ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF PLANT EXTRACTS FROM *STERCULIA AFRICANA, ACACIA SIEBERIANA* AND *CASSIA ABBREVIATA ssp. ABBREVIATA*

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN NATURAL PRODUCTS TECHNOLOGY AND VALUE ADDITION OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

2017

EXTENDED ABSTRACT

The study of self-medication in non-human primates sheds new light on the complex interaction of animal, plant and parasite. The main objective of this study was to evaluate the antimicrobial properties and phytochemical profile of crude extracts from *Sterculia Africana, Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata*, plants present in the yellow baboon diet in Mikumi National Park, Tanzania. Specifically aimed at assessing antibacterial activity of the crude extracts through *in vitro* studies using standard strains and to establish the phytochemical profile of the crude extracts using chromatography methods. Minimum Inhibitory Concentration (MIC) technique was employed to assess antibacterial activity whereas Thin layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) techniques were used to assess the plants' chemical profile. *Acacia sieberiana and Cassia abbreviata ssp. abbreviata* showed the lowest MIC values of 0.31mg/ml against the Gram negative strains whereas 0.63 mg/ml was the lowest value against the Gram positive strains used in this study. Total extraction was done by maceration and the highest extraction yields of 9.66% and 6.22% were obtained from the root bark of *Cassia abbreviata ssp. abbreviata* and the leaves of *Acacia sieberiana* respectively. Findings from Thin Layer Chromatography (TLC) indicated presence of saturated and unsaturated compounds while colour reactions with Vanillin reagent inferred presence of triterpene group of compounds in the ethanolic crude plant extracts. The chemical profile obtained from the HPLC for the plant extracts was comparable to the one from the TLC profile. Antibacterial studies revealed presence of pharmacological activity in the crude plant extracts suggesting that non–human primates feed on the nutrient poor parts of these plants for self-medication. Chromatography analysis offers a starting point in isolation of pure compounds for the purpose of drug development since these plant extracts exhibit activity against bacteria of medical and veterinary importance.

DECLARATION

I, **Kirabo Irene**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted to any other institution.

_____ _____

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The above declaration is confirmed by;

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Prof Robinson. H. Mdegela Date

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ACKNOWLEDGEMENTS

I first of all thank God, The Almighty, through whom we live, move and have our being. I thank the INTRA-ACP mobility scholarship project for funding my studies with special thanks to Prof. Dominic Kambarage, the Ministry of Health, Uganda for granting me a study leave that has enabled me to pursue this degree programme. I am grateful to my supervisors, Dr. Faith Mabiki, you moved so much around to ensure that my research is a success, Prof. Robinson Mdegela and Mr. Obbo Christopher. The technical team at the Chemistry Laboratory, at Solomon Mahlangu campus in Mazimbu especially Mr. Mwesongo James, Madam Annet, Ms Anna, your help to me was invaluable.

I thank the INTRA- ACP family, a group of different people from different countries and walks of life, the Natural Products Technology and Value Addition class. It's been a real pleasure knowing and working with you. Finally, my mother, Ms. Catherine. Olivia Mufumba, my husband, Mr. Geofrey Mugabi for their sacrifice, encouragement, financial and moral support.

DEDICATION

To my mother, Ms. Catherine Olivia Mufumba, you worked so hard to build the foundation and pillars of my education. My husband and best friend, Mr. Geofrey Mugabi, you are a tower of strength, and to Gabriel Christopher, you are my heartbeat in this final year of Research, making me smile without reason.

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LIST OF ABBREVIATIONS

AIDS	Acquired Immuno Deficiency Syndrome
amu	atomic mass unit
AR	Antimicrobial resistance
ATCC	American Type Culture Collection
C	Carbon
DMSO	Dimethyl sulfoxide
FA	Fatty Acid
GC-MS	Gas chromatography- Mass Spectrophotometer
HIV	Human Immunodeficiency Virus
^{1}H- NMR	Proton Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
INT	IodoNitroTetrazolium
MIC	Minimum Inhibitory Concentration
NIAID	National Institute of Allergy and Infectious Diseases
Nm	Nano metres
TAG	Triacylglycerol
TLC	Thin Layer Chromatography
UV	Ultraviolet
WHO	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Ingestion of plant parts supposedly for their bioactive properties has been widely reported across the animal kingdom (Huffman, 1989, 1993, 1996, 2001). Zoopharmacognosy is a term that was devised to refer to self-medication in animals particularly in non-human primates. The study of self-medication in non-human primates sheds new light on the complex interactions of animal, plant and parasite (Huffman, 1996, 2001). A variety of non-nutritional plant secondary metabolites (small molecules of molecular weight < 1500 amu, produced by an organism, but not strictly necessary for the survival of the organism for example toxic materials providing defence against predators (Sarker and Nahar, 2007) and nutrient-poor bark is found in the primate diet yet little is known about the possible medicinal consequences of their ingestion.

Whether present in the human or non- human primate diet, plants as food standardised extracts or pure compounds provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. The numerous structurally diverse compounds found in them serve as a unique source for novel drugs and several of the well-known drugs have had their origin in nature (Tagboto and Townson, 2001). The present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants present in the non- human primate diet. Microbial resistance resulting from selective pressure of antimicrobial use, misuse of antimicrobial agents, societal and technologic changes that enhance the transmission of drug resistant organisms in humans and animals has grown into a great public health concern (Cos *et al.*, 2006; Olivier *et al.*,

2010; AR threats report, 2013). This phenomenon has resulted into severe reduction in efficacies of commonly used antibacterial, anti-parasitic, antiviral and antifungal drugs. The resistance mechanisms involved are complex and not clear. Of concern are the spectra of pathogenic bacteria that are continuously widening and so are the spectra of diseases they cause in humans and animals (AR threats report, 2013). Last resort drugs for severe bacterial infections, are neither affordable nor accessible to populations in resource-poor nations. In such settings, individual subjects infected with bacteria resistant to specific antibacterial drugs, have an increased risk of worse clinical outcomes and higher probability of death (Okeke *et al.,* 2005). To compound all that, a huge knowledge gap exists about the magnitude of the problem, a gross under reporting and no new therapeutic options on the horizon to replace those that become ineffective or to protect the efficacy of existing drugs (Gislene *et al.*, 2000).

In light of this, *Sterculia africana, Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata*, plants chosen solely on the basis of their consumption by non -human primates in Mikumi National Park, Tanzania were studied. Literature search revealed that *Sterculia africana* is one of the sources of non- traditional seed oils in Botswana (Mitei *et al.*, 2008) and is widely used in African Traditional Medicine (Mitei *et al.,* 2008, Mainen *et al*., 2007, PROTA4U, 2016). In Somalia a decoction of the crushed fresh roots of *Sterculia africana* is drunk as an antihelmintic where as a root decoction is taken against back pain, hernia and dizziness, a root infusion is drunk as an aphrodisiac, and leaf decoctions are drunk against fungal infections and convulsions (Mainen *et al.*, 2007). In some parts of East Africa, the roots, stem bark and leaves are boiled and the vapour inhaled for the treatment of influenza and fever (PROTA4U). Whereas a root bark decoction is drunk by women for the treatment of postnatal and stomach pains, a leaf infusion is drunk against cough and chest complaints.A fruit decoction is drunk to relieve

pain during pregnancy and after giving birth (PROTA4U). Additionally, in Malawi the irritant hairs along the splitting point of the fruits are burnt and the ash used as an ointment for the treatment of eye infections, especially in babies (PROTA4U, 2016).

Acacia sieberiana has been utilized in ethno-medicine for treatment of skin eruptions, rheumatic pains and in treatment of syphilis, gastritis, cough, fever, ringworm, leprosy, epilepsy, dysentery, mouth ulcers, as a vermicide and contraceptive (Obidah *et al.*, 2009). Different communities in Ethopia and South Africa also traditionally utilise *Acacia sieberiana* for the treatment of various ailments including inflammation, tiredness, joint pains, bilharzia, fever and enemas (Doka and Yagi, 2009, Elgorashi *et al*., 2003). The stem and root back extract both rich in tannins, are used in treating schistosomiasis, fever, stomach ache, jaundice, cough, sexual impotence, haemorrhoids, syphilis, uterine problems and to improve lactation after child birth (Christiana *et al.*, 2012). Studies done on *Acacia sieberiana* reveal that it is effective against *Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Mycobacterium avium.* Preliminary cold water extracts screened for phytochemicals showed presence of saponins, tannins, cardiac glycosides, flavonoids and anthraquinones (Burkill, 1995; Mahdi *et al.*, 2013). In Bukoba and Morogoro, Tanzania the root barks of *Cassia abbreviata* are used for treatment of oral and vaginal candidiasis particularly in HIV/AIDS patients (Hamza *et al,* 2006; Runyoro *et al.*, 2006).

1.2 Zoopharmacognosy

Mankind has for a long time developed drug therapies from observing animals utilise medicinal plants that were previously unknown or believed to be poisonous (Huffman, 2001). In Tanzania about a century ago a medicine man, Babu Kalunde, learnt an important treatment that saved the lives of many people in his village who were suffering

an epidemic of a dysentery-like illness. He discovered the potential medicinal value of a plant known to the WaTongwe (a Kitongwe speaking tribe from areas around Lake Tanganyika in Kigoma district) as *mulengelele* by observing a similarly sick young porcupine ingest the roots of the plant (Huffman, 2001). Preceding this observation the people of this village had avoided this plant because it was believed to be highly poisonous. He narrated his observations of the porcupine and ingested small doses of the plant and thereafter proceeded to persuade them to use the plant on the sick. To this day, the WaTongwe use the roots of *mulengelele* as medicine to treat gonorrhoea and syphilis (Huffman, 2001).

The term zoopharmacognosy was coined in the 1990s, to describe self-medication by animals in general and non- human primates in particular (Glander, 1994). Studies on the white and red Colobus monkeys, baboons, chimpanzees and lowland gorillas among others have provided evidence of self-medication by non-human primates (Huffman, 1997, 2001). New light has been shed on the rather complex interaction between plant, parasite and animals by studies on animal self-medication. Behavioural, ecological, and pharmacological studies have shown that the non-human primate diet contains a variety of nutrient poor plant parts which may be consumed because of their secondary compounds (Glander, 1994; Huffman, 1997). Chemical investigations of the medicinal hypothesis have found that the bitter pith of *Vernonia amygdalina* have chemical compounds that are apparently responsible for the control of nematode infections (Glander, 1994). *Diospyros abyssinica, Uvariopsis congensis, Albizia grandibracteata,* and *Trichilia rubescens* are only a few of the plants present in the non- human primate diet that have exhibited biological activity. However, there still exists a dearth of knowledge on the microbial activity of some of the plants present in their diet. Non-human primates are infected with the closest relatives of important human pathogens. It has been established that 27.5 % of

parasites found in wild primates, have also been reported in humans, given our genetic relatedness with non-human primates, we perhaps select the same plants when showing similar symptoms of illness (Wolfe *et al.*, 1998; Pedersen *et al.*, 2005; Su *et al.*, 2013). Scholars familiar with the use of plants as effective drugs by humans worldwide should not be amazed that non-human primates as well, exploit medicinal plants that are available to them from the natural pharmacopoeia found in their habitat (Glander, 1994).

1.3 Plants as Medicinal Sources

For many centuries, plants have been a valuable source of natural products in maintaining human health, especially in the last decade, when more intensive studies have been carried out on natural therapies (Gislene *et al.*, 2000). When compared to humans and other animals, plants lack a specific immune system and therefore depend on a cocktail of chemicals to protect them against parasites and predators (Hammerschmidt, 1999). The knowledge of this has enabled humans to harness plants to treat diseases and in the last century, pure compounds from plants have been isolated and developed into pharmaceutical drugs. As a matter of fact 25% of prescription drugs and 11% of drugs considered essential by WHO are derived from plants plus a large number of drugs are precursor compounds obtained from plants (Rates, 2001).

Plants synthesise over 100 000 small molecules, most of which have antimicrobial activity (Dixon, 2001). To avoid selective pressure and development of resistance, these antibiotic molecules are only produced when induced by pathogen attack (Hammerschmidt, 1999). In addition, the antibiotic molecules do not have specific cellular targets in the pathogens, are of low efficacy, act in synergy, target bacterial virulence rather than bacterial growth and work in concert with multi-drug-resistance pump inhibitors (Tegros, 2002; Gibbons, 2004; Hung, 2005; Lewis, 2006).

The diversity of medicinal plants that provided therapy from antiquity continues to be used traditionally against infections in all countries where pathogenic microbes are endemic (Fabricant and Farnsworth, 2001; Al-Musayeib, 2012). A considerable number of their extracts and isolated compounds have been shown to possess significant anti-parasitic activities (Kuete and Efferths, 2010). Therefore natural products are still a major potential source of innovative therapeutic agents for various conditions, including infectious diseases, as they represent an unmet source of chemical diversity (Clardy and Walsh, 2004).

1.4 Antibacterial Resistance

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Antibacterial resistance is a worldwide problem and one of the most serious health threats facing mankind in the 21st Century. Bacterial pathogens have become resistant to multiple types or classes of antibiotics and antibacterial resistant microbes can now cross international boundaries with ease (AR threats report, 2013). In most cases, antibiotic-resistant infections require prolonged and/or costlier treatments, extended hospital stays, necessitate additional doctor visits and healthcare use and result in greater disability and death compared to infections that are easily treatable with antibiotics. The WHO (2014) reported for the first time a commencement of the regional surveillance of antibacterial resistance and indicated the deteriorating efficacies in commonly used antibacterial drugs and the escalating resistance in the nine major pathogenic bacteria of international concern. All bacteria of concern show resistance to at least one common drug and in some, there is already resistance to carbapenems, the last resort treatment in severe infection (AR threats report, 2013). In its antibacterial resistance programme the National Institute of Allergy and Infectious Diseases (NIAIDs) also reported the escalating antibiotic resistance situation and the

urgent need for novel antibiotics NIAIDs (2014). Unfortunately, the available therapies for each of these diseases (caused by bacteria of medical importance) are meagre and have severely limited utility in disadvantaged settings due to high costs, low efficacy, high toxicity, poor compliance and the ever evolving resistance to mono-therapeutic drugs (Nwaka and Ridley, 2003). Worse still, some of these drugs are old, imposing the urgent need to search for new, safer, more effective and cheaper drug molecules and also for new leads with new mechanisms of action (Hoet *et al.*, 2003; Nwaka *et al.*, 2009; Brun *et al.*, 2010).

1.5 Problem Statement and Justification

Zoopharmacognosy as a source of information for alternative natural therapies has not been adequately exploited to search for safer and more efficacious drugs. A knowledge gap exists about the antimicrobial and phytochemical properties of most of the plants present in the non- human primate diet which consists of over 250 000 flowering plants. Studies on *Acacia sieberiana*, *Sterculia africana* and *Cassia abbreviata ssp. abbreviata* will; provide data on the spectra of microbes susceptible to plant extracts from the non-human primate diet. Findings will also contribute to the existing non –human primate foraging theory, cognition, conservation of these plants fed on while providing cognizance about the mechanisms of food selection as well as discovery of new therapeutic leads. Phytochemistry findings may also avail supplementary knowledge on the group of compounds found in these plants in order to provide a baseline for separation of pure compounds or formulation of standardised mixtures. These findings may also advance zoopharmacognosy as an alternative source of new therapeutic leads that are safe and effective to mitigate the microbial resistance phenomena.

Researchers knowledgeable about the use of plants as medicines by humans should not be surprised that non-human primates can also exploit plants for the same purpose. While it may not be possible to pharmacologically analyse or carry out phytochemical screening for each one, special attention should be accorded to plants fed on by non- human primates exhibiting potential illness symptoms as mentioned by Krief *et al.* (2006) such as but not limited to a lack of appetite, intestinal disorder and coughing. This research was undertaken to establish the phytochemical profile as well as study the antimicrobial properties of *Cassia abbreviata, Acacia sieberiana* and *Sterculia africana* against Gram positive and Gram negative bacteria of medical and veterinary concern using extracts from a single solvent.

1.6 Objectives of this Study

1.6.1 Overall objective

To evaluate the antimicrobial properties and phytochemical profile of crude extracts from *Sterculia Africana, Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata.*

1.6.2 Specific objectives

i. To assess the antibacterial activity of the crude extracts *in vitro* using standard strains.

ii. To establish the phytochemical profile of the crude extracts using chromatography methods.

1.7 Materials and Methods

1.7.1 Study design

This study was designed as an experimental study.

1.7.2 Sample collection

It is vital to consider climatic conditions due to the fact that they can interfere with the final biosynthesis particularly the final concentration of bioactive compounds (Mabiki *et al.*, 2013). With this in mind, all samples for both antimicrobial and phytochemical studies were obtained from the same location and at the same time. This was done in order to minimise possible errors caused by variations in time of collection as well as location. Samples were collected in the month of September during a dry spell when *Sterculia africana* had shed its leaves and so these were not studied.

The samples were collected and authenticated with the help a Botanist from the University of Dar es salaam and were assigned voucher specimen numbers FMM 3704, 3705 and 3706 for *Acacia sieberiana, Sterculia africana* and *Cassia abbreviata ssp. abbreviata* respectively.

1.7.3 Laboratory analysis

1.7.3.1 Determination of the phytochemical profile of the crude plant extracts

(a) Preparation of samples for analysis

Total extraction was done using maceration technique with ethanol as the menstruum. Samples were soaked in ethanol for 72 hrs with occasional shaking, the combination of ethanol and sample was strained and then filtered. The marc was re-soaked for another 72 hours in ethanol in order to optimize extraction. Extracting the plant material in 96% ethanol enabled us to obtain a wide range of compounds with different polarities as well as preserve it from microbiological decay.

(b) Thin layer chromatography (TLC)

The sample extracts were spotted on silica gel coated aluminium plates known as Thin Layer Chromatograph (TLC) paper and allowed to develop under different mobile phases

such as 80% Petroleum ether and 20% ethyl acetate so as to obtain maximum separation of compounds within the plant extracts to separate. When the TLC plate had fully developed, compounds were identified under natural light and unsaturated compounds identified under UV light at 254 nm and 365nm. Vanillin reagent was used for the colour reactions. (Refer to Paper II). Spray reactions with Vanillin reagent gave blue, red, purple, pink colours that denote presence of triterpenoids, saponins, alcohols and sterols (Wagner et al., 1984)

(c) High performance Liquid chromatography

This technique was employed to determine the nature of compounds in each plant extract in order to compare with the observed TLC chemical profile. The question to be answered by running the HPLC was whether an extract mainly consisted of polar or non-polar compounds observed by the pattern of eluted peaks. A low gradient system was employed with an acetonitrile/methanol mobile phase at 254nm using a reverse phase C-18 column for separation (Mabiki *et al.,* 2013).

1.7.3.2 Assessing the anti-bacterial activity of plant crude extracts

In evaluating the anti-bacterial activity of the crude plant extracts, both agar well diffusion method and Minimum Inhibitory Concentration (MIC) were used. However, Agar well diffusion method was only used preliminarily to determine whether the plant extracts had any biological activity. Agar well diffusion technique is limited by diffusivity of components and is better used for quantification purposes (Mabiki *et al.*, 2013). Consequently, MIC method was used due to its sensitivity to small concentrations of sample extracts in determining bactericidal effects.

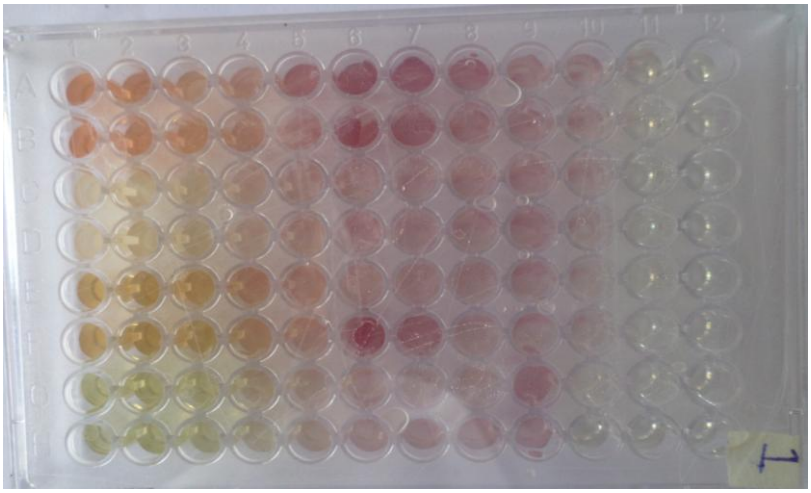

Figure 1.1: Picture of a microtitre plate used during the determination of minimum inhibitory concentration of *Staphylococcus aureus*. Picture of microtiter plate taken after 2 hours of incubation

1.8 Dissertation Organisation

This dissertation has been developed in 'publishable papers format' comprising of three chapters. The first chapter consists of the extended abstract, introduction as well as the overall subject studies presenting description of the commonality of the concepts across the different papers presented in this dissertation.

Chapter two and three have a paper each following the arrangement of the outlined specific objectives; '*In vitro* antibacterial activity of plant extracts of *Sterculia africana*, *Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata*, plants ingested by primates for self- medication in Mikumi National Park, Tanzania (Paper 1).' Chapter three has Paper 2 titled, 'Chemical profile of extracts from selected plants used for self-medication by non-human primates in Mikumi National Park, Tanzania.' Chapter four consists of general results and discussion, conclusion, recommendations and references.

REFERENCES

Ahmad, I. and Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *Journal of Ethnopharmacology* 74(2): 113–123.

Al-Musayeib, N. M., Mothana, R. A., Matheeussen, A., Cos, P. and Maes, L. (2012). *In vitro* antiplasmodial, antileishmanial and trypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsular region. *BMC Complementary and Alternative Medicine*. [https://www.ncbi.nlm.nih.gov /pmc/articles/PMC3493369/pdf/1472-6882-12-49.pdf.] site visited on 16/11/2017.

Antimicrobial Resistance threats report, (2013). [https://www.cdc.gov/drugresistance/ threat-report-2013/index.html] site visited on15/11/2017.

Benayache, S., Benayache, F. and Benyahia, S. (2001). Leaf Oils of some Eucalyptus Species Growing in Algeria. *Journal of Essential Oil Research* 13: 210-213.

Burkill, H. M. (1995). The useful plants of west tropical Africa. *Royal Botanic Garden* 3: 199 – 201.

Christiana, J. D., Ishaku, L. E., Nkechi, V. O., Jurbe, G. G., Olumola, O. O., Micah, S. M. and Sunday, M. (2012). Antidiarrheal evaluation of aqueous and ethanolic leaf extracts of acacia sieberiana DC. (Fabaceae) in Albino Rats. *Asian Journal of Experimental Biological Sciences* 3(4): 779 – 803.

Clardy, J. and Walsh, C. (2004). Lessons from natural molecules. *Nature* 432: 829–837.

Cohen, M. L. (1992). Epidemiology of drug resistance: *Implications for a Post Antimicrobial Era Science* 257: 1050 – 1055.

Corbett, Y., Herrera, L., Gonzalez, J., Cubilla, L., Capson, T. L., Coley, D. P., Kursar, A. T., Romero, I. L. and Ortega-Barria, E. (2004). A novel DNA-based microflourimetric method to evaluate antimalarial drug activity. *American Journal of Tropical Medicine Hygiene* 70(2): 119–124.

Cos, P., Vlictinck, A. J., VandenBerghe, D. and Macs, L. (2006). Anti-infective potential of natural products: How to develop a stronger *in vitro* proof of concept. *Journal of Ethnopharmacology* 16: 290 – 302.

Dixon, R. A. (2001) Natural products and disease resistance. *Nature* 411: 843–847.

Doka, I. G. and Yagi, S. M. (2009). Ethnobotanical survey of medicinal plants in West Kordofan (Western Sudan). *Ethnobotanical Leaflet* 13: 1409-1416.

Elgorashi, E. E., Taylor, J. L.S., Standen, A.J., Kimpe, N. D. and Verschaeve, L. (2003). Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicology Letters* 143(2003): 195-207.

Fabricant, D. S. and Farnsworth, N. R. (2001). The Value of Plants Used in Traditional Medicine for Drug Discovery. *Environmental Health Perspectives* 109: 1

Gibbons, S. (2004). Anti-staphylococcal plant natural products. *Natural Products Report* 21: 263–277.

Gislene, G. F. N., Juliana, L., Paulo, C. F. and Giuliana, L. S. (2000). Antibacterial Activity of Plant Extracts and Phytochemicals on Antibiotic Resistant Bacteria. *Brazilian Journal of Microbiology* 31: 247-256.

Glander, E. K. (1994). *Eating on the Wild Side.* The University of Arizona Press, London. 239pp.

Hammerschmidt, R. (1999). Induced disease resistance: how do induced plants stop pathogens? *Physiological and Molecular Plant Pathology 55*: 77–84.

Hamza, O. J. M., Van den Bout-van den Beukel, C. J. P., Matee, M. I. N., Moshi, M. J., Mikx, F. H. M., Semani, H. O., Mbwambo, Z. H. and Van der ven, A. J. (2006). Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infection. *Journal of Ethno Pharmacology* 108: 124 – 132.

Huffman, M. A. (1997). Current Evidence for Self-Medication in Primates: A multidisciplinary perspective. *Yearbook of Physical Anthropology* 40: 171–200.

Huffman, M. A. (2001). Self-medicative behaviour in the African great apes: An evolutionary perspective into the origins of human traditional medicine. *BioScience* 51(8): 651 – 661.

Hung, D. T., Shakhnovich, E. A., Pierson, E. and Mekalanos, J. J. (2005) Antimicrobial resistance in bacteria. *Science* 310: 670–674 (2005).

Kuete, V. and Efferth, T. (2010). Cameroonian medicinal plants: Pharmacology and derived natural products. *Frontiers in Pharmacology* 1(123): 1 – 19.

Lewis, K. and Ausubel, F. M. (2006). Prospects for plant-derived antibacterials. *Nature Biotechnology* 24: 1504 – 1507.

Mabiki, P. F., Magadula, J. J., Mdegela, H. R. and Mosha, D. R. (2013). Optimisation of extraction conditions and phytochemical screening of root extract of *Synadenium glaucescens* Pax. *International Journal of Chemistry* 5(4): 103 – 112.

Mabiki, P. F., Mdegela, H. R., Mosha, D. R. and Magadula. J. J. (2013). *In ovo* antiviral activity of *Synadenium glaucescens* (pax) crude extracts on Newcastle disease virus. *Journal of Medicinal Plants Research* 7(14): 863 – 870.

Mahdi, H., Palmina, K. and Tony, C. (2013). Analysis of commercial vegetable tannin materials and related polyphenols of selected acacia species. *Journal of Forest Products and Industry* 2(1): 21 – 28.

Mainen, J. M., Carolien, J. P., Vanden, B. and Omar, J. M. (2007). Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *African Journal of Traditional Complementary and Alternative Medicine* 2: 219 – 225.

Martins-Melo, F. R., Alencar, C. H., Ramos, A. N. Jr. and Heukelbach, J. (2012). Epidemiology of mortality related to Chagas' disease in Brazil, 1999–2007. *PLoS Neglected Tropical Diseases* 6(2): 1 – 8.

Mitei, Y. C., Ngila, J. C., Yeboah, S. O., Wessjohann, L. and Schmidt, J. (2008). NMR, GC–MS and ESI-FTICR-MS profiling of fatty acids and triacylglycerols in some Botswana seed oils. *Journal of American Oil Chemists' Society* 85: 1021–1032.

Mwila, M. and Shiv, P. (2015). Antimicrobial activity and potency of *cassia abbreviata oliv* stem bark extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(6): 426 – 428.

Obidah, W., Saad, U. A. and Wurocheke, A. U. (2009). Toxic effects of Aqueous Stem Bark of Cassia sieberiana on Some Biochemical Parameters in Rats. *African Journal of Biochemistry Research* 3(5): 229 – 231.

Okeke, I. N., Laxmaninarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., Pablos-Mendez, A. and Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part 1: Recent trends and current status. *Lancet Infectious Diseases* 5: 481 – 493.

Olivier, C., Williams-Jones, B., Doize, B. and Ozdemir, V. (2010). *Containing Global Antibiotic Resistance: Ethical Drug Promotion in the Developing World in Antimicrobial Resistance in Developing Countries.* Springer Science and Business Media, Canada. 524pp.

Papadopoulou, K., Melton, R. E., Leggerr, M., Daniels, M. J. and Osbourne, A. E. (1999). Compromised disease resistance in saponin-deficient plants. *Proceedings from the National Academy of Sciences* 96: 12923–12928.

Pedersen, A., Poss, M., Nunn, C. L., Cunningham, A. and Altizer, S. (2005). Patterns of host specificity and transmission among parasites of free-living primates. *International Journal for Parasitology* 35: 647 – 657.

PROTA4U (2016). Plant resources of tropical Africa. [https://www.prota4u.org] site visited on 20/11/2016.

Rates, S. M. K. (2001). Plants as a source of drugs. *Toxicon* 39: 603 – 613.

Reddy, J. and Jose, B. (2010). Evaluation of antibacterial activity of the leaf essential oil of *Costus Pictus D.* Don. from South India. *International Journal of Current Pharmaceutical Research* 2(3): 68 – 70.

Runyoro, D. K. B., Ngassapa, O. D., Matee, M. I. N., Joseph, C. C. and Moshi, M. J. (2006). Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. *Journal of Ethnopharmacology* 106: 158 – 165.

Santiago-Alarcon, D., Diana C., Ricklefs, R. E., Patricia G. and Parker, P. G. (2010). Phylogenetic relationships of haemosporidian parasites in New World Columbiformes, with emphasis on the endemic Galapagos dove. *International Journal for Parasitology* 40: 463–470.

Satyajit, D. S. and Lutfun, N. (2007). *Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry* John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England. 283pp.

Su, H., Su, Y. and Huffman, M. A. (2013). Leaf swallowing and parasitic infection of the Chinese lesser civet Viverriculaindica in north-eastern Taiwan. *Zoological Studies* 52(22): 1 – 8.

Tagboto, S. and Townson, S. (2001). Antiparasitic properties of medicinal plants and other naturally occurring products. *Advances in Parasitology* 50: 199-295.

WHO (2014). Fact sheet N°259. Trypanosomiasis, (human African sleeping sickness), Fact sheet N°259. [www.who.int/mediacentre/factsheets/fs259/en/] site visited on 10/09/2011.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. and Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerging Infectious Diseases* 4(2): 149–158.

CHAPTER TWO

PAPER 1

In vitro antibacterial activity of plant extracts of *Sterculia Africana, Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata* used by non- human primates for self-medication in Mikumi National Park, Tanzania

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Target Journal: *International journal of Public Health and epidemiology*

Abstract

That animals in general and non- human primates in particular self-medicate has been widely reported, however little is still known about the pharmacological activity of the plants present in their diet. This study was undertaken in order to evaluate the *In vitro* antibacterial activity of the stem and root bark as well as leaf extracts of *Sterculia Africana, Acacia sieberiana* and *Cassia abbreviata* ssp. *abbreviata*. These plants were observed to be fed on by yellow baboons (*Papio cynocephalus*) in Mikumi National Park, Tanzania. Plant extracts were tested against both Gram positive and Gram negative bacteria of medical and veterinary importance employing a modified agar well diffusion method and Minimum Inhibitory Concentration (MIC) technique. *Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata* showed the lowest MIC values of 0.31mg/ml for both *Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumoniae* (ATCC 13883) strains. The lowest MIC value for Gram positive strains was 0.63mg/ml demonstrated by *Cassia abbreviata ssp. abbreviata* against *Staphylococcus aureus* (ATCC 25923). The highest susceptibility to the ethanol plant extracts was exhibited by *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* some of which are examples of microbes that affect both humans and non- human primates. These findings demonstrate that the plant extracts from *Sterculia Africana, Acacia sieberiana* and *Cassia abbreviata* ssp. *abbreviata* have antibacterial activity. Interesting to note is that the lowest crude plant extract MIC of 0.31mg/ml was only 31-fold weaker than that Gentamicin, a standard drug.

Key words: *Papio cynocephalus,* zoopharmacognosy, *sterculiaceae*, *Caesalpiniaceae*, *mimosaceae*

2.0 Introduction

There is a growing body of evidence to support the theory that animals self-medicate by ingesting plants of both nutritional and medicinal value (Huffman, 1997, 2001; Wolfe *et al.*, 1998; Carrai *et al*., 2003; Su *et al.*, 2013). A Tanzanian medicine man, Babu Kalunde centuries ago was able to treat a dysentery-like illness by observing a porcupine with similar symptoms ingest the roots of *mulengelele*, a plant previously believed to be toxic (Huffman, 2001). Some of the evidence for self-medication by African great apes includes the infrequent intake of plant species which are not a regular part of the diet, restriction of plant use to seasons or other periods associated with high risk of parasitic infection (Huffman *et al*., 1990, 1997; Wrangham, 1995), illness or parasite infection of the individual at the time of ingestion of a putative medicinal plant (Huffman and Seifu, 1989; Huffman *et al*., 1997; Wrangham, 1995), and a subsequent positive change in this condition following ingestion (Huffman *et al.*, 1993, 1996b). Furthermore, it has been suggested that compounds found in the ordinary diet of animals may have important positive effects on health and may prevent risks of infection and illness (Huffman, 1997; Huffman *et al.,* 1998). Chemical investigations of the self-medication hypothesis have found that the bitter pith of *Vernonia amygdalina* has chemical compounds that apparently are responsible for the control of nematode infections (Glander, 1994). *Diospyros abyssinica, Uvariopsis congensis, Albizia grandibracteata, and Trichilia rubescens* are only a few of the plants present in the non-primate diet that have been tested for biological activity (Cousins and Huffman., 2002; Rodrigues *et al*., 2016).

Among the plants observed to be fed on by yellow baboons (*Papio cynocephalus*) are; *Acacia sieberiana* (*Mimosaceae* family), *Sterculia Africana* (*Sterculiaceae* family) and *Cassia abbreviate ssp. abbreviata* (*Caesalpiniaceae* family) which are the focus of this study. A number of studies have been done on *Cassia abbreviata* oliv, a close relative of

C*assia abbreviata ssp. abbreviata* however it is important to note that *Cassia abbreviata ssp abbreviata* is endemic to Morogoro region from where this sample was taken (Maroyi, 2011; Mwila and Shiv, 2015). In Bukoba and Morogoro, Tanzania the root bark of *Cassia abbreviata ssp. abbreviata* are used in the treatment of oral and vaginal candidiasis particularly in HIV/AIDS patients (Hamza *et al.,* 2006; Runyoro *et al.,* 2006).

Sterculia africana is valued in the study area for traditional worship and is one of the plants associated with ancestral sacrifices in Tanzania (Luoga *et al.*, 2000). *Sterculia africana* root is used to treat Asthma in communities around Lake Victoria region in Tanzania (Otieno *et al.,* 2011) and also possesses strong antifungal activity (Hamza *et al*, 2006). The Maale and Ari communities in Ethopia utilize *Sterculia africana* to treat vomiting and food poisoning as well as to treat fever in the Blue Nile state, Sudan (Gibreel *et al.*, 2013; Kidane *et al.,* 2014). Literature search revealed that *Sterculia africana* is one of the sources of non- traditional seed oils in Botswana (Mitei *et al.,* 2008) and is widely used in African traditional medicine. In Somalia a decoction of the crushed fresh roots is drunk as an anthelmintic. In Tanzania a root decoction is taken against back pain, hernia and dizziness, a root infusion is drunk as an aphrodisiac, and leaf decoctions are drunk against fungal infections and convulsions (Mainen *et al.,* 2007). In parts East Africa the roots, bark and leaves are boiled and the vapour inhaled for the treatment of influenza and fever. In Namibia a root or bark decoction is drunk by women for the treatment of postnatal and stomach pains, a leaf infusion is drunk against cough and chest complaints, and a fruit decoction is drunk to relieve pain during pregnancy and after giving birth. In Malawi the irritant hairs along the splitting point of the fruits are recorded to be burnt and the ash used as an ointment for the treatment of eye infections, especially in babies (PROTA4U, 2016).

On the other hand, *Acacia sieberiana* has been utilized traditionally for treatment of skin eruptions, rheumatic pains, and in treatment of syphilis, gastritis, cough, fever, ringworm, leprosy, epilepsy, dysentery, mouth ulcers, as vermicide and contraceptive (Obidah *et al*., 2009). Communities in South Africa and Ethiopia traditionally utilise *Acacia sieberiana* for the treatment of various ailment including inflammation, tiredness, joint pains, bilharzia, fever, enemas (Doka and Yagi, 2009; Elgorashi *et al*., 2003). The stem and root back extract both rich in tannins are used in treating schistosomiasis, fever, stomach ache, jaundice, cough, sexual impotence, erectile dysfunction, haemorrhoids, syphilis, uterine problems and to improve lactation after child birth (Christiana *et al.*, 2012). Studies done on *Acacia sieberiana* reveal that it is effective against *Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Mycobacterium avium.* Preliminary phytochemical screening showed presence of saponins, tannins, cardiac glycosides, flavonoids and anthraquinones (Burkill, 1995; Mahdi *et al.,* 2013) the crushed pods are used in the treatment of hypertension in the Blue Nile state in Sudan (Gibreel *et al.*, 2013).

This study was undertaken to establish the phytochemical profile as well as study the antibacterial activity of *Cassia abbreviata ssp. abbreviata, Acacia sieberiana, Sterculia africana* against Gram positive and Gram negative bacteria of medical and veterinary concern.

2.1 Materials and Methods

Plant collection and processing

Sterculia africana, *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* were chosen solely on the basis that they were observed to be eaten by yellow baboons in Mikumi National Park, Tanzania shown in Figure 2.1 below. On three separate occasions while

observing yellow baboons, only one or two as opposed to the whole group, would feed on the leaves or stem bark of these plants then join up with the rest. A video of this phenomenon was recorded on one of these visits. In this study, the roots of the mentioned plants were tested as well even though they were not observed to be eaten along with the leaves of *Cassia abbreviata ssp. abbreviata*. These samples were collected in the month of September during a dry spell when *Sterculia africana* had shed its leaves and so these were not studied.

Plant materials were collected from areas around Sokoine University of Agriculture main campus and areas around Mikumi National Park in Morogoro region, Tanzania.

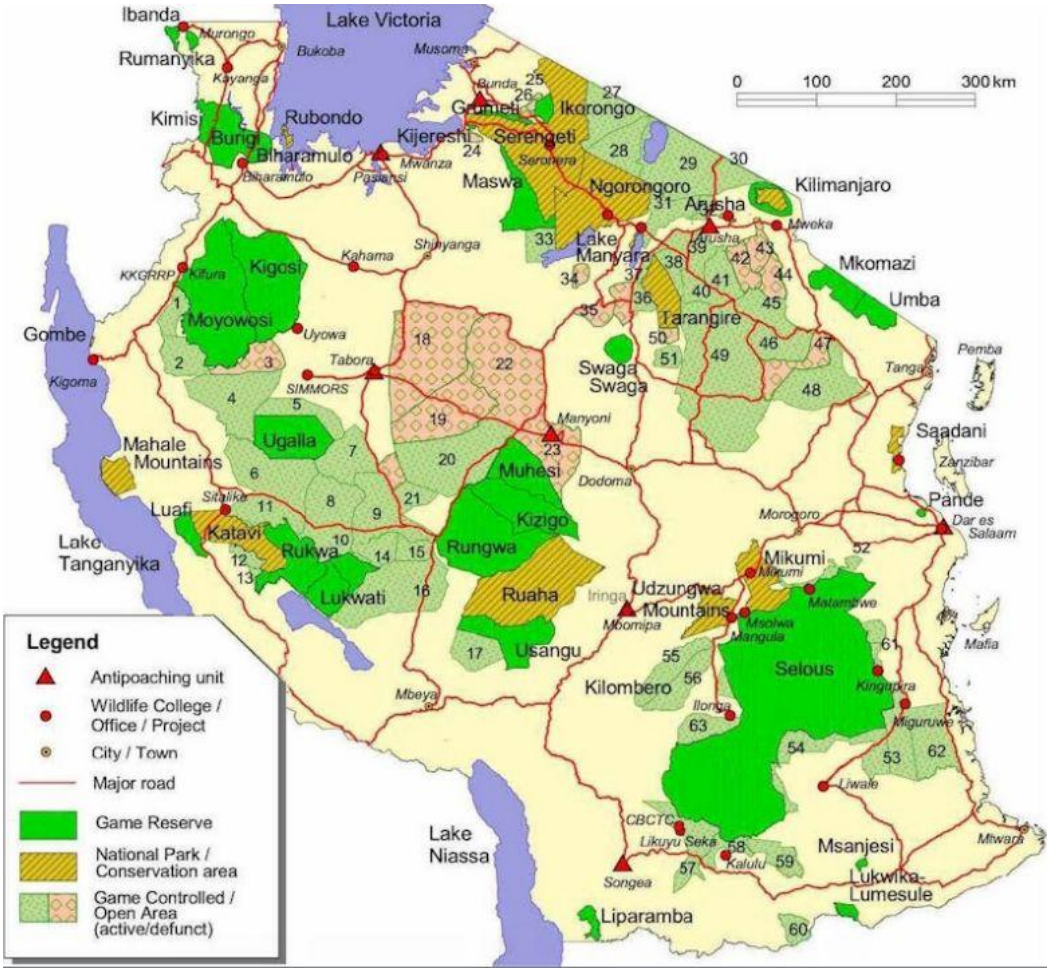

Figure 2.1: A map of Tanzania showing the location of Mikumi National Park and Morogoro region

The samples were collected during morning hours and authenticated with the help of a Botanist from the University of Dar es Salaam and were assigned voucher specimen numbers FMM 3704, 3705 and 3706 for *Acacia sieberiana*, *Sterculia africana* and *Cassia abbreviata ssp. abbreviata* respectively. From *Sterculia Africana,* the root bark and stem bark were collected while *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* samples constituted of the stem bark, root bark and leaves. The 8 samples were cleaned, the stem bark and root bark were cut into smaller pieces to allow for better drying in air. The samples were then pulverised to a particle size of 1mm.

2.2 Extraction of Samples

In the Chemistry laboratory at Mazimbu Campus, one sample was handled at a time in order to avoid cross contamination. Using a Yanhe Analytical Electronic Balance, the dry samples were each weighed into a separate clean, marked plastic containers of known weight and the sample weight recorded. The menstruum used was ethanol (Sigma-Aldrich) Analytical grade, which was added till all the sample was fully soaked and macerated for 3 days with constant shaking. The samples were then strained and filtered using 110mm whatman filter paper and excess menstruum evaporated using a rotary evaporator then finally transferred to dry in an oven (Shel Lab) till constant weight. The marc was re-soaked in fresh ethanol for 3 days and treated as the previous samples then dried before being added to their corresponding samples. The crude plant extracts were then coded as shown in Table 2.1 and stored at room temperature before use for subsequent analysis.

Table 2.1: Codes of the extracts used during this study

Name of plant	Code of plant	Plant part (p p)	Code of pp
Acacia sieberiana	A	Leaves	A.L
		Stem bark	A.S
		Root bark	A.R
Cassia abbreviata ssp. abbreviata	B	Leaves	B.L
		Stem bark	B.S
		Root bark	B.R
Sterculia africana	C	Stem bark	C.S
		Root bark	C. R

2.3 Antibacterial Screening

Test microorganisms

Agar well diffusion method was used for preliminary analysis to screen for samples with antibacterial activity and thereafter MIC was used for analysis.

Bacterial strains used in this study are standardized by the American Type Cell Collection (ATCC/Manassas, VA/USA) and constituted of Gram negative and Gram positive bacteria. The Gram negative bacteria tested were; *Salmonella paratyphi* (ATCC 9150), *Klebsiella pneumoniae* (ATCC 13883), *Shigella sonnei* (ATCC 25931), *Enterobacter cloacae* (ATCC 23355), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922). Gram positive bacteria tested were; *Enterococcus feacalis* (ATCC 51299) and *Staphylococcus aureus* (ATCC 25923). Bacterial culture cells were maintained at 37°C on Muller-Hinton (MH) agar on slants until required. All microbial species used were supplied by the Department of Microbiology, Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania.

2.4 Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of extract that inhibits growth of test microorganisms. A modified method by Mabiki *et al*. (2013) was employed in determining the MIC of the different sample extracts. Sterile 100 µl of Muller Hinton broth was measured into each well of a 96- well microtiter plate (Mabiki *et al*., 2013). 100 µl of 10mg/ml of extract prepared in Dimethyl sulfoxide (DMSO) was added to row 1 and mixed using a micropipette. This was followed by serial dilution to row 8 with the additional 100 µl there in discarded. One column was used for sterility control (no culture was added), another column was used as a negative control (100 µl of DMSO without extract) and another column used as the positive control with Gentamicin (0.01mg/ml) making a total of 12 rows. A 0.5 McFarland standard suspension of test bacteria was made in nutrient broth from which 100 µl of the final inoculums containing approximately 1×10^{8} cfu/ml was added to each well except the sterility control to make a final volume of 200 µl. The experiments were performed in duplicate. The microtiter plates were sealed in a plastic film and then incubated at 37°C in a humidified incubator

for 18 hrs. After incubation, 40 µl of 0.2 mg/ml Iodonitrotetrazolium (INT) was added to each well and the microtiter plates incubated for another 2 hours before removal for observation. The development of a purple colour resulting from the formation of the red/purple formazan was an indication of growth (positive indicator of cell viability). Decrease in the intensity of the red/purple formazan colour was indicative of inhibition of growth of test microorganisms.

2.5 Results

The MIC results tabulated in Table 2.2 below showed that the crude plant extracts were active against all the test microorganisms. The lowest MIC value was 5mg/ml as compared against 0.01mg/ml the standard drug, Gentamicin. The negative control showed development of a purple colour resulting from formation of the purple formazan which is a positive indicator of cell viability as shown in Figure 1.1, row 9 whereas the sterility control showed no colour change as shown in Figure 1.1, row10, an indication of absence of test microorganisms. Minimum Inhibitory Concentration ranged from 0.31mg/ml to 5mg/ml with 0.31mg/ml, the lowest value denoting the greatest antibacterial efficacy from A.R, A.S, B.R and B.L plant parts. Gram-negative bacteria in the ethanol extracts had higher MIC values as opposed to the Gram positive bacteria. A.R, A.S and B.L demonstrated the lowest MIC values of 0.31mg/ml for both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains. The lowest MIC value for Gram positive strains was 0.63mg/ ml demonstrated by B.R and B.L against *Staphylococcus aureus.* Overall, plants A and B showed lower MIC values as opposed to plant C. The experiments were performed in duplicate and the average value tabulated in Table 2.2 below.

The duplicate results performed had no significant difference making statistical data analysis inapplicable.

Table 2.2: Minimum Inhibitory concentration (mg/ml) of plant extracts.

Test organism	Code of plant part							
	C.R	**C.S**	**A.R**	**A.S**	**A.L**	**B.R**	**B.S**	**B.L**
E.coli (ATCC 25922)	2.50	2.50	2.50	1.25	0.63	1.25	1.25	0.63
S. paratyphi (ATCC 9150)	1.25	1.25	0.31	0.31	0.63	0.63	1.25	0.63
K. pneumoniae (ATCC 13883)	1.25	2.50	5.00	1.25	5.00	2.50	2.50	5.00
S. sonnei (ATCC 25931)	5.00	5.00	5.00	5.00	0.63	2.50	2.50	1.25
E. cloacae (ATCC 23355)	5.00	1.25	2.50	2.50	2.50	2.50	2.50	1.25
P. aeruginosa (ATCC 27853)	1.25	1.25	0.31	0.31	0.63	0.63	1.25	0.63
S. aureus (ATCC 25923)	1.25	1.25	1.25	1.25	1.25	0.63	1.25	0.63
E. faecalis (ATCC 51299)	1.25	2.50	5.00	1.25	5.00	2.50	2.50	5.00

2.6 Discussion

Cassia abbreviata ssp. abbreviata, *Acacia sieberiana* and *Sterculia africana* extracts possess antibacterial activity against the test strains as shown from the results of the *in vitro* experiment in Table 2.2. *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* exhibited better activity against the test microorganisms as compared to *Sterculia africana* with *Cassia abbreviata ssp. abbreviata* showing the lowest MIC value (0.31mg/ml) of the three plants. The positive control was a standard drug, Gentamicin (0.01mg/ ml), an aminoglycoside targeting the bacterial ribosome. Interestingly, the lowest crude plant extract MIC of 0.31mg/ml was only 31-fold weaker than Gentamicin. The crude plant extracts performed better than the negative control to a concentration of 0.313mg/ml of extract. Other studies reported on *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* concur with these findings (Christiana *et al.,* 2012; Maroyi, 2011; Mwila and Shiv, 2015). A viable challenge in interpreting self-medication is differentiating between

plants ingested for their nutritional value but are laden with medicinal benefits and plants ingested solely for their medicinal benefits. This challenge exists in traditional human societies where medicine and food are of the same origin. For instance traditional spices and condiments of daily Asian cuisine, such as marine algae, ginger root, turmeric and herbs also play an important role in suppressing viral and parasite infections (Ramesh *et al*., 2013, Huffman, 1997). Results show that different concentrations of the plant extracts were required to inhibit the growth of different microbes due to the difference in potency of the plant extracts attributed to phytochemicals present, environment of growth or extraction method used. The variation in the susceptibility of microorganisms could also be attributed to their intrinsic properties that are related to the permeability of their cell surface to the plants extracts. Their pharmacological effect could therefore be experienced with repeated ingestion or work in synergy.

This study provides evidence that the yellow baboon forage on similar plants also used in ethno medicine as seen from previous literature reviewed (Maroyi, 2011; Gibreel *et al.*, 2013; Kidane *et al.*, 2014; Mwila and Shiv, 2015). 27.5% of microbes that affect non-human primates affect humans as well, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* being only a few examples of such microbes, a factor attributed to our phylogenetic closeness (Glander, 1994; Wolfe *et al*., 1998; Pedersen *et al.*, 2005; Su *et al*., 2013;). It should therefore not come as a surprise that human and non-human primates perhaps select similar plants when challenged with similar illnesses. It is known that non-human primates feed on a great variety of plant species, however it is prudent to take note of the ones that are fed on infrequently and or by isolated cases in an uncommon manner as was the case with the yellow baboons in Mikumi National Park. It may not be possible to pharmacologically analyse or carry out phytochemical screening for each one, however, special attention should be accorded to plants fed on by non- human primates exhibiting

potential illness symptoms or in uncommon feeding behaviour as mentioned by Krief *et al*. (2006) such as but not limited to; a lack of appetite, intestinal disorder and coughing.

2.7 Conclusion

The results of the *in vitro* experiment in Table 2.2 demonstrate that ethanol extracts from the different plant parts of *Cassia abbreviata ssp. abbreviata*, *Acacia sieberiana* and *Sterculia africana* exhibit antibacterial activity against the test microbes. Some of these microorganisms are of medical and veterinary importance because they affect both humans and non-human primates. Additionally, these findings contribute to the foraging theory that suggests that the diet of non- human primates contains plants that are fed on for self-medication. Their diet could therefore act as a sieve through which plants fed on in uncommon manner by the non- human primates are tested. A combination of long term dietary data, pharmacological studies and analysis of plant chemistry may lead to discovery of new therapeutic leads. In addition to that, conservation of the plants fed on is vital for primate conservation because of the pharmacological benefits obtained from these medicinal trees. These findings therefore propose zoopharmacognosy as an alternative field in search for new therapeutic leads and drug discovery and further suggest that non-human primates feed on the non-nutritive parts of these plants for their pharmacological benefits.

2.8 Acknowledgement

I wish to genuinely thank INTRA-ACP mobility scholarship and RISE- AFNNET for partially funding this research, the Faculty of Veterinary Medicine, Sokoine University of Agriculture for facilitating this study. I wish to thank in a special way Mr. Mwesongo James, Mr. Frank Mbago and Ms Anna Mpanyakavili, your help was invaluable.

2.9 References

Burkill, H. M. (1995). The useful plants of west tropical Africa. *Royal Botanic Garden* 3: 199 – 201.

carrai, V., Borgognini-Tarli, S. M., Huffman, M. A. and, Bardi, M. (2003). Increase in tannin consumption by sifaka (Propithecus verreauxi verreauxi) females during the birth season: a case for self-medication in prosimians? *Primates* 44: 61–66.

Christiana, J. D., Ishaku, L. E., Nkechi, V. O., Jurbe, G. G., Olumola, O. O., Micah, S. M. and Sunday, M. (2012). Antidiarrheal evaluation of aqueous and ethanolic leaf extracts of acacia sieberiana DC. (Fabaceae) in Albino Rats. *Asian Journal of Experimental Biological Sciences* 3(4): 779 – 803.

Doka, I. G. and Yagi, S. M. (2009). Ethnobotanical survey of medicinal plants in West Kordofan (Western Sudan). *Ethnobotanical Leaflet* 13: 1409-1416.

Don, C. and Huffman, M. A. (2002). Medicinal properties in the diet of gorillas: an Ethno-pharmacological evaluation. *African Study Monographs* 23(2): 65-89

Elgorashi, E. E., Taylor, J. L.S., Standen, A.J., Kimpe, N. D. and Verschaeve, L. (2003). Screening of medicinal plants used in South African traditional medicine for genotoxic effects. *Toxicology Letters* 143(2003): 195-207.

Gibreel, H. H., Maha A. Y., Kordofani, E., Warrag, I. and Hoyam, O. A. (2013). Medicinal value and ecotaxonomy of the flora of Blue Nile State-Sudan. *Journal of Chemical and Pharmaceutical Research* 5(2): 36 – 43.

Glander, E. K. (1994). *Eating on the Wild Side.* The University of Arizona Press, London. 235pp.

Hamza, O. J. M., Van den Bout-van den Beukel, C. J. P., Matee, M. I. N., Moshi, M. J., Mikx, F. H. M., Semani, H. O., Mbwambo, Z. H. and Van der ven, A. J. (2006). Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infection. *Journal of Ethno Pharmacology* 108: 124 – 132.

Huffman, M. A. (1997). Current evidence for self-medication in primates: A multidisciplinary perspective. *Yearbook of Physical Anthropology* 40: 171–200.

Huffman, M. A. (1998). Control of nematode infections by African great apes: a new paradigm for treating parasite infection with natural medicines? *American Association of Veterinary Parasitologists* 20(2): 3-7.

Huffman, M. A. (2001). Self-medicative behaviour in the African great apes: An evolutionary perspective into the origins of human traditional medicine. *BioScience* 51(8): 651 – 661.

Huffman, M. A. and Seifu, M. (1989). Observations on the illness and consumption of a possibly medicinal plant Vernonia amygdalina by a wild chimpanzee in the Mahale Mountains, Tanzania. Primates 30(1): 51-63.

Huffman, M. A., Gotoh, S., Izutsu, D., Koshimizu, K. and Kalunde, M. S. (1993). Further observations on the use of the medicinal plant, Vernonia amygdalina (Del) by a wild chimpanzee, its possible effect on parasite load, and its phytochemistry. *African Study Monographs* 14(4): 227-240.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M. and Yoshida, K. (1997). Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains National Park, Tanzania. *Primates* 38: 111-125.

Huffman, M. A., Page, J. E., Sukhdeo, M. V. K., Gotoh, S., Kalunde, M. S., Chandrasiri, T. and Towers, G. H. N. (1996). Leaf-swallowing by chimpanzees, a behavioral adaptation for the control of strongyle nematode infections. *International Journal of Primatolology* 17(4): 475-503.

Kidane, B., Tinde van, A., Laurentius, J. G., van der, M. and Zemede, A. (2014). Use and management of traditional medicinal plants by Maale and Ari ethnic communities in southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine* 10: 46 – 61.

Krief, S., Huffman, M. A., Sevenet, T., Hladik, C., Grellier, P., Loiseau, P. M. and Wrangham, R.W. (2006). Bioactive Properties of Plant Species Ingested by Chimpanzees (Pan troglodytesschweinfurthii) in the Kibale National Park, Uganda. *American Journal of Primatology* 68: 51–71.

Luoga, E. J., Witkowski, E. T. F. and Balkwill, K. (2000). Differential utilization and ethnobotany of trees in Kitulanghalo forest reserve and surrounding communal lands, Eastern Tanzania. *Economic Botany* 54(3): 328 – 343.

Mabiki, P. F., Mdegela, H. R., Mosha, D. R. and Magadula. J. J. (2013). *Inovo* antiviral activity of *Synadenium glaucescens* (pax) crude extracts on Newcastle disease virus. *Journal of Medicinal Plants Research* 7(14): 863 – 870.

Mahdi, H., Palmina, K. and Tony, C. (2013). Analysis of commercial vegetable tannin materials and related polyphenols of selected acacia species. *Journal of Forest Products and Industry* 2(1): 21 – 28.

Mainen, J. M., Carolien, J. P., Vanden, B. and Omar, J. M. (2007). Brine shrimp toxicity evaluation of some Tanzanian plants used traditionally for the treatment of fungal infections. *African Journal of Traditional Complementary and Alternative Medicine* 2: 219 – 225.

Maroyi, A. (2011). An ethnobotanical survey of medicinal plants used by the people in Nhema communal area, Zimbabwe. *Journal of Ethnopharmacology* 136: 347 – 354.

Mitei, Y. C., Ngila, J. C., Yeboah, S. O., Wessjohann, L. and Schmidt, J. (2008). NMR, GC–MS and ESI-FTICR-MS profiling of fatty acids and triacylglycerols in some Botswana Seed Oils. *Journal of American Oil Chemists' Society* 85:1021–1032.

Mongalo, N. I. and Mafoko, B. J. (2013). Cassia abbreviata Oliv. A review of its ethnomedicinal uses, toxicology, phytochemistry, possible propagation techniques and Pharmacology. *African Journal of Pharmacy and Pharmacology 7*(45): 2901 – 2906.

Mwila, M. and Shiv, P. (2015). Antimicrobial activity and potency of *cassia abbreviataoliv* stem bark extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 7(6): 426 – 428.

Obidah, W., Saad, U. A. and Wurocheke, A. U. (2009). Toxic effects of Aqueous Stem Bark of Cassia sieberiana on Some Biochemical Parameters in Rats. *African Journal of Biochemistry Research* 3(5): 229 – 231.

Otieno, J. N., Magadula, J. J., Kakudidi, E., Kirimhuzya, C., Orodho, J. and Okemo, P. (2011). Use of ethnobotanical criteria for conservation assessment of plants used for respiratory diseases in Lake Victoria region, Tanzania. *International Journal of Biodiversity and Conservation* 3(11): 610 – 617.

Papadopoulou, K., Melton, R. E., Leggerr, M., Daniels, M. J. and Osbourne, A. E. (1999). Compromised disease resistance in saponin-deficient plants. *Proceedings from the National Academy of Sciences* 96: 12923–12928.

Pedersen, A., Poss, M., Nunn, C. L., Cunningham, A. and Altizer, S. (2005). Patterns of host specificity and transmission among parasites of free-living primates. *International Journal for Parasitology* 35: 647 – 657.

Runyoro, D. K. B., Ngassapa, O. D., Matee, M. I. N., Joseph, C. C. and Moshi, M. J. (2006). Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. *Journal of Ethnopharmacology* 106: 158 – 165.

Su, H., Su, Y. and Huffman, M. A. (2013). Leaf swallowing and parasitic infection of the Chinese lesser civet Viverriculaindica in north-eastern Taiwan. *Zoological Studies* 52(22): 1 - 8.

Vijayameena, C., Submachine, G., Loganayagi, M. and Ramesh, B. (2013). Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in Annona muricata. *International Journal of Current Microbiology Applied Science* 2(1): 1 – 8.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. and Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerging Infectious Diseases* 4(2): 149 –158.

Wrangham, R. W. (1995). Relationship of chimpanzee leaf-swallowing to a tapeworm infection. American *Journal of Primatology* 37: 297-303.

CHAPTER THREE

PAPER II

Chemical profile of crude extracts from selected plants used for self-medication by non- human primates in Mikumi National Park, Tanzania

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Target Journal: *International journal of Chemistry*

Abstract

This study was undertaken to evaluate the chemical profile of *Sterculia africana, Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* plants present in the non – human primate diet using HPLC and TLC analytical techniques. Polar and non-polar solvents where employed in running both HPLC and TLC methods. The ethanolic crude plant extracts demonstrated presence of saturated and unsaturated compounds when viewed under natural light and UV light at 254nm and 365nm. Colour reactions with Vanillin reagent revealed likelihood of the presence of terpernoids and saponins group of compounds. HPLC method confirmed presence of highly polar, polar and medium polar compounds in the crude plant extracts as earlier observed when running TLC. The peaks of leaf extracts from *Acacia sieberiana* and *Cassia abbreviata ssp. abbreviata*, showed great similarity in time of elution, an observation attributed to the fact that *Acacia sieberiana* (*Fabaceae*) and *Cassia abbreviata ssp. abbreviata* (*caesalpiniaceae*) belong to one large family of leguminosae.

Key words: ***Sterculia africana, Cassia abbreviata ssp. abbreviata, Acacia sieberiana***

3.0 Introduction

In the 1990s, the term zoopharmacognosy was coined to describe self-medication by animals in general and by non- human primates in particular. Studies on the white and red Colobus monkeys, baboons, chimpanzees and lowland gorillas among others have provided evidence of self-medication in animals particularly in non-human primates (Huffman, 1997, 2001).

The chemical profile of three plants; *Acacia sieberiana* (*mimosaceae* family), *Cassia abbreviata ssp. abbreviata* (*caesalpiniaceae* family) and *Sterculia africana* (*Sterculiaceae* family) observed to be consumed by yellow baboons in Mikumi National Park, Morogoro region in Tanzania was evaluated. In Botswana, the seeds of *Sterculia africana* have been tested for fatty acid (FA) and 95 triacylglycerol (TAG) profiles. GC–MS and 1H-NMR analyses showed the FA profiles for *S. africana* containing significant amounts of cyclic FAs of approximately 19.9% (Mitei *et al.*, 2008). On the other hand, preliminary phytochemical screening of *Acacia sieberiana* have shown presence of saponins, tannins, cardiac glycosides, flavonoids and anthraquinones (Burkill, 1995; Anisa, 2010; Mahdi *et al.*, 2013). HPLC analysis of the leaves of this plant showed the presence of two flavonols and a flavone (Anisa, 2010). The seeds of *Acacia sieberiana* were reported to contain 4% concentration of fixed oils with a composition of 44% oleic acids and 31% palmitic acids. The bark was also reported to contain about 3.8% of condensed tannins, 4.9% and 5.1% catechin (Shittu *et al.*, 2010; Mahdi *et al.*, 2013).

A number of studies have been done on *Cassia abbreviata* oliv, a close relative of C*assia abbreviata* Oliv. however it is important to note that *Cassia abbreviata ssp. abbreviata* is endemic to Morogoro region from where this sample was taken. A variety of non-nutritional plant secondary compounds and nutrient-poor bark is found in the primate diet,

yet little is known about the chemical composition of the plants found in the non-human primate diet (Cousins and Huffman., 2002; Rodrigues *et al*., 2016). An understanding of the analysis of plant chemistry, underlying wildlife behaviour combined with long term dietary data may lead to new chemotherapeutic drugs (Wolfe *et al.*, 1998). This study was undertaken in order to evaluate the phytochemical profile of these three plants consumed by non- human primates in Mikumi National Park, Tanzania to contribute to the knowledge on the chemistry of plants consumed in the non- human primate diet leading to possible isolation of pure compounds.

3.1 Materials and Methods

3.1.1 Plant collection and processing

Sterculia africana, Cassia abbreviata ssp. abbreviata and *Acacia sieberiana* were chosen solely on the basis that they were observed to be eaten by non- human primates in uncharacteristic feeding behaviour, in Mikumi National Park, Tanzania.

The fresh plants were collected from areas around Mikumi National Park and Sokoine University of Agriculture main campus found in Morogoro region, Tanzania the location of which is shown on a map in Figure 2.1.

In an effort to minimise possible errors brought by variations in time of collection as well as location, samples were obtained from the same location and at the same time. The samples were collected and authenticated with the help of a Botanist from the University of Dar es salaam and were assigned voucher specimen numbers FMM 3704, 3705 and 3706 for *Acacia sieberiana, Sterculia africana* and *Cassia abbreviata* respectively. From *Sterculia Africana,* the root bark and stem bark were collected while *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* constituted of the stem bark, root bark and leaves. The 8 samples were cleaned, the stem bark and root bark were cut into smaller pieces to

allow for better drying in air. The samples were then pulverised to a particle size of 1mm in readiness for use.

3.2 Extraction of Crude Samples

3.2.1 Total extraction technique by maceration

In the laboratory, one sample was handled at a time in order to avoid cross contamination. The dry samples were each weighed into a separate clean, marked container and the initial weight recorded. Total extraction was done by use of maceration technique using ethanol as the menstruum which was added till the entire sample was fully soaked. Samples were soaked in ethanol for 72 hrs with occasional shaking, the combination of ethanol and sample was strained, filtered and dried using a rotary evaporator to obtain 8 crude extracts. The marc was re-soaked for another 72 hours in ethanol in order to optimize extraction and treated as the previous samples and dried before adding them to their corresponding samples. Extracting the plant material in 96% Ethanol enabled us to get a wide range of compounds with different polarity as well as preserve it from microbiological decay. The crude plant extracts were then coded as shown in Table 3.1 and stored at room temperature before Phytochemistry screening was carried out.

Table 3.1: Codes of the extracts used during this study

Name of plant	Code of plant	Plant part (p p)	Code of pp
Acacia sieberiana	A	Leaves	A.L
		Stem bark	A.S
		Root bark	A.R
Cassia abbreviata ssp. abbreviata	B	Leaves	B.L
		Stem bark	B.S
		Root bark	B.R
Sterculia Africana	C	Stem bark	C.S
		Root bark	C. R

3.2.2 Thin layer chromatography

TLC plates used as the stationary phase were analytical TLC conducted on a TLC silica gel $60F_{254}$ pre coated alumina sheets (Merck). Each of the eight plant extracts were spotted on TLC plates and developed under different polar solvent systems. A sheet of paper was used to saturate the atmosphere inside the chamber with the solvent phase in order to get better separation with the plant extracts. Visualization of developed TLC plates was achieved using UV (254 and 365 nm) and vanillin-sulphuric acid spray. Spray reaction with Vanillin reagent gives blue, red, purple, pink colours that denote presence of triterpenoids, saponins, alcohols and sterols (Wagner *et al*., 1984).

3.2.3 High performance Liquid chromatography

Ten milligrams of dry ethanolic extract was dissolved in 10ml of HPLC grade methanol/ acetonitrile and then filtered with a 0.2 µm microfilter. The filtrate was then analysed using an HPLC (Shimadzu, 20AD) fitted with an auto sampler and an SPA UV detector at 254 nm using a reversed-phase supelco C-18 column (150 x 4.60 mm and particle size of 5 µm) with the column temperature set at 40°C. The sample injection volume was set at 1µL and flow rate of 1mL/min. The mobile phase constituted of 2 solvents; Solvent B: Methanol and Solvent C: Acetonitrile. A low gradient elution system of changing solvents was used: 0–15 min, 5% B; 15–20 min, 95% B; 20-25 min, 5% B (Mabiki *et al.*, 2013).

3.3 Results and Discussion

Solvents of varying polarities were used to prepare the mobile phase used. The table below presents a summary from one of the experiments using 30% methanol/ ethyl acetate. Results from colour reactions using vanillin reagent followed by observation at 254nm, 365nm are presented in Table 3.2 below.

Table 3.2: Phytochemical profile of crude plant extracts run using 30% methanol/ethyl acetate

Plant part	Number of compounds		Colour reaction with vanillin reagent	Inference from colour reaction
	At 254 nm	At 365 nm		
A.L	1	4	Green	Triterpenoids
A.S	4	1	Pink, blue/purple	Saponins, Triterpenoids
A.R	5	3	Pink, blue/ purple	Saponins, Triterpenoids
B.L	4	2	Green	
B.S	4	1	Pink	Saponins
B.R	3	1	Pink, blue/ purple	Saponins, Triterpenoids
C.S	3	1	Pink, blue/ purple	Saponins, Triterpenoids
C.R	4	1	Pink, blue/ purple	Saponins, Triterpenoids

From the results obtained in Table 3.2, the number of compounds observed on the TLC paper for plant part A.S at 254nm is 4 and 1 observed at 365nm. 4 compounds were observed for plant part B.L at 254nm and 2 compounds observed at 365nm. On spraying with Vanillin reagent, pink and blue/purple compounds were observed for plant part A.S inferring presence of Triterpenoids and saponins. When Plant part B.L was sprayed with vanillin reagent, green compounds were observed inferring presence of triterpenoid compounds. (Wagner et., 1984)

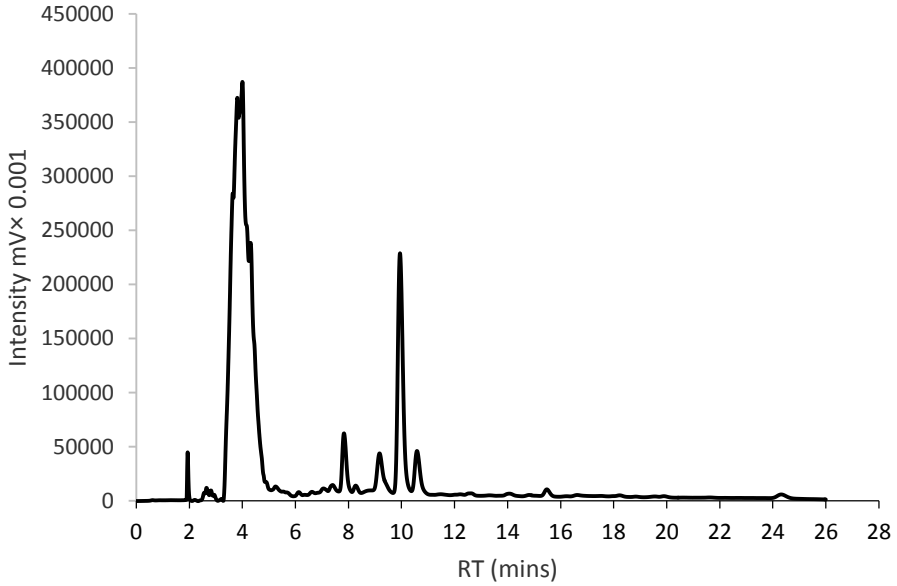

Figure 3.1: A chromatogram showing eluted peaks of crude extract B.L

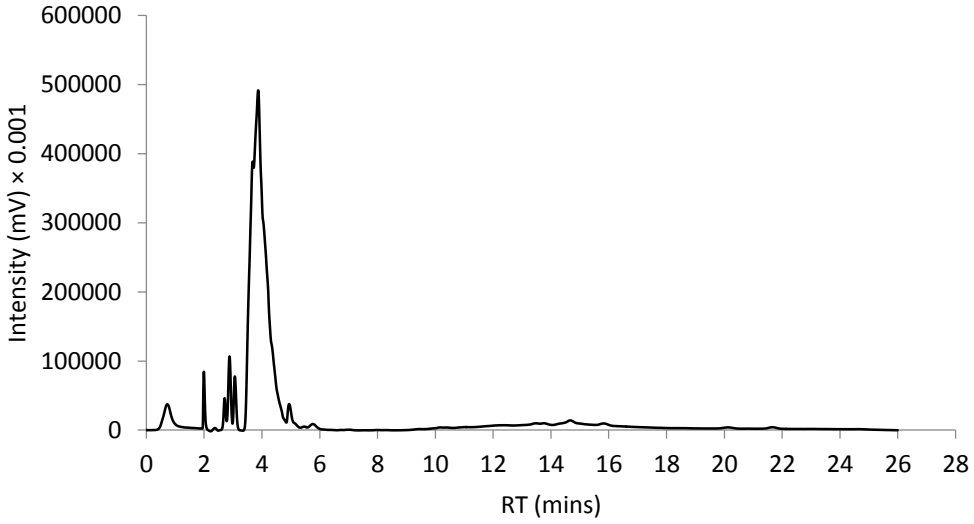

Figure 3.2: A chromatogram showing eluted peaks of crude extract A.S

From the HPLC results obtained in Figure 3.1 of extract B.L has eluted peaks at 1.5 minutes and at 4 minutes, other peaks were eluted at 8, 9, 10 and 11 minutes. Figure 3.2 of crude plant extract A.S eluted peaks at 1.0, 2.0, 2.5, 3.0, 3.5, 4 and 5 minutes.

Since a reverse phase column and a low pressure gradient system were employed in this separation, Figure 3.1 of the chromatogram from B.L shows that peaks were all eluted in the first 15 minutes with a mobile phase composition of 5% methanol and 95% Acetonitrile. Figure 3.2 of chromatogram A.S shows peaks eluted before 5 minutes and others between 8 and 11 minutes with the running time set at 25 minutes since there were no peaks eluted after 18 minutes. Crude extract B.L consists of both highly polar and medium polar compounds. A.S on the other hand has mainly highly polar compounds with all its peaks eluted in the first 5 minutes. The leaves, root and stem barks of these three plants namely; B.R, A.L, A.R, C.S, C.R contain polar and medium polar compounds as evidenced from the eluted peaks. The chromatograms obtained of A.L and B.L extracts show that peaks obtained have a great similarity in time of elution, which had been observed earlier while carrying out TLC. This could be attributed to the fact that *Acacia sieberiana* (*Fabaceae*) and *Cassia abbreviata ssp. abbreviata* (*caesalpiniaceae*) belong to one large family of leguminosae.

HPLC and TLC results were comparable. A total of 6 compounds were observed for Plant part B. L under UV light at 254nm and 365nm and also about 7 peaks are shown on the HPLC chromatograms of the same compound. The HPLC method confirmed presence of highly polar, polar and medium polar compounds present in the crude plant extracts as earlier observed when running TLC.

The TLC technique revealed presence saponins which are the starting points for the semi-synthesis of steroidal drugs, these metabolites are highly sought after by the

pharmaceutical industry. There is a growing interest in natural triterpenoids due to their wide spectrum of biological activities. Presence of these compounds in the plant extracts may be responsible for medicinal activity. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, parasitic and protozoal infections and above all, for their cytostatic effects

3.4 Conclusion

Observations from running TLC method confirmed presence of both saturated and unsaturated compounds as viewed under natural light, UV light at 254 nm and 365 nm respectively.

Observations from TLC results show presence of triterpene group of compounds which include sterols and triterpenes, which can accumulate as glycosides (saponins). Saponins are glycosylated (aglycone = sapogenin) secondary metabolites found in a variety of plant species. Their surface-active properties are what distinguish these compounds from other glycosides (Papadopoulou *et al.*, 1999).

Further chemical analysis could yield pure compounds that may possess therapeutic benefits.

3.5 Acknowledgement

I wish to genuinely thank INTRA-ACP mobility scholarship, RISE- AFNNET for partially funding this research, the Faculty of Veterinary Medicine, Sokoine University of Agriculture for facilitating this study. I wish to thank Mr. Frank Mbago and Ms Anna Mpanyakavili, your help was invaluable.

3.6 References

Anisa, S. (2010). Antimicrobial activity and chemical profile of traditional medicinal plants indigenous to Southern Africa used to treat respiratory tract infection. Thesis for Award of PhD Degree at University of Witwatersrand, Johannesburg, South Africa. 84pp.

Burkill, H. M. (1995). The useful plants of west tropical Africa. *Royal Botanic Garden* 3: 199 – 201.

Huffman, M. A. (1997). Current evidence for self-medication in primates: a multidisciplinary perspective. *Yearbook of Physical Anthropology* 40: 171–200.

Huffman, M. A. (2001). Self-medicative behaviour in the African great apes: An evolutionary perspective into the origins of human traditional medicine. *BioScience* 51(8): 651 – 661.

Mahdi, H., Palmina, K. and Tony, C. (2013). Analysis of commercial vegetable tannin materials and related polyphenols of selected Acacia species. *Journal of Forest Products and Industry* 2(1): 21 – 28.

Mitei, Y. C., Ngila, J. C., Yeboah, S. O., Wessjohann, L. and Schmidt, J. (2008). NMR, GC–MS and ESI-FTICR-MS profiling of fatty acids and triacylglycerols in some Botswana seed oils. *Journal of American Oil Chemists' Society* 85: 1021–1032.

Shittu, A. O., Oyi, A. R. and Onaolapo, J. A. (2010). Isolation, characterisation and compaction properties of acacia sieberiana gum in chloroquine and metronidazole tablet formulation. *International Journal of Pharmaceutical and Biomedical Research* 1(4): 149 – 153.

Wagner H., Bladt S. and Zgainski E. M. (1984). *Plant Drug Analysis.* Springer, Berlin, Germany. 320pp.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. and Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerging Infectious Diseases* 4(2): 149 – 158.

CHAPTER FOUR

4.0 GENERAL RESULTS, DISCUSSION AND CONCLUSION

4.1 General Results and Discussion

For the antibacterial study, MIC results tabulated in Table 2.2 showed that the crude plant extracts were active against all the test microorganisms. The lowest MIC value was 5mg/ml as compared against 0.01mg/ml the standard drug, Gentamicin. The negative control showed development of a purple colour resulting from formation of the purple formazan which is a positive indicator of cell viability as shown in Figure 1.1, row 9 whereas the sterility control showed no colour change as shown in Figure 1.1, row10, an indication of absence of test microorganisms. Minimum Inhibitory Concentration ranged from 0.31mg/ml to 5mg/ml with 0.31mg/ml, the lowest value denoting the greatest antibacterial efficacy from A.R, A.S, B.R and B.L plant parts. Gram-negative bacteria in the ethanol extracts had higher MIC values as opposed to the Gram positive bacteria. A.R, A.S and B.L demonstrated the lowest MIC values of 0.31mg/ml for both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains. The lowest MIC value for Gram positive strains was 0.63mg/ ml demonstrated by B.R and B.L against *Staphylococcus aureus.* Overall, plants A and B showed lower MIC values as opposed to plant C. *Cassia abbreviata ssp. abbreviata*, *Acacia sieberiana* and *Sterculia africana* extracts possess antibacterial activity against the test strains as shown from the results of the *in vitro* experiment in Table 2.2. *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* exhibited better activity against the test microorganisms as compared to *Sterculia africana* with *Cassia abbreviata ssp. abbreviata* showing the lowest MIC value (0.31mg/ml) of the three plants. The positive control was a standard drug, Gentamicin (0.01mg/ ml), an aminoglycoside targeting the bacterial ribosome. Interestingly, the lowest crude plant extract MIC of 0.31mg/ml was only 31-fold weaker than Gentamicin. The crude plant

extracts performed better than the negative control to a concentration of 0.313mg/ml of extract. Other studies reported on *Cassia abbreviata ssp. abbreviata* and *Acacia sieberiana* concur with these findings (Christiana *et al.*, 2012; Maroyi, 2011; Mwila and Shiv, 2015). A viable challenge in interpreting self-medication is differentiating between plants ingested for their nutritional value but are laden with medicinal benefits and plants ingested solely for their medicinal benefits. This challenge exists in traditional human societies where medicine and food are of the same origin. For instance traditional spices and condiments of daily Asian cuisine, such as marine algae, ginger root, turmeric and herbs also play an important role in suppressing viral and parasite infections (Ramesh *et al*., 2013, Huffman, 1997). Results show that different concentrations of the plant extracts were required to inhibit the growth of different microbes due to the difference in potency of the plant extracts attributed to phytochemicals present, environment of growth or extraction method used. The variation in the susceptibility of microorganisms could also be attributed to their intrinsic properties that are related to the permeability of their cell surface to the plants extracts. Their pharmacological effect could therefore be experienced with repeated ingestion or work in synergy.

This study provides evidence that the yellow baboon forage on similar plants also used in ethno medicine as seen from previous literature reviewed (Maroyi, 2011; Gibreel *et al.*, 2013; Kidane *et al.*, 2014; Mwila and Shiv, 2015). 27.5% of microbes that affect non-human primates affect humans as well, *Staphylococcus aureus*, *Salmonella* and *Escherichia coli* being only a few examples of such microbes, a factor attributed to our phylogenetic closeness (Glander, 1994; Wolfe *et al*., 1998; Pedersen *et al.*, 2005; Su *et al*., 2013;). It should therefore not come as a surprise that human and non-human primates perhaps select similar plants when challenged with similar illnesses. It is known that non-human primates feed on a great variety of plant species, however it is prudent to take note

of the ones that are fed on infrequently and or by isolated cases in an uncommon manner as was the case with the yellow baboons in Mikumi National Park. It may not be possible to pharmacologically analyse or carry out phytochemical screening for each one, however, special attention should be accorded to plants fed on by non- human primates exhibiting potential illness symptoms or in uncommon feeding behaviour as mentioned by Krief *et al*. (2006) such as but not limited to; a lack of appetite, intestinal disorder and coughing.

The chemical profile of *Cassia abbreviata ssp. abbreviata*, *Acacia sieberiana* and *Sterculia africana* is drawn from results obtained in Table 3.2, the number of compounds observed on the TLC paper for plant part A.S at 254nm is 4 and 1 observed at 365nm. 4 compounds were observed for plant part B.L at 254nm and 2 compounds observed at 365nm. On spraying with Vanillin reagent, pink and blue/purple compounds were observed for plant part A.S inferring presence of Triterpenoids and saponins. When Plant part B.L was sprayed with vanillin reagent, green compounds were observed inferring presence of triterpenoid compounds (Wagner *et al.*, 1984).

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The TLC technique revealed presence saponins which are the starting points for the semi-synthesis of steroidal drugs, these metabolites are highly sought after by the pharmaceutical industry. There is a growing interest in natural triterpenoids due to their wide spectrum of biological activities. Presence of these compounds in the plant extracts may be responsible for medicinal activity. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, parasitic and protozoal infections and above all, for their cytostatic effects

4.2 Conclusion

This study shows that the diet of non- human primates contains plants with antibacterial activity that may be used for self-medication and their diet could therefore act as a sieve through which plants fed on in uncommon manner are tested for antimicrobial activity. Observations from TLC results show presence of triterpene group of compounds which include sterols and triterpenes, which can accumulate as glycosides (saponins). Saponins are glycosylated (aglycone = sapogenin) secondary metabolites found in a variety of plant species. Their surface-active properties are what distinguish these compounds from other glycosides (Papadopoulou *et al.,* 1999).

These findings therefore propose zoopharmacognosy as an alternative field in search for new therapeutic leads and drug discovery and suggest that non-human primates feed on the non-nutritive parts of these plants for their pharmacological benefits.

They also provide more information on the spectra of microbes susceptible to plant extracts from the non- human primate diet.

Additionally, these findings contribute to the existing non –human primate foraging theory and cognizance about the mechanisms of food selection.

4.3 Recommendations

The existing knowledge gaps observed in self-medication in non-human primates could be reduced through long term collection of dietary data, pharmacological studies and analysis of plant chemistry.

Plants fed on by non- human primates should be conserved because they are not only vital to non-human primate preservation but to humans as well because of their pharmacological benefits.

Column chromatography and further separation should be done for purposes of structural identification to avail supplementary knowledge on the group of compounds found in these plants and act as a baseline for separation of pure compounds or formulation of standardised mixtures.

References

Glander. E. K. (1994). *Eating on the Wild Side.* The University of Arizona Press, London. 239pp.

Huffman, M. A., Gotoh, S., Turner, L. A., Hamai, M., Yoshida, K. (1997). Seasonal trends in intestinal nematode infection and medicinal plant use among chimpanzees in the Mahale Mountains National Park, Tanzania. *Primates* 38: 111-125.

Pedersen, A., Poss, M., Nunn, C. L., Cunningham, A. and Altizer, S. (2005). Patterns of host specificity and transmission among parasites of free-living primates. *International Journal for Parasitology* 35: 647 – 657.

Su, H., Su, Y. and Huffman, M. A. (2013). Leaf swallowing and parasitic infection of the Chinese lesser civet Viverriculaindica in north-eastern Taiwan. *Zoological Studies* 52(22): 1 - 8.

Vijayameena, C., Submachine, G., Loganayagi, M. and Ramesh, B. (2013). Phytochemical screening and assessment of antibacterial activity for the bioactive compounds in Annona muricata. *International Journal of Current Microbiology Applied Science* 2(1): 1 – 8.

Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A. and Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerging Infectious Diseases* 4(2): 149 – 158.