COMPARATIVE ANALYSIS OF *Pavetta crassipes* AND *Pavetta schumanniana* AS ETHNOMEDICINES FROM DEDZA AND MZIMBA IN MALAWI

MSc THESIS (FORESTRY AND ENVIRONMENTAL MANAGEMENT)

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MZUZU UNIVERSITY

JULY, 2024

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BSc (Environmental Management)

A THESIS SUBMITTED TO THE FACULTY OF ENVIRONMENTAL SCIENCES, DEPARTMENT OF FORESTRY AND ENVIRONMENTAL MANAGEMENT, IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER OF SCIENCE (MSc) DEGREE IN FORESTRY AND ENVIRONMENTAL MANAGEMENT

JULY, 2024

DECLARATION

I, Mwayi Chirwa, hereby declare that the work on which this thesis is based is my own original work (except where acknowledgements indicate otherwise) and that neither the whole work or part of it has been, is being or is to be submitted for another degree in this, or any other University.

Mwayi Chirwa

Signature: ……

Date: ……

CERTIFICATE OF APPROVAL

We the undersigned certify that this thesis represents the student's own work and effort and that, to the best of our knowledge, it has not been submitted for any other academic awards within Mzuzu University or elsewhere.

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DEDICATION

I dedicate this work to my dear son, Boaz Chibaka, and my dear husband, Mr. W. Chibaka who believed in my abilities, and continually encouraged me in my studies.

ACKNOWLEDGEMENT

I thank The Almighty God who has been the source of my strength, sound health and grace to be connected to the right people during the entire period of my study. I sincerely thank my supervisors, Associate Professor Jarret Mhango, Associate Professor John Kamanula and Mr. Twalibu Tandwe. I consider it a great privilege to be trained and mentored by these great scientists. I appreciate the help of the Late Associated Professor Chimuleke Yagontha Munthali, the Late Dr. Dominic Gondwe, Dr. Dalo Njera and Brave Mwanza who assisted me in my work in various ways throughout my study.

I also thank Mr. Elvis Manda and Mr. Winfrey Kayange of Chemistry and Biology laboratories respectively of Mzuzu University for their assistance in preparation of solutions and setting up instrumentation for data collection. I specially acknowledge Mr. Njala, Forestry Officer for Mtakataka and all staff members of Mtakataka Forestry Department for their assistance during field data collection. God should bless them abundantly.

I sincerely thank the Ministry of Education Science and Technology for supporting my research (partially) through Innovation Grant Scheme for Tertiary Students in Malawi and Research Grant for Tertiary Student in Malawi. Funding for this study was provided (in part) by University of Livingstonia. This afforded me a great opportunity to conduct experiments that were expensive. I also thank Mzuzu University for administering the program. My special thanks also go to all lecturers in the Forestry and Environmental Management Department for their support throughout my study.

I sincerely thank my parents, Mr. and Mrs. Chirwa, for the education they gave me and for being a great source of inspiration. Thanks also to my siblings; my two sisters, Maureen and Mercy, and my two brothers, Phillip and Saul. You have always showed me your love and support. I also acknowledge my friends, Leah Ngoma Mhango and Wezzie Nyasulu, who helped me in many ways while I was pursuing this program.

ABSTRACT

Pavetta crassipes and *P. schumanniana* are some of the neglected and underutilised, yet useful medicinal plants in Malawi. In regions where *P. crassipes* and *P. schumanniana* leaves are traditionally used for medicinal purposes, there is lack of scientific understanding regarding their therapeutic efficacy, chemical composition, and antimicrobial properties. The objective of the study was to compare ethno medicinal use, phytochemical composition and antimicrobial activity of *P. crassipes* and *P. schumanniana* plant leaves. The study was conducted in March, 2022. This study used both qualitative and quantitative methods. In ethno medicinal survey, simple random and snowball sampling procedures were used to select sites and respondents respectively. Data were analysed using SPSS version 20 and STATA version 16. Chi-square test was used to determine significant differences or associations in parameters of interest between the two plant species. For phytochemical analyses, qualitative chemical methods of analysis were used to study phytochemical compounds. For microbial effects, the diffusion disc method was used to assess antimicrobial activity of the leaf extracts against *E coli* and *S. aureus*. The zone of inhibition of *P. crassipes* and *P. schumanniana* aqueous leaf extract in mm were entered into STATA version 16, and thereafter were subjected to One Way Analysis of Variance (ANOVA). Some diseases that were reported to be managed by *P. schumanniana* and were common whilst others were specific to one plant species. *P. schumanniana* was commonly used to manage cough while *P. crassipes* was commonly used as libido enhancer. Phytochemical analysis revealed the presence of bioactive compounds in *P. crassipes* leaves which were steroids, tannins, anthocyanin and anthraquinones, while *P. schumanniana* leaves contained saponins, terpenoids/steroids, tannins and anthocyanins. Alkaloids and flavonoids were not detected in both plant species. The data showed a concentration dependent efficacy on *E. coli* while *S. aureus* was resistant to both species. The most effective concentration (100 µg/mL) of *P. crassipes and P. schumanniana aqueous* leaf extract recorded highest mean zone of inhibition of 8.10±0.89 mm ($p = 0.915$) and 7.73±0.67 mm ($p = 0.999$) for *P. crassipes* and *P. schumanniana,* respectively. This study validates the traditional uses of *Pavetta schumanniana* and *Pavetta crassipes*, highlights their potential as sources of antimicrobial agents, and provides a foundation for further research into their bioactive compounds.

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ACRONYMS

Acronym	Meaning
ABC	ATP Binding Cassette
ASL	Above Sea Level
ATP	Adenosine Triphosphate
CFU	Colony Forming Units
COVID-19	Corona Virus Disease of 2019
DFO	District Forestry Office
DNA	Deoxyribonucleic Acid
EGCG	Epigallocatechin Gallate
FAO	Food and Agriculture Organization
GLUT-4	Glucose Transporter - 4
HCL	Hydrochloric acid
HDL	High Density Lipoprotein
HepG2	Human Hepatocellular Liver Carcinoma Cell Line
HPV	Human Papilloma Virus
HIV/AIDS	Human Immuno-Deficiency Virus/Acquire Immune Deficiency Virus
IMF	Intermediate Moisture Foods
Inos	Inducible Nitric Acid Oxide Synthase
IR	Insulin Resistance
ITK	Indigenous Traditional Knowledge
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide

MBC	Minimum Bactericidal Concentration
MIC	Minimum Inhibition Concentration
MG	Malawi Government
MGDS	Malawi Growth Development Strategy
MOLT	Nuclear Factor Kappa-Light-Chain Enhancer of Activated B Cells
MRSA	Methicillin Resistant Staphylococcus Aureus
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
PUFA	Polyunsaturated Fatty Acid
RNA	Ribonucleic Acid
SDGs	Sustainable Development Goals
STZ	Streptozoticin
TTP	Thrombotic Thrombocytopenic Purpura
UTI	Urinary Tract Infection
UV	Ultraviolet Light
VT	Verotoxin

DEFINITION OF TERMS

Term		Definition
Antimicrobial	:	An agent that destroys or inhibits growth of microorganism that might cause diseases.
Bactericidal:		The agent that kills bacteria
Bacteriostatic	:	The agent that inhibits the growth of bacteria i.e. it keeps them in stationary phase of growth

Minimum bactericidal concentration: The lowest concentration of an antibacterial agent required to kill a particular bacterium

Minimum inhibition concentration: The lowest concentration of an antibacterial agent that prevents visible growth of bacteria after overnight incubation

Traditional knowledge: Indigenous information passed down from one generation to another or among one generation

Phytochemical compounds: Natural active compounds found in plants which prevent pathogens from causing diseases.

Purposive sampling: A non-probability sampling technique used by researcher to find respondents in a population

Sensitive: The efficacy of plants extract to control bacteria.

Snowballing: Sampling technique used to identify respondents in a population.

Susceptibility: Response in controlling bacteria by plant extract or antibiotic.

Zone of inhibition: The diameter surrounding presence of bacteria, a sign of controlling bacteria by the use of plant extract or antibiotic.

CHAPTER ONE: INTRODUCTION

1.1 Background information

The importance of herbs in the management of human ailments cannot be overstated. In the plant kingdom, most of the plants have medicinal values and their application especially in traditional medicine is currently well acknowledged and established as a viable profession (Balasundram, Sundram and Samman, 2006). It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases (Parekh and Chanda, 2010). Scientific evidence has shown that many plants have medicinal properties to treat diseases and alleviate symptoms or prevent diseases(Lai and Roy, 2012; Shang *et al.*, 2019).

Study findings have reported presence of phytochemicals in medicinal plants. These chemical compounds are also known as secondary plant metabolites and they provide health benefits to humans. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). Phytochemicals primarily protect plants from disease and damage, and also contribute to the plant's colour, aroma and flavor. Phytochemicals accumulate in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed (Costa *et al.*, 1999; Okunlola *et al.*, 2017). The levels of these phytochemicals vary from plant to plant depending upon the variety and climatic growing conditions (Narasinga, 2003; Kamanula *et al.*, 2017). In the genus Pavetta, *Pavetta crassipes* and *Pavetta schumanniana* are two of the many medicinal plants found in Malawi although their medicinal attributes have not been well documented to date. They remain neglected and underutilized as valuable sources of medicine among other uses.

1.1.1 *Pavetta crassipes* and *Pavetta schumanniana*

Pavetta crassipes K. Schum. (Rubiaceae) is a low glabrous shrub of the savannah with stout sub-quadrangular branches covered with pale corky bark which splits and falls off and it can grow 1.8 meters tall (Daget, 2002). Leaves are usually clustered near the apices of the branches, paired, or occasionally ternate or quadrate, glabrous; blades 8-30 × 1.3-7.5 cm, linear to narrowly elongate-oblong or oblanceolate, rounded or sometimes obtuse at the apex, obtuse to attenuate at the base; lateral nerves in 8-11 main pairs (Daget, 2002). Inflorescences corymbose, crowded, (3)7.5-17 cm across (excluding corollas). Flowers are white and green, pedicellate, with shortly dentate, glabrous

tubular calyx, glabrous tubular corolla about 12-18 mm long. Fruit black, shiny, 6-8 mm in diameter; pedicels slightly accrescent; calyx limb persistent. Seeds greyish-brown, 5-5.5 mm wide, slightly rugulose on convex face (Daget, 2002; Zerbo *et al.*, 2011).

Pavetta schumanniana F. Hoffm. ex K. Schum (Rubiaceae) is a much-branched shrub or tree up to 4 m high (Verstraete *et al.*, 2011). Leaves are usually opposite, but often whorled with triangular stipules between them, large, broadest towards the tip. They have a rough leathery texture and are finely and densely hairy below. Venation is raised on the underside, and the leaves tend to have round dark bacterial nodules scattered throughout the leaf surface (Bridson, 2001). *Pavetta schumanniana* flowers are usually dense white fragrant clusters. The calyx lobes are toothed, with sharp tips. Fruits are mostly produced in summer. They are round and fleshy, ± 8 mm in diameter. At first they are green, but they change to glossy black after reaching maturity (Bridson, 2001).

1.1.2 Ethno medicinal information of *Pavetta crassipes* and *Pavetta schumanniana*

The leaves of *P. crassipes* are used to treat schistosomiasis or haematuria, malaria, splenosis, fever, conjunctivitis, syphilis sores, diarrhoea, stiffness, weakness, respiratory diseases, hypertension (Daget, 2002; Zerbo *et al.*, 2011). The roots of *P. crassipes* are used for treating gonorrhea, in some cases as a laxative in times of constipation (Daget, 2002; Mponda, 2012). The bark of *P. crassipes* is used for treating snake bite, febrifuge and thyroid stimulating while roots and leaves of *P. crassipes* are used to treat fever, vitamin deficiency and kwashiorkor, and fruits are used as vermifuge (Zerbo *et al.*, 2008; Mponda, 2012). In Nigeria, leaves of *P. crassipes* are used as medicine in the management of respiratory infections and abdominal disorders (Aliyu *et al.*, 2008; Zerbo *et al.*, 2008). The leaves are also utilized locally in Tanzania for treating gonorrhea (Aliyu *et al.*, 2008).

Alkaloid extracts from the plants have been shown to have significant anti-malarial activity (Sanon *et al.*, 2003). The ethanol extract has been shown to lower the blood pressures of cats and rats in a dose dependent manner (Amos *et al.*, 1998, 2004; Bello, Ndukwe and Audu, 2014). A bioactive flavonoid extract has been shown to have antimicrobial properties (Bello *et al.*, 2011; Bello, Ndukwe and Audu, 2014). In Zimbabwe, leaves of *Pavetta schumanniana* are used as medicine in the management of respiratory infections such as coughing. It is also used for treating infertility and venereal diseases in women (Bridson, 2001).

Undocumented results indicate that some people in Enukweni, Mzimba districts in Malawi use leaves of *Pavetta schumanniana* to treat different ailments which include respiratory problems, coughing, malaria and sexually transited diseases. Despite the wide use of *P. crassipes* and *P. schumanniana*, information on their efficacy as well as chemical composition is not fully investigated and documented in Malawi. In addition, the global demand for medicinal plants is still increasing exponentially, as people are becoming aware of the potency and side effects of synthetic drugs (Verma and Singh, 2008; Street and Prinsloo, 2013). Malawi has a very small share of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants. Therefore, it is imperative to conduct this study.

Generally, phytochemical production in plants varies because of different factors such as: genetics, physiological variations which include organ development, pollinator activity circle, type of plant material, type of secretory structure, seasonal variations and mechanical or chemical injury (Chanli, 2012; Tiwari and Cummins, 2013; Drahansky *et al.*, 2016). Phytochemical composition is also influenced by geographic variation and environmental conditions which include climate, pollution, diseases and pests and edaphic factors (Figueiredo *et al.*, 2008). Genetic factors and evolution also influence chemical composition in plants. Genetic and hybridization studies reported that the composition of phytochemicals is under genetic control (Figueiredo *et al.*, 2008). Despite that species of the same genus possess similar traits, chemical composition in *P. crassipes* and *P. schumanniana* may be different because of geographic variations and environmental conditions (Figueiredo *et al.*, 2008). Therefore, it is hypothesized that in spite of P. *crassipes* and *P. Schumanniana* belonging to the same genus, they are used differently in the two districts under study. The current study therefore investigated the uses of *P. crassipes* and *P. schumanniana* in managing different ailments by the communities in their area of occurrence. *P. crassipes* and *P. schumanniana* are found in several parts of Malawi and many other African countries but due to geographical differences, it is important to known their chemical composition as may be influenced by their geographical origin. These plants are already locally used in processed form as powder, concoction and fresh leaves to treat several ailments (Mponda, 2012), therefore the significance of ethno medicinal use of these species cannot be overstated. These factors, among others, made this study imperative.

1.1.3 Phytochemical compounds of *Pavetta crassipes* and *Pavetta schumanniana*

Pavetta crassipes and *P. schumanniana* leaves like other medicinal plants contain phytochemicals, which are natural plant chemicals which provide health benefits to humans (Bridson, 2001). Phytochemicals are natural products for example, tannin, vitamins, terpenoids, phenolic acids, lignin, flavonoids, anthraquinones, anthocyanins and alkaloids (Shad *et al.*, 2014; Nyamai *et al.*, 2016a), that are found in medicinal plants. Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). The levels of these phytochemicals vary from plant to plant depending upon the variety and climatic growing conditions (Narasinga, 2003). Knowledge of phytochemical variation is very important to understand the biological activities of the plant species and their relevance in the management of different ailments.

Phytochemicals vary among plants belonging to the same genus although they may possess similar traits (Figueiredo *et al.*, 2008). The hypothesis was that phytochemical composition of *P. crassipes* and *P. schumanniana* leaves may vary if obtained from different geographic regions. However, no systematic studies in Malawi have been done to compare the phytochemical composition of *P. crassipes* and *P. schumanniana* leaves which makes the species effective against a diversity of pathogens. The current study was a way of verifying the medicinal properties of *P. crassipes* and *P. schumanniana* leaves, through antimicrobial assessments as they are reported by users to be effective in curing a number of diseases.

Medicinal plants have important contributions in the health care system of local communities as the main source of medicine for the majority of the rural population. About 60 % of the world population and 80% of the population of developing world, Malawi inclusive, rely on traditional medicine (Amber *et al.*, 2017). Similarly, more than 4.5 billion people in the developing world, including Malawi, rely on medicinal plants as components of their health care (Aggarwal *et al.*, 2006). Recent WHO report on traditional and complementary medicine reported that 41 of the 47 Member States in the WHO African Region (87%) formally acknowledged the use of T&CM by their populations (WHO, 2019). This observation could be attributed to several factors including inaccessibility to modern drugs, the low modern-drug purchasing power within the populations living in the rural areas, in addition to side effects posed by synthetic drugs (Bhat, Kumar and Bussmann, 2013). Moreover, some of the local remedies are effective and, as such, there is no need for anything different (Lifongo *et al.*, 2014). Therefore, it is importantly to know the phytochemistry

of these plant species that are already in use by rural people in Malawi. Even though similar studies were done elsewhere but it is important to study them because chemical composition is influenced by several factors such as genetic, geographic variations and climatic conditions of an area (Figueiredo *et al.*, 2008). This was deemed an important factor worth studying.

1.1.4 Antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana*

Phytochemical constituents that protect plants from environmental hazards such as pollution, stress, drought, ultra violet exposure and pathogenic attack have found applications in human medicine (Nyamai *et al.*, 2016a). Phytochemical constituents from plants control the growth of microbes such as bacteria, fungi, parasites and viruses (Nascimento *et al.*, 2000; Bello *et al.*, 2011; Ločárek *et al.*, 2015).

Several studies have reported that *P. crassipes* leaves contain phytochemicals such as flavonoids, terpenoids, saponins, alkaloids, anthocyanins, anthraquinone, steroids and tannins. Flavonoids have been reported to exert multiple biological properties including antimicrobial, cytotoxicity, anti-inflammatory as well as anti-tumor activities; but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Tapas, Sakarkar and Kakde, 2008).

Tannins are known to be antimicrobial agents against fungi, viruses, yeast and bacteria (Borrelli and Izzo, 2000), anti-diabetic (Kang *et al.*, 2011) and anti-tumor agents (Cassidy, Hanley and Lamuela-Raventos, 2000). Terpenoids possess anti-oxidant, antimicrobial, anti-tumor, anti-inflammatory and anti-hepatotoxic properties (Yang *et al.*, 2008; Prakash, 2017; Guimarães *et al.*, 2019). Saponins exhibit anti-phlogistic, anti-allergic, cytotoxic, anti-tumor, anti-viral, anti-hepatotoxic, molluscicidal, anti-bacterial, anti-parasitic, anti-fungal activities, and immunoadjuvant activities (Francis, Wolf-Watz and Forsberg, 2002; Barbosa, 2014). Alkaloids have been reported to demonstrate antioxidant activity responsible for various biological activities including anti-diabetic activity (Cassidy, Hanley and Lamuela-Raventos, 2000; Gupta *et al.*, 2011; Abdirahman *et al.*, 2015) and anti-arrythmic, anti-hypertensive, anti-cancer, anti-bacterial, anti-fungal and anti-malarial activity (Cassidy, Hanley and Lamuela-Raventos, 2000; Kwan and Achike, 2002; Raskin *et al.*, 2005; Gupta *et al.*, 2011; Ločárek *et al.*, 2015; Mabhiza, Chitemerere and Mukanganyama, 2016). Anthocyanins have several health benefits, such as prevention of chronic diseases, antimicrobial, anti-oxidative and anti-inflammatory effects as well as improving vision and memory (Khoo, Lim and Azlan, 2019; Shang *et al.*, 2019; Winter and Bickford, 2019). Anthraquinones were

reported to have anti-plasmodial, anti-tuberculosis, anti-fungal and anti-cancer activity (Kanokmedhakul, Kanokmedhakul and Phatchana, 2005).

Although anti-bacterial activity of *P. schumanniana* leaf extract has not been reported elsewhere in Malawi, however, studies revealed *that Morinda tinctoria, Mussaenda frondosa, Psychotria gardneri* and *Psychotria stenophylla* which are related species of the same family (Rubiaceae) inhibit the growth of bacteria, such as *Saccharomyces cereisiae; Ustilago maydis, Escherichia coli; Micrococcus luteus; Bacillus subtilis, Bacillus cereus* and *Aspergillus niger* (Jayasinghe *et al.*, 2002). Studies have reported that *P crassipes* leaf extract controls the growth of bacteria such as *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Proteus species, Neisseria gonorrhea,* and *Pseudomonas aeruginosa* and others, which cause various diseases (Baldé *et al.*, 2010; Mustapha and Bala, 2010; Bello *et al.*, 2011). These findings have not been ascertained for the two species under study in Malawi. This study is therefore vital since it will evaluate the antimicrobial activity of *P. crassipes* and *P. schumanniana* leaf extract against selected gram positive and gram negative type of bacteria to validate the use of the species in treatment of many ailments.

Bacteria are normal flora found in human body with potential to cause diseases depending on virulent factors such as toxins, surface coats that inhibit phagocytosis, and surface receptors that bind to host cells (Peterson, 2013). Generally, bacteria can be grouped into two; Gram positive and Gram negative. Gram positive type of bacteria have a thick and multilayered peptidoglycan layer is bacteria and thick cell wall of 20-80 nm while Gram negative bacteria have thin and single-layered peptidoglycan layer and thin cell wall of 5-10 nm with outer membrane acting as a barrier to toxic compounds including anti-biotic (Panawala, 2017). *Staphylococcus aureus* is one of the gram-positive type of bacteria and can cause skin and soft tissue infections, food poisoning and toxic shocks (Kotzekidou, Giannakidis and Boulamatsis, 2008). *Escherichia coli* is a gram negative type of bacteria can cause infections and treating with antibiotics can be difficult since they develop resistance (Panawala, 2017). *Escherichia coli* are pathogenic and causes diarrhoea. According to traditional knowledge, *P. crassipes* and *P. schumanniana* cure several diseases including bacterial infections such as diarrhoea, sexual transmitted diseases, and many others (Mponda, 2012). Therefore, evaluating the antimicrobial activity of *P. crassipes* and *P. schumanniana* leaves extract against selected gram positive and gram negative type of bacteria might be of potential use in treating these selected bacteria and manufacturing pharmaceutical products out of them.

From the foregoing, *P. crassipes* and *P. schumanniana* appear to be multipurpose plant species with diverse uses in ethno medicine. With the high prevalence of Human Immunodeficiency Virus (HIV) and Acquired Immuno Deficiency Syndrome (AIDS) in most African countries including Malawi, this species may offer some hope as a source of relief from opportunistic diseases which affect people who are living with HIV/AIDS as well as Corona Virus Disease of 2019 (COVID-19). In spite of all the medicinal related claims about *P. crassipes* and *Pavetta schumanniana* in other countries, documentation regarding the medicinal use especially ethno medicinal, phytochemical properties and antimicrobial activity of the species is by far limited in Malawi. It is important that a study of the plant species be carried out with the aim of filling the identified gaps.

1.2 Problem Statement

Access to Western medicine is a big challenge to many people living in rural areas in developing countries such as Malawi. To overcome this problem, people use medicinal plants to treat different ailments. Sometimes where western medicine is available, some people supplement western medicine with herbal medicine. For example, some people in Enukweni and Mtakataka, Mzimba and Dedza districts, respectively, use *P. crassipes* and *P. schumanniana* leaves to treat different ailments which include respiratory problems, coughing, malaria and sexually transited diseases (Mponda, 2012). In regions where *P. crassipes* and *P. schumanniana* leaves are traditionally used for medicinal purposes, there is lack of scientific understanding regarding their therapeutic efficacy, chemical composition, and antimicrobial properties. This knowledge gap hinders the integration of traditional medicine into modern health care systems and may overlook potentially valuable treatments for infectious diseases. Thus, this research aims to address this gap by investigating the ethno medicinal uses of *P. crassipes* and *P. schumanniana,* conducting chemical analyses to identify their bioactive constituents, and assessing their antimicrobial activity against clinically important pathogens like bacteria. By bridging traditional knowledge with scientific validation, this study seeks to unlock the therapeutic potential of these plants for the benefit of local communities. Apart from that, this study will contribute to the conservation of traditional knowledge and development of new anti-microbial agents. In addition, the global demand for medicinal plants is still increasing exponentially, as people are becoming aware of the potency and side effect of synthetic drugs (Verma and Singh, 2008; Street and Prinsloo, 2013). Malawi has a very small share of this ever-growing global market. To compete with the growing market, there is urgency to expeditiously utilize and scientifically validate more medicinally useful plants. Therefore, it is

imperative to conduct this study to understand the therapeutic benefits and risks, and to utilize opportunities for developing new drugs.

1.3 Aims and objectives of the study

1.3.1 General Objective

The main objective of this study was to make a comparative analysis of ethnomedicine, phytochemical composition and antimicrobial activity of *P. crassipes* and *P. schumanniana* plant leaves.

1.3.2 Specific Objectives

i. To evaluate ethno medicinal attributes of *P. crassipes* and *P. schumanniana* plant leaves.

ii. To analyses phytochemical compounds in the leaves of *P. crassipes* and *P. schumanniana* plant leaves.

iii. To evaluate antimicrobial activities of *P. crassipes* and *P. schumanniana* aqueous leaf extracts.

1.3.3 Alternate Hypothesis

i. H_{a1}: There are significant differences in the ethno medicinal attributes between *P. crassipes* and *P. schumanniana* as observed through traditional knowledge and practices

ii. H_{a2}: The leaves of *P. crassipes* and *P. schumanniana* contain different phytochemicals of medicinal value (Alkaloids, Saponins, Flavonoids, Tannins, Anthocyanins, Anthraquionones, and Terpenoids).

iii. H_{a3}: *Pavetta crassipes and Pavetta schumanniana* leaf extracts exhibit significantly different antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*

1.4 Significance of the study

The proposed study advances one of the overall objectives of the Malawi Forest Policy (GoM, 2016) that advocates that communities neighboring to forests need to benefit from the forest resources to reduce poverty levels through improved and sustained financial benefits and other livelihoods outcomes including food, biomass, shelter and health from forests. In addition, this study supports Sustainable Development Goals (SDGs), goal 3, first part of target 3.b, which support research and development of vaccines and medicines for the communicable and non-communicable diseases that primarily affect developing countries. This study also aligns with Pillar number 1 agricultural

transformation and commercialisation of Malawi's agricultural sector including the development of drugs. It is also in line with Enabler 1 Human capital Development since the study involves research in the fields of ethnobotany, phytochemistry and microbiology contributing to the development of human capital. It also aligns with Enabler 2 Science, technology and innovation since the study employs modern science techniques such as phytochemical analysis and antimicrobial activity testing. This study also aligns with the Malawi First Ten Year Implementation plan in particular MIP priorities 1-5 that's agriculture and industrialisation, Human capital development, Health, environmental and natural resources, and science, technology and innovation. Study findings will help different stakeholders to come together and see how best to harness medicine from this species, therefore it is imperative to carry out this research.

CHAPTER TWO: LITERATURE REVIEW

2.1 Botanical description of *Pavetta crassipes* and *Pavetta schumanniana*

Pavetta crassipes K. Schum. (Rubiaceae) is a low glabrous shrub of the savannah with stout sub-quadrangular branches covered with pale corky bark which splits and falls off and it can grow 1.8 meters tall (Daget, 2002). Leaves are usually clustered near the apices of the branches, paired, or occasionally ternate or quadrate, glabrous; blades 8-30 × 1.3-7.5 cm, linear to narrowly elongate-oblong or oblanceolate, rounded or sometimes obtuse at the apex, obtuse to attenuate at the base; lateral nerves in 8-11 main pairs (Daget, 2002). Inflorescences corymbose, crowded 7.5-17 cm across (excluding corollas). Flowers are white and green, pedicellate, with shortly dentate, glabrous tubular calyx, glabrous tubular corolla about 12-18 mm long. Fruit black, shiny, 6-8 mm in diameter; pedicels slightly accrescent; calyx limb persistent. Seeds greyish-brown, 5-5.5 mm wide, slightly rugulose on convex face (Daget, 2002; Zerbo *et al.*, 2011).

Pavetta schumanniana F. Hoffm. ex K. Schum (Rubiaceae) is a much-branched shrub or tree up to 4 m high (Verstraete *et al.*, 2011). Leaves are usually opposite, but often whorled with triangular stipules between them, large, broadest towards the tip. They have a rough leathery texture and are finely and densely hairy below. Venation is raised on the underside, and the leaves tend to have round dark bacterial nodules scattered throughout the leaf surface (Bridson, 2001). *P. schumanniana* flowers are usually dense white fragrant clusters. The calyx lobes are toothed, with sharp tips. Fruits are mostly produced in summer. They are round and fleshy, ± 8 mm in diameter. At first they are green, but they change to glossy black after reaching maturity (Bridson, 2001).

2.2 Occurrence of *Pavetta crassipes* and *Pavetta schumanniana*

2.2.1 *Pavetta crassipes*

Pavetta crassipes is found in Benin, Burkina Faso, Burundi, Central African Republic, Côte ivory, Ethiopia, Ghana, Guinea, Malawi, Mali, Mozambique, Niger, Nigeria, Tanzania, Zambia. From Senegal to Cameroon, as far as Sudan, tropical Africa (Daget, 2002; Zerbo *et al.*, 2011). It is usually found in high rainfall and warm areas. In English, *P. crassipes* is known as gland leaf brides bush, in vernacular languages it is called *Mzubula*, Mchoka, *Manjaatali*, *Lilaka*, *lyanyombe*, *Lilime*, *lyanyombe* and *Mpewu*.

2.2.2 *Pavetta schumanniana*

Pavetta schumanniana is found in Angola, Botswana, Namibia, Cameroon, Central Africa Republic, Democratic Republic of the Congo, Rwanda, Burundi, Tanzania, Malawi, Mozambique, Zambia, Zimbabwe and South Africa. It is usually found in Miombo and other types of deciduous woodland and wooded grassland and also on rocky hillsides (Bridson, 2001). These places are characterized by hot summers and warm (certainly frost-free) winters, or by a relative lack of discernible seasons. Rainfall in the seasonal parts of the range of this species is mostly in summer; in the more tropical areas it is monsoonal. The quantity of rain is moderate, as these areas are neither very dry nor moist. In English, *P. schumanniana* is known as poison bride's bush or poison pavetta, in vernacular languages it is called *Mpambo, Mpumba, Chikolowanga, Njiliti* and *Nachumba.*

2.3 Uses of *Pavetta crassipes* and *Pavetta schumanniana*

2.3.1 *Pavetta crassipes*

In Nigeria, leaves of *P. crassipes* are used as medicine in the management of respiratory infections and abdominal disorders (Daget, 2002; Aliyu *et al.*, 2008; Zerbo *et al.*, 2011). The leaves of *P. crassipes* are also utilized by local people in Tanzania for treating gonorrhea (Daget, 2002; Aliyu *et al.*, 2008). In Central and some parts of Southern Africa including Malawi, the acid infusion of the leaves is taken as a cough remedy, dried ground leaves as libido enhancers in men, roots are used for snake bites and bark as a purgative (Mponda, 2012). Alkaloid extracts from the plants have been shown to have significant anti-malarial activity (Sanon *et al.*, 2003). The ethanol extract has been shown to lower the blood pressures of cats and rats in a dose dependent manner (Amos *et al.*, 1998).

The leaves of *P. crassipes* are used to treat schistosomiasis or haematuria, malaria, splenosis, fever, conjunctivitis, syphilis sores, diarrhoea, stiffness, weakness, respiratory diseases, hypertension (Daget, 2002; Zerbo *et al.*, 2011). The roots of *P. crassipes* are used for treating gonorrhea, in some cases as a laxative in times of constipation (Daget, 2002; Mponda, 2012). The bark of *P. crassipes* is used for treating snake bite, febrifuge and thyroid stimulating while roots and leaves of *P. crassipes* are used to treat fever, vitamin deficiency and kwashiorkor, and fruits are used as vermifuge (Zerbo *et al.*, 2008; Mponda, 2012). In Nigeria, leaves of *P. crassipes* are used as medicine in the management of respiratory infections and abdominal disorders (Aliyu *et al.*, 2008; Zerbo *et al.*, 2008). The leaves are also utilized locally in Tanzania for treating gonorrhea (Aliyu *et al.*, 2008).

2.3.2 *Pavetta schumanniana*

In Zimbabwe, leaves of *P. schumanniana* are used as medicine in the management of respiratory infections such as coughing. It is also used for treating infertility and venereal diseases in women (Bridson, 2001). Generally, there is limited data on the uses of *P. schumanniana*. However, since it belongs to the same genus, the hypothesis of this study is that it may likely have medicinal properties of significance for use in the medical/pharmaceutical fraternity.

2.4 Classes of phytochemicals that are usually found in medicinal plants

There is no specific classification of phytochemicals so far because of the wide variety of them. As a result, phytochemicals are usually classified differently by various researchers depending on their structure and function. This literature review 7 main groups of phytochemicals namely: phenols or phenolic compounds, terpenoids, alkaloids, saponins, cardiac glycosides, sterols and essential oils (Doughari, 2012; Nyamai *et al.*, 2016a).

2.4.1 Phenolic Compounds

Phenolic compounds are phytochemicals that have one or more aromatic rings with at least one hydroxyl group where the (-OH) bonds directly to an aromatic hydrocarbon group. He further reported that phenol (C_6H_5OH) is the simplest class of this group of natural compounds. Phenolic compounds are a large and complex group of secondary chemical metabolites found in plants (Walton, Mayer and Narbad, 2003; Dai and Mumper, 2010). In plants, they play a protective role by minimizing the effect of aggression by predators, parasites and also protect plants from ultraviolet radiation hence they are known as defensive compound (Dai and Mumper, 2010). Phenolics are very common in fruits, cereals, legumes and vegetables (Walton, Mayer and Narbad, 2003). Phenolic compounds show several properties that are beneficial to human health, and their antioxidant properties are crucial in determining their role as protecting agents against free radical-mediated disease processes (Nyamai *et al.*, 2016a). Plant phenolics include flavonoids, phenolic acids, stilbenes, lignans and tannins (Mukundi *et al.*, 2015). Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytoconstituents (Table 1).

Table 1: Occurrences and role of major classes of phytochemicals

Class of Phytochemical	Occurrence as natural product (%)	Role in health care
Phenolics	45	Anti-oxidants, anti-cancerous, cytotoxicants, antimicrobials and vasodilating
Terpenoids and Steroids	27	Antimicrobials, detoxifying agents, strengtheners, anti-rheumatics, anti-malarial, hepaticidal
Alkaloids	18	Neuropharmaceuticals, anti-cancerous, sedatives, anti-microbials, insecticidal
Other chemicals	10	Anti-inflammatory, Immunostimulating

Adapted from Koche et al., 2018

2.4.1.1 Flavonoids

Flavonoids are low molecular weight polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks. Flavonoids have been reported to exert multiple biological properties including antimicrobial, cytotoxicity, anti- inflammatory as well as antitumor activities; but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Tapas, Sakarkar and Kakde, 2008). In addition, flavonoids have been reported to have ant hyperglycemic effect (Muriithi *et al.*, 2015). Genistein (Figure 1), is used to treat pancreatic cancer. This done by combining genistein, an isoflavone with cisplatin, a cytostatic drug that induces the apoptosis of BxPC-3 pancreatic carcinoma cells and also reduces the multiplication of the cancerous cells (Middleton, Kandaswami and Theoharides, 2000; Arika *et al.*, 2015). The combination of genistein and arsenic trioxide activates caspase-3 and increases cytochrome-c release thus increases apoptosis in human leukemia cells hence it has been concluded that flavonoids prevent the development of blood cancer (Romani *et al.*, 2004). These two compounds

also work synergistically to stimulate apoptosis and reduce cell viability of hepatocellular carcinoma cell lines (Wang, Hao and Chen, 2007). It has been discovered that genistein suppresses glucose uptake in hormone-dependent and hormone-independent breast cancer cell lines and induces overexpression of glucose-regulated protein involved in cell viability (Mertens-Talcott, Talcott and Percival, 2003; Sharma, Hupp and Tepe, 2007).

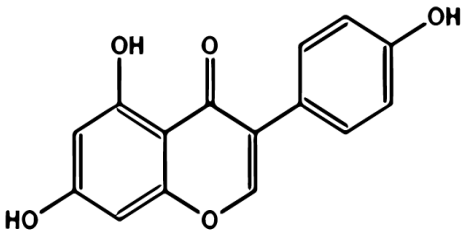


Figure 1: Genistein

Several studies reported that members of the flavonoid group such as Cyanidin-o-galactoside (Figure 2), cyanidin-3-o-rutinoside (Figure 3), procyanidin B5 (Figure 4) and robinetinidol-(4-α-8) catechin-(6,4-α) robinetinol and their derivatives they inhibit cell proliferation and have free radical scavenging activity (Nyamai *et al.*, 2015). The capacity of flavonoids to act as antioxidants primarily depends on their molecular structure. It has been reported that the position of hydroxyl groups and other features in the chemical structure of flavonoids are responsible for their antioxidant and free radical scavenging activities. As a result, flavonoids constitute a wide range of chemical compounds that play an important role in protecting biological systems against the harmful effects of oxidative processes on macromolecules such as carbohydrates, proteins, lipids and DNA (Seeram *et al.*, 2006).

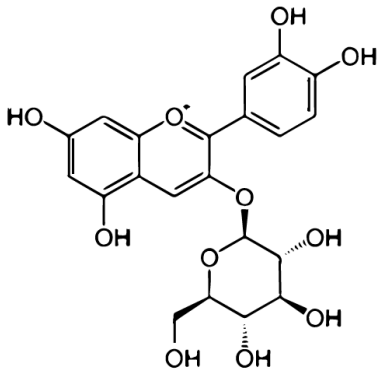


Figure 2: Cyanidin-o-galactoside

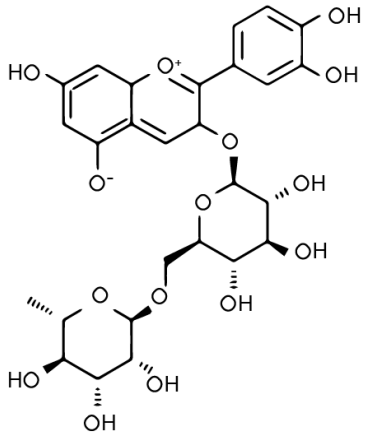


Figure 3: Cyanidin-3-o-rutinoside

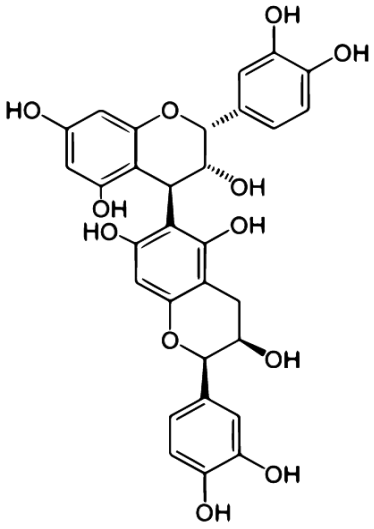


Figure 4: Procyanidin B5

Flavonoids have been used in the treatment of chronic cardiac insufficiency and hypertension because they have the ability to block the activation of necrosis factor kappa-B which has the ability to normalize blood pressure and the function of the heart (Lim *et al.*, 2006). Flavonoids like flavone C-glycoside (Figure 5), kakonein (Figure 6) and caesalpin P improves the function of pancreatic islet cells and have diabetic activity (Mohammad *et al.*, 2006). Quercetin has been shown to induce apoptosis in mouse pre-adipocytes and to inhibit adipogenesis (Majumdar *et al.*, 2009). The polyhydroxylated flavonol, myricetin (Figure 7), enhances lipogenesis and glucose uptake in the adipocytes and flavanoid, myricetin has demonstrated insulinomimetic properties (Kuhar, Imran

and Singh, 2007). This compound, however, does not have any negative impact on insulin receptor auto-phosphorylation. An in vitro experiment that was conducted (Zhang *et al.*, 2008) demonstrated that Epicatechin (Figure 8) and its active principles facilitate the release of insulin through conversion of pro-insulin to insulin. It has been shown that the flavonoid and flavonoid glycosides cause pancreatic beta cell regranulation and have been used in clinical treatment of diabetes due to improved sensitivity of insulin (Seeram *et al.*, 2006).

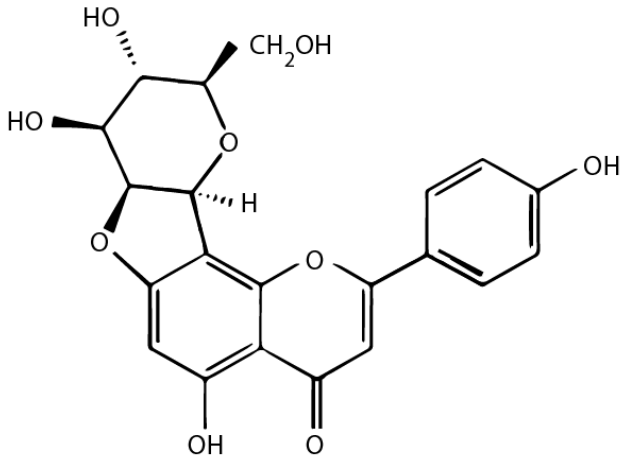


Figure 5: Flavone C-glycoside

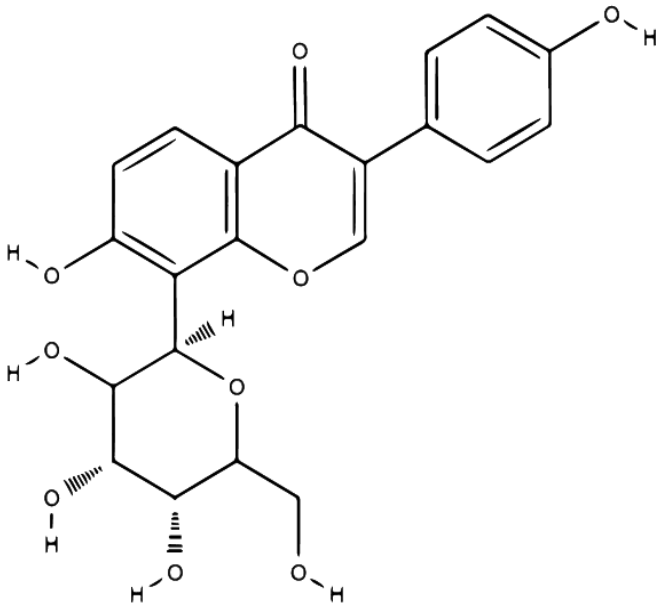


Figure 6: Kakonein

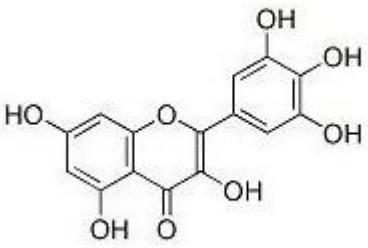


Figure 7: Myricetin

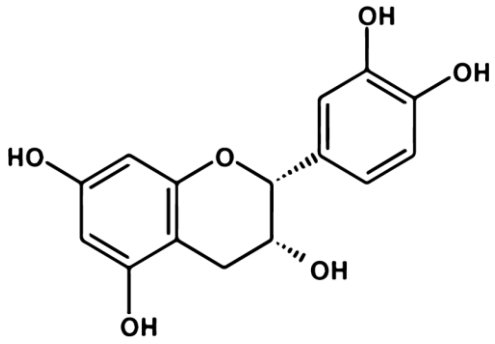


Figure 8: Epicatechin

2.4.1.2 Phenolic acids

The term “phenolic acids”, in general, designates phenols that possess one carboxylic acid functional group. Phenolic acids are aromatic acid, a phenolic acid is known to have a wide range of therapeutic effect against diseases like diabetes, cancer, neurodegenerative, cardiovascular and inflammatory diseases (Ronchetti *et al.*, 2006; Lampiasi and Montana, 2016). It has been reported that these therapeutic effects are a result of antioxidant activity of this phenolic acid (Joshi, Kuszynski and Bagchi, 2005). Ferulic acid (Figure 9) prevents lipid peroxidation and scavenges superoxide free ion radicals. The structural characteristics of phenolic acids help them confer the antioxidant properties. These compounds have a phenolic nucleus and an unsaturated side chain that reacts with other molecules to form a resonance stabilized phenoxy group (Iwase *et al.*, 2000). Reactive radicals collide with these compounds gaining a hydrogen atom and forming a phenoxy radical. Phenolic acids and their ester derivatives reduce the level of inflammatory mediators like tumor necrosis factor-alpha, prostaglandin E2 (Appendino *et al.*, 2006). Ferulic acid also lowers the expression and inhibits function of Inducible Nitric Oxide Synthase (INOS) in cells that are activated by bacterial endotoxin liposaccharide (Srinivasan, Sudheer and Menon, 2007). Ferulic

acid derivatives have been reported to suppress the activity of cyclooxygenase-2 promoter activity in human colon cancer DLD-1 cells through the β-galactosidase reporter gene assay system (Ronchetti *et al.*, 2006; Lampiasi and Montana, 2016).

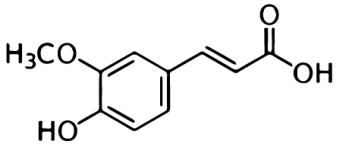


Figure 9: Ferulic acid

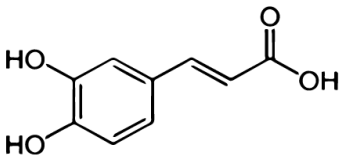


Figure 10: Caffeic acid

Diabetes is an endocrine disorder that is characterized by hyperglycemia leading to oxidative stress due to the over production of free radicals (Piero *et al.*, 2015). Phenolic acids protect the streptozin from toxicity by neutralizing the free radicals which are produced in the pancreas by streptozotocin (Uttara *et al.*, 2009). The decrease in toxicity and oxidative stress in the pancreatic cells has positive impacts on the beta cells hence reproduce rapidly and secrete more insulin (Kang *et al.*, 2011). Increased insulin secretion lowers glucose levels due to increased glucose utilization by extra hepatic tissues. Phenolic acids are also reported to protect proteins, Deoxynucleic Acid (DNA) and lipids from oxidative stress thus exerting anticancer properties; and they restore normal homeostasis by inducing apoptosis in cancer cells (Delmas *et al.*, 2006; Kang *et al.*, 2011). These chemical compounds also scavenge deleterious radicals and chain reactions, and suppress radiation-induced oxidative reactions hence they preserve the physiological integrity of cells (Uttara *et al.*, 2009).

Study findings have shown that phenolic acids provide protection against polyunsaturated fatty acids (PUFA) and alcohol (Zerbo *et al.*, 2008) induced toxicity and also enables the body to overcome deleterious effects of PUFA and alcohol (Zerbo *et al.*, 2008). Same authors pointed out that phenolic acids have the capacity of quenching the lipid peroxidative chain and scavenging free radicals as a result they preserve the integrity of cells exposed to alcohol stress. The mechanism of action is through the abstraction of hydrogen ion (H^+) by hydroperoxyl and hydroxyl radicals from

a free phenolic substrate to form a phenoxyl radical which then forms products that are excreted in bile. Alzheimer is a neurodegenerative disease that is characterized by free radical-mediated oxidative stress in brain cells (Uttara *et al.*, 2009). This oxidative stress mainly is caused by reactive nitrogen species and reactive oxygen species can lead to neuronal dysfunction, Riboxynucleic Acid (RNA) and DNA oxidation and lipid peroxidation. Phenolic acids are reported to reduce the probability of oxidative attack on proteins hence they prevent their modification through oxidative reactions (Delmas *et al.*, 2006). Nicotine causes oxidative cellular injury by increasing lipid peroxidation, and this is a major cause of several smoking-related diseases. Phenolic acids, however, they tend to reduce the negative health impact of nicotine by increasing the endogenous antioxidant defense system, reversing the damage caused by nicotine and protecting cells from oxidative damage (Uttara *et al.*, 2009). These compounds protect the membrane by quenching the free radicals, improving the antioxidant status and inhibiting the leakage of marker enzymes into circulation (Delmas *et al.*, 2006).

2.4.1.3 Stilbenes

Stilbenes are a family of secondary metabolites derived from phenylpropanoid pathway that consist a trans-ethene double bond substituted with a phenyl on both carbon atoms of the double bond (Nyamai *et al.*, 2016a). Stilbenes are reported to have the ability of inhibiting the development of cancerous cell. Same authors further pointed out that the mechanism of action of these compounds is through inhibition of the cellular events associated with tumor initiation, promotion and progression by inducing quinone reductase enzyme that catalyses the reactions of detoxifying carcinogens thus acts as an anti-mutagen. Stilbenes inhibit the arachidonic acid pathway that leads to the formation of prostaglandins that activate carcinogenesis and stimulate cancer cell growth by inhibiting the hydroperoxidase activity of cyclooxygenase (Dai and Mumper, 2010). Same authors noted that as stilbenes inhibit the arachidonic acid pathway, they also demonstrate anti-inflammatory activities. Stilbene inhibits the development of preneoplastic lesions as a result they slow the progression of carcinogenesis however in a dose dependent manner (Ronchetti *et al.*, 2006). Stilbenes are also reported to inhibit DNA synthesis and duplication and lymphocyte proliferation during immunosuppressive therapies (Uttara *et al.*, 2009). Resveratrol (Figure 11) and ellagic acid are also known to induce apoptosis and have antiproliferative activity on human leukemia cells. It has been reported that curcumin (Figure 12) and resveratrol synergistically inhibits growth of p53-negative and p53-positive human colon cancer cells (Ronchetti *et al.*, 2006).

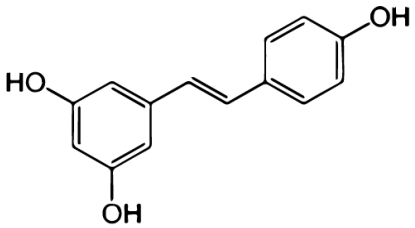


Figure 11: Resveratrol

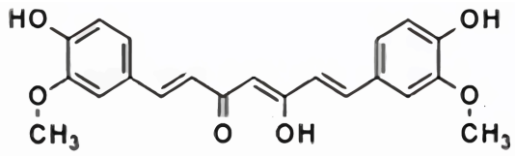


Figure 12: Curcumin

2.4.1.4 Lignans

Lignans are plant polyphenolic compounds derived from phenylalanine through dimerization of substituted cinnamic acid (Figure 13) alcohols (Nyamai *et al.*, 2016a). Study findings from in vitro experiment have shown that lignans have the ability of reducing cell proliferation in colon cancer cells and they exhibit anticancer properties (Delmas *et al.*, 2006). They further discovered that these compounds inhibit metastatic secondary tumors and decrease levels of colon cancer markers in rat models. Lignans are also reported to suppress the receptor binding of platelet activating factor and inhibit the replication of human immunodeficiency virus at the integration stage (Delmas *et al.*, 2006). These compounds are also known to inhibit tumor necrosis factor-alpha from lipopolysaccharide-triggered murine microphage (Delmas *et al.*, 2006). Necrosis is the death of most or all of the cells in tissue or an organ due to disease, injury, or failure of the blood supply (Nyamai *et al.*, 2016a).

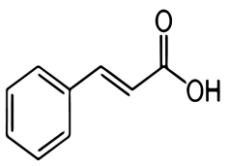


Figure 13: Cinnamic acid

2.4.1.5 Tannins

Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species (Doughari, 2012). They are found in abundance in the tree bark, wood, fruit, fruit pod, leaves and roots and also in plant gall (Nyamai *et al.*, 2016b). Tannins can be classified into two broad groups, hydrolysable tannins and condensed tannins. The tannin epigallo-catechin-3-gallate (Figure 14) is reported to exhibit anti-diabetic activity (Gao *et al.*, 2004). It has been reported that all forms of tannins participate in the management of glucose level in blood (Kang *et al.*, 2011). Tannin has been shown to stimulate the receptor cells to utilize carbohydrate (Doughari, 2012). Ellagic acid (Figure 15) and quercetin are anti-leukemia chemical compounds which act synergistically to reduce viability, proliferation and trigger apoptosis of human leukemia cells (Gao *et al.*, 2004). Study findings have shown that ellagic acid and resveratrol effectively inhibit skin tumorgenesis in mice (Cassidy, Hanley and Lamuela-Raventos, 2000).

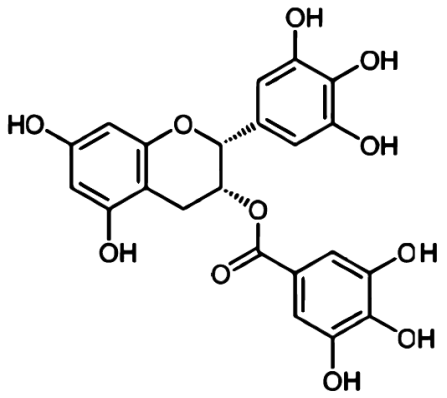


Figure 14: Epigallo-catechin-3-gallate

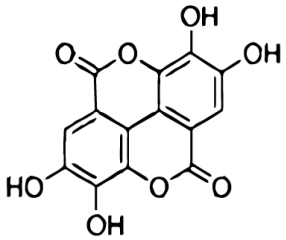


Figure 15: Ellagic acid

Tannins have got anti-bacterial, anti-tumor, anti-cancer, anti- carcinogenic anti-oxidant and anti-mutagenic activities (Koche *et al.*, 2010). In addition, (Kambewankako, 2005) also reported that tannins have many chemical activities which include anthelminthic, anti HIV, antiviral, pesticide, psychotropic, and prevents formation of cancer cells. Report by (Mukundi *et al.*, 2015) showed that tannins have antibacterial, anticancer and anti-inflammation activities. However, the production and accumulation of tannins are affected by light intensity (Akula and Ravisharkar, 2011). The higher the light intensity the higher the production of tannins

2.4.2 Alkaloids

Alkaloids are natural product that contains heterocyclic nitrogen atoms and are basic in character. The name of alkaloids derives from the word "alkaline" and it was used to describe any nitrogen-containing base (Chaurasiya *et al.*, 2008). Alkaloids are naturally produced and have a bitter taste. For example, the alkaloid quinine is one of the most bitter tasting substances known and is significantly bitter ($1x10^{-5}$) at a molar concentration (Bassoli, Borgonovo and Busnelli, 2007).

Alkaloids are known to have blood glucose lowering activity (Pelletier, 1996). Alkaloids tetrandine (Figure 16) and barbering (Figure 17) have been reported to demonstrate antioxidant activity responsible for various biological activities including anti-diabetic activity (Cassidy, Hanley and Lamuela-Raventos, 2000; Gupta *et al.*, 2011; Abdirahman *et al.*, 2015). The alkaloids l-ephedrine (Figure 18) of Ephedra distachya herbs play a crucial role in the restoration and regeneration of atrophied pancreatic islets that induces the secretion of insulin which result in hypoglycemic condition in diabetic mice (Piero *et al.*, 2015). Alkaloids are also known to be anti-arrythmic effects, antihypertensive effects, anticancer and anti-malarial activity (Kwan and Achike, 2002; Raskin *et al.*, 2005; Dholi *et al.*, 2011; Verma *et al.*, 2013; Abdirahman *et al.*, 2015).

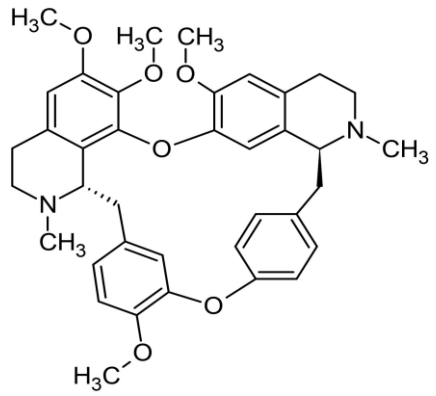


Figure 16: Tetrandine

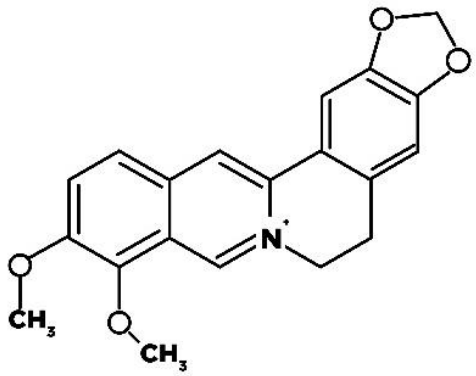


Figure 17: Barbering

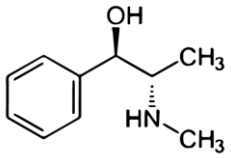


Figure 18: Alkaloids l-ephedrine

Generally, alkaloids are widely used for treating various diseases because of their pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine) (Wink and Latz-Brüning, 1994). These are just a few example illustrating the great economic importance of this group of phytochemicals. Some alkaloids have stimulant property as caffeine and nicotine, morphine is used as the analgesic and quinine as the antimalarial drug.

Alkaloids are reported to have neuro-protective, cholinergic and antioxidant activities in Alzheimer's disease (Nyamai *et al.*, 2016a). These chemical compounds enhances memory and cognitive- activities on Alzheimer's patients (O'Brien, Carrasco-Pozo and Speisky, 2006). Same authors further discovered that the therapeutic effect of these chemical compounds is achieved by restricting oxidative stress and inflammatory reactions, enhancing cholinergic transmission, elevating estrogen and other neurotropic agents and preventing β-amyloid toxicity formation. It is also important to note that alkaloids inhibit the production of acetylcholinesterase enzyme (Guruayoorappan *et al.*, 2014). Inhibition of this enzyme enhances the activity of acetylcholine which is a chemical that play an important role in the management of Alzheimer's disease (Guruayoorappan *et al.*, 2014). Tetramethylpyrazine (Figure 19), an amide alkaloid is known to elicit hypotensive effects by inhibiting platelet aggregation and vasoconstriction (Guruayoorappan

et al., 2014). This alkaloid is also reported to cause inotropic and chronotropic responses on isolated atria (Guruayoorappan *et al.*, 2014). Due to vasodilatory effects of tetramethylpyrazine, it has been used in the treatment of occlusive cerebral arteriolar diseases (O'Brien, Carrasco-Pozo and Speisky, 2006). Alkaloids have also been reported to have antimicrobial, cytotoxic and trypanocidal activity (Nyamai *et al.*, 2016b). They further discovered that these compounds act by intercalating DNA thus impairing replication and transcription which result into frame-shift mutations. Alkaloids have both antimicrobial and trypanocidal properties such that they have the ability to wipe out microorganisms by inhibiting protein biosynthesis and by interacting with neuroreceptors (Francis, Wolf-Watz and Forsberg, 2002).

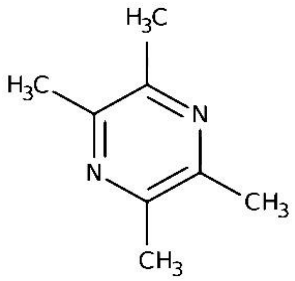


Figure 19: Tetramethylpyrazine

2.4.3 Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. Saponins possess 'soaplike' behaviour in water, i.e. they produce foam hence the name "saponin" (Doughari, 2012). Saponins occur either as steroid alkaloids, glycosides of triterpenoids or steroids, and they are known to have hypocholesterolaemic, immunostimulant, hypoglycemic effect and anticarcinogenic properties (Ros, 2000). He further pointed out that hypoglycemic effect of saponins is due to their ability to stimulate pancreatic β-cells, inhibit glucose transport across the brush border cells of the small intestines and suppress the transfer of glucose from the stomach to the small intestines. Saponins are also reported to inhibit gastric emptying in a dose dependent manner (Tan and Vanitha, 2012). These chemical compounds are known to lower cholesterol levels by forming large micelles that are then excreted in bile. Saponins also have the ability to lower serum levels of low density lipoproteins-cholesterol and decrease absorption of cholesterol in the intestines (Chung, 2004).

Saponins have been reported that they play a crucial role in body defense against diseases by acting as adjuvants in enhancing antibody production and in the stimulation of cell mediated immune system (Nyamai *et al.*, 2016b). These chemical compounds are reported to possess mediating immune-stimulant effects by inducing the production of interferon and interleukin through their interaction with antigen-presenting cells (Guruayoorappan *et al.*, 2014). Same authors further noted that saponins possess anti-cancer properties. Their mode of action is through tumor cell growth inhibition by apoptosis in leukemia cell line and by cell cycle arrest in breast cancer cell line. In addition, they exert anti-proliferative effect to prostate carcinoma cells by inducing apoptosis and cell cycle arrest at G1 phase. Apoptosis is induced by the stimulation of cytochrome c-caspase pathway (Dastmalchi *et al.*, 2007).

Saponins are also reported to lower the risk of cancer and other chronic diseases; and they are effective for both hormone dependent and non-hormone dependent cancer (Hostanska *et al.*, 2005). Saponins are also reported to exert antifungal and hypocholesterolemic effects (Nyamai *et al.*, 2016b). These effects are a result of their combination with bile acids which form micellar aggregates (Nyamai *et al.*, 2016b). Saponins prevent lipid peroxidation hence they protect the liver from hyperlipemia and liver injury (Lee *et al.*, 2004). These authors also pointed out that the mechanism of action of these chemical compounds in this case is through inhibition of lipid peroxide peroxidation and inhibition of lipid peroxide production. It has been discovered that saponins inhibit HIV infection in vitro in addition to having antitumor properties (Jassim and Naji, 2003). They also noted that these effects are attributed to the prevention effect of HIV-induced cell fusion but have no direct effect on reverse transcriptase activity of the virus. Saponins have been reported to have the ability to prevent various diseases in human beings by scavenging superoxide on oxygen radicals that are known to be responsible for the development and initiation of several diseases (Zhu *et al.*, 2004).

2.4.4 Terpenoids

The terpenoids are a class of natural products which have been derived from five-carbon isoprene units mainly isopentenyl pyrophosphate and its isomer dimethylallyl pyrophosphate by terpene synthases (Nyamai *et al.*, 2016b). Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons (Gao *et al.*, 2004). Terpenoids have antioxidant properties and also interact with most regulatory proteins. In modern science, terpenes are used as inhibitors of Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells (NF-kB) (Piero *et al.*, 2015). NF-kB system is a cytoplasmic sensor that responds

to various internal and external signals like genotoxic stress and hypoxia as well as disturbances in the immune system (Mertens-Talcott, Talcott and Percival, 2003). NF-kB also plays a crucial role in the development of cellular resistance against apoptosis and anti-apoptotic signaling (Newman *et al.*, 2008). They also discovered that most terpenes in plants occur as terpene derivatives (terpenoids). Sesquiterpenoids are the main tepernes and are known to have NF-kB signaling inhibitory effect while triterpenoids and diterpenoids are reported to have several potent inhibitors of NF-kB signaling system (Mertens-Talcott, Talcott and Percival, 2003).

Aucubin (Figure 20), a monoterpenoid that occurs in plants as a glycoside derivative prevents the nuclear translocation of P65 subunit of NF-kB complex in stimulated mast cells and also inhibits the degradation of IkBa protein (Piero *et al.*, 2015). Several studies have shown that aucubin and linalool (Figure 21) possess anti-tumor and anti-inflammatory property and they also play a protective role against hepatoxicity (Yang *et al.*, 2008). Limonene (Figure 22) and its derivative perillyl alcohol are reported to have inhibitory effect on pancreatic and mammary tumors (Yang *et al.*, 2008). Same authors also pointed out that these two compounds are also known to inhibit proliferation and metastasis of gastric cancer. A terpene extracted from confer trees, α-Pinene (Figure 23), is known to inhibit translocation of NF-kB or p65 protein into nuclei of LPS-stimulated cells (Salminen *et al.*, 2008). Helenalin A (Figure 24), a sesquiterpene inhibits DNA binding of NF-kB and the transcription of NF-kB-dependent genes by alkylating the p65 subunits of NF-kB complex (Mertens-Talcott, Talcott and Percival, 2003; Yang *et al.*, 2008). Artemisinin (Figure 25), a lactone extracted from *Artemisia annua* is mainly used as an anti-malarial drug but it is also used as an antifungal, anticancer, immunosuppressive and antiangiogenesis properties (Salminen *et al.*, 2008; Yang *et al.*, 2008).

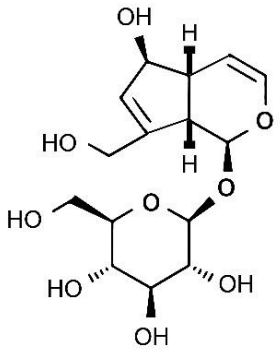


Figure 20: Aucubin

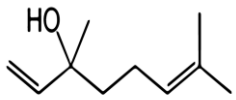


Figure 21: Linalool

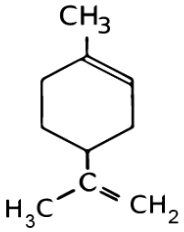


Figure 22: Limonene

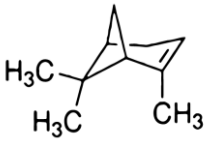


Figure 23: α-Pinene

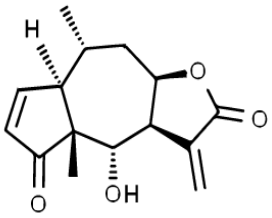


Figure 24: Helenalin A

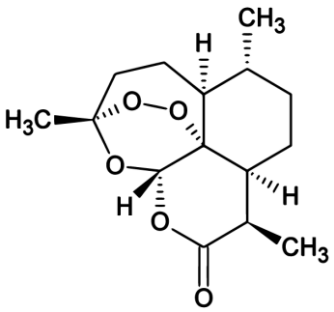


Figure 25: Artemisinin

Study findings have shown that terpenoids improve the skin tone, increases the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply (Liu, 2004; Aggarwal *et al.*, 2006). Terpenoids also improve lung function (Mujoo *et al.*, 2001). Terpenoids possess anti-diabetic property such that the leaves and seeds of *S. spectabilis* are used in the treatment of diabetes because of the presence of phytochemicals including terpenoids (Grace *et al.*, 2015). They also discovered that terpenoids reduce diastolic blood pressure and lower the sugar level in blood in hypertensive and diabetic patients respectively. The anthraquionone (Figure 26) in the plant extracts of *Polygonum multiflorum* have been used in treating peripheral neuropathy, a complication associated with diabetes mellitus (McPartland and Russo, 2001). In addition, Terpenoids are used to produce hormones such as corticosteroids which improve fertility in men and are also active against bacteria (Karou *et al.*, 2011; Guimarães *et al.*, 2019).

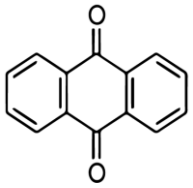


Figure 26: Anthraquionone

2.4.5 Steroids/sterols

Phytosterols are subgroup of steroids that have structures and functions similar to cholesterol (Nyamai *et al.*, 2016b). Phytosterols in plants act as substrates for the synthesis of secondary metabolites, regulate permeability and fluidity of cell membranes and also act as biogenic precursors of growth factors (Patel and Thompson, 2006). Phytosterols occurs either as sterols or stanols (the saturated forms of sterols) (Nyamai *et al.*, 2016b). Same authors also pointed out that usually the absorption of sterols in the intestines is higher than that of stanols resulting to lower concentrations in blood serum. It is also important to note that phytosterols inhibit absorption of cholesterol in the intestines (Hendriks *et al.*, 1999). Phytosterols and cholesterol require Niemann-Pick C1-like protein for their entry in the intestine cells (Katan *et al.*, 2003). Same authors further noted that cholesterol is esterified in the enterocytes by acetyl-coenzyme A acetyltransferase-2 enzyme and are packed into chylomicrons and transported to the lymphatic system. ATP-Binding Cassete (ABC) transporters pump phytosterols and non-esterified cholesterols back to the intestinal lumen (Katan *et al.*, 2003). This process lowers the amount of cholesterol assimilated in the system (Hendriks *et al.*, 1999; O'Neill, Sanders and Thompson, 2005). Study findings have shown that

phytosterol intake leads to up to 15% reduction of low density lipoprotein (LDL-cholesterol). Intake of plant stanols reduces both plant sterol and cholesterol concentrations in the serum (Vanhanen *et al.*, 1993). Genetic differences in sterol metabolism and amount of phytosterols determines the effectiveness of cholesterol lowering by phytosterol supplements (Yamamoto *et al.*, 1991).

β-Sitosterol (Figure 27) is the main phytosterol in plants and it is also found in human serum together with its glycoside at lower concentrations (Ibekwe *et al.*, 2012, 2018). β-sitosterol and β-sitosterol glycoside have been reported to reduce occurrences of inflammatory diseases and carcinogen-induced cancer. Several study findings have shown that β-sitosterol and β-sitosterol glycoside have insulin releasing effect, anti-complement and antipyretic activity.(Raicht *et al.*, 1980; Micallef and Garg, 2008; Zerbo *et al.*, 2008). β-sitosterol and its glycoside work synergistically in immune modulating activities on conditions that are non-infectious such as rheumatoid arthritis and allergies and chronic infectious diseases like tuberculosis and Human Papilloma Virus (Kulkarni Harshal and Lohar Prakash, 2014). They further discovered that a mixture of the two with higher concentrations of β-sitosterol is known to influence the high production of T-lymphocytes after these cells are activated by mitogens in vitro. (Kulkarni Harshal and Lohar Prakash, 2014) also noted that these phytosterols are shown to increase the rapid production of TH1-type helper cells while inhibiting TH2-type helper cells. In addition, phytosterols inhibit the secretion of (Interleukin) IL-4 but increase the secretion of Interferon-Gamma (IFN-g) and IL-2. This specificity towards certain T-helper cells implies that this mixture has significant modulatory and regulatory activities in conditions where enhancement of TH1-helper cell is important for the clearance of pathogens. The mixture is also reported to increase the lytic ability of natural killer cells to cancer cell lines in vitro (Bouic *et al.*, 1996). β-sitosterol and its glycoside have anti-inflammatory activity as they inhibit both tumor necrosis factor alpha and interleukin-6 in a dose dependent manner (Nyamai *et al.*, 2016a).

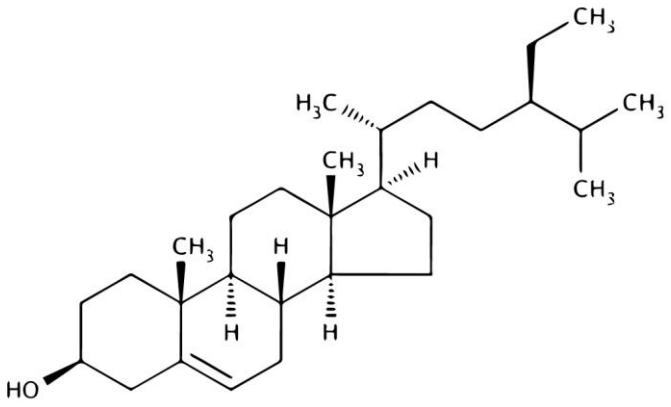


Figure 27: β-Sitosterol

2.4.6 Cardiac Glycosides

Plant steroids (or steroid glycosides) also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Perne *et al.*, 2009). Cardiac glycosides are plant secondary metabolites that have a glycoside unit and they act on the contractile action of the cardiac muscle (Doughari, 2012). The later has made these chemical compounds useful in treating cardiac arrhythmias and congestive heart failure as they increase contractile force. Digitalis, which is one of the cardiac glycosides, is commonly used both traditionally and in modern medicine (Yeh *et al.*, 2003). This glycoside contains two glycosides; digitoxin (Figure 28) and digoxin (Figure 29) whose structures differ only by an extra hydroxyl group on digoxin. The mechanism of action of cardiac glycosides is by inhibition of Na^+, K^+-ATPase resulting to decreased intracellular K^+ ions and increased intracellular Ca^{2+} and Na^+ ions (Perne *et al.*, 2009). They further noted that digitalis directly inhibits rapid reproduction of androgen dependent and androgen independent prostate cancer cell lines by initiating apoptosis and increasing intracellular Ca^{2+}. Several study findings have shown significant inhibition of cell growth in androgen dependent prostate cancer cells by ouabain (Figure 30) (Newman *et al.*, 2008). It has been reported that oleandrin (Figure 31) and bufalin (Figure 32) have apoptotic effect on normal leukocytes (Zerbo *et al.*, 2008).

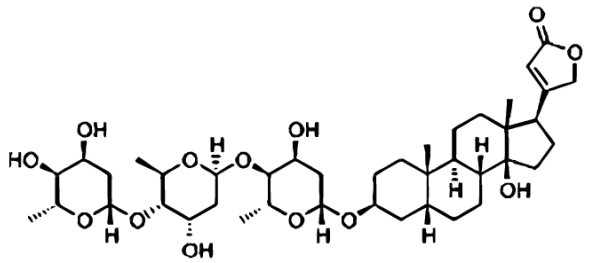


Figure 28: Digitoxin

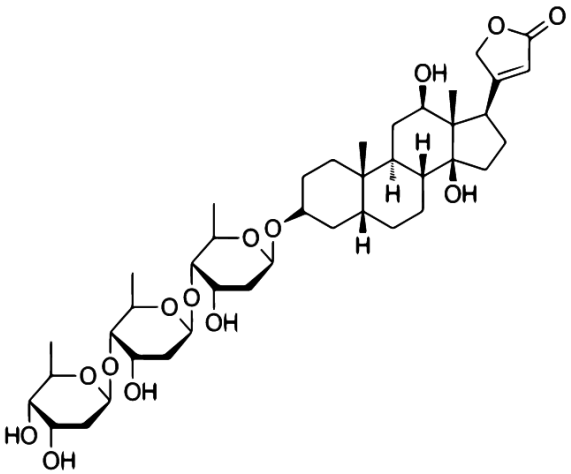


Figure 29: Digoxin

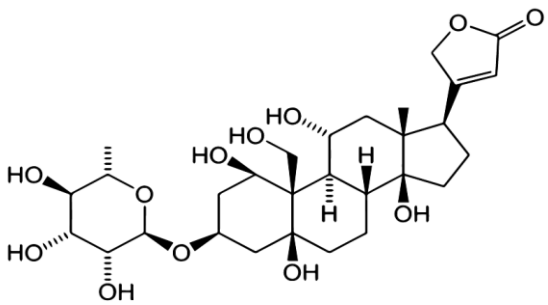


Figure 30: Ouabain

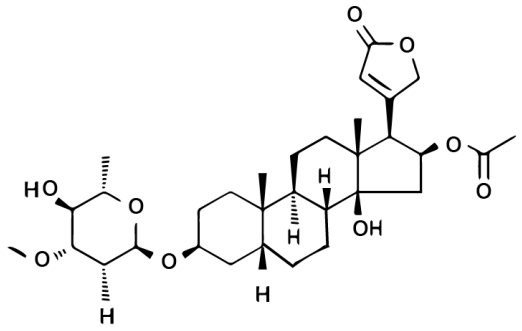


Figure 31: Oleandrin

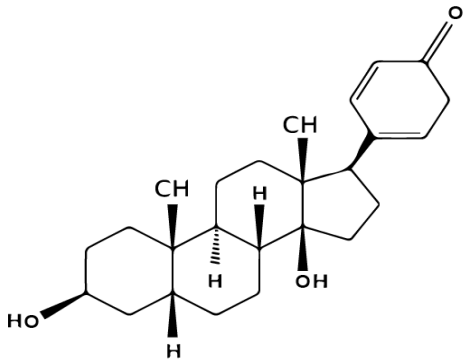


Figure 32: Bufalin

Cardiac glycosides have been reported to prevent the development of prostate cancer by inhibiting the four genes that are overexpressed in prostate cancer cells including the prevention of apoptosis by inhibitor survivin and transcription factors (Newman *et al.*, 2008). They further discovered that digitoxin suppresses hypersecretion of IL8, a protein that is known to be responsible for lung inflammation thus inhibiting activation of the NF-B signaling pathway in cystic fibrosis. These compounds have been reported to exert cytotoxic effects in both cell lines derived in advanced cancer and normal prostate epithelial cells. Oleandrin, a glycoside derived from oleander, is known to induce apoptosis by sustaining Ca^{2+} increase that precedes release of cytochrome c from mitochondrion and caspase activation (Hartmann, 1998). Oleandrin causes cell arrest at G2-M phase of the cell cycle in a dose dependent manner. In general, oleandrin has the ability to inhibit cell growth and tumor cell proliferation.

2.4.7 Essential oils

Essential oils are the odorous and volatile products of various plant and animal species that have the tendency of evaporating on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils (Kalemba and Kunicka, 2005). Essential oils mostly

contribute to the odoriferous constituents or *'essences'* of the aromatic plants that are used abundantly in enhancing the aroma of some spices (Kalemba and Kunicka, 2005). Essential oils are secreted either directly by the plant protoplasm or by the hydrolysis of some glycosides (Bruna *et al.*, 2014). Plant structures associated with the secretion of essential oils include: glandular hairs (Lamiaceae e.g. *Lavandula angustifolia*), oil tubes (or vittae) (Apiaceae eg. *Foeniculum vulgare*, and *Pimpinella anisum* (Aniseed), modified parenchymal cells (Piperaceae e.g. *Piper nigrum* - Black pepper), Schizogenous or lysigenum passages (Rutaceae e.g. *Pinus palustris* - Pine oil) (Moreira, Alvarez and Ponce, 2016). Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavour and odour. Examples of volatile oils include amygdaline (volatile oil of bitter almond), sinigrin (volatile oil of black mustard), and eugenol occurring as gein (volatile oil of *Geum urbanum*) (Figure 33) (Moreira, Alvarez and Ponce, 2016).

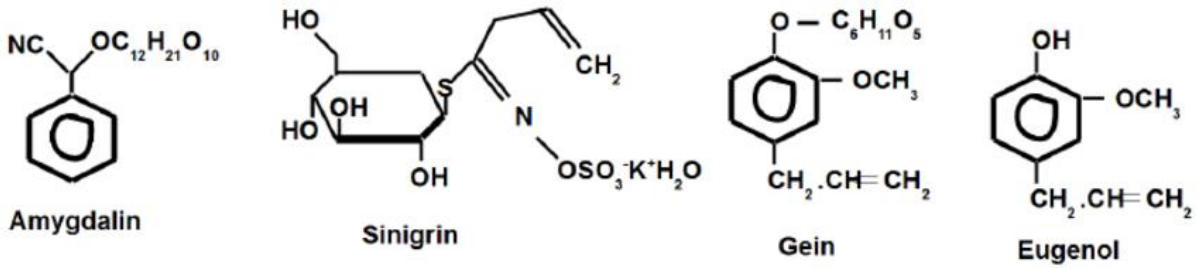


Figure 33: Basic structures of some pharmacologically important plant derived essential oils

2.4.8 Phytochemicals present in *Pavetta crassipes* and *Pavetta schumanniana*

Literature has revealed that *P. crassipes* contain these chemical constituents in the leaves; tannins, saponins, alkaloids, flavonoids, reducing sugars, carbohydrates, proteins, amino acids, organic acids (citric, ascorbic), sterols, anthraquinones and terpenes/steroids (Sanon *et al.*, 2003; Amos *et al.*, 2004; Baldé *et al.*, 2010; Ibekwe *et al.*, 2012; Lifongo *et al.*, 2014; Olutayo *et al.*, 2018). However, (Aliyu *et al.*, 2008) reported that saponins were not present in *P. crassipes* leaves. Similarly, (Osuntokun and Ajayi, 2014) reported that steroids were not present in *P. crassipes* leaves. This may be due to several factors that affects the production and composition of phytochemicals in plant. There are several factors that influence secondary metabolite composition these include physiological variations; environmental conditions; geographic variation; genetic factors and evolution; pre-harvest and post-harvest food processing operations and storage (Chanli,

2012; Jones, Stefanelli and Tomkins, 2015; Drahansky *et al.*, 2016; Liu *et al.*, 2016). Information on phytochemical composition of *P. schumanniana* were not available for both Malawi and elsewhere. This indicates that there is knowledge gap hence this present study is imperative to investigate phytochemical composition of *P. schumanniana.* There is also evidence gap hence this novel study is overbearing since studies conducted on *P. crassipes* plants from elsewhere cannot be generalized in the Malawian context, looking at the fact that secondary metabolite production is influenced by several factors (Figueiredo *et al.*, 2008).

2.5 Biological Activities of Phytochemicals

Several study findings show that phytochemicals may reduce the risk of coronary heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol, reducing the synthesis or absorption of cholesterol, normalizing blood pressure and clotting, and improving arterial elasticity (Hahn, 1998; Cohen *et al.*, 2006; Wadhera *et al.*, 2016). In addition, phytochemicals detoxify substances that cause cancer. They appear to neutralize free radicals, inhibit enzymes that activate carcinogens, and activate enzymes that detoxify carcinogens (Bianchini and Vainio, 2001). For example, some research findings demonstrate that genistein prevents the formation of new capillaries that are needed for tumor growth and metastasis (Hark, Deen and Morrison, 2003; Tsubura *et al.*, 2012; Pizzino *et al.*, 2017). The physiologic properties of relatively few phytochemicals are well studied and understood; and many studies have been conducted to establish their possible role in preventing or treating cancer and heart disease (Bianchini and Vainio, 2001; Cohen *et al.*, 2006; Koche *et al.*, 2010; Wadhera *et al.*, 2016; Pizzino *et al.*, 2017). There has been an increased awareness that phytochemicals help in the prevention and treatment of diabetes, high blood pressure, and macular degeneration (Cassidy, Hanley and Lamuela-Raventos, 2000; Mujoo *et al.*, 2001; Mukundi *et al.*, 2015). In addition, phytochemicals have an antimicrobial property. They show different modes of action against bacterial strains such as interference with the phospholipids bilayer of the cell membrane which has as a consequence permeability increase and loss of cellular constituents; damage of the enzymes involved in the production of cellular energy and synthesis of structural components; and destruction or inactivation of genetic material (Nweze, Okafor and Njoku, 2004). In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Kothari, 2004). Some specific modes of actions are outlined below.

2.5.1 Antimicrobial activity

Phytochemical constituents that protect plants from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack have found applications in human medicine (Nyamai *et al.*, 2016a). Some phytochemicals such as phenolic acids they help significantly in the reduction of particular adherence of organisms to the cell's lining of the bladder, and the teeth, which ultimately lowers the incidence of urinary-tract infections (UTI) and the usual dental caries (Nascimento *et al.*, 2000). Phytochemicals they also exert either bacteriostatic or bactericidal activity on microbes. Study findings from research conducted by Jakhetia *et al.*, (2010) reported that the volatile gas phase of combinations of *Cinnamon* oil and clove oil inhibited the growth of spoilage fungi, yeast and bacteria normally found on Intermediate Moisture Foods (IMF) when combined with a modified atmosphere comprising a high concentration of carbon dioxide (40%) and low concentration of oxygen (<0.05%). It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varies from researcher to researcher. This is because concentration of phytochemicals of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study (Jakhetia *et al.*, 2010).

2.5.1.1 Antimicrobial, and anti-protozoal activity of *P. crassipes* and *P. schumanniana* leaves

An in vitro experiment noted that methanol and dichloromethane extracts from *P. crassipes* leaves had antiproliferative properties against microbes (*Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*), protozoans (*Trypanosoma cruzi*, *Trypanosoma brucei*, *Leishmania infantum* and *Plasmodium falciparum*) (Baldé *et al.*, 2010). Similarly, it was reported that chloroform and ethanol extracts of *P. crassipes* leaves inhibits the growth of some bacterial respiratory pathogens (Mustapha and Bala, 2007). Comparatively, the anti-bacterial activity which Mustapha and Bala (2010) observed was reported to be lower than what (Balde *et al*., 2010) observed in their study and this may be attributed to different climatic conditions where the plants were corrected and studied and because of other factors too. The concentration of phytochemicals of the same plant species can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial (Figueiredo *et al.*, 2008; Jakhetia *et al.*, 2010). In addition, the analyses revealed that the components present in the alkaloid extract of *P. crassipes* leaves are responsible for its anti-protozoal and cytotoxic efficacy (Bello *et al.*, 2011). The anti-bacterial activity of the

P. crassipes leaves have been strongly linked with the presence of bioactive compounds especially the flavonoids which is one of the principal phytochemical components of the *P. crassipes* leaves (Bello *et al.*, 2011). In addition, crude hot water extracts from eight medicinal plants collected in Togo, West Africa, were examined for anti-malarial properties against *Plasmodium falciparum* using an in vitro test. A preliminary phytochemical screening of the leaf extract of *P. crassipes* leaves revealed the presence of flavonoids, tannins and anthraquinones as possible candidates for such inhibitory substances (Amos *et al.*, 1998). Literature for antimicrobial activity of *P. schumanniana* leaves could not be found.

2.5.2 Anti-ulcer activity

Plants extracts have been reported to inhibit both the growth of *Helicobacter pylori in-vitro* as well as its urease activity. Similarly, aqueous leaf extracts from *Moringa oleifera* showed anti-ulcer effect (Choudhary, Bodakhe and Gupta, 2013). It has been established that most plant extracts possess antiulcer activity because of the presence of various phytochemicals including methanol, flavonoids, saponins and tannins (Amos *et al.*, 1998; Borrelli and Izzo, 2000; Choudhary, Bodakhe and Gupta, 2013). It was also reported that the efficiency of some extracts in liquid medium and at low pH levels enhances their potency even in the human stomach (Jakhetia *et al.*, 2010).

2.5.2.1 Anti-ulcer activity of *Pavetta crassipes* and *P. schumanniana* leaves

It has been reported that most *P. crassipes* leaf extracts possess anti-ulcer activity because of the presence of various phytochemicals including flavonoids, saponins and tannins (Borrelli and Izzo, 2000). These chemicals are also found in *P. crassipes* leaves hence it has the potential of possessing anti-ulcer properties (Osuntokun and Ajayi, 2014). Therefore, there need to study this property in detail. Literature for anti-ulcer activity of *P. schumanniana* leaves could not be found.

2.5.3 Anti-diabetic activity

Cinnamaldehyde, a phytochemical constituent extracts has been reported to exhibit significant anti-hyperglycemic effect resulting in the lowering of both total cholesterol and triglyceride levels and, at the same time, increasing high density lipoprotein (HDL)-cholesterol in streptozotocin induced diabetic rats (Jakhetia *et al.*, 2010). This investigation revealed the potential of cinnamaldehyde for use as a natural oral agent, with both hypoglycaemic and hypolipidemic effects. Recent reports indicate that *Cinnamon* extract and polyphenols with procyanidin type-A polymers exhibit the potential to increase the amount of TTP (Thrombotic Thrombocytopenic Purpura), IR (Insulin Resistance), and GLUT4 (Glucose Transporter-4) in 3T3-L1 Adipocytes (Cao, Polansky and

Anderson, 2007; Anderson, 2008; Jakhetia *et al.*, 2010). It was suggested that the mechanism of *Cinnamon*'s insulin-like activity may be in part due to increase in the amounts of TTP, IR, and GLUT4. *Cinnamon* polyphenols may have additional roles as anti-inflammatory and/or anti-angiogenesis agents (Jakhetia *et al.*, 2010).

2.5.3.1 Anti-diabetic activity of *P. crassipes* and *P. schumanniana* leaves

Alkaloids are known to have blood glucose lowering activity (Pelletier, 1996; Cassidy, Hanley and Lamuela-Raventos, 2000; Yang *et al.*, 2001). Some Rubiaceae species including *Morinda lucida* and *Nauclea latifolia* have been screened for anti-diabetic activity and it was reported that the aqueous and ethanolic extracts significantly lowered the fasting blood glucose levels of the diabetic rats in a dose-dependent manner; however, the aqueous extract did not significantly lower the glucose levels of normoglycaemic rats (Gidado *et al.*, 2008). Similarly, it was reported that the methanol extract of the *Morinda lucida* exerted a dose-dependent hypoglycaemic activity in normal rats within 4 hours after oral administration (Olajide, Olumayokuna *et al.*, 1999). In hyperglycaemic rats, the extract produced a significant anti-diabetic effect from day 3 after oral administration. Furthermore, the aqueous extract of the roots of the plant, exhibited potent hypoglycaemic effects in both norm glycemic and alloxan-induced diabetic mice by oral administration (Kamanyi, Njamen and Nkeh, 1994). Anti-diabetic activity may also be attributed to the presence of tannins. Tannins exhibits anti-diabetic activity and all their forms participate in the management of glucose level in blood (Kang *et al.*, 2011). Tannin has been shown to stimulate the receptor cells to utilize carbohydrate (Doughari, 2012). Since *P. crassipes* and *P. schumanniana* belongs to the gunus, they might also possess anti-diabetic activity.

2.5.4 Anti-inflammatory activity

Essential oil from twigs of *Cinnamomum osmoploeum* commonly known as indigenous Cinnamon has excellent anti- inflammatory activities and cytotoxicity against HepG2 (Human Hepatocellular Liver Carcinoma Cell Line) cells. Other studies also indicated that the constituents of *C. osmophloeum* twig exhibited excellent anti-inflammatory activities in suppressing nitric oxide production by LPS (Lipopolysaccharide)-stimulated macrophages (Tung *et al.*, 2008, 2010).

2.5.4.1 Anti-inflammatory activity of *P. crassipes* and *P. schumanniana* leaves

Several studies have reported the presence of flavonoids in *P. crassipes* (Bello *et al.*, 2011; Bello, Ndukwe and Audu, 2014; Osuntokun and Ajayi, 2014). Flavonoids have been reported to exert multiple biological properties including antimicrobial, cytotoxicity, anti-inflammatory as well as

anti-tumor activities; but the best-described property of almost every group of flavonoids is their capacity to act as powerful anti-oxidants which can protect the human body from free radicals and reactive oxygen species (Tapas, Sakarkar and Kakde, 2008). Literature for anti-inflammatory activity of *P. schumanniana* could not be found. There is need to conduct studies on the anti-inflammatory activity of *P. crassipes* and *P. schumanniana* since there is limited information on the subject.

2.5.5 Anti-carcinogenesis

Polyphenols are among the diverse phytochemicals that have the potential in the inhibition of carcinogenesis (Doughari, 2012). He further discovered that phenolic acids usually significantly minimize the formation of the specific cancer-promoting nitrosamines from the dietary nitrites and nitrates. Similarly, glucosinolates from different vegetable sources as broccoli, cabbage, cauliflower, and Brussel sprouts exert a substantial protective support against the colon cancer (Liu *et al.*, 2014). Several study findings have shown that regular consumption of Brussel sprouts by human subjects up to 300 g per day, miraculously causes a very fast (say within a span of 3 weeks) appreciable enhancement in the glutathione-S-transferase, and a subsequent noticeable reduction in the urinary concentration of a specific purine metabolite that serves as a marker of DNA-degradation in cancer (Doughari, 2012). Isothiocyanates and the indole-3-carbinols do interfere categorically in the metabolism of carcinogens thus causing inhibition of procarcinogen activation, and thereby inducing the 'phase-II' enzymes known as NADPH quinone reductase or glutathione S-transferase, that specifically detoxify the selected electrophilic metabolites which are capable of changing the structure of nucleic acids. Sulforaphane which is found in large quantities in broccoli has been proved to be an extremely potent phase-2 enzyme inducer (Liu *et al.*, 2014). It predominantly causes specific cell-cycle arrest and also the apoptosis of the neoplasm (cancer) cells. Sulforaphane categorically produces D-gluconolactone which has been established to be a significant inhibitor of breast cancer (Liu *et al.*, 2014). He further pointed out that indole-3-carbinol, most vital and important indole present in broccoli, specifically inhibits the Human Papilloma Virus (HPV) that causes uterine cancer by blocking the estrogen receptors specifically present in the breast cancer and it also inhibit prostate cancer. Phytosterols inhibits the development of tumors in colon, breast, and prostate glands; even though the precise and exact mechanisms whereby the said blockade actually takes place are not yet well understood, yet they seem to change drastically the ensuing cell-membrane transfer in the phenomenon of neoplasm growth and thereby reduce the inflammation significantly (Liu *et al.*, 2014).

2.5.5.1 Anti-carcinogenesis activity of *P. crassipes* and *P. schumanniana* leaves

An in vitro experiment indicated that methanol and dichloromethane extracts from *P. crassipes* leaves had anti-proliferative properties against cancerous cell (U373, PC3, MXT and A549) and normal cell lines (NHDF and MRC-5) (Baldé *et al.*, 2010). These analyses revealed that the components present in the alkaloid extract of *Pavetta crassipes* leaves are responsible for its cytotoxic efficacy. On the one hand, it was reported that flavonoids, which are also present in *P. crassipes* leaves, reduces the multiplication of the cancerous cells (Abdirahman *et al.*, 2015; Arika *et al.*, 2015). In addition, saponins which are also present in *P. crassipes* leaves, have anti-carcinogenic properties (Ros, 2000). Literature for anti-carcinogenesis activity of *P. schumanniana* could not be found.

2.5.6 Antioxidants

Anti-oxidants protect cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage (Mattson and Cheng, 2006). It has been discovered that natural antioxidants play a significant role in health maintenance and prevention of the chronic and degenerative diseases such as atherosclerosis, cardiac and cerebral ischema, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage and ageing (Zerbo *et al.*, 2008). Antioxidants exert their activity by reacting with the 'free-oxygen radicals' of which the end products of the reactions are fairly 'stable radicals'. The free radicals are metastable chemical species which have the tendency of trapping electrons from the molecules in the immediate surroundings. These radicals if not scavenged effectively in time, they may destroy very important biological molecules like lipids, proteins including those present in all membranes, mitochondria and, the DNA resulting in abnormalities leading to disease conditions (Zerbo *et al.*, 2008). Thus, free radicals are involved in a number of diseases including; tumour inflammation, hemorrhagic shock, atherosclerosis, diabetes, infertility, gastrointestinal ulcerogenesis, asthma, rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, neurodegenerative diseases (e.g. parkinsonism, Alzheimer's diseases), AIDS and even early senescence. Several studies reported that the human body produces insufficient amount of antioxidants which are essential for preventing oxidative stress (Holst and Williamson, 2008; Uddin *et al.*, 2008; Zerbo *et al.*, 2008; Chothani and Mishra, 2012). Therefore this deficiency has to be compensated by making use of natural exogenous antioxidants, such as vitamin C, vitamin E, flavones, carotene and natural products in

plants (Lindberg Madsen and Bertelsen, 1995; Rice-Evans, Miller and Paganga, 1997; Diplock *et al.*, 1998; Srinivasan, Sudheer and Menon, 2007).

Phytochemicals, which are biologically active, naturally occurring chemical compounds found in plants, contain a wide variety of free radicals scavenging compounds including phenols, flavonoids, vitamins, terpenoids that are rich in antioxidant activity (Lindberg Madsen and Bertelsen, 1995; Cai, Sun and Corke, 2003; Srinivasan, Sudheer and Menon, 2007; Holst and Williamson, 2008). Many plants, citrus fruits and leafy vegetables are the source of ascorbic acid, vitamin E, caratenoids, flavanols and phenolics which have the ability to scavenge the free radicals in human body (Hertog *et al.*, 1993; Anderson *et al.*, 2001). Significant antioxidant properties have been recorded in phytochemicals that are responsible for the reduction in the occurrence of many diseases. Many dietary polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than vitamins E or C, and thus might contribute significantly to protective effects *in vivo* (Rice-Evans, Miller and Paganga, 1997; Jayasri, Mathew and Radha, 2009). They further discovered that methanol extract of cinnamon contains a number of antioxidant compounds which can effectively scavenge reactive oxygen species including superoxide anions and hydroxyl radicals as well as other free radicals *in vitro*. The fruit of cinnamon, an under-utilized and unconventional part of the plant, contains a good amount of phenolic antioxidants to counteract the damaging effects of free radicals and may protect against mutagenesis (Cao, Polansky and Anderson, 2007; Jakhetia *et al.*, 2010; Tung *et al.*, 2010).

Antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and do so by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process (Cai, Sun and Corke, 2003). Due to safety concerns of synthetic compounds, food industries are trying their very best to find natural anti-oxidants to replace synthetic compounds (Srinivasan, Sudheer and Menon, 2007). Generally, there has been a growing trend in consumer preferences for natural antioxidants, all of which has given more energy to explore natural sources of antioxidants.

2.5.6.1 Antioxidant activity of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Saponins that are found in *P. crassipes* leaves possess antioxidant property and have been reported to have the ability to prevent various diseases in human beings by scavenging superoxide on oxygen radicals that are known to be responsible for the development and initiation of several diseases (Zhu *et al.*, 2004; Olutayo *et al.*, 2018). Other phytochemical that was found in *P. crassipes* leaves and

have antioxidant property are flavonoids (Seeram *et al.*, 2006; Bello *et al.*, 2011; Osuntokun and Ajayi, 2014; Nyamai *et al.*, 2015). Literature for anti-oxidant activity of *P. schumanniana* leaves could not be found.

2.5.7 Multi-functional targets

Multiple molecular targets of dietary phytochemicals have been studied and identified from pro- and anti-apoptotic proteins, cell cycle proteins, cell adhesion molecules, protein kinases, transcription factors to metastasis and cell growth pathways (Donaldson, 2004; Liu, 2004; Aggarwal *et al.*, 2006; Holmes, 2006). Phytochemicals such as epigallocatechin-3-gallate (EGCG) from green tea, curcumin from turmeric, and resveratrol from red wine tend to have impact on different molecules as a result there is no tangible information on the mechanisms of action of phytochemicals from those plants (Francis, Wolf-Watz and Forsberg, 2002). The multi-target nature of phytochemicals has positive outcome on overcoming the problem of cancer drug resistance (Doughari, 2012). This multi-faceted mode of action hinders the cancer cell's ability to develop resistance to the phytochemicals, for example Epigallocatechin Gallate (EGCG) has inhibitory effects on the extracellular release of verotoxin from *E. coli* and similarly ethanol pericarp extracts of *Punica granatum* inhibited verotoxin production in periplasmic space and cell supernatant. Therefore, the mechanisms responsible for this inhibition are yet to be understood, however the active compounds from the plant are thought to interfere with the transcriptional and translational processes of the bacterial cell (Voravuthikunchai and Kitpipit, 2005).

2.5.7.1 Multi-functional targets of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Extracts of *P. crassipes* leaves have been found to have several biological activities such anti-bacterial, anti-protozoal, anti-diabetic, anti-inflammatory and anti-oxidant activities (Sanon *et al.*, 2003; Baldé *et al.*, 2010; Mustapha and Bala, 2010; Bello *et al.*, 2011; Bello, Ndukwe and Audu, 2014; Osuntokun and Ajayi, 2014; Ibekwe *et al.*, 2018; Olutayo *et al.*, 2018). Literature for multi-functional targets of *P. schumanniana* leaves could not be found.

CHAPTER THREE: MATERIALS AND METHODS

In this study, the study area covered Dedza district in Central region and Mzimba district in Northern Region in Malawi. These areas were selected because of expected availability of the two species as guided by the Mzuzu botanical gardens, and respondents had knowledge on the utilisation of the plants. Neither of the two sites had both *Pavetta schumanniana* and *Pavetta crassipes* present. As a result though distinct species, *P. schumanniana* and *P. crassipes*, were compared at the genus level, despite being found in different locations, Mzimba and Dedza districts, respectively.

Mzimba district is the largest district in Malawi. It is bordered by Rumphi to the North, Nkhata Bay to the East, Kasungu to the South and Zambia to the West. The district has a total area of 10,430 square kilometers. Mzimba is largely covered with indigenous and woodland forests, predominantly semi-evergreen woodlands of *Brachystegia Julbernardia* and *Erythrophloem*, especially in Mpherembe-Euthini zone. There are thickets of *Combretum, Commophora* and *Euphorbia* interspersed with the *Brachystegia* woodlands at the extreme northern part of the district of which Enukweni is within this region, where it borders with Rumphi district. Plantation forests of *Pinus* and *Eucalyptus* species around Viphya and Chikangawa, grasslands with forest remnants, dry grasslands with fallow or regenerating shrubs and seasonal grasslands are common in some parts of the district (GoM, 2018). This study focused on Mzimba district in Northern Malawi. Mzimba district is the largest district in the country. It has an estimated population of 870,000 and a population density of 70 persons per square kilometer (lower than the average national population density of 139 persons per square kilometer). The district is covered mainly with medium to light textured but moderately fertile soils with eutric-fersialic soil characteristics. The sandy-loam and loamy soils have moderate to good drainage and are suitable for growing tobacco and maize. The district has a warm tropical climate. The average monthly maximum temperature varies from 27°C to 33°C with the month of November being the hottest. The average monthly minimum temperatures range from 0°C to 10°C with June and July (winter season) being the coldest. The mean monthly temperature varies from 15.5°C to 19.8°C. Annual rainfall ranges from 650 to 1300 mm (Mzimba District Planning Department 2008).

Dedza district is located in the Central region of Malawi about 86 kms South of Lilongwe City, the capital city. It is the largest district in the Central Region of Malawi covering a total area of 3,624 square kilometers which is about 4 % of the Malawi's total land area (94276 square kilometers). It borders Mangochi district to the west, Lilongwe district to the north, Salima district to the north-east and it shares its north-eastern boundary with Mozambique's Calomue Border Post in the Tete

Province. The district is divided into three topographic zones namely; Lilongwe plain (altitude 1100-1300m), the Dedza highlands (1200-2200m) and the Dedza escarpments (1000-1500m). Dedza, Mtakataka experiences a cool climate with mean annual temperatures ranging from 14°C to 21°C. The annual rainfall for Dedza District ranges from 800mm to 1200mm and falls between Mid-November to mid-April. (*Dedza District socio-economic profile*, 1999).

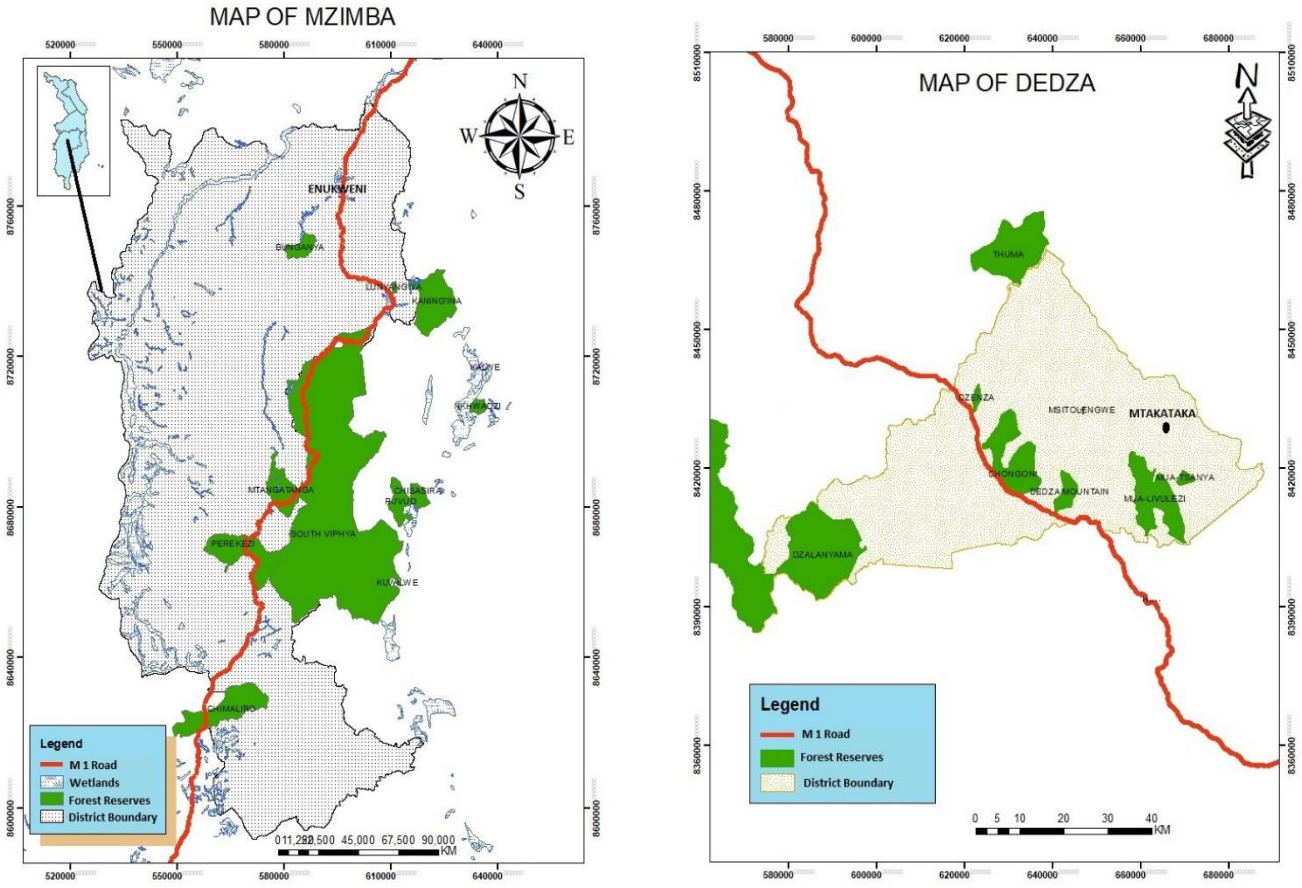


Figure 34: Maps of Mzimba and Dedza districts

3.1 Ethno medicinal uses of *Pavetta crassipes* and *Pavetta schumanniana* leaves in selected populations in Dedza and Mzimba districts

3.1.1 Research design and sampling procedure

The study used both qualitative and quantitative methods. Using mixed methods in this study allowed for a comprehensive understanding based on the research objectives. A comprehensive understanding, in this context, involves gaining insights from multiple perspectives and methodologies. Ethnomedicine digs into the traditional uses, capturing traditional knowledge passed down through generations. Phytochemical analysis helps to identify specific chemical compounds in the plants, providing insight into potential therapeutic agents. Antimicrobial analyses

assess the efficacy of the plant species against microorganisms contributing to their medicinal relevance. By combining both qualitative and quantitative methods, the researcher was able to have a holistic view, bridging cultural knowledge with scientific analysis for a more robust interpretation of the plant species' therapeutic potential. In addition to that quantitative research design was preferred because questionnaires had both open and closed-ended questions and sample size was large enough to accommodate statistical analyses.

3.1.1.1 Sampling procedure for ethno medicinal use of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Purposive sampling (Tongco, 2007; Singh and Masuku, 2014; Glen, 2015) and snowballing (Naderifar, Goli and Ghaljaie, 2017) procedures were used to select sites and respondents, respectively. Snowballing is also known as chain referral sampling (Naderifar, Goli and Ghaljaie, 2017). In this study, purposive and snowballing sampling techniques were relevant because the study targeted only those who used *P. crassipes* and *P. schumanniana* leaves. Purposive sampling was used to select districts and sites with known existence of the species while snowballing technique was used to identify individuals to be interviewed starting from key informants. The researcher contacted District Forestry Officers (DFO) of the districts who referred her to local leaders of the respective sites. The first known interviewee who were using *P. crassipes* and *P. schumanniana* leaves would reveal the next member to be interviewed who also used *P. crassipes* and *P. schumanniana* leaves and so on until a point of similarity in responses was reached which was the cutting point for respondents covered.

Simple random sampling was used to select five villages. In Dedza district, respondents were selected from Ndelema, Kamchamba, Kalindiza, Lunguzi and Kanyera while in Mzimba district respondents were from Zigodo, Matemanga, Kajawa Nyasulu, Chilolo and Munthali. A total of 258 respondents were interviewed, of which 34% were males and 66% were females. Out of this number (258) respondents, 4% were local leaders, 5% were traditional healers and 2% were birth attendants. These were key informants (KI) in all two sites.

3.1.2.2 Data collection for ethno medicinal use of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Data for ethno medicinal use of *P. crassipes* and *P. schumanniana* leaves was collected from 10 villages in two districts in Malawi (Figure 34). The data collection exercise was carried out from September 2022 to December 2022 using semi structured questionnaire-guided interviews. A semi structured questionnaire was used to collect data from individuals who used *P. crassipes* and *P.*

schumanniana leaves, while for the key informants such as herbalists, traditional birth attendants, and local leaders, a key informant checklist was used to collect data to validate or contradict data which was collected from individual participants. The questionnaire was pretested to five herbalists in Mzimba districts during a reconnaissance survey and thereafter the questionnaire was modified accordingly before the real survey. Questionnaire which was translated in Chichewa and Tumbuka in Dedza and Mzimba Districts respectively. Cultural consideration, back translation and pilot testing were among the factors that the researcher considered in order to translate the questionnaires from English to Chichewa and Tumbuka without missing meaning. Questionnaire was administered to individuals in form of a discussion and according to the topics relevant to this study. The major questions were: What was the use of *P. crassipes* and *P. schumanniana*? Which parts of *P. crassipes* and *P. schumanniana* is mostly used? What type of diseases were cured by *P. crassipes* and *P. schumanniana* leaves? How *P. crassipes* and *P. schumanniana* leaves are prepared to cure the diseases mentioned? How was *P. crassipes* and *P. schumanniana* medicine administered? How much of *P. crassipes* and *P. schumanniana* leaves is harvested to cure the diseases mentioned? Key informants were used to verify the collected information especially on diseases treated and the preparation of the medicine. A check list (Appendix C) was used.

3.2 Phytochemical analysis of *Pavetta crassipes* and *Pavetta schumanniana* leaf extracts

3.2.1 Sample collection and processing

The plant specimens of *P. crassipes* and *P. schumanniana* (2 kg from each site) were collected randomly from Mtakataka (Dedza) and Enukweni (Mzimba) on 20th February and 16th March 2022, respectively after identification and verification was done by the National Botanic Gardens (Mzuzu) staff. The researcher had an opportunity to look at *P. crassipes* and *P. schumanniana* specimens at botanical gardens with voucher numbers 494A and 1056 respectively (Appendix J). The leaves were carried in sacks to ensure that they were flesh. Fresh leaves of *P. crassipes* and *P. schumanniana* (2 kg from each site) were collected from Mtakataka (Dedza) and Enukweni (Mzimba) on 20th February and 16th March 2022, respectively. The leaves were dried under shade at room temperature. After drying for two weeks, each portion of the sample was weighed on an analytical balance (Model number: ATY-224) (Appendix I) and ground in a food blender to powder (Figure 35). The powdered plant material was sieved using a 0.5 mm size sieve (Model number: IKA 2939000), and was stored in 250 mL glass bottles which were stoppered. Glass bottles were labelled for easy identification and they were kept in cool, dry place with no direct light or heat. The processing of the plant material was done in Chemistry Laboratory of Mzuzu University.

Figure 35: Researcher grinding *P. crassipes* and *P. schumanniana* leaves using a food blender

3.2.2 Chemicals, reagents and instrumentation

The following chemicals and reagents were of analytical grade (AR) and were used for qualitative phytochemical screening analyses: bismuth sub nitrate, concentrated hydrochloric acid (32% v/v), sodium hydroxide, potassium iodide, concentrated ammonia (25% v/v), chloroform, diethyl ether, acetic anhydride, acetic acid, concentrated sulphuric acid and ferric chloride. The equipment that was used in the analyses include; water bath (Model number: AVI- 410) (Appendix G), suction machine (Model number: TID-15) (Appendix G) for obtaining crude extracts of plant samples, and analytical balance was used for weighing plant samples used for phytochemical screening tests.

3.2.3 Phytochemical screening of plant samples

Standard chemical tests were used to determine the presence of alkaloids, saponins, flavonoids, terpenoids tannins, anthocyanins and anthraquinones in the plant samples (Harborne, 1998). All the phytochemical screening was done at Mzuzu University Chemistry Laboratory.

3.2.3.1 Preparation of testing reagents

3.2.3.1.1 Dragendorff's reagent

Dragendorff reagent preparation

Potassium iodide (40 g) was weighed and dissolved in 50 mL distilled water. After dissolution, the solution was quantitatively transferred into a 100 mL volumetric flask and kept in an amber glass bottle (250 mL) and labelled (solution A). Bismuth nitrate (1.7 g) was weighed and dissolved in 50 mL of acetic acid: water solution (80 mL: 20 mL). The solution was quantitatively transferred into

a 100 mL volumetric flask and diluted to a 100 mL mark with acetic acid: water solution (80 mL: 20mL) (solution B).

Dragenndorff reagent mixture

Dragenndorff reagent mixture was prepared by mixing 5 mL Dagendorff A + 5 mL of Dragendorff B + 20 mL concentrated acetic acid and 70 mL distilled water, to make a 100 mL Dragendorff solution. This solution was the one that was used for alkaloids testing.

3.2.3.1.2 Dilute aqueous hydrochloric acid solution (5% v/v)

Concentrated hydrochloric acid (32% v/v, 12.50 mL) was added to distilled water (50 mL) and more distilled water was added up to 250 mL mark of the volumetric flask. The solution was shaken gently to obtain a homogenous solution.

3.2.3.1.3 Dilute aqueous hydrochloric acid solution (10% v/v)

Concentrated hydrochloric acid (32% v/v, 20 mL) was added to distilled water (50 mL) and more distilled water was added up to 200 mL mark of the volumetric flask. The solution was shaken gently to obtain a homogenous solution.

3.2.3.1.4 Dilute aqueous ammonia solution (10% v/v)

Concentrated ammonia (10 mL) was pipetted into a 100 mL glass bottle containing 50 mL distilled water. The solution was stirred thoroughly using a glass rod, transferred into a 100 mL volumetric flask and diluted to a 100 mL mark with distilled water.

3.2.3.1.5 Aqueous ferric chloride solution (0.5 % w/v)

A sample of ferric chloride (0.5 g) was dissolved in distilled water (40 mL) and quantitatively transferred into 100 mL volumetric flask and diluted to a 100 mL mark with distilled water.

3.2.3.1.6 Chloroform ether (1:3)

Chloroform (10 mL) and diethyl ether (30 mL) were pipetted separately into a 100 mL volumetric flask. The flask was swirled to obtain a homogeneous solution and covered with a stopper.

3.2.3.1.7 Sodium hydroxide (10% w/v)

Sodium hydroxide (2 g) was measured and dissolved in 10 mL of distilled water and the solution was diluted to 20 mL with distilled water.

3.2.3.1.8 Aqueous hydrochloric acid solution (2 M)

Hydrochloric acid (32% v/v, 196 mL) was measured and poured into a 1 L volumetric flask containing 200 mL deionised water. The flask was shaken thoroughly and diluted to 1000 mL with distilled water in a 1L volumetric flask.

3.2.3.2 Chemical tests of phytochemicals

3.2.3.2.1 Alkaloids tests

A test for the presence of alkaloids was done using Dragendorff's reagent. A dried powdered sample (5 g) was macerated in 50 mL 5% (v/v) hydrochloric acid solution for 24 hours. After 24 hours, the mixture was filtered using a suction machine (Model number: TID-15). To 1 mL of the filtrate, in a test tube, 10 drops of a mixture of Dragendorff's reagents were added drop wise. Absence of the red precipitates were taken as an indication for the absence of alkaloid (Harborne, 1998).

3.2.3.2.2 Saponins test

Powdered plant material (1.0 g) was weighed and macerated in 20 mL of distilled water. The mixture was stirred using a glass stirring rod and left to stand for 24 hrs. The Whatman filter paper No. 1 was used to filter the extract and the filtrate (10 mL) was transferred into a 16 mm by 160 mm test tube then vigorous shaking for 10 seconds followeds. The mixture was left to stand for 5 minutes. The persistent foaming after 5 minutes indicated the presence of saponins. The depth of froth gave a rough idea of the levels of saponins in the samples. The depth greater than 1 mm was indicative of strong presence of saponins (Harborne, 1998; Shad *et al.*, 2014).

3.2.3.2.3 Terpenoids/steroids test

From dried powdered sample of each plant material 1 g was weighed using analytical balance (Model number: ATY-224) (Appendix I) and macerated in 20 mL of diethyl ether in a stoppered conical flask for 48 hours. The conical flask was stoppered tightly to avoid volatilization of the solvent. After 48 hours, the mixture was filtered using Whatman filter paper No. 1. Then 10 drops of the filtrate were transferred into porcelain crucible and evaporated to dryness on a water bath. After the residue cooled, 10 drops of concentrated sulphuric acid were added and the colour produced was recorded. Other 10 drops of filtrate were placed in a porcelain crucible and dried in a water bath. After the residue cooled, ten drops of concentrated acetic anhydride were added followed by 10 drops of concentrated sulphuric acid. The appearance of green to blue colours indicated the presence of steroids in the plant sample.

3.2.3.2.4 Flavonoids test

From dried powdered sample of each plant material, 2 g was weighed using analytical balance (Model number: ATY-224) and macerated in 50 mL of distilled water and the mixture was left for 24 hours. After 24 hrs, the mixture was filtered. To 2 mL of the filtrate, 0.5 mL of concentrated hydrochloric acid, 0.5 mL of methanol and 0.5 mL of distilled water (1:1:1 ratio) were added into a 16 mm test tube followed by 0.5 g of magnesium turnings. Absence of pink, red or magenta colour was taken as an indication for the absence of flavonoids.

3.2.3.2.5 Tannins test

Powdered plant material (1.0 g) was weighed and macerated in 20 mL of distilled water and the mixture was left to stand for 24 hours. The mixture was filtered and two to five drops of aqueous ferric chloride solution (0.1 % m/v) were added. A brownish green to blue-black coloration observed indicated the presence of tannins.

3.2.3.2.6 Anthocyanin test

Each powdered plant material (1.0 g) was weighed separately and macerated in 20 mL of distilled water and the mixture was left to stand for 24 hours. After 24 hrs, the mixture was filtered and 2 mL of filtrate was poured into a separate test tube where 5 mL of the prepared aqueous hydrochloric acid solution (2 M) was added. The mixture was heated in a water bath for 30 minutes (Harborne, 1998). Pink, red to brown solution indicated the presence of anthocyanins.

3. 2.3.2.7 Anthraquinones test

From dried powdered sample of each plant material, 2 g was weighed using analytical balance (Model number: ATY-224) and placed in a conical flask. The sample was moistened with aqueous hydrochloric acid solution (10 % v/v, 5 mL) first and then macerated in 15 mL of chloroform-ether solution (1-3 ratio). The mixture was left for 24 hrs. After 24 hrs, the mixture was filtered and 1 mL of the filtrate was poured into a test tube (16 mm long). After that, the filtrate in the test tube was treated with aqueous sodium hydroxide solution (10 % v/v, 1 mL) and the mixture was shaken well to obtain a homogeneous solution. The mixtures were shaken and presence of pink, red or violet colour on the bottom of a test tube was an indication of anthraquinones (Sofowora, 1996; Guta *et al.*, 2016).

3.2.4. Qualitative scores assessment of phytochemical compounds in *Pavetta crassipes a*nd *Pavetta schumanniana* leaves

The dried crude aqueous extracts of *P. crassipes a*nd *P. schumanniana* leaves were screened for the presence of alkaloids, saponins, terpenoids/sterols, tannnins, anthraquinones and anthocyanins using the standard chemical tests as described in section 3.2.3.2. The concentration of phytochemicals was assessed using qualitative scores (+++, ++, +, -) where +++ denoted strong concentration, ++ representing moderate concentration, + indicating weak concentration and – indicating absence of a particular phytochemical.

3.3 Antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract against Bacteria

In studying the efficacy of *P. crassipes* and *P. schumanniana* leaf extract against microbial activity, an aqueous leaf extract was applied to selected gram-positive and gram-negative bacteria *Escherichia coli* and *Staphylococcus aureus* in order to observe the reaction.

3.3.1 Study area of antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaves

The study was conducted at Mzuzu University Biology laboratory. *P. crassipes* and *P. schumanniana* leaf powder which was stored in airtight glass bottles which was used for phytochemical analyses was also used for antimicrobial analyses.

3.3.2 Sampling procedure of antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract

The antimicrobial activity of *P. crassipes* and *P. schumanniana* leaf aqueous extract against two different strains of bacteria (gram-positive and gram-negative types of bacteria) was carried out as described in the following protocols:

3.3.2.1 Processing of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract for bacterial treatment

About 50 g of *P. crassipes* and *P. schumanniana* leaf powder from the storage bottles was weighed on an analytical balance (Model number: ATY-224, calibrated to four decimal places) and was soaked in 1000 mL of distilled cold water for 24 hours (Harborne, 1998). After 24 hours, the mixture was filtered with a suction machine (Model number: TID-15) using Whatman's filter paper number one. The filtrates were collected in a sterilized 250 milliliter (mL) predetermined mass beakers. The

filtrate was evaporated using an oven (Model number: 920) (Appendix I) at 60-80° C until only residues remained in the beaker. The beaker containing the residues of plant extract was removed from the oven, and was placed in a desiccator to cool and remove extra moisture. The beaker with residues was weighed and the mass of the extract was recorded. The resulting powder was dissolved and diluted to the required concentrations (25, 50, 75 and 100 µg/mL).

3.3.2.2 *Pavetta crassipes* and *Pavetta schumanniana* leaf extract and ciprofloxacin antibiotic dilution

From the masses of the *P. crassipes* and *P. schumanniana* leaf extracts obtained from the process explained in 3.2.1, 1.0 g for each sample was weighed and dissolved in a small (50 mL) beaker separately with small amounts of sterilized distilled water. Glass rod was used for stirring until *P. crassipes* and *P. schumanniana* leaf extracts were completely dissolved. The plant extract solution was then poured into 1000 mL volumetric flask and distilled water was added to the 1000 mL mark, making 1000 mg/L. Preparation of, 25 mg/ L, 50 mg/ L, 75 mg/ L and 100 mg/ L were made by pipetting 2.5 mL, 5.0 mL, 7.5mL or 10.0 mL of diluted plant extract, respectively. From 1000 mg/L into 100 volumetric flasks and distilled water was added to the 100 mL mark. These made 25 micrograms/ milliliter (µg/mL), 50 µg/mL, 75 µg/mL and 100 µg/ mL, respectively.

Ciprofloxacin, a commercial antibiotic in tablet form bought from a private pharmacy, was used as a positive control while sterilized distilled water was used as a negative control. A 500 mg Ciprofloxacin tablet was dissolved into a 500 mL volumetric flask with small amount of sterilized distilled water. A glass rod was used for stirring until Ciproflaxin was completely dissolved. More sterilized distilled water was added to the 500 mL mark. The solution was homogenized by shaking the volumetric flask. Diluted antibiotic of 1.25 mL was pipetted into 250 mL volumetric flask and sterile distilled water was also added to 500 mL mark, making a concentration of 5µg/mL. The antibiotic was used as a positive control for all treatments. Ciprofloxacin was used since it is a broad spectrum antibiotic which is effective for both on gram- negative and gram-positive bacteria (Akinyemi, Oluwa and Omomigbehin, 2006). Ciprofloxacin was used as standard antibiotic (control) at a concentration of 5 µg/ mL.

3.3.2.3 Preparation and impregnation of diffusion discs

Evaluation of antimicrobial activity for the *P. crassipes* and *P. schumanniana* leaf extract was done by disc diffusion method. To prepare diffusion discs, Whatman filter paper number 1 (Qualitative circle 90 mm) was punched using a punching machine (Appendix H) of 6 mm size. The punched

small papers were used as diffusion discs. The diffusion discs were sterilized by the manual autoclave at 121° C >15 psi for 15 minutes in screwed up bottles (McCartney bottles). Bottles containing discs were left to cool in the autoclave before removing and utilizing them.

After sterilizing the diffusion discs, they were impregnated with 25 mL of *P. crassipes* and *P. schumanniana* leaf concentrations, these were: 25 µg/ mL, 50µg/ mL, 75 µg/ mL and 100 µg/ mL (Table 2). Then the diffusion discs were dried in an oven in separate 90 mm sterilized glass petri-dishes at 50° C for 5 minutes.

Calculations of the concentrations used were determined as shown below:

$$V_1 = \frac{C_2 \, x V_2}{C_2}$$ **Equation 1**

Where:

V_1= Volume required in mL

V_2= Volume of 100 mL

C_1= Concentration of *P. crassipes* and *P. schumanniana* leaf aqueous extract in 1000 mg/L

C_2= Concentration of *P. crassipes* and *P. schumanniana* leaf aqueous extract in 25 mg/L

Later the units were changed from milligram per liter (mg/L) to microgram/milliliter (µg/mL) as shown in Table 2.

Table 2: Concentration of *P. crassipes* and *P. schumanniana* leaf extract used

Volume pipetted (V_1) in mL	**Final volume (V_2) in ml**	**Concentration required (µg/mL)**
25	100	250
50	100	500
75	100	750
100	100	100

3.3.2.4 Preparation of Barium sulphate suspension

To prepare Barium sulphate suspension, 1.175 g of Barium chloride was weighed on an analytical balance (Model number ATY-224) using a 50 mL predetermined mass beaker. A small amount of sterile distilled water was added to the beaker containing 1.175 g of Barium chloride and it was

shaken until the solute was completely dissolved. The solution was transferred into 100 mL sterile volumetric flasks and sterile distilled water was added gradually followed by stirring up to 100 mL mark. The volumetric flask was capped with a top and shaken vigorously to homogenize the solution thoroughly. A sterile pipette was used to measure 0.5 mL of 1.175% Bacl2 aqueous solution and added to 99.5 mL of 1% (v/v) sulphuric acid (H_2SO_4), this process was done with constant stirring to make the solution equivalent to 0.5 McFarland standard.

The Barium sulfate suspension (4-6 mL aliquots) was transferred into sterile screw cap tubes of the same size. The tubes were tightly sealed and stored in the dark at room temperature of 25° Celsius until the solution was used. The ringer's saline solution was made by dissolving ringer's tablet (one) into 500 mL of sterile distilled water using 1L volumetric flask. The solution was shaken well to homogenize it. The saline solution was placed into screwed up bottles (McCartney bottles each containing 60 ml of the ringer's solution). The bottles containing ringer's saline solution were sterilized by auto-claving for 15 minutes at 121° C > 15psi.

3.3.2.5 Collection of test organisms

The test bacteria used in this study were *Staphylococcus aureus* and *Escherichia coli*. They were obtained from Department of Medical Microbiology in Mzuzu Central Hospital. Biochemical tests were carried out to confirm all the test bacterial strains. Bacterial strains were isolated on sterile nutrient agar slants and taken to Microbiology Laboratory of Mzuzu University using a cooler box of ice packs. At Microbiology Laboratory all slants of test organisms were kept at 4° C waiting for sub-culturing procedure.

3.3.3 Experimental design and treatments

The antimicrobial study of *P. crassipes* and *P. schumanniana* leaf extract used a completely randomized design. The antimicrobial study had four treatments which comprised of: two types of bacteria species (*E. coli* and *S. aureus*), two types of plant species (*P. crassipes* and *P. schumanniana*), four concentrations of plant leaf extract (25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL), and two types of control (positive and negative) (Table 3). Ciprofloxacin standard antibiotic of dilution of 5µg/mL was used as a positive control while as sterile distilled water was used as a negative control. Concentration of 5µg/mL was used as minimum inhibition concentration for all the two types of bacteria. In Table 3, AB means antibiotic, DW means Sterile distilled water.

Table 3: Treatment levels (types of bacteria, plant extract) on each type of bacterium

Types of bacteria	**Plant species**	**Plant lea extract concentrations (µg/mL)**	**Control**	
			Positive	**Negative**
EC	P. Cr	25	AB	DW
		50	AB	DW
SA		75	AB	DW
		100	AB	DW
EC	P. Sc	25	AB	DW
		50	AB	DW
SA		75	AB	DW
		100	AB	DW

Key: Bacteria type EC = *E. coli,* SA = *S. aureus,* P. Cr = *P. crassipes,* P.Sc = *P. schumanniana* AB means antibiotic (ciprofloxacin), and DW means sterilized distilled water**.**

3.3.3.1 Preparation of bacteria culture and sub-culturing of bacteria

To prepare bacteria culture, Vile red blood agar (VRBA) and MacConkey were made according to the manufacturer's instructions. The made MacConkey was autoclaved by the manual autoclave (Appendix G) for 15 minutes at 121°C > 15psi.

Prepared culture media about 15 to 20 mL of VRBA and MacConkey were poured into 90 mm petri dishes and were allowed to cool at body temperature of 37° C in the laminar flow. The remaining medium was put in the water bath (Appendix F) at 45° C (Model number: AVI- 410) to avoid solidifying. Sub-culturing was done by scooping the contents from nutrient agar slants using a wire loop and rubbing it on the surface of the solid media in prepared petri dishes. Each time the wire loop was used, it was sterilized by a spirit burner. The petri dishes left to set in the laminar flow. After 15 minutes, petri dishes were put in the incubator (Appendices H, Model number: AVI-412) upside down at 35°C for 24 hours so that bacteria could multiply. The pure-cultured isolated colonies were put into bottles containing 60 mL of 0.9% normal saline solution (Ryan and Ray, 2004).

3.3.3.2 Preparation of Standard Inoculums

The turbidity of the actively growing broth culture was adjusted with sterile 0.9% normal saline solution to obtain turbidity, optically comparable to that of the 0.5 McFarland standard. The result was a suspension containing approximately 1.5 x 10^8 colony forming units (cfu) or spores/mL of each bacterium (Marasini *et al.*, 2015). The procedure was done to all the two types of bacteria in the presence of adequate light, to visually compare the inoculum tube and the 0.5 McFarland standards without difficulties. A card with white background was used to effectively ensure that the colour (turbidity) was the same for both 0.9% normal saline solution with bacteria and the McFarland standard solutions (Ayele, Regasa and Delesa, 2015).

3.3.3.3 Inoculation of the test plates

After the standardization of McFarland standards, turbidity test was done. Two (2) mL each of inoculum suspension was pipetted into petri dishes containing VRBA and MacConkey Agar medium for *E. coli* and *S. aureus* respectively. After the inoculum was placed in the petri-dishes by pouring method, each impregnated disc representing sterilized distilled water (DW), antibiotic (AB) and four types of concentration (100 µg/mL, 75 µg/mL, 50 µg/mL and 25 µg/mL was presented in each petri dish with a sterilized forceps. The six discs were placed in the petri dish anti- clock wise, having a mark in the sides of the petri dish marking the first discs. The discs were pressed down with forceps to ensure complete contact with the agar surface. The forceps were soaked in methylated spirit and then sterilized by Bunsen burner frame each time the forceps were used. The covers of the petri dish were well labeled indicating type of plant species (*P. crassipes* and *P. schumanniana* leaf extract), type of medium, date of culturing, initial of the researcher and type of treatment. The dish was rotated approximate 60^0 each time to ensure an even distribution of an inoculum before left to dry in the laminar flow (Appendix I) for 3 to 5 minutes. Drying of the inoculum was done to avoid spilling in the incubator. The remaining discs were stored in the refrigerator at 4-8 degrees C. The petri dishes were placed in the incubator upside down at 37^0 C for 24 hours within 15 minutes after application. After 24 hours, inhibition zones were measured using a digital-caliper (150mm Model 500). The inhibition zones were equated to the nearest millimeter and the figures were recorded in the laboratory note book. Each bacterium was recorded on a separate page.

3.3.3.4 Determination of Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibition concentration of the precipitate was carried out on the microorganisms that were susceptible to it and was carried out using the modification of the dilution method for the determination of MIC and MBC. This was done by diluting leaf extracts of *P. crassipes* and *P. schumanniana* into various concentrations, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, 25, 27.5, 30, 35, 40, 45 and 50 µg/ml in sterile nutrient broth in test tubes (Chikezie, 2017). Using standard wire loop, a loopful (10 µl) of *E. coli* culture of 0.5 McFarland standard was inoculated into test tubes containing 1 ml of the various concentrations of *P. crassipes* and *P. schumanniana* leaf extract in nutrient broth (EUCAST, 2003). Similarly, this was repeated for *S. aureus*. The tubes were incubated at 37°C for 18 to 24 h and thereafter observed for growth or turbidity. The lowest concentration of the tubes that were clear was recorded as the MIC. Subsequently, a loopful of broth from each test tube not showing growth, was inoculated into nutrient agar plate. Thereafter, equal volumes of sterile nutrient broth were added into the test tube cultures and incubated further for 24 hours at 37°C. Then, the tubes and agar plates were examined for growth or turbidity using unaided eye (CLSI, 2012). The lowest concentration of the tubes, 25 µg/mL, that were clear was recorded as the MBC. This was carried out in order to determine whether the microorganisms could be completely killed or their growth could only be inhibited.

3.4 Data analysis

3.4.1 Data analysis for social use of *Pavetta crassipes* and *Pavetta schumanniana* leaves

The questionnaire was coded and then data collected from two sites was entered into the Statistical Package for Social Scientists (SPSS) version 20. Data was analysed using SPSS version 20 to compute frequencies and percentages for different parameters such as demographic characteristics of the respondents, parts of the plant mostly used, availability of the plant species, diseases treated by the plant species, mode of administering and method of preparation of *P. crassipes* and *P. schumanniana* leaves. In addition, Chi-square test was used to determine statically significant differences in parameters of interest between the two plant species at significant level of 0.05 significant level.

3.4.2 Qualitative analysis of phytochemicals in *Pavetta crassipes* and *Pavetta schumanniana* leaves

Data for objective 2 was analysed using qualitative chemical methods of analysis by assessing the presence or absence of phytochemicals in the plant material (see 3.2.4).

3.4.3 Data analysis of antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract

The zone of inhibition of *P. crassipes* and *P. schumanniana* aqueous leaf extract in mm were entered into STATA version 16. Shapiro Wilk test for normal data was used to see if data was normally distributed, and thereafter it was subjected to ANOVA.

3.5. Research ethics

Ethical approval was granted by Mzuzu University Research Ethics Committee (MZUNIREC) with approval number MZUNIREC/DOR/21/47. Informed consent was obtained from all participants in the survey prior to interviews. Informed consent entailed informing the respondents about the purpose of the survey and the benefits and had to express their willingness to participate or not. Participants were also at liberty to stop their involvement in the research at any time they felt to do so. No monetary incentives were given to the participants for participating in the research project. Participants were assured that the data will be used for academic purposes only and will be treated in complete confidential manner.

CHAPTER FOUR: RESULTS

4.1 Ethno medicinal uses of *Pavetta crassipes* and *Pavetta schumanniana* leaves in Dedza and Mzimba

4.1.1 Medicinal uses of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Pavetta schumanniana and *Pavetta crassipes* leaves were claimed to treat 8 and 9 diseases in Mzimba and Dedza districts, respectively (Figure 36). Statistical analysis, Person chi-square, exhibited significant differences (X^2 = 122.969, df = 11, p = 0.000) in terms the percentage of respondents and how the two plants species are used to treat diseases in the two study sites. Majority of the respondents in Mzimba use *P. schumanniana* to manage coughing while in Dedza they mostly use *P. crassipes* to enhance libido.

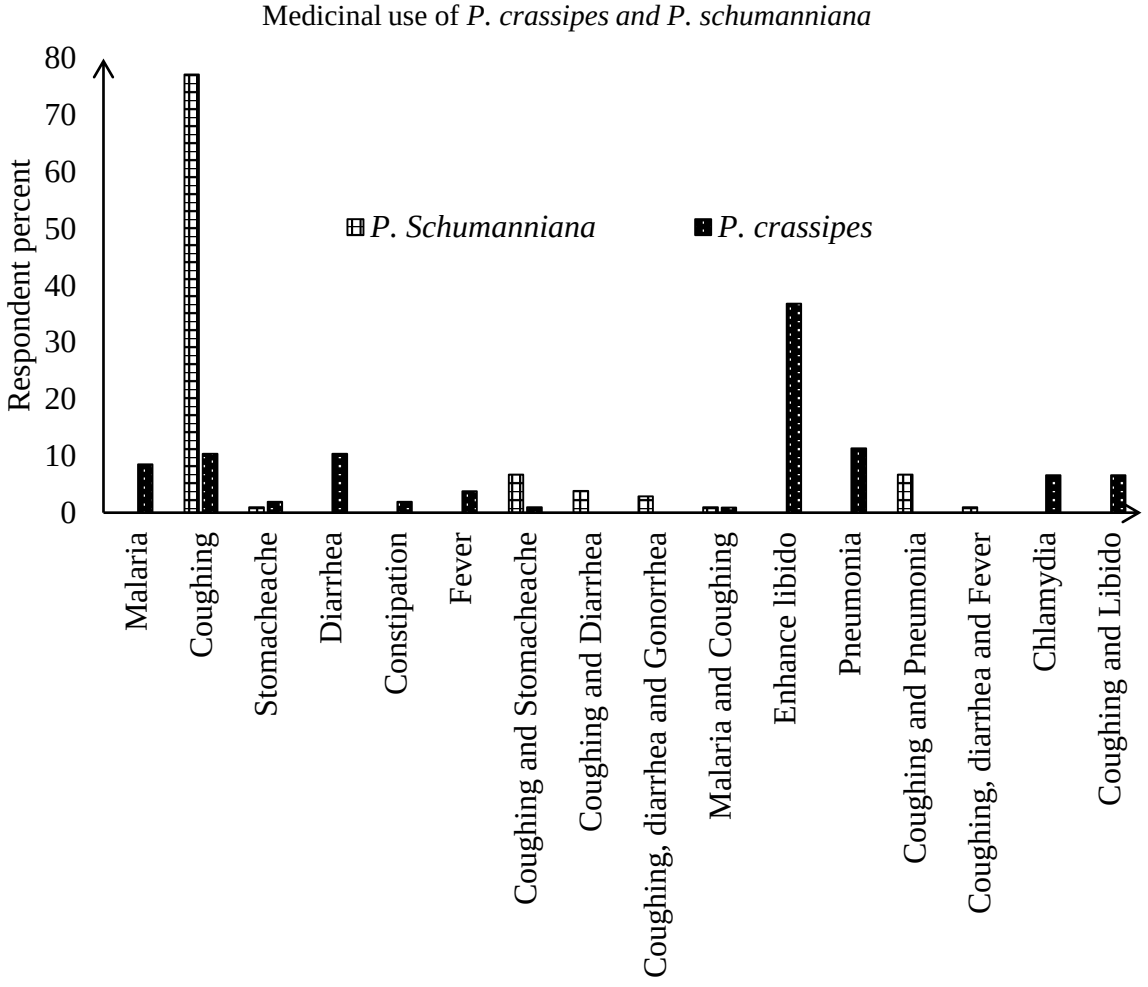


Figure 36: Medicinal uses of *P. crassipes* and *P. schumanniana* leaves

The study also investigated on how local communities prepare *P. schumanniana* and *P. crassipes*

leaves medicine to treat various ailments mentioned in the section above. The results indicated significant differences between sites in the method of preparing medicine from *P. schumanniana* and *P. crassipes* leaves ($X^2 = 129.443$, df = 4, p = 0.000). In general, results showed that in Mzimba and Dedza prepared *schumanniana* and *P. crassipes* leaves medicine as powder and fresh leaves (Figure 37). However, the majority of respondents in Mzimba (99 %) used fresh leaves of *P. schumanniana*, while in Dedza (55 %) widely prepared it as a powder.

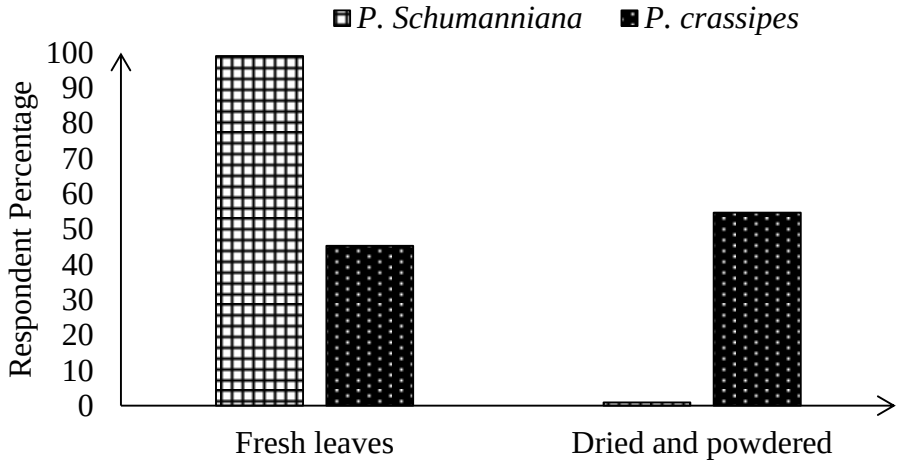


Figure 37: Method of preparing medicine from *P. schumanniana* and *P. crassipes* leaves

Study also investigated whether *P. schumanniana* and *P. crassipes* leaves are used alone or in combination with other ingredients (medicinal plants). Study findings revealed that both *P. schumanniana* and *P. crassipes* leaves are mostly used to manage ailment alone (Figure 38).

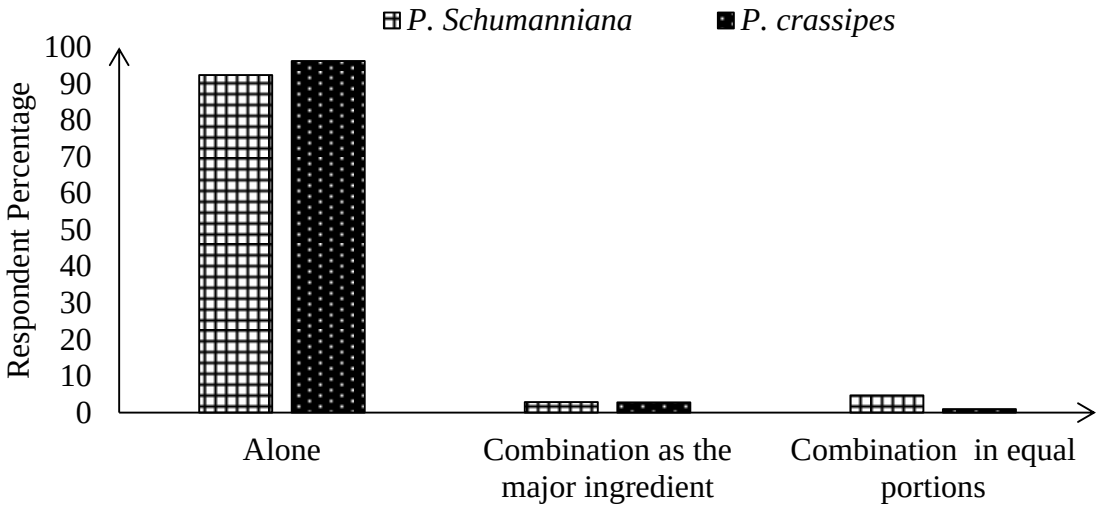


Figure 38: Utilisation of *P. schumanniana* and *P. crassipes* leaves

The study also investigated on how local communities prepare *P. schumanniana* and *P. crassipes* leaves medicine to treat various ailments mentioned above. Results demonstrated significant difference $X^2 = 3.015$, df =1, p = 0.083 on the percentage of respondents and their mode of administration of *P. schumanniana* and *P. crassipes* leaves medicine in Mzimba and Dedza districts. Results revealed 2 modes of administering *P. schumanniana* and *P. crassipes* leaves medicine and oral method of administration was dominant (100 %, 97 %) in Mzimba and Dedza respectively (Figure 39).

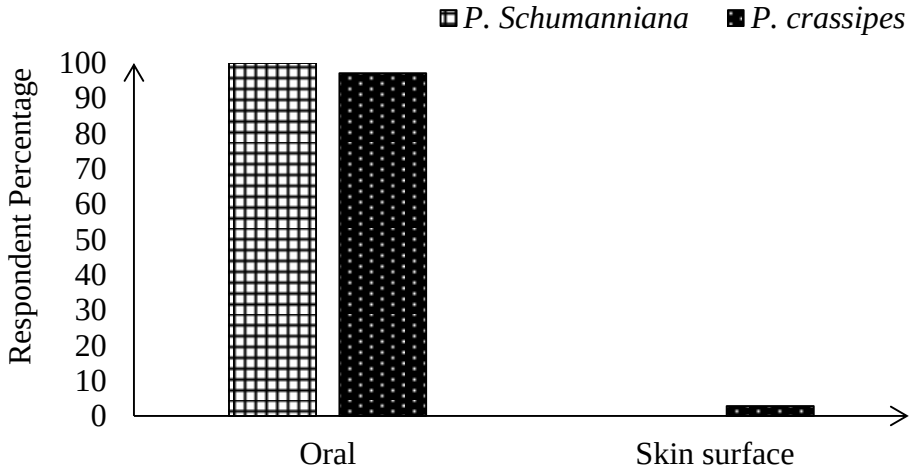


Figure 39: Mode of administration of *P. schumanniana* and *P. crassipes* leaves

The study also investigated on the frequency of repondents and their intake of leaves of *P.*

schumanniana and *P. crassipes* as medicine to treat various ailments mentioned above. Results revealed significant difference $X^2 = 29.997$, df =4, p = 0.000 on the frequency of *P. schumanniana* and *P. crassipes* leaves medicine intake. The majority of the respondent in Mzimba (50) mentioned thrice daily while in Dedza (47 %) mentioned twice daily (Figure 40).

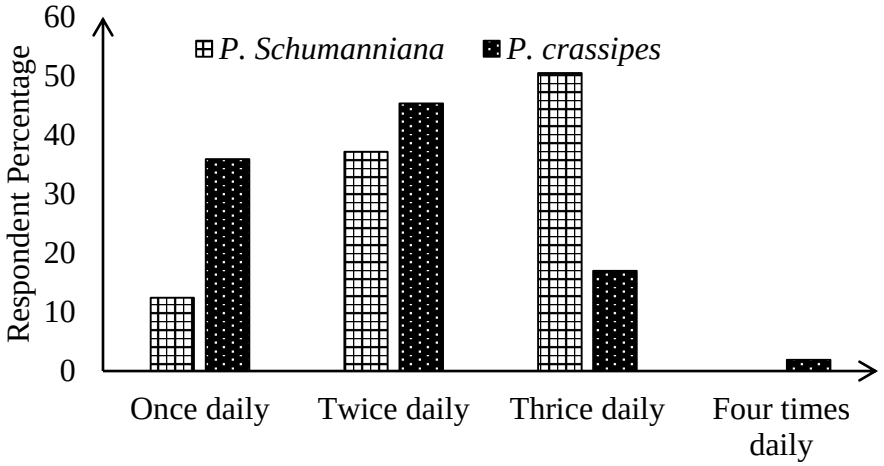


Figure 40: The frequency of *P. schumanniana* and *P. crassipes* leaves medicine intake

The study also investigated on the reported side effects *P. schumanniana* and *P. crassipes* leaves medicine by the users. The majority of the respondents in Mzimba and Dedza said that *P. schumanniana* and *P. crassipes* leaves medicine has no side effects (98 % and 92 %), respectively. Only few people mentioned sweating (1 %) and dry mouth (1 %) in Mzimba, and sweating (5 %) and dizziness (3 %) in Dedza (Figure 41) as experienced side effects.

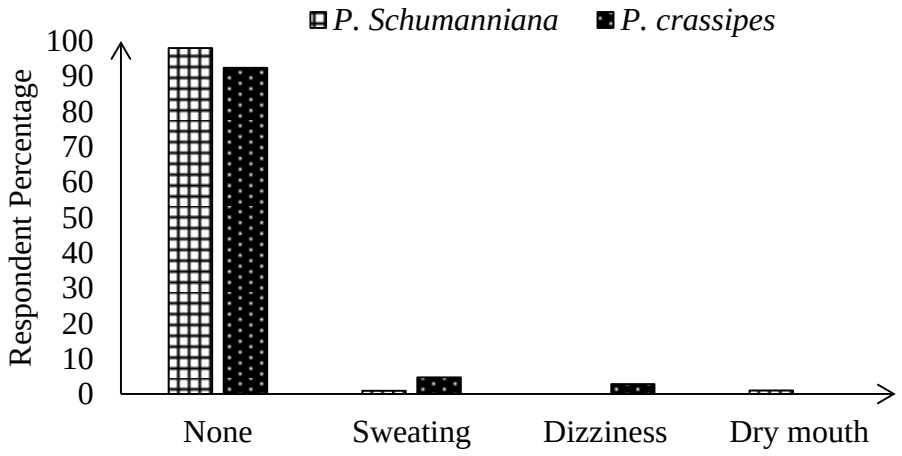


Figure 41: The side effects *P. schumanniana* and *P. crassipes* leaves medicine

4.1.2 Availability of *Pavetta crassipes* and *Pavetta schumanniana* leaves

Results revealed no significant difference on respondent's perception on the availability of *P. crassipes* and *P. schumanniana* plants between Mzimba and Dedza, respectively. In general, the majority of respondents in Mzimba (63 %) said *P. schumanniana* is moderately scarce and in Dedza (64 %) said *P. crassipes* was moderately scarce (Figure 42).

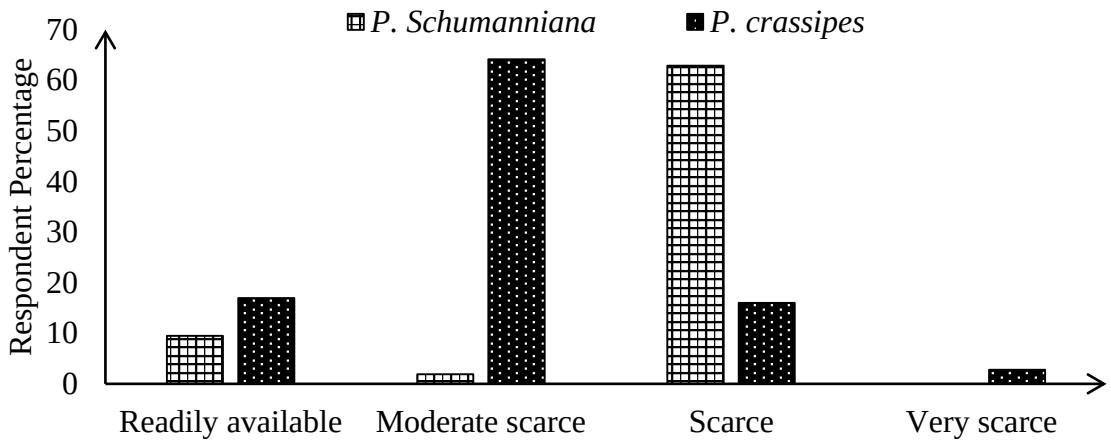


Figure 42: Availability of *P. crassipes* and *P. schumanniana* plants

Results showcased highly significant difference X^2 =72.061, df =4, $p < 0.001$ on the amount of *P. crassipes* and *P. schumanniana* plants material harvest for medicinal preparation in Mzimba and Dedza respectively. The majority of respondents in Mzimba (99%) and Dedza (47%) mentioned handful of plant leaf material (Figure 43). The quantities were estimated in wet weight.

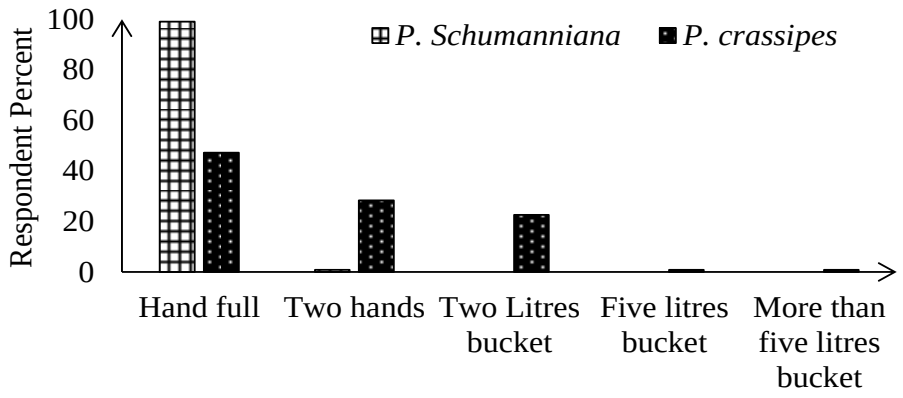


Figure 43: Amount of *P. crassipes* and *P. schumanniana* plants material harvest for medicinal preparation

4.1.3 Traditional knowledge of *Pavetta crassipes* and *Pavetta schumanniana* leaves

The study also investigated on how local communities pass on traditional knowledge of the medicinal uses of *P. schumanniana* and *P. crassipes* leaves in Mzimba and Dedza districts respectively. Results revealed highly significant difference X^2 =72.061, df =4, $p < 0.001$ on how they got informed about the medicinal uses of *P. schumanniana* and *P. crassipes* leaves in Mzimba and Dedza districts, respectively. The majority of respondents in Mzimba (60 %) and Dedza (66 %) mentioned grandparents as the source of traditional knowledge of *P. schumanniana* and *P. crassipes* leaves in Mzimba and Dedza districts respectively (Figure 44).

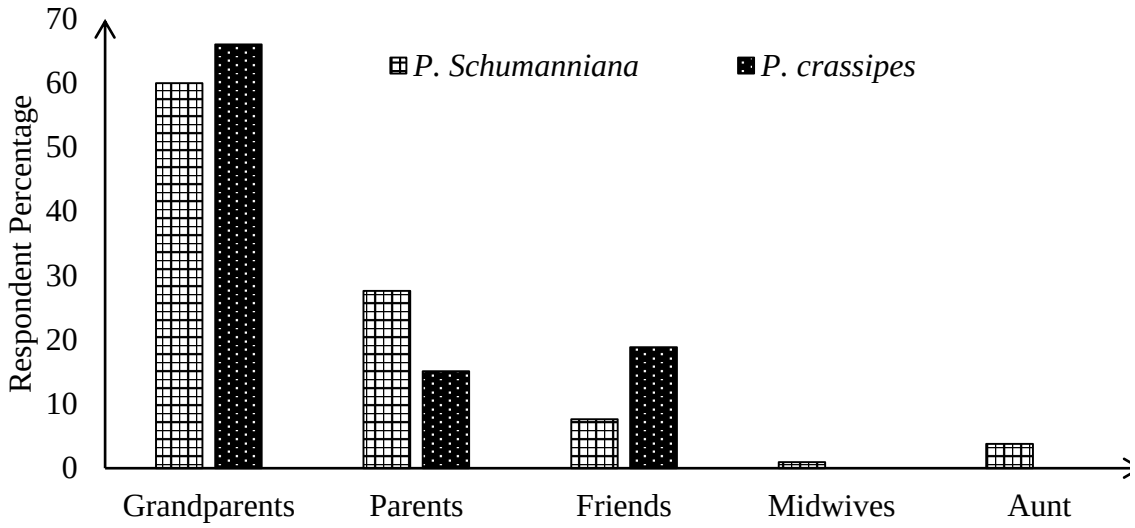


Figure 44: Traditional knowledge of the medicinal uses of *P. schumanniana a*nd *P. crassipes* leaves

Results showed that there was no significant difference on how people share traditional knowledge of medicinal uses of *P. schumanniana* and *P. crassipes* leaves in Mzimba and Dedza districts, respectively. The majority of respondents in Mzimba (60 %) and Dedza (66 %) mentioned that they shared with their friends the traditional knowledge of medicinal use *P. schumanniana* and *P. crassipes* leaves (Figure 45).

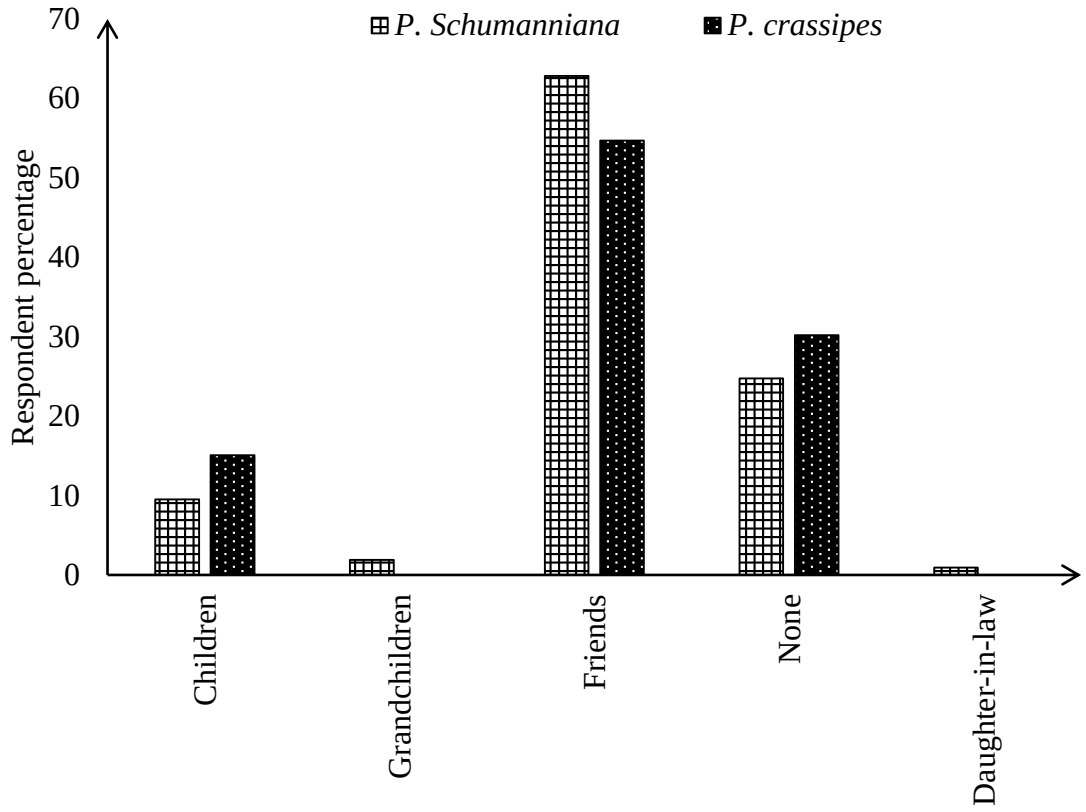


Figure 45: Transfer of traditional knowledge of medicinal uses of *P. schumanniana* and *P. crassipes* leaves

4.2 Phytochemical analysis of *Pavetta crassipes* and *Pavetta schumanniana* leaf extracts

The study has revealed that there were similarities and differences on type and concentration of phytochemical compounds found in *P. crassipes* and *P. schumanniana* leaf extracts between the two species (Table 4). The phytochemical compounds present in *P. crassipes* leaves were steroids, tannins, anthocynins and anthraquinones. The phytochemical compounds present in *P. schumanniana* leaves were saponins, terpenoids/steroids, tannins and anthocynins. Despite the differences, both species had strong presence (+++) of terpenoids/steroids and tannins. Apart from that there were weak presence (+) of anthocyanins in *P. crassipes* and strong presence (+++) of anthocynins in *P. schumanniana*. Moderate presence (++) of anthraquinones were found in *P. crassipes* and were absent in *P. schumanniana*. Weak presence (+) of saponins were found in *P. schumanniana* and were absent in *P. crassipes*. In addition, alkaloids and flavonoids were absent in *P. crassipes* and *P. schumanniana*.

Table 4: Phytochemical compounds found in *P. crassipes and P. schumanniana* leaf extracts

Species	**Phytochemical compounds**						
	Terpenoids/ Steroids	Saponins	Alkaloids	Tannins	Flavonoids	Anthocyanins	Anthraquinones
Pavetta crassipes	+++	-	-	+++	-	+	++
Pavetta schumanniana	+++	+	-	+++	-	+++	-

Key: +++ means strong presence, ++ means moderate presence, + means weak presence, - means absent

4.3 Antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract against Bacteria

4.3.1 Efficacy of *P. crassipes and P. schumanniana leaf* extracts on antibacterial activities

Regarding the microbial effects of *P. crassipes and P. schumanniana leaf extract* on selected types of bacteria species, the data showed a concentration dependent efficacy on *E. coli* while for *S. aureus* there was no inhibition. As shown on Table 5, the most effective *P. crassipes and P. schumanniana* aqueous leaf extract concentrations were 100 µg/mL with highest recorded mean zone of inhibition of 8.10±0.89 mm (p = 0.999) and 7.73±1.17 mm (p = 0.999), compared to concentrations of 75 µg/mL and 50 µg/mL with mean zones of inhibition of 5.77±0.68 mm (p = 0.431) and 4.33±0.55 mm (p = <0.001) for *P. crassipes* and 5.50±0.97 mm (p = 0.999) and 4.17±1.09 mm (p = 0.368), for *P. schumanniana* respectively. The least zone of inhibitions 3.07 ± 0.52 mm (p = 0.999) and 2.83 ± 0.99 mm (p = 0.955) were recorded for 25 µg/mL of both *P. crassipes* and *P. schumanniana* aqueous leaf extract concentrations respectively. The mean zone of inhibition of ciprofloxacin/antibiotic (positive control) was 18.27 ± 0.55 mm while distilled water (negative control) recorded 0.667 ± 0.547 mm.

4.3.2 Sensitivity of *Pavetta crassipes* and *Pavetta schumanniana* aqueous leaf extract on individual bacteria type (*E. coli* and *S. aureus*)

With regard to individual bacteria species type, both *P. crassipes and P. schumanniana aqueous leaf* extracts showed inhibition on *Escherichia coli* gram negative bacteria, while on *Staphylococcus aureus*, a gram positive bacteria there was no inhibition.

4.3.3 MIC and MBC of *Pavetta crassipes* and *Pavetta schumanniana* aqueous leaf extract against *E. coli* and *S. aureus*

The minimum inhibition concentrations and minimum bactericidal concentrations for both *P. crassipes and P. schumanniana aqueous* leaf extracts against *E. coli* were 12.5 and 25 µg/mL (Table 6). In this study it was observed that the MIC of plant extracts of two plant species were lower than the MBC, suggesting that the plant extracts were bacteriostatic at lower concentration and bactericidal at higher concentration. On the other hand, it was not possible to determine the minimum inhibition concentrations and minimum bactericidal concentrations for both *P. crassipes and P. schumanniana aqueous* leaf extracts against *S. aureus* because the plant species were not active on the concentrations tested. This study recommends further studies using higher concentrations because there is a possibility that the aqueous leaf extracts of two plant species were not effective in inhibiting *S. aureus* because the tested concentrations were low.

Table 5: MIC and MBC of *P. crassipes* and *P. schumanniana* aqueous leaf extract against *E. coli* and *S. aureus*

Plant Species	Organism	MIC	MBC
Pavetta crassipes	*E. coli*	12.5	25
	S. aureus	NA	NA
Pavetta schumanniana	*E. coli*	12.5	25
	S. aureus	NA	NA

NA = Not Active on the concentrations tested

CHAPTER FIVE: DISCUSSION

5.1 Ethno medicinal uses of *Pavetta crassipes* and *Pavetta schumanniana* leaves in selected populations in Dedza and Mzimba

5.1.1 Medicinal uses of *Pavetta crassipes* and *Pavetta schumanniana* leaves

The findings on medicinal use of *P. crassipes and P Schumanniana* plant leaves in the two districts signify the importance of conducting investigations on ethno medicinal use of plant species where the two species under study were said to be used to treat various types of diseases. Since *P. schumanniana* belongs to the genus like most examples cited above, the findings in this study suggest its potential in possessing medicinal properties. Study findings concur with previously reports which documented the pharmacological activities of *P. schumanniana* included management of respiratory infections such as coughing, treating infertility and venereal diseases in women (Bridson, 2001). The following pharmacological activities have been reported on the *P. crassipes* plant; anti-plasmodial/malarial activity (Sanon *et al.*, 2003), hypotensive activity (Amos *et al.*, 2004), inhibitory effects on gastrointestinal and uterine smooth muscles (Amos *et al.*, 1998) and in vitro antiprotozoal, antimicrobial and antitumor activities (Baldé *et al.*, 2010). The leaves of *P. crassipes* are used to treat schistosomiasis or haematuria, malaria, splenosis, fever, conjunctivitis, syphilis sores, diarrhoea, stiffness, weakness, respiratory diseases, hypertension (Daget, 2002; Zerbo *et al.*, 2011).

In Central and some parts of Southern Africa including Malawi, the acid infusion of the leaves is taken as a cough remedy, dried ground leaves as libido enhancers in men, roots are used for snake bites and bark as a purgative. Similarity in diseases recorded in different countries might imply that traditional knowledge in *P. crassipes* herbal medicine is wide spread globally. Such results give confidence on the use of the species as a genuine medicinal plant. The leaves are also utilized by locally in Tanzania for treating gonorrhea (Daget, 2002; Aliyu *et al.*, 2008).

Other parts reported to be used include: roots of *P. crassipes.* used for treating gonorrhea, in some cases as a laxative in times of constipation (Daget, 2002; Mponda, 2012). The bark of *P. crassipes* is used for treating snake bite, febrifuge and thyroid stimulating while roots and leaves of *P. crassipes* are used to treat fever, vitamin deficiency and kwashiorkor, and fruits are used as vermifuge (Zerbo *et al.*, 2008;

Mponda, 2012). In Nigeria, leaves of *P. crassipes* are used as medicine in the management of respiratory infections and abdominal disorders (Daget, 2002; Aliyu *et al.*, 2008; Zerbo *et al.*, 2011).

Results have shown that there are two preferred methods of preparing medicines from *P. crassipes* and *P. schumanniana* leaves namely; powder and fresh leaves. In addition, results showed that the most preferred method of preparing medicine in Dedza is powder while in Mzimba is fresh leaves for *P. crassipes* and *P. schumanniana* leaves respectively. This suggests that fresh leaves might be an easier and quicker method of preparing the *P. schumanniana* leaves as compared with processing powder. On the other hand, powder form was the most preferred methods for preparing *P. crassipes*. This is because *P. crassipes* leaves were likely to be consumed frequently because of its acclaimed aphrodisiac properties hence powdered plant leaf material was preferred due to its longer shelf life compared to fresh leaves. However, sanitation and quality of water is an issue that needs investigation to ensure that users are safe. This study suggests further research to investigate microbial contamination on *P. crassipes* and *P. schumanniana* leaf drug preparations in the study areas.

The majority of respondents in this study preferred using *P. crassipes* and *P. schumanniana* leaves only when managing different ailments (Figure 36) and this is in line with study findings of Mponda *et al*., 2012. In addition, the major mode of administering drug of *P. crassipes* and *P. schumanniana* leaves was oral (Figure 39), just as studies by Lifongo (2014); Aliyu *et al*. (2015); Mponda *et al*. (2012) also found similar results. This implies that the major mode of administering *P. crassipes* and *P. schumanniana* leaves drugs was through the mouth (oral) using a teaspoon depending on prescription. In administering the medicine, there is no agreed unit of measure, for example the measure of *P. crassipes* and *P. schumanniana* leaves medicine for both adult and infants was a teaspoon. On frequency of taking the drug study findings have shown that for *P. crassipes* leaves drug the majority were taking the drug twice daily, followed by those that were taking it once daily, then thrice daily and the least were those that were taking the drug four times daily (Figure 40). On the other hand, *P. shumanniana* leaves drug, the majority were taking it thrice daily, followed by those that were taking it twice daily and the least were those that were taking the drug once daily. On the toxicity, the present study has revealed that *P. crassipes* and *P. schumanniana* leaves drugs do not have serious side effects (Figure 41) compared to conventional drugs. In addition, the majority reported that the drugs do not have side effects this is in line with what Lifongo (2014) reported that traditional medicinal do not have

serious side effects compared to synthetic drugs.

It is also important to note that due to the culture of secrecy in traditional knowledge systems some people were not comfortable to disclose sensitive information on other diseases treated by *P. crassipes* and *P. schumanniana* leaves. This could have resulted in missing some important ailments which are also treated by *P. crassipes* and *P. schumanniana* leaves. Therefore, for further research studies, it is commendable to use Traditional Medicine Association of Malawi (TMAM) to capture the possible hidden ailments during the survey.

5.1.2 Availability of *Pavetta crassipes* and *Pavetta schumanniana* plants

Apart from ethno medicinal uses, the study also investigated on the availability of *P. crassipes* and *P. schumanniana* plants (Figure 42). Respondents indicated that both *P. crassipes* and *P. schumanniana* plants are moderately scarce in Mzimba and Dedza Districts. It is therefore imperative to study methods of domesticating the plant species so that the plants can be readily available to people but most importantly to reduce pressure on the available forest resources. Study findings have shown that the majority of respondents in Mzimba and Dedza harvests only a handful of plant leaf material when they want to prepare medicine (Figure 43). Since small amount of plant material is used to prepare medicine, it implies that if domesticated, sustainable utilisation might be possible because plant harvesting may not exceed regeneration capacity of the plants.

5.1.3 Traditional knowledge of *Pavetta crassipes* and *Pavetta schumanniana* leaves

The study also investigated on how local communities passed on traditional knowledge of the medicinal uses of *P. schumanniana* and *P. crassipes* leaves in Mzimba and Dedza districts respectively. Study findings have shown that mostly grandparents were the source of traditional knowledge of *P. schumanniana* and *P. crassipe*s leaves utilisation in Mzimba and Dedza districts respectively (Figure 44). Similarly, study findings also revealed that traditional knowledge on medicinal uses *P. schumanniana* and *P. crassipes* leaves was mostly shared among friends (Figure 45). Traditional knowledge of medicinal uses in most cases is restricted to traditional healers and elder community members (Amber *et al.*, 2017). This knowledge is at the verge of extinction because younger generation is not taking interest in its learning and preservation process (Aziz *et al.*, 2018). On the contrary, study

findings of this novel research have reported that traditional knowledge on the medicinal uses of *P. crassipes* and *P. schumanniana* leaves was shared among friends. However, there is still need to investigate and document traditional knowledge on medicinal uses of *P. schumanniana* and *P. crassipe*s leaves in other districts because we might be missing valuable information.

5.2 Phytochemical analysis of *Pavetta crassipes* and *Pavetta schumanniana* leaf extracts

Strong presence (+++) of terpenoids were found in leaf extracts of both *P. crassipes* and *P. schumanniana.* The observed similarities in phytochemical compounds amongst the two species could be attributed to being species of the same genus (Figueiredo *et al.*, 2008). On the other hand, the observed variations in phytochemical compounds amongst the two species could be attributed to differences in the environmental factors such as temperature, type of soil and precipitation (Jakhetia *et al.*, 2010; Liu *et al.*, 2016).

Pavetta crassipes leaf samples from Mtakataka in Dedza might have been subjected to a cool climate with mean annual temperatures ranging from 14°C to 21°C and annual rainfall ranging from 800mm to 1200mm. On the other hand, *P. schumanniana* leaf samples from Enukweni in Mzimba might have been subjected to a warm tropical climate with average monthly maximum temperatures ranging from 27°C to 33°C and average monthly minimum temperatures ranging from 0°C to 10°C during winter season. Annual rainfall ranges from 650 to 1300 mm (Mzimba District Planning Department, 2008). In addition, Enukweni, Mzimba has medium to light textured sandy-loam and loamy moderately fertile soils with eutric-fersialic soil characteristics while Mtakataka, Dedza has sandy loam relatively fertile alluvial soils (*Dedza District socio-economic profile*, 1999).

This study has reported strong presence (+++) of steroids in both *P. crassipes* and *P. schumanniana* leaf extracts. This might be because the two species belong to the same genus and leaf samples were a combination of young to mature leaves. Study conducted by (Taylor *et al.*, 2001) reported that the composition and concentrations of phytochemical compounds in plants, such as terpenoids/steroids, vary with age of the plant. The younger the plant the less production of the steroids and the older the plant the higher the production. Terpenoids and steroids are responsible for sex hormones, this explains why *P. crassipes* leaves were used to enhance libido in Mtakataka, Dedza. This study therefore suggests that more studies should be conducted to investigate these claims. This study also reports that the leaves

of *P. crassipes* and *P. schumanniana* have the potential of development of pharmaceutical products such as anti-viral drugs and libido enhancers.

There was also strong presence (+++) of tannins in both *P. crassipes* and *P. schumanniana* leaf extracts. This study could not establish the cause of similarity in the phytochemicals found in *Pavetta crassipes* and *P. schumanniana* leaf extracts since Enukweni and Mtakataka have different climatic conditions despite that the two species belong to the same genus. However, one possible explanation for the similarity may be that samples of *P. crassipes* and *P. schumanniana* leaves were exposed to heat wave which hit almost all the districts in Malawi in 2020. Study by (Akula and Ravisharkar, 2011) reported that the production and accumulation of tannins are affected by light intensity. The higher the light intensity the higher the production of tannins. This implies that climate change might affect phytochemical production and accumulation in medicinal plants. Similarly, study by (Kumar *et al.*, 2017) reported that extreme temperatures and drought cause high production of tannins in plants due to stress. This study therefore suggests more studies should be conducted to see how climate change as well as temporary changes in precipitation and light intensity affect phytochemical production in medicinal plants.

Strong presence (+++) of anthocyanins were found in *P. schumanniana* leaf extract compared to *P. crassipes* leaf extract which had weak presence of anthocyanins (+). Chan *et al.*, (2010), reported that *Melastoma malabathricum* cell cultures incubated at a lower temperature range (20 ± 2°C) grew better and had higher anthocyanin production than those grown at 26 ± 2°C and 29 ± 2°C. Optimum temperature (25°C) maximizes the anthocyanin yield as demonstrated in cell cultures of *Perilla frutescens* (Zhong and Yoshida, 1993) and strawberry (Zhang *et al.*, 1997). Similarly, Akula and Ravishankar (2011) reported that lower temperature favours anthocyanin accumulation, but reduces cell growth. This study cannot conclude that temperature was the possible cause of variations because Mzimba has a warm tropical climate while Dedza has a cool climate therefore strong presence of anthocyanins were supposed to be observed in *P. crassipes*. Therefore, possible cause of this variation might be stress. When plants are stressed, secondary metabolite production may increase because growth is often inhibited more than photosynthesis, and the carbon fixed is predominantly allocated to secondary metabolites (Akula and Ravisharkar, 2011). Similarly, the *Daucus carota* callus subjected to phosphate stress produced 7.2% dry weight anthocyanin against 5.4% dry weight (distilled water) in

the control (Rajendran, Ravishankar and Venkataraman, 1992). Anthocyanins have antioxidant and antimicrobial activities (Pervaiz *et al.*, 2017). Akula and Ravishankar, 2011 reported that anthocyanins are used for food colour and have anti-age related neurological disorder and anti-cardiovascular activities.

There was also moderate presence (++) of anthraquinones in *P. schumanniana* leaf extract and anthraquinones were absent (-) in *P. crassipes* leaf extract. Simpson and Amos (2017) reported that anthraquinones are found in several angiosperms families including rubiaceae. Similarly, Aliyu *et al.*, (2008) reported the presence of anthraquinones in methanol extract. However, Olutayo *et al.,* (2018) reported that anthraquinones were absent in methanol extract. Therefore, possible cause of variation in this novel study might be climatic conditions, as well as soil type but not extraction methods. Anthraquinones are used as laxatives and possess antibacterial, antiparasitic, insecticidal, fungicidal and antiviral properties (Malik and Miiller, 2016). Anthraquinones are active components of many plant blends which are used as medicines and exhibit laxative, diuretic, immunomodulatory and anticancer effects (Gupta and Yadav, 2019).

5.3 Antimicrobial activity of *Pavetta crassipes* and *Pavetta schumanniana* leaf extract against Bacteria

5.3.1 Efficacy of *Pavetta crassipes and Pavetta schumanniana leaf* extract on antibacterial activity

Both *P. crassipes and P. schumanniana leaf extracts* were not effective in inhibiting the growth of *S. aureus,* gram positive bacteria. Distilled water did not work, proving that *P. crassipes* and *P. schumanniana* leaf extracts had an antibiotic effect on gram negative bacteria. This conclude that the two plant species were effective in inhibiting the growth of gram negative bacteria at all concentrations. Controlling the growth of gram negative bacteria by *P. crassipes* and *P. schumanniana* aqueous leaf extracts at the highest concentrations of 100 µg/mL was less effective compared ciprofloxacin an antibiotic with the same concentration. The effectiveness of *P. crassipes* and *P. schumanniana* aqueous leaf extracts against the growth of gram negative bacteria tended to increase with increase in concentration. The results suggested that *P. crassipes* and *P. schumanniana* aqueous leaf extracts could be used to control the growth of gram negative bacteria, and its effectiveness increases with the increase in

concentration. Therefore, this study recommends antimicrobial studies of the two plants species using higher concentrations of the plant extracts.

The results of this study agree with Mustapha and Bala (2010) who reported the effectiveness of *Pavetta crassipes* was a function of concentration of the extract. For example, 8,000 µg/mL was more effective than (1,000 µg/mL, 2,000 µg/mL and 5,000 µg/mL). Similarly, Osuntokun and Ajayi (2014) reported that the aqueous and ethanol and acetyl acetate extracts of 20, 40 and 60 mg/mL (20,000 µg/mL 40,000 µg/mL and 60,000 µg/mL) of each extract of *P. crassipes* leaves respectively had larger zone of inhibition at 60 mg/mL. In addition, the report by Gidado *et al.*, 2008 also indicated that the effectiveness of plant extract of *Morinda lucida* and *Nauclea latifolia,* of the same family Rubiaceae, increased with increase in concentration. Based on the current findings, *P. crassipes* and *P. schumanniana* leaves have the potential for further exploration to produce antimicrobial drug leads for the development of antibiotics and disinfectants and associated dosage forms.

5.3.2 Sensitivity of *Pavetta crassipes* and *Pavetta schumanniana* aqueous leaf extract on *E. coli* and *S. aureus*

Pavetta crassipes and *P. schumanniana* aqueous leaf extract inhibited the growth of *E. coli* while *S. aureus* was not inhibited. Phytochemical studies revealed the presence of tannins and anthocyanins in both *P. crassipes* and *P. schumanniana* were responsible for the anti-microbial properties (Aliyu *et al.*, 2008; Bello *et al.*, 2011). The antimicrobial activity of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelop proteins (Koche *et al.*, 2010). In addition, Bello *et. al.*, (2014) reported that *P. crassipes* precipitate had remarkable activity at 50 mg/ml against six of the ten microorganisms tested with zones of inhibition between 15 to 22 mm but it could not inhibit the growth of *Staphylococcus aureus, Bacillus subtilis, Salmonella typhii* and *Candida albicans*.

The antibacterial activity of the *P. crassipes* has been strongly linked with the presence of bioactive compounds especially the flavonoids, which is one of the principal phytochemical components of the plant (Osuntokun and Ajayi, 2014). The antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall (Koche *et al.*, 2010). The compound was reported to inhibit the growth of *P. aeruginosa, K. pneumonia, S. pyogenes* and *E. coli*. Phytochemical analysis in this study shown absence of flavonoids in both *P. crassipes* and

P. schumanniana. The absence of flavonoids in the extracts of the two plant species may have attributed to lack of effect against *S. aureus*.

5.3.3 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Pavetta crassipes* and *Pavetta schumanniana* aqueous leaf extract against *E. coli* and *S. aureus*

Minimum Inhibitory Concentration and MBC studies showed that both *P. crasspes* and *P. schumanniana* leaf extracts inhibited the growth of *E. coli* both at concentration of 12.5 µg/mL with an MBC at 25 µg/mL while *S. aureus* exhibited resistance. Bello *et al.,* (2014) MIC and MBC studies showed that the precipitate inhibited the growths of *Streptococcus pyogenes, Klebsiella pneumoniae* and *Neisseria gonorrhoea* at concentration of 12.5 mg/ml with an MBC at 25 mg/ml while *Corynebacterium ulcerans, E. coli* and *Pseudomonas aeruginosa* were all inhibited at concentration of 6.25 mg/ml with corresponding MBC at 12.5 mg/ml. The minimum inhibitory concentration of *E. coli* reported by Bello *et al.,* (2014) indicated that a precipitate from *P. crassipes* was more pronounced than this study. This may be attributed to different climatic conditions where the plants were found. This may also be due to solvents and or method of extraction used (Rios and Recio, 2005). Similarly, the MIC and MBC values obtained showed that methanol extract of *Acacia albida* was most potent against the MRSA isolates (MIC, 3 mgmL-1 and MBC, 4 mgmL-1) than other plants extracts which exhibited MIC 4 mgmL-1 and MBC 5 mgmL-1 except *Boscia senegalensis* which rather displayed high MIC, 5 mgmL-1 and MBC, 6 mgmL-1 (Aliyu *et al*., 2008).The fact that both *P. crassipes* and *P. schumanniana* leaf extracts shown antibacterial activity against *E. coli*, it is a good indication that the plants hold a great potential for the isolation of antimicrobial compounds which could be used in the development of drugs.

The reports suggesting that *P. crassipes* treats various ailments might be attributed to phytochemical constituents such as tannins, saponins, alkaloids, flavonoids, sterols, anthraquinones and terpenes/steroids (Amos *et al.,* 1998; Osuntokun and Ajayi, 2014) which are found in the plant leaves. Information on phytochemical composition of *P. schumanniana* plant species was not available for both Malawi and elsewhere until this study. Present results have revealed the presence of steroids, tannins, anthocynins and anthraquinones in *P. crassipes* leaves while in *P. schumanniana* leaves have saponins, terpenoids/steroids, tannins and anthocynins. The ability of *P. crassipes* to enhance libido

might be attributed to the presence of terpenoids which are reported to assist in producing sex hormones such as testosterone in which enhances libido (Hillier and Lathe, 2019). The evidence validates the effectiveness of *P. crassipes* leaves in enhancing libido in both men and women as claimed by the respondents in this study and others. In addition, the report by Sanon (2003) indicated that alkaloid extracts from *P. crassipes* leaves exhibited in vitro antiplasmodial/antimalarial activities hence used to treat malaria.

Anthraquinones have been reported to exhibit antimalarial properties (Malik and Miiller, 2016). Tannins and anthraquinones are anti-bacterial (Malik and Miiller, 2016; Koche, Shirsat and Kawale, 2018) and are responsible for the treatment of diarrhoea, cough, pneumonia, and sexually transmitted diseases such as Gonorrhea and Chlamydia. With the results stated above, *P. crassipes* and *P. schumanniana* leaves contains phytochemicals which justify their use to treat several diseases as were reported by communities in this study. Thus, *P. crassipes* and *P. schumanniana* leaves may be used to develop pharmaceutical products that could be sold just like other herbal medicines on the market as concoction made into syrup, powder and/or tablets or other suitable formulations after validation studies are done on their efficacy among other things. This initiative may improve the livelihood of the rural communities in Malawi since they may earn money by selling the processed pharmaceutical products therefore it is imperative to investigate methods of domesticating these two plant species.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Pavetta schumanniana and *P. crassipes* leaves have been found to be used by local people to manage a total of 8 and 9 diseases in Mzimba and Dedza districts, respectively. In Mzimba the majority of respondents used *P. schumanniana* to treat coughs while *P. crassipes* is used by the majority of repondents to enhance libido in Dedza. Powder and fresh leaves were the common formulations prepared as medicine from *P. schumanniana and P. crassipes* leaves respectively. Furthermore, both *P. schumanniana* and *P. crassipes* leaves were mostly used to manage ailments alone. The majority of the people preferred using *P. crassipes* and *P. schumanniana* leaves alone and not in combination with other traditional medicine as a major or minor ingredient or in equal proportions when managing different ailments. On toxicity, results have shown that *P. schumanniana* and *P. crassipes* leaves medicine have no side effects except sweating and dry mouth from *P. schumanniana* by a few people and sweating and dizziness from use of *P. crassipes* by another few respondents. Study findings have shown that mostly grandparents were the source of traditional knowledge of *P. schumanniana* and *P. crassipe*s leaves utilisation in Mzimba and Dedza districts, respectively.

The study has unraveled the presence of steroids, tannins, anthocynins and anthraquinones in *P. crassipes* leaves; and saponins, terpenoids/steroids, tannins and anthocynins *P. schumanniana* leaves which validates the use of the plant species to treat different ailments The study has revealed that there were similarities and differences on type and concentration of phytochemical compounds found between *P. crassipes* and *P. schumanniana* leaf extracts. The observed similarities in phytochemical compounds between the two species could be attributed to being species of the same genus. On the other hand, the observed variations in phytochemical compounds amongst the two species could be attributed to differences in the environmental factors such as temperature, type of soil and precipitation where the two species were growing.

Regarding the microbial effects of *P. crassipes and P. schumanniana* leaf extract on selected types of bacteria species, results have shown a concentration dependent efficacy on *E. coli* while for *S. aureus* there was no inhibition. Furthermore, the minimum inhibition concentrations and minimum

bactericidal concentrations for both *P. crassipes and P. schumanniana aqueous* leaf extracts against *E. coli* were 12.5 and 25 µg/mL. The study has shown the ability of *P. crassipes and P. schumanniana* leaf extract to control gram-negative type of bacteria, *E coli*, at a higher concentration compared to ciprofloxacin a common antibiotic prescribed in hospitals.

6.2 Recommendations

Recommendations to forestry department and botanical gardens:

To begin with domestication of the species. In fostering the domestication of the species, a comprehensive approach should be employed, considering factors such as habit suitability, nutritional requirements, and sustainable management practices. Collaborative efforts involving communities, researchers, and conservationists are pivotal in ensuring the responsible domestication of species, safeguarding biodiversity and promoting ecological balance.

Training local healers using a culturally sensitive and context specific curriculum is essential. Tailoring educational programs to encompass traditional healing practices alongside modern medical knowledge can empower local healers to contribute meaningfully to health care delivery. This approach not only respects cultural heritage but also enhances community health by merging traditional with contemporary medical insights.

Documentation of traditional knowledge pertaining to the utilization of *P schumanniana* and *P. crassipes* holds profound significance in preservation and promotion of the rich tapestry of traditions embedded in tribal cultures. Through meticulous documentation, generations can safeguard their unique practices, wisdom, and ecological insights in relation to the two plant species. This process not only serves as a repository for traditional knowledge but also facilitates intergenerational transmission, fostering a sense of identity and continuity. Documenting this traditional knowledge also provides a comprehensive understanding of the potential economic value of the two plant species.

Recommendations for people in Dedza and Mzimba:

When using *P. schumanniana* and *P. crassipes* for medicinal purposes, they should be cautious about potential toxicity, as some plants can have adverse effects if consumed improperly. Seek

guidance from local experts to understand the correct dosage and preparation methods to mitigate any risks associated with these medicinal plants.

Further research studies are recommended on the following:

1. Analysis of phytochemical compounds should be carried out using different extraction/assessment methods.
2. *Pavetta crassipes* and *Pavetta schumanniana* from the same region or regions with similar environmental conditions should be investigated and compared to elucidate their similarities and differences, potentially revealing insights into their evolutionary adaptations, phytochemical profiles, and antimicrobial properties.
3. Antimicrobial research of the two species using higher concentrations of the plant extracts
4. The effect of genotype, age, precipitation, soil variation and season of harvesting on phytochemical composition should be investigated.
5. The relationship of bacterial load and concentration of the extract should be modelled.
6. Study covering a wider geographical area in Malawi where ever the two species are found in the same location.
7. Future studies should investigate the effectiveness of particular phytochemical compounds found in the plant species on specific diseases.
8. Further studies should also quantify the presence of the compounds in the two plant species since the current study did a qualitative assessment on presence or absence of the compounds.

6.3 Challenges met in the study

1. Advanced assessment to quantify phytochemical constituents were not done because of financial challenges. Nevertheless, a quantitative analysis of the phytochemicals has been recommended for future studies.

2. Absence of both species in the same location made the study to focus on comparisons based on the genus rather than the species level. Further studies have been recommended on *Pavetta crassipes* and *Pavetta schumanniana* from the same region or regions with similar environmental conditions to elucidate their similarities and differences.

REFERENCES

Abdirahman, Y. *et al.* (2015) 'In-Vivo Antidiabetic Activity and Safety of The Aqueous Stem Bark Extract of Kleinia squarrosa', *Journal of Diabetes & Metabolism*, 06(09), pp. 1–11. doi: 10.4172/2155-6156.1000601.

Aggarwal, B. B. *et al.* (2006) 'From traditional Ayurvedic medicine to modern medicine: Identification of therapeutic targets for suppression of inflammation and cancer', *Expert Opinion on Therapeutic Targets*, 10(1), pp. 87–118. doi: 10.1517/14728222.10.1.87.

Akinyemi, K. O., Oluwa, O. K. and Omomigbehin, E. O. (2006) 'Antimicrobial activity of crude extracts of the three medicinal plants used in South-West Nigerian folk medicine on some food borne bacterial pathogens', *African Journal of Traditional, Complementary and Alternative Medicines*, 3(4), pp. 13–22. doi: 10.4314/ajtcam.v3i4.31173.

Akula, R. and Ravisharkar, G. (2011) 'Influence of Abiotic Stress Signals on Secondary Metabolites in Plants', *Plant Signaling & Behavior*, 6(11), pp. 1720–1731.

Aliyu, A. B. *et al.* (2008) 'Activity of Plant Extracts Used in Northern Nigerian Traditional Medicine Against Methicillin-Resistant Staphylococcus Aureus (MRSA)', *Nigerian Journal of Pharmaceutical Sciences*, 7(1), pp. 1–8.

Amber, R. *et al.* (2017) 'A Review on Antiviral Activity of the Himalayan Medicinal Plants Traditionally Used to Treat Bronchitis and Related Symptoms', *Journal of Pharmacy and Pharmacology*, 69(2), pp. 109–122. doi: 10.1111/jphp.12669.

Amos, S. *et al.* (1998) 'Inhibitory Effects of the Aqueous Extract of Pavetta Crassipes Leaves on

Gastrointestinal and Uterine Smooth Muscle Preparations Isolated from Rabbits, Guinea Pigs and Rats', *Journal of Ethnopharmacology*. doi: 10.1016/S0378-8741(98)00046-4.

Amos, S. *et al.* (2004) 'Behavioural effect of Pavetta crassipes extract on rodents', *Pharmacology Biochemistry and Behavior*. doi: 10.1016/j.pbb.2004.01.020.

Anderson, K. J. *et al.* (2001) 'Biochemical and Molecular Action of Nutrients Walnut Polyphenolics Inhibit In Vitro Human Plasma and LDL Oxidation 1 , 2', *The Journal of Nutrition*.

Anderson, R. A. (2008) 'Chromium and polyphenols from cinnamon improve insulin sensitivity', in *Proceedings of the Nutrition Society*. doi: 10.1017/S0029665108006010.

Appendino, G. *et al.* (2006) 'A meroterpenoid NF-κB inhibitor and drimane sesquiterpenoids from asafetida', *Journal of Natural Products*. doi: 10.1021/np0600954.

Arika, W. *et al.* (2015) 'In Vivo Antidiabetic Activity of the Aqueous Leaf Extract of Croton macrostachyus in Alloxan Induced Diabetic Mice', *Pharmaceutica Analytica Acta*, 6(11), pp. 1–6. doi: 10.4172/2153-2435.1000447.

Ayele, T., Regasa, M. and Delesa, D. (2015) 'Antibacterial and Antagonistic Activity of Selected Traditional Medicinal Plants and Herbs from East Wollega Zone against Clinical Isolated Human Pathogens', *Science Technology and Arts Research Journal*, 4(3), pp. 175–179. doi: 10.1088/1751-8113/44/8/085201.

Aziz, M. A. *et al.* (2018) 'Traditional uses of medicinal plants practiced by the indigenous communities at Mohmand Agency, FATA, Pakistan', *Journal of Ethnobiology and Ethnomedicine*. Journal of Ethnobiology and Ethnomedicine, 14(1), pp. 1–16. doi:

10.1186/s13002-017-0204-5.

Balasundram, N., Sundram, K. and Samman, S. (2006) 'Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses', *Food Chemistry*. doi: 10.1016/j.foodchem.2005.07.042.

Baldé, E. S. *et al.* (2010) 'In vitro antiprotozoal, antimicrobial and antitumor activity of Pavetta crassipes K. Schum leaf extracts', *Journal of Ethnopharmacology*. doi: 10.1016/j.jep.2010.05.042.

Barbosa, A. (2014) 'Saponins as immunoadjuvant agent: A review', *African Journal of Pharmacy and Pharmacology*, 8(41), pp. 1049–1057. doi: 10.5897/AJPP2014.4136.

Bassoli, A., Borgonovo, G. and Busnelli, G. (2007) 'Alkaloids and the Bitter Taste', in *Modern Alkaloids: Structure, Isolation, Synthesis and Biology*. doi: 10.1002/9783527621071.ch3.

Bello, I. A. *et al.* (2011) 'A bioactive flavonoid from Pavetta crassipes K. Schum', *Organic and Medicinal Chemistry Letters*, 1(1), p. 14. doi: 10.1186/2191-2858-1-14.

Bello, I. A., Ndukwe, G. I. and Audu, O. T. (2014) 'Phytochemical analysis and biological activity of a precipitate from Pavetta crassipes', *Journal of Medicinal Plants Research*, 8(6), pp. 285–287. doi: 10.5897/jmpr10.088.

Bhat, J. A., Kumar, M. and Bussmann, R. W. (2013) 'Ecological status and traditional knowledge of medicinal plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya, India', *Journal of Ethnobiology and Ethnomedicine*, 9(1), pp. 1–18. doi: 10.1186/1746-4269-9-1.

Bianchini, F. and Vainio, H. (2001) 'Allium vegetables and organosulfur compounds: Do they help

prevent cancer?', *Environmental Health Perspectives*. doi: 10.1289/ehp.01109893.

Borrelli, F. and Izzo, A. A. (2000) 'The plant kingdom as a source of anti-ulcer remedies', *Phytotherapy Research*. doi: 10.1002/1099-1573(200012)14:8<581::AID-PTR776>3.0.CO;2-S.

Bouic, P. J. D. *et al.* (1996) 'Beta-sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: Implications for their use as an immunomodulatory vitamin combination', *International Journal of Immunopharmacology*. doi: 10.1016/S0192-0561(97)85551-8.

Brenan, J. P. M., Watt, J. M. and Breyer-Brandwijk, M. G. (1964) 'Medicinal and Poisonous Plants of Southern and Eastern AfricaThe Medicinal and Poisonous Plants of Southern and Eastern Africa', *Kew Bulletin*. doi: 10.2307/4113816.

Bridson, D. M. (2001) 'Additional notes on Pavetta (Rubiaceae: Pavetteae) from Tropical Eastern and Southern Africa', *Kew Bulletin*. doi: 10.2307/4117685.

Bruna, F. *et al.* (2014) 'Antimicrobial activity of essential oils', *Journal of Essential Oil Research*, 26(1), pp. 34–40. doi: 10.1080/10412905.2013.860409.

Cai, Y., Sun, M. and Corke, H. (2003) 'Antioxidant activity of betalains from plants of the Amaranthaceae', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf030045u.

Cao, H., Polansky, M. M. and Anderson, R. A. (2007) 'Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3-L1 adipocytes', *Archives of Biochemistry and Biophysics*. doi: 10.1016/j.abb.2006.12.034.

Cassidy, A., Hanley, B. and Lamuela-Raventos, R. M. (2000) 'Isoflavones, lignans and stilbenes - Origins, metabolism and potential importance to human health', *Journal of the Science of Food and Agriculture*. doi: 10.1002/(SICI)1097-0010(20000515)80:7<1044::AID-JSFA586>3.0.CO;2-N.

Chan, L. K. *et al.* (2010) 'Effects of abiotic stress on biomass and anthocyanin production in cell cultures of Melastoma malabathricum', *Biological Research*, 43(1), pp. 127–135. doi: 10.4067/S0716-97602010000100014.

Chanli, H. (2012) 'Factors Affecting Phytochemical Composition and Antioxidant Activity of Ontario Vegetable Crops', pp. 19–23. Available at: http://atrium.lib.uoguelph.ca:8080/xmlui/handle/10214/3592.

Chaurasiya, N. Das *et al.* (2008) 'Analysis of withanolides in root and leaf of Withania somnifera by HPLC with photodiode array and evaporative light scattering detection', *Phytochemical Analysis*. doi: 10.1002/pca.1029.

Chikezie, I. O. (2017) 'Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method', *African Journal of Microbiology Research*, 11(23), pp. 977–980. doi: 10.5897/ajmr2017.8545.

Chothani, D. L. and Mishra, S. H. (2012) 'In vitro anti-oxidant activity of Ruellia tuberosa root extracts', *Free Radicals and Antioxidants*, 2(4), pp. 38–44. doi: 10.5530/ax.2012.4.7.

Choudhary, M. K., Bodakhe, S. H. and Gupta, S. K. (2013) 'Assessment of the antiulcer potential of moringa oleifera root-bark extract in rats', *JAMS Journal of Acupuncture and Meridian Studies*.

doi: 10.1016/j.jams.2013.07.003.

Chung, M. (2004) 'Vitamins, Supplements, Herbal Medicines, and Arrhythmias', *Cardiology in Review*.

Cohen, J. C. *et al.* (2006) 'Sequence variations in PCSK9, low LDL, and protection against coronary heart disease', *New England Journal of Medicine*. doi: 10.1056/NEJMoa054013.

Costa, M. A. *et al.* (1999) 'Toward Engineering the Metabolic Pathways of Cancer-Preventing Lignans in Cereal Grains and Other Crops', in *Phytochemicals in Human Health Protection, Nutrition, and Plant Defense*. doi: 10.1007/978-1-4615-4689-4_4.

Daget, P. (2002) *Analyse d'ouvrage: Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest, de Michel Arbonnier*, *Acta Botanica Gallica*. doi: 10.1080/12538078.2001.10515914.

Dai, J. and Mumper, R. J. (2010) 'Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties', *Molecules*, 15(10), pp. 7313–7352. doi: 10.3390/molecules15107313.

Dastmalchi, K. *et al.* (2007) 'Plants as potential sources for drug development against Alzheimer's disease', *International Journal of Biomedical and Pharmaceutical Sciences*.

Dedza District socio-economic profile (1999). [Malawi : Dedza District Assembly,.

Delmas, D. *et al.* (2006) 'Resveratrol as a Chemopreventive Agent: A Promising Molecule for Fighting Cancer', *Current Drug Targets*. doi: 10.2174/138945006776359331.

Dholi, S. K. *et al.* (2011) 'Invivo antidiabetic evaluation of neem leaf extract in alloxan induced rats', *Journal of Applied Pharmaceutical Science*, 1(4), pp. 100–105.

Diplock, A. T. *et al.* (1998) 'Functional food science and defence against reactive oxidative species', *British Journal of Nutrition*, 80(S1), pp. S77–S112. doi: 10.1079/bjn19980106.

Donaldson, M. S. (2004) 'Nutrition and cancer: A review of the evidence for an anti-cancer diet', *Nutrition Journal*. doi: 10.1186/1475-2891-3-19.

Doughari, H. J. (2012) 'Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents', *Phytochemicals - A Global Perspective of Their Role in Nutrition and Health*. doi: 10.5772/26052.

Drahansky, M. *et al.* (2016) 'We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %', *Intech*, i(tourism), p. 13. doi: http://dx.doi.org/10.5772/57353.

EUCAST, E. C. for A. S. T. (2003) 'Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution European', *Clinical Microbiology and Infection*, 9(8), pp. 1–7.

Figueiredo, A. C. *et al.* (2008) 'Factors affecting secondary metabolite production in plants: Volatile components and essential oils', *Flavour and Fragrance Journal*. doi: 10.1002/ffj.1875.

Francis, M. S., Wolf-Watz, H. and Forsberg, Å. (2002) 'Regulation of type III secretion systems', *Current Opinion in Microbiology*. doi: 10.1016/S1369-5274(02)00301-6.

Gao, X. *et al.* (2004) 'Immunomodulatory activity of curcumin: Suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production in vitro', *Biochemical Pharmacology*. doi: 10.1016/j.bcp.2004.03.015.

Gidado, A. *et al.* (2008) 'Hypoglycaemic activity of Nauclea latifolia sm. (Rubiaceae) in experimental animals', *African Journal of Traditional, Complementary and Alternative Medicines*. doi: 10.4314/ajtcam.v5i2.31274.

Glen, S. (2015) *'Purposive Sampling (Deliberate Sampling)', Statisticshowto*.

GoM (2016) 'Malawi Government National Forest Policy', (June), p. 60. Available at: http://www.unpei.org/sites/default/files/dmdocuments/Malawi Government National Forest Policy - June 2016.pdf.

GoM (2018) 'ESIA Report for the Proposed Improvement of Mzimba Turn Off-Mzuzu-kacheche (M1) Rad'.

Grace, N. *et al.* (2015) 'Utilisation Of Weed Species As Sources Of Traditional Medicines In Central Kenya Resumen', (December), pp. 1–16.

Guimarães, A. C. *et al.* (2019) 'Antibacterial activity of terpenes and terpenoids present in essential oils', *Molecules*. doi: 10.3390/molecules24132471.

Gupta, R. *et al.* (2011) 'Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia', *Journal of Diabetes*, 3(1), pp. 29–37. doi: 10.1111/j.1753-0407.2010.00107.x.

Guruayoorappan, C. *et al.* (2014) *Preface, Anticancer Properties of Fruits and Vegetables: A Scientific Review*. doi: 10.1142/9789814508896.

Guta, D. *et al.* (2016) 'Socio-Economic Importance, Abundance and Phytochemistry of Jateorhiza palmata (Lam.) Miers a Medicinal Plant in Nsanje, Malawi', *International Journal of Scientific*

Research in Agricultural Sciences, 3(3), pp. 73–83. doi: 10.12983/ijsras-2016-p0073-0083.

Hahn, N. I. (1998) 'Are phytoestrogens nature's cure for what ails us? A look at the research', *Journal of the American Dietetic Association*. doi: 10.1016/S0002-8223(98)00223-5.

Harborne, J. B. (1998) 'Phytochemical Methods A Guide To Modern Tecniques Of Plant Analysis, Third Edition', *Chapman & Hall*. doi: 10.1017/CBO9781107415324.004.

Hark, L., Deen, D. and Morrison, G. (2003) *Medical Nutrition and Disease: A Case-Based Approach*, *WD info*. doi: 10.1002/ejoc.201200111.

Hartmann, M. A. (1998) 'Plant sterols and the membrane environment', *Trends in Plant Science*. doi: 10.1016/S1360-1385(98)01233-3.

Hasler, C. M. and Blumberg, J. B. (1999) 'Symposium on phytochemicals: Biochemistry and physiology: Introduction', *Journal of Nutrition*, 129(3), pp. 756–757.

Hendriks, H. F. J. *et al.* (1999) 'Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects', *European Journal of Clinical Nutrition*, 53(4), pp. 319–327. doi: 10.1038/sj.ejcn.1600728.

Hertog, M. G. L. *et al.* (1993) 'Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study', *The Lancet*, 342(8878), pp. 1007–1011. doi: 10.1016/0140-6736(93)92876-U.

Hillier, S. G. and Lathe, R. (2019) 'Terpenes, hormones and life: Isoprene rule revisited', *Journal of Endocrinology*, 242(2), pp. R9–R22. doi: 10.1530/JOE-19-0084.

Holmes, S. (2006) ‘Nutrition and the prevention of cancer.’, *The journal of family health care.* doi: 10.1079/phn2003588.

Holst, B. and Williamson, G. (2008) ‘Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants’, *Current Opinion in Biotechnology*, 19(2), pp. 73–82. doi: 10.1016/j.copbio.2008.03.003.

Hostanska, K. *et al.* (2005) ‘Apoptosis of human prostate androgen-dependent and -independent carcinoma cells induced by an isopropanolic extract of black cohosh involves degradation of cytokeratin (CK) 18’, *Anticancer Research*, 25(1 A), pp. 139–147.

Ibekwe, N. N. *et al.* (2012) ‘In vitro antimycobacterial studies on the leaf extracts and fractions of Pavetta crassipes K . Schum’, *African Journal of Pure and Applied Chemistry*, 6(2), pp. 55–58. doi: 10.5897/AJPAC11.094.

Ibekwe, N. N. *et al.* (2018) ‘Chemical constituents and antimycobacterial studies of the leaf extracts of Pavetta crassipes K. Schum’, *Tropical Plant Research*, 5(1), pp. 88–95. doi: 10.22271/tpr.2018.v5.i1.013.

Iwase, Y. *et al.* (2000) ‘Inhibitory effect of flavonoids from Citrus plants on Epstein-Barr virus activation and two-stage carcinogenesis of skin tumors’, *Cancer Letters*. doi: 10.1016/S0304-3835(00)00386-4.

Jakhetia, V. *et al.* (2010) ‘Cinnamon: A Pharmacological Review’, *Journal of Advanced Scientific Research*.

Jassim, S. A. A. and Naji, M. A. (2003) ‘Novel antiviral agents: A medicinal plant perspective’,

Journal of Applied Microbiology. doi: 10.1046/j.1365-2672.2003.02026.x.

Jayasinghe, U. L. B. *et al.* (2002) 'Antimicrobial activity of some Sri Lankan Rubiaceae and Meliaceae', *Fitoterapia*. doi: 10.1016/S0367-326X(02)00122-3.

Jayasri, M. A., Mathew, L. and Radha, A. (2009) 'Report International Journal of Integrative Biology A journal for biology beyond borders A report on the antioxidant activity of leaves and rhizomes of Costus pictus D. Don', 5(1), pp. 20–26.

Jones, R. B., Stefanelli, D. and Tomkins, R. B. (2015) 'Pre-harvest and post-harvest factors affecting ascorbic acid and carotenoid content in fruits and vegetables', *Acta Horticulturae*. doi: 10.17660/ActaHortic.2015.1106.6.

Joshi, S., Kuszynski, C. and Bagchi, D. (2005) 'The Cellular and Molecular Basis of Health Benefits of Grape Seed Proanthocyanidin Extract', *Current Pharmaceutical Biotechnology*. doi: 10.2174/1389201013378725.

Kalemba, D. and Kunicka, A. (2005) 'Antibacterial and Antifungal Properties of Essential Oils', *Current Medicinal Chemistry*. doi: 10.2174/0929867033457719.

Kamanula, J. F., Belmain, S. R., Hall, D. R., Farman, D. I., Goyder, D. J., & Mvumi, B. M. (2017). Chemical variation and insecticidal activity of Lippia javanica (Burm. f.) Spreng essential oil against Sitophilus zeamais Motschulsky. *Industrial Crops and Products*, 110, 75-82. doi: 10.1016/j.indcrop.2017.06.034

Kamanyi, A., Njamen, D. and Nkeh, B. (1994) 'Hypoglycaemic properties of the aqueous root extract of Morinda lucida (Benth) (Rubiaceae). Studies in the mouse', *Phytotherapy Research*. doi:

10.1002/ptr.2650080612.

Kambewankako, Y. (2005) ‘The Preliminary Assessment of the Resource Base of Jateorhiza Species (Calumba Root) in Malawi.’, *Occassional Paper No. 12*, p. 2005.

Kang, N. J. *et al.* (2011) ‘Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis’, *Pharmacology and Therapeutics*. doi: 10.1016/j.pharmthera.2011.02.004.

Kanokmedhakul, K., Kanokmedhakul, S. and Phatchana, R. (2005) ‘Biological activity of Anthraquinones and Triterpenoids from Prismatomeris fragrans’, *Journal of Ethnopharmacology*. doi: 10.1016/j.jep.2005.03.018.

Karou, S. D. *et al.* (2011) ‘Sub-Saharan Rubiaceae: A Review of Their Traditional Uses, Phytochemistry and Biological Activities’, *Pakistan Journal of Biological Sciences*, pp. 149–169. doi: 10.3923/pjbs.2011.149.169.

Katan, M. B. *et al.* (2003) ‘Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels’, *Mayo Clinic Proceedings*, 78(8), pp. 965–978. doi: 10.4065/78.8.965.

Khoo, H., Lim, S. and Azlan, A. (2019) ‘Evidence-Based Therapeutic Effects of Anthocyanins from Foods’, *Pakistan Journal of Nutrition*. doi: 10.3923/pjn.2019.1.11.

Koche, D. *et al.* (2010) ‘Phytochemical screening of eight traditionally used ethnomedicinal plants from Akola district (MS) India’, *International Journal of Pharma and Bio Sciences*.

Koche, D., Shirsat, R. and Kawale, M. (2018) ‘An Overerview of Major Classes of Phytochemicals: Their Types and Role in Disease Prevention’, *Hislopia Journal*, 9(1), p. 2016.

Kothari, C. R. (2004) *Research Methodology: Methods and Techniques*.

Kotzekidou, P., Giannakidis, P. and Boulamatsis, A. (2008) 'Antimicrobial activity of some plant extracts and essential oils against foodborne pathogens in vitro and on the fate of inoculated pathogens in chocolate', *LWT - Food Science and Technology*. doi: 10.1016/j.lwt.2007.01.016.

Kuhar, M., Imran, S. and Singh, N. (2007) 'Curcumin and Quercetin Combined with Cisplatin to Induce Apoptosis in Human Laryngeal Carcinoma Hep-2 Cells through the Mitochondrial Pathway', *Journal of Cancer Molecules*.

Kulkarni Harshal, C. and Lohar Prakash, S. (2014) 'Comparative studies on antioxidant and antipyretic activities of leaf extracts of cassia fistula, Psida cordifolia and Aegel marmelos', *Research Journal of Biotechnology,* 9(2), pp. 60–64.

Kumar, S. *et al.* (2017) 'Effect of climate change on phytochemical diversity, total phenolic content and in vitro activity of antioxidant Aloe vera (L.) Burm.f', 10(60). doi: 10:1186/s13104-017-2385-3.

Kwan, C. and Achike, F. I. (2002) 'Tetrandrine_and_related_bis-benzylisoquinoline_alkaloids from medicinal herbs: cardiovascular effects and mechanisms of action', 23(12), pp. 1057–1068.

Lai, P. and Roy, J. (2012) 'Antimicrobial and Chemopreventive Properties of Herbs and Spices', *Current Medicinal Chemistry,* 47(2), pp. 271–282. doi: 10.2174/0929867043365107.

Lampiasi, N. and Montana, G. (2016) 'The molecular events behind ferulic acid mediated modulation of IL-6 expression in LPS-activated Raw 264.7 cells', *Immunobiology*. doi: 10.1016/j.imbio.2015.11.001.

Lee, K. J. *et al.* (2004) 'Protective effect of saponins derived from roots of Platycodon grandiflorum on tert-butyl hydroperoxide-induced oxidative hepatotoxicity', *Toxicology Letters*. doi: 10.1016/j.toxlet.2003.12.002.

Lifongo, L. L. *et al.* (2014) 'A Bioactivity Versus Ethnobotanical Survey of Medicinal Plants from Nigeria, West Africa', *Natural Products and Bioprospecting*, 4(1), pp. 1–19. doi: 10.1007/s13659-014-0005-7.

Lim, H. A. *et al.* (2006) 'Genistein induces glucose-regulated protein 78 in mammary tumor cells', *Journal of Medicinal Food*, 9(1), pp. 28–32. doi: 10.1089/jmf.2006.9.28.

Lindberg Madsen, H. and Bertelsen, G. (1995) 'Spices as antioxidants', *Trends in Food Science and Technology*. doi: 10.1016/S0924-2244(00)89112-8.

Liu, L. *et al.* (2014) 'A Sesquiterpene Lactone from a Medicinal Herb Inhibits Proinflammatory Activity of TNF-α by Inhibiting Ubiquitin-Conjugating Enzyme UbcH5', *Chemistry and Biology*. Elsevier Ltd, 21(10), pp. 1341–1350. doi: 10.1016/j.chembiol.2014.07.021.

Liu, R. H. (2004) 'Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action', *The Journal of Nutrition*, 134(12), pp. 3479S-3485S. doi: 10.1093/jn/134.12.3479s.

Liu, W. *et al.* (2016) 'Influence of environmental factors on the active substance production and antioxidant activity in Potentilla fruticosa L. and its quality assessment', *Scientific Reports*, 6(July), pp. 1–18. doi: 10.1038/srep28591.

Locarek, M. *et al.* (2015) 'Antifungal and Antibacterial Activity of Extracts and Alkaloids of Selected Amaryllidaceae Species', *Natural product communications*. doi:

10.1177/1934578x1501000912.

Mabhiza, D., Chitemerere, T. and Mukanganyama, S. (2016) ‘ Antibacterial Properties of Alkaloid Extracts from Callistemon citrinus and Vernonia adoensis against Staphylococcus aureus and Pseudomonas aeruginosa ’, *International Journal of Medicinal Chemistry*. doi: 10.1155/2016/6304163.

Majumdar, A. P. N. *et al.* (2009) ‘Curcumin synergizes with resveratrol to inhibit colon cancer’, *Nutrition and Cancer*. doi: 10.1080/01635580902752262.

Malik, M. and Miiller, E. (2016) ‘Anthraquinones as Pharmacological Tools and Drugs.’, *Pharmaceutical Chemistry 1*, 4(36), pp. 705–748. Available at: doi: 10.1002/med2139.

Marasini, B. P. *et al.* (2015) ‘Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria’, *BioMed Research International*, 16, pp. 1–27. Available at: http://eprints.ums.ac.id/37501/6/BAB II.pdf.

Mattson, M. P. and Cheng, A. (2006) ‘Neurohormetic phytochemicals: low-dose toxins that induce adaptive neuronal stress responses’, *Trends in Neurosciences*. doi: 10.1016/j.tins.2006.09.001.

McPartland, J. M. and Russo, E. B. (2001) ‘Cannabis and Cannabis Extracts’, *Journal of Cannabis Therapeutics*. doi: 10.1300/j175v01n03_08.

Mertens-Talcott, S. U., Talcott, S. T. and Percival, S. S. (2003) ‘Low Concentrations of Quercetin and Ellagic Acid Synergistically Influence Proliferation, Cytotoxicity and Apoptosis in MOLT-4 Human Leukemia Cells–’, *The Journal of Nutrition*, 133(8), pp. 2669–2674. doi: 10.1093/jn/133.8.2669.

Micallef, M. A. and Garg, M. L. (2008) 'The Lipid-Lowering Effects of Phytosterols and (n-3) Polyunsaturated Fatty Acids Are Synergistic and Complementary in Hyperlipidemic Men and Women', *The Journal of Nutrition*. doi: 10.1093/jn/138.6.1086.

Middleton, E., Kandaswami, C. and Theoharides, T. C. (2000) 'The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer', *Pharmacological Reviews*.

Mohammad, R. M. *et al.* (2006) 'Cisplatin-induced antitumor activity is potentiated by the soy isoflavone genistein in BxPC-3 pancreatic tumor xenografts', *Cancer*. doi: 10.1002/cncr.21731.

Moreira, M. R., Alvarez, M. V. and Ponce, A. G. (2016) 'Essential oils', in *Postharvest Management Approaches for Maintaining Quality of Fresh Produce*. doi: 10.1007/978-3-319-23582-0_7.

Mponda, J. S. (2012) 'Study of ethnobotany and phytochemistry of Pavetta crassipes leaves and Calotropis procera bark from Malawi. Master's degree thesis. Taipei Medical University, 104p.', p. 2012.

Mujoo, K. *et al.* (2001) 'Triterpenoid saponins from Acacia victoriae (Bentham) decrease tumor cell proliferation and induce apoptosis', *Cancer Research*, 61(14), pp. 5486–5490.

Mukundi, M. J. *et al.* (2015) 'In Vivo Anti-diabetic Effects of Aqueous Leaf Extracts of Rhoicissus tridentata in Alloxan Induced Diabetic Mice', *Journal of Developing Drugs*, 04(03). doi: 10.4172/2329-6631.1000131.

Muriithi, N. J. *et al.* (2015) 'Determination of Hematological Effects of Methanolic Leaf Extract of Vernonia lasiopus in Normal Mice', *Journal of Blood & Lymph*, 05(03). doi: 10.4172/2165-

7831.1000139.

Mustapha, Y. and Bala, M. (2007) 'Antimicrobial activity of leaf extracts of Pavetta crassipes (Hutch) against some respiratory tract pathogens', *World Science,* 2(2), pp. 32–33.

Mustapha, Y. and Bala, M. . (2010) 'Antimicrobial activity of leaf extracts of *Pavetta crassipes* (hutch) against some respiratory tract pathogens.', *Science World Journal*. doi: 10.4314/swj.v2i2.51736.

Mzimba District Planning Department, 2008. Malawi Socio-ecomomic profile

Naderifar, M., Goli, H. and Ghaljaie, F. (2017) 'Snowball Sampling: A Purposeful Method of Sampling in Qualitative Research', *Strides in Development of Medical Education,* 14(3), pp. 1–7. doi: 10.5812/sdme.67670.

Narasinga, B. S. (2003) 'Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention', *Asia Pacific Journal of Clinical Nutrition,* 12(1), pp. 9–22.

Nascimento, G. G. F. *et al.* (2000) 'Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria', *Brazilian Journal of Microbiology,* 31(4), pp. 247–256. doi: 10.1590/S1517-83822000000400003.

Newman, R. A. *et al.* (2008) 'Cardiac glycosides as novel cancer therapeutic agents', *Molecular Interventions,* 8(1), pp. 36–49. doi: 10.1124/mi.8.1.8.

Nweze, E., Okafor, J. and Njoku, O. (2004) 'Antimicrobial Activities Of Methanolic Extracts Of *Trema guineensis* (Schumm And Thorn) And *Morinda lucida* Benth Used In Nigerian', *Bio-Research*. doi: 10.4314/br.v2i1.28540.

Nyamai, D. *et al.* (2015) 'Pharmacognosy & Natural Products Phytochemical Profile of Prunus africana Stem Bark from Kenya', *J Pharmacogn Nat Prod*, 1(1), pp. 1–8. doi: 10.4172/jpnp.1000110.

Nyamai, D. *et al.* (2016a) 'Medicinally Important Phytochemicals: An Untapped Research Avenue', *Research & Reviews: Journal of Pharmacognosy and Phytochemistry*, 4(1), pp. 35–49. Available at: http://www.rroij.com/abstract.php?abstract_id=67696.

Nyamai, D. *et al.* (2016b) 'Research and Reviews : Journal of Pharmacognosy and Phytochemistry Medicinally Important Phytochemicals : An Untapped Research Avenue', *Jprpc*, 4(1).

O'Brien, P., Carrasco-Pozo, C. and Speisky, H. (2006) 'Boldine and its antioxidant or health-promoting properties', *Chemico-Biological Interactions*. doi: 10.1016/j.cbi.2005.09.002.

O'Neill, F. H., Sanders, T. A. B. and Thompson, G. R. (2005) 'Comparison of efficacy of plant stanol ester and sterol ester: Short-term and longer-term studies', *American Journal of Cardiology*. doi: 10.1016/j.amjcard.2005.03.017.

Okunlola, G. O. *et al.* (2017) 'Comparative Study of the Phytochemical Contents of Cochorus Olitorius and Amaranthus Hybridus At Different Stages of Growth', *Annales of West University of Timisoara. Series of Biology*, 20(1), p. 43.

Olajide, Olumayokuna, A. *et al.* (1999) 'Evaluation of the Anti-diabetic Property of Morinda lucida Leaves in Streptozotocin-diabetic Rats', *Journal of Pharmacy and Pharmacology*. doi: 10.1211/0022357991776903.

Olutayo, O. *et al.* (2018) 'Phytochemical and antioxidant properties of some Nigerian medicinal plants

Phytochemical and antioxidant properties of some Nigerian medicinal plants', (January 2011). doi: 10.5251/ajsir.2013.4.3.328.332.

Osuntokun, O. and Ajayi, A. (2014) 'Analysis of Four Nigerian Medicinal Plants on some Clinical Microorganisms', 2(5), pp. 457–461. doi: 10.1038/nbt1082.CITATIONS.

Panawala, L. (2017) *Difference Between Gram Positive and Gram Negative Bacteria Stunning images of cells Discover how scientists use Main Difference – Gram Positive vs Gram Negative Bacteria, Pediaa*.

Parekh, J. and Chanda, S. (2010) 'Antibacterial and phytochemical studies on twelve species of Indian medicinal plants', *African Journal of Biomedical Research*, 10(2), pp. 175–181. doi: 10.4314/ajbr.v10i2.50624.

Patel, M. D. and Thompson, P. D. (2006) 'Phytosterols and vascular disease', *Atherosclerosis*. doi: 10.1016/j.atherosclerosis.2005.10.026.

Pelletier, S. W. (1996) *Alkaloids:Chemical & Biological perspecties Voulme 10*. Edited by S. wiliam Pelletier. New York: Pergamon.

Perne, A. *et al.* (2009) 'Cardiac glycosides induce cell death in human cells by inhibiting general protein synthesis', *PLoS ONE*. doi: 10.1371/journal.pone.0008292.

Pervaiz, T. *et al.* (2017) 'Naturally Occurring Anthocyanin, Structure, Functions and Biosynthetic Pathway in Fruit Plants', *Journal of Plant Biochemistry & Physiology*, 05(02). doi: 10.4172/2329-9029.1000187.

Peterson, J. W. (2013) 'Medical Microbiology/Chapter 7 Bacterial Pathogenesis', *Medical*

Microbiology. 4th edition. doi: 10.1101/cshperspect.a012476.

Piero, N. M. *et al.* (2015) 'In Vivo Antidiabetic Activity and Safety In Rats of Cissampelos pareira Traditionally Used In The Management of Diabetes Mellitus In Embu County, Kenya', *Journal of Drug Metabolism & Toxicology*, 06(03). doi: 10.4172/2157-7609.1000184.

Pizzino, G. *et al.* (2017) 'Oxidative Stress: Harms and Benefits for Human Health', *Oxidative Medicine and Cellular Longevity*. doi: 10.1155/2017/8416763.

Prakash, V. (2017) 'Terpenoids as source of anti-inflammatory compounds', *Asian Journal of Pharmaceutical and Clinical Research*. doi: 10.22159/ajpcr.2017.v10i3.16435.

Raicht, R. F. *et al.* (1980) 'Protective Effect of Plant Sterols against Chemically Induced Colon Tumors in Rats', *Cancer Research*, 40(2), pp. 403–405.

Rajendran, L., Ravishankar, G. A. and Venkataraman, L. . (1992) 'Anthocyanin production in callus cultures ofDaucus carota as influenced by nutrient stress and osmoticum.', *Biotechnology Lettuce*, 14, pp. 707–712. doi: https://doi.org/10.1007/BF01021647.

Raskin, J. *et al.* (2005) 'A double-blind, randomized multicenter trial comparing duloxetine with placebo in the management of diabetic peripheral neuropathic pain', *Pain Medicine*, 6(5), pp. 346–356. doi: 10.1111/j.1526-4637.2005.00061.x.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. (1997) 'Antioxidant properties of phenolic compounds', *Trends in Plant Science*. doi: 10.1016/S1360-1385(97)01018-2.

Rios, J.L., & Recio, M.C. (2005). Medicinal Plants and Antimicrobial Activity. Journal of Ethnopharmacology, 100, 80-84. doi: 10.1016/j.jep.2005.04.025

Romani, A. *et al.* (2004) 'Evaluation of antioxidant effect of different extracts of Myrtus communis L', *Free Radical Research*, 38(1), pp. 97–103. doi: 10.1080/10715760310001625609.

Ronchetti, D. *et al.* (2006) 'Modulation of iNOS expression by a nitric oxide-releasing derivative of the natural antioxidant ferulic acid in activated RAW 264.7 macrophages', *European Journal of Pharmacology*. doi: 10.1016/j.ejphar.2005.12.034.

Ros, E. (2000) 'Intestinal absorption of triglyceride and cholesterol. Dietary and pharmacological inhibition to reduce cardiovascular risk', *Atherosclerosis*. doi: 10.1016/S0021-9150(00)00456-1.

Ryan, K. J. and Ray, C. G. (2004) *Sherris medical microbiology. 4th Edition*. McGraw Hill.

Salminen, A. *et al.* (2008) 'Terpenoids: Natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential', *Cellular and Molecular Life Sciences*, 65(19), pp. 2979–2999. doi: 10.1007/s00018-008-8103-5.

Sanon, S. *et al.* (2003) 'Antiplasmodial activity of alkaloid extracts from Pavetta crassipes (K. Schum) and Acanthospermum hispidum (DC), two plants used in traditional medicine in Burkina Faso', *Parasitology Research*. doi: 10.1007/s00436-003-0859-9.

Seeram, N. P. *et al.* (2006) 'Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro', *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf061750g.

Shad, A. A. *et al.* (2014) 'Phytochemical and biological activities of four wild medicinal plants', *Scientific World Journal*, 2014(November). doi: 10.1155/2014/857363.

Shang, Y. *et al.* (2019) 'Anthocyanins: Novel Antioxidants in Diseases Prevention and Human Health', in *Flavonoids_ AColoring Model for Cheering up Life*. doi: http://dx.doi.org/10.5772/57353.

Sharma, V., Hupp, C. and Tepe, J. (2007) 'Enhancement of Chemotherapeutic Efficacy by Small Molecule Inhibition of NF-κ B and Checkpoint Kinases', *Current Medicinal Chemistry*. doi: 10.2174/092986707780362844.

Singh, A. and Masuku, M. (2014) 'Sampling Techniques & Determination of Sample Size in Applied Statistics Research: an Overview', *Ijecm.Co.Uk*.

Simpson, & Amos, (2017). Properties, health effects, and applications of lignans as food bioactive ingredients.*Handbook of Food Bioactive Ingredients.* doi:10.1007/9783030814045.

Sofowora, A. (1996) 'Research on medicinal plants and traditional medicine in Africa', *Journal of Alternative and Complementary Medicine*. doi: 10.1089/acm.1996.2.365.

Srinivasan, M., Sudheer, A. R. and Menon, V. P. (2007) 'Ferulic acid: Therapeutic potential through its antioxidant property', *Journal of Clinical Biochemistry and Nutrition*. doi: 10.3164/jcbn.40.92.

Street, R. A. and Prinsloo, G. (2013) 'Commercially important medicinal plants of South Africa: A review', *Journal of Chemistry*. doi: 10.1155/2013/205048.

Tan, B. and Vanitha, J. (2012) 'Immunomodulatory and Antimicrobial Effects of Some Traditional Chinese Medicinal Herbs: A Review', *Current Medicinal Chemistry*, 11(11), pp. 1423–1430. doi: 10.2174/0929867043365161.

Tapas, A., Sakarkar, D. and Kakde, R. (2008) 'Flavonoids as Nutraceuticals: A Review', *Tropical Journal of Pharmaceutical Research*, 7(3). doi: 10.4314/tjpr.v7i3.14693.

Taylor, J. L. S. *et al.* (2001) 'Towards the scientific validation of traditional medicine plants', *Plant growth Regulation*, 34(1), pp. 23–37.

Tiwari, U. and Cummins, E. (2013) 'Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations', *Food Research International*. doi: 10.1016/j.foodres.2011.09.007.

Tongco, M. D. C. (2007) 'Purposive sampling as a tool for informant selection. Ethnobotany research and applications', *Ethnobotany Research and Applications*. doi: 10.17348/era.5.0.147-158.

Tsubura, A. *et al.* (2012) 'Anticancer Effects of Garlic and Garlic-derived Compounds for Breast Cancer Control', *Anti-Cancer Agents in Medicinal Chemistry*. doi: 10.2174/187152011795347441.

Tung, Y. T. *et al.* (2008) 'Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (Cinnamomum osmophloeum) twigs', *Bioresource Technology*. doi: 10.1016/j.biortech.2007.07.050.

Tung, Y. T. *et al.* (2010) 'Anti-inflammatory activities of essential oils and their constituents from different provenances of indigenous cinnamon (Cinnamomum osmophloeum) leaves', *Pharmaceutical Biology*. doi: 10.3109/13880200903527728.

Uddin, S. N. *et al.* (2008) 'Antioxidant and Antibacterial Activities of Trema cannabina', *Middle-East Journal of Scientific Research*, 3(2), pp. 105–108.

Uttara, B. *et al.* (2009) 'Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options', *Current Neuropharmacology*, 7(1), pp. 65–74. doi: 10.2174/157015909787602823.

Vanhanen, H. T. *et al.* (1993) 'Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment', *Journal of Lipid Research*, 34(9), pp. 1535–1544.

Verma, A. *et al.* (2013) 'Nitrogen Containing Privileged Structures and their Solid Phase Combinatorial Synthesis', *Combinatorial Chemistry & High Throughput Screening*. doi: 10.2174/1386207311316050003.

Verma, S. and Singh, S. P. (2008) 'Current and future status of herbal medicines', *Veterinary World*. doi: 10.5455/vetworld.2008.347-350.

Verstraete, B. *et al.* (2011) 'Endophytic bacteria in toxic south african plants: Identification, phylogeny and possible involvement in gousiekte', *PLoS ONE*. doi: 10.1371/journal.pone.0019265.

Voravuthikunchai, S. P. and Kitpipit, L. (2005) 'Activity of medicinal plant extracts against hospital isolates of methicillin-resistant Staphylococcus aureus', *Clinical Microbiology and Infection*. European Society of Clinical Infectious Diseases, 11(6), pp. 510–512. doi: 10.1111/j.1469-0691.2005.01104.x.

Wadhera, R. K. *et al.* (2016) 'A review of low-density lipoprotein cholesterol, treatment strategies, and its impact on cardiovascular disease morbidity and mortality', *Journal of Clinical*

Lipidology. doi: 10.1016/j.jacl.2015.11.010.

Walton, N. J., Mayer, M. J. and Narbad, A. (2003) 'Molecules of Interest: Vanillin', *ChemInform*. doi: 10.1002/chin.200338253.

Wang, M. W., Hao, X. and Chen, K. (2007) 'Biological screening of natural products and drug innovation in China', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 362(1482), pp. 1093–1105. doi: 10.1098/rstb.2007.2036.

World Health Organization. (2019). Introduction. In WHO global report on traditional and complementary medicine 2019 (pp. 1-10).

Wink, M. and Latz-Brüning, B. (1994) 'Allelopathic Properties of Alkaloids and Other Natural Products', in. doi: 10.1021/bk-1995-0582.ch008.

Winter, A. N. and Bickford, P. C. (2019) 'Anthocyanins and their metabolites as therapeutic agents for neurodegenerative disease', *Antioxidants*. doi: 10.3390/antiox8090333.

Yamamoto, M. *et al.* (1991) 'Agricultural and Biological Chemistry Anti-inflammatory Active Constituents of Aloe arborescens Miller Anti-inflammatory Active Constituents of Aloe arborescens Miller', *Kazuya Nakagomi & Hiroyuki Nakazawa Agricultural and Biological Chemistry Agric. Bioi. Chem*, 556(556), pp. 1627–1629. doi: 10.1080/00021369.1991.10870794.

Yang, C. S. *et al.* (2001) 'Inhibition of Carcinogenesis by Dietary Polyphenolic Compounds', *Annual Review of Nutrition*. doi: 10.1089/109662000416311.

Yang, J. Y. *et al.* (2008) 'Enhanced inhibition of adipogenesis and induction of apoptosis in 3T3-L1

adipocytes with combinations of resveratrol and quercetin', *Life Sciences*. doi: 10.1016/j.lfs.2008.03.003.

Yeh, J. Y. *et al.* (2003) 'Effects of bufalin and cinobufagin on the proliferation of androgen dependent and independent prostate cancer cells', *Prostate*, 54(2), pp. 112–124. doi: 10.1002/pros.10172.

Zerbo, P. *et al.* (2008) 'Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties', *Cancer Research*. European Society of Clinical Infectious Diseases, 6(1), p. 60. doi: 10.2174/0929867043365161.

Zerbo, P. *et al.* (2011) 'Plantes médicinales et pratiques médicales au Burkina Faso : cas des Sanan', *Bois et Forets des Tropiques*, 65(307), pp. 41–53. doi: 10.19182/bft2011.307.a20481.

Zhang, W. *et al.* (1997) 'Effect of temperature and its shift on growth and anthocyanin production in suspension cultures of strawberry cells. Plant Science', *Plant Science*, (127), pp. 207–214.

Zhang, Y. *et al.* (2008) 'Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties', in *Journal of Agricultural and Food Chemistry*. doi: 10.1021/jf071989c.

Zhong, J. J. and Yoshida, T. (1993) 'Effects of temperature on cell growth and anthocyanin production in suspension cultures of Perilla frutescens', *Journal of Fermentation and Bioengineering*, 76(6), pp. 530–531. doi: 10.1016/0922-338X(93)90255-7.

Zhu, Y. Z. *et al.* (2004) 'Antioxidants in Chinese herbal medicines: A biochemical perspective', *Natural Product Reports*. doi: 10.1039/b304821g.

APPENDICES

Appendix A: Questionnaire for *Pavetta schumanniana* Enukweni

SECTION A: DEMOGRAPHIC CHARACTERISTICS

1. Demographic information

Attribute	Response
Gender	1. Male 2. Female
Age	1.15-25 2. 26-35 3. 36-45 4. 46-55 5. 55 above
Marital status	1. Single 2. Married 3. Separated 4. Divorced 5. Widow 6.Widower
Household head	1. Male headed 2. Female headed 3. Child headed 4.Other
Household members	1.1 to 3 2. 4 to 6 3. 7 to 10 4. Above 10
Education	1. Primary level 2. Secondary level 3.Tertiary level 4. Never
Occupation	1. Farmer 2. Employed 3. Self-employed 4. Piece works 5. None
Tribe	1. Ngoni 2. Chewa 3. Tumbuka 4. Tonga 5. Other (Specify)_____

PART B: KNOWLEDGE OF THE PLANT

2. Local name(s) (in vernacular) _____

3. Habit

   1. Tree 2. Shrub 3. Herb 4. Climber

4. Habitat

   1. Farm land 2. Forest 3. Garden 4. Others (Specify)_____

5. How did you get to know that the plant has medicinal value?

   1. Grandparents 2. Parents 3. Friends 4. Midwives 5. Aunt 6. Other (Specify)_____

6. Who else did you tell about this medicinal plant?

   1. Children 2. Grandchildren 3. Friends 4. Midwives 5. None

6. Daughter-in-law 7. Others (Specify)_____

7. Which part(s) of the plant do you mostly use?

   1. Leaves 2. Stems 3. Flowers 4. Fruits 5. Roots 6. Bark

8. At what stage do you harvest the leaves?

   1. Juvenile 2. Flowering period 3. Fruiting period 4. Mature leaves 5. Any green leaves

9. Why do you harvest at this stage?

   1. It is easy to process at this stage 2. We were taught to harvest at this time by our grandparents 3. They work best at this time 4. We were advised to harvest at this time by friends 5. We were advised to harvest at this time by Parents 6. No apparent reasons 7. Other (specify)_____

10. Indicate availability of *P. crassipes*

    1. Readily available 2. Moderate scarce 3. Scarce 4. Very Scarce

11. Do you harvest from one tree/shrub or more?

    1. One shrub 2. Two shrubs 3. Three shrubs 4. 4 Shrubs 5. More than 4 shrubs

12. How much do you harvest for a particular need?

    1. Hand full 2. Two hands full 3. Bucket (2L) 4. Bucket (5L) 5. More than 5L bucket

13. Do you use it

    1. Alone 2. In combination as the major ingredient 3. In combination as the minor ingredient 4. In equal portions

14. Indicate your preferred method of processing the leaves after collection?

    1. No processing/Green leaves 2. Shade drying 3. Sun drying 4. Smoking 5. Oven drying 6. Other (specify)_____

15. Why do you prefer this method of processing?

1. Medicine works best after using this method 2. We were taught by grandparents to process using this method 3. It is easy 4. We were advised by friends to process this way 5. We were advised by relatives to process this way 6. No apparent reason 7. Other (specify)_____

16. How long does it take you to process the plant leaves?

1. 5 minutes or less 2. 6-10 min 3. 11-15 min 4. 16-20 min 5. 21-25 min 6. 26-30 min 7 More than 30 min

17. How long do you store the plant leaf material?

1. A day 2. Two days 3. Three days 4. Four days 5. Five days 6. Six days 7. Seven days

18. Name of disease(s) treated

1. Malaria 2. Coughing 3. Diarrhea 4. Constipation 5. Fever 6. Stomachache
7. Chlamydia 8. Pneumonia 9. Libido 10. Other (specify)_____

19. Frequency of drug intake

1. Once daily 2. Twice daily 3. Thrice daily 4. 4 times daily 5. More than 4 times daily

20. Toxicity

1. None. Sweating 3. Nausea 4. Dizziness 5. Dry mouth 6. Diarrhea 7. Constipation 8. Rash 9. Other (specify)_____

21. Mode of preparation of medicine for specific diseases

Diseases	Mode of preparation of medicine	Crude preparation	Mode of Administration

22. How many times do you harvest the plant material to the point the patient heals?

1. Once 2. Twice 3. Thrice 4. Until the patient gets better 5. Other (specify) _____

THANK YOU

Appendix B: Questionnaire for *Pavetta crassipes* Mtakataka

SECTION A: DEMOGRAPHIC CHARACTERISTICS

1. Demographic information

Attribute	Response
Gender	1. Male 2. Female
Age	1.15-25 2. 26-35 3. 36-45 4. 46-55 5. 55 above
Marital status	1. Single 2. Married 3. Separated 4. Divorced 5. Widow 6.Widower
Household head	1. Male headed 2. Female headed 3. Child headed 4.Other
Household members	1.1 to 3 2. 4 to 6 3. 7 to 10 4. Above 10
Education	1. Primary level 2. Secondary level 3.Tertiary level 4. Never
Occupation	1. Farmer 2. Employed 3. Self-employed 4. Piece works 5. None
Tribe	1. Ngoni 2. Chewa 3. Tumbuka 4. Tonga 5. Other (Specify)_____

PART B: KNOWLEDGE OF THE PLANT

2. Local name(s) (in vernacular) _____

3. Habit

   1. Tree 2. Shrub 3. Herb 4. Climber

4. Habitat

   1. Farm land 2. Forest 3. Garden 4. Others (Specify)_____

5. How did you get to know that the plant has medicinal value?

   1. Grandparents 2. Parents 3. Friends 4. Midwives 5. Aunt 6. Other (Specify)_____

6. Who else did you tell about this medicinal plant?

   1. Children 2. Grandchildren 3. Friends 4. Midwives 5. None

6. Daughter-in-law 7. Others (Specify)_____

7. Which part(s) of the plant do you mostly use?

   1. Leaves 2. Stems 3. Flowers 4. Fruits 5. Roots 6. Bark

8. At what stage do you harvest the leaves?

   1. Juvenile 2. Flowering period 3. Fruiting period 4. Mature leaves 5. Any green leaves

9. Why do you harvest at this stage?

   1. It is easy to process at this stage 2. We were taught to harvest at this time by our grandparents 3. They work best at this time 4. We were advised to harvest at this time by friends 5. We were advised to harvest at this time by Parents 6. No apparent reasons 7. Other (specify)_____

10. Indicate availability of *P. crassipes*

    1. Readily available 2. Moderate scarce 3. Scarce 4. Very Scarce

11. Do you harvest from one tree/shrub or more?

    1. One shrub 2. Two shrubs 3. Three shrubs 4. 4 Shrubs 5. More than 4 shrubs

12. How much do you harvest for a particular need?

    1. Hand full 2. Two hands full 3. Bucket (2L) 4. Bucket (5L) 5. More than 5L bucket

13. Do you use it

    1. Alone 2. In combination as the major ingredient 3. In combination as the minor ingredient 4. In equal portions

14. Indicate your preferred method of processing the leaves after collection?

    1. No processing/Green leaves 2. Shade drying 3. Sun drying 4. Smoking 5. Oven drying 6. Other (specify)_____

15. Why do you prefer this method of processing?

1. Medicine works best after using this method 2. We were taught by grandparents to process using this method 3. It is easy 4. We were advised by friends to process this way 5. We were advised by relatives to process this way 6. No apparent reason 7. Other (specify)_____

16. How long does it take you to process the plant leaves (Fresh leaves)?

1. 5 min or less 2. 6-10 min 3. 11-15 min 4. 16-20 min 5. 21-25 min 6. 26-30 min 7 More than 30 min

17. How long does it take you to process the plant leaves (dried leaves)?

1. A day 2. Two days 3. Three days 4. Four days 5. Five days 6. Six days 7. Seven days

18. How long do you store the plant leaf material (fresh leaves)?

1. A day 2. Two days 3. Three days 4. Four days 5. Five days 6. Six days 7. Seven days

19. How long do you store the plant leaf material (dried leaves)?

1. One weak 2. Two weeks 3. Three weeks 4. Four weeks 5. More than 4 weeks

20. Name of disease(s) treated

1. Malaria 2. Coughing 3. Diarrhea 4. Constipation 5. Fever 6. Stomachache
7. Chlamydia 8. Pneumonia 9. Libido 10. Other (specify)_____

21. Frequency

1. Once daily 2. Twice daily 3. Thrice daily 4. 4 times daily 5. More than 4 times daily

22. Toxicity

1. None. Sweating 3. Nausea 4. Dizziness 5. Dry mouth 6. Diarrhea 7. Constipation 8. Rash 9. Other (specify)_____

23. Mode of preparation of medicine for specific diseases

Diseases	**Mode of preparation of medicine**	**Crude preparation**	**Mode of Administration**

24. How many times do you harvest the plant material to the point the patient heals?

1. Once 2. Twice 3. Thrice 4. Until the patient gets better 5. Other (specify) _____

THANK YOU

Appendix C: Checklist

Check list for ethno medicinal information of *P. crassipes*/*P. schumanniana* leaves

1. What are the uses of *P. crassipes*/*P. schumanniana* leaves?
2. What are the diseases treated by *P. crassipes*/*P. schumanniana* leaves?
3. Explain how *P. crassipes*/*P. schumanniana* leaves are prepared to cure disease(s)?
4. How is the medicine administered to a patient?
5. Where do you harvest *P. crassipes*/*P. schumanniana* leaves from (wild/domesticated)?
6. How readily available is *P. crassipes*/*P. schumanniana*?

Appendix D: Demographic data of respondents (Enukweni, n=105)

Demographic parameter	Value	Total Respondents using *P. schumanniana*	Percentage of respondents (%)	P-value
Tribe	Ngoni	32	30.5	0.000
	Chewa	2	1.9	
	Tumbuka	68	64.8	
	Tonga	2	1.9	
	Nkhonde	1	1	
Gender	Male	26	25.7	0.000
	Female	79	74.5	
Marital status	Married	77	73.3	0.000
	Single	2	1.9	
	Separated	5	4.8	
	Widow	21	20.0	
Household size	< 4	7	6.7	0.000
	4-6	44	41.9	
	7-10	40	38.1	
	>10	14	13.3	
Age	15-25	5	4.8	0.034
	26-35	17	16.2	
	35-45	25	23.8	
	45-50	23	21.9	
	> 50	35	33.3	
Education	None	2	1.9	0.000
	Primary	78	74.3	
	Secondary	22	21.0	
	Tertiary	3	2.9	
Occupation	Farmer	96	91.4	0.000
	Employed	3	2.9	
	Self-Employed	5	4.8	
	Business	1	1.0	

Appendix E: Demographic data of respondents (Mtakataka, n=106)

Demographic parameter	Value	Total Respondents using *P. crassipes*	Percentage of respondents (%)	P-value
Tribe	Ngoni	27	25.5	0.000
	Chewa	79	74.5	
Gender	Male	30	28.3	0.000
	Female	76	71.7	
Marital status	Single	19	17.9	0.000
	Married	78	73.6	
	Separated	3	2.8	
	Widow	6	5.7	
Household size	< 4	18	17.0	0.000
	4-6	52	49.1	
	7-10	21	19.8	
	>10	15	14.2	
Age	15-25	11	10.4	0.000
	26-35	29	27.4	
	35-45	28	26.4	
	45-50	19	17.9	
	> 50	19	17.9	
Education	None	21	19.8	0.000
	Primary	73	68.9	
	Secondary	9	8.5	
	Tertiary	3	2.8	
Occupation	Farmer	90	84.9	0.000
	Employed	1	0.9	
	Piece works	3	2.8	
	Self-Employed	11	10.4	
	Business	1	0.9	

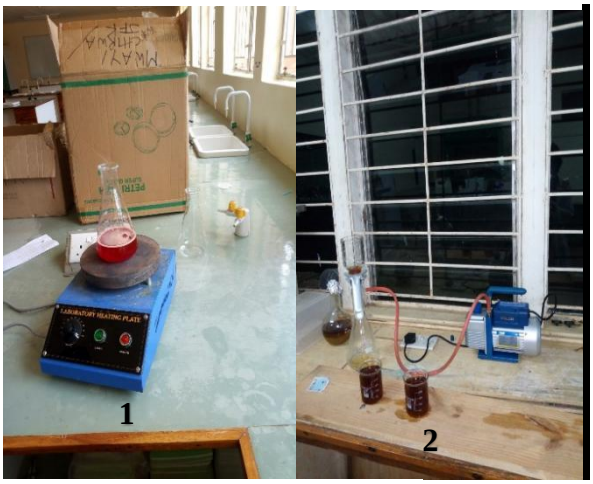
Appendix F: Shapiro Wilk Test for normal data for *P. schumanniana* and *P. crassipes*

Plant species	Concentration	Mean	± SD	W	p - value
Pavetta crassipes	25	3.07	0.52	0.997	0.999
	50	4.33	0.547	0.821	<0.001
	75	5.77	0.679	0.966	0.431
	100	8.1	0.885	0.993	0.999
	P Control	18.63	2.71	0.979	0.799
	N Control	6.67	0.547	0.894	0.006
Pavetta schumanniana	25	2.83	0.986	0.999	0.955
	50	4.17	1.085	0	0.368
	75	5.5	0.974	0.431	0.999
	100	7.73	1.172	0.999	0.074
	P Control	18.63	2.71	0.978	0.799
	N Control	6.67	0.547	0.893	0.006

W = Shapiro Wilk test for normal data

* = Significant at 5% level of significance, otherwise not significant

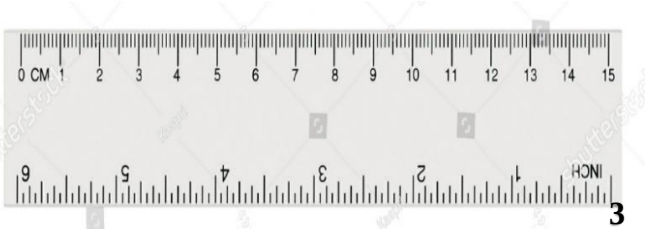
Appendix G: Laboratory equipment: Hot plate, Suction machine, Autoclave and Water bath



4

1 = Hot plate, 2 = Suction machine, 3 = Autoclave and 4 = Water bath

Appendix H: Laboratory equipment: Food blender, Punching machine and 15 cm ruler

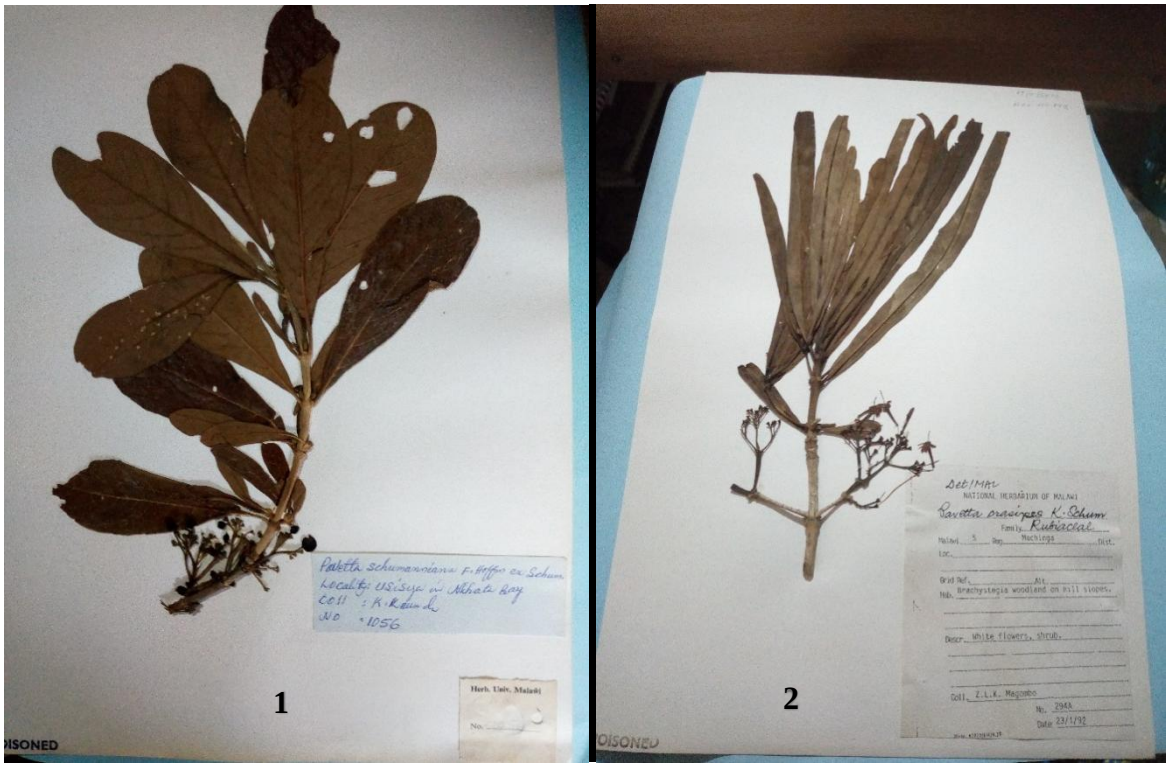


1 = Food blender, 2 = Punching machine and 3 = 15 cm ruler

Appendix I: Laboratory equipment: Laminar flow, desiccator, oven and an analytical balance

1 = Laminar flow, 2 = Desiccator, 3 = Oven and 4 = Analytical balance

Appendix J: Photos of *Pavetta schumanniana* and *Pavetta crassipes* specimens captured at Mzuzu Botanical gardens



1 = *Pavetta schumanniana* specimen and 2 = *Pavetta crassipes* specimen

Appendix K: *Pavetta schumanniana* and *Pavetta crassipes* powdered leaf material in the Laboratory

Appendix L: Consent form

Department of Forestry and Environmental Management

Mzuzu University

P/Bag 201, Mzuzu 2, Malawi

Informed Consent Form for Research in Ethnomedicine, Phytochemical Analysis and Anti-Microbial Activities of *Pavetta crassipes* and *Pavetta schumanniana* Leaf Extracts

Introduction

I am Mwayi Chirwa, Mzuzu University student under the Department of Forestry and Environmental Management. I am doing research on **Ethnomedicine, Phytochemical Analysis and Anti-Microbial Activities of *Pavetta crassipes* and *Pavetta schumanniana* Leaf Extracts.** This consent form may contain words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask.

Purpose of the research

This research aims to analyses ethnomedicine, phytochemical composition and antimicrobial activity of *P. crassipes* and *P. schumanniana* plant leaves.

Type of Research Intervention

This research will involve your participation in a group discussion and/or individual interview.

Participant Selection

You are being invited to take part in this research because of your awareness regarding the plant's usage.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate nothing will change. You may skip any question and move on to the next question.

Duration

The research takes place over 12 months in total.

Risks

You do not have to answer any question or take part in the discussion/interview/survey if you feel the question(s) are too personal or if talking about them makes you uncomfortable.)

Reimbursements

You will not be provided any incentive to take part in the research.

Sharing the Results

If you like, a summary of the results of the study will be sent to you. The knowledge that we get from this research will be shared with your community. We will also publish the results so other interested people may learn from the research.

Right to Ask Questions and Report Concerns

You have the right to ask questions about this research study and to have those questions answered by me before, during or after the research. If you have any further questions about the study, at any time feel free to contact me, Mwayi Chirwa at mwayi.chirwa@gmail.com or by telephone at +265886308969. If you have any other concerns about your rights as a research participant, you may contact Head of Department, Forest and Environmental Management, P/Bag 201, Mzuzu.

This proposal has been reviewed and approved by MZUNIREC, which is a committee whose task is to make sure that research participants are protected from harm. If you wish to find more, contact Mr. G. Mbwele, MZUNIREC Secretariat, Mzuzu University Research Ethics Committee, P/Bag 201,

Luwinga, Mzuzu 2; E-mail address: mzunirec@mzuni.ac.mw. Do you have any questions?

Part II: Certificate of Consent

I have been invited to participate in research about **Ethnomedicine, Phytochemical Analysis and Anti-microbial Activities of *Pavetta crassipes* and *Pavetta schumanniana* Leaf Extracts**.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

Print Name of Participant_____
Signature of Participant _____
Date _____
Day/month/year

If illiterate [1]

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness_____ **Thumb print of participant**
Signature of witness _____
Date _____
Day/month/year

1 A literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the research project. I confirm the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Signature of Researcher /person taking the consent_____

Date _____

Day/month/year

Appendix M: Questionnaire for *Pavetta crassipes* Mtakataka

GAWO LOYAMBA: ZAMBIRI ZOKHUZA MUNTHU

1. Zambiri zokhudza Munthu

Attribute	Response
Jenda	1. Mamuna 2. Mkazi
Zaka	1.15-25 2. 26-35 3. 36-45 4. 46-55 5. 55 above
Banja	1. Wosakwatila 2. Wokwatila 3. Wolekana 4. wosudzulidwa 5. Mkazi wamasiye 6. Mamuna wamasiye
Mutu wabanja	1. Mamuna 2. Mkazi 3. Mwana 4. Wina
Mamembala apabanja	1.1 to 3 2. 4 to 6 3. 7 to 10 4. Above 10
Maphuzilo	1. Pulayimale 2. Sekondale 3.Sukulu yaukachenjede 4. Sindinaphunzire
Ntchito	1. Mlimi 2. Yolembedwa 3. Yodzilemba ekha 4. Zamaganyu 5. Palibe
Fuko	1. Ngoni 2. Chewa 3. Tumbuka 4. Tonga 5. Ena (Tchulani)_____

GAWO LACHIWIRI: KUDZIWA ZA ZOMELA

2. Dzina lake pa Chichewa _____

3. Mtundu

   1. Mtengo 2. Zitsamba 3. Zitsamba zopanda thima 4. Zokwera/Kuyanga

4. Malo okhala

   1. Munda 2. Nkhalango 3. Dimba 4. Tchulani malo ena _____

5. Munadziwa bwanji kuti zomerazi ndizogwiraso ngati mankhwala?

   1. Agogo 2. Makolo 3. Azanga 4. Azamba 5. Azakhali 6. Ena (Tchulani)_____

6. Ndi enaso ati munawauzapo zachitsamba chamankhwalachi?

   1. Ana 2. Adzukulu 3. Azanga 4. Azamba 5. Ayi 6. Mpongozi 7.Ena (Tchulani)_____

7. Ndi mbali iti yazomela imene mumakonda kugwiritsa ntchito?

   1. Masamba 2. Thima/Thuthu/Tsinde 3. Maluwa 4. Zipatso 5. Mizu 6. Makungwa

8. Mumayamba kukolora masamba atafika msinkhu wanji

   1. Ali ang'ono 2. Akamatulutsa maluwa 3. Nthawi yobala yazipatso 4. Masamba okwima 5. Masamba onse obiliwira

9. Mchifukwa chani mumakolora nthawi imeneyi?

   1. Amakhala osavuta kuwakonza 2. Tinaphuzitsidwa kutero ndi agogo anthu 3. Amagwira bwino ntchito nthawi imeneyi4. Tinalangizidwa ndi azanthu kukolora panthawiyi 5. Tinalangizidwa ndi makolo kukolora panthawiyi 6. Palibe chifukwa chenicheni 7. Zina (Tchulani)_____

10. Sonyezani kapezekedwe ka *Manja atali*

    1. Ndiopezeka mosavuta 2. Wosowa pang'ono 3. Wosowa 4. Ndiwosowa kwambiri

11. Mumakolola pa mtengo umodzi kapena yambiri?

    1. Umodzi 2. Iwili 3. Itatu 4. Inayi 5. Yoposela inayi

12. Mumakolora wochuluka bwanji mukafuna kugwiritsa ntchito?

    1. Odzadza dzanja 2. Manja awiri odzadza 3. Ka beseni (2L) 4. Ka beseni (5L) 5. Oposela ka beseni ka 5L.

13. Mumawagwiritsa ntchito bwanji?

    1. Pawokha 2. Mophatikiza ndi ena koma iwowo ambiri 3. Mophatikiza ndi ena koma iwowo ochepa 4. Pa mlingo/muyezo ofanana.

14. Sonyezani njira yomwe mumakonda pokoza masamba anu mukatchola?

    1. Wosakoza/Wobiliwira 2. Kuyanika pa nthuzi 3. Kuyanika pa dzuwa 4. Kuwamba 5. Kuwumika mu uvuni 6. Zina (Thulani)_____

15. Mchifukwa chani mumakonda njira imeneyi?

1. Mankhwala amagwira bwino tikagwiritsa njira imeneyi 2. Tinaphuzitsidwa njira imeneyi ndi agogo anthu 3. Ndiyosavuta/Yophweka 4. Tinalangizidwa ndi azathu kakozedwe kameneka 5. Tinalangizidwa ndi abale kakozedwe 6. Popanda chifukwa chenicheni 7. Zina (Tchulani)_____

16. Zimakutengerani nthawi yaitali bwanji kukoza masamba a awisi/wosawuma?

1. Mphindi 5 kapena kucheperapo 2. Mphindi 6-10 3. Mphindi 11-15 4. Mphindi 16-20 5. Mphindi 21-25 6. Mphindi 26-30 7. Mphindi zoposela 30

17. Zimakutengerani nthawi yaitali bwanji kukoza masamba owumika?

1. Tsiku limodzi 2. Masiku awiri 3. Masiku atatu 4. Masiku anayi 5. Masiku asanu 6. Masiku asanu ndilimodzi 7. Masiku asanu ndi awiri

18. Mumasunga kwa nthawi yaitali bwanji masamba anu anakali awisi?

1. Tsiku limodzi 2. Masiku awiri 3. Masiku atatu 4. Masiku anayi
5. Masiku asanu 6. Masiku asanu ndilimodzi 7. Masiku asanu ndi awiri

19. Mumasunga kwa nthawi yaitali bwanji masamba anu ali owumika?

1. Sabata limodzi 2. Masabata awiri 3. Masabata atatu 4. Masabata anayi
5. Kuposela masabata anayi.

20. Dzina la matenda amene akufunika kuchizidwa?

1. Malungo 2. Chifuwa 3. Kutsekula m'mimba 4. Kudzimbidwa 5. Kutentha thupi. 6. Kupweteka kwa m'mimba 7. Mauka 8. Chibayo 9. Mphamvu kuchipinda 10. Zina (Tchulani)_____

21. Mumamwa pafupipafupi bwanji?

1. Kamodzi patsiku 2. Kawiri patsiku 3. Katatu patsiku 4. Kanayi patsiku
5. Kuposela kanayi patsiku

22. Kawopsedwe kake mukamwa

1. Palibe 2. Kutuluka thukuta 3. Nseru 4. Chizungulire 5. Kuuma mkamwa6. Kutsekula m'mimba 7. Kudzimbidwa 8. Zidzolo/Zotupa pa khungu 9. Zina (Tchulani)_____

23. Njira yokozela mankhwala popeleka kwa/pa matenda odziwikilatu

Matenda	**Njira yokozela mankwala**	**Njira yokozela mankwala yopanda ukadaula weniweni.**	**Njira yakapelekedwe kake**

24. Mumakolola kangati masamba/mizu/tsinde kufikila odwalayo atachila?

1. Kamodzi 2. Kawiri 3. Katatu 4. Mpaka odwla atapeza bwino 5. Zina (Tchulani) _____

ZIKOMO

Appendix N: Questionnaire for *Pavetta schumanniana* Enukweni

CHIGAWA CHAKWAMBA. VINANDI VAKUKHWASYANA NA MUNTHU

1. **Vinandi vakukhwasyana na munthu**

KAWIRO	**MAZGOLO**
Gula la Wanthu	1. Mwanalume, 2. Mwanakazi
Vyaka	1. 15-25, 2. 26-35, 3. 36-45, 4. 46-55 5. 55 kukwela kuchanya
Banja	1. Wambula kutola or kutengwa, 2. Wakutola panyakhe kutengwa, 3. Banja lilikumala, 4. Chokolo chanakazi, 6. Chokolo chanalume
Mlongozgi wa Banja	1. Mwanalume 2. Mwanakazi, .3. Wana, 4. Vinyakhe
Unandi wa wanthu pa Banja	1. 1 mpaka 3, 2. 4 mpaka 6, 3. 7 mpaka 9, 4. Kulutilila pa 10.
Masambiro	1. Kulekezgela 8, 2. Fomu 1 mpaka 4, 3. Yunivesite, 4. Nindaluteko ku sukulu
Ntchito	1. Ulimi, 2. Pa ntchito, 3. Kujilemba mwekha, 4. Maganyu, 5. Palije
Mtundu	1. Mungoni, 2. Mu chewa, 3. Mutumbuka, 4. Mutonga 5. Chinyakhe(longosalani)_____

CHIGAWA CHACHIWIRI. KAMANYIRO KA KHUNI

2. Dzina la khuni muchiyowoyelo chinu?_____

3. Kasi lili mugulu ndini lamakhwala?

   1. Khuni

4. Uko khuni likusangika

   Kumunda 2. Kuthondo 3. Mudimba 4. Zgolo linyakhe(zunulani)_____

5. Mukamanya uli kuti khuni ili linamakhwala?

   1. Agogo 2. Wapapi 3. Wabwezi 4. Wazamba 5. Ankhazi 6. Zgolo linyakhe (zunulani)_____

6. Mulikuphalilaposo anjani za nkhuni ili?

   1.Wana 2. Wazukulu 3. Wabwezi 4. Wazamba 5. Palije 6. Mkamwana 7. Zgolo linyakhe (zunulani)_____

7. Nchigawa ntchini cha khuni ili icho mukugwiliska ntchito chomene?

   1. Mahamba 2. Mithavu 3. Maluwa 4. Vipaso, 5. Misisi 6. Vikwa

8. Mahamba yake mukuyamba kukolola nyengo uli?

   1. Tuchoko/tuteta 2. Pala nkhuni lina maluwa 3. Pala nkhuni lina vipaso 4. Pala yakhoma

   5. Mahamba yali yose ya girini.

9. Ntchifukwa uli mukukolola pa nyengo iyi?

   1. Vikuwa vambula kusuzga kupanga makhwala pa nyengo iyi
   2. Tikaphalilika kuti titolenge pa nyengo iyi na wazigogo withu
   3. Yakugwila ntchito makola pa nyengo iyi
   4. Tikalongozgeka kuti tikolole pa nyengo nawa bwezi withu
   5. Tikalongozgeka kuti tikolole pa nyengo iyi nawa papi
   6. Palije chifukwa cheneko

10. Tiphalilani za kasangikilo ka khuni ili

    1. Likusangika bweka 2. Likuzgewa padoko 3. Ndakuzgewa 4. Ndakuzgewa chomene

11. Ka munkhwala mukutolanga ku khuni limoza panji yanandi?

    1. Limodza 2. Yawiri 3. Yatatu 4. Yanayi 5. Makuni yanandi

12. Kasi mukutola unandi uli kuti vikwanile panyengo iyo?

    1.Kukaoko waka 2. Maoko yawiri kuzula 3. Mukapelo ka malita yawiri 4. Mukapelo ka 5 litazi 5. Kujumpha kapelo ka 5 litazi.

13. Kasi mukugwiliska ntchito

    1. Chekha 2. Kusazga na vinyakhe ilo kuwalinandi 3. Kusazga inyakhe ilo kuwa nga lakuonjezela waka 4. Ili hafu na yanyakheso hafu.

14. Longolani nthowa iyo mukutenga kupanga pala mwatola waka kula ku thondo mahamba?

    1. Pakuwevya kunozga kulikose

    2. Tikuyaomika mahamba mu mthudzi

    3. Tikuyanika mahamba kumu hanya

    4.Tikuyawika ku josi

    5. Tikuyawika mu uvuni kuti yaomile

    6. Zgolo linyakhe (zunulani)_____

15. Ka si mukutemwelachi nthowa iyi yakapangilo?

    1.Makhwala yakugwila makola ntchito pala tangwiliska ntchito nthowa iyi

    2. Tikasambizgika na agogo withu kuti tigwiliske ntchito iyi

    3. Njipusu

    4. Tikalongozgeka nawa nyithu kuti tikugwiliske ntchito nthowa iyi

    5.Tikalongozgeka nawa bali withu kuti tipangenge nthena

6. Palije chifukwa cheneko cheneko

7. Zgolo linyakhe (zunulani)_____

16. Kasi vikumutolelani nyengo itali uli kuti mahamba yawisi aya yawe makhwala?

1. 5 minisi panji kuchepela apa 2. 6 mpaka 10 minisi 3. 11 mpaka 15 minisi 4. 16 mpaka 20 minisi 5. 21 mpaka 25 minisi 6. 26 mpaka 30 minisi 7. Kujumphila pa maminisi 30.

17. Kasi vikukutolelani ngengo itali uli kuti mupange mahamba yakomila aya kuwa makhwala

1. zuwa limodza 2. Mazuwa yawiri 3. Mazuwa yatatu 4. Mazuwa yanayi 5. Mazuwa yankhonde 6. Mazuwa yankhonde na limoza 7. Mazuwa yankhonde na limodza

18. Kasi mahamba yawisi mukayasunga nyengo itali uli?

1. zuwa limodza 2. Mazuwa yawiri 3. Mazuwa yatatu 4. Mazuwa yanayi 5. Mazuwa yankhonde 6. Mazuwa yankhonde na limoza 7. Mazuwa yankhonde na limodza

19. Kasi mahamba yakomila mukuyasunga nyengo itali uli

1. Sabata imoza 2. Sabata ziwiri 3. Sabata Zitatu 4. Sabata zinayi 5. Kujumpha masabata yanayi.

20. Zina la nthenda iyo mukugwiliska ntchito makhwala aya

1.Maleriya 2. Chikhoso 3. Pamoyo 4. Kuthyumba 5. Mphepo 6. Munthumbo, 7. Mauka 8. Chilaso 9. Suzgo lakuchipinda 10. Zgolo linyakhe (zunulani)_____

21. Mukugwiliska ntchito kalinga

1. Kamoza pa zuwa, 2. Kawiri pa zuwa, 3. Katatu pazuwa, 4. Kanayi pazuwa, 5. Kujumpha ka nayi pazuwa

22. Viwonekelo vakhe

1. Palije 2. Kufoma 3. Museru 4. Chizgumbo 5. Komila mumulomo 6. Kufumila 7. Kuthyumba 8. Vakufuma nthupi 9. Zgolo linyakhe (zunulani)_____

23. Nthowa yaka pangilo ka makhwala kuyana na nthenda

NTHENDA	**NTHOWA YAKAPANGILO MAKHWALA**	**NTHOWA YAKUNOZGENA MAKHWALA**	**NTHOWA YAKA GWILISKILO NTCHITO**

24. Kasi makhwala mukutola kalinga ku nkhuni kuti mpaka munthu wachile?

1. Kamoza 2. Kawiri 3. Katatu 4 Mpaka muluwali wachile 5. Zgolo linyakhe (zunulani)_____

YEWO

Appendix O: Ethical Approval Letter

MZUZU UNIVERSITY

Mzuzu University Private Bag
201 L u w I n g a

M z u z u 2
M A L A W I
TEL: 01 320 722

DIRECTORATE OF

MZUZU UNIVERSITY RESEARCH ETHICS COMMITTEE (MZUNIREC)

Ref No: MZUNIREC/DOR/21/47 **18th Oct. 2021.**

Mwayi Chirwa,
University of Livingstonia,
P. O. Box 37,
Rumphi.

Email: mwayi.chirwa@gmail.com

Dear Mwayi Chirwa,

RESEARCH ETHICS AND REGULATORY APPROVAL AND PERMIT FOR PROTOCOL REF NO. MZUNIREC/DOR/21/47: ETHNOMEDICINE, PHYTOCHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF *Pavetta crassipes* AND *Pavetta schumanniana* LEAVES FROM SELECTED POPULATIONS OF DEDZA AND MZIMBA

Having satisfied all the relevant ethical and regulatory requirements, I am pleased to inform you that the above referred research protocol has officially been approved. You are now permitted to proceed with its implementation. Should there be any amendments to the approved protocol in the course of implementing it, you shall be required to seek approval of such amendments before implementation of the same.

This approval is valid for one year from the date of issuance of this approval. If the study goes beyond one year, an annual approval for continuation shall be required to be sought from the Mzuzu University Research Ethics Committee (MZUNIREC) in a format that is available at the Secretariat. Once the study is finalised, you are required to furnish the Committee with a final report of the study. The Committee reserves the right to carry out compliance inspection of this

approved protocol at any time as may be deemed by it. As such, you are expected to properly maintain all study documents including consent forms.

Wishing you a successful implementation of your study

Yours Sincerely,

Gift Mbwele

MZUZU UNIVERSITY RESEARCH ETHICS ADMINISTRATOR

For: CHAIRMAN OF MZUNI