vary due to their diverse chemical functionalities (Bello *et al*., 2016; Dewick, 2009). In that regard, it is important to investigate and analyze phytocomponents of medicinal plants in order to discern the chemical functionalities as well as the possible mode of action of bioactive compounds. Following antimicrobial activities of extracts worked it was worth identifying phytochemicals found in methanolic leaf and root extracts (Mlozi *et al*., 2020).

Consistently, this study attested the presence of the tannins, steroids, terpenes, flavonoids and glycosides from methanolic leaf and root extracts of *Tephrosia vogelii* (Table 3). Also, this study on phytochemical screening revealed the absence of alkaloids and anthraquinones suggesting that these medicinal properties of *Tephrosia vogelii* found particularly in Tanzania may be branded mainly by tannins, steroids, terpenes, flavonoids and glycosides. On the other hand, the chemical nature of the phytochemicals analysed from this medicinal plant

clearly coincides with the antimicrobial activities and ectoparasitic activities reported in the previous studies (Alamgir, 2017; Arif *et al*., 2011; Badri *et al*., 2017; Kenechukwu *et al*., 2012; Irawan *et al*., 2018; Makoshi & Arowolo, 2011; Mbunde *et al*., 2016; Mlozi *et al*., 2020; Murtaza *et al*., 2015; Nneka & Jude, 2012; Okpogba *et al*., 2019; Omodamiro & Amechi, 2013; Pietta, 2000; Priya *et al*., 2014; Ramawat & Mérillon, 2013; Sahu *et al*., 2014; Samuel *et al*., 2019; Sasidharan *et al*., 2011; Singh *et al*., 2017; Swamy *et al*., 2015; Trakranrungsie, 2011).

Table 3: Qualitative phytochemical test of methanolic leaf and root extracts

Nature of tested phytochemicals	**Results of phytochemical test of extracts screened**	
	TV-R, ME	**TV-L, ME**
Tannins	+	+
Terpenes	+	+
Saponins	+	+
Flavonoids	+	+
Alkaloids	-	-
Glycosides	+	+
Steroids	+	+
Anthraquinones	-	-

Key: (+) = present; (-) = absent

Nevertheless, the GC-MS spectroscopic technique was used for isolation and identification of the phytochemical compounds in the same way as reported in previously studies (Das *et al*., 2014; Harborne, 1973; Sweeney, 2014; Ukwubile *et al*., 2019; Zahra *et al*., 2019). The technique was particularly useful to identify the phytochemical compounds from the bioactive compounds of methanolic extracts of *Tephrosia vogelii* by matching the data of GC-MS chromatograms with the available NIST and Wiley library databases. As a result, ten (**1-10**) phytochemical compounds were identified from the methanolic root and leaf extracts of *Tephrosia vogelii* (Fig. 14) using the GC-MS exploration in reference to molecular weights (*m/z*). Among ten phytocompounds, eight compounds were common in both roots and leaves (Tables 4 and 5). The two flavonoids (**4** and **10),** were ascertained from the methanolic leaf

extracts but could not be found in the methanolic root extracts. Five of them, **1**, **2**, **3**, **7** and **8** are reported for the first in this species.

Table 4: Ten compounds (**1-10**) identified from methanolic leaf extracts of *Tephrosia vogelii*

Cpd	Retention Time	m/z	Formula	Name	Nature of Compounds
1.	17.192	329.21	$C_{18}H_{16}O_6$	Demethylmunduserone	Isoflavonoids
2.	17.676	357.20	$C_{19}H_{18}O_7$	Sumatrol	Isoflavonoids
3.	17.805	270.08	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Fatty acids
4.	17.885	270.28	$C_{16}H_{14}O_4$	5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one	Flavonoid
5.	18.754	254.23	$C_{15}H_{26}O_3$	(4R,5R,8S,8aS)-5-isopropyl-8-methyl-3-methylene-decahydroazulene-4,5,8-triol	Terpenoids (sesquiterpenes)
6.	19.298	394.32	$C_{23}H_{22}O_6$	Deguelin	Isoflavonoids
7.	19.561	256.26	$C_{16}H_{32}O_2$	Hexadecanoic acid	Fatty acids
8.	19.887	343.22	$C_{19}H_{18}O_6$	Munduserone	Isoflavonoids
9.	20.58	410.41	$C_{23}H_{22}O_7$	Tephrosin	Isoflavonoids
10.	20.621	338.65	$C_{21}H_{22}O4$	7-hydroxy-5-methoxy-8-(3-methylbut-2-enyl)-2-phenyl-2,3-dihydrochromen-4-one	Flavonoid aglycones

Molecular weights and chemical structures of all identified compounds were established based on mass spectrometry (mass spectra of *m/z* in the attached appendix 4). The sesquiterpenes (**5)** and rotenoids (**6** and **9**) identified are the phytochemicals that have also been reported from leaves of *Tephrosia vogelii* in the previous studies (Stevenson *et al*.,

2012; Wei *et al*., 2009). Similarly, the flavonoids, **4** and **10**, were reported from the same species in Malawi by Stevenson and co-workers 2012 (Stevenson *et al*., 2012). Thus, the phytocompounds **4**, **5**, **6**, **9** and **10** are not new from *Tephrosia vogelii*. Remarkably, however, it is the first time to report compounds **1, 2** and **8** in the *Tephrosia vogelii* though such rotenoids have previously been reported from *Tephrosia pentaphylla* and *Tephrosia fulvinervis*. It is noted that compound **2** was reported from leaves of *Tephrosia pentaphylla* while compounds **1** and **8** were reported from roots of *Tephrosia fulvinervis* (Dagne *et al*., 1989). Certainly, similar phytocompounds had never been reported in *Tephrosia vogelii.* Interestingly, the three species shares the genus *Tephrosia* suggesting that they have similar capabilities of synthesizing analogous secondary metabolites particularly **1**, **2** and **8**.

Table 5: Eight compounds (**1-3, 5-9**) from methanolic root extracts of *Tephrosia vogelii*

Cpd	Retention Time	m/z	Formula	Name	Nature of Compounds
1.	17.192	329.21	$C_{18}H_{16}O_6$	Demethylmunduserone	Isoflavonoids
2.	17.676	357.20	$C_{19}H_{18}O_7$	Sumatrol	Isoflavonoids
3.	17.805	270.08	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester	Fatty acids
5.	18.754	254.23	$C_{15}H_{26}O_3$	(4R,5R,8S,8aS)-5-isopropyl-8-methyl-3-methylene-decahydroazulene-4,5,8-triol	Terpenoids (sesquiterpenes)
6.	19.298	394.32	$C_{23}H_{22}O_6$	Deguelin	Isoflavonoids
7.	19.561	256.26	$C_{16}H_{32}O_2$	Hexadecanoic acid	Fatty acids
8.	19.887	343.22	$C_{19}H_{18}O_6$	Munduserone	Isoflavonoids
9.	20.58	410.41	$C_{23}H_{22}O_7$	Tephrosin	Isoflavonoids

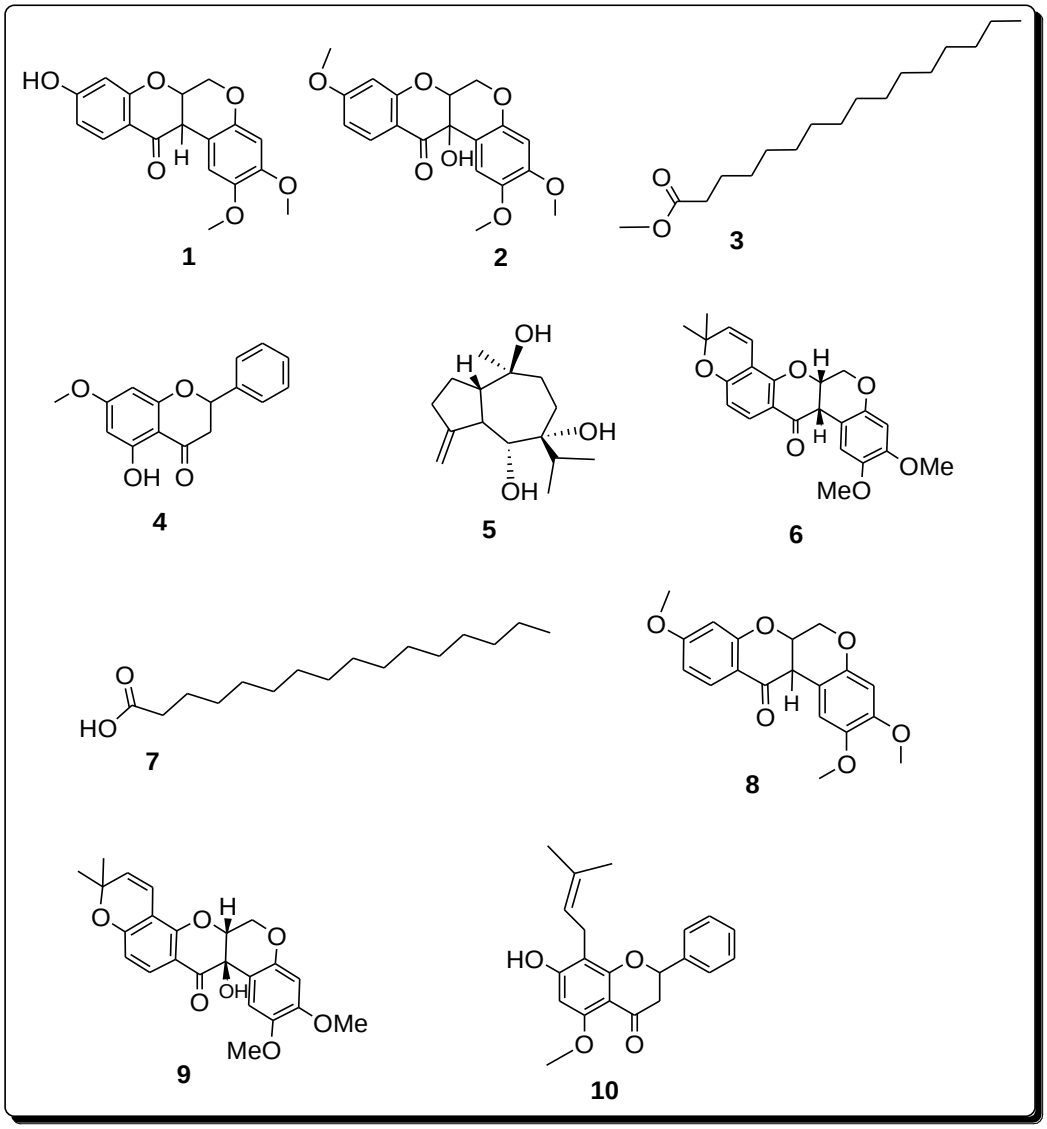


Figure 14: Shows chemical structures of the identified compounds of *Tephrosia vogelii*

Furthermore, this is the first time to report the fatty acids, **3** and **7**, from both the leaf and roots of *Tephrosia vogelii* although they have previously been reported from other plant species such as *Archidendron bubalinum* (Irawan *et al*., 2018). Therefore, the GC-MS guided analysis of *Tephrosia vogelii* methanolic extracts in comparison with the previous studies has showed to us that the genus *Tephrosia* is one of the renowned genera which are a potential natural resource of therapeutic bioactive compounds. It consists of species characterized by richness in rotenoids and flavonoids as well as other classes such as terpenoids which are responsible for its medicinal nature and value against various diseases (Touqeer *et al*., 2013).

On the medicinal value perspective, the identified fatty acids (**3** and **7)** reported to have therapeutic roles ranging from antibacterial, antifungal, anti-inflammatory to antioxidant activities (Aparna *et al*., 2012; Cartron *et al*., 2014; Chandrasekaran *et al*., 2008, 2011;

Kabara *et al*., 1972). Basically, the terpenoids, with respect to sesquiterpenes (**5)** in this study are known to greatly contribute to the medicinal therapeutic values of the medicinal plant which include: anti-hyperglycemic activity, anti-inflammatory activity, anti-parasitic activity, enhancer of skin permeation for many drugs across cell membrane, anti-viral activity, anticancer activity and antimicrobial activities (Ramawat & Mérillon, 2013). Moreover, rotenoids are mostly reported phytochemicals that are used for management of larvae, pests and insects (Dzenda *et al*., 2008; Inalegwu & Sodipo, 2015; Kalume *et al*., 2012; Kidukuli *et al*., 2015; Li *et al*., 2015; Samuel *et al*., 2019; Touqeer *et al*., 2013). Additionally, they have been reported to exhibit biological activities that have paved the way to find the remedy of infectious and non-infectious diseases (Inalegwu & Sodipo, 2015; Makoshi & Arowolo, 2011; Samuel *et al*., 2019; Swamy *et al*., 2015).

The flavonoids (**4** and **10**) are even important compounds due to their broad biological potencies on anticancer activities, antioxidants, antimicrobial activities and activating neural-brain (Atanasov *et al*., 2015; Dewick, 2009; Maheswari *et al*., 2016; Marinova *et al*., 2005; Pietta, 2000; Prabha *et al*., 2014; Slimestad & Verheul, 2009; Yao *et al*., 2004). The tannins, steroids and saponins are similarly important phytocompounds in the therapeutic regime. The tannins have been reported to exhibit neuronal activity, anticancer activity, antimicrobial activities and treatment of constipation (Hussain *et al*., 2019; Singh & Kumar, 2019; Hwang, 2018). Apart from antimicrobial activity, saponins have shown to exhibit hepatopathy activity in assisting the body cell to recover from injuries (Patel *et al*., 2013).

These pieces of evidence generated from our GC-MS analysis and literature suggest that among other, the presence of identified flavonoids, sesquiterpenes and fatty acids contributes largely to the antimicrobial activities (Mlozi *et al*., 2020). Moreover, these phytocompounds sustenance reported ethnopharmacological possessions of *Tephrosia vogelii*. Thus, presence of these important potential phytochemical compounds even prompted further step of drug formulation or development from this medicinal plant.

4.3 Toxicity Evaluation

Medicinal plants are good candidates for the health care system as herbal medicines against diseases perpetrating human beings and animals' husbandry. Concurrently, their worth sources are attested beyond doubts in recent years whereby many researchers have paid more attention to the exploitation of herbal medicines for development of antimicrobial products

(Amy *et al*., 2000; Ekor, 2014). However, little is done to carry out toxicity evaluation of such herbal medicines. The toxicity evaluation of herbal medicines is necessary because evidence has shown that some herbal bioactive agents have negative effects (Bello *et al*., 2016). It is scientific procedures to undertake toxicity assesement of chemical substances to check their safety before approval for application to the global users for healthy benefity of human and animals (Worth, 2018).

Accordingly, this study determined the toxicity of methanolic root and leaf extracts of *Tephrosia vogelii* regarding the previous reported ethnopharmacological potentiality with the focus of drug formulation. Explicitly, the lethality (LD_{50}) and sub-acute toxicity of methanolic leaf and root extracts of *Tephrosia vogelii* were evaluated using the animal model. The results intended to establish a toxicity profile that would serve as a guide and/or precaution to usage of *Tephrosia vogelii* as the medicinal plant and drug development for curing various human diseases. The clinical signs are presented in Tables 4.5 and 4.6 while the histopathological examinations are presented in Figures 15, 16, and 17. On the other hand, the phytochemical screened and determined phytochemical compounds present in the methanolic root and leaf extracts were responsively associated with the toxicity manifestation in the subjected rats.

As for LD_{50} assessment; the methanolic root and leaf extracts of *Tephrosia vogelii* administered at the doses of 600, 1200, 2000, and 5000 mg/kg did not exhibit any mortality in the rats within 72 hours. However, sedation was observed as a clinical toxicity manifestation for the animals administered with both root extracts and leaf extracts at 5000 mg/kg body weight (Table 6). The root and leaf extract dose at 5000 mg/kg caused a sedative sign for two out of three treated rats, and the experiential signs witnessed just half an hour after administration. The clinical sign disappeared with two hours. Sedation suggested the presence of toxicants in the extracts such as rotenoids which are commonly responsible for the bioactivities reported in the same plant species (Caboni *et al*., 2004; Ibrahim *et al*., 2000; Ling, 2003). The rotenoids especially rotenone and tephrosin are flavonoids (Isoflavonoids) ever been reported to exhibit poisonous effects on insects, fishes, and ectoparasite (Caboni *et al*., 2004). Therefore, probably the observed sedation signs could be attributable by the flavonoids whose presence in the *Tephrosia vogelii* extracts is more likely.

On the other hand, the lethal dose could not be established because doses administered did not cause any death for rats exposed to the doses. Apparently, these results give the

impression that the lethal doses for both extract types must be higher than 5000 mg/kg body weight, which is the highest reference dose (Caboni *et al*., 2004). In this regard, extracts may be considered safe up to the tested levels of 5000 mg/kg body weight (Caboni *et al*., 2004). Since the LD_{50} test of methanolic root and leaf extracts could not exhibit dreadful outcomes from all doses subjected, then it can be reasoned that compounds in the extracts probably do not express any deleterious effects on the rats at single doses and below 5000 mg/kg body weight.

Table 6: Assessments during lethality (LD_{50}) test, recorded observations for 1 – 72 hours

Parameters for Assessment	**Dose (mg/kg body weight)**							
	TV-R, ME 600	**TV-R, ME 1200**	**TV-R, ME 2000**	**TV-R, ME 5000**	**TV-L, ME 600**	**TV-L, ME 1200**	**TV-L, ME 2000**	**TV-L, ME 5000**
Feeding	N	N	N	N	N	N	N	N
Fur condition	N	N	N	N	N	N	N	N
Eye colour	N	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N	N
Locomotion	N	N	N	N	N	N	N	N
Sedation	N	N	N	Ab	N	N	N	Ab
Survive or Death	S	S	S	S	S	S	S	S

Key: "Normal (N) or Abnormal (Ab)"; "Survive (S) or Death (D)"

Although the physical appearances of rats suggested that the extracts doses were not lethal, histopathological examinations were vital to check for any predicaments that could be occurring at a cellular level. To achieve this, a sub-acute toxicity evaluation experiment was conducted. The clinical signs in subjected animals following sub-acute toxicity assessments were immediately recorded after oral dose administration (Table 7). The signs were important indicators to explain the physical effects of doses on animals. Interestingly, even the sub-acute toxicity assessments could not exert any physical abnormality among set clinical signs. This means the subjected animals did not indicate any toxicity symptoms for doses below 2000 mg/kg body weight.

Table 7: Assessments of Sub-acute toxicity study, recorded observations from day 1-28

Parameters for Assessment	Dose (mg/kg)						
	TV-R, ME 600	TV-R, ME 1200	TV-R, ME 2000	TV-L, ME 600	TV-L, ME 1200	TV-L, ME 2000	Control group
Feed intake	N	N	N	N	N	N	N
Water intake	N	N	N	N	N	N	N
Fur condition	N	N	N	N	N	N	N
Eye colour	N	N	N	N	N	N	N
Convulsion	N	N	N	N	N	N	N
Locomotion	N	N	N	N	N	N	N
Sedation	N	N	N	N	N	N	N
Aggressiveness	N	N	N	N	N	N	N
Behaviour	N	N	N	N	N	N	N
Survive or Death	S	S	S	S	S	S	S

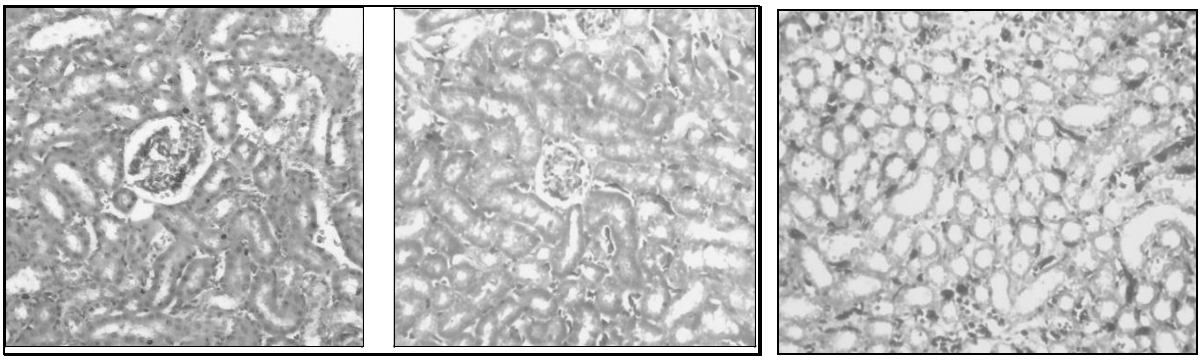
Key: "Normal (N) or Abnormal (Ab)"; "Survive (S) or Death (D)"

The histopathological examination of hepatic-renal tissues or cell architectures after repetitive dosage regime of *Tephrosia vogelii* in rats was conducted to predict sub-acute toxicity effects on humans (Olson *et al*., 2000). The treated rats' liver and kidney organs were macroscopically observed normal as compared to the control animal group. Consistently, the histopathological examination of the kidneys treated with both methanolic leaf and root extracts did not exhibit any histopathological degeneration of tissue or cells because there was no deleterious alteration of the cellular machinery as compared to the control (Fig. 15). Therefore, the tissues and cells exposed to the doses of leaf and root extracts of *Tephrosia vogelii* administered remained consistently unaffected across the experiments which would be interpreted that the doses caused no deleterious effects on the kidneys; and these results are congruent with other studies (Kilonzo *et al*., 2016). Similarly, the histopathological examination of the liver treated with methanolic leaf extracts doses of 600, 1200 and 2000 mg/kg body weight of *Tephrosia vogelii* for all assessed animals revealed that the tissues and

cells were normal (Fig. 16-B & 17-B), because were not different from the control group (Fig. 16-A & 17-A). Thus, the methanolic leaf extracts doses did not cause harm to both subjected kidney and liver organs. Correspondingly, the methanolic root extracts doses of 600 and 1200 mg/kg body weight of *Tephrosia vogelii* revealed normal liver cells from the treated animals as compared to the control animal group (Fig. 16-C & 17-C).

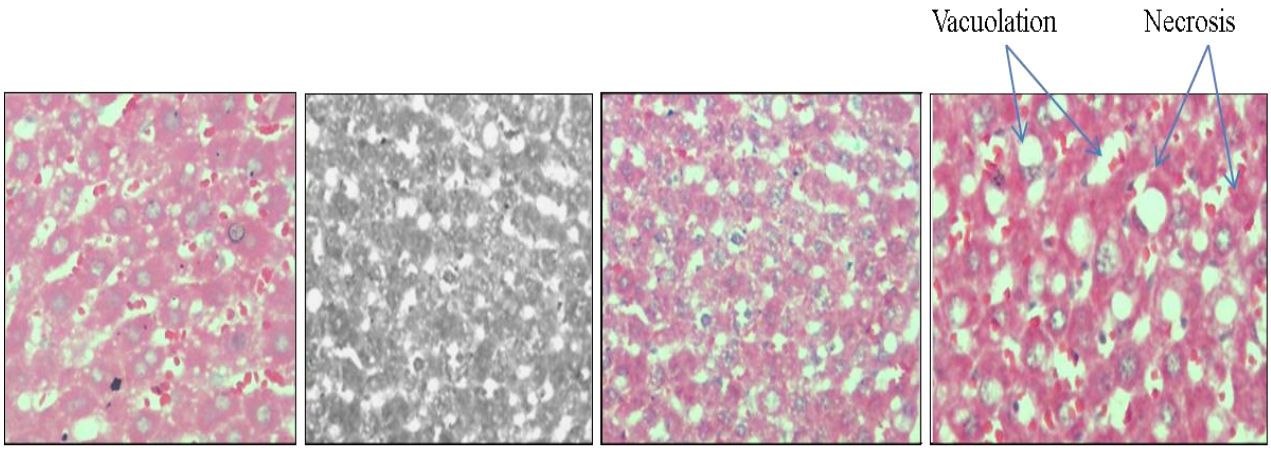
On the contrary, the dose of 2000 mg/kg body weight of methanolic root extract caused hepatic vacuolation (inflammations) and hepatic necrosis on day 14 (Fig. 16-D). This adverse phenomenon could be attributed to a large quantity (content) of the poisoning metabolites contained in the 2000 mg/kg of methanolic root extracts. Hence suggesting that compound-cell interactions were magnified at the high dose administered for a prolonged time (Shorinwa & Monsi, 2020). Thus, histopathological findings from the liver signposted that administered lower doses (600 and 1200 mg/kg) of methanolic root extracts of *Tephrosia vogelii* were not hepatotoxic as compared to 2000 mg/kg body weight which was inflammatory and necrotic to the liver after dose ingestion in 14 days. Interestingly, extending the duration without administration of the same dose to another two weeks (between 15-28 days), resulted in the disappearance of inflammations and necrosis in the hepatocytes. Therefore, there was a complete cellular recovery on the 28^{th} day of the histopathological assays.

Typically, the cellular recovery suggested that the adverse effects of the bioactive compounds at 2000 mg/kg body weight dose on the hepatocytes were temporary (Fig. 17-C). This suggests further that the toxicity is influenced by high concentrations of the compound(s) whose toxicity fades as time increases (Kifayatullah *et al*., 2015). Perhaps, the toxicity disappearance of the compound(s) may be due to the cell self-protection mechanism, which might be increasing over time. Despite the ability of the hepatocytes to recover after two weeks of the dose administration, still, both observed inflammations and necrosis within two weeks should serve as a reminder that the 2000 mg/kg body weight dose and above may not be safe if administered for long term dose regime.



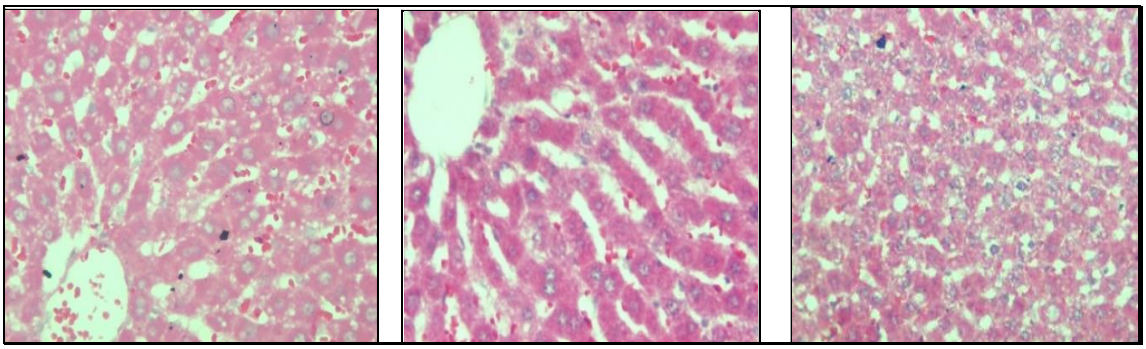
(A) Normal (B) Kidney vs TV-R, ME (C) Kidney vs TV-L, ME

Figure 15: Histopathological examination of kidney section parts (H & E stained under 10x magnification power) where the slide; (A) represent control (normal) (B) represent kidney tissues treated with TV-R, ME at doses of 600, 1200 and 2000 mg/kg (C) represent kidney tissues treated with TV-L, ME at doses of 600, 1200 and 2000 mg/kg body weight



(A) Normal (B) Liver vs TV-L, ME (C) Liver vs TV-R, ME (1) (D) Liver vs TV-R, ME (2)

Figure 16: Histopathological slides of Liver sections at day 14 (H & E stained under 20x magnification power) where the slide (A) represent control (normal) (B) represent liver tissues treated with TV-L, ME at doses of 600, 1200 and 2000 mg/kg (C) represent liver tissues treated with TV-R, ME at doses of 600 and 1200 mg/kg) (D) represent liver treated with TV-R, ME at doses of 2000 mg/kg body weight.



(A) Normal (B) Liver vs TV-L, ME (C) Liver vs TV-R, ME

Figure 17: Histopathological examination of liver section at day 28, (H&E stained under 20x magnification power) where the slide (A) represent Liver tissues of control (B) Liver tissues treated with TV-L, ME of doses 600, 1200 and 2000 mg/kg (C) Liver treated with TV-R, ME of doses 600, 1200 and 2000 mg/kg body weight.

The toxicity of a substance in human or animal is measured in terms of the extent to which that substance causes harm in the cells or tissues or organ. Toxins can be taken through ingestion or created by body physiological reactions (Olaniyan *et al*., 2016). Regarding their roles, the liver and the kidney are vital organs used as toxicity indices for evaluating the toxicity of pharmaceutical drugs and plant extracts (Joseph *et al*., 2015; Olaniyan *et al*., 2016). For instance, since the liver and the kidney are detoxifying organs, they are the first targets of toxicants and their adverse effects such as degenerations and alterations (Bello *et al*., 2016). Degenerations and alterations of tissue morphology such as hepatic vacuolation, necrosis, and hyperplasia are common evidence for the presence of the toxic substances in these detoxifying organs and they are responsible for liver and kidney damage or impairment (Emerole *et al*., 1981). Therefore, toxicity evaluation conducted in the laboratory rats stand as a guide on the impacts to human using the plants though might be slight difference.

In this study, observed hepatic necrosis in the liver was indicative of cell deaths accompanied by the presence of Kupffer cells (Emerole *et al*., 1981; Maregesi *et al*., 2016). Notably, several Kupffer cells (hepatic macrophages) emerged on the lining of the walls of the sinusoids of the liver treated with methanolic root extracts at the dose of 2000 mg/kg at day 14. Probably, these Kupffer cells were playing a protective role of hepatocytes through secretion of immune-regulatory mediators as well as orchestration of a cooperative system in the liver (Emerole *et al*., 1981). Apparently, the presence of numerous hepatic macrophages promotes the protection of the hepatocytes from toxic extracts and can be used

as a toxicity alarm of a given dose; it promotes the removal of senescent red blood cells and debris from portal blood flow (Bello *et al*., 2016). The resultant hepatic vacuolation, characterized by cellular inflammations and nucleus shrinks, occurs as a cell self-protection mechanism from poisons and toxicants (Emerole *et al*., 1981).

Therefore, hepatic necrosis and vacuolation indicated that the hepatocytes were affected by chemical compound(s) contained in the root extracts at the dose of 2000 mg/kg of *Tephrosia vogelii* administered to rats for 14 days although time prolongation without the same dose to 28 days led to recovery and disappearance of hepatic necrosis and vacuolation. The quick tissue regeneration and disappearance of hepatic necrosis and vacuolation suggested that the toxicants were either degraded or detoxified by the cell self-protection mechanism over time. Consequently, the dose did not demonstrate lethality. Although there was no lethality, the hepatic necrosis and vacuolation manifested by the dose of 2000 mg/kg of the methanolic root extracts warn that this dose or above may not be safe. The secondary metabolites such as saponins, and terpenes might contribute toxicity of such hepatic inflammations and necrosis (Patel *et al*., 2013). The toxicity manifestation of methanolic root extracts provides another avenue for anticancer studies of the same extracts. Accordingly, the methanolic leaf extracts were considered safe for antifungal agent formulation.

4.4 Formulation and Evaluation of Herbal Cream for Topical Application

The herbal creams play a great role in the treatment of infected human topical skins (Moldovan *et al*., 2017; Premkumar *et al*., 2014; Shankar *et al*., 2016). Of course, skin care is vital due to its role of protecting the body from external environment messes especially microbial infections (Shankar *et al*., 2016). Skin care and treatment is indispensable against microbial infections especially fungal skin infections to avoid serious damages such as skin cancers and deaths (Academy & Dermatology, 2017; Premkumar *et al*., 2014; WHO, 2020). It is in this vein that herbal cream formulations $CBTV_1$, $CBTV_2$ and $CBTV_3$ were prepared (Fig. 18) as a trajectory to fight human microbial skin infections. In this study, three different concentrations of herbal creams were formulated and evaluated for their physicochemical properties (Table 9) and effectiveness against three pathogens: *C. albicans, E. coli* and *S. aureus* (Table 8). The *C. albicans* and *S. aureus* were subjected as representative of pathogenic microbes causing topical skin infections because they are the most notorious and common skin-infection pathogens (Cartron *et al*., 2014; Schlecht *et al*., 2015). The effectiveness of bioactive secondary metabolites contents contained in the cream formulation

was measured in terms of their ability to inhibit these pathogens via the antimicrobial activities measured from the zones of inhibitions. The zones of inhibition following *in vitro* antimicrobial activities of the herbal creams were found to range from 6.0 to 12.5 mm diameter (Table 8). Correspondingly, these zones of inhibition signposted that effectiveness of formulated herbal cream varied from low to high. These variations could be attributable to the diversity and varied concentrations of the bioactive contents present in the creams.

In fact, the degrees of performance of the herbal creams formulated varied from species to species between all assayed microbes. It is important to note that the cream base (drug vehicle) was used as the control in all bioassays of the creams against the selected pathogens. The control cream had no inhibitory effects on pathogens because it did not affect their growth while the standard drugs demonstrated highest zones of inhibitions. The $CBTV_1$ exhibited lowest antimicrobial activity because it showed the smallest zones of inhibitions of all across the tested microbes. Consistently, $CBTV_2$ exhibited moderate effectiveness as compared to $CBTV_1$ and $CBTV_{3.}$ In the same way, $CBTV_3$ exhibited highest antimicrobial activity against assayed selected pathogens particularly *S. aureus* and *C. albicans*. The *E. coli* demonstrated some resistance to both herbal cream formulations.

Nevertheless, the antimicrobial activity patterns of herbal creams revealed that the drug (active ingredients) might have both microcidal and microstatic effects (Kumar & Jha, 2017). On the other hand, Microstatic (bacteriostatic and fungistatic) effects were greatly observed regarding zones of inhibition against the tested pathogens. The microstatic effect of herbal creams was manifested by the fact that the microorganisms continued to grow even after the inhibition zones were formed (Eloff, 1998). However, the study revealed a very pronounced and promising performance of herbal creams with highest concentration, $CBTV_3$. This performance is probably due to the presence of antimicrobial agents such as terpenes, fatty acids, saponins and flavonoids characterized by active moiety and lipophilic features contained in $CBTV_3$ in high concentrations (Abdel-Naime *et al*., 2019; Arif *et al*., 2011; Elibol *et al*., 2011; Gemeda *et al*., 2018; Gendimenico *et al*., 2015; McNulty, 2017; Pietta, 2000; Ramawat & Mérillon, 2013; Shah *et al*., 1992; Soković *et al*., 2013; Tiwari *et al*., 2011).

With regard to the MICs as a reference of formulation concentrations (Mlozi *et al*., 2020), we think that there are both positive and negative effects the plant creams have exerted on the microbes but the determinants of such effects are still unclear and they are subject to

further studies. However, from the *in vitro* work it is very clear that sample $CBTV_3$ of herbal cream formulation had shown that the cream could be an ideal antimicrobial candidate because it exhibited the highest antimicrobial activity of all creams against microbes known to cause human skin infections. Henceforth, we expect that $CBTV_3$ might be an effective cream formulation for treating topical skin disease pathology. However, *in vivo* evidence using animal model and subsequently carrying out clinical trials in order to check for its effectiveness to treat human skin infections is needed..

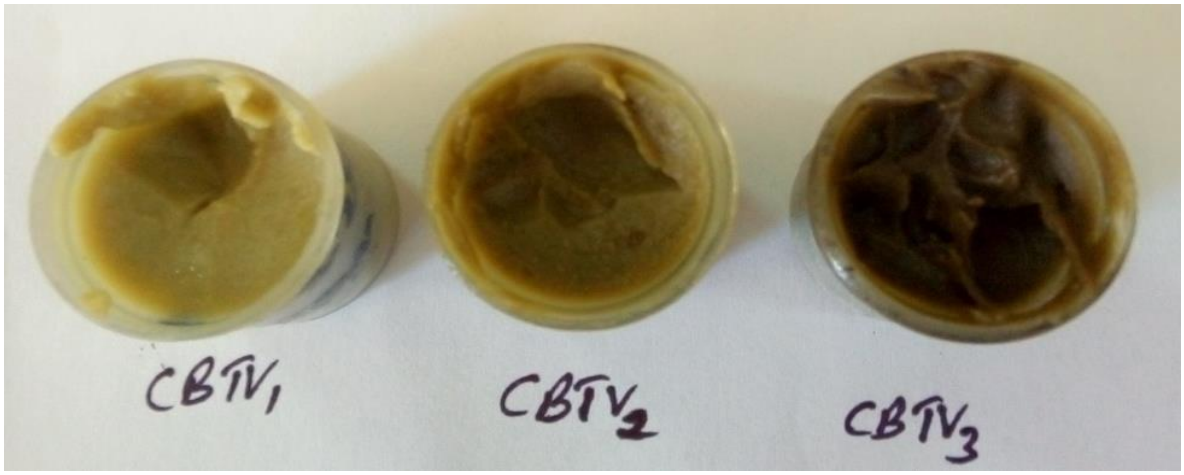


Figure 18: Formulated herbal creams from methanolic leaf extracts of *Tephrosia vogelii*

Table 8: *In vitro* antimicrobial activity of the tested cream samples

Microbial Species (microorganisms)	**Mean Zones of Inhibitions (mm) for Tested Herbal Cream**				
	Ciprofloxacin/ Fluconazole	**Cream base (-ve control)**	**$CBTV_1$**	**$CBTV_2$**	**$CBTV_3$**
Staphylococcus aureus	21.5	0	6.0	8.5	12.5
Escherichia coli	16.5	0	6.0	6.0	6.5
Candida albicans	17.5	0	6.0	7.0	10.5

The *in vivo* experiments to assess the formulated herbal cream ($CBTV_3$) using mice revealed positive impact against *Candida albicans.* The hairs of the mice were standing immediate after application of the clotrimazole and $CBTV_3$ suggesting that the mice reacted to the active ingredients of clotrimazole and CBTV3 with the skin. Such a reaction lasted from 5-10

minutes. The mice treated with standard drug (clotrimazole cream) and formulated herbal creams could not contract the fungal skin infections. Moreover, three out of four mice treated with direct methanolic leaf extracts were observed to be free from skin infections. By calculation, 3 out of 4 implies as 0.75 which equal to seventy per cent. Therefore, this suggests that performance of extracts was 75% as compared to formulated cream in the *in vivo* test, hence supporting more effort on herbal formulation of medicinal plant extracts. On the other hand, the skin of all group 1 mice which was not treated with drugs (negative control group) indicated to be infected. Thus, in this instance the antifungal agent as herbal cream product, $CBTV_3$, proved to have an effective antifungal activity against *C. albicans*; hence promising for further human clinical trials for authenticity therapy delivery. However, optimization of the formulated herbal drug and related quality will be equally significant to be improved prior to clinical trials (Shah *et al*., 1992).

Table 9: Physicochemical assessment of the herbal cream formulations

Assessed Parameter	**Recorded Physical Observation**		
	$CBTV_1$ (0.05%)	**$CBTV_2$ (0.10 %)**	**$CBTV_3$ (0.25 %)**
Colour	Light green	Light green	Deep green
Solubility	Warm water, methanol and ethanol	Warm water, methanol and ethanol	Warm water, methanol and ethanol
Odour	Aroma smell	Aroma smell	Aroma smell
Homogeneity	Homogenous	Homogenous	Homogenous
Washability	water	Water	Water
Texture	Smooth	Smooth	Smooth
Stability at 4 ^oC, 25 ^oC and 37 ^oC	Stable	Stable	Stable
pH	5.4	5.5	5.7

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Drug discovery and development from medicinal plants have been taking a significant trajectory in recent years. It has involved systematic steps especially bio-guided approach to come up with formulations or synthetic drugs. Developing an understanding of bioactivities, safety and phytochemicals of medicinal plants before drug formulations or development is highly necessary for advancement in this area. For instance, to ensure applicability of developed antimicrobial agents, the attention was invested on antimicrobial findings, toxicity study of bioactive extracts and identification of phytocompounds from the bioactive extracts.

The systematic biological assays of *Tephrosia vogelii* extracts proved that the plant species has remarkable antimicrobial potential against fungal and bacterial diseases. For example, the lower MICs values exerted by leaf extracts suggest that *Tephrosia vogelii* leaf plant material contains active compounds against *Candida albicans* and *Cryptococcus neoformans*. Also, MICs of the methanolic root and leaf extracts of *Tephrosia vogelii* suggest that they contain potent compounds which expressed to inhibit growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi*. Overall, findings from antifungal and antibacterial activities of bioactives against *Candida albicans*, *Cryptococcus neoformans*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella typhi* revealed that the methanolic leaf and root extracts of *Tephrosia vogelii* are potential source of antimicrobial agents for development of pharmaceutical drugs. Moreover, these results shed light and may attract the attention to increase cultivation of *Tephrosia vogelii* for antifungal and antibacterial applications in addition to management of ecto-parasite and pest as well as fishing applications.

Chemical characterization of secondary metabolites established the bioactive phytochemical profile, which have corroborated the biological activities obtained in the study. From GC-MS analysis revealed ten potential bioactive compounds namely: demethylmunduserone, tephrosin, 7-hydroxy-5-methoxy-8-(3-methylbut-2-enyl)-2-phenyl-2,3-dihydrochromen-4-one, sumatrol, 5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one, munduserone, hexadecanoic acid, (4R,5R,8S,8aS)-5-isopropyl-8-methyl-3-methylene-decahydroazulene-4,5,8-triol, deguelin hexadecanoic acid, and methyl ester. These phyto-compounds from

Tephrosia vogelii concurred with flavonoids, terpenes, steroids, saponins and rotenoids. Nevertheless, antimicrobial activities of bioactive secondary metabolites from *Tephrosia vogelii* extracts were enough evidence that this plant is a rich and reliable natural resource from which antimicrobial agents against infectious and non-infectious diseases could be discovered.

Toxicity evaluation of herbal medicines is paramount in order to provide safety assurance of their subsequent used herbal products. The magnitude of toxicity of the drug(s) is determined by their lethality and degeneration they cause on the vital organs particularly the liver and the kidney tissues or cells. In this study, the *in vivo* toxicity evaluation of methanolic root and leaf extracts of *Tephrosia vogelii* revealed no mortality cases even at the highest dose of 5000 mg/kg body weight of the albino rats suggesting that the extracts were not deadly. Histopathological examination of kidney and liver did not show any alterations in tissue morphology for the methanolic leaf extracts doses of 600, 1200 and 2000 mg/kg body weight. On the other hand, the methanolic root extracts did not cause any impairment or abnormality in the kidney in all administered dosages but exhibited toxicity in the liver at the dosage of 2000 mg/kg body weight. Nevertheless, the methanolic root extracts of *Tephrosia vogelii* at the higher dose provoke for more studies especially research on searching for anticancer agents. For that reason, the toxicity evaluation results backed up the toxicity profile of *Tephrosia vogelii* that may be useful to health practitioners using *Tephrosia vogelii* as herbal medicine from which scientists could do drug formulation. According, our toxicity evaluation data of both methanolic leaf and root extracts of *Tephrosia vogelii*, we clearly learned that the leaf dose is ideal for the formulation of antimicrobial products.

Regarding medicinal properties and evident pharmacological activity of *Tephrosia vogelii*, it has been vital for us to search for antimicrobial agents from this herbal plant so as to overcome trending bacterial and fungal skin infections. It is in this ground that herbal cream formulations were conducted. The performance of the herbal creams formulated from methanolic leaf extracts of *Tephrosia vogelii* through *in vitro* and *in vivo* tested was carried to observe effectiveness. The *In vitro* antimicrobial activity indicated the capability for treatment of skin infections as antifungal and antibacterial agents for topical therapy. The *in vivo* test of herbal cream ($CBTV_3$) demonstrated that the product is strongly effective against *Candida albicans*. Indeed, the herbal cream formulations of *Tephrosia vogelii* exhibited antimicrobial capability as a result of its bioactive contents such as flavonoids, tannins and

terpenoids. The bioactive compounds seem to have active moiety with either microstatic or microcidal effects. Thus, this study substantiated the ability of *Tephrosia vogelii* herbal cream ($CBTV_3$) which could potentially be used to combat fungal skin infections in humans. Nonetheless, further clinical studies will be needed to authenticate therapeutic prospective of the antimicrobial herbal cream product formulated. Of course, to the best of our knowledge this developed herbal cream prototype from *Tephrosia vogelii* Hook.f is hereby reported for the first time.

5.2 Recommendations

The isolation and identification of active pure phytochemical compounds is needed so that total or semi-synthesis can be done in the laboratory. The use of a combination of spectroscopic techniques such as nuclear magnetic resonance (NMR), infra-red spectroscopy (IR) and mass spectroscopy (MS) will enable more elucidation of compound in case of isolated compounds.

Since secondary metabolites vary due to geographical location, season, age and parts of plants, I commend that more studies be conducted on this medicinal plant by considering variation of plant age and varying stresses regarding different regions so as to study bioactive compounds produced.

Moreover, in future studies, it is indispensable to identify bioactive compounds from the methanolic root extracts for which a concentration higher than 2000 mg/kg causes hepatic necrosis and vacuolation. Also, how such compounds cause hepatic necrosis and vacuolation needs to be investigated in future because such findings could probably lead to the discovery of secondary metabolite which may be useful for anticancer agents.

The herbal cream formulated in this plant needs more optimization to find out diverse effectiveness and efficiencies prior to human clinical trials. Furthermore, *in vivo* clinical trials in human and drug evaluation for the formulated cream (product) is important for future drug improvement in order to safeguard the public safety before approval for topical delivery in the large community.

REFERENCES

Abdel-Naime, W. A., Fahim, J. R., Fouad, M. A., & Kamel, M. S. (2019). Antibacterial, antifungal, and GC–MS studies of *Melissa officinalis*. *South African Journal of Botany, 124*, 228–234. https://doi.org/10.1016/j.sajb.2019.05.011

Abdellatif, A. A. H., Alawadh, S. H., Bouazzaoui, A., Alhowail, A. H., & Mohammed, H. A. (2020). Anthocyanins rich pomegranate cream as a topical formulation with anti-aging activity. *Journal of Dermatological Treatment*, 1–8. https://doi.org/10.1080/09546634.2020.1721418

Ahmad, F., Anwar, F., & Hira, S. (2016). Review on medicinal importance of Fabaceae family. *Pharmacologyonline, 3*, 151–156.

Ajala, T. O., Femi-Oyewo, M. N., Odeku, O. A., Aina, O. O., Saba, A. B., & Oridupa, O. O. (2016). The physicochemical, safety and antimicrobial properties of *Phyllanthus amarus* herbal cream and ointment. *Journal of Pharmaceutical Investigation, 46*(2), 169–178. https://doi.org/10.1007/s40005-015-0226-8

Alamgir, A. N. M. (2017). Therapeutic use of medicinal plants and their extracts. In *Progress in Drug Research, 73*. https://doi.org/10.1007/978-3-319-63862-1_4

Ali, H. S., Shehab, N. A., & Mohamed, E. B. (2013). Formulation and evaluation of herbal cream from *Ziziphus spina* leaves extract. *International Research Journal of Pharmacy, 4*(6), 44–48. https://doi.org/10.7897/2230-8407.04610

American Academy of Dematology. (2017). Skin infections by the numbers. In *American Academy of Dermatology* (pp. 18–346).

Amy, M. H., DeWitt, B. A., & Lukes, A. L. (2000). Potential interactions between alternative therapies and warfarin. *American Journal of Health-System Pharmacy, 57*(13), 1221–1227. https://doi.org/10.1093/ajhp/57.13.1221

Animal Welfare Act*: Enacted by Parliamentary of the United Republic of Tanzania*. (2008).

Anjarwalla, P., Chepkoech, D., Kinyanjui, D., Belmain, S., Jamnadass, R., & Stevenson, P. C. (2015). Kipeperushi cha mimea inayotumika kama dawa ya kudhibiti wadudu: *Tephrosia vogelii* Hook.f. In *World Agroforestry Centre*. World Agroforestry Centre. http://projects.nri.org/options/images/Tephrosia_vogelii_Hook._f.-2.pdf. Accessed 10 October, 2020.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C., & Haridas, M. (2012). Anti-Inflammatory Property of n-Hexadecanoic Acid: Structural Evidence and Kinetic Assessment. *Chemical Biology and Drug Design*, *80*(3), 434–439. https://doi.org/10.1111/j.1747-0285.2012.01418.x

Arendrup, M. C. (2014). Update on antifungal resistance in *Aspergillus* and *Candida*. *Clinical Microbiology and Infection*, *20*(6), 42–48. https://doi.org/10.1111/1469-0691.12513

Arif, T., Mandal, T. K., & Dabur, R. (2011). Natural products: Anti-fungal agents derived from plants. In *Opportunity, challenge and scope of natural products in medicinal chemistry* (pp. 283–311). Research Signpost. https://www.researchgate.net/publication/236244385_9_Natural_products_Anti-fungal_agents_derived_from_plants. Accessed 10 October, 2020.

Atanasov, A. G., Waltenberger, B., Pferschy-wenzig, E., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E. H., Rollinger, J. M., Schuster, D., Breuss, J. M., Bochkov, V., Mihovilovic, M. D., Kopp, B., Bauer, R., Dirsch, V. M., & Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, *33*(8), 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Badiee, P. (2017). Antifungal resistance in cancer patients. *Iranian Journal of Medical Sciences, 17*, 727-735. https://doi.org/10.1186/s12879-017-2825-7

Badri, A. M., Garbi, M. I., Gmaraldeen, S. M., Magzoub, A. A., Ibrahim, I. T., Saleh, M. S., Kabbashi, A. S., & Mohamed, S. G. (2017). Antioxidant activity and phytochemical screening of *Prosopis juliflora* leaves extract. *Advancement in Medicinal Plant Research*, *5*(3), 37–40. https://doi.org/10.30918/AMPR.53.17.020

Balls, M., Goldberg, A. M., Fentem, J. H., Broadhead, C. L., Burch, R. L., Balls, M., Goldberg, A. M., Broadhead, J. H., Burch, C. L., & Festing, R. L. (1995). The Three Rs: The Way Forward. *Alternative to Laboratory Animal*, *23*, 838–866. https://www.wellbeingintlstudies repository.org/acwp_arte/58. Accessed 10 October, 2020

Banerjee, K., Thiagarajan, N., & Thiagarajan, P. (2019). Formulation and characterization of a *Helianthus annuus* - Alkyl polyglucoside emulsion cream for topical applications. *Journal of Cosmetic Dermatology*, *18*(2), 628–637. https://doi.org/10.1111/jocd.12756

Bank, W. (2017). Drud-resistant infection: A threat to our economic future. *World Bank Group*. http://documents1.worldbank.org/curated/en/323311493396993758/pdf/final-report.pdf. Accessed 10 January, 2021.

Bauer, A., Kirby, W., Sherris, J. C., & Turck, M. (1966). Antibiotic sus- ceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, *45*(4), 49–52. https://doi.org/https://doi.org/10.1093/ ajcp/45.4_ts.493

Beardsley, J., Halliday, C. L., Chen, S. C., & Sorrell, T. C. (2018). Responding to the emergence of antifungal drug resistance: Perspectives from the bench and the bedside. *Future Microbiology*, *13*, 1175–1191.

Bello, I., Bakkouri, A. S., Tabana, Y. M., Al-hindi, B., Al-Mansoub, M. A., Mahmud, R., & Asmawi, M. Z. (2016). Acute and sub-acute toxicity evaluation of the methanolic extract of *Alstonia scholaris* stem bark. *Medical Sciences*, *4*(4), 1–14. https://doi.org/10.3390/ medsci4010004

Benedict, K., Jackson, B. R., Chiller, T., & Beer, K. D. (2019). Estimation of direct healthcare costs of fungal diseases in the United States. *Clinical Infectious Diseases*, *68*(11), 1791–1797. https://doi.org/10.1093/cid/ciy776.

Bongomin, F., Gago, S., Oladele, R. O., & Denning, D. (2017). Global and Multi-National Prevalence of Fungal Diseases - Estimate Precision. *Journal of Fungi*, *3*(57), 1–29. https://doi.org/10.3390/jof3040057

Caboni, P., Sherer, T. B., Zhang, N., Taylor, G., Greenamyre, J. T., & Casida, J. E. (2004). Rotenone, Deguelin, Their Metabolites, and the Rat Model of Parkinson's Disease. *Chemical Research in Toxicol*ogy, *17*, 1540–1548. https://doi.org/10.1021/tx049867r

Carolus, H., Van-Dyck, K., & Van-Dijck, P. (2019). *Candida albicans* and *Staphylococcus* Species: A Threatening Twosome. *Frontiers in Microbiology*, *10*, 1–8. https://doi.org/10.3389/fmicb.2019.02162

Cartron, M. L., England, S. R., Chiriac, A. I., Josten, M., Turner, R., Rauter, Y., Hurd, A., Sahl, H. G., Jones, S., & Foster, S. J. (2014). Bactericidal activity of the human skin fatty acid *cis*-6-hexadecanoic acid on *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *58*(7), 3599–3609. https://doi.org/10.1128/AAC.01043-13

Casadevall, A. (2018). Fungal Diseases in the 21st Century: The Near and Far Horizons. *Pathogens and Immunity*, *3*(2), 183. https://doi.org/10.20411/pai.v3i2.249

CDC. (2019a). *Drug-Resistant: Candida species*. The Centre of Disease Control and Prevention. https://www.cdc.gov/drugresistance/pdf/threats-report/candida-508.pdf

CDC. (2019b). *Fungal Diseases*. The Centers for Disease Control and Prevention. https://www.cdc.gov/fungal/diseases/candidiasis/index.html

Chandrasekaran, M., Kannathasan, K., & Venkatesalu, V. (2008). Antimicrobial activity of fatty acid methyl esters of some members of chenopodiaceae. *Journal of Biosciences*, *63*(5–6), 331–336. https://doi.org/10.1515/znc-2008-5-604

Chandrasekaran, M., Senthilkumar, A., & Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences*, *15*, 775–780.

Chen, M. X., Alexander, K. S., & Baki, G. (2016). Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. *Journal of Pharmaceutics*, *2016*, 1–10. https://doi.org/10.1155/2016/5754349

Chikoi, R., Nyawale, H. A., & Mghanga, F. P. (2018). Magnitude and Associated Risk Factors of Superficial Skin Fungal Infection Among Primary School Children in Southern Tanzania Study Design and Setting. *Cureus*, *10*(7), 1–12. https://doi.org/10.7759/cureus.2993

Chinedu, E., Arome, D., & Ameh, F. S. (2013). A new method for determining acute toxicity in animal models. *Toxicology International*, *20*(3), 224–226. https://doi.org/10.4103/0971-6580.121674

Cole, D. C., Govender, N. P., Chakrabarti, A., Sacarlal, J., & Denning, D. W. (2017). Improvement of fungal disease identification and management: combined health systems and public health approaches. *The Lancet Infectious Diseases*, *17*(12), e412–e419. https://doi.org/10.1016/S1473-3099(17)30308-0

Dagne, E., Yenesew, A., & Waterman, P. G. (1989). Flavonoids and isoflavonoids from *Tephrosia fulvinervis* and *Tephrosia pentaphylla*. *Phytochemistry*, *28*(11), 3207–3210. https://doi.org/10.1016/0031-9422(89)80308-5

Danby, S. G., Draelos, Z. D., Gold, L. F. S., Cha, A., Vlahos, B., Aikman, L., Sanders, P., Wu-Linhares, D., & Cork, M. J. (2020). Vehicles for atopic dermatitis therapies: more than just a placebo. *Journal of Dermatological Treatment*, 1–14. https://doi.org/10.1080/09546634.2020.1789050

Das, S., Vasudeva, N., & Sharma, S. (2014). Chemical composition of ethanol extract of *Macrotyloma uniflorum* (Lam.) Verdc. using GC-MS spectroscopy. *Organic and Medicinal Chemistry Letters*, *4*(1), 2–5. https://doi.org/10.1186/s13588-014-0013-y

Denning, D. W. (2017). Calling upon all public health mycologists: To accompany the country burden papers from 14 countries. *European Journal of Clinical Microbiology and Infectious Diseases*, *36*, 923–924. https://doi.org/10.1007/s10096-017-2909-8

Denning, D. W., Kneale, M., Sobel, J. D., & Rautemaa-richardson, R. (2018). Review of Global burden of recurrent vulvovaginal candidiasis: A systematic review. *The Lancet Infectious Diseases*, *18*(11), 1–9. https://doi.org/10.1016/S1473-3099(18)30103-8

Dewick, P. M. (2009). Medicinal Natural Product: A Biosynthetic Approach, 3rd Edition. In *John Wiley and Sons Ltd*. https://doi.org/DOI:10.1002/9780470742761

Dzenda, T., Ayo, J. A., Adelaiye, A. B., & Adaudi, A. O. (2008). Ethno-medical and veterinary uses of *Tephrosia vogelii* Hook.f.: A Review. *Nigerian Veterinary Journal*, *28*(3), 24–39. https://doi.org/10.4314/nvj.v28i3.3564

Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, *4*, 1–10. https://doi.org/10.3389/fphar.2013.00177

Elibol, B., Ezer, T., Kara, R., Celik, G. Y., Colak, E., Yuv, G., & Colak, E. (2011). Antifungal and antibacterial effects of some acrocarpic mosses. *African Journal of Biotechnology*, *10*(6), 986–989.

Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, *64*(8), 711–713. https://doi.org/10.1055/s-2006-957563

Emerole, G., Thabrew, M. I., Anosa, V., & Okorie, D. A. (1981). Structure-activity relationship in the toxicity of some naturally occuring coumarins-chalepin, imperatorin and oxypeucedanine. *Toxicology*, *20*, 71–80.

Evans, D., & Robinson, M. (2011). What is histopathology? In *Oxford University Press*. Oxford University Press.

Faini, D., Maokola, W., Furrer, H., Hatz, C., Battegay, M., Tanner, M., Denning, D. W., & Letang, E. (2015). Burden of serious fungal infections in Tanzania. *Mycoses*, *58*, 70–79. https://doi.org/10.1111/myc.12390

Fatima, S., Zaman, R., Haider, N., Shamsi, S., & Alam, A. (2017). Design and development of Unani anti-inflammatory cream. *Journal of Ayurveda and Integrative Medicine*, *8*(3), 140–144. https://doi.org/10.1016/j.jaim.2017.03.002

Fausto, A., Rodrigues, M. L., & Coelho, C. (2019). The still underestimated problem of fungal diseases worldwide. *Frontiers in Microbiology*, *10*, 1–5. https://doi.org/10.3389/fmicb.2019.00214

Gadzirayi, C. T., Mutandwa, E., Mwale, M., & Chindundu, T. (2009). Utilization of *Tephrosia vogelii* in controlling ticks in dairy cows by small-scale commercial farmers in Zimbabwe. *African Journal of Biotechnology*, *8*(17), 4134–4136. https://doi.org/10.5897/AJB2009.000-9396

Garcia-Solache, M. A., & Casadevall, A. (2010). Global warming will bring new fungal diseases for mammals. *MBio*, *1*(1), 1–3. https://doi.org/10.1128/mBio.00061-10

Gbadamosi, I. T., & Erinoso, S. M. (2016). A review of twenty ethnobotanicals used in the management of breast cancer in Abeokuta, Ogun State, Nigeria. *African Journal of Pharmacy and Pharmacology*, *10*(27), 546–564. https://doi.org/10.5897/AJPP2015.4327

Gemeda, N., Tadele, A., Lemma, H., Girma, B., Addis, G., Tesfaye, B., Abebe, A., Gemechu, W., Yirsaw, K., Teka, F., Haile, C., Amano, A., Woldkidan, S., Geleta, B., & Debella, A. (2018). Development, Characterization, and Evaluation of Novel Broad-Spectrum Antimicrobial Topical Formulations from *Cymbopogon martini* (Roxb.) W. Watson Essential Oil. *Evidence-Based Complementary and Alternative Medicine*, *2018*, 1–16. https://doi.org/10.1155/2018/9812093

Gendimenico, J. L., Gendimenico, G. J., Cauwenbergh, G., & Mezick, J. A. (2015). Dermatotherapeutic Agents. In *Ullmann's Encyclopedia of Industrial Chemistry* (pp. 1–20). https://doi.org/10.1002/14356007.a08_301.pub3

Gibbs, S. (1996). Skin disease and socioeconomic conditions in rural Africa: Tanzania. *International Journal of Dermatology*, *35*(9), 633–639. https://doi.org/10.1111/j.1365-4362.1996.tb03687.x

Gupta, P. D., & Birdi, T. J. (2017). Development of botanicals to combat antibiotic resistance. *Journal of Ayurveda and Integrative Medicine*, *8*(4), 266–275. https://doi.org/10.1016/j.jaim.2017.05.004

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, *27*(1), 1–93. https://doi.org/10.1016/j.mam.2005.07.008

Harborne, J. B. (1973). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. In *British Library Cataloguing in Publication Data*. Chapman and Hall Ltd. https://doi.org/10.1007/978-94-009-5921-7

Herman, R. A., Wang, J. Z., Amuzu, P., Shittu, S., Wu, F. A., & Wang, J. (2020). Evaluation of inhibitory activities of two medicinal plant extracts *Parkia biglobosa* and *Lonicera japonica* against spoilage microorganisms isolated from mulberry fruit. *Journal of Food Processing and Preservation*, *e14630*, 1–11. https://doi.org/10.1111/jfpp.14630

Hisham, A., John, S., Al-Shuaily, W., Asai, T., & Fujimoto, Y. (2006). (+)-Apollineanin: A new flavanone from *Tephrosia apollinea*. *Natural Product Research*, *20*(12), 1046–1052. https://doi.org/10.1080/14786410500399714

Hudzicki, J. (2016). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. In *American Society For Microbiology*.

Hussain, G., Huang, J., Rasul, A., Anwar, H., Imran, A., Maqbool, J., Razzaq, A., Aziz, N., Makhdoom, E. H., Konuk, M., & Sun, T. (2019). Putative roles of plant-derived tannins in neurodegenerative and neuropsychiatry disorders: An updated review. *Molecules*, *24*, 1–16. https://doi.org/10.3390/molecules24122213

Ibrahim, B., M'batchi, B., Mounzeo, H., Bourobou, H. P. B., & Posso, P. (2000). Effect of *Tephrosia vogelii* and *Justicia extensa* on *Tilapia nilotica* in vivo. *Journal of Ethnopharmacology*, *69*, 99–104. https://doi.org/10.1016/S0378-8741(99)00036-7

Inalegwu, B., & Sodipo, O. A. (2015). Antimicrobial and foam forming activities of extracts and purified saponins of leaves of *Tephrosia vogelii*. *European Journal of Experimental Biology*, *5*(5), 49–53.

Irawan, C., Foliatini., Hanafi., Sulistiawaty, L., & Sukiman, M. (2018). Volatile compound analysis using GC-MS, phytochemical screening and antioxidant activities of the husk of "Julang-jaling" (*Archidendron bubalinum* (Jack) I. C. Nielsen) from Lampung, Indonesia. *Pharmacognosy Journal*, *10*(1), 92–98. https://doi.org/10.5530/pj.2018.1.17

Joseph, O., Kihdze, T. J., Katusiime, B., Imanirampa, L., Bajunirwe, F., Ganafa, A. A., Oloro, J., Tanayen, J. K., Barbra, K., Lawrence, I., Paul, W., Francis, B., & Amon, A. G. (2015). Toxicity of four herbs used in erectile dysfunction: *Mondia whiteii*, *Cola acuminata*, *Urtica massaica*, and *Tarenna graveolens* in male rats. *African Journal of Pharmacy and Pharmacology*, *9*(30), 756–763. https://doi.org/10.5897/AJPP2015.4299

Kabara, J. J., Swieczkowski, D. M., Conley, A. J., & Truant, J. P. (1972). Fatty Acids and Derivatives as Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy*, *2*(1), 23–28. https://doi.org/10.1016/B978-1-4377-2702-9.00294-4

Kalume, M. K., Losson, B., Angenot, L., Tits, M., Wauters, J. N., Frédérich, M., & Saegerman, C. (2012). Rotenoid content and in vitro acaricidal activity of *Tephrosia vogelii* leaf extract on the tick *Rhipicephalus appendiculatus*. *Veterinary Parasitology*, *190*(1–2), 204–209. https://doi.org/10.1016/j.vetpar.2012.06.015

Karimkhani, C., Dellavalle, R. P., Coffeng, L. E., Flohr, C., Hay, R. J., Langan, S. M., Nsoesie, E. O., Ferrari, A. J., Erskine, H. E., Silverberg, J. I., Vos, T., & Naghavi, M. (2017). Global skin disease morbidity and mortality: An update from the global burden of disease study 2013. *JAMA Dermatology*, *153*(5), 406–412. https://doi.org/10.1001/jamadermatol.2016.5538

Kenechukwu, F. C., Mbah, C. J., Momoh, M. A., Chime, S. A., Umeyor, C. E., & Ogbonna, J. D. N. (2012). Pharmacological justification for the ethnomedical use of *Clausena anisata* root-bark extract in the management of epilepsy. *Journal of Applied Pharmaceutical Science*, *2*(9), 36–40. https://doi.org/10.7324/JAPS.2012.2907

Kidukuli, A. W., Maregesi, S. M., Saria, J., Otieno, N. J., Lawi, Y., Nondo, R. S., Innocent, E. M., Mlimbila, J., Mihale, J., & Moshi, M. J. (2015). Larvicidal efficacy of some *Tephrosia* species extracts against *Anopheles Gambiae* Ss and *Culex Quinquefasciatus* Say. *Spatula DD*, *5*(1), 21–25. https://doi.org/10.5455/spatula.20150625013601

Kifayatullah, M., Mustafa, M. S., Sengupta, P., Sarker, M. R., Das, A., & Das, S. K. (2015). Evaluation of the acute and sub-acute toxicity of the ethanolic extract of *Pericampylus glaucus* (Lam.) Merr. in BALB/c mice. *Journal of Acute Disease*, *4*(4), 309–315. https://doi.org/10.1016/j.joad.2015.06.010

Kilonzo, M., Ndakidemi, P. A., & Chacha, M. (2016). Acute oral toxicity study of *Mystroxylon aethiopicum* root bark aqueous extract in albino mice. *Journal of Medicinal Plants Research*, *10*(37), 656–661. https://doi.org/10.5897/JMPR2016.6245

Kontoyiannis, D. P. (2017). Antifungal Resistance: An Emerging Reality and Global Challenge. *The Journal of Infectious Diseases*, *216*(Suppl 3), 431–435. https://doi.org/10.1093/infdis/jix179

Kujur, R. S., Singh, V., Ram, M., Yadava, H. N., Singh, K. K., Kumari, S., & Roy, B. K. (2010). Antidiabetic activity and phytochemical screening of crude extract of *Stevia rebaudiana* in alloxan-induced diabetic rats. *Pharmacognosy Research*, *2*(4), 258–263. https://doi.org/10.4103/0974-8490.69128

Kumar, A., & Jha, A. (2017). Drug Development Strategies. In *Anticandidal Agents* (pp. 63–71). Elsevier Inc. https://doi.org/10.1016/b978-0-12-811311-0.00007-7

Kumar, S. N., & Thampi, N. (2015). Phytochemical screening and characterization of the bioactive compounds from the leaves of H*yptis suaveolens* and S*pathodea campanulata*. *Journal of Chemical and Pharmaceutical Research*, *7*(7), 840–850.

Labar, A. S., Millman, J. S., Ruebush, E., Opintan, J. A., Bishar, R. A., Aboderin, A. O., Newman, M. J., Lamikanra, A., & Okeke, I. N. (2012). Regional dissemination of a trimethoprim-resistance gene cassette via a successful transposable element. *PLoS ONE*, *7*(5). https://doi.org/10.1371/journal.pone.0038142

Li, W., Huang, C., Wang, K., Fu, J., Cheng, D., & Zhang, Z. (2015). Laboratory evaluation of aqueous leaf extract of *Tephrosia vogelii* against larvae of *Aedes albopictus* (Diptera: Culicidae) and non-target aquatic organisms. *Acta Tropica*, *146*, 36–41. https://doi.org/10.1016/j.actatropica.2015.02.004

Limper, A. H., Adenis, A., Le, T., & Harrison, T. S. (2017). Fungal infections in HIV/AIDS. *The Lancet Infectious Diseases*, *17*(11), e412–e419. https://doi.org/10.1016/S1473-3099(17)30303-1

Ling, N. (2003). Rotenone - A review of its toxicity and use for fisheries management. In *Science for Conservation 211*. Department of Conservation.

Lockhart, S. R., Etienne, K. A., Vallabhaneni, S., Farooqi, J., Govender, N. P., Colombo, A. L., Calvo, B., Cuomo, C. A., Desjardins, C. A., Berkow, E. L., Castanheira, M., Magobo, R. E., Jabeen, K., Asghar, R. J., Meis, J. F., Jackson, B., Chiller, T., & Anastasia, P. (2017). Simultaneous emergence of multidrug resistant *Candida auris* on three continents confirmed by whole genome sequencing and epidemiological analyses. *Clinical Infectious Diseases*, *64*, 134–140. https://doi.org/10.1093/cid/ciw691

Maheswari, U., Sridevi, S. K. S., Umamaheswari, S., Uma, C., Reddy, M., & Kalkura, S. N. (2016). Flavonoids: Therapeutic Potential of Natural Pharmacological Agents. *International Journal of Pharmaceutical Sciences and Research*, *7*(10), 3924–3930. https://doi.org/10.13040/IJPSR.0975-8232.7(10).3924-30

Mahomoodally, M. F., Gurib-Fakim, A., & Subratty, A. H. (2005). Antimicrobial activities and phytochemical profiles of endemic medicinal plants of Mauritius. *Pharmaceutical Biology*, *43*(3), 237–242. https://doi.org/10.1080/13880200590928825

Majinda, R. R. T., Abegaz, B. M., Bezabih, M., Ngadjui, B. T., Wanjala, C. C. W., Mdee, L. K., Bojase, G., Silayo, A., Masesane, I., & Yeboah, S. O. (2001). Recent results from natural product research at the University of Botswana. *Pure and Applied Chemistry*, *73*(7), 1197–1208.

Makoshi, M., & Arowolo, R. O. A. (2011). Therapeutic effects of *Tephrosia vogelii* ointment in the treatment of bovine dermatophilosis. *Journal of Veterinary Medicine and Animal Health*, *3*(4), 51–55.

Marango, S. N., Khayeka-Wandabwa, C., Makwali, J. A., Jumba, B. N., Choge, J. K., Adino, E. O., & Anjili, C. O. (2017). Experimental therapeutic assays of *Tephrosia vogelii* against Leishmania major infection in murine model: In vitro and in vivo. *BMC Research Notes*, *10*(1), 1–12. https://doi.org/10.1186/s13104-017-3022-x

Maregesi, S. M., Mwakigonja, A. R., & Urio, P. (2016). Toxicity evaluation of Abrus precatorius seeds collected from Bunda District, Tanzania. *Scholars Academic Journal of Pharmacy*, *5*(10), 399–405. https://doi.org/10.21276/sajp.2016.5.10.3

Maregesi, S. M., Ngassapa, O. D., Pieters, L., & Vlietinck, A. J. (2007). Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases. *Journal of Ethnopharmacology*, *113*(3), 457–470. https://doi.org/10.1016/j.jep.2007.07.006

Marinova, D., Ribarova, F., & Atanassova, M. (2005). Total phenolics and flavonoids in Bulgarian fruits and vegetables. *Journal of the University of Chemical Technology and Metallurgy*, *40*(3), 255–260.

Masevhe, N. A., McGaw, L. J., & Eloff, J. N. (2015). The traditional use of plants to manage candidiasis and related infections in Venda, South Africa. *Journal of Ethnopharmacology*, *168*, 364–372. https://doi.org/10.1016/j.jep.2015.03.046

Mbunde, M. V. N., Innocent, E., Mabiki, F., Andersson, P. G., Victor, M., Mbunde, N., Innocent, E., & Mabiki, F. (2016). Ethnobotanical survey and toxicity evaluation of medicinal plants used for fungal remedy in the Southern Highlands of Tanzania. *Journal of Intercultural Ethnopharmacology*, *6*(1), 84–96. https://doi.org/10.5455/jice. 20161222103956

McManus, D. S. (2017). Antifungal Drugs. In *Side Effects of Drugs Annual* (1st Edition), *39*, 245–258. Elsevier B.V. https://doi.org/10.1016/bs.seda.2017.06.031

McNulty, C. (2017). Fungal skin and nail infections: Diagnosis and laboratory investigation. In *Quick reference guide for primary care: For consultation and local adaptation* (pp. 1–13). Public Health England.

Mlozi, S. H., Mmongoyo, J. A., & Chacha, M. (2020). Antimicrobial activities of *Tephrosia vogelii* against selected pathogenic fungi and bacteria strains. *Mycology*, *11*(1), 49–55. https://doi.org/10.1080/21501203.2019.1705929

Moldovan, M., Lahmar, A., Bogdan, C., Parauan, S., Tomuţă, I., & Crişan, M. (2017). Formulation and evaluation of a water-in-oil cream containing herbal active ingredients and ferulic acid. *Clujul Medical*, *90*(2), 212–219. https://doi.org/10.15386/cjmed-668

Morris, J. B. (1999). Legume genetic resources with novel "value added" industrial and pharmaceutical use. *Proceedings of the Fourth National Symposium New Crops and New Uses: Biodiversity and Agricultural Sustainability*, 196–201.

Murtaza, G., Mukhtar, M., & Sarfraz, A. (2015). A Review: Antifungal Potentials of Medicinal Plants. *Journal of Bioresource Management*, *2*(2). https://doi.org/10.35691/ jbm.5102.0018

Mwaura, L., Stevenson, P. C., Ofori, D. A., Anjarwalla, P., & Jamnadass, R. (2013). *Pesticidal Plant Leaflet*: *Tephrosia vogelii* Hook.f. World Agroforestry Centre; World Agroforestry Centre.

Nanthini, K., Kanakavalli, K., & Kaliyamurthy, V. (2014). Acute and Sub Acute Toxicity Study on Siddha Drug from *Mandoora chooranam*. *International Journal of Pharmaceutical & Biological Archives*, *5*(2), 86–91.

Nneka, V., & Jude, A. (2012). Antimicrobial Properties and Phytochemical Analysis of Methanolic Extracts of *Aframomum melegueta* and *Zingiber officinale* on Fungal Diseases of Tomato Fruit. *Journal of Natural Sciences Research*, *2*(6), 10–16.

OECD. (2001). *OECD Guideline for Testing of Chemicals: OECD 423*.

OECD. (2008). *OECD Guideline for Testing of Chemicals: OECD 407* (pp. 1–13).

Okeke, I. N., Aboderin, O. A., Byarugaba, D. K., Ojo, K. K., & Opintan, J. A. (2007). Growing problem of multidrug-resistant enteric pathogens in Africa. *Emerging Infectious Diseases*, *13*(11), 1640–1646. https://doi.org/10.3201/eid1311.070674

Okpogba, T. C., Sobowale, A. A., & Gbadamosi, I. (2019). Control of some Penicillium and Aspergillus rots of *Discorea alata* Poir and *Discorea rotundata* L. using extracts of *Xylopia aethiopica* (Dunal.) Linn. and *Syzygium aromaticum* (Linn.) Merr. *African Journal of Plant Scince*, *13*, 113–124. https://doi.org/10.5897/AJPS2019.1764

Olaniyan, J. M., Muhammad, H. L., Makun, H. A., Busari, M. B., & Abdullah, A. S. (2016). Acute and sub-acute toxicity studies of aqueous and methanol extracts of *Nelsonia campestris* in rats. *Journal of Acute Disease*, *5*(1), 62–70. https://doi.org/10.1016/j.joad.2015.08.006

Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., & Deun, K. V. (2000). Concordance of the Toxicity of Pharmaceuticals in Humans and in Animals. *Regulatory Toxicology and Pharmacology*, *32*, 56–67. https://doi.org/10.1006/rtph.2000.1399

Omodamiro, O. D., & Amechi, U. (2013). The phytochemical content, antioxidant, antimicrobial and anti-inflammatory activities of *Lycopersicon esculentum* (Tomato). *Asian Journal of Plant Science and Research*, *3*(5), 70–81.

Onwukaeme, D. N., Ikuegbvweha, T. B., & Asonye, C. C. (2007). Evaluation of Phytochemical Constituents, Antibacterial Activities and Effect of Exudate of *Pycanthus Angolensis* Weld Warb (Myristicaceae) on Corneal Ulcers in Rabbits. *Tropical Journal of Pharmaceutical Research*, *6*, 725–730.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R., & Anthony, S. (2009). *Tephrosia vogelii* Hook.f Fabaceae - Papilionoideae. Agroforestry Database. http://apps.worldagroforestry.org/treedb/AFTPDFS/*Tephrosia_vogelii*.PDF

Patel, S., Nag, M. K., Daharwal, S. J., Singh, M. R., & Singh, D. (2013). Plant toxins: An overview. *Research Journal of Pharmacology and Pharmacodynamics*, *5*(5), 283–288.

Perlin, D. S., Rautemaa-richardson, R., & Alastruey-izquierdo, A. (2017). The global problem of antifungal resistance: Prevalence, mechanisms, and management. *The Lancet Infectious Diseases*, *17*, e383–e392. https://doi.org/10.1016/S1473-3099(17)30316-X

Pietta, P. (2000). Flavonoids as Antioxidants. *Journal of Natural Products*, *63*, 1035–1042.

Porwal, M., Khan, N. A., & Maheshwari, K. K. (2017). Evaluation of Acute and Subacute Oral Toxicity Induced by Ethanolic Extract of *Marsdenia tenacissima* Leaves in Experimental Rats. *Scientia Pharmaceutica*, *85*(29), 1–11. https://doi.org/10.3390/scipharm85030029

Prabha, D. S., Dahms, H. U., & Malliga, P. (2014). P harmacological potentials of phenolic compounds from *Prosopis* spp.: A review. *Journal of Coastal Life Medicine*, *2*(11), 918–924. https://doi.org/10.12980/JCLM.2.2014J27

Premkumar, A., Muthukumaran, T., & Ganesan, V. (2014). Formulation and Evaluation of Cream Containing Antifungal Agents, Antibacterial Agents and Corticosteroids. *Hygeia: Journal for Drugs and Medicines*, *6*(2), 5–16. https://doi.org/10.15254/H.J.D.Med.6.2014.131

Prescott, M. J., & Lidster, K. (2017). Improving quality of science through better animal welfare: The NC3Rs strategy. *Alternatives to Laboratory Animals*, *46*(4), 152–156. https://doi.org/10.1038/laban.1217

Priya, S., Nethaji, S., & Sindhuja, B. (2014). GC-MS analysis of some bioactive constituents of D*iospyros virginiana*. *Research Journal of Pharmacy & Technology*, *7*(4), 429–432.

Rahman, A. H. M., & Parvin, M. I. A. (2014). Study of Medicinal Uses on Fabaceae Family at Rajshahi, Bangladesh. *Research in Plant Sciences*, *2*(1), 6–8. https://doi.org/10.12691/plant-2-1-2

Rajasingham, R., Smith, R. M., Park, B. J., Jarvis, J. N., Govender, N. P., Chiller, T. M., Denning, D. W., Loyse, A., & Boulware, D. R. (2017). Global Burden of Disease of HIV-Associated Cryptococcal Meningitis: An Updated Analysis. *The Lancet Infectious Diseases*, *17*(8), 873–881. https://doi.org/10.1016/S1473-3099(17)30243-8

Ramawat, K. G., & Mérillon, J. M. (2013). Terpenes: Chemistry, Biological Role, and Therapeutic Applications. In *Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes* (pp. 2667–2691). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-642-22144-6_120

Redfield, R. R. (2019). Antibiotic resistance threats in the United States. In *Centers for Disease Control and Prevention*. https://doi.org/CS239559-B

Rodrigues, M. L., & Albuquerque, P. C. (2018). Searching for a change: The need for increased support for public health and research on fungal diseases. *PLoS Neglected Tropical Diseases*, *12*(6), 1–5. https://doi.org/10.1371/journal.pntd.0006479

Russell, D. A., Freudenreich, J. J., Ciardiello, J. J., Sore, H. F., & Spring, D. R. (2017). Stereocontrolled semi-syntheses of deguelin and tephrosin. *Organic and Biomolecular Chemistry*, *15*(7), 1593–1596. https://doi.org/10.1039/c6ob02659a

Sahu, M., Vermaand, D., & Harris, K. K. (2014). Phytochemical Analysis of the Leaf, Stem and Seed Extracts of *Cajanus cajan* L. (Dicotyledoneae: Fabaceae). *World Journal of Pharmacy and Pharmaceutical Sciences*, *3*(8), 694–733.

Samuel, V. J., Mahesh, A. R., & Murugan, V. (2019). Phytochemical and pharmacological aspects of *Tephrosia* genus: A brief review. *Journal of Applied Pharmaceutical Science*, *9*(3), 117–125. https://doi.org/10.7324/JAPS.2019.90317

Sanguinetti, M., Posteraro, B., & Lass-Flörl, C. (2015). Antifungal drug resistance among *Candida* species: mechanisms and clinical impact. *Mycoses*, *58*(S2), 2–13. https://doi.org/10.1111/myc.12330

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., Latha, L. Y., Bedong-semeling, J., & Nasi, B. A. (2011). Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Africa Journal of Traditional, Complementary and Alternative Medicines*, *8*(1), 1–10.

Satimia, F. T., Mcbride, S. R., & Leppard, B. (1998). Prevalence of Skin Disease in Rural Tanzania and Factors Influencing the Choice of Health Care, Modern or Traditional. *Archieves Dermatology*, *134*, 1363–1366. https://doi.org/10.1001/archderm.134.11.1363

Schlecht, L. M., Peters, B. M., Krom, B. P., Freiberg, J. A., Hänsch, G. M., Filler, S. G., Jabra-Rizk, M. A., & Shirtliff, M. E. (2015). Systemic *Staphylococcus aureus* infection mediated by *Candida albicans* hyphal invasion of mucosal tissue. *Microbiology*, *161*, 168–181. https://doi.org/10.1099/mic.0.083485-0

Shah, V. P., Behl, C. R., Flynn, G. L., Higuchi, W. I., & Schaefer, H. (1992). Principles and criteria in the development and optimization of topical therapeutic products. *International Journai of Pharmaceutics*, *82*, 21–28.

Shankar, R., Sarangi, B., Gupta, R., & Pathak, K. (2016). Formulation and characterization of polyherbal cream for skin manifestations. *Journal of Asian Association of Schools of Pharmacy*, *1*, 360–366. https://doi.org/10.1021/jp107865rn

Shorinwa, O. A., & Monsi, B. (2020). Toxicological implications of the fruit of *Harungana madagascariensis* on wistar rats. *Clinical Phytoscience*, *6*(2), 1–9. https://doi.org/10.1186/s40816-019-0145-8

Silverberg, J. I., Vos, T., & Naghavi, M. (2017). Global Skin Disease Morbidity and Mortality: An Update from the Global Burden of Disease Study 2013. *JAMA Dermatology*, *44113*(5), 406–412. https://doi.org/10.1001/jamadermatol.2016.5538

Singh, A. P., & Kumar, S. (2019). Applications of Tannins in Industry. In *Tannins - Structural Properties, Biological Properties and Current Knowledge* (pp. 1–19). https://doi.org/10.5772/intechopen.85984

Singh, R., Hussain, A. A., Ahmad, G. A., Prakash, M. R., Vyas, M., Ahmad, G. H., Ali, S., Ranu, S. C., Allaie, A. H., Ganaie, A. A., Prakash, R., Vyas, M., Ganaie, H. A., & Ali, S. (2017). Antifungal activity of medicinal plants with special reference to antidermatophytic activity: A review. *The Pharma Innovation Journal*, *6*(11), 251–259.

Slimestad, R., & Verheul, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* Mill.) cultivars. *Journal of Science, Food and Agriculture*, *89*, 1255–1270. https://doi.org/10.1002/jsfa.3605

Society, M. (2016). Human Fungal Diseases. *Human Fungal Diseases. Microbiology Society*, 1–2.

Soković, M. D., Glamočlija, J. M., & Ćirić, A. D. (2013). Natural Products from Plants and Fungi as Fungicides. In *Fungicides - Showcases of Integrated Plant Disease Management from Around the World* (pp. 187–232). https://doi.org/10.5772/50277

Stevenson, P. C., & Belmain, S. R. (2017). *Tephrosia vogelii*: A pesticide of the future for African farming. *Boletín SEEA*, *2*, 19–22.

Stevenson, P. C., Kite, G. C., Lewis, G. P., Forest, F., Nyirenda, S. P., Belmain, S. R., Sileshi, G. W., & Veitch, N. C. (2012). Distinct chemotypes of *Tephrosia vogelii* and implications for their use in pest control and soil enrichment. *Phytochemistry*, *78*, 135–146. https://doi.org/10.1016/j.phytochem.2012.02.025

Swamy, A. T., Obey, J. K., Erick, T., & Edwin, M. O. (2015). *In vitro* antibacterial activity of ethanolic-aqua extract of *Tephrosia vogelii* bark against laboratory strains of selected microorganisms. *Baraton Interdisciplinary Research Journal*, *5*, 133–139.

Sweeney, D. L. (2014). A Data Structure for Rapid Mass Spectral Searching. *Mass Spectrometry*, *3*(3), 1–9. https://doi.org/10.5702/massspectrometry.s0035

Tabassum, N., & Hamdani, M. (2014). Plants used to treat skin diseases. *Pharmacognosy Reviews*, *8*(15), 52–61. https://doi.org/10.4103/0973-7847.125531

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical Screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, *1*(1), 98–106.

Tole, T. T., & Neme, A.B. (2019). Chemical Studies of *Tephrosia vogelii* and *Commiphora schimperi* Occurring in Ethiopia. *Modern Chemistry*. *7*(3), 45-53. https://doi.org/ 10.11648/ j.mc.20190703.11

Touqeer, S., Saeed, M. A., & Ajaib, M. (2013). A Review on the Phytochemistry and Pharmacology of Genus Tephrosia. *Phytopharmacology*, *4*(3), 598–637.

Trakranrungsie, N. (2011). Plant derived antifungals-trends and potential applications in veterinary medicine: A mini-review. *Communicating Current Research and Technological Advances*, 1195–1204.

Ukwubile, C. A., Ahmed, A., Katsayal, U. A., Ya'u, J., & Mejida, S. (2019). GC–MS analysis of bioactive compounds from *Melastomastrum capitatum* (Vahl) Fern. leaf methanol extract: An anticancer plant. *Scientific African*, *3*, 10–17. https://doi.org/ 10.1016/j.sciaf.2019.e00059

UNDP. (2015). Sustainable Development Goals. *UNDP*.

Vandeputte, P., Ferrari, S., & Coste, A. T. (2012). Antifungal resistance and new strategies to control fungal infections. *International Journal of Microbiology*, *2012*, 1–27. https:// doi.org/10.1155/2012/713687

Wei, H. H., Xu, H. H., Xie, H. H., Xu, L. X., & Wei, X. Y. (2009). Sesquiterpenes and lignans from *Tephrosia vogelii*. *Helvetica Chimica Acta*, *92*(2), 370–374. https:// doi.org/10.1002/hlca.200800272

WHO. (2015). Global action plan on antimicrobial resistance. In *World Health Organisation* (Issue May).

WHO. (2018). Annex 1 WHO guidelines on good herbal processing practices for herbal medicines. In *WHO Technical Report Series, No. 1010*.

WHO. (2020). *Bulletin of the World Health Organization Growing awareness of skin disease starts flurry of initiatives*. World Health Organisation. https://www.who.int/bulletin/ volumes/83/12/news41205/en

Wiederhold, N. P. (2017). Antifungal resistance: current trends and future strategies to combat. *Infection and Drug Resistance*, *10*, 249–259. https://doi.org/https://doi.org/10.2147/IDR.S124918

Worth, A. P. (2018). Types of Toxicity and Applications of Toxicity Testing. In *The History of Alternative Test Methods in Toxicology* (pp. 7–10). Elsevier Inc. https://doi.org/10.1016/B978-0-12-813697-3.00002-0

Yadav, M. K., Singh, S. K., Singh, M., Mishra, S. S., & Singh, A. K. (2019). In Vivo Toxicity Study of Ethanolic Extracts of *Evolvulus alsinoides* & *Centella asiatica* in Swiss Albino Mice. *Open Access Macedonian Journal of Medical Sciences*, *7*(7), 1071–1076. https://doi.org/10.3889/oamjms.2019.209

Yao, L. H., Jiang, Y. M., Shi, J., Thomas-barberan, F. A., Datta, A., Chen, S., & Singanusong, R. (2004). Flavonoids in Food and Their Health Benefits. *Plant Foods for Human Nutrition*, *59*, 113–122. https://doi.org/10.1007/s11130-004-0049-7

Yarnell, E. (2005). Dosing Botanical Medicines. *Journal of Herbal Pharmacotherapy*, *5*(1), 73–78. https://doi.org/10.1300/J157v05n01_08

Hwang, D. Y. (2018). Therapeutic Role of Natural Products Containing Tannin for Treatment of Constipation. In *Constipation* (pp. 1–19). https://doi.org/10.5772/intechopen.81837

Zahra, G., Khadijeh, B., Hossein, D., & Ali, S. (2019). Essential oil composition of two *Scutellaria* species from Iran. *Journal of Traditional Chinese Medical Sciences*, *6*(3), 244–253. https://doi.org/10.1016/j.jtcms.2019.07.003

APPENDICES

Appendix 1: Ethical clearance certificate

Kibong'oto Infectious Diseases Hospital- Nelson Mandela African Institution of Science and Technology- Centre for Educational Development in Health, Arusha (KIDH-NM-AIST-CEDHA) -KNCHREC

RESEARCH ETHICAL CLEARANCE CERTIFICATE

Research Proposal No:

KNCHREC Ref No. 00022 **28th OCTOBER 2019**

Study Title: Antifungal Investigations and Toxicity Evaluation of *Tephrosia vogelii* Hook. F.

Study Area: MBEYA & ARUSHA REGION & NM_AIST

PI Name: Stephano Hanolo

Co-Investigator:

Institutions: **NM-AIST** School of Life Science and Bio-Engineering (LiSBE) of the Nelson Mandela African Institution of Science and Technology

The Proposal has been approved by KNCHREC on 18TH OCTOBER 2019

1. Subject to this approval you will be required to submit your progress report to the KNCHREC, National Institute for Medical Research, and Ministry of Health Community Development Gender Elderly and Children
2. Publication of your findings is subject to presentation to the KNCREC and NIMR Approval.
3. Copies of final publication should be made available to KNCHREC, National Institute of Medical Research and Ministry of Health Community Development Gender Elderly and Children.

Duration of Study Renewal: Subject to Renewal within ONE YEAR

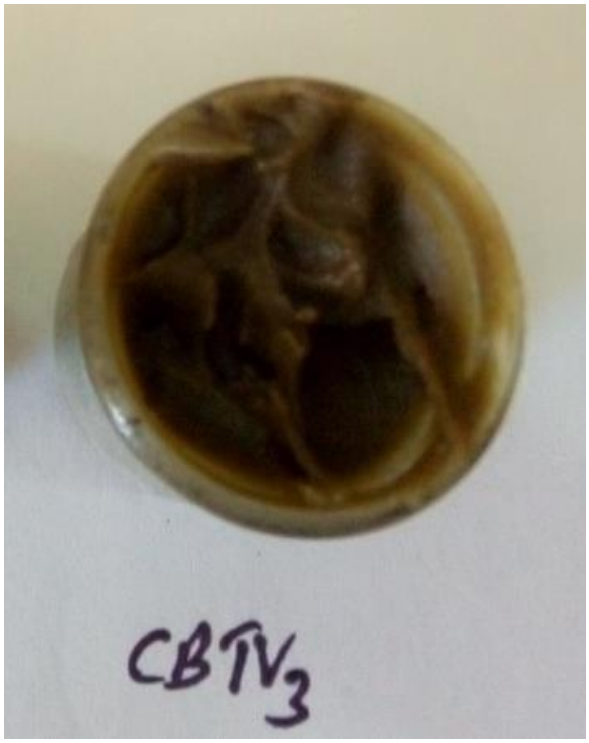
Span From: 18th OCTOBER 2019 to 17TH OCTOBER 2020.

.....

Mr. Simon Njeya
Secretary
KNCHREC

Chairperson
KNCHREC

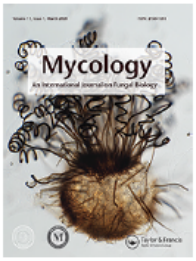
Appendix 2: Research output: Formulated herbal cream for topical application



The product is ready for human clinical trials

Appendix 3: Research output: Journal papers

1. Paper published (https://doi.org/10.1080/21501203.2019.1705929)



Taylor & Francis

Mycology

An International Journal on Fungal Biology

ISSN: 2150-1203 (Print) 2150-1211 (Online) Journal homepage: https://www.tandfonline.com/loi/tmyc20

Antimicrobial activities of *Tephrosia vogelii* against selected pathogenic fungi and bacteria strains

Stephano Hanolo Mlozi, Juma A. Mmongoyo & Musa Chacha

To cite this article: Stephano Hanolo Mlozi, Juma A. Mmongoyo & Musa Chacha (2020) Antimicrobial activities of *Tephrosia vogelii* against selected pathogenic fungi and bacteria strains, Mycology, 11:1, 49-55, DOI: 10.1080/21501203.2019.1705929

To link to this article: https://doi.org/10.1080/21501203.2019.1705929

Published online: 19 Dec 2019.

Stephano Hanolo Mlozi[a,b], Juma A. Mmongoyo[b] and Musa Chacha[a]

[a]School of Life Science and Bioengineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania; [b]Department of Chemistry, University of Dar es Salaam, Mkwawa University College of Education, Iringa, Tanzania

ABSTRACT

Candida albicans and *Cryptococcus neoformans* are dangerous pathogens causing fungal diseases. *C. albicans* and *C. neoformans* developed resistance to fungicides such as fluconazole. Similarly, pathogenic bacteria *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Salmonella typhi* have become resistant to antibiotcs such as methicillin. Thus, searching for alternative antimicrobial agents is inevitable. *Tephrosia vogelii* used traditionally for management of fungal and bacterial diseases is potential source of antimicrobial agents. It is in this vein that, antimicrobial activities of leaf and root extracts of *T. vogelii* were evaluated against *C. albicans* (ATCC 90028), *C. neoformans* (clinical isolate), *S. aureus* (ATCC25923), *E. coli* (ATCC29953), *K. pneumoniae* (ATCC 700603) and *S. typhi* (NCTC 8385). A two-fold serial dilution method using the sterilised 96 wells of polystyrene microlitre plates used to determine the minimum inhibitory concentration (MIC) of extracts. Hexane and dichloromethane extracts exhibited the lowest activity against fungi strains with MICs >10 mg/mL. Root and leaf methanolic extracts exhibited activity at MICs of 5 and 1.25 mg/mL, respectively, against both tested fungi. Dichloromethane and methanolic extracts exhibited antibacterial activity with MICs ranging from 2.5 - 10 mg/mL and 0.625 - 5 mg/mL, respectively. Antimicrobial activities of the extracts of *T. vogelii* revealed potentiality of bioactives against fungal and bacterial diseases.

ARTICLE HISTORY
Received 18 September 2019
Accepted 13 November 2019

KEYWORDS
Tephrosia vogelii; antifungal agents; antibacterial agents; methanolic extracts

2. Paper Published (https://doi.org/10.1186/s40816-020-00216-6)

Mlozi *et al. Clinical Phytoscience* (2020) 6:73
https://doi.org/10.1186/s40816-020-00216-6

Clinical Phytoscience

ORIGINAL CONTRIBUTION Open Access

The in vivo toxicity evaluation of leaf and root methanolic extracts of *Tephrosia vogelii* Hook.f using animal model

Stephano Hanolo Mlozi[1,2*], Juma A. Mmongoyo[2] and Musa Chacha[1]

Abstract

Background: Traditionally, herbal medicines are commonly used to cure several diseases since immemorial of human life. Nevertheless, the safety of some traditionally used medicinal plants is uncertain. Since *Tephrosia vogelii* Hook.f is a traditionally used medicinal plant, the effects of its extracts were evaluated on lethality (LD_{50}) and sub-acute toxicity in this study.

Methods: Phytochemistry screening and an in vivo toxicity evaluation of leaf and root methanolic extracts of *T. vogelii* using laboratory albino rats were conducted. Methanolic extracts of doses 600, 1200, 2000 and 5000 mg/kg body weights were administered single dose in rats to observe deaths within 72 h in order to determine the LD_{50}. Methanolic extracts doses of 600, 1200 and 2000 mg/kg body weights were consecutively administered for 14 days in order to evaluate sub-acute toxicity.

Results: Tannins, steroids, terpenoids, flavonoids and saponins were identified in the phytochemical screening. The LD_{50} experiments revealed zero deaths of rats for the administered doses, 600 to 5000 mg/kg body weight. Histopathological examination of liver and kidney for sub-acute toxicity test showed safety at all doses except root methanolic extracts dose of 2000 mg/kg which exhibited necrosis and vacuolation of liver cells on the 14th day. Nonetheless, hepatic necrosis and hepatic vacuolation disappeared upon time elongation without dose administration to 28th day.

Conclusion: The conducted toxicity evaluation of methanolic leaf and root extracts in albino rats revealed no deleterious effects, henceforth, suggesting that *T. vogelii* could be safe to users using it as a medicinal plant.

Keywords: Herbal medicines, *Tephrosia vogelii* Hook.f, Methanolic extracts, Lethality, Sub-acute toxicity

3. Manuscripts submitted to Journals: Under Review

Journal of Traditional Chinese Medical Sciences

GC-MS Analysis of Bioactive Phytochemicals from Methanolic Leaf and Root Extracts of Tephrosia vogelii

--Manuscript Draft--

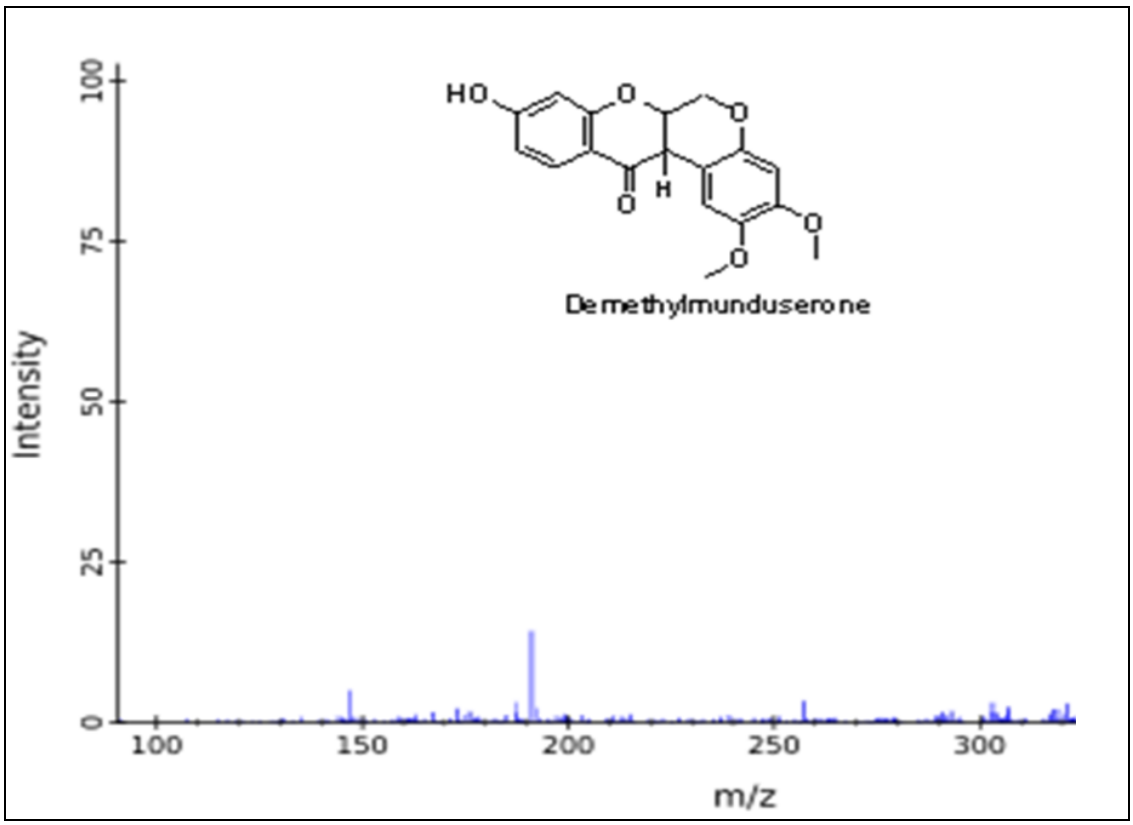
Manuscript Number:	JTCMS-D-YR-01063
Article Type:	Full Length Article
Keywords:	Tephrosia vogelii; medicinal plants; bioactives; GC-MS analysis; phytochemicals
Corresponding Author:	Stephano Hanolo Mlozi Nelson Mandela African Institute of Science and Technology School of Life Sciences and Bio-Engineering Arusha, TANZANIA, UNITED REPUBLIC OF
First Author:	Stephano Hanolo Mlozi, MSc & PhD Candidate
Order of Authors:	Stephano Hanolo Mlozi, MSc & PhD Candidate
	Juma A. Mmongoyo, PhD
	Musa Chacha, PhD
Abstract:	Objective Tephrosia vogelii is a medicinal plant known for its ethnomedical properties and pharmacological inferences. Its ability to synthesize bioactive secondary metabolites is undoubtedly what brands its medicinal possessions and value. The bioactive secondary metabolites from this species could be important agents for drug development against fungal infections. Discerning the secondary metabolites of such medicinal plant is indispensable. Thus, the objective of this study was to determine phytochemical compounds from methanolic root and leaf extracts of T. vogelii . Methods Phytochemical screening was conducted to determine nature of secondary metabolites while the Gas Chromatography-Mass Spectrometry, (GC-MS) was conducted to determine specific phyto-compounds present in the methanolic root and leaf extracts. Phytochemical compounds were ascertained based on molecular weights (m/z) acquired from GC-MS chromatograms. Compounds were established through interpretation of spectral peaks and comparing data with stored databases from National Institute Standard and Technique (NIST) library.

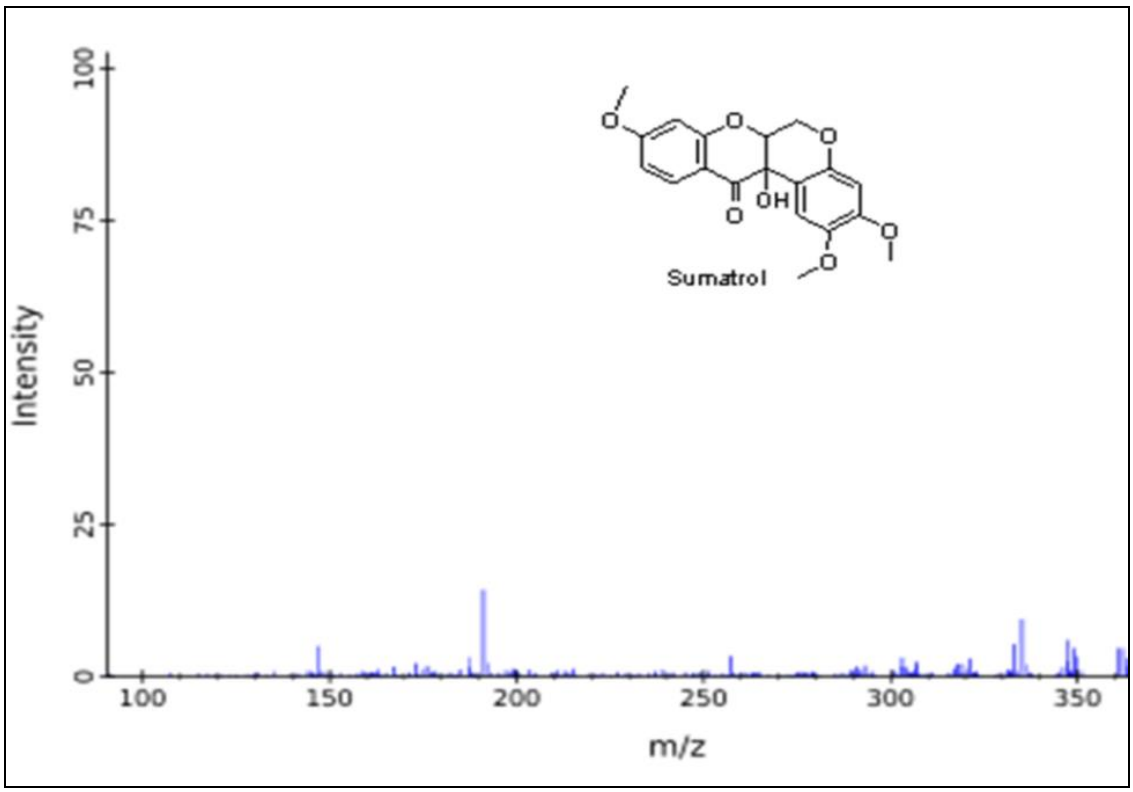
4. Manuscripts submitted to Journals: Under Review

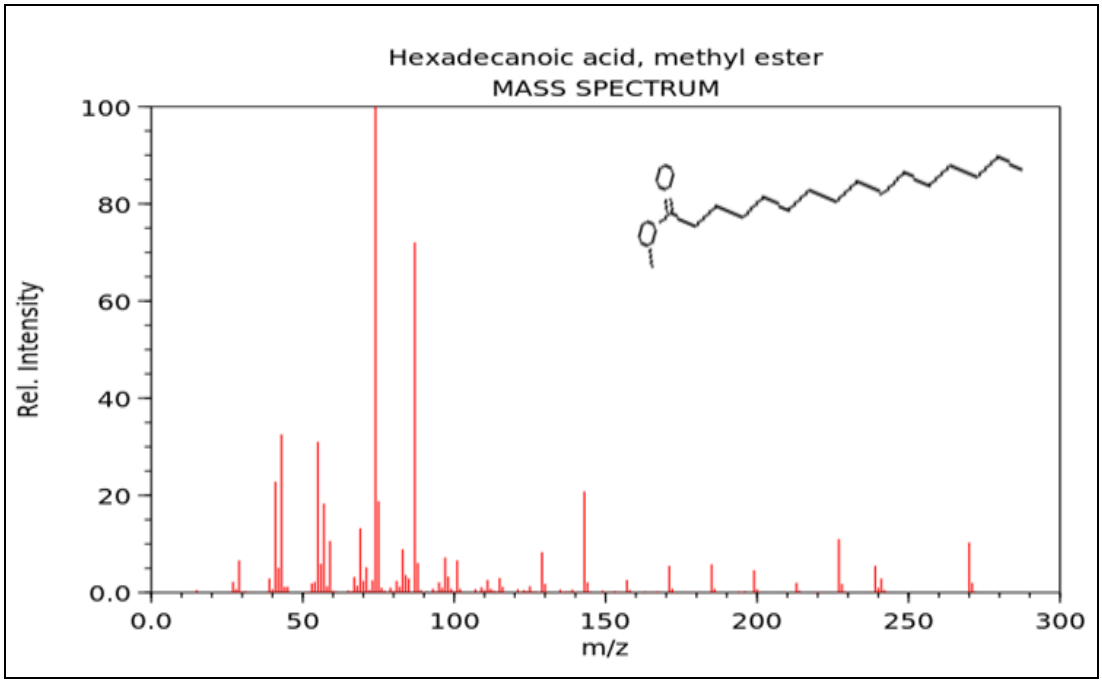
Formulation and Evaluation of Herbal Cream from Methanolic Leaf Extracts of Tephrosia vogelii Hook.f for Topical Application

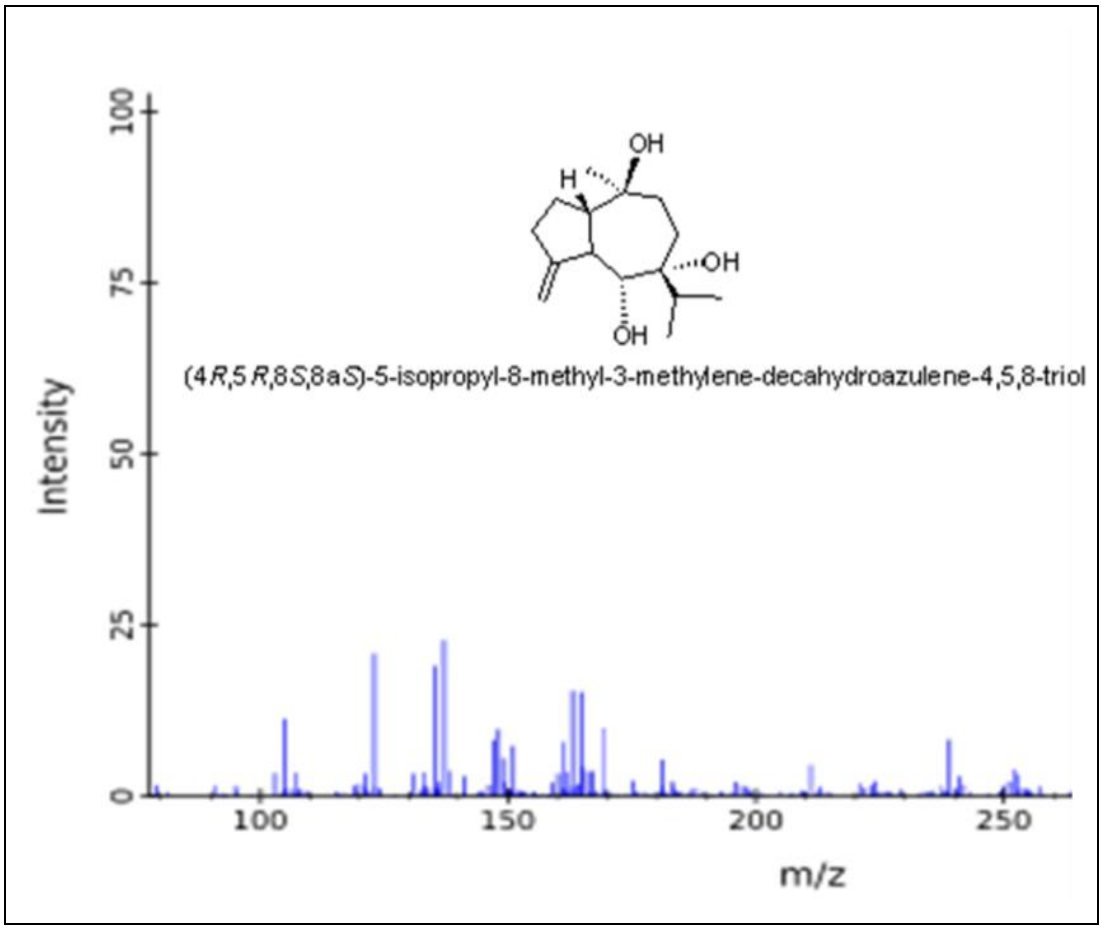
Journal:	*Journal of Dermatological Treatment*
Manuscript ID	2020-JDT-OR-1077
Manuscript Type:	Original Article
Date Submitted by the Author:	14-Oct-2020
Complete List of Authors:	Mlozi, Stephano; Nelson Mandela African Institute of Science and Technology, ; Mkwawa University College of Education Faculty of Science, Chemistry
Keywords:	Tephrosia vogelii Hook.f, herbal cream, disc diffusion, skin infection,, topical therapy

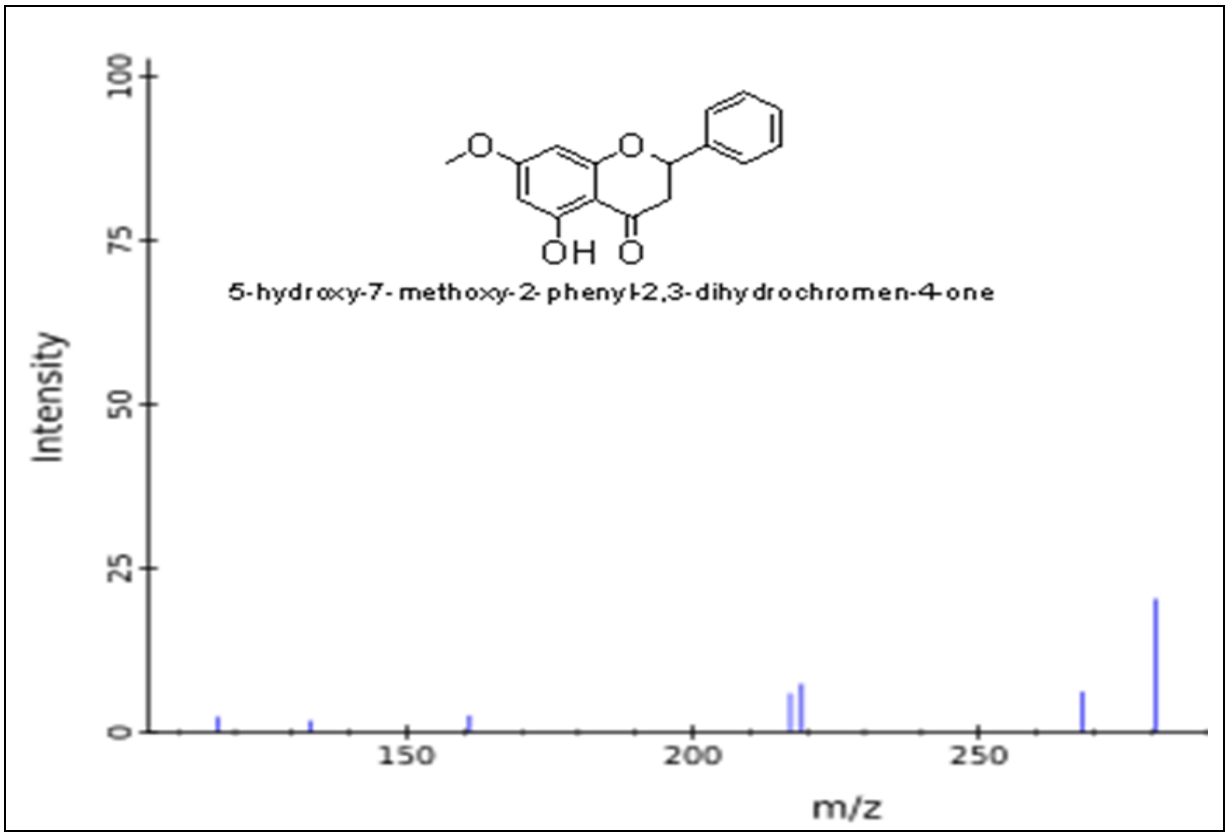
Appendix 4: Mass spectra of phytochemical compounds identified from *Tephrosia vogelii*

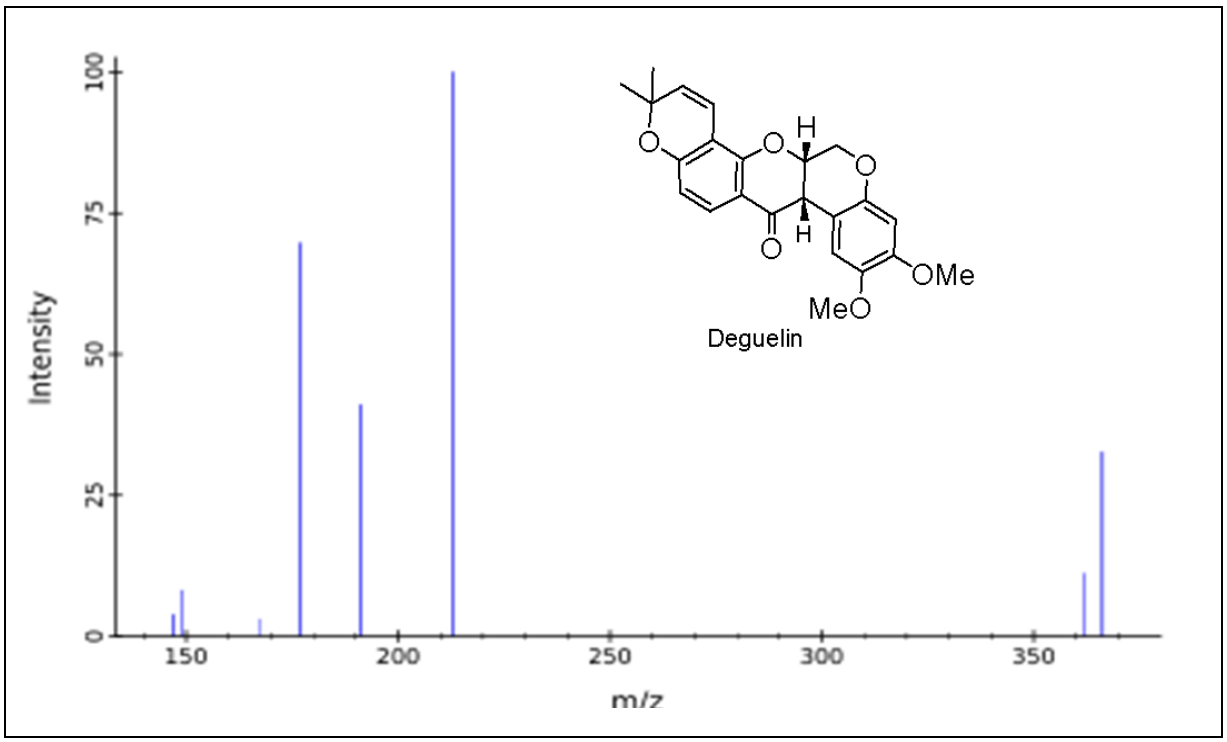


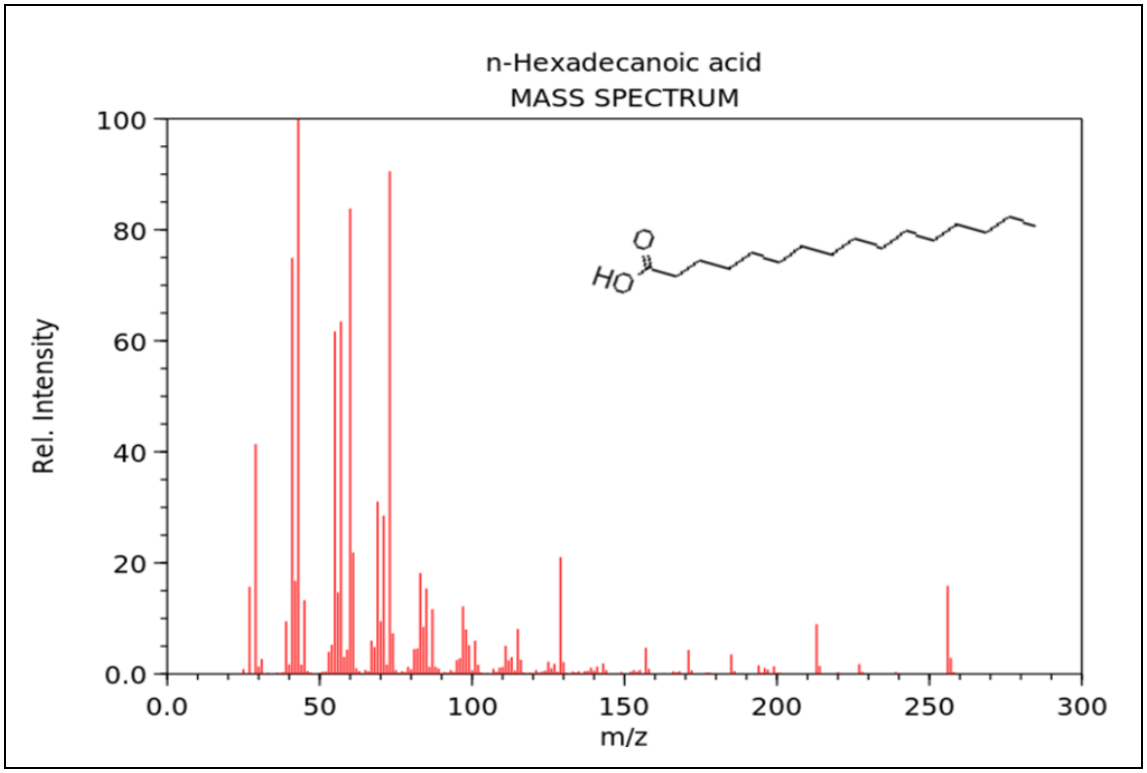


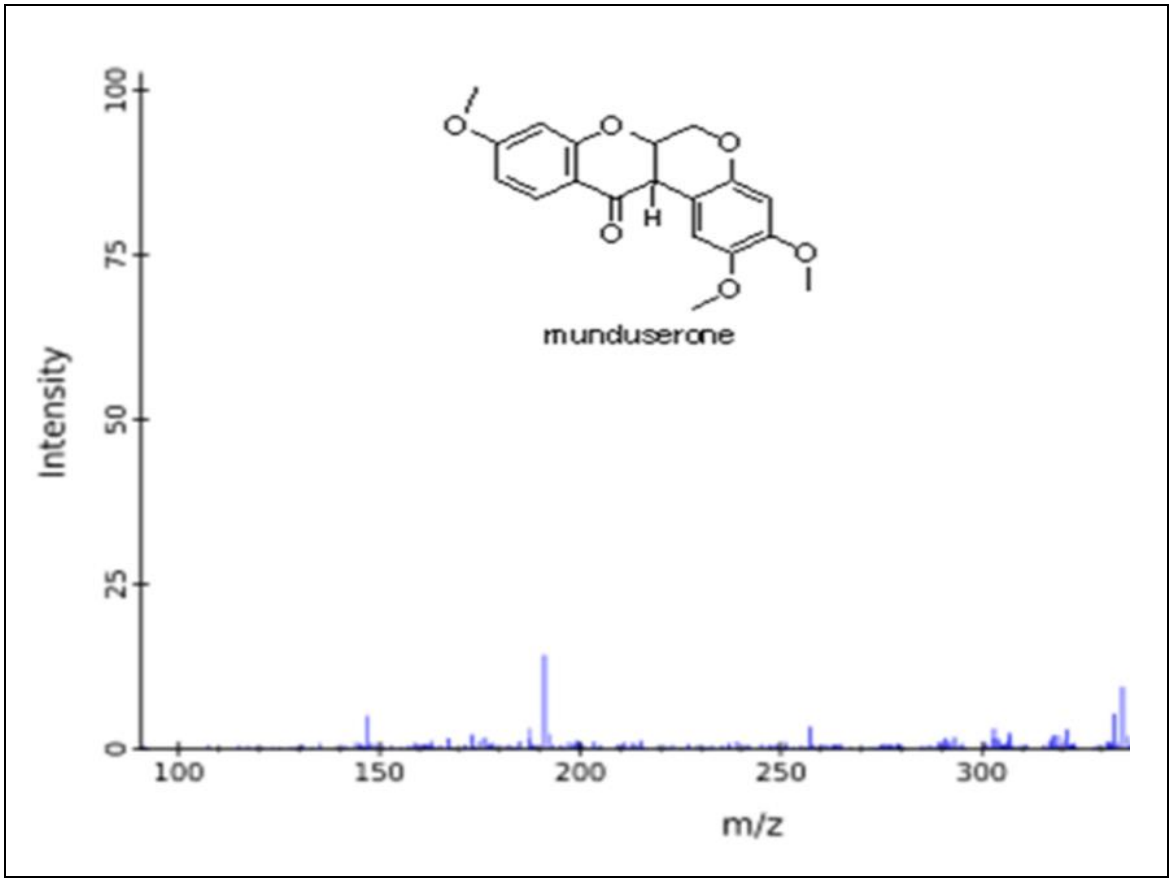

Hexadecanoic acid, methyl ester
MASS SPECTRUM
Rel. Intensity
m/z
100
80
60
40
20
0.0
0.0
50
100
150
200
250
300
O
O

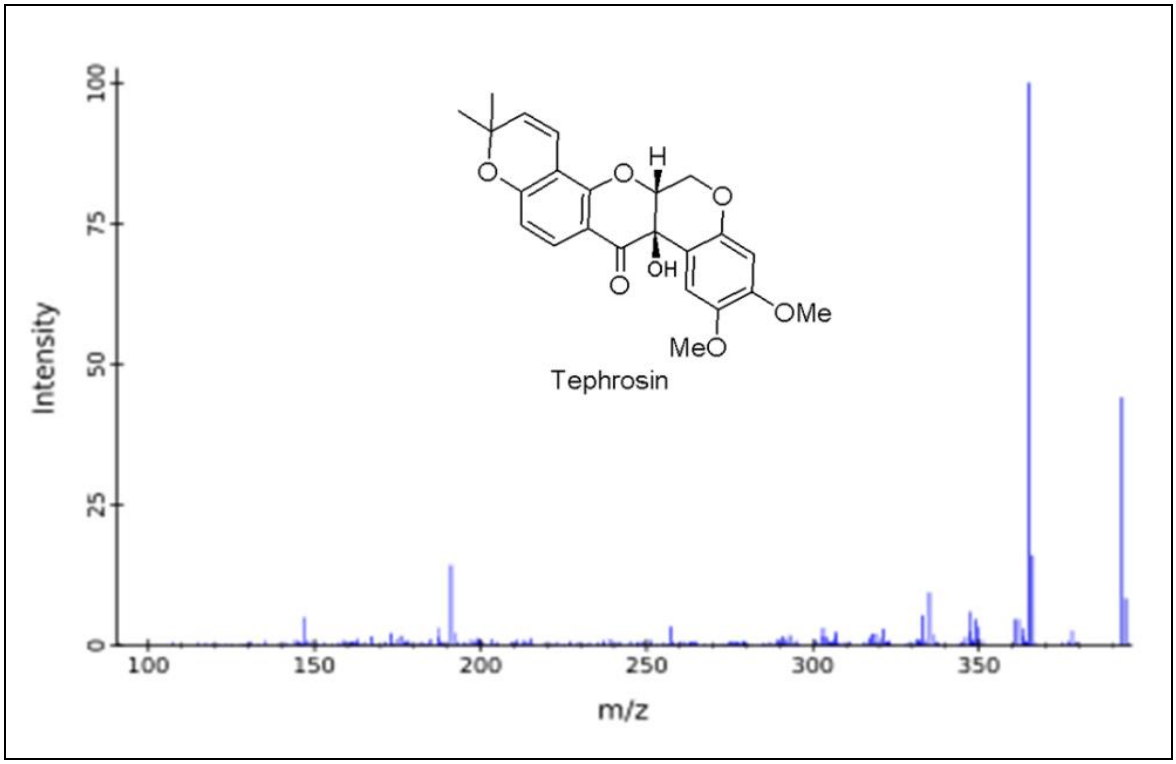

Intensity
m/z
100
75
50
25
0
100
150
200
250
OH
H
OH
OH
(4R,5R,8S,8aS)-5-isopropyl-8-methyl-3-methylene-decahydroazulene-4,5,8-triol

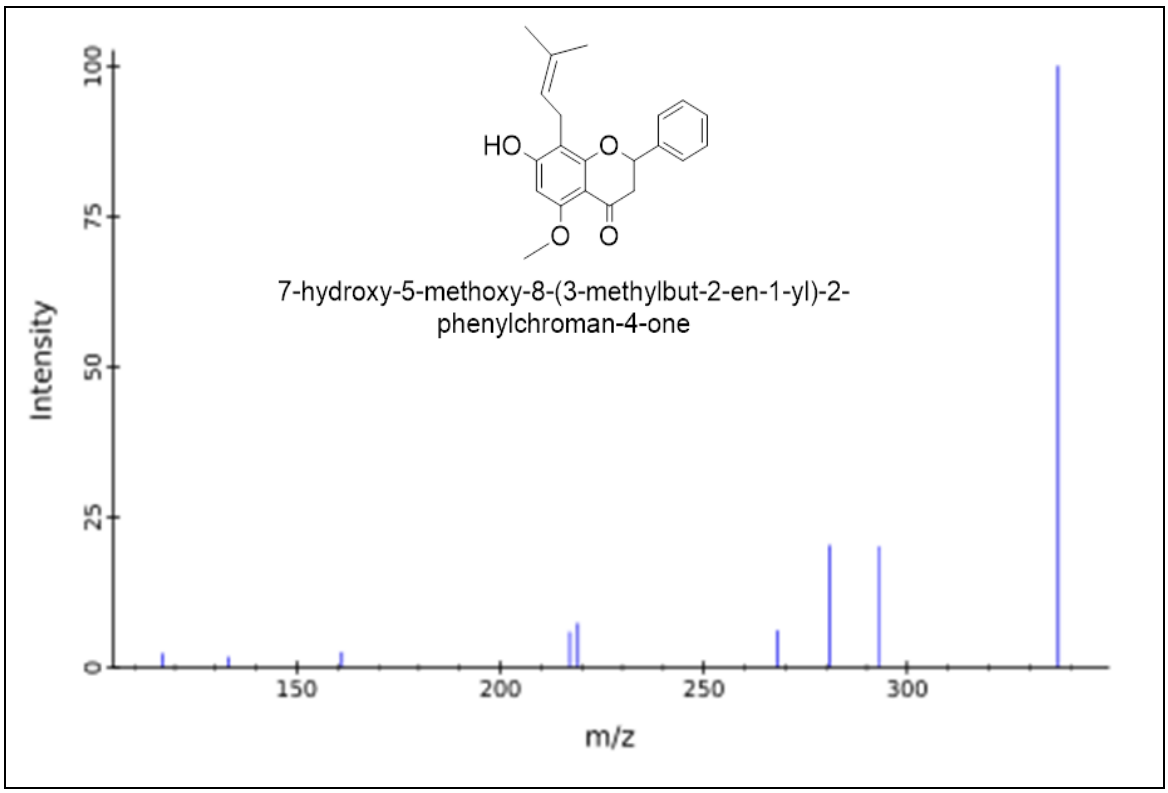

Intensity
100
75
50
25
0
150
200
250
m/z
OH
O
O
O
5-hydroxy-7-methoxy-2-phenyl-2,3-dihydrochromen-4-one


Intensity
100
75
50
25
0
150
200
250
300
350
m/z
O
O
H
O
H
O
OMe
MeO
Deguelin


n-Hexadecanoic acid
MASS SPECTRUM
Rel. Intensity
m/z
100
80
60
40
20
0.0
0.0
50
100
150
200
250
300
O
HO


munduserone
Intensity
m/z
100
75
50
25
0
100
150
200
250
300
O
H


Intensity
100
75
50
25
0
100
150
200
250
300
350
m/z
H
O
O
O
OH
OMe
MeO
Tephrosin


Intensity
100
75
50
25
0
150
200
250
300
m/z
HO
O
O
O
7-hydroxy-5-methoxy-8-(3-methylbut-2-en-1-yl)-2-
phenylchroman-4-one