ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF SELECTED TANZANIAN MEDICINAL PLANTS

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By

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A Dissertation Submitted in Fulfilment of the Requirements for the Degree of Masters of Pharmacy (Medicinal Chemistry) of

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled; **"Antibacterial activity and, Phytochemical screening of selected Tanzanian medicinal plants",** in (partial) fulfilment of the requirements for the degree of Master of Pharmacy (Medicinal Chemistry) of Muhimbili University of Health and Allied Sciences.

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……28/10/2019……

Date

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I, **Paul Malaba**, declare that this **dissertation** is my original work and it has not been presented nor will it be presented to any other University for similar or any other degree award.

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DEDICATION

This dissertation is dedicated to my family and friends. Thank you for your love, prayers, tireless support and understanding.

ABSTRACT

Background: With the currently limited number of antibacterial drugs and the ever increasing antimicrobial resistance, it is imperative that new antibacterial drugs be continuously discovered. Natural sources especially plants are cheaper, rich and diverse sources of potentially active and safe antimicrobials. Guided searches especially ethno-botanical surveys greatly focus this process since plants screening for bioactivities is guided by the ethno-medicinal applications of the plants, making it resources-effective.

Purpose of the study: This study aimed to investigate the antibacterial activities of plants used for infectious conditions among the local communities in Southern highlands of Tanzania. The study also aimed to determine the phytochemical compositions of the active extracts and development of their thin layer chromatography (TLC) profiles with subsequent bioautography.

Methods: A total of eight plant samples were collected from Njombe and Iringa regions following ethno-medicinal information obtained from the indigenous. The collected parts were air dried before pulverization and extraction by repeated cold maceration using 80% aqueous ethanol. After drying *in vacuo,* the obtained extracts were tested for antibacterial activities by broth microdilution assay against both standard and clinical isolates of bacteria. Standard bacteria were of the American Type Culture Collection (ATCC) namely *Escherichia coli (*ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhi* (ATCC 8385) and *Staphylococcus aureus* (ATCC 25923). The clinical isolates included; *Styphylococcus aureus,* methicillin resistant *Staphylococcus aureus* (MRSA)*, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi*. Antibacterial activities were determined in terms of minimum inhibitory concentrations (MICs) obtained as the mean values of duplicate assays. Phytochemical screening, TLC profiling and bioautography were subsequently done for the most active extracts.

Results: Among the eight plant samples, the crude extracts of four (4) of them exhibited the best antibacterial activities, whereby; *Sorindeia madagascariensis* leaves showed the highest activity (MIC of 0.193 ± 0.00 mg/ml) followed by *S. madagascariensis* roots, *Mucuna stans* leaves and *Albizia harveyi* leaves displaying MICs of 0.289 ± 0.14, 0.77 ± 0.00, and 1.54 ±

0.00 mg/ml respectively. All four plant samples were active against the test bacteria, including the MRSA with *S. madagascariensis* leaves inhibiting it at 0.193 ± 0.00mg/ml. Tannins, phenolics, flavonoids, saponins and glycosides were the mostly detected phytochemical groups among the extracts. TLC profiles of the most active samples, revealed high proportions of polar components compared to less polar components. Subsequent bioautography suggested the activity to reside in the highly polar fractions of the plants.

Conclusion: This study revealed the unpublished antibacterial potentials of the selected plants. The findings partly establish the scientific basis for ethno-medicinal applications of the plants among the indigenous of the collection sites. More importantly, it is evident that fractionation of and isolation of active molecules from these crude extracts should be done since may greatly improve the observed activities and provide lead molecules for newer antibacterial drugs. For the respective plants, the parts that were not tested in this study, specifically; the fruits, flowers, stem- and root-barks should also be explored as they may have better antibacterial activities than what this study reports.

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LIST OF ABBREVIATIONS

AMR	Antimicrobial resistance
ASM	American Society of Microbiology
Bpharm	Bachelor degree of Pharmacy
DMSO	Dimethylsulfoxide
HIV	Human Immunodeficiency Virus
INT	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium
ITM	Institute of Traditional Medicine-MUHAS
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicilin Resistant *Staphylococcus aureus*
MUHAS	Muhimbili University of Health and Allied Sciences
RSV	Respiratory Syncytial Virus
TLC	Thin Layer Chromatography
WHO	World Health Organization
ZOI	Zone of inhibition

DEFINITION OF TERMS

Antimicrobial resistance (AMR): The ability of an organism to develop strains that are impervious to specific threats to their existence (International Epidemiological Association, 2014). Precisely, it is the ability of microbes to grow in the presence of a chemical (drug) that would normally kill them or limit their growth (NIAD, 2019).

Bioautography: A technique in which organic compounds are separated by thin layer chromatography and characterized by studying their effects on microorganisms (Dictionary.com, no date).

Efflux pumps: Gene coded cell membrane proteins responsible for ejection of antimicrobial molecules from microbes.

Endemic: Refers to persistent occurrence of a disease in a given area with either stable or fluctuating frequency.

Ethno-medicinal: Related or concerned with how different cultures view disease and how they treat or prevent(Merriam-Webster, no date)

Inoculation: Addition or application of microorganisms into/onto growth medium(ASM and Cavalieri, 2005).

Minimum Inhibitory Concentration (MIC): It is the lowest concentration of the antimicrobial agent that inhibits a given microorganism from multiplying and producing visible growth in the test system (ASM and Cavalieri, 2005). .

Phytoconstituents/Phytochemicals/Phytochemical components: These are chemical compounds that occur naturally in plants (Varma, 2016).

Phytomedicine: The scientific investigation of the medicinal properties of plants or specific plant extracts; the evaluation and use of herbal medicines on pharmacological principles (Oxford-online-dictionary, no date).

Quorum sensing (QS): Communication among bacterial cells involving production and detection of diffusible signal molecules. It is a density-dependent system that regulates the bacterial expression of specific genes, whose products modify the local host environment favouring the invasion and persistence of the pathogen (Savoia, 2012).

Sporadic: Refers to irregular occurrence of a disease in a given area with unpredictable frequency.

Traditional Healer: A person in a primitive society who uses long-established methods passed down from one healer to another to treat a person suffering from various illnesses, many of which have psychological underpinnings (Segen's Medical Dictionary, 2012). They use rituals and herbal remedies in treatment of various illnesses(White and Dandurand, no date).

Zone of inhibition(ZOI): An area of growth inhibition around a point source, within a lawn of cultured organisms on a solid medium, due to the action of a growth-inhibitory substance, such as an antimicrobial agent, present at the source(Medical Dictionary for the Health Professions and Nursing, 2012).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Infectious diseases:

Infectious diseases also referred to as communicable diseases are illnesses due to specific infectious agents that arise through transmission of such agents from infected persons, animals or reservoirs to susceptible hosts (International Epidemiological Association, 2014). Infectious agents range from viruses, bacteria, fungi, protozoa to helminths, and all these cause a variety of diseases depending on pathogen- and host-related factors (Charles A Janeway *et al.*, 2001). Poor living conditions are highly linked to infectious diseases. In low income societies; malaria, HIV/AIDS, pneumonia and tuberculosis are endemic while tend to be more sporadic in high income societies (Lerner and Lerner, 2008; Gupta and Guin, 2010). Currently, respiratory tract infections, diarrhoeal diseases, HIV, Malaria and Tuberculosis are the top five causes of death and morbidity in low-income societies at prevalence of 9.4%, 7.2%, 5.5%, 4.6% and 4.2% respectively (WHO, 2018a).

Infectious diseases burden:

Despite the progressive decrease in global burden of infectious diseases from around 24% to 16% of all deaths globally, in 2010 and 2016 respectively, (WHO, 2018a), they continue to impact several regions of the World especially the low income societies (Bhutta *et al.*, 2014; WHO, 2018a). Such societies are even more affected by what is called a double burden, since deaths from non-infectious diseases too are progressively increasing (Marquez and Farrington, 2012; Kushitor and Boatemaa, 2018).

Antimicrobial resistance:

Although infectious diseases have been a big problem mostly for the low-income societies, they are turning to be a major global concern. It has been noted by multilateral organs that, the tremendous success in containment of infectious diseases will ultimately be lost as a result of the alarmingly increasing rates of antimicrobial resistance (Review on Antimicrobial Resistance, 2015; European Commission, 2017; Word Bank, 2017; WEF, 2018; WHO,

2018b). The worst parts are; first, antimicrobial resistance is inevitable since even rational use of antimicrobials provides for resistance development as a natural protective process among microbes (Review on Antimicrobial Resistance, 2016). Secondly, antimicrobial resistance does not respect international borders, which turns infectious diseases a global concern (WHO, 2018b). Adding to that, high levels of antimicrobial resistance have been reported in both high- and low- income countries with isolation of microorganisms resistant to antimicrobials of last resort like carbapenems and colistin (Reardon, 2017; WHO, 2017b). The common and reasonably affordable antibacterial drugs have been reported to face high levels of resistance. For example, resistance rates of 51% and 65% against penicillins and ciprofloxacin respectively have been reported (WHO, 2018a).

Economic Impact of AMR:

With the observed rates of AMR, the World Bank in March 2017 estimated that AMR would exert a drag on global GDP of between 1.1 and 3.8 percentage points between now and 2050 which calls for corrective measures around the World (Word Bank, 2017; WEF, 2018). The World Bank stresses that; International trade may be heavily affected if AMR spreads uncontained. Output and trade in livestock and livestock products are especially vulnerable to AMR impacts as livestock production in low-income countries would decline the most, with a possible 11 percent loss by 2050 (Word Bank, 2017).

Corrective measures and the current challenges:

In response to the growing threat of infectious diseases in form of AMR, various international and national agencies have taken advocacy to tackle that problem. Several measures have been proposed including; reduction of unnecessary prescriptions of antimicrobials, controlled veterinary use of antimicrobials, support of national health care systems, promoting and scaling up of research and development of newer antimicrobials (Review on Antimicrobial Resistance, 2015, 2016; Word Bank, 2017; WEF, 2018; WHO, 2018c).

To streamline the search for relevant antimicrobials, the WHO has named several priority pathogens against which newer antimicrobials should be developed. Together with the old-priority diseases like Tuberculosis, HIV/AIDS and Malaria, another list of priority pathogenic

bacteria has recently been released. Carbapenem–resistant strains of *Acinetobacter baumannii, Pseudonomonous aeruginosa* and Enterobacteriaceae (*Klebsiella pneumoniae, Eschrichia coli, Proteus spp, Enterobacter spp* and others) have been ranked as critical priority pathogens. Methicillin resistant, vancomycin intermediate and resistant *Staphylococcus aureus*, Fluoroquinolone resistant *Salmonella spp* and others have been ranked as high priority pathogens. Penicillin-non-susceptible *Streptococcus pneumoniae,* ampicillin-resistant *Haemophilus influenza* and fluoroquinolone-resistant *Shigella spp* have been ranked as medium priority pathogens (WHO, 2017b).

Despite the urgency of antimicrobial resistance, the current rate of discovery of newer antimicrobials does not give enough hope. Recently, the discovery and development of new antibacterials have largely slowed down because of factors like; scientific barriers to drug discovery, regulatory challenges, and diminishing returns on investment, forcing major drug companies to scale back or abandon their antibiotic researches (Review on Antimicrobial Resistance, 2016; The Pew Charitable Trusts, 2016). It is also reported that, nearly all antimicrobials available in the market are based on classes discovered back in 1980s. This aggravates the chances for several antimicrobials turning obsolete in the near future as a result of cross-resistances caused by close similarities among the antimicrobials. (The Pew Charitable Trusts, 2016; WHO, 2017a; WEF, 2018).

As of May 2017, about 51 antibiotics and 11 biologicals were at different stages of clinical development with 42 constituting new chemical entities that target various priority pathogens including tuberculosis (WHO, 2017a). However, this move has been reported to be insufficient to curb antimicrobial resistance since qualitative and experiential analysis predicts that, only one or two anti-Gram-negative agents in that pipeline will probably make it to the market in five years from 2017. And for Gram positive pathogens, recent market authorization of newer classes like oxazolidinones and cyclic peptides is still not enough to keep up with the expected evolution of resistance (Review on Antimicrobial Resistance, 2015; WHO, 2017a). For those reasons, the WHO insists that, more investment is needed in basic science, drug discovery and clinical development, especially for the critical priority Gram-negative

carbapenem-resistant pathogens *P. aeruginosa, A. baumannii* and the Enterobateriaceae (WHO, 2017a).

Plants as potential sources of newer antimicrobials:

Among many available approaches to newer antimicrobials, the use of natural sources has been of paramount advantage. Plants are capable of generating several compounds evidenced by several novel drugs in market with their origin in plants (Abdallah, 2011; Katiyar *et al.*, 2012; Savoia, 2012). It is estimated, about 25% of drugs originate or at least mimic compounds of plant origin (Gurib-Fakim, 2006). Reputable drugs like quinine, artemisinins and digoxin, vincristine, vinblastine, paclitaxel and several others find their origin in plants (Katiyar *et al.*, 2012; Savoia, 2012).

Recently, studies have shown that, plants offer a variety of molecules with diversified antimicrobial modes of action (Savoia, 2012). Compounds of plant origin like hiperenone A, hypercalin B and hyperphorin have shown direct antibacterial activities to resistant bacteria like *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Osman *et al.*, 2012; Savoia, 2012; Shiu *et al.*, 2012).

Apart from giving molecules with direct antibacterial activities, plants have been found to possess molecules with modulating actions on antimicrobial resistance profiles of microbes (Rates, 2001; Abdallah, 2011; Savoia, 2012). Plant based molecules with inhibitory activities on; bacterial efflux pumps, bacterial quorum sensing and biofilms have been isolated. Despite all these potentials, it is just a small fraction of plants, estimated to be not more than 10% that has been tapped by human civilization worldwide. This signifies the need for further exploration of plants in drug discovery (Newman, Cragg and Snader, 2003; Osman *et al.*, 2012; Savoia, 2012).

Ethno-botanical surveys as a reliable approach and this study:

One of the approaches upon searching for therapeutically potential agents from plants is ethno-medicinal approach which is also referred to as ethno-directed bio-rational approach (Rates, 2001; Savoia, 2012). In this case, searching is guided by a thorough study of the folklore of the plants (ethno-medicine) through ethno-botanical surveys, followed by

confirmation of the observed traditional claims in accepted experimental systems rather than unguided screening for bioactivities of such plants (Rates, 2001; Katiyar *et al.*, 2012). It is also argued that, this mode of drug discovery is likely to give more safer drugs backed up by a long history of use by local communities (Katiyar *et al.*, 2012).

In respect of the above, eight (8) plant samples found in Njombe and Iringa regions were investigated for their antimicrobial potentials. The selected plants are all claimed to be used in infectious conditions especially those of the skin namely; boils, abscess and folliculitis. These plants included; *Albizia harvey* (leaves and roots), *Mucuna stans* (leaves), *Elaeodendron buchananii* (stem bark), *Leonotis nepetifolia* (leaves), *Sorindeia madagascariensis* (leaves and roots) and *Parinari curatellifolia* leaves. After preliminary screening for antibacterial activity, *Sorindeia madagascariensis* Baill (Anacardiace) both leaves and roots, *Mucuna stans* Baker (Fabaceae) leaves and *Albizia harvey*i E. Fourn (Fabaceae) leaves were selected for further studies following the preliminary superior antibacterial activities they exhibited.

***Sorindeia madagascariensis* Baill (Anacardiace)**

Belonging to the family Anacardiaceae, *Sorindeia madagascariensis* is an evergreen 20m tall tree or a multi-stem shrub native of Africa especially Tanzania, Kenya, Malawi, Mozambique, Madagascar and Somalia. In Tanzania, it is largely distributed along the coastal natural forest and in the Southern highlands (Breteler, 1999). Its local uses range from edible uses (fruits), construction and fuel (wood) and medicinal uses. Published ethnomedicinal uses of the plant include; treatment of Malaria, Tuberculosis, Schistosomiasis, hookworm and menstruation problems (Donkeng Donfack *et al.*, 2014).

***Mucuna stans* Baker (Fabaceae)**

Mucuna stans is a highly branched erect shrub, with pressed grey-silky hairs native of East and Central Africa, Mozambique, Malawi, Zambia, Zimbabwe, and Somalia, Ethiopia, Angola and Equatorial Guinea (*Mucuna stans in Global Plants on JSTOR*, no date). Less is published about its uses in such communities. However, more information is published about the prototype plant of the genus; *Mucuna pruriens.* Being in one genus, the two plants might have parallel behaviours and local uses. *Mucuna pruriens* finds numerous edible uses as well as

reputable ethno-medicinal applications. These include treatment of roundworms, as a diuretic, treatment of elephantiasis and as a rubefacient (Tiwari and Goyal, 2018). Other applications include; treatment of bacterial infections (Lampariello *et al.*, 2012), as an aphrodisiac(K umar *et al.*, 1994), treatment of Parkinson's disease (Dhanasekaran, Tharakan and Manyam, 2008). These applications may be true for *Mucuna stans* too, given the possible phylogenetic relatedness of the two plants. Extensive published literature on antibacterial and other bioactivities of *Mucuna pruriens* is also available while that of *Mucuna stans* is not available.

***Albizia harvey*i Fourn (Fabaceae)**

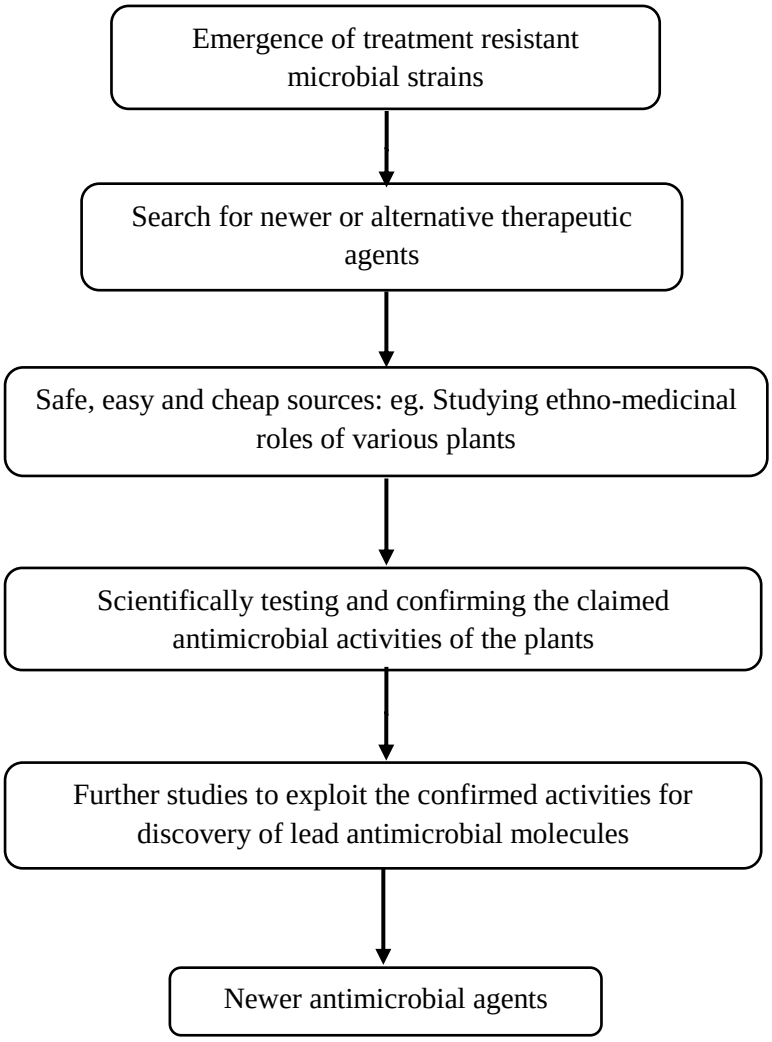
It is a tree that grows up to 15 m with nearly rounded crown. It is native of the Tropical Africa; Zimbabwe, Tanzania, Zambia, Swaziland, Mozambique, Namibia, Kenya, South Africa and DR Congo (*Plants of Ngorongoro Crater, Tanzania)*, no date; Thippeswamy *et al.*, 2015). Its local uses range from mostly construction and fuel as well as ethno-medicinal applications. Published ethno-medicinal uses include treatment of epilepsy, urinary tract infections, vomiting and infertility (Huxley, Griffiths and Royal Horticultural Society (Great Britain), 1992)

1.2 Problem Statement

Considering the emerging treatment challenge in form of drug resistant strains of bacteria, continuous search for newer antibacterial agents is inevitable (WEF, 2018). Plants continue to be a better option as sources of newer or alternative antimicrobials especially in resource-limited settings since they are cheaply available and offer a wide array of compounds that can serve as antibacterials or at least precursor molecules (Katiyar *et al.*, 2012). Furthermore, drug discovery from plants partly assures the safety of the obtained drugs as histories of local utilization of the plants as drugs without posing any noticeable problems give supporting backups (Rates, 2001; Vlietinck *et al.*, 2015). Regular ethno-botanical surveys coupled with scientific confirmation of survey findings is therefore indispensable approach towards discovery of newer or alternative antibacterial drugs. This study therefore embarked on screening for antibacterial activities of the selected plants having suggestive ethno-medicinal applications with an intention of coming up with useful information towards discovery of active lead molecules for development of new drugs.

1.3 Conceptual framework

Emerging challenges in treatment of microbial infections especially development of resistant strains call for immediate actions like continued search for newer or alternative therapeutic agents (Review on Antimicrobial Resistance, 2015; World Economic Forum, 2018). Exploring nature by using ethno-medicinal surveys and testing the reported claims can give an easier way of discovering potential antimicrobials from natural sources particularly plants (Katiyar *et al.*, 2012; WHO, 2018c).



Scheme 1: Discovery of antibacterial drugs from plants guided by ethno-medicine

1.4 Rationale

Findings from this study are essential, partly in confirming the long history of traditional use of the plants in treatment of infectious conditions in the collection sites and more importantly to provide the basis for further investigations like isolation and characterization of lead compounds for newer antibacterial agents.

1.5 Research questions

1. Do the selected plant species possess antibacterial activities?
2. What are the phytochemical components in the active plant extracts?
3. What are the TLC profiles of the active plant extracts?
4. What are the bioautograms of the active plant extracts?

1.6 Objectives of the study

1.6.1 Broad Objective

To evaluate the antibacterial activities and phytochemical composition of extracts from selected Tanzanian medicinal plants.

1.6.2 Specific Objectives

1. To evaluate the *in vitro* antibacterial activities of extracts from selected plant species.
2. To determine the phytochemical composition of the active plant extracts.
3. To develop the TLC profiles of the active plant extracts.
4. To determine the bioautograms of the active plant extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

Drug discovery from natural sources particularly plants comes with numerous benefits including; low costs, directed discovery through ethno-botanical surveys, safety assurance backed up by long history of use of the given plants by a given society and increased diversity of molecules that cannot be attained by the still sophisticated artificial synthetic systems (Katiyar *et al.*, 2012). To make it efficient, drug discovery form natural sources is normally preceeded by ethnobotanical surveys whereby, the local medicinal applications of several plants are learnt and the given plants are collected and tested for the claimed medicinal attributes. The highly qualifying plants are then used to obtain useful lead molecules which can be used directly or modified to enhance their activities (Rates, 2001; Katiyar *et al.*, 2012).

In this study, eight plant samples were collected and screened for antibacterial activities afterwhich, three of them constituting four samples were selected for subsequent studies. These included; *Sorindeia madagascariensis* Baill (Anacardiace) both leaves and roots, *Mucuna stans* Baker (Fabaceae) leaves and *Albizia harvey*i E. Fourn (Fabaceae) leaves. Limited or at least related published literature on the antibacterial activities and phytochemical attributes of these plants is available.

Sorindeia madagascariensis appears not to be well studied in terms of its antibacterial activity although its little literature on other bioactivities is available including its potential antifungal activity at MIC of 0.625mg/ml (Victor *et al.*, 2019). Its Cameroonian congener, *S. juglandifolia* has been reported to possess a strong antimycobacterial activity at 3.9 µg/ml (Donkeng Donfack *et al.*, 2014). Phenolics namely; 2,3,6-trihydroxy benzoic acid and 2,3,6-trihydroxy methyl benzoate were found to be responsible for the antimycobacterial activity of *S. juglandifolia*. Another member of this genus in Nigeria namely; *S. warneckei* was long reported to possess antibacterial activity against several bacteria (Taiwo, Xu and Lee, 1999).

Apart from the limited literature on the antibacterial activities of the genus, reports on other bioactivities are available and mostly the antiplasmodial activity of *Sorindeia juglandifolia* (Boyom *et al.*, 2012) whereby isolated phenolics again were found to be responsible for the observed activity. Generally, *Sorindeia* constitutes a genus that is not extensively explored in terms of its bioactivities and its phytochemical attributes. However, the currently available literature signifies the antimicrobial potentials of this genus.

Mucuna stans (Fabaceae) is one of the congeners of the prototype of the genus, *Mucuna pruriens* which is extensively studied contrary to *Mucuna stans* and the other members. Reports on the antibacterial activities of *M. stans* are not available. Of recent, is the report on its antifungal activity at MIC of 0.117-0.313 mg/ml, (Victor *et al.*, 2019). The antibacterial activities of *Mucuna pruriens* have been widely reported from its several parts; mostly the leaves (Salau and Odeleye, 2007; Mastan *et al.*, 2009) and seeds (Ifeanyi *et al.*, 2014).

Apart from antibacterial activity, the seeds of *Mucuna pruriens* have been reported to possess; aphrodisiac (Kumar *et al.*, 1994), ant-parkinsonism (Dhanasekaran, Tharakan and Manyam, 2008), antioxidant (Lampariello *et al.*, 2012), hypolipidemic (Herrera Chalé *et al.*, 2016) and several other activities being referred to as Magic Velvet beans. Phytochemically, the seed has been reported to contain 3-(3,4- dihydroxyphenyl)-l-alanine (levodopa), gallic acid and beta-sitosterol. Other phytoconstituents reported include; mucunine, prurienine and mucunadine. Tetrahydroquinoline alkaloids are also reported in the seeds. Its leaves and roots have been reported to contain indole-3-alkylamines-N,N-dimethyltryptamine (Lampariello *et al.*, 2012).

Published reports on the antibacterial activity of *Albizia harveyi* are also limited although reports on its antioxidant activity are available. Polyphenolics isolated from the plants have been reported to be responsible for its antioxidant activity (Sobeh *et al.*, 2017). The antibacterial activities of several other *Albizia* species have however been reported with some species exhibiting good antibacterial activities. *Albizia odoratissima* and *Albizia julibrissin* displayed low MICs of 136 µg/ml (Banothu *et al.*, 2017) and 65 µg/ml (Rajalakshmi and Senthil, 2014) respectively. An alkalodal fraction of the seeds of *Albizia bernieri* of

Madagascar have been reported to inhibit bacteria at MIC between 10 and 1000 µg/ml (Randriamampianina *et al.*, 2017).

Stem bark of an Ethiopian *Albizia lebbeck* was found to possess both antibacterial and antioxidant activities having a strong antibacterial activity with MIC of 10 µg/ml (Abriham and Paulos, 2016). The leaves of the same plant had previously been reported in Nigeria to have antibacterial activity at MIC of 50mg/ml (Sheyin *et al.*, 2015). The leaves of both, *Albizia amara* (Shubha *et al.*, 2014) and *Albizia procera* have also been reported to possess antibacterial activity with the latter being reported to also have analgesic and depressive activities (Khatoon *et al.*, 2014). Compounds isolated from several *Albizia* species have displayed several bioactivities. Flavonoids isolated from *A. julibrissin* were reported to be responsible for the antibacterial activities of the plant (Rajalakshmi and Senthil, 2014). Other compounds isolated from *Albizia* species have exhibited; anticancer, antidiabetic and anti-inflammatory activities. Generally, glycosides, saponins, tannins, flavonoids and terpenoids are the mostly isolated classes of constituents from this genus (Kokila, Priyadharshini and Sujatha, 2013).

Whenever a crude extracts is found to show strong bioactivity, the ultimatum is normally to fractionate the extract and isolate the active molecules. This process can be done either, blindly by trying different solvent systems which is normally tedious and time consuming or by using bio-guided fractionation and isolation which is precise and convenient (Altemimi *et al.*, 2017).

Bioautography is one of the bio-guiding techniques whereby a well separated/eluted extract spot on TLC plate is tested to see the components (spots) possessing the target bioactivity in the extract. Such impression guides the selection of solvent system that will give fraction (s) with most of the active molecules (Dewanjee *et al.*, 2015). When well optimised, the technique is resources saving.

Direct, agar overlay, and contact; are the three variants of bioautography. In direct bioautography, a suspension of microorganisms in nutrient broth is applied directly on a pre-developed TLC plate, incubated at specified conditions of temperature, humidity and time, followed by detection of areas of inhibition of growth mostly through staining. Areas of inhibition of growth correspond to spots with active molecules (Dewanjee *et al.*, 2015). Limited growth of organisms caused by drying of broth has been reported to be the limiting factor for direct bio-autography which can be alleviated by using facilities with controlled humidity.

In agar overlay, a thin layer of molten agar is applied on the surface of a developed TLC and left to harden. The agar can be pre-seeded with microorganisms when cool but still molten or inoculated after hardening on the chromatogram. This is followed by incubation and growth inhibition-detection as described for direct bioautography. In contact bioautography, a chromatogram is placed face down on an inoculated agar for a specified period to allow diffusion of molecules into an inoculated agar followed by incubation and growth detection. Limited diffusion of extract-molecules has been reported to be a challenge for both agar overlay and contact bioautography and need to be well optimised for good results to be obtained (Cleidson Valgas; *et al.*, 2007; Choma and Grzelak, 2011; Dewanjee *et al.*, 2015).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Plant material collection

A total of eight plant samples were collected from Njombe and Iringa regions of Tanzania between December 2018 and January 2019. These included; the leaves of; *Albizia harveyi, Mucuna stans, Leonotis nepetifolia,* and *Sorindeia madagascariensis.* Others were the roots of *Sorindeia madagascariensis* and *Albizia harveyi* and the stem bark of *Elaeodendron buchananii.*

After collection, the plant samples were first allowed to air-dry at the collection sites in order to reduce the chances for deterioration during transport (Evans, 2009; Shah and Seth, 2010). After arrival at the laboratory, the samples were further air-dried for adequate drying (Aboltins and Kic, 2016). Concurrently, voucher specimens were prepared and deposited in the herbarium at the Institute of Traditional Medicine (ITM) of MUHAS.

3.2 Extraction

This was done in the Medicinal Chemistry laboratory of the School of Pharmacy at MUHAS (SoP-MUHAS).

3.2.1 Solvents

Absolute Ethanol which was then mixed with distilled water to make 80% ethanol solution was used for extraction of all mentioned plant samples separately (Narendra *et al.*, 2015).

3.2.2 Extraction of the plant samples

After adequate air-drying (Muller and Heindl, 2006), plant samples were separately pulverized and the respective course powders (40 mesh) were subjected to extraction by cold maceration for 96 hours (4 days). There were intermittent agitations (12 hourly) to enhance extraction. After 96 hours, the extracts were filtered, collected in separate clean containers and stored below ambient temperature (Ogueke *et al.*, 2007). Such obtained extracts were subsequently concentrated using rotary evaporator at 45^{o}C, and the amorphous concentrates were kept in

airtight containers and refrigerated until their use (Al-Mughrabi, 2003; Bandar *et al.*, 2013; Yeo *et al.*, 2014).

3.3 Antibacterial activity studies

These were carried in the Pharmaceutical Microbiology Laboratory of the SoP-MUHAS. All extracts were tested for antibacterial activities by broth micro-dilution assay.

3.3.1 Test microorganisms

Test bacteria were selected based on both, the WHO priority pathogens list and their prevalence in causation of diseases (WHO, 2017b). These included standard and clinical isolates of bacteria and were all donated by the Pharmaceutical Microbiology Laboratory of the SoP-MUHAS. Standard bacteria included; the American Type Culture Collection (ATCC) namely *Escherichia coli (*ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603) and *Pseudomonas aeruginosa* (ATCC 27853). Other standard bacteria included Salmonella typhi (ATCC 8385) and *Staphylococcus aureus* (ATCC 25923). Clinical isolates included; *Styphylococcus aureus, MRSA and Klebsiella pneumonia.* Other clinical isolates included *Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi*.

3.3.2 Determination of the antibacterial activities

Determination of antibacterial activities of the plants was done by using the method of 2-fold broth microdilution whereby the minimum inhibitory concetrations (MICs) of the plants against the test bacteria were determined (ASM and Cavalieri, 2005). All eight (8) plant samples were screened for antibacterial activities at a concentration range of 3 to 0.023mg/ml (Ríos and Recio, 2005; Kuete, 2010).

96 wells microtitre plates were used for MIC determination and each plate was dedicated to a single bacterium. Mueller Hinton broth (Oxoid 1640936) sterilized and kept in sterile containers was used as a nutrient and diluent. Before setting for MICs determination, test bacteria were sub-cultured for 24 hours on fresh nutrient agar (Techno Pharmchem, India) in order to reactivate them (ASM and Cavalieri, 2005). Plant sample stock solutions were prepared by using the respective broth and solubilization was aided by DMSO at 20% of the

resulting extract solution. Ciprofloxacin and 20% DMSO were used as positive and negative controls respectively. Ciprofloxacin being one of the mostly used antibacterials and being at risk of running obsolete due to antimicrobial resistance (WHO, 2018b) was preferencially selected. Its test concentration was set between 52 µg/ml and 0.4µg/ml since most susceptible bacteria are sensitive within or below that range of concetration (Bayer HealthCare, no date).

Upon setting for MIC determination, 100 µl of Muller Hinton broth were added into all wells of labelled microtitre plates by using multi-channel micropipettes. Subsequently, 100µl of crude extracts were added in the first wells of the plates and mixed to make a total volume of 200µl. From such wells, 100µl were drawn and added to the next wells and the process continued to constitute the two-fold serial dilutions down the rows of the wells. Such two-fold serial dilutions gave a concentration range of 3 mg/ml to 0.023mg/ml along the given rows of the microtitre plates (ASM and Cavalieri, 2005; Pisano *et al.*, 2016).

0.5 MacFarland-equivalent (approximately 1.0 x 10^5 colon forming units) suspensions of the test bacteria in Muller Hinton broth were prepared by adjustments of turbidity to that of the 0.5 MacFarland turbidity standard. Wells of microtitre plates were then inoculated with the suspensions of the test bacteria. The inoculated plates were ultimately incubated at 37^{o}C for 24 hours after which the plates were observed for inhibition of growth of microorganisms. Detection of growth inhibition was through visualization aided by iodonitrotetrazolium (INT) chloride (Sigma-Aldrich). Upon visualization, 30µl of 0.4mg/ml INT(Sigma-Aldrich, 2018) were added in the wells followed by re-incubation for 30 minutes. After incubation, growth inhibition was considered when no color changes occurred, whereas color changes to purple or pink signified active growth of bacteria. The lowest concentrations inhibiting growth of microorganisms in the wells were taken as the MICs. (ASM and Cavalieri, 2005).

MICs determination was repeated one time for the plant samples that showed the best activities among the screened plants and the results were expressed as means and standard deviations of the MICs of the individual plants against the respective test bacteria.

3.4 Phytochemical screening of the active extracts

This was qualitatively done for extracts showing the best antibacterial activities. Standard phytochemical procedures as adopted from (Akinyemi, Oluwa and Omomigbehin, 2006; Evans, 2009; Rao, Abdurrazak and Mohd, 2016) were carried as follows;

Testing for alkaloids: 0.5g of extract dissolved 1% HCl was boiled, filtered for proteinaceous precipitates followed by treatment with Meyer's reagent. Formation of a yellowish brown precipitate indicated the presence of alkaloids.

Test for saponins: 0.5g of extract was dissolved in 20ml of distilled water and shaken in graduated cylinder for 15 minutes. The formation of one centimeter layer of foam indicated the presence of saponins.

Testing for phenols: To 0.5g of extract in 1 ml of distilled water in a test tube, 1 or 2 drops of Iron(III) chloride were added. Formation of a blue, red or purple color indicated the presence of phenols.

Testing for flavonoids: To 0.5g of extract one to five drops of concentrated HCl were added. Immediate formation of a red colour indicated the presence of flavonoids.

Testing for glycosides: 0.5g of extract in 1 ml of distilled water in a test tube were added with a few drops of dilute NaOH. Formation of a yellowish color indicated the presence of glycosides.

Testing for terpenoids: 0.5g of extract was added into 2 ml of chloroform contained in a test tube. 3 ml of concentrated H_2SO_4 were then added resulting into formation of a layer. Formation of a reddish brown color at the interface indicated the presence of terpenoids.

Testing for tannins: 0.5g of extract was placed in a test tube and then 1 ml of 5% Iron(III) chloride solution was added. Formation of a greenish-black precipitate indicated the presence of tannins.

3.5 Thin Layer Chromatography (TLC) profiling

This was done only for the best two samples in respect of the exhibited antibacterial activities. Different solvent systems were tested using analytical grade solvents and aluminium silica gel 60 254 TLC plates (Merck Germany). TLC plates were cut into 10 by 5 cm dimensions, spotting was done by using 4µl microcapillary tubes. Adequate chamber saturation was ensured by using Whatman grade 1 filter papers. Visualization was done by using both long and short UV light and derivatization by iodine and vanillin (15g of vanillin, 1% H_2SO_4 in ethanol)(*TLC Stains*, no date).

Three solvent systems were developed to acquire separation in the regions of low polarity, medium polarity, and high polarity. Different solvent combinations were tried and those that gave good separations in each region were then used to develop several copies of TLC plates, of which some were derivatized for visualization and recording and others for bioautography.

3.6 Bioautography

This was done in order to get guidance on the polarity range or region of the compounds responsible for activity in the crude extracts which can help upon fractionation and isolation of active molecules. Agar overlay method was adopted whereby, the developed non-derivatized TLC plates were used. Molten Muller Hinton Agar (CONDA) was poured on the surface of developed TLC plate and allowed to solidify (Dewanjee *et al.*, 2015). Previously selected and sub-cultured bacteria were prepared into 0.5 McFarland-equivalent suspension using normal saline. These were then used to inoculate the hardened agar on the surface of TLC plates supported in trays.

Purposely, a few bacteria namely; Standard *S. aureus*, MRSA and clinical isolate *P. aeruginosa* were used as representative bacteria at this preliminary step and by considering their previously observed susceptibility towards the plant extracts. The inoculated plates were incubated for 24 hours after which they were sprayed with 0.4 mg/ml Iodonitrotetrzolium chloride (Sigma-Aldrich, 2018) solution to aid detection of areas of active growth and inhibition based on colour changes. The sprayed plates were re-incubated for 30 minutes after which colour changes were carefully observed whereby the regions that had a purple or pink

colour meant organisms were actively growing and the parts without purple colour meant growth inhibition.

3.7 Ethical clearance

Ethical clearance was obtained from MUHAS, Research and Publications Committee. Appropriate Laboratory and Environment-protective practices were adhered to throughout the study time.

3.8 Study limitations and mitigation

Limited study time and funds to buy more solvents and materials shortened the scope of this study as advanced procedures like fractionation, isolation and characterization of active molecules could not be done.

CHAPTER FOUR

4.0 RESULTS

4.1 Antibacterial activities of the active plant extracts in mg/ml

Antibacterial activities of plant extracts expressed as minimum inhibitory concentrations (MICs) and their respective standard deviations are presented in table 4.1. Photographs of the developed microtitre plates for the; clinical isolate of *P. aeruginosa*, MRSA, standard *S. aureus* ATCC 2592 and standard *K. pneumoniae* ATCC are presented in figure 4.1.

Table 4.1: Minimum inhibitory concentrations (MIC) observed of the active plants: in mg/ml

Organism	SML	SMR	MSL	AHL	20 % DMSO (Negative control)	CFN (positive control)
Std *S. aureus*	0.193 ± 0.00	0.385 ± 0.00	0.77 ± 0.00	1.54 ± 0.00	+	0.0064 ± 0.00
Std *E. coli*	0.578 ± 0.27	0.77 ± 0.00	0.77 ± 0.00	2.31± 1.09	+	< 0.0005
Std *K. pneumoniae*	0.77 ± 0.00	0.77 ± 0.00	1.16 ± 0.54	2.31± 1.09	+	0.0129 ± 0.01
Std. *S. typhi*	0.635 ± 0.19	1.16 ± 0.54	1.16 ± 0.54	1.54 ± 0.00	+	0.0032 ± 0.00
Std *P. aeruginosa*	0.77 ± 0.00	1.16 ± 0.54	3.08 ± 0.00	>3.08	+	0.0024 ± 0.00
CI *S. aureus*	0.193 ± 0.00	0.289± 0.14	0.77 ± 0.00	1.54 ± 0.00	+	0.0129 ± 0.00
MRSA	0.193 ± 0.00	0.385 ± 0.00	1.16 ± 0.54	1.54 ± 0.00	+	> 0.0515
CI *P. aeruginosa*	1.16 ± 0.54	1.16 ± 0.54	2.31± 1.09	1.54 ± 0.00	+	0.0016 ± 0.00
CI. *S. typhi*	0.578 ± 0.27	1.54 ± 0.00	1.54 ± 0.00	3.08 ± 0.00	+	0.0048 ± 0.00
CI *K. pneumoniae*	0.77 ± 0.00	1.54 ± 0.00	2.31± 1.09	1.54 ± 0.00	+	0.0024 ± 0.00
CI *E. coli*	0.385 ± 0.00	0.77 ± 0.00	1.16 ± 0.54	>3.08	+	0.0032 ± 0.00

AHL = *A. harveyi* leaves, MSL = *Mucuna stans* leaves, SML = *S. madagascariensis* leaves, SMR = *S. madagascariensis* roots, CFN = Ciprofloxacin, DMSO = Dimethylsulfoxide, + = Active growth of organisms, Std = Standard bacteria, American Type Culture Collection, CI = Clinical Isolate. MRSA =Methicillin resistant *Staphylococuss aureus*, > means MIC is greater than the highest set concentration, < means MIC is lower than the lowest set concentration.

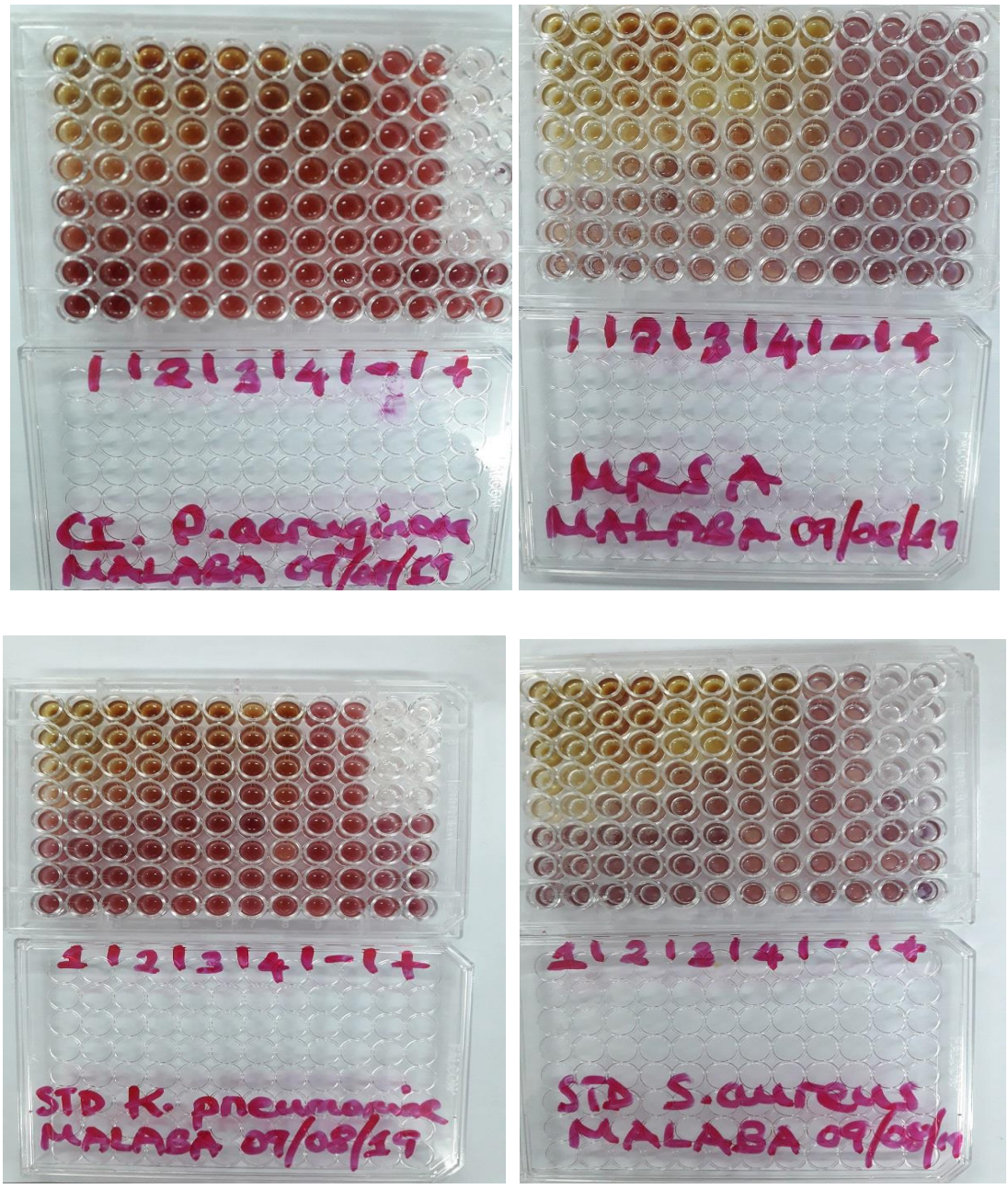


Figure 4.1: Microtitre plates for some of the test bacteria: Purplish colour means actively growing microorganisms, clear or yellowish colour means growth inhibition. 1= *Sorindeia madagascariensis* leaves, 2 = *Sorindeia madagascariensis* roots, 3 = *Mucuna stans* leaves, 4 = *Albizia harveyi* leaves, (-) = Negative control (20% DMSO) and (+) = Positive control (Ciprofloxacin)

4.2 Phytochemical analysis of the active plant samples

Phytochemical screening results are presented in table 4.2. Difference in colour intensities among the tested samples were visually compared to give impression on relative amounts of the detected phytoconstituents among the plant extracts.

Table 4.2: Phytochemical groups detected in the selected four plant samples

Phytochemical group	SML	SMR	MSL	AHL
Tannins	+	+	++	+
Saponins	++	+	+	+
Flavonoids	+	–	+	+
Steroids	–	–	–	+
Terpenoids	++	+	+	+
Phenols	++	+	+	+
Alkaloids	–	–	–	–
Glycosides	++	++	–	+

AHL = *A. harvey* leaves, MSL = *Mucuna stans* leaves, SML = *S. madagascariensis* leaves, SMR = *S. madagascariensis* roots, + means the phytochemical group is detected(present), - means the phytochemical group is not detected(not present), ++ means the observed color was so intense to signify high amount of the phytochemical group detected compared to the other samples.

4.3 Thin Layer Chromatography (TLC) profiling;

This was done for two samples which were most active namely; the leaves and roots of *Sorindeia madagascariensis*. After a series of trials, three solvent systems were found optimal for elution of phytochemical groups present in the selected plant samples as tabulated below;

Table 4.3: Established solvent systems for TLC profiles of the most active samples

S/N	ORDER OF ELUTION	SELECTED SOLVENT SYSTEM
1	First elution	Ethylacetate : Methanol (19:1)
2	Second elution	Ethylacetate : Isobutanol : Water (16:2:2)
3	Third elution	Methanol : Water (16:4) + 3 drops of glacial acetic acid

The resulting chromatograms are presented as visualized in daylight following derivatization by iodine vapour and vanillin solution (*TLC Stains*, no date)

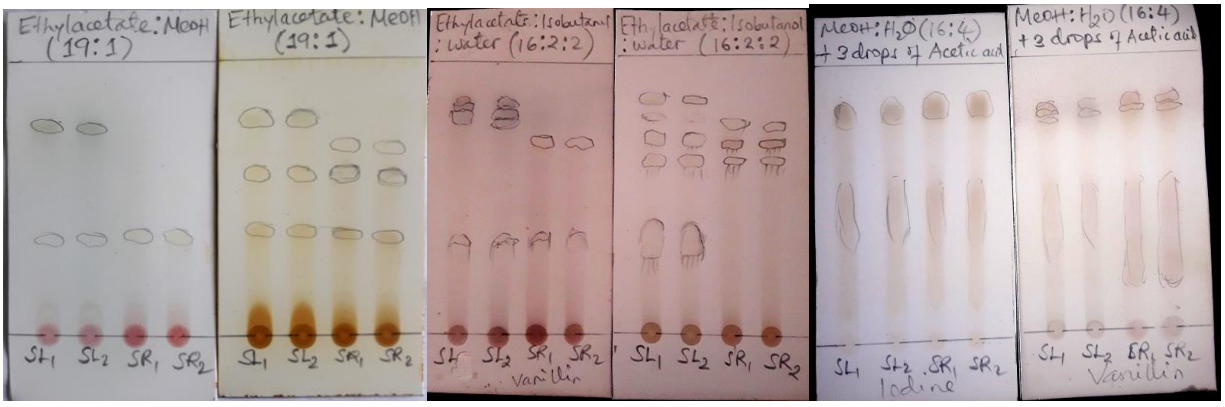


Figure 4.2: Developed TLC plates for the three solvents systems: SL and SR stand for *Sorindeia madagascariensis* leaves and roots respectively. Derivatized by vanillin and iodine followed by visualization in daylight.

4.4 Bioautography

Results for bioautography indicated activity to be confined in the most polar components as display by the bioautograms below.

1 **2** **3**

Figure 4.3: Representative bioautograms: 1 and 2 are bioautograms of the standard *S. aureus* at first and second elution respectively. 3 is the bioautogram of the MRSA at the third (highest polarity) elution. Purple/pink colour means active growth of bacteria, yellow/white colour means growth inhibition. SL= *Sorindeia madagascariensis* leaves, SR = *Sorindeia madagascariensis* roots and Cipro = Ciprofloxacin, positive control (Solvent systems: 1 = Ethylacetate:Methanol, 19:1, 2 = Ethylacetate:Isobutanol:H_2O, 16:2:2, 3 = Methanol:H_2O 16:4 plus 3 drops of glacial acetic acid)

CHAPTER FIVE

5.0 DISCUSSION

Despite the activities being moderate (0.1<MIC< 0.625mg/ml) to weak (MIC>0.625mg/ml) (Kuete, 2010), this study revealed the unknown antibacterial potentials of the crude extracts of the selected plants. All plants exhibited antibacterial activities against most of the test bacteria including the selected resistant strains. *Sorindeia madagascariensis* leaf extract exhibited the best activity among all extracts, against all tested bacteria. Its highest activity was observed against; the standard *S. aureus* ATCC 25923, clinical isolate of *S.aureus* and MRSA at MIC of 0.193 ± 0.00mg/ml and its lowest activity was against the clinical isolate of *P. aeruginosa* at MIC of 1.16 ± 0.54 mg/ml. Roots of the same plant showed the second best activity against the; standard *S. aureus*, clinical isolate *S. aureus* and MRSA at MICs of 0.385 ± 0.00, 0.289 ± 0.14 and 0.385 ± 0.00 mg/ml respectively. Its lowest activity was against the clinical isolates of *K. pneumoniae*, and *S. typhi* at MIC of 1.54 ± 0.00 mg/ml. Published literature on the antibacterial activity of the genus *Sorindeia* and specifically *S. madagascariensis* is very limited. However, these findings parallel the reported antibacterial activities of very few species in this genus. Specifically, molecules isolated from the Cameroonian *Sorindeia juglandifolia* have been reported to possess strong antimycobacterial activity at MIC of 3.9 μg/ml (Donkeng Donfack *et al.*, 2014).

Mucuna stans (Fabaceae) leaves exhibited the third best activity displaying its highest activity against the standard isolates of *S. aureus* ATCC 25923, and *E. coli* ATCC 25922 as well as the clinical isolate of *S. aureus* at MIC of 0.77±0.00mg/ml. Its least activity was observed against the standard *P. aeruginosa* at MIC of 3.08 ± 0.00 mg/ml. These findings are at present not supported by published literature on the antibacterial activity of *Mucuna stans* although reports on antibacterial activity of the congener plant; *Mucuna pruriens* exist. The leaves (Salau and Odeleye, 2007; Mastan *et al.*, 2009) and seeds (Ifeanyi *et al.*, 2014) of *Mucuna pruriens* have been reported to exhibit antibacterial activities.

On the other side, *Albizia harveyi* (Fabaceae) leaves displayed the least antibacterial activity against several test bacteria, mostly at MIC of 1.54 ± 0.00mg/ml. However, it failed to inhibit two of the test bacteria namely; *Pseudomonas aeruginosa* ATCC 27853 and the clinical isolate of *E. coli*. Growth was observed up to the wells with the highest set concentration signifying the MICs for the two bacteria to be beyond the highest set test concentration (>3.08mg/ml). Published reports on the antibacterial effects of *Albizia harveyi* do not exist. However, the antibacterial activities of several other *Albizia* species have been reported with some species exhibiting significant to moderate (Kuete, 2010) antibacterial activities. For instance, *Albizia julibrissin* and *Albizia odoratissima* have been reported to exhibit antibacterial activities at MICs of 65µg/ml (Rajalakshmi and Senthil, 2014) and 136µg/ml (Banothu *et al.*, 2017) respectively. Stem bark of an Ethiopian *Albizia lebbeck* was found to possess a significant antibacterial activity with MIC of 10 µg/ml (Abriham and Paulos, 2016). The leaves of the same plant had previously been reported in Nigeria to have a weak antibacterial activity at MIC of 50mg/ml (Sheyin *et al.*, 2015). The leaves of both, *Albizia amara* (Shubha *et al.*, 2014) and *Albizia procera* have also been reported to possess antibacterial activities (Khatoon *et al.*, 2014). All these reports are in support of what this study revealed of *Albizia harveyi* since plants in the same genus are likely to possess similar or closely related biochemical properties.

Ciprofloxacin was used as the positive control based on its availability and its public significance as one of the most affordable antibacterials (WHO, 2018b). It inhibited all test bacteria except the MRSA which was resistant up to the highest set concentration of the drug (0.0515mg/ml). Its activity was generally higher than those of the of the plants, as it inhibited all susceptible bacteria at MICs between 15 to 6000-fold less than those observed for the crude extracts of the plants. Fractionation and isolation of active molecules from the crude extracts of the plants may further improve the MICs of the plants to parallel those of the positive control (Etame *et al.*, 2018).

The standard *S. aureus* ATCC 25923, the clinical isolate *S. aureus* and MRSA were the mostly inhibited bacteria by all plant extracts at MIC range of 0.193 ± 0.00 to 1.54 ± 0.00 mg/ml. This supports the application of these plants in treatment of infectious skin conditions like boils and carbuncles among the local communities in Southern highlands and coastal areas of Tanzania (unpublished information). Such skin conditions are mainly caused by gram positive bacteria especially *Staphylococcus aureus* (Carey, Schuster and McGowan, 2007). The most significant observation was the high susceptibility of the MRSA to the crude extracts, particularly the leaves of *S. madagascarensis* at 0.193 ± 0.00 mg/ml while completely resistant to the positive control drug, ciprofloxacin. This means bioactive molecules from *S. madagascarensis* may give leads against drug resistant bacteria particularly MRSA strains.

Gram negative bacteria were generally less susceptible to inhibition by the crude extracts with *Pseudomonas aeruginosa* ATCC 27853 and the clinical isolate *E. coli* displaying the least susceptibility. This can be explained by the generally known, inherent less susceptibility of gram negative bacteria to antibacterial molecules caused by their protective structural composition (Miller, 2016).

Tannins, flavonoids, saponins, phenolics, glycosides and terpenoids detected in most of the plant samples may be responsible for the observed antibacterial activities (Varma, 2016). *Sorindeia madagascariensis* leaves were observed to contain comparably high quantities of such phytochemicals based on colour intensity upon detection. Specifically, phenolics were among the largely detected phytochemical groups in the leaves of *S. madagascariensis,* and *s*imilar compounds, namely; 2,3,6-trihydroxy benzoic acid and 2,3,6-trihydroxy methyl benzoate have been found to be responsible for antimycobacterial activity of the fruits of *Sorindeia juglandifolia* (Donkeng Donfack *et al.*, 2014). With this observation, it is likely that similar compounds may be responsible for the antibacterial activity of the leaves of *S. madagascariensis.* The other phytochemicals of *S. madagascariensis* may also have added or sole effects on the antibacterial effects of the plant as most phytochemicals detected like; saponin, tannins and flavonoid from different plants have been reported to possess

antibacterial activities (Akiyama, 2001; Lamb and Cushnie, 2005; Akinyemi, Oluwa and Omomigbehin, 2006; Varma, 2016).

Compared to *S. madagascariensis* leaves, such phytochemical groups were fairly and variably detected in the other plants. Tannins and phenolics present in *Mucuna stans* leaves may be responsible for its observed activity as the later are highly linked to the antibacterial effects of other *Mucuna* species (Tiwari and Goyal, 2018).

Tannins, saponins and flavonoids detected in fair amounts in *Albizia harveyi* leaves may be associated with the observed activity of the plant. A flavonoidal fraction has been linked to the activity of *Albizia julibrissin* (Rajalakshmi and Senthil, 2014), which suggests the extension of such activity to other species of the genus since the antibacterial activities of flavonoids like kaempferol and several others are well known and extensively studied (Xie *et al.*, 2014).

Upon TLC profiling for the most active two plant samples namely *S. madagascariensis* leaves and roots, it was observed that, the components of the two samples were largely polar demanding solvent systems of moderate to high polarity for separation to occur. Furthermore, alkalization of the medium largely halted elution and separation, whereas acidification largely promoted elution of components. This implies the majority of phytochemicals in the plant extracts contain ionizable acidic functional groups. This correlated with intense detection of phenolics and tannins which are partially ionisable acidic compounds. Acidifying the medium makes most of such groups less ionized while alkalization causes ionization of such groups which in turn makes them more adherent on silica and difficult to elute (*Tailing in TLC*, no date).

Bioautography of the leaves and roots of *S. madagascariensis* showed the most polar molecules of the extracts to be responsible for the antibacterial activity displaying clear zones of inhibition around the spots eluted by more polar solvent systems but not around the spots eluted by the less polar solvent systems. However, the active components of extracts exhibited limited antibacterial activities with narrower zones of inhibition than those of the control drug. This can be an outcome of limited diffusion of the extract-molecules in Muller-Hinton agar compared to the molecules of the control drug (Dewanjee *et al.*, 2015).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The crude extracts of all selected plants exhibited antibacterial activities. *Sorindeia madagascariensis* leaves and roots displayed moderate activities, with their highest activities being at MICs of 0.193 ± 0.00 and 0.289 ± 0.14 mg/ml respectively. The leaves of both *Mucuna stans* and *Albizia harveyi* displayed weak antibacterial activities with their best activities at MICs of 0.77 ± 0.00 and 1.54 ± 0.00 mg/ml respectively. Specifically, the crude extract of *S. madagascariensis* leaves exhibited its highest activity (0.193 ± 0.00 mg/ml) against the; standard *S. aureus* ATCC 25923, clinical isolate of *S.aureus* and MRSA, although the later was completely resistant to the control drug, ciprofloxacin. The other plant extracts were all active against MRSA at MICs from 0.289 ± 0.14 mg/ml to 1.54 ± 0.00 mg/ml.

Phytochemical screening largely revealed tannins, phenolics, flavonoids, saponins and glycosides among the plants. The high intensity of colour upon detection of saponins, terpenoids, phenols and glycosides in the leaf extract of *S. madagascariensis*, signifies high contents of the respective phytoconstituents, and that may be linked to its observed superior antibacterial activity among the tested plants. The detected phytochemical groups were fairly and variably detected in the extracts of the rest of the plants.

Thin layer chromatography done for *S. madagascariensis* leaves and roots suggested high content of very polar components than non-polar components.

Bioautography suggested the antibacterial activity of *S. madagascariensis* to be contained in the most polar components.

Conclusively, all study objectives were met and the findings have partly, established the scientific basis for ethno-medicinal applications of the selected plants among the indigenous of the collection sites. More importantly, the findings give insight towards realization of further interventions like fractionation of extracts and isolation of molecules for antibacterial drugs discovery.

6.2 Recommendations

With the observed antibacterial activities of the crude extracts of the selected plants particularly the leaves and roots of *Soriendeia madagascariensis*, there should be further studies entailing fractionation of the crude extracts and isolation of active molecules, in order to deploy the antibacterial potentials of the plants in the course of discovery of new antibacterial drugs. The developed TLC profiles and bioautograms can be directly used or further improved to guide fractionation and isolation of active molecules from the plant.

For all plants, the parts that were not tested in this study, specifically, the fruits, flowers, stem- and root-barks should also be explored as they may have better antibacterial activities than what this study reports.

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