ETHNOPHARMACOLOGICAL AND TOXICOLOGICAL STUDY OF MEDICINAL PLANTS USED AGAINST RESPIRATORY INFECTIONS IN KISUMU EAST SUB- COUNTY

James Kiamba Mailu, (BPharm)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS FOR MASTER OF SCIENCE DEGREE OF UNIVERSITY OF NAIROBI

(PHARMACOLOGY AND TOXICOLOGY)

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY, FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF NAIROBI

2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

James Kiamba Mailu, BPharm.  Date. 29.06.2021

This thesis has been submitted for examination with our approval as University supervisors.

1. Dr. Joseph M. Nguta, BVM, MSc, PhD (UoN). Date: 30.06.2021

   Department of Public Health Pharmacology and Toxicology, University of Nairobi.

2. Prof. James M. Mbaria, BVM, MSc, PhD (UoN). Date: 1/07/2021

   Department of Public Health Pharmacology and Toxicology, University of Nairobi.

DEDICATION

To my lovely wife Judy Wanjiru Wairimu and son Dylan Musyimi Kiamba for their immeasurable love and support, throughout the period of study. They have been a great inspiration and motivation encouraging me to work on the project.

ACKNOWLEDGEMENT

I would like to thank God for good health, wisdom and favor he has bestowed on me. I would also like to express my sincere gratitude to my supervisors Dr. Joseph M. Nguta and Prof. James Mbaria for their overwhelming support, advice and critical inputs throughout the study.

My special thanks to Mr. Peter Olewe and Mr. Kimeu Musembi for their help in identifying the plants collected in the study area. Further, I express my appreciation to Mr. Mainga and Mr. Maloba for their guidance and invaluable assistance during the various experiments in the laboratory. They were of great help during extraction process, screening for phytochemical constituent, antimicrobial screening and toxicity bioassay.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ALRI	Acute Lower Respiratory Infection
ARI	Acute Respiratory infection
ATCC	American Type Culture Collection
BACUC	Biosafety, Animal Care and Use Committee
COPD	Chronic obstructive pulmonary disease
CTMDR	Centre for Traditional Medicine and Drug Research
DMSO	Dimethyl sulfoxide
EMA	European Medical Agency (EMA)
FDA	Food and Drug Administration
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Statistics
LC_{50}	Median Lethal Dose
LRTI	Lower respiratory tract infections
NHIF	National Hospital Insurance Fund
PHPT	Public Health, Pharmacology and Toxicology
RFC	Relative frequency of citation

RSV	Respiratory Syncytial Virus
SARI	Severe Acute Respiratory Infection
WHO	World Health Organization

ABSTRACT

Respiratory diseases are a major cause of mortality in developing countries. Poor access to healthcare in rural areas makes many people in such communities to rely on traditional medicine. The current study was designed to address the following objectives: identify medicinal plants used to manage respiratory diseases in Kisumu East Sub-County, analyze the phytochemical composition of the plant extracts, establish the *in- vitro* antimicrobial activity of the crude extracts and evaluate *in-vitro* cytotoxicity of crude plants extracts against *Artemia salina* Leach (*Artemiidae*) larvae. An ethnobotanical survey was conducted in Kisumu East Sub -County. Semi- structured questionnaires were used to collect information from 30 Traditional Medicine Practitioners (TMPs). Sociodemographic characteristics of informants, local names of plants used, habit, active parts, indications, method of preparation, routes of administration, scientific identity and conservation status were recorded. A literature search was conducted via PubMed, Google Scholar, and Research Gate to identify reported activities of the plants. Three plants parts namely, the root of *Acanthus polystachius* Delile (Acanthaceae), the root bark of *Keetia gueinzii* (Sond.) Bridson (Rubiaceae) and the root tuber of *Rhynchosia elegans* A. Rich. (Leguminosae) were collected using standard methodologies. The parts were extracted using double distilled water, 100% acetone, and 100% methanol. The aqueous, acetonic and methanolic crude extracts were subjected to secondary phytochemical screening; antimicrobial activity against *Staphylococcus aureus* (*S. aureus*), *Bacillus cereus* (*B. cereus*), *Escherichia coli* (*E. coli*), and *Candida albicans* (*C. albicans*)*;* and cytotoxic potential using the brine shrimp lethality assay. Standard methods were adopted for efficacy, safety evaluation and for identification of secondary metabolites in the test crude extracts. Majority of the TMPs were female and comprised of 26 practitioners (86.7%). From the survey, 45 plant species, belonging to 43 genera and 28 families were identified. Leguminosae and Rutaceae were the most dominant plant families. Leaves were mentioned by 19 respondents (32.8%), hence were the most commonly used, while trees were mentioned by 20 respondents (44.4%) and comprised the commonest habitat. A literature search established that at least 43/45 plant species had reported pharmacological activities. Qualitative phytochemical analysis of aqueous extracts of *Keetia gueinzii* showed presence of flavonoids, glycosides, phenols, saponins and tannins. Aqueous extracts of *Acanthus polystachius* contained saponins, phenols and tannins. Results from the antimicrobial evaluation showed the extracts had varying degrees of activity against the tested microorganisms. Aqueous and acetonic root bark extracts of *Keetia gueinzii* had the lowest Minimum Inhibitory Concentration (MIC) of 12.5 mg/ml against *Staphylococcus aureus*. The methanolic extract of *Keetia gueinzii* had the lowest MIC value of 100 mg/ml against *Candida albicans.* Brine lethality results showed that aqueous, acetonic and methanolic extracts of *Rhynchosia elegans* A. Rich. were toxic with LC_{50} values of 422.09 µg/ml, 175.77 µg/ml and 168.76 µg/ml respectively. Acetonic and methanolic extracts of *Acanthus polystachius* were also toxic with a LC_{50} value of 195.17 µg/ml and 174.26 µg/ml respectively. All extracts of *Keetia gueinzii* were non- toxic. This serves as the first report on the phytochemical composition, antimicrobial and cytotoxic activity of *Acanthus polystachius, Keetia gueinzii* and *Rhynchosia elegans.* With the exception of the root barks of *Keetia gueinzii,* the use of all other plants tested are limited by safety concerns. In their current crude form, these plants are not recommended for the management of microbial infections. However, the root bark of *Keetia gueinzii* warrants further research.

Keywords: Respiratory infections, phytochemical, antimicrobial, cytotoxicity.

CHAPTER ONE

INTRODUCTION

1.1. Background information

Respiratory diseases comprise various conditions affecting the bronchi, lungs and nasal passages. These illnesses can be classified as upper and lower respiratory tract infections. They can be further grouped as acute or chronic respiratory infections. Acute infections include pneumonia and bronchitis while chronic infections include chronic obstructive disease and asthma. Respiratory infections are a health concern globally (Wang *et al*., 2016). In the year 2016, about six million people died due to Lower Respiratory Tract Infections (LRTI) and Chronic obstructive pulmonary disease (COPD) with lung cancer; trachea and bronchus cancer claiming 1.7 million lives (World Health Organization, 2018).

The burden of communicable diseases is high in Sub Saharan Africa (Murray *et al*., 2012). Lower Respiratory Tract Infections (LRTI), diarrheal diseases and Acquired Immunodeficiency Syndrome (AIDS) contribute a considerable percentage of mortality for all ages (World Health Organization, 2018). The Kenya Human Resource for Health (HRH) strategy 2014- 2018 highlights skin diseases, respiratory diseases, accidents, malaria and diarrhea among the top five causes of outpatient morbidity accounting for about 70 percent of reported deaths. According to World Health Organization (WHO) Kenya Country Profile 2014, chronic respiratory diseases and cancer are among the main causes of premature mortality.

It is therefore important to find preventive and curative measures including drug alternatives to mitigate this continued mortality due to respiratory diseases despite presence of a wide range of commercially available remedies. In Sub Saharan Africa, there is an increase in consumption of

antimicrobials despite little knowledge on resistance patterns of common pathogenic bacteria (Ayukekbong et al., 2017).

There is an increase in use of medicinal plants in management of various illnesses worldwide (Ruhsam and Hollingsworth, 2018). In countries such as China, Germany and France, extracts of plants are being used as prescription drugs (Ji *et al*., 2017). In Kenya, communities especially from the poor rural areas rely on traditional herbal medicine (Kigen et al., 2013). This is attributed to proximity of herbal practitioners, affordability and cultural preference. With continued failure of conventional drugs in management of diseases including asthma, cancer, diabetes and malaria, considerable interest should be given to herbal medicine as a possible alternative. This study aimed at investigating ethnopharmacological utilization, validation of anecdotal efficacy and evaluation of the safety profile of medicinal plants used in management of respiratory tract infections in Kisumu East Sub- County. Communities in this locality use locally available medicinal plants, such as *Ajuga remota* Wall ex. Benth. (Lamiaceae) against many illnesses. The community has a wealth of ethnopharmacological knowledge, which can be scientifically exploited in a collaborative manner, in search of novel ligands against respiratory tract illnesses. This will ultimately address Sustainable Development Goal (SDG) number 3, which aims at global alleviation of illnesses, while addressing easy accessibility to affordable medicines to all Kenyan citizens, as envisaged in the "big four agenda".

1.2. Problem statement

Respiratory diseases are a leading cause of death and disability globally. Premature mortality from chronic respiratory diseases is high in poorly resourced health systems (Collaborators,

2020). Acute respiratory infection including pneumonia constitutes a substantial disease burden in older adults > 65 years (Shi, et al., 2020). Tuberculosis is still considered as an emerging infectious disease (Nguta et al., 2015). In the year 2018, Kenya recorded between 11000- 30000 deaths attributed to tuberculosis infection. (WHO, 2019). Kisumu county has been reported to have a high level of multi-drug resistant tuberculosis. (Odongo et al., 2017). This continued morbidity and mortality attributed to respiratory diseases warrants investigation to find possible remedies.

1.3. Justification

During the year 2011, acute upper respiratory infections were a major cause of inpatient morbidity for children aged 5 years and below. According to the Kenya National Bureau of Statistics (KNBS) economic survey (2013), tuberculosis and pneumonia were responsible for 13.7 percent of total deaths in that year compared to AIDS which was 5.39 percent in Nyanza region. The KNBS economic survey (2020) reported that in the year 2019, respiratory system disease was the leading cause of morbidity in Kenya accounting for 25% of all disease incidence. It further reported that pneumonia incidence for the period 2015- 2019 stood at 2.58%. Pneumonia was reported to be the leading cause children under- five admissions to health facilities with 21,383 (18.7%) cases for the period 2017- 2019. These reports indicate the need to find drug alternatives. Herbal medicines are increasingly being used in developed and developing countries and alternatives to the conventional methods of management should be studied further (McKay and Blumberg, 2006). In Sub Saharan Africa, herbal remedies play a key role in the health care system and it's of great benefit that their contribution in treatment of disease be studied (Omoruyi et al., 2012). This serve as a good entry point and a great wealth of knowledge could be attained.

1.4. Objectives

1.4.1. Broad objective

To document and evaluate pharmacological and toxicological profile of medicinal plants used to manage respiratory diseases in Kisumu East Sub- County.

1.4.2. Specific objectives

1. To identify and document medicinal plants used to manage respiratory diseases in Kisumu East Sub-County.
2. To analyze the phytochemical composition of the plant extracts.
3. To establish the *in- vitro* antimicrobial activity of the crude extracts.
4. To evaluate *in-vitro* cytotoxicity of crude plants extracts against *Artemia salina* larvae.

1.5. Hypothesis

Crude plant extracts from some selected plants are rich in phytochemical constituents, possess significant *in- vitro* antimicrobial activity on test bacteria and are non-toxic to *Artemia salina* larvae.

CHAPTER TWO

LITERATURE REVIEW

2.1. Ethnobotany and ethnopharmacology

In 1896, John Harshberger coined the term ethnobotany. Ethnobotany refers to study of plant use by man. It is a broad discipline as it encompasses medicinal plants and natural products including food, plants used for rituals, building materials for houses etc. It further includes aspects of biochemistry, pharmacognosy, toxicology, medicine and history. Over the years, there has been need to improve compilation of ethnobotanical data collection by incorporating suitable quantitative methods. These methods aid in describing the variables quantitatively leading to generation of quality information (Phillips, 1996).

Ethnopharmacology is an interdisciplinary exploration of biologically active agents traditionally used by a particular community. It involves bioscientific study of indigenous drugs. Ethnopharmacology represent diverse intellectual traditions including botany, pharmacology and anthropology. Ethnopharmacology strives to bridge the gap between natural sciences and symbolic and cognitive aspects. People select plants based on their pharmacological properties and the symbolic power that they believe is in a plant. Some plants have been claimed to be used to expel evil, fend off spirits and as luck charms (Van Andel et al., 2013). Factors that could contribute a plant to be considered magical include; an associated medicinal use; a perceived connection with ancestors; its habitat; growth form, shape, or color and sacred status (Van Andel et al., 2013). The leaf of *Aloe megalacantha* Bark. (Aloaceae) and the seeds of *Peganum harmala* L. (Zygophyllaceae) has reportedly been used for evil eye (Ghorbani et al., 2006;Teklayet al.,

2013). The roots of *Clerodendrum myricoides subsp. namibiense* R. Fern. (Lamiaceae) has been reportedly used for evil spirits (Teklayet al., 2013).

Ethnopharmacology strives to develop improved preparations that can be utilized by members of the local community. Determination of bioactive compounds from plants and evaluation of their pharmacological and toxicological profile helps in validation of their ethnomedicinal use.

Various ethnobotanical studies have reported plants used for respiratory ailments. A study conducted in Lake Victoria region; Tanzania reported the use of *Albizia versicolor* Welw. Ex Oliv. (Fabaceae), *Balanites aegyptiaca* (L.) Delile (Zygophyllaceae), *Cajanus cajan* (L.) Druce (Fabaceae), *Securidaca longipendunculata* Fres (Polygalaceae). and *Diospyros fischeri* Gürke (Ebenaceae) in management of cough (Otieno *et al.*, 2011).

Decoction of stem bark of *Olax scandens* Roxb. (Olacaceae) has been reported to be used in treatment of cough (Duraipandiyan *et al.,* 2006). The role of medicinal plants in management of respiratory illnesses should be fully exploited to enable derivation of new compounds to aid to address economic and health challenges associated with infectious and non-infectious illnesses.

A study by Suliman *et al.*, (2010) reported the use of *Artemisia. Afra* Jacq. (Asteraceae) and Zanthoxylum. *capense* Harv. (Rutaceae) in febrile conditions and treatment for colds. The fresh leaves of *Artemisia. Afra* Jacq. are inserted into the nostrils, inhaled or infused to treat upper and lower respiratory tract infections while a concoction of *Artemisia. Afra* Jacq. and *Warburgia salutaris* (G. Bertol.) Chiov. (Canellaceae) is used in management of acute bronchitis, coughs from colds or flu, fever.

2.2. Respiratory infections

2.2.1. Prevalence and burden of respiratory infections

Infectious diseases are a major cause of death in developing countries (Lozano et al., 2010). Global Burden of Disease Study 2017 ranked LRTIs 4th among the leading cause of early death in 2017 (Arthur, 2014). Respiratory diseases negatively impact individual's productivity (Wang et al., 2016). Acute respiratory infections have contributed to economic loss with increase in utilization of healthcare resources and reduced productivity. This results in increase in out-of-pocket cost negatively impacting an already poor population (Peasah et al., 2015).

In Africa, respiratory tract infections are important causes of death. According to World Health Organization (WHO) estimates, infectious diseases account for 65% of all deaths (Bates *et al.*, 2013). In Sub-Saharan Africa, surveillance for influenza has been limited by poor public health infrastructure and others competing health priorities including tuberculosis and malaria (Katz et al., 2014). In Kenya, pneumonia is a public health problem responsible for 19-23% of deaths among children below the age of 5 years (Tornheim *et al.*, 2007). A literature review of studies conducted in Kenya showed that hospitalizations of children with influenza was 2-3 times higher than in United States (Emukule et al., 2015). It has been reported that the number of hospitalized influenza- associated Severe Acute Respiratory Infection (SARI) cases annually ranged 6,882-7,836 for persons > 5years old with the number of non-hospitalized influenza-associated cases being higher than the hospitalized cases (Fuller et al., 2013). In Western Kenya, a hospital-based surveillance identified 6.9% and 13.8% of inpatients and outpatient children aged 0-4 years respectively had influenza (McMorrow *et al.*, 2015).

Studies have reported pneumonia as an important cause of childhood mortality and kills far more children than human immunodeficiency virus or malaria (Ferkol and Schraufnagel, 2014; Zar and Ferkol, 2014).

A study conducted in Kenyatta National Hospital and Kibera South Hospital quantified the cost of screening and further evaluation of asthma in Kenya at $4.23 and $53 in public and private hospital respectively. The estimated cost for management of mild and severe asthma was $67.93 and $146.74 respectively compared to $295.45 and $ 879.08 in private facilities. The cost for management of COPD was $372.45 and $1530.06 in public and private hospital respectively (Subramanian et al., 2018). This is similar to other studies conducted which showed a substantial cost in management of asthma. A study conducted in the USA reported that the economic cost of asthma in the year 2013 was $81.9 billion (Nurmagambetov et al., 2018).

With a substantial proportion of health care being provided by the private sector, it is imperative that there is need for review of National Hospital Insurance Fund (NHIF) to cater for all costs in private sector reducing patients' out-of-pocket payments for health services (Chuma et al., 2012). Factoring the burden of infectious diseases worldwide, the ever-changing disease patterns and emergence of resistance to various drug molecules, demand has increased for new antimicrobial agents (Kariuki et al., 2012). In view of this, there should be increased funding for basic research towards novel remedies against respiratory-related diseases, antimicrobial resistance and emerging infectious diseases.

2.2.2. Etiology

Bacterial, mycobacterial, fungal and viral pathogens are known causative agents of respiratory tract infections. Etiological agents responsible for Acute Lower Respiratory Infection (ALRIs) include bacteria, viruses, and fungi. Human coronavirus, Respiratory syncytial virus (RSV), human rhinoviruses and human influenza viruses have been identified among patients with ALRI (Feng et al., 2014).

A study in Dhaka city reported that in symptomatic cases of viral respiratory infections, the predominant virus detected were *human rhinovirus*, RSV and *adenovirus* accounting for 31.5%, 31% and 7% respectively. *Streptococcus pneumonia* was the most frequently isolated bacterial pathogen (9%), followed by *Klebsiella pneumoniae* (5.5%) and *Hemophilus influenzae* (1.5%) (Bhuyan et al., 2017).

In addition to the etiological agents, other factors including temperature and humidity have been shown to have an influence on respiratory disease (Jati and Ginandjar, 2017). Activities such as smoking and exposure to biomass smoke are considered important risk factor for COPD. This is of importance as the tendency among the young generation to engage in smoking will likely lead to increase in incidences of COPD (Richardson et al., 2014).

The continuous advancement in vaccine development and vaccination against influenza and pneumococcus is a welcome relief and will likely prevent the substantial ARI cases for persons living in rural African setup (Feikin *et al.*, 2012).

2.3. Traditional medicine and medicinal plants

2.3.1. Introduction

Ethnopharmacological knowledge serves as a systemic approach that can aid in discovery of new, safer and affordable medicines. Documentation of herbal medications in Kenya is insufficient (Nguta et al., 2010). With continued infrastructural development, cultural lands and wild vegetation are being utilized and it is imperative to document plants of medicinal value for posterity (Meragiaw, 2016). In the past, natural products from plant biodiversity have been less intensively investigated (Nguta *et al.*, 2016). Ethnobotanical studies have reported various plants used for managing respiratory ailments including; *Rhoicissus tridentata* subsp. *cuneifolia* (Eckl. & Zeyh.) (Vitaceae) (Nanyingi et al., 2008), *Strychnos henningsii Gilg* (Loganiaceae) (Kaingua et al., 2014), *Metha longifolia L.* (Lamiaceae) (Asadbeigi et al., 2014), *Clematis brachiata* Thunb. (Ranunculaceae) (York et al., 2011) among many others. The appeal for herbal medicine has been occassioned in some measure by the rising concern on the safety of convectional drugs (Scott et al., 2010). With health seeking behavior being a mix of different medical systems, it is prudent to incorporate traditional medicinal knowledge and conservation strategies to complement established healthcare systems.

2.3.2. Plants as a source of medicine

Plants are known to be sources of medicines and around 2000 new plants species are identified annually (Willis, 2017). These increase in demand and utilization of herbal drug and natural health products has led to rapid use of medicinal plants globally (Cole et al., 2007). The demand of plant species witnessed an increase of between 8% to 15 % yearly in Asia, North America and

Europe (Ross, 2007). In the United States, one hundred and eighteen of the top 150 prescription medicines are from natural sources (Chen et al., 2016). In India herbal industry, 95% of traditional medicinal plants used and more than a quarter of prescribed drugs in developed countries are sourced from the wild (Hamilton, 2004).

Despite popularity in their use, there has been challenges associated with plants as a source of new drug molecules. The continuous modification in plant taxonomy has led to difficulty in identification of plants (David et al., 2015). In addition, the population of many medicinal plants found naturally have been facing pressure from environmental degradation, climate change, urbanization, deforestation, high population and uncontrolled harvesting from the wild (Lata et al., 2010). The estimated loss of plant species is as high as 1000 times the natural rate of extinction and there is a loss of one or more potential drug every 2 years (Chen et al., 2016). Twenty percent of wild sources of these plant species has been destroyed due to increased plant consumption and human population expansion. This loss of species and destruction of habitat risks extinction of medicinal plants in Tanzania and Uganda as reported by Zerabruk and Yirga (2012), in Kenya Hamilton (2008), in China and India Heywood and Iriondo (2003).

Overexploitation and indiscriminate collection affect the population of species. However, this does not provide sufficient explanation to individual species susceptibility or resilience to harvest pressure. Some biological characters that correlate with risk of extinction include rate of growth, distribution, habitat, population, species diversity and reproductive system (Chen et al., 2016).

2.3.3. Drug development from natural products

Bioprospecting focuses on the development of pharmaceutical products based on the chemical, biological and diversity of the different ecosystems. It involves collection of biogenic samples

comprising of microorganisms and animals. These are then analyzed for biological and chemical activity. Entities showing promising activity are isolated and serve as drugs or template of new drugs.

Most plants of medicinal value are endemic species and their properties are attributed to presence of secondary phytoconstituents. These metabolites respond to stimuli in natural environments which may not be replicated under culture conditions in controlled laboratory setting (Figueiredo and Grelle, 2009). Validation of their use has led to increase in use of phyto-therapeutic agents in disease management in the last decade (Younis et al., 2018). Naturally derived products including paclitaxel, podophyllotoxin, or vinblastine have proven to be active compounds and have been in use for a period of time. However, compounds from these natural sources accumulate over long periods of growth and therefore cannot meet market demands (Staniek et al., 2014).

Knowledge of interaction of a drug candidate with target site is key in its optimization for pharmacological activity. However, challenges in determining the precise molecular mechanism of action of natural products exist (Corson and Crews, 2007). Once a natural product is found promising by exhibiting pharmacological activity, challenge of availability arises as large quantities are required for characterization of pharmacological activity (Atanasov et al., 2015). Currently, the pharmaceutical industry has shifted interest towards biological big molecules in favor of small molecule-based drug (Mander and Liu, 2010). However, there is a drawback of high costs for biologicals and this increases pressure on national health insurance systems. Stringent regulations pertaining patentability of natural products and seasonal variation of

chemical composition of plant material has further led to already present challenges of use of natural products in development of new drugs (Corson and Crews, 2007; David et al., 2015).

2.4. Phytochemical constituents, solvent extraction and compound isolation

2.4.1. Phytochemical constituents and their reported biological activities

Medicinal plants contain natural products which possess pharmacological activity in human body systems. These secondary metabolites include flavonoids, tannins, withanolides among many others. These metabolites defend plants against abiotic stresses, pathogenic microbes and a variety of herbivores (Mazid et al., 2011). Azadirachtin, present in *Azadirachta indica* A. Juss (Meliacea) is known to act as a defense mechanism against insects (Njoku et al., 2011).

Phytochemical analysis of medicinal plants is of great importance in not only the pharma industry but other related industries. Analysis of phytoconstituents coupled with knowledge of disease-causing microorganisms may offer new opportunities in management of disease (McGaw and Eloff, 2008). Presence of antioxidant bioactive molecules in plants is of interest among players in food industry and scientist in health sector (Olajuyigbe and Afolayan, 2011). Antioxidant activity is attributed to polyphenols present in plant materials which protects the body against reactive oxygen species (Burton *et al.*, 1985; Oliveira *et al.*, 2008; Gülçin *et al*., 2011;Sharma *et al.*, 2012).

Terpenoids and flavonoids are known to possess antioxidants, anti-inflammatory, antiviral, anti-bacterial, and anticancer properties (Iloki-Assanga et al., 2015). Terpenoids have been reported to have antimicrobial properties (Scortichini and Rossi, 1991; Griffin *et al.*, 1999; Singh and Singh, 2003). Terpenoids inhibit larval attachment and metamorphosis of the barnacle *Amphibalanus*

Amphitrite Darwin (Balanidae) (Hirota *et al.,* 1996). Iboga-type indole alkaloid coronaridine possess antileishmanial activity inhibiting promastigote and amastigotes (Delorenzi *et al.,* 2001).

Flavonoids possess antimicrobial properties and are produced in response to microbial infestation (Hossain *et al.,* 2013). Their antimicrobial activity is due to a combination of complexion with extra-cellular, soluble proteins and bacterial cells (Maiyo et al., 2010). Withanolides present in roots of Withania somnifera subsp. obtusifolia (Tackh.) Abedin, Al- Yahya, Chaudhary & J.S. Mossa (Solanaceae) crude extracts have been reported to have a role in promoting memory and learning in animal models (Dhanani et al., 2017).

Other secondary metabolites have been characterized including more than 8000 phenolic compounds. Phenolics exhibit various properties, such as vasodilatory effect, anti-inflammatory, anti-allergenic, anti-atherogenic, anti-microbial, cardio protective and anti-viral. Antioxidant activity is attributed to their redox properties, where they act as reducing agents. (Tan et al., 2013; Altemimi et al., 2017). Moreover, studies have shown that medicinal plants containing polyphenols have neuroprotective effects and may represent a potential source for drug development against Parkinson's disease. (Ngoungoure et al., 2019).

Saponins have been shown to exhibit tonic and stimulating activities (Edeoga et al., 2005). Saponins have been reportedly used in treatment of hypercholesterolemia, hyperglycemia, cancer and weight loss (Manjunatha, 2006). Further non-biological activity of saponins has been reported with relation to use of *Carica papaya var. bady* Aké Assi (Caricaceae) as a soap substitute (Njoku et al., 2011). Inhibitory effect on gram-positive organisms and anti-inflammatory effects of saponins have been reported (Estrada *et al.,* 2000; Oyekunle *et al.,* 2006).

Cardiac glycosides act on smooth muscle of the vascular system and indirectly influence electrical activities of the heart. They work by inhibiting Na^+/K^+-ATPase and are used for management of atrial arrhythmia and heart failure. This explains their rationale for use in the treatment of hypertension (Olaleye, 2007).They have also shown anticancer activity and consequently potential use in cancer therapy (Prassas and Diamandis, 2008).

Alkaloids have been reported to interfere with cell division, possess analgesic, anti-asthmatic and anti-anaphylactic properties and are used as anesthetic agents (Stærk *et al.,* 2002).. Alkaloid constituents in crude ethanolic extracts of bark of *Galipea officinalis* J. Hancock (Rutaceae) have been reported to possess antimycobacterial *(*Houghton *et al.,* 1999).Alkaloid insecticidal activity has been reported against common fruit fly, *Drosophila melanogaster* Meigen (Drosophilidae) larvae (Riaz et al., 2018). Cytotoxic activity of alkaloids has also been reported (Kim et al., 2001).

Tannins possess useful therapeutic benefits. They are known to exhibit antimicrobial activity, anticancer activity, antioxidant activity and radical scavenging activity (Ghosh, 2015). Tannins acts via deprivation of iron, formation of hydrogen bonds and interaction with vital proteins to exert antimicrobial activities (Njume *et al.,* 2009)Tannins have protein-precipitating and vasoconstriction effect which has been found to be advantageous in preventing ulcer development. Herbs containing tannins are known astringents used in treatment of various intestinal disorders such as dysentery and diarrhea and persistent cough (Akinpelu and Onakoya, 2006; Sultana et al., 2016).

2.4.2. Extraction, Fractionation, Isolation, Structure Elucidation and Characterization of bioactive natural product molecules

Extraction is a crucial and essential step that aids in recovery and purification of active constituents found in plant materials. The extraction process aims to achieve high extraction yield, high quality and concentration of target compound (Tan *et al.*, 2013). Nature of extraction solvent, temperature, period of soaking and method of extraction has an influence on the extraction yield (Nyong et al., 2009; Azwanida, 2015). Selection of suitable method of extraction is essential in upscaling from bench scale to pilot scale level. Commonly used extraction techniques that can be employed include infusion, maceration, hot continuous extraction and percolation (Dhanani et al., 2017).

In practice, the method used in the preparation of bioactive ingredients is key in determining the phytochemical constituent extracted and their health benefit in treatment (Ngo et al., 2017). For example, preparing a decoction using potable water might extract a group of anti-inflammatory steroids to treat arthritis, yet the same plant if extracted in alcohol could yield different antibacterial alkaloids. A study by Ngo et al, (2017) reported that 50% acetone was the best solvent for extraction of saponins, 100% acetone was best for extraction of proanthocyanidins and 50% (v/v) methanol was best for extraction of total phenolic content. Additional factors having an influence on phytochemical composition include genetic factors, rainfall, the nature of the soil, humidity and temperature.

Various attempts of isolation of the compounds have been carried out. Isolation and determination of active components aids in the study of their pharmacological, pharmacokinetic and

toxicological mechanisms. Constituents present in the crude extracts including protein, sugars and fats hinders isolation and measurement of the active constituents. Chromatographic techniques have extensively been employed to isolate these phytoconstituents. Column chromatography is a widely used method. Mass spectrometry and infrared spectroscopy have the additional advantage of possibility of elucidation of chemical structure.

Despite spirited efforts to isolate and characterize chemical compounds present in crude plant extracts, there has been concerns regarding their effectiveness. Activity of isolated active constituents has reportedly been found to be reduced as compared to that of crude extracts (Rasoanaivo et al., *2*011). This has promoted focus on use of the plant extract wholesomely without need for isolation of the active moieties.

2.5. Toxicity studies

Toxicological screening is an important step in development of new drugs and chemical moieties. Toxicity test are designed to examine specific end point events including cardiotoxicity, skin/eye irritation and determination of No Observed Adverse Effect Level. Toxicological studies have various advantages including; ability to measure various responses, can assess effect of host characteristics e.g. age, genetics, other modifiers including diet. They in addition offer the potential to study mechanisms of development symptoms.

Qualitative and quantitative analysis of toxins can be done to ascertain the nature of toxin. Some of the quantitative techniques that can be employed include colorimetric and Ultraviolet-Visible spectrophotometric analysis, gas chromatography, infrared spectroscopy and immunoassay techniques (Parasuraman, 2011). However, disadvantages associated with toxicological studies

exist including: uncertainty in the relevance of animal response to human response and the concentration of exposure and time frames are to some extent different from those experienced by humans.

Some of the naturally derived plant secondary metabolites are toxic and may exhibit mutagenic or genotoxic potential. Negative effects associated with use of traditional medicinal plants in management of human illness is due to the presence of toxic contaminants/chemicals, over-dosage and inadequate knowledge of by-products contained in some plants which may possess harmful effects (Hamidi et al., 2014). Cytotoxic components present in plant extracts may mask detection of bioactive constituents which may possess desired activity. For example, saponins have detergent activity which can interfere with results of cell-based assay as they cause cell lysis (Henrich and Beutler, 2013).

2.5.1. Brine Shrimp Lethality (BSL) Studies

Brine shrimp lethality test is an example of a biological assay that can be used to evaluate botanicals. Brine shrimp studies are routinely used for screening of extracts and isolated compounds. The test involves exposing larvae of *Artemia salina* to test sample in marine salt solution (3.3%) and % mortality calculated after 24 hours in a Finney computer program. It is a useful bench top assay which is cheap and easy to perform (Mclaughlin et al., 1998). The test is used to evaluate toxicity and/or general bioactivity of different formulations (Olowa and Nuñeza, 2013).

Various studies have shown that there is a correlation between LC_{50} values obtained with Brine Shrimp Lethality Assay and Acute Oral Toxicity Assay in Mice (Naidu et al., 2014). This

observation is in agreement with earlier observations (Nguta et al., 2011). Other advantages of the test include; rapidity of the test; simplicity as it requires no special training; low requirements; robustness, - inexpensiveness, - high degree of repeatability. A drawback of the study is that it does not provide sufficient information on possible mechanism of action of toxicity.

Broad applicability of the Brine Shrimp Lethality Assay is achieved through adherence to some criteria including experimental conditions, geographical region of the cysts and age of *Artemia* nauplii. Biological assays with species of *Artemia franciscana* Kellog (Artemiidae) and *Thamnocephalus platyurus* Packard (Thamnocephalidae) have been employed in recent studies. These tests serve as preliminary assessment of toxicity (Hamidi et al., 2014).

CHAPTER THREE

MATERIAL AND METHODS

3.1. Ethnobotanical survey

3.1.1. Study area.

The study was conducted in Kisumu East Sub-County, Kisumu County, Kenya (Figure 3.1).

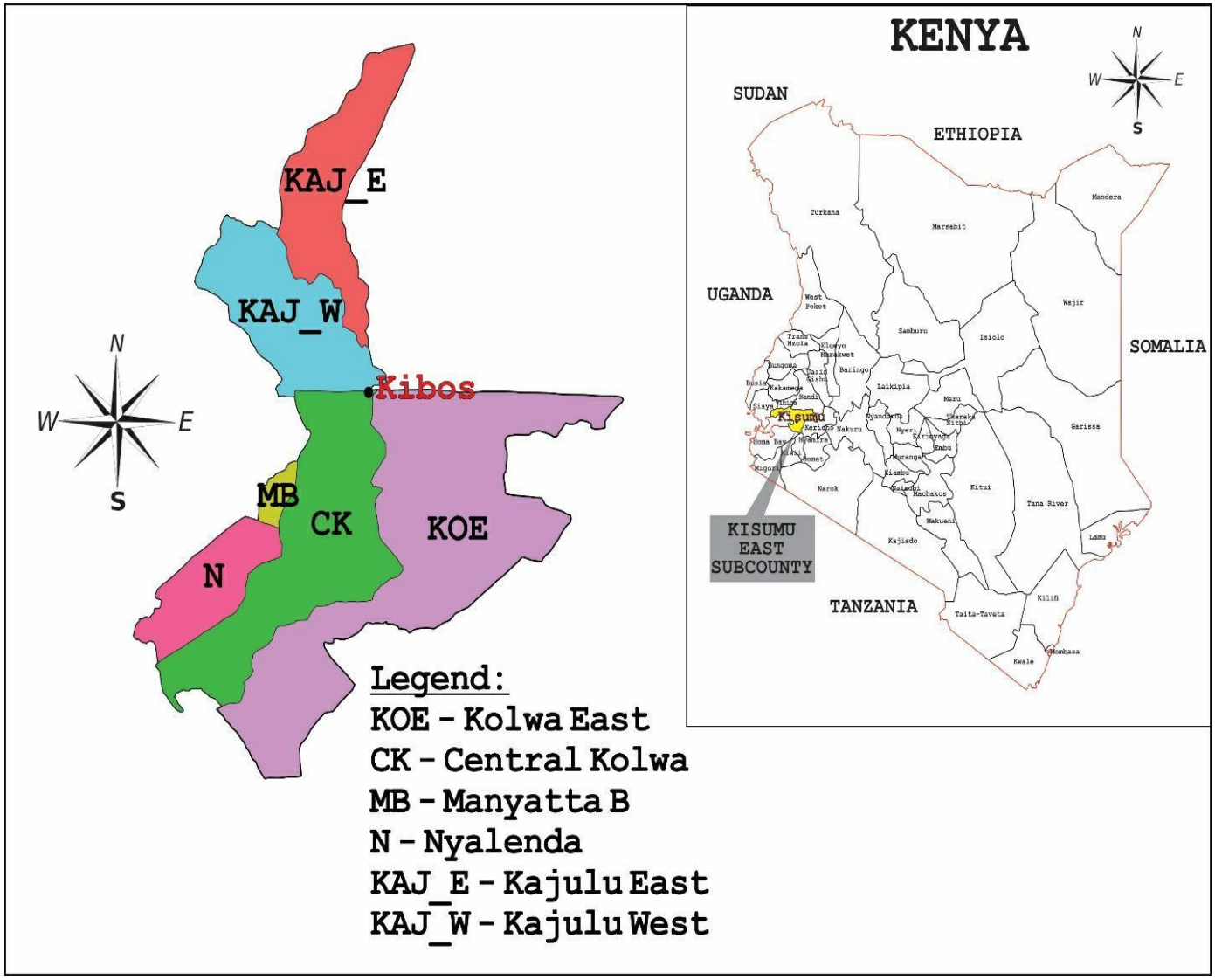


Figure 3. 1: Map of Kenya showing Kisumu East Sub- County and Kisumu County.

The Sub- County is 365 Km West of Nairobi. It covers an area of 135.9 square kilometers and lies within longitudes 33°20'E and 35°20'E and latitudes 0°20'South and 0°50'South. The Sub –

County is comprised of Kajulu, Kolwa Central, Kolwa East, Manyatta B, and Nyalenda A administrative ward.

Its population based on the 2019 Kenya Population and Housing Census is 220,977 (Kenya National Bureau of Statistics, 2019). The area receives annual rainfall of between 1200mm and 1300mm with temperature range of between 20^{o}C to 35^{o}C. Residents engage in various economic activity including fish farming, agriculture including sugar factories, poultry and livestock farming (County Government of Kisumu, 2013).

3.1.2 Study design

A cross sectional survey was conducted to determine ethno medicinal plants used to manage respiratory infection in Kisumu East Sub-County. Non probability convenient sampling was used to identify traditional practitioners with good ethno medicinal knowledge. Through the local area chief, traditional practitioners were identified and convenient sampling was used to identify other practitioners.

3.1.3. Ethical approval of the study

Ethical clearance was sought from Faculty of Veterinary Medicine Biosafety, Animal Care and Use Committee (FVM-BACUC) Ref No. FVM- BACUC/2019/210 (Appendix I) and NACOSTI License No: NACOSTI/P/20/3004 (Appendix II). The local chief was informed of the study and approved it. The participants were informed on the scope of the study, after which they signed a written consent (Appendix III) and willingly participated in the study.

3.1.4. Data collection

Data was collected between March and September, 2019. Semi-structured questionnaires were used for data collection. The target respondents comprised of traditional practitioners rich in ethnomedicinal knowledge of plants used to manage respiratory infections. Thirty local traditional practitioners from both genders were selected from 3 wards: Kolwa Central, Kolwa East, and Kajulu. The interviews were conducted in Swahili and in Luo. A taxonomist familiar with the local flora and dialect was recruited. To ensure confidentiality, respondents were interviewed individually. The study sought to answer:

1) Which plant species and plant parts are used in preparation of remedies for respiratory infections? It was assumed that respiratory illnesses were a common occurrence in the study area and the local community had acquired rich ethnopharmacological knowledge over time.

2) How are the remedies prepared? In the same breath, the supposition was that the study community have inherited a wealth of experience from their fore fathers on the best methods of herbal preparations against lung diseases.

3) What respiratory illnesses are treated in the area? The study community was assumed to have an excellent ethno diagnostic skill in reference to respiratory tract infections.

4) What are the routes of administration? Just as information, communication and technology, in addition to movement of people have drastically changed ethnomedicinal practices, the supposition was that the Kisumu community have a clear knowledge on

those routes that have over the years, been shown to yield the best results upon herbal formulation exposure in positively diagnosed patients.

3.1.5. Collection and identification of plant specimens

Several trips were made to collect specimen of plants mentioned in Kajulu hills, herbalist homesteads and its environs with the help of a taxonomist. Kajulu hills was rich in medicinal plants and served as a key source of medicinal plants in the study area. Identification of plants specimens was done by a taxonomist in Maseno University and voucher specimens deposited at the University of Nairobi Herbarium. University of Nairobi taxonomist and literature survey further confirmed the identity of collected plants.

3.1.6. Data analysis

Descriptive analysis of data was done using frequencies and percentages for the social demographic data of the respondents. Ethnobotanical data was analyzed using Relative Frequency of Citation (RFC).

3.1.6.1. Relative Frequency of Citation

The measure was calculated to determine how many traditional medicine practitioners realized a particular plant species was worth mentioning (Tardio and Pardo-de- Santayana, 2008). The value was calculated using the formula below:

$$\text{RFCs} = \frac{FCs}{N} = \sum_{i=i1}^{iN} URi/N$$

Fc is the number of respondents who cited a particular species and N is the total number of respondents.

3.2. Plant collection and preparation

A literature search conducted via Google Scholar and Research Gate found that 3 plants; *Keetia gueinzii*, *Acanthus polystachyus* and *Rhynchosia elegans* had little/no phytochemical, pharmacological and cytotoxic work on done on them despite their use among various communities. The root bark of *K. gueinzii*, roots of A. *polystachyus* and root tuber of *R. elegans* were collected in June 2019 in Kajulu hills, Kajulu west ward, Kisumu East Sub-County (Appendix V and VI). The plant parts were properly cleaned with running water and left to dry. The root bark of *K. gueinzii* was scraped while the whole root of *A. polystachyus* and root tuber of *R. elegans* were chopped into small pieces and left to dry for 4 weeks. They were then ground to a fine powder, weighed on a Mettler PM4600 balance and weights recorded. The ground powders were then stored in plastic bags and placed in tightly lidded plastic containers.

3.3. Phytochemical constituents of root bark of *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber of R*hynchosia elegans* A. Rich.

3.3.1. Preparation of crude extracts and yield determination

3.3.1.1. Organic extraction

For organic extraction, 100% methanol and 100% acetone were used. 500 g of each of the three powdered plant materials were separately macerated with 500 ml of the respective solvent for 72

h. It was then filtered to remove coarse residues. The filtrate was further passed through filter paper No. 1 MN615 (Macherey-Nagel). The resulting filtrate was concentrated on rota-vapor Buchi-R, under reduced pressure at 50 ^{0}C. The extract was transferred to glass bottles previously weighed. The glass bottles were covered with perforated aluminum foil then placed in a sand bath and left for 48h to allow slow and complete evaporation of the organic extractive solvent. The dried extracts in the glass containers were kept at 4 ^{0}C awaiting analysis (Naz et al., 2017; Mostafa et al., 2018).

3.1.1.2. Aqueous extraction

165 g of powdered plant part was put into a 2 L conical flask and 1 L of distilled water added until complete dissolution. The conical flask was then placed in a water bath at 90 ^{0}C and incubated for 1 h with intermittent shaking. This was the ideal conditions for maximum extraction without risk of denaturation. The process was repeated twice to ensure full extraction. The contents were filtered and the supernatant (0.5 L) placed into 1 L freeze drying flasks. The flasks were coated (frozen) with dry ice that had been dissolved in acetone until all the liquid was gone. They were then freeze dried using a Buchi Lyovapor freeze drier operating at 0.5 mbar and -104^{0}C. The freeze-dried powder was recovered and put into glass sample containers that were air tight and kept in a cool dry place (Yong et al., 2013; Tadesse et al., 2017).

3.1.1.3. Determination of yield

Glass vials containing the crude plant extracts were weighed using an analytical balance (Mettler AE 163) and weights recorded.

Yield of the samples was determined by:

$$\frac{[(\text{Weight of glass bottle + extract}) - (\text{Weight of empty glass bottle})] \times 100}{\text{Weight of powdered plant part}}$$

3.3.2. Phytochemical evaluation of aqueous, acetonic and methanolic crude plant extracts

Crude plant extracts were phytochemically analyzed for presence of alkaloids, phenols, saponins, flavonoids, glycosides, terpenoids and tannins using standard methods. The results were evaluated by visual inspection and observed color change or precipitation recorded.

3.3.2.1. Test for alkaloids

Dragendorff's reagent was prepared accordingly (Pascaline et al., 2011). To 10 g of extract, 4 ml of 1% hydrochloric acid was added and placed in a water bath for 5 minutes. To 2 ml of the filtrate, Dragendorff's reagent was added. Appearance of an orange red precipitate was considered positive for alkaloids (Iqbal et al., 2015)

3.3.2.2. Test for saponins

500 mg of extract was mixed with 10 ml of distilled water and shaken for 30 s. After standing for 30 minutes, froth persistence above the surface of the liquid confirmed the test to be positive for saponins (Pandey, 2015)

3.3.2.3. Test for phenols

2 ml of ferric chloride solution was mixed with 1 g of crude extract. Appearance of a blue- green precipitate is a positive test for phenols (Jaradat et al., 2015).

3.3.2.4. Test for flavonoids

500 mg of the extract was mixed with 2 ml of dilute sodium hydroxide. Appearance of a yellow colour which turned colourless on addition of 2 drops of diluted acid was considered a positive test for flavonoids (Jaradat et al., 2015).

3.3.2.5. Test for glycosides

0.5 ml of extract was mixed with 2 ml of acetic acid glacial with 2 drops of 2% Ferric chloride solution. To this, 1 ml concentrated sulphuric acid was added. Appearance of a brown ring between the layers was considered positive for cardiac steroidal glycoside (Jaradat et al., 2015).

3.3.2.6. Test of terpenoids

100 mg of extract was shaken with 2 ml of chloroform and concentrated Sulphuric acid (H_2SO_4). Reddish-brown coloration at the interface was considered a positive test for terpenoids (Iqbal et al., 2015).

3.3.2.7. Test for tannins

10 ml of distilled water was mixed with 500 mg of the extract and stirred. Two drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Appearance of blue green precipitate was considered to be a positive test for tannins (Usman et al., 2009).

3.4. In-vitro antimicrobial activity of root bark of *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber of *Rhynchosia elegans* A. Rich.

3.4.1. Collection and preparations of crude test extracts

Plant materials were collected and prepared as describe earlier in section 3.2.1.

3.4.2. Reference strains of microorganisms and culture conditions

The reference strains for screening were *Staphylococcus aureus (*ATCC 25923), *Candida albicans (*ATCC 10231), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778). Bacteria were obtained from stock cultures from the department of Public Health, Pharmacology and Toxicology (PHPT), Faculty of Veterinary Medicine, University of Nairobi. Freshly growing strains of bacteria were obtained by growing bacteria in Blood agar and incubated at 37 ^{o}C for 24 h. Fungal stock cultures were sub cultured in Sabouraud dextrose broth at 35^{o}C for 48 hours. The test strains were suspended in sterile saline to give a final density of 1.5 X 10^6 cfu/ml of bacteria or 1.5 x 10^5 spores/ml (The et al., 2017).

3.4.3. Standard antimicrobial drugs

Gentamycin powder and benzyl penicillin were used as reference standards for bacteria while nystatin was used as a reference for antifungal activity.

3.4.4. Minimum Inhibitory Concentration value (MIC values) determination

Broth dilution technique was conducted as adopted by Teke *et al.* (2013) with modifications. Sixteen hundred grams of respective crude plant extracts were put in 4 ml of pre-sterilized Muller Hinton Broth and Sabouraud Dextrose Broth for bacteria and fungi respectively contained in a

sterilized 10 ml test tube. Each test tube was clearly labeled and put in a test tube rack. Serial two-fold dilutions of plant extracts were made. A total of 6 dilutions: 400, 200, 100, 50, 25 and 12.5 mg/ml were prepared. Using a sterile 1 ml pipette, 0.1 ml of bacterial and fungal suspension was dispensed into the test tubes. A test tube containing Muller Hinton Broth without extract and 0.1 ml of the inoculum was used as a negative control. They were incubated at 37 ^{0}C for 24 h for and 35^{o}C for 48 h bacteria and fungi respectively. Experiments were performed in triplicates. Two-fold dilution of standard antimicrobials amoxicillin, gentamycin and nystatin were used as positive controls. After incubation period, visual turbidity was observed. The Minimum Inhibitory Concentration (MIC) was determined from readings on culture plates after incubation.

3.4.5. Determination of minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC)

A sterile pipette was used to draw 0.1 ml from the two lowest concentrations of the plant extract exhibiting invisible growth from the MIC test tubes and sub cultured onto Muller Hinton Broth sterile agar plates and incubated accordingly. Microbial growth was examined for the respective plant extract and corresponding concentration.

3.5. Evaluation of cytotoxicity of root bark of *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber *Rhynchosia elegans* A. Rich. against brine shrimp (A*rtemia salina*) larvae.

3.5.1. Hatching of Brine Shrimp (*Artemia salina)* Larvae

Artemia salina eggs were hatched in a dish partitioned into two compartments by a perforated plastic divider containing artificial sea water. Artificial sea water was prepared by dissolving 33 g of sea salt (Meersalz, Germany) in 1L of distilled water. One compartment was darkened by placing a plastic cover. 50 g of brine shrimp eggs were sprinkled in this compartment. The other compartment was illuminated by leaving the lid ajar under natural light next to a window. It was incubated in room temperature (23-29 ^{0}C) for 48 h. Phototropic nauplii attracted to the illuminated side were collected for the brine shrimp lethality test.

3.5.2. Preparation of test crude extracts and cytotoxic drug for bioassay

Aqueous and organic crude plants extracts were prepared accordingly. Briefly, 50 mg of organic extracts were dissolved in 0.1 ml of 0.01% Dimethyl sulfoxide (DMSO) followed by dilution with distilled water to make 5 ml stock solution (10,000 µg/ml). The DMSO concentration used is too low to be associated with solvent carry-over effects as reported in literature (Nguta et al., 2011). The same concentration of aqueous extracts was prepared by dissolving 50 mg in 5 ml of distilled water. Stock solutions of Vincristine Sulphate injection USP (10,000 µg/ml) were also prepared.

3.5.3. Bioassay of *Artemia Salina* Larvae

Brine Shrimp Lethality bioassay was done as described by Meyer et al., (1982). Three dilutions were prepared by transferring 5 ml of 1000 µg/ml, 100 µg/ml and 10 µg/ml of the plant extracts to a set of five graduated tubes. Using a pipette, 10 shrimps were transferred into vials. Five graduated vials were set for each dilution and a further five for the controls. Stock solution of the

positive control vincristine sulphate injection USP (10,000 µg/ml) was also prepared. The negative control group contained artificial sea water and brine shrimp larvae only while the positive control group contained vincristine sulphate and brine shrimp larvae. The tubes were left under illumination at room temperature. After 24 h, surviving larvae were counted with aid of a magnifying glass. The mortality endpoint was marked with absence of forward motion during 30 s of observation.

The percentage mortality was determined for each dilution and controls. Abbot formula was used to correct data where control deaths occurred, as follows: % deaths = [(Test-control)/control * 100]. (Nguta et al., 2012).

3.5.4. Statistical analysis

Lethal dose was calculated by linear regression analysis (Naz et al., 2017). Mean results of mortality percentage (converted to probits using Probit Transformation Table) versus the Log_{10} concentrations was plotted using Microsoft Excel 2019 spreadsheet application which formulated the regression equations and LC_{50} values calculated (Meyer et al., 1982; Clarkson et al., 2004; Lei and Sun, 2018).

CHAPTER FOUR

RESULTS

4.1. Ethnobotanical survey

4.1.1. Social demographic information

Summary of socio-demographic information of the respondents.is provided in Table 4.1 below.

Table 4. 1: Demographic profile of respondents (n=30)

Biodata	Frequency	Percentage (%)
Sex		
Male	4	13.3
Female	26	86.7
Age		
31 – 40	5	16.7
41 – 50	1	3.3
51 – 60	5	16.7
61 – 70	13	43.3
71 - >	6	20
Education		
None	17	56.7
Basic	12	40
Secondary	1	3.3
Years of experience as healer		
Between 1 – 10 years	5	16.7
Between 11 – 20 years	8	26.7
Between 21 – 30 years	9	30
Between 31 – 40 years	4	13.3
Between 41 – 50 years	2	6.7
51 - > years	2	6.7

Thirty traditional medicinal practitioners were involved in the study. A total of 26 (86.7%) of the respondents were females while 4 (13.3%) were male. Majority of the respondents 13 (43.3%) were between the age of 61- 70 years. A total of 17 (56.7%) of the respondents had no formal education. The respondents had a mean of 26 years of practice.

4.1.2. Diversity of medicinal plants identified and their uses

Summary of the reported medicinal plants is shown in Table 4.2. The table shows Family, scientific name, voucher No., local name, part used, habit and status.

Table 4. 2: Plants used in managing respiratory infections in Kisumu East Sub- County

Family	Scientific name	Local name	Voucher No.	Habitat	Status	Part used	Condition	Mode of preparation/ Route of administration	RFC
Acanthaceae	*Acanthus polystachius* Delile.		JM2019/ 284/003	Shrub	Wild	R	Cough	D/O	0.07
Asphodelaceae	*Aloe kedongensis* Reynolds	Ogaka	JM2019/ 194/030	Shrub	Wild	L	Asthma, Pneumonia	C/O	0.23
Amaryllidaceae	*Allium sativum* fo. p*ekinense* (Prokhanov) Makino	Otungu	JM2019/ 194/031	Herb	Cultivated	Bu	Allergies	Chew or C/O	0.03
Anacardiaceae	*Rhus natalensis* var. *comorensis* Engl.	Sagla	JM2019/ 194/021	Shrub	Wild	R	Asthma	C/O	0.07
Apiaceae	*Steganotaenia araliacea* Hochst.	Nyanian g-liech	JM2019/ 118/006	Tree	Wild	R/ SB	Pneumonia	D/O	0.03
Apocynaceae	*Carissa edulis* var. *ambungana* Pichon	Ochuoga	JM2019/ 194/022	Shrub	Wild	R	Common cold, pneumonia, asthma	D/O	0.67

Asteraceae	Artemisia subsect. annua (Rydb.) Krasnob.	Nyumba	JM2019/269/001	Herb	Wild or cultivated	L	Asthma	D/O	0.03
	Microglossa pyrifolia (Lam.) Kuntze	Nyabung -odide	JM2019/194/006	Shrub	Wild	L/R	Cough	M, C/O	0.07
	Tithonia diversifolia subsp. g*labriuscula* S.F. Blake	Mafua/maua	JM2019/194/012	Shrub	Wild	SB/L	Asthma	C/O	0.03
Bignoniaceae	*Kigelia africana* subsp. *moosa (Sprague) Bidgood & Verdc.*	Yago	JM2019/194/003	Tree	Wild or cultivated	Fr/SB	Pneumonia	D/O	0.3
Burseraceae	*Commiphora Africana* var. *venosa* (Mattick) Govaerts	Arupiny	JM2019/194/007	Tree	Wild	R	Pneumonia	D/O	0.17
Canellaceae	*Warburgia salutaris* (G. Bertol.) Chiov.	Abaki	JM2019/244/001	Tree	Wild or cultivated	SB	Asthma, allergy, chest pain, pneumonia	D/O	0.47
Caricaceae	*Carica papaya* var. *bady* Aké Assi	Apoyo	JM2019/269/002	Tree	Cultivated	R/L	Bronchitis	D/O	0.07
Combretaceae	*Terminalia glabrata* var. *brownii* Fosberg & Sachet	Minera/Manera	JM2019//058/016	Tree	Wild or cultivated	SB	Asthma, pneumonia, common cold	D/O	0.2
Convolvulaceae	*Ipomoea kituiensis v*ar. *hirsutissima* Verdc.	Obinju	JM2019/194/028	Shrub	Wild	L	Cough	D/O	0.03

Ebenaceae	*Euclea divinorum* Hiern.	Ochol	JM2019/194/023	Shrub	Wild	R	Pneumonia, asthma	D/O	0.73
Euphorbiaceae	*Croton megalocarpus* Hutch.	Ofunja muri	JM2019/194/015	Tree	Wild	L	Pneumonia	D/O	0.17
	Croton dichogamous Pax.	Rachar	JM2019/178/001	Tree	Wild	R	Asthma	D/O	0.1
Hypericaceae	*Harungana madagascariensis* Lam. Ex Poir	Aremo	JM2019/058/005	Tree	Wild	L	Cough	D/O	0.2
Iridaceae	*Gladiolus dalenii* var. *andongensis* (Baker) Goldblattex Geerinck	Obuya	JM2019/284/001	Corm	Wild	Corm	Asthma, allergy	P/N	0.1
Lamiaceae	*Clerodendrum myricoides* subsp. *namibiense* R. Fern.	Okwero gweno/sangla	JM2019/058/021	Shrub	Wild	R/L	Pneumonia, asthma	D/O	0.17
	Plectranthus barbatus var. *grandis* (L.H Cramer) Lukhoba & A. J. Paton	Okita	JM2019/058/009	Shrub	Wild	L	Asthma, pneumonia, allergy	D/O	0.33
	Vitex doniana Sweet	Kalemba	JM2019/194/009	Tree	Wild	L/SB	Allergies, common cold	D/O	0.03
Leguminosae	*Acacia robusta* subsp. *usambarensis* (Taub.) Brenan	Otiep	JM2019/214/001	Tree	Wild	SB/RB	Bronchial obstruction	C/O	0.03
	Albizia zygia (DC.) J.F..Macbr.	Oturbam	JM2019/224/002	Tree	Wild	SB	Pneumonia	D/O	0.1

	Rhynchosia elegans A. Rich.	Jandarusi/Jandalusi	JM2019/284/002	Herb	Wild	Rt	Cough	C/O	0.03
	Tamarindus indica L.	Chwaa	JM2019/194/018	Tree	Wild or cultivated	Fr/SB	Cough, general body malaise	D/O	0.03
	Tylosema fassoglense (Kotschy ex Schweinf.) Torre & Hillc.	Ombasa	JM2019/194/016	Climber	Wild	R	Flu, pneumonia, asthma	D/O	0.67
Meliaceae	*Azadirachta indica* A. Juss	Mwarubaine	JM2019/269/003	Tree	Wild or cultivated	L	Cough	D/O	0.3
	Khaya senegalensis (Desr.) A. Juss.	Tido	JM2019/194/019	Tree	Wild	SB	Common cold, cough	D/O	0.47
Molluginaceae	*Mollugo nudicaulis* Lam.	Ataro	JM2019/138/001	Herb	Wild	L	Cough	Chewed or D/O	0.03
Moringaceae	*Moringa oleifera* Lam.		JM2019/269/004	Tree	Cultivated	L	General body malaise	D/O	0.13
Myrtaceae	*Eucalptus camaldulensis* subsp. *arida* Brooker & M.W.McDonald	Bao	JM2019/269/005	Tree	Wild or cultivated	L	Common cold	D/O	0.33
	Syzygium cumini var. *obtusifolium* (Roxb.) K.K. Khanna	Jamna	JM2019/194/008	Shrub	Wild	SB	Cough	C/O	0.03
Olacaceae	Ximenia Americana var. argentinensis DeFilipps	Olemo	JM2019/269/006	Shrub	Wild	R/SB	Cough	C/O	0.07
Ranunculaceae	*Clematis hirsuta* var. *junodii* (Burtt Davy) W.T Wang	Achogo	JM2019/269/007	Climber	Wild	L	Common cold	D/O	0.1
Rubiaceae	*Gardenia ternifolia* var. *goetzei* (Stapf	Rayudhi	JM2019/194/014	Shrub	Wild	R	Cough, Pneumonia	D/O	0.13

	& Hutch.) Verdc.								
	Keetia gueinzii (Sond.) Bridson	Atego	JM2019/ 264/001	Shrub	Wild	RB	Asthma, pneumonia, coughing, allergy	P/N	0.2
Rutaceae	*Harrisonia abyssinica* Oliv.	Pedo	JM2019/ 194/001	Shrub	Wild	R	Cough, pneumonia, asthma	D/O	0.6
	Teclea nobilis Delile	Madat/midat	JM2019/ 194/024	Tree	Wild	R/L	Asthma, common cold	D/O	0.2
	Toddalia asiatica var. *parva* Z.M. Tan	Ajua/Nyalwet-kwach	JM2019/ 194/017	Shrub	Wild	L/R	Common cold, pneumonia, throat infection	C/O	0.33
	Zanthoxylum chalybeum var. molle Kokwaro	Roko	JM2019/ 269/008	Tree	Wild	SB/ RB	Pneumonia	D/O	0.03
	Zanthoxylum gilletii (De Wild.) P.G Waterman	Sogo-maitha	JM2019/ 224/001	Tree	Wild or cultivated	SB	Asthma, pneumonia, coughing, General body malaise	D/O	0.5
Vitaceae	*Cissus rotundifolia* var. *ferrugineopubescens* Verdc.	Minya/katera	JM2019/ 194/026	Climber	Wild	L	Throat infection, pneumonia, coughing	D/O	0.2
	Rhoicissus revoilii Planch.	Rabong'o	JM2019/ 269/009	Shrub	Wild	Rt	General body malaise	D/O	0.17

RFC: Relative Frequency of citation, SB: stem bark; R: roots; L: leaves; Rt: root tuber; RB: root bark; Fr: fruit; Bu: bulb; C: concoction (prepared by boiling plant parts of different plants in water); D: decoction(prepared by boiling part in water); P: powdered (Plant part is dried and ground); M: macerate(Plant part is soaked in water); I: inhale; O: oral

Forty-five plant species belonging to 43 genera distributed among 28 families were reported to be used in herbal preparations for managing respiratory ailments. All the plants were identified by their local names except *A. polystachius* that was not identified by its local name. It was referred to as 'Nyanandi' depicting that it may have originated from the neighbouring Nandi County.

Figure 4.1 below shows the distribution of the plant families reported in this study.

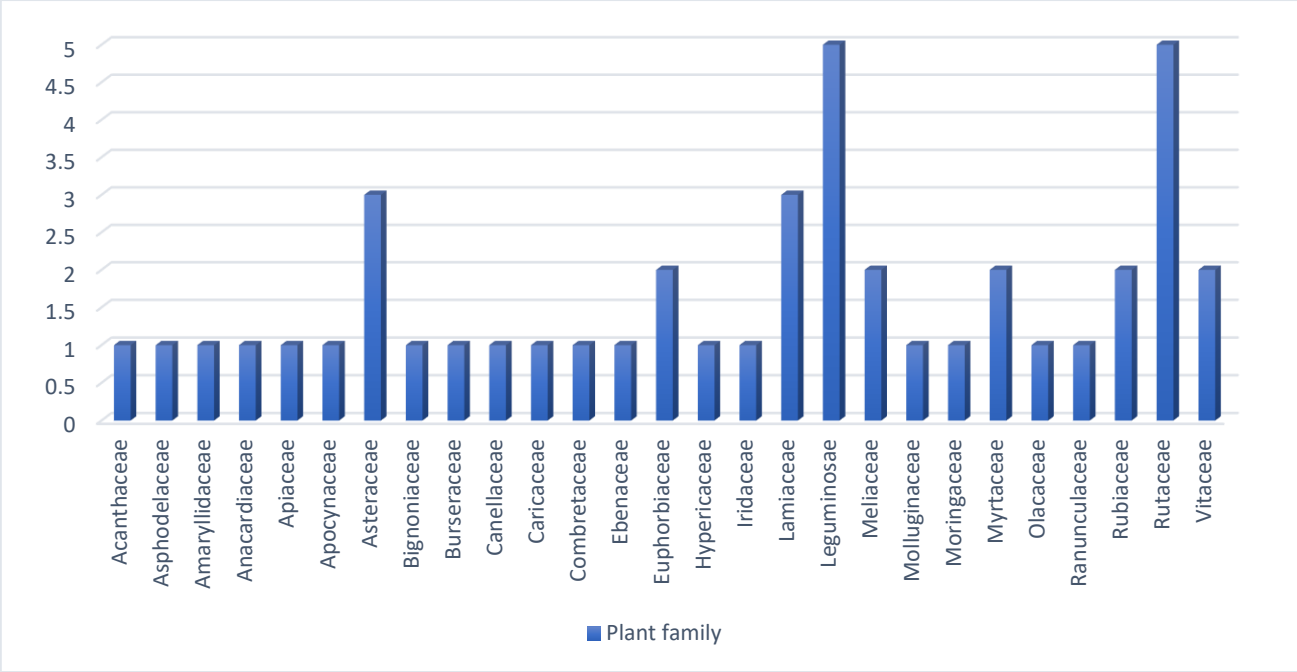
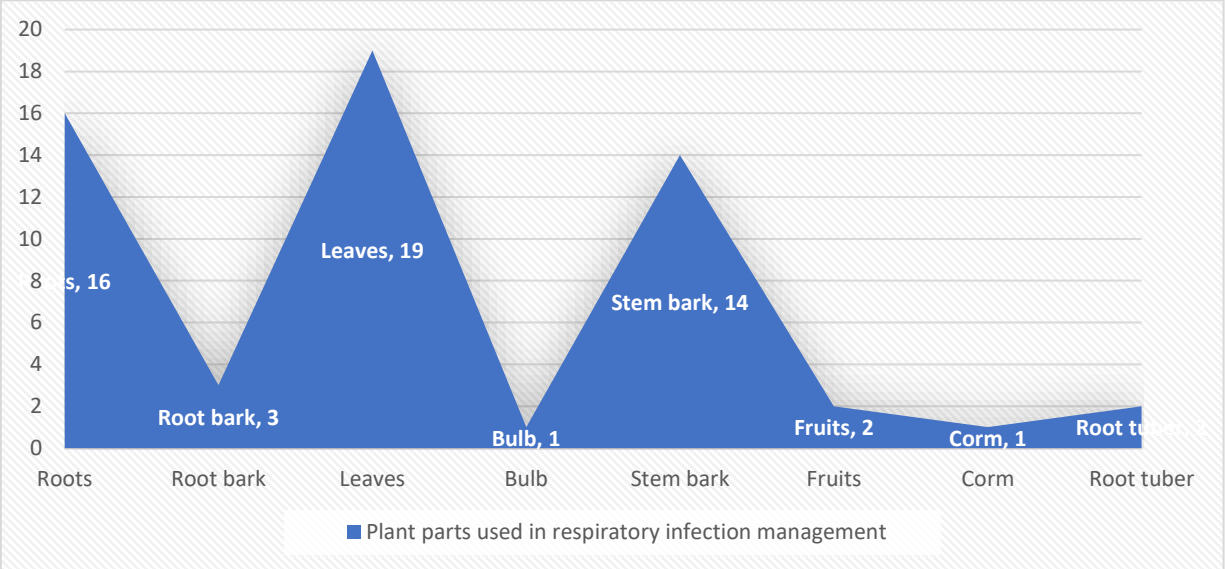


Figure 4. 1: Distribution of plant families used in managing respiratory infections in Kisumu East Sub- County, Kenya

The families Rutaceae and Leguminosae were dominant with 5 species each. The families Lamiaceae and Asteraceae had 3 species each. Of the identified plant species, they comprised; trees 20 (44.4%), shrubs 17 (37.8%), herbs 4 (8.9%), climbers 3 (6.7%) and corms 1 (2.2%).

Majority of the plants were sourced from the wild 42 (79.2%) while some were grown within the traditional practitioner's homestead 11 (20.8%). *Euclea divinorum*, *Tylosema fassoglense* and *Carissa edulis* were the most cited plants with Relative Frequency of Citation (RFC) values of 0.73, 0.67, and 0.67 respectively. Figure 4.2 below provides a summary of frequency of plant parts mentioned by the respondents used in preparation of remedies used for respiratory



infections.

Figure 4. 2: Plant parts used in management of respiratory infections

Leaves were the most frequently mentioned by the 19 Traditional Medicine Practitioners (TMPs) (32.8%). Roots accounted for 16 (27.6 %) with the stem bark accounting for 14 (24.1%). The other plant parts accounted for the remaining 15.5%.

4.1.3. Dosage, mode of preparation and route of administration

The common methods for preparation of herbal remedies were decoction 33 (68.8%), concoction 10 (20.8%) and chewing 2 (4.2%). Decoction were prepared by boiling a plant part of a particular

plant species in water while concoctions were prepared by boiling plant parts of different plants species in water. Decoctions and concoctions would be prepared by the herbalist. Alternatively, the herbalist would instruct the patient on how to prepare the remedy. Prepared decoctions and concoctions were sold to patients in plastic bottles varying between 500 ml to 2 L.

Other methods included cold maceration and powdering. The plants would be harvested, air dried and then crushed into powder. Some would be taken to the local market and ground to powder.

The major route of administration was the oral route. For management of common cold, a blanket would be used to cover the patient and a pot emanating with steam from boiling leaves of *Eucalyptus camaldulensis* for a few minutes after which the patient takes 2 teaspoons of the decoction. The herbalists reported that remedies rarely failed and failure was attributed to patients not following instructions.

4.1.4. Previous reported ethno medicinal uses and pharmacological activity of the medicinal plants documented in this study.

Of the 45 identified plant species, 43 have been reported to have various ethnomedicinal uses and pharmacological activity. This is summarized in Table 4.3 below.

Table 4. 3: Literature review of medicinal plants used for respiratory illnesses in Kisumu East Sub -County

Plant name	Reported traditional use	Reported pharmacological/chemical activity
Acanthus polystachius Delile	Malaria (Asnake et al., 2016).	Wound healing activity (Demilew et al., 2018). Antimalarial activity (Derebe and Wubetu, 2019)
Aloe kedongensis Reynolds	Malaria (Pascaline et al., 2011).	Antiplasmodial, leishmanicidal activity (Kigondu et al., 2009).
Allium sativum fo. *pekinense* (Prokhanov) Makino	Malaria, wound disinfectant, intestinal infections (Kasali et al., 2014). Cold (Kankara et al., 2015). Aphrodisiac (Singh et al., 2012).	Antimicrobial activity (Benkeblia, 2004). Antiparasitic, antiviral activity (Ankri and Mirelman, 1999).
Rhus natalensis var. *comorensis* Engl.	Diarrhea, influenza (Kareru et al., 2008). Respiratory disorders, malaria (Kimondo et al., 2015).	Antinociceptive (Kariuki et al., 2012). Aqueous extract possessed antibacterial activity (Kareru et al., 2008).
Steganotaenia araliacea Hochst.	Skin diseases (Taddese et al., 2003). Tuberculosis (Bunalema et al., 2014).	Antibacterial (Lino and Deogracious, 2006). Diuretic activity however causes damage to vital organs (Agunu et al., 2005).
Carissa edulis var. *ambungana* Pichon	Respiratory infections (Kariuki and Njoroge, 2011) Chest pains (Nedi et al., 2004; Kigen et al., 2019)	Antibacterial (Abdu et al., 2008). Presence of alkaloids, sterols and resins (Abdu et al., 2008)
Artemisia subsect. annua (Rydb.) Krasnob.	Fever (Kankara et al., 2015).	Antimicrobial (Tajehmiri et al., 2014). Camphor (Bilia et al., 2014). Methanolic leaf extract had antioxidant (Iqbal et al., 2012).
Microglossa pyrifolia (Lam.) Kuntze.	Ovarian cyst (Kasali et al., 2014) Malaria (Guédé et al., 2010; Kasali et al., 2014).	Antioxidant activity, Flavonoids (Akimanya et al., 2015).
Tithonia diversifolia subsp. *glabriuscula* S.F. Blake	Diabetes, malaria (Mwanauta et al., 2014; Passoni et al., 2013). Abscesses, snake bites (Passoni et al., 2013).	Ethanolic extracts of aerial parts have ant plasmodial activity (Elufioye and Agbedahunsi, 2004). Aqueous extract of stem had antibacterial and antifungal activities (Liasu and Ayandele, 2008). Tagitinine C has anti- plasmodium activity (Goffin *et al*., 2002).
Kigelia africana subsp. *moosa* (Sprague) Bidgood & Verdc.	Pneumonia (Chenia, 2013). Tuberculosis (Bunalema et al., 2014). Measles in children (Kigen et al., 2019)	Stem bark and fruits extracts exhibited antibacterial activities (Grace et al., 2002). Antifungal (Owolabi et al., 2007). Aqueous fruit extracts had antifungal and anti-giardial activity and anticancer properties

		(Arkhipov et al., 2014).
Commiphora Africana var. *venosa* (Mattick) Govaerts	Malaria, fever (Nadembega et al., 2011). Swollen testicles and abdominal pains (Kigen et al., 2019). Pneumonia (Kareru et al., 2008).	Root extract possessed antifungal activity (Akor and Anjorin, 2009).
Warburgia salutaris (G. Bertol) Chiov	Cough (Maroyi, 2013). Yellow fever (Kuglerova et al., 2011). Common cold, malaria (Coopoosamy and Naidoo, 2012). Aspergillosis (Otang et al., 2012).	Acetone extract showed fungicidal activity Fusarium species (Samie and Mashau, 2013). Antimycobacterial activity (; Rabe and Van Staden, 2000; Clarkson et al., 2007).
Carica papaya var. *bady* Aké Assi	Malaria, liver disease (Asnake et al., 2016). Tuberculosis (Bunalema et al., 2014). Malaria (Ngarivhume et al., 2015; Agbodeka et al., 2016). Fever (Kankara *et al*, 2015).	Antibacterial activity. Glycosides, phenols present (Doughari et al., 2007).
Terminalia glabrata var. *brownii* Fosberg & Sachet	Cough, bronchitis (Khalid et al., , 2012; Salih et al., 2017) Allergy, Diabetes, malaria (Kareru et al., 2008; Salih et al., 2017) Clotting agent, coughs and joint stiffness (Kaigongi and Musila, 2015).	Anti-fertility effect (Kamita et al., 2014). Aqueous extract of bark possessed antibacterial (Kareru et al., 2008). Flavonoids, triterpenoids (Kamita et al., 2014)
Ipomoea kituiensis var. *hirsutissima* Verdc.	Constipation, digestive disorders (Kaigongi and Musila, 2015).	Methanol in DCM (1:1v/v) leaves extract showed acaricidal activity (Onyango, 2016).
Euclea divinorum Hiern.	Stomachache (Kidane et al., 2014). Bleeding (Cheikhyoussef et al., 2011). Diarrhea, typhoid, stroke (Kamau et al.,2016). Toothache (Kaigongi and Musila, 2015).	Contractile activity (*Kaingu* et al., 2012).
Croton megalocarpus Hutch.	Influenza, pneumonia (Kamau et al., 2016).	Antibacterial, antifungal. Presence of triterpenes, chalcones (Kisangau et al., 2007). Antifungal (Kiswii et al., 2014).
Croton dichogamous Pax	Chest congestion (Kipkore et al., 2014). Pesticidal activity (Qwarse et al., 2018) Chest pains (Kigen et al., 2019). Threatened abortion, infertility (Kaingu et al., 2013).	No reports
Harungana	GIT disorders (Okoli et al.,	Activity against *S. Typhimurium* (Kengni et al.,

madagascariensis Lam. Ex Poir	2002)	2013). Astilbin (Moulari et al., 2006). Presence of triterpenoids, phenols (Kengni et al., 2013).
Gladiolus dalenii var. *andongensis* (Baker) Goldblattex Geerinck	Epilepsy, Nasopharyngeal infection, (Ngoupaye et al., 2014).	Antibacterial (Gbadamosi, 2012). Antifungal activity against *Aspergillus niger* (Odhiambo et al., 2010). Presence of alkaloids, saponins, cardenolides (Gbadamosi, 2012)
Clerodendrum myricoides subsp. *namibiense* R. Fern.	Malaria (Moshi et al., 2010). Febrile convulsions, Abdominal colic (Moshi et al., *2012*). Respiratory infections (Kariuki and Njoroge, 2011). Pneumonia (Kareru et al., 2008).	Organic solvent root extract had antibacterial and antifungal activity (Njeru et al., 2016). Antibacterial activity (Matu and van Staden, 2003; Kareru et al., 2008). Anti-plasmodial activity (Deressa et al., 2010)..
Plectranthus barbatus var. *grandis* (L.H Cramer) Lukhoba & A. J. Paton	Abdominal pain, diarrhea (Kigen et al., 2016). Tuberculosis (Bunalema et al., 2014). Malaria (Nguta et al., 2010). Wounds, swelling, joint pain, stomach problems, malaria (wanjiku Ngari, 2010). Asthma (Yashaswini and Vasundhara, 2011).	Larvicidal properties (Govindarajan et al., 2016). α-pinene, manool (Kerntopf et al., 2002). Anticonvulsant activity (Borges Fernandes et al., 2012).
Vitex doniana Sweet	Diabetes, ulcers (Osuagwu and Eme, 2013). Malaria, Measles (Lagnika et al., 2012). Gastroenteritis, Diarrhea (Fadeyi et al., *2*013). Diuretic, diabetes (Muanda et al., 2011)	Antimicrobial properties (Kilani, 2006; Ali et al., 2017). Aqueous leaf extract possessed antioxidant activity (Agbafor and Nwachukwu, 2011). Presence of alkaloids, anthraquinones, flavonoids (Agbafor and Nwachukwu, 2011). Hydroalcoholic Stem bark extract had wound healing properties (Amégbor et al., 2012).
Acacia robusta subsp. *usambarensis* (Taub.) Brenan	Malaria (Belayneh et al., 2012). Fibroids (Kaingu et al., 2013).	Antifungal (Hamza et al., 2006).
Albizia zygia (DC.) J.J. Macbr.	Antimalarial (Abdalla and Laatsch, 2012; Kokila et al., 2013). Anticancer (Appiah- Opong et al., 2016). Cough, fever (Oloyede and Oguniade, 2013). Bronchial disease, fever (Okpo et al., 2016).	Antimicrobial activity. Presence of alkaloid, saponin, (Oloyede and Oguniade, 2013). Anti-inflammatory, antioxidant (Olarbi et al., 2016).
Rhynchosia elegans A. Rich.	Malaria, common cold, fever (Asnake et al., 2016).	No report
Tamarindus indica L.	Malaria (Ali et al., 2004; Pierre et al., 2011). Constipation, jaundice (Khalid et al., 2012). Aphrodisiac (Singh et al., 2012). General wellbeing (Kankara et	Antibacterial (Doughari, 2006; Abukakar et al., 2008). Presence of tannins, phlobatamins, alkaloids, saponins, sesquiterpenes (Doughari, 2006).

	al., 2015). Sexually Transmitted Infections (Kaigongi and Musila, 2015).	
Tylosema fassoglense (Kotschy ex Schweinf.) Torre & Hillc.	Cancer (Kigen et al., 2016).	Methanol extracts had antibacterial activity (Adongo et al., 2012). Antifungal (Ochanga and Chacha, 2016).
Azadirachta indica A. Juss	Malaria (Ali et al., 2004; Njoroge and Bussmann, 2006) Scabies, control blood sugar levels (Singh et al.,2012). Tuberculosis (Bunalema et al., 2014).	Antibacterial (Akpuaka et al., 2013) Antioxidant, antibacterial. Triterpenoids, steroids (Pandey et al., 2014).
Khaya senegalensis (Desr.) A. Juss	Diabetes (Karou et al., 2011). Hepatic inflammations, sinusitis (Khalid et al., 2012). Malaria (Agbodeka et al., 2016).	Antibacterial (Ugoh et al., 2014). Ethyl acetate extract had hypoglycemic activity (Muhammad et al., 2016). Aqueous extract showed hepato- protective and hepatotoxicity effects (Muhammad et al., 2015). Ethanolic extract possessed antioxidant activity (Ibrahim et al., 2014). Presence of anthroquinones (Ugoh et al., 2014).
Mollugo nudicaulis Lam.	Jaundice (Nagesh and Shanthamma, 2011).	Antioxidant activity (Rameshkumar and Sivasudha, 2012). Whole plant had antidiabetic properties (Sindhu et al., 2010). Presence of steroids, reducing sugars (Rameshkumar and Sivasudha, 2012).
Moringa oleifera Lam.	Malnutrition (Nadembega et al., 2011). Tuberculosis (Bunalema et al., 2014). Loss of memory, prostate cancer (Kamau et al., 2016). Flu, asthma, hypertension, malaria (Kasolo et al., 2010).	Antibacterial (Caceres et al., 1991). Flavonoids, terpenoids, steroids (Kasolo et al., 2010).
Eucalptus camaldulensis subsp. arida Brooker & M.W.McDonald	Tuberculosis (Bunalema et al., 2014). Malaria, liver disorders (Nadembega et al., 2011). Respiratory tract congestion, chronic bronchitis, coughing, tuberculosis (Basak and Candan, 2010).	Antibacterial (Ghalem and Mohamed, 2008). (Adeniyi et al., 2009) Chloroform leaf extract had activity against Antimycobacterial (Gemechu et al., 2013).
Syzygium cumini var. *obtusifolium* (Roxb.) K.K. Khanna	Asthma, bronchitis, sore throat (Ayyanar and Subash-Babu, 2012). Coughing, diabetes, dysentery, ringworm, inflammation (Swami et al.,2012). Diarrhea, dysentery, wounds, constipation (Singh et al., 2012).	Bark extract exhibited anti-inflammatory activity (Muruganandan et al., 2001). Aqueous extract had hypoglycemic and antihyperglycemic activity (Saravanan and Pari, 2008).
Ximenia Americana var. argentinensis DeFilipps	Throat infection, amenorrhea, pain (Le et al., 2012).	Root and stem bark extract had antimicrobial activity (James et al., 2007). Methanolic stem bark extract had antioxidant activity (Maikai et al.,

		2010). Tannins, cyanogenetic glycosides (Ogunleye and Ibitoye, 2003). Terpenoids, glycosides, steroids, phenols and triterpenoids (Kamita et al., 2014).
Clematis hirsuta var. *junodii* (Burtt Davy) W.T Wang	Colds, cleanser (Kamau et al., 2016). Common cold, chest problems (wanjiku Ngari, 2010).	Antifungal activity against *Candida albicans* (Cos et al., 2002).
Gardenia ternifolia var. *goetzei* (Stapf & Hutch.) Verdc.	Hypertension (Karou et al., 2011). Treat dysentery, urinary tract infections (Silva et al., 1996).	Antimicrobial activity (Silva et al., 1996). Root bark extract exhibited *in -vivo* antiplasmodial activity (Nureye et al., 2018). Ethanolic extract exhibited viricidal activity (Silva et al., 1997).
Keetia gueinzii (Sond.) Bridson	Malaria (Njoroge and Bussmann, 2006).	No reports
Harrisonia abyssinica Oliv.	Arthritis, STIs (Kimondo et al., 2015). Stomach ache, coughs, malaria (Kaigongi and Musila, 2015). Malaria (Nguta et al., 2010).	Antifungal activity (Fabry et al., 1996). Antiviral, antifungal, antibacterial and molluscicidal activity (Balde et al., 1995).
Teclea nobilis Delile	Antipyretic (Wabe et al., 2011). Common cold, pneumonia, chest pain (wanjiku Ngari, 2010). Pneumonia and arthritis (Kigen et al., 2019).	Antipyretic, analgesic activities, anti-inflammatory (Mascolo et al., 1988; Al Rehaily et al., 2001). Anti-caseinolytic activity (Chayamiti et al., 2013).
Toddalia asiatica var. *parva* Z.M. Tan	Sore throat, Malaria (Orwa et al., 2008). Fever, stomach ache (Orwa et al., 2013). Common colds, cancer, (Kigen et al., 2016). Tuberculosis (Bunalema et al., 2014). Pneumonia (wanjiku Ngari, 2010). Colds, respiratory diseases (Kamau et al., 2016). Malaria and respiratory illnesses (Kigen et al., 2019).	Larvicidal activity (Borah et al., 2010). Antifungal activity against Candida albicans (Maobe et al., 2013). Anti-inflammation (Kariuki et al., 2013).
Zanthoxylum chalybeum var. molle Kokwaro	Tuberculosis (Bunalema et al., 2014). Malaria (Njoroge and Bussmann, 2006). Pneumonia (wanjiku Ngari, 2010). Cough, cervical cancer (Tugume *et al*., 2016).	Antibacterial (Matu and van Staden, 2003; Nguta and Kiraithe, 2019). Antihyperglycemic activity (Kimani et al., 2015). Anti-plasmodial activity (Kiraithe et al., 2016).
Zanthoxylum gilletii (De Wild.) P.G. Waterman	Malaria (Guédé et al., 2010).	Anti-plasmodial (Omosa and Okemwa, 2017). (E)-β-ocimene, E-nerolidol (Affouet et al., 2012). Sanguinarine, 8-Methylnorchelerythrine (Gaya et al., 2013).

Cissus rotundifolia var. *ferrugineopubescens* Verdc.	Contraception (Kaingu et al., 2013). Pain (Matu and van Staden, 2003). Malaria, liver disease and otitis (Hussein and Dhabe, 2018). Malaria (Ali et al., 2004).	Antibacterial activity (Alzoreky and Nakahara, 2003). Aqueous leaf extracts had hypoglycemic activity (Al-Mehdar and Al-Battah, 2016).
Rhoicissus revoilii Planch	Pneumonia, tonsillitis (Thoithi et al., 2014).	Ethanolic extract had good antifungal activity against *Candida albicans* (Thoithi et al., 2014).

4.2. Preliminary phytochemical analysis of root bark *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber of *Rhynchosia elegans* A. Rich.

4.2.1. Solvent extraction yields

Acetonic, methanolic and aqueous extraction yields are given in Table 4.1 below.

Table 4. 4: Percentage extraction yield of extraction solvents

Scientific name	**Extraction yield**		
	Aqueous (%)	**Acetone (%)**	**Methanol(%)**
Acanthus polystachius Delile	21.23	4.27	10.14
Keetia gueinzii (Sond.) Bridson	17.8	4.93	10.91
Rhynchosia elegans A. Rich.	20.21	0.78	5.18

The aqueous extracts from all the plant parts had a higher yield, followed by methanolic extract. Acetonic extract had the lowest yield. Aqueous extracts of *A. polystachyus* and *R. elegans* gave the highest recovery of 21.23% and 20.21% respectively, while *K. gueinzii* aqueous extract had a yield of 17.8%. The methanolic extract of *K. gueinzii* and *A. polystachyus* had a yield of 10.19% and 10.14% respectively. Acetone had the least yield recovery across the plant species, with *R. elegans* recording the lowest yield of 0.78%. *K. gueinzii* and *A. polystachyus* had an extraction yield of 4.93% and 4.27% respectively. The yield of extraction increased with polarity of the solvent used. The extraction yield of acetone was lowest as compared to absolute methanol and distilled water. This is an agreement with previous studies that reported extraction of 100% acetone to be lowest (Do et al., 2014).

4.2.2. Phytochemical screening

Screening of acetonic, methanolic and aqueous extracts of root bark of *K. gueinzii*, roots of *A. polystachius* and root tuber of R. *elegans* revealed presence of various phytoconstituents as presented in Table 4.5 below.

Table 4. 5: Preliminary phytochemical screening of crude root, root bark, and root tuber extracts of *Acanthus polystachius*, *Keetia gueinzii*, and *Rhynchosia elegans* respectively

Plant metabolite	Tests	Root extracts of *Acanthus polystachyus*			Root bark extracts of *Keetia gueinzii*			Root tuber extracts of *Rhynchosia elegans*		
		AQ	ACT	MEOH	AQ	ACT	MEOH	AQ	ACT	MEOH
Alkaloids	Dragendorrf's test	-	+	+	-	-	+	-	+	-
Flavonoids	Alkaline reagent test	-	+	+	+	-	-	-	-	-
Glycosides	Keller-killiani test	+	+	+	+	+	-	-	+	-
Phenols	Ferric chloride test	+	+	+	+	+	+	+	+	+
Saponins	Froth test	+	-	+	+	-	+	+	-	+
Tannins	Ferric chloride test	+	-	-	+	+	+	+	+	-
Terpenoids	Salkowski test	-	-	+	-	-	-	+	-	-

AQ: Aqueous extract; ACT: Acetone extract; MEOH: Methanol extract; +: metabolite present; -: metabolite absent

Methanolic extracts of *A. polystachyus* contained alkaloids, flavonoids, glycosides, phenols, saponins and terpenoids while the methanolic extract of *R. elegans* contained saponins and phenols. Methanolic extracts of *K. gueinzii* alkaloids, phenols, saponins and tannins.

Aqueous extracts of *K. gueinzii* possessed flavonoids, glycosides, phenols, saponins and tannins while extracts of *R. elegans* and *A. polystachius* contained saponins, phenols and tannins.

Acetonic extract of *A. polystachyus* contained alkaloids, phenols, flavonoids and glycosides while *K. gueinzii* contained phenols, glycosides and tannins.

4.3. *In-vitro* antimicrobial activity of root bark of *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber of *Rhynchosia elegans* A. Rich.

4.3.1. Minimum Inhibitory Concentration (MIC) Values

Antimicrobial results of the plant extracts against the four tested microorganisms are shown in Table 4.6.

Table 4. 6: Minimum inhibitory, Bactericidal, and Fungicidal concentrations of crude root, root bark, and root tuber extracts of *Acanthus polystachius, Keetia gueinzii, and Rhynchosia elegans* respectively.

Type of pathogen	Plant extract	MIC	MBC	MFC	MBC/MIC	MFC/MIC
Bacillus	*Acanthus*					
Cereus	*polystachyus*					
(Gram +ve)	AQ	25	50	-	2	-
	ACT	12.5	12.5	-	1	-
	MEOH	100	200	-	2	-
	Keetia gueinzii					
	AQ					
	ACT	50	100	-	2	-
	MEOH	12.5	12.5	-	1	-
		25	50	-	2	-
	Rhynchosia					
	elegans					
	AQ	ND	ND	-	ND	-
	ACT	*	*	-	*	-
	MEOH	200	400	-	2	-
Staphylococcus	*Acanthus*					
aureus	*polystachyus*					
(Gram +ve)	AQ	ND	ND	-	ND	-
	ACT	ND	ND	-	ND	-
	MEOH	200	400	-	2	-
	Keetia gueinzii					

	AQ					
	ACT	12.5	12.5	-	1	-
	MEOH	12.5	12.5	-	1	-
		25	50	-	2	-
	Rhynchosia					
	elegans					-
	AQ					-
	ACT	ND	ND	-	ND	-
	MEOH	*	*	-	*	
		200	400	-	2	
	Amoxicillin	0.00156	0.00156	-	1	-
Escherichia coli	*Acanthus*					
(Gram –ve)	*polystachyus*					
	AQ	ND	ND	-	ND	-
	ACT	ND	ND	-	ND	-
	MEOH	200	400	-	2	-
	Keetia gueinzii					
	AQ					
	ACT	200	400	-	2	-
	MEOH	200	400	-	2	-
		200	400	-	2	-
	Rhynchosia					
	elegans					
	AQ	ND	ND	-	ND	-

	ACT	*	*	-	*	-
	MEOH	ND	ND	-	ND	-
	Gentamycin	0.00156	0.00156	-	1	-
Candida albicans	*Acanthus polystachyus*					
	AQ	200	-	400	-	2
	ACT	ND	-	ND	-	ND
	MEOH	200	-	400	-	2
	Keetia gueinzii					
	AQ					
	ACT	ND	-	ND	-	ND
	MEOH	200	-	400	-	2
		100	-	200	-	2
	Rhynchosia elegans					
	AQ	ND	-	ND	-	ND
	ACT	*	-	*	-	*
	MEOH	ND	-	ND	-	ND
	Nystatin	0.00156	-	0.00156	-	1

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum fungicidal concentration; AQ: aqueous extract; ACT: acetone extract; MEOH: methanol extract; ND: Not determined (Did not display activity at the tested concentrations).; * Extract not subjected to antimicrobial screening. 0.00156 mg/ml was the lowest test concentration used for the positive controls.

Acetonic and methanolic extract of *K. gueinzii* showed broad spectrum activity against the test microorganism. MIC values of the extracts tested against *S. aureus* showed that acetonic and aqueous root bark extracts of *K. gueinzii* had the lowest MIC of 12.5 mg/ml. Methanolic extracts of *A. polystachius* and *R. elegans* showed activity against *S. aureus* with MIC values of 200 mg/ml.

All extracts exhibited activity against *B. cereus* at the tested concentrations with the exception of aqueous extract of *R. elegans*. Aqueous extract of *R. elegans* did not show activity against any of the microorganisms at the tested concentrations. The acetonic root bark extract of *K. gueinzii* and root extract of *A. polystachius* against *B. cereus* had a MIC value of 12.5 mg/ml. Aqueous extract of *A. polystachius* and methanolic extract of *K. gueinzii* activity against *B. cereus* had a MBC of 50 mg/ml.

Aqueous, acetonic and methanolic extract of *K. gueinzii* possessed activity against *E. coli* with a MIC value of 200 mg/ml. Methanolic extract *A. polystachyus* showed activity against *E. coli* with MIC of 200 mg/ml.

Aqueous extract of *A. polystachyus* possessed activity against *C. albicans* with a MFC of 400mg/ml. Methanolic extract of *K. gueinzii* had the lowest MIC value of 100mg/ml against *C. albicans* and a MFC value of 200 mg/ml. Acetonic extract of *R. elegans* was not subjected to antimicrobial testing due to a very low extraction yield.

Bacteriostatic activity was determined by MBC/MIC values of >4 while bactericidal activity with MBC/MIC values of < 4 (Djeussi et al., 2013). Fungistatic activity was determined by MFC/MIC values of >4 while fungicidal activity with MFC/MIC values of < 4 (Hazen, 1998; de Castro et al.,

2015). Based on the calculated values of ratios of MBC/MIC, aqueous, acetonic and methanolic extract of *K. gueinzii* were considered bactericidal against all the tested bacterial with values of < 4. Methanolic extract of *K. gueinzii* and *A. polystachyus* were considered fungicidal with MFC/MIC values of < 4.

4.4. Evaluation of cytotoxicity of root bark of *Keetia gueinzii* (Sond.) Bridson, roots of *Acanthus polystachius* Delile and root tuber of *Rhynchosia elegans* A. Rich. against brine shrimp (A*rtemia salina*) larvae.

4.4.1. Acute toxicity of the crude extracts

Results showed that aqueous extracts of root bark of *K. gueinzii* and root of *A. polystachyus* were non-toxic. However, aqueous root tuber extract of *R. elegans* with a LC_{50} of 422.09 µg/ml was considered to be moderately toxic to toxic against brine shrimp larvae, as tabulated in table 4.7. below.

Table 4. 7: Brine shrimp cytotoxicity of Dimethyl sulfoxide, vincristine sulphate and crude

			Mortality per test dose				Toxicity	
Plant species	**Part used**	**Solvent Used**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**	**LC_{50} (µg/ml)**	**Meyer's toxicity index**	**Clarkson's toxicity index**
Acanthus polystachius Delile	Roots	Acetone	0	1	50	195.17 (109.11-349.08)	Toxic	Moderately toxic
		Distilled Water	0	0	0	No death	Non-toxic	Non-toxic
		Methanol	0	6	49	174.26 (93.37-325.23)	Toxic	Moderately toxic
Keetia gueinzii (Sond.) Bridson	Root bark	Acetone	0	0	0	No death	Non-toxic	Non-toxic
		Distilled Water	0	0	3	148,735,210.7 (2,807,216.20-7,880,462,816)	Non-toxic	Non-toxic
		Methanol	0	0	1	1.0×10^{16} (2.0×10^{12}-5.1×10^{19})	Non-toxic	Non-toxic
Rhynchosia elegans A. Rich.	Root tuber	Acetone	0	2	50	175.77 (98.27-314.39)	Toxic	Moderately toxic
		Distilled Water	0	0	45	422.09 (199.52-893.59)	Toxic	Moderately toxic
		Methanol	0	5	50	168.76 (94.35-301.85)	Toxic	Moderately toxic
0.1% Dimethyl sulfoxide	_	_	0	0	0	No death	Non-toxic	Non-toxic
Vincristine sulphate	_	_	8	31	50	45.77 (19.75-106.06)	Toxic	Highly toxic

extracts of *Acanthus polystachius, Keetia gueinzii,* and *Rhynchosia elegans*

Methanolic extract of *K. gueinzii* was non-toxic with a LC_{50} value of 1.0×10^{16} µg/ml. However, methanolic extract of *A. polystachyus* and *R. elegans* with LC_{50} values of 174.26 µg/ml and 168.76 µg/ml were considered moderately toxic to toxic.

Acetonic extract of *K. gueinzii* was found to be non-toxic. *A. polystachyus* and *R. elegans* extracts were toxic with LC_{50} values of 195.17 µg/ml and 175.77 µg/ml respectively.

CHAPTER FIVE

DISCUSSION

5.1. Ethnobotanical survey

Continuous and timely documentation of medicinal plants used by communities' aids in preservation of ethnomedicinal knowledge. Within the study locality, majority of practitioners belonged to the older age group. This age group is considered to be a more likely custodian of traditional ethno medicinal knowledge and their advanced age shows the potential risk of loss of this information (Lambert et al., 2011; Mukungu et al., 2016). Inexperience among the youth with regard to traditional medicinal knowledge further impacts negatively on their acceptance by the community (Mukungu et al., 2016). The. Males were fewer as they resorted to other ventures to supplement income from herbal practice. It has been reported that a major portion of herbal practitioners practice medicine as a part time (Parthiban et al., 2016).

Majority of the respondents interviewed had little or no formal education. This serves to further reinforce the fact that herbal practice is a field left to the less educated members of the community (Mukungu et al., 2016). It serves to argue that younger people are not keen on learning the craft of herbal medicine. The extensive year of experience of male and female practitioners in the study serves to explain their rich experience and sharp expertise with regards to medicinal plants utilization.

Most of the plants cited belonged to the Leguminosae family. This observation is different from a similar study that reported Asteraceae as the dominant family of plant used for management of respiratory diseases in Pakistan (Alamgeer et al., 2018). The family Leguminosae is widely

distributed and is one of the largest plant families globally (Christenhusz and Byng, 2016). The dominance of Leguminosae family use for preparation of remedies in the study locality could be attributed to its worldwide distribution and presence of various secondary metabolites which are known to have pharmacological activity. This argument has been shared by other authors (Alamgeer et al., 2018).

The predominance of trees in the study could be attributed to their large presence and ease of availability. Trees are known to be more resistant to season variations and drought (Ahmed and Bassuony, 2009; Khan et al., 2013; Alamgeer et al., 2018). Leaves had the highest frequency of use which was also reported in similar studies. Leaves are important sites for photosynthesis and this could explain their high frequency of use (Mukungu et al., 2016; Alamgeer et al., 2018).

Over reliance of wilds plants poses a threat as some species are utilized to extinction (Kankara et al., 2015). It was observed that some plants were being uprooted during collection. This could be attributed to poor conservation and harvesting practices, scarcity of plant species and the extensive drought period. This is of concern as over usage of root parts and stem bark pose danger to plant biodiversity (Maroyi, 2013).

The dosage of indigenous remedy depended on the indication, severity of the illness and the years of experience of the practitioners. The practitioners emphasized on the importance of following laid down instructions on the preparation and administration of the herbal remedy. Dosage of the remedy was measured using teaspoons and tablespoons. Decoctions and concoctions were measured as cupful/glassful. However, there was ambiguity on the appropriate cup size and the sizes ranged between 300 ml to 500 ml. Previous studies have reported on this ambiguity

(Kankara et al., 2015). The herbalist reported that remedies rarely failed and had very minimal side effects. However, the capacity of the herbalist in identifying adverse effects of the remedies cannot be ascertained.

Various studies have been conducted in the country in an effort to conserve ethnomedicinal knowledge including; Kisumu (Kokwaro and Johns, 1998; Orwa et al., 2007), Kakamega (Mukungu et al., 2016), Lake Victoria region (Otieno et al., 2011) and Msambweni (Nguta et al., 2010). This serves as the first study that documents plants of medicinal value used for respiratory infection in Kisumu East Sub-County.

There is agreement with other herbal practitioners on the use of plants identified in the study in management of illnesses. *Carissa edulis* has been reportedly used in management of respiratory infections (Kariuki and Njoroge, 2011). Leaves of *Moringa oleifera* have been used for flu, asthma and hypertension (Basak and Candan, 2010). *Clematis hirsuta* has been reported for cold, body cleanser and chest problems (Kamau et al., 2016). *Harrisonia abyssinica* has been reported for use in management of cough and malaria (Nguta et al., 2010; Kaigongi and Musila, 2015). This overlap in utility suggests that there is agreement among practitioners from different geographical locations with regards to the use of medicinal plants.

From literature review, there is minimal or no pharmacological evaluation of *A. polystachius, K. gueinzii* and *R. elegans. A. polystachyus* has been reported in management of malaria and scorpion bites (Asnake et al., 2016; Demilew et al., 2018). *R. elegans* root has been used in treatment of backleg disease, anthrax and amoebiasis (Kidane et al., 2014). The leaves have been reported in treatment of malaria, fever and cold (Asnake et al., 2016).

5.2. Phytochemical constituents

Medicinal plants possess curative properties due to presence of various secondary metabolites (Pandey, 2015). It is of great interest that this serves as the first report on phytochemical composition of the above crude plant extracts.

Quantitative estimates of percentage crude yields were different. Variation in yields could be attributed to concentration of compounds, their polarity, structure of compounds, particle size of plant material which would have contributed to surface to volume ratio, types of compounds present, plant part used and compound similarities. Concentration of plant constituents varies with plant part used and across plant species and geographical location (Figueiredo et al., 2008).

The current study serves as the first report on the phytochemical constitution of *K. gueinzii, A. polystachyus* and *R. elegans*. Phytochemical screening showed that the roots, root bark and root tuber of the test plant materials contained various chemical constituents. Alkaloids, saponins, phenols, flavonoids and tannins were present in the plant parts. The variation in phytochemical present could be attributed to differences in solubility of the phytoconstituents in the various extraction solvents (Ngo et al., 2017).

The biological activity of a plant extract is dependent on the phytoconstituents present. These chemical constituents have been known to possess biological activity which is of health benefit. Flavonoids have been reported to exhibit analgesic, antimicrobial and anti-allergic activity (Wintola and Afolayan, 2015). Antioxidant properties of flavonoids have contributed to the treatment of several diseases including microbial infections, cancer, liver dysfunction and

cardiovascular disease (Mohammed et al., 2013). Phenols are known to possess antimicrobial, anti-inflammatory and antioxidant activities (Amoo et al., 2011).

These reported activities attributable to the phytochemicals will further serves as areas for further research in an effort to understand observed chemical and pharmacological properties of the plant extracts. Further work to quantify these phytoconstituents is recommended

5.3. Antimicrobial properties of various test extracts against test microorganisms

In -vitro antimicrobial activity assays are an important step in validating ethnomedicinal use of plants. In these study, organic and aqueous crude plant extracts showed varying degree of antimicrobial activity. The MIC value ranged from 12.5 mg/ml to 200 mg/ml.

The MBC for the aqueous and acetonic extract of *K. gueinzii* against *S. aureus* was 12.5 mg/mL, with the methanolic extract at 50 mg/ml. Acetonic crude extracts of *K. gueinzii* and *A. polystachyus* had MBC values of 12.5 mg/ml. These findings are of interest as *S. aureus* and *B. cereus* are known to being resistant to various antibiotics. *S. aureus* and *B. cereus* produce enterotoxins that can lead to septicemia and enteritis. The antimicrobial properties of *K. gueinzii* crude extracts could be attributable to presence of alkaloids, saponins, phenols, flavonoids and tannins. Phenols are known to possess antimicrobial, antioxidant and anti-inflammatory activities (Amoo et al., 2011; Jaberian, et al., 2013). Tannins present could also explain their activity against *S. aureus* as reported in earlier studies (Akiyama et al., 2001). They have been reported to inhibit growth of S. *aureus* and *P. aeruginosa* with MIC values of 4.01 mg/ml and 8.20 mg/ml respectively (Rodrigues et al., 2014).

The MFC of methanolic and acetonic crude extracts of *K. gueinzii* against *C. albicans* was 200 mg/ml and 400 mg/ml respectively. Likewise, acetonic and methanolic crude extract of *A. polystachyus* had MFC of 400 mg/ml each. These could be attributed to presence of phenols. Thymol, a phenol derivative has been shown to reduce biofilm mass formed by *C. albicans* significantly reducing the number of viable cells (Guo *et al.*, 2009; Vasconcelos et al., 2014). Carvacrol, a phenol, has exhibited activity against strains of *C. neoformans*. It acts by causing fungal membrane instability. Antibacterial and antioxidant activity of carvacol has also been reported (Jaberian et al., 2013; Nóbrega et al., 2016). This could explain the observed fungicidal activity of acetonic and methanolic extracts of *K. gueinzii* against *C. albicans.*

Aqueous extract of *R. elegans* showed no activity against any of the tested microorganisms despite the presence of phenols, tannins, saponins and terpenoids which are known to possess antimicrobial properties (Mastelic et al., 2005). Bioactivity cannot be completely ruled out. Observed lack of bioactivity could be due to antagonistic effects of other constituents in the extract or low concentration of bioactive constituents. The crude extract could possibly have activity against other microorganisms that cause respiratory ailments. Furthermore, antimicrobial effects of the plants could be due to immunostimulant activity, or compounds present that require activation by enzymes to bring about therapeutic effect *in-vivo* (Madikizela et al., 2013).

Methanolic extract of *R. elegans* showed no activity against gram-negative bacteria and fungi but showed activity against gram positive bacteria. This could be attributed to presence of saponins which have been reported to possess inhibitory effect on gram-positive organism but not on gram negative organism and the fungi (Oyekunle et al., 2006). However, Acaciaside A and B, two

acylated bisglycoside saponins have reportedly exhibited antifungal and antibacterial activities (Mandal et al., 2005).

5.4. Cytotoxicity evaluation of crude extracts

Evaluation of toxicity of plant extracts is important in order to ascertain safety on administration. It is of importance to note that these calculations offer a significant preliminary data for further toxicity testing (Hamidi et al., 2014).

Findings from the current study reports for the first-time cytotoxicity of the crude extracts derived from *K. gueinzii*, *A. polystachyus* and *R. elegans*. The degree of lethality of the extract was proportional to concentration of the extracts. Aqueous crude extract of *R. elegans* was considered moderately toxic to toxic with an LC_{50} value of 422.09 µg/ml. This brings concern on continued use of decoctions and concoctions that containing the said plant. The traditional medicinal practitioners did not report any adverse effects of use of the plants apart from diarrhea. This could be attributed to poor hygiene and potential toxicity of the plants as shown from the results. Lack of reported adverse effects of *R. elegans* could be attributed to its use in small quantities during preparation of concoctions in management of respiratory infections in the study area. However, its use should be carried out with caution as the extent of cumulative toxicity of the plant is not known. Consideration should be made for dose adjustment of the plant extracts before administration. Substitute of the plant should also be considered as other plants have been equally mentioned to be used for respiratory infections. It is important to note that toxic plant extracts may have medicinal potential which may not be antimicrobial. A study by Sautron and Cock,

(2014) demonstrated that toxicity in bioassays using *A. franciscana* nauplii may indicate anti-cancer potential.

Acetonic extracts of *A. polystachyus* and *R. elegans* were considered moderately toxic to toxic and further *in-vivo* studies are recommended to ascertain extent of toxicity. This suggest that there is presence of potent cytotoxic components. Presence of alkaloids in the crude extracts could be attributed to the reported cytotoxicity. Previous studies have demonstrated the cytotoxic and anticancer activity of alkaloids (Lamchouri et al., 2000; Ma et al., 2004). Herbalists from the study area could still be trusted with the information since they prepare decoctions and concoctions using water, which according to the current study, was not toxic.

Aqueous, methanolic and acetonic extracts from the root bark extracts of *K. gueinzii* showed that they were non-toxic. The aqueous and methanolic extracts had LC_{50} values greater than 1,000 µg/ml. This suggest that antimicrobial activity of the preparation is not related to its inherent cytotoxicity. This further suggest that *K. gueinzii* can be safely used in management of respiratory infections. Further work is recommended to quantify and separate pure compounds. We recommend for further acute toxicity testing on animal model to correlate the two methods of toxicity evaluation. Clinical studies should be conducted to have a better understanding of their toxicity profile.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The Luo community resident in Kisumu East Sub – County had a wealth of ethnomedicinal knowledge. Forty-five plant species were identified and documented for management of respiratory diseases. Roots, root barks and root tubers were predominantly used in the preparation of herbal remedies by the herbalist in the study area which threatens the ecological survival of some of the plants species used. Tested crude plant extracts of root bark of *Keetia gueinzii*, root of *Acanthus polystachyus* and root tuber of *Rhynchosia elegans* showed presence of various phytoconstituents. Phenols were detected in all the crude extracts of the studied plants.

K. gueinzii extracts exhibited antimicrobial activity at the tested concentrations. Methanolic extract of *A. polystachius* showed antimicrobial activity with acetonic and aqueous extracts exhibiting activity against *B. cereus* and *C. albicans.* Aqueous and organic crude extracts of *Keetia gueinzii* were all nontoxic to Artemia salina larvae and therefore considered safe for use in management of respiratory illness. All extracts of Rhynchosia elegans were toxic to Artemia salina larvae and it use in treatment in human population should be done while exercising caution.

6.2. Recommendations

Efforts should be made to documentation and preservation of ethnomedicinal knowledge within the study locality. Further studies should be conducted to quantify phytochemicals present and other phytoconstituents present when other extractive solvents are used. In addition, further antimicrobial evaluation studies should be carried out to validate the use of these plants. Some plants were being used as decoctions and concoctions against respiratory infections but did not appear to possess antibacterial or antifungal activity at the tested concentration.

Present observations have shown that some of the tested crude extracts were toxic. It is recommended that *in-vivo* studies be conducted to determine the possible risk of continued use of *R. elegans* to the patients. Results of this study serve as an opportune starting point to further investigate the studied and commonly used medicinal plants amongst the Luo community for possible isolation of active moieties to combat ever rising concern of resistance to antimicrobials. Due to increase of new and emerging diseases, there is an urgent need of new agents with diverse novel mechanisms of action and chemical structures (Cheesman et al., 2017).

REFERENCES

Abdalla, M. A., and **Laatsch, H. (2012)**. Flavonoids from Sudanese *Albizia zygia* (Leguminosae, subfamily Mimosoideae), a plant with antimalarial potency. *African Journal of Traditional, Complementary and Alternative Medicines*, ***9*(1)**, 56-58.

Abdu, K. B., Khan, M. E., and **Rumah, M. M. (2008).** Antimicrobial activity and phytochemical screening of extracts from the root bark of *Carissa edulis*, against human/animal pathogens. *Continental Journal of Tropical Medicine, **2***, 1.

Abukakar, M. G., Ukwuani, A. N., and **Shehu, R. A. (2008).** Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. *Asian Journal of. Biochemistry, **3*(2)**, 134-138.

Adeniyi, C. A., Lawal, T. O., and **Mahady, G. B. (2009).** In vitro susceptibility of *Helicobacter pylori* to extracts of *Eucalyptus camaldulensis* and *Eucalyptus torelliana*. *Pharmaceutical Biology, **47*(1)**, 99-102.

Adongo, J. O., Omolo, J. O., Njue, A. W., and **Matofari, J. W. (2012).** Antimicrobial activity of the root extracts of *Tylosema fassoglensis* Schweinf. Torre & Hillc (Caesalpiniaceae). *Science Journal of Microbiology*.

Affouet, K. M., Tonzibo, Z. F., Attioua, B. K., and **Chalchat, J. C. (2012).** Chemical Investigations of Volatile Oils from Aromatic Plants Growing in Côte d'ivoire: *Harisonia*

abyssinica Oliv., *Canarium schwerfurthii* Engl. *Zanthoxylum gilletti* (De wild) Waterm. and *Zanthoxylum zanthoxyloides* Lam. *Analytical Chemistry Letters, 2*(6).

Agbafor, K. N., and **Nwachukwu, N. (2011).** Phytochemical analysis and antioxidant property of leaf extracts of *Vitex doniana* and *Mucuna pruriens*. *Biochemistry Research International, 2011*.

Agbodeka, K., Gbekley, H.E., Karou, S.D., Anani, K., Agbonon, A., Tchacondo, T., Batawila, K., Simpore, J. and **Gbeassor, M., (2016).** Ethnobotanical study of medicinal plants used for the treatment of malaria in the plateau region, Togo. *Pharmacognosy Research, **8*** **(Suppl 1)**, S12.

Agunu, A., Abdurahman, E. M., Andrew, G. O., and **Muhammed, Z. (2005).** Diuretic activity of the stem-bark extracts of *Steganotaenia araliacea* hochst [Apiaceae]. *Journal of Ethnopharmacology, **96*****(3)**, 471-475.

Ahmed, A. A., and **Bassuony, N. I. (2009).** Importance of medical herbs in animal feeding. *World Journal of Agriculture Science,* **5(4)**, 456-465.

Akimanya, A., Midiwo, J.O., Matasyoh, J., Okanga, F., Masila, V.M., Walker, L., Tekwani, B.L., Muhammad, I. and **Omosa, L.K., (2015).** Two polymethoxylated flavonoids with antioxidant activities and a rearranged clerodane diterpenoid from the leaf exudates of Microglossa pyrifolia. *Phytochemistry Letters, **11**,* 183-187.

Akinpelu, D. A., and **Onakoya, T. M. (2006).** Antimicrobial activities of medicinal plants used in folklore remedies in south-western. *African journal of Biotechnology, **5*****(11)**.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T., and **Iwatsuki, K. (2001)**. Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, **48(4)**, 487-491.

Akor, J. S., and **Anjorin, T. S. (2009)**. Phytochemical and antimicrobial studies of *Commiphora africana* root extracts. *International Journal of Agriculture and Biology*, ***11*(6)**, 795-797.

Akpuaka, A., Ekwenchi, M. M., Dashak, D. A., and **Dildar, A. (2013)**. Biological activities of characterized isolates of n-hexane extract of *Azadirachta indica* A. Juss (Neem) leaves. *Nature and Science*, ***11*(5)**, 141-147.

Al Rehaily, A. J., El Tahir, K. E., Mossa, J. S., and **Rafatullah, S. (2001).** Pharmacological studies of various extracts and the major constituent, lupeol, obtained from hexane extract of *Teclea nobilis* in rodents. *Natural Product Sciences*, ***7*(3)**, 76-82.

Alamgeer, Younis, W., Asif, H., Sharif, A., Riaz, H., Bukhari, I. A., and **Assiri, A. M. (2018).** Traditional medicinal plants used for respiratory disorders in Pakistan: a review of the ethno-medicinal and pharmacological evidence. *Chinese Medicine*, **13(1)**,48.

Ali, A. A., Al- Rahwi, K., and **Lindequist, U. (2004)**. Some medicinal plants used in Yemeni Herbal Medicine to treat. *African journal of Traditional, Complementary and Alternative Medicines*, ***1*(1)**, 72-76.

Ali, M., Aminu, F., and **Ibrahim, I. (2017).** In-vitro assessment of antibacterial activity and phytochemical screening of *Vitex doniana* on clinical isolate of *Salmonella typhi*. *International Journal of Advanced Academic Research*, ***3*(1)**.

Al-Mehdar, A. A., and **Al-Battah, A. M. (2016).** Evaluation of Hypoglycemic Activity of *Boswellia carterii* and *Cissus rotundifolia* in Streptozotocin/Nicotinamide-Induced Diabetic Rats. *Yemeni Journal for Medical Sciences, **10***, 30-38.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and **Lightfoot, D. A. (2017)**. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants, **6*** **(4)**, 42.

Alzoreky, N. S., and **Nakahara, K. (2003)**. Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology, **80*** **(3)**, 223-230.

Amégbor, K., Metowogo, K., Eklu-Gadegbeku, K., Agbonon, A., Aklikokou, K. A., Napo-Koura, G., and **Gbeassor, M. (2012).** Preliminary evaluation of the wound healing effect of *Vitex doniana* sweet (Verbenaceae) in mice. *African Journal of Traditional, Complementary and Alternative Medicines, **9*****(4)**, 584-590.

Amoo, S. O., Ndhlala, A. R., Finnie, J. F., and **Van Staden, J. (2011)**. Antifungal, acetylcholinesterase inhibition, antioxidant and phytochemical properties of three Barleria species. *South African Journal of Botany, **77*****(2)**, 435-445.

Angerhofer, C. K., Guinaudeau, H., Wongpanich, V., Pezzuto, J. M., and **Cordell, G. A. (1999).** Antiplasmodial and cytotoxic activity of natural bisbenzylisoquinoline alkaloids. *Journal of Natural Products*, **62(1)**, 59-66.

Ankri, S., and **Mirelman, D. (1999).** Antimicrobial properties of allicin from garlic. *Microbes and Infection, **1*****(2),*** 125-129.

Appiah- Opong, R., Asante, I. K., Safo, D. O., Tuffour, I., Ofori-Attah, E., Uto, T., and **Nyarko, A. K. (2016).** Cytotoxic effects of *Albizia zygia* (DC) JF Macbr, a Ghanaian medicinal plant, against human T-lymphoblast-like leukemia, prostate and breast cancer cell lines. *International Journal of Pharmacy and Pharmaceutical Sciences, **8*****(5)**, 392-6.

Arkhipov, A., Sirdaarta, J., Rayan, P., McDonnell, P. A., and **Cock, I. E. (2014).** An examination of the antibacterial, antifungal, anti-Giardial and anticancer properties of *Kigelia africana* fruit extracts. *Pharmacognosy Communications, **4*****(3)**.

Arthur, M. (2014). Institute for Health Metrics and Evaluation. *Nursing Standard (2014+), **28*****(42)**, 32.

Asadbeigi, M., Mohammadi, T., Rafieian-Kopaei, M., Saki, K., Bahmani, M., and **Delfan, M. (2014).** Traditional effects of medicinal plants in the treatment of respiratory diseases and disorders: an ethnobotanical study in the Urmia. *Asian Pacific Journal of Tropical Medicine, **7***, S364 -S368.

Asnake, S., Teklehaymanot, T., Hymete, A., Erko, B., and **Giday, M. (2016).** Antimalarial medicinal plants used by Gumuz people of Mandura Woreda, Benishangul-Gumuz Regional State, Ethiopia. *Indian Journal of Traditional Knowledge, **15*****(4)**, 546-552.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H. and **Rollinger,**

J.M., (2015). Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances, **33*****(8)**, 1582-1614.

Ayukekbong, J. A., Ntemgwa, M., and **Atabe, A. N. (2017).** The threat of antimicrobial resistance in developing countries: causes and control strategies. *Antimicrobial Resistance & Infection Control, **6*****(1)**, 47.

Ayyanar, M., and **Subash-Babu, P. (2012).** *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asian Pacific Journal of Tropical Biomedicine, **2*****(3)**, 240-246.

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medicinal and Aromatic Plants, **4*****(196)**, 2167-0412.

Balde, A. M., Pieters, L., De Bruyne, T., Geerts, S., Vanden Berghe, D., and **Vlietinck, A. (1995)**. Biological investigations on *Harrisonia abyssinica*. *Phytomedicine, **1*****(4)**, 299-302.

Basak, S. S., and **Candan, F. (2010).** Chemical composition and in vitro antioxidant and antidiabetic activities of *Eucalyptus camaldulensis* Dehnh. essential oil. *Journal of the Iranian Chemical Society, **7*****(1)**, 216-226.

Bates, M., Mudenda, V., Mwaba, P., and **Zumla, A. (2013)**. Deaths due to respiratory tract infections in Africa: a review of autopsy studies. *Current Opinion in Pulmonary Medicine, **19*****(3)**, 229-237.

Belayneh, A., Asfaw, Z., Demissew, S., and **Bussa, N. F. (2012).** Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of Babile Wereda, Eastern Ethiopia. *Journal of Ethnobiology and Ethnomedicine,* ***8*****(1)**, 42.

Benkeblia, N. (2004). Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *LWT-Food Science and Technology,* ***37*****(2)**, 263-268.

Bhuyan, G.S., Hossain, M.A., Sarker, S.K., Rahat, A., Islam, M.T., Haque, T.N., Begum, N., Qadri, S.K., Muraduzzaman, A.K.M., Islam, N.N. and **Islam, M.S., (2017).** Bacterial and viral pathogen spectra of acute respiratory infections in under-5 children in hospital settings in Dhaka city. *PloS One,* ***12*****(3)**.

Bilia , A. R., Santomauro, F., Sacco, C., Bergonzi, M. C., and **Donato, R. (2014).** Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. *Evidence-Based Complementary and Alternative Medicine, 2014*.

Borah, R., Kalita, M. C., Kar, A., and **Talukdar, A. K. (2010)**. Larvicidal efficacy of *Toddalia asiatica* (Linn.) Lam against two mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. *African Journal of Biotechnology,* ***9*****(17)**, 2527-2530.

Borges Fernandes, L. C., Campos Camara, C., and **Soto-Blanco, B. (2012).** Anticonvulsant activity of extracts of *Plectranthus barbatus* leaves in mice. *Evidence-Based Complementary and Alternative Medicine, 2012*.

Bunalema, L., Obakiro, S., Tabuti, J. R., and **Waako, P. (2014)**. Knowledge on plants used traditionally in the treatment of tuberculosis in Uganda. *Journal of Ethnopharmacology*, ***151*(2)**, 999-1004.

Burton, G. W., Doba, T., Gabe, E., Hughes, L., Lee, F. L., Prasad, L., and **Ingold, K. U. (1985)**. Autoxidation of biological molecules. 4. Maximizing the antioxidant activity of phenols. *Journal of the American Chemical Society*, ***107*(24)**, 7053-7065.

Caceres, A., Cabrera, O., Morales, O., Mollinedo, P., and **Mendia, P. (1991).** Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity. *Journal of Ethnopharmacology*, ***33*(3)**, 213-216.

Carballo, J. L., Hernández-Inda, Z. L., Pérez, P., and **García-Grávalos, M. D. (2002).** A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology*, ***2*(1)**, 17.

Chayamiti, T., Mwenje, E., and **Mahamadi, C. (2013)**. Spectrophotometric study of the anti-caseinolytic activity of root extracts of *Teclea nobilis* and V*epris zambesiaca* on *Bitis arietans* venom. *African Journal of Pharmacy and Pharmacology*, ***7*(21)**, 1420-1425.

Cheikhyoussef, A., Shapi, M., Matengu, K., and **Ashekele, H. M. (2011)**. Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *Journal of Ethnobiology and Ethnomedicine*, ***7*(1)**, 10.

Chen, S. L., Yu, H., Luo, H. M., Wu, Q., Li, C. F., and **Steinmetz, A. (2016)**. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese Medicine, **11*(1)**, 37.

Chenia, H. Y. (2013). Anti-quorum sensing potential of crude *Kigelia africana* fruit extracts. *Sensors, **13*(3)**, 2802-2817.

Christenhusz, M. J., and **Byng, J. W. (2016)**. The number of known plants species in the world and its annual increase. *Phytotaxa*, **261(3)**, 201-217.

Chuma, J., Maina, T., and **Ataguba, J. (2012)**. Does the distribution of health care benefits in Kenya meet the principles of universal coverage? *BMC Public Health, **12*(1)**, 20.

Clarkson, C., Madikane, E. V., Hansen, S. H., Smith, P. J., and **Jaroszewski, J. W. (2007)**. HPLC-SPE-NMR characterization of sesquiterpenes in an antimycobacterial fraction from *Warburgia salutaris. Planta Medica, **73*(06)**, 578-584.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J. and **Folb, P. I. (2004).** In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of Ethnopharmacology*, *92*(2-3), 177-191

Cole, I. B., Saxena, P. K., and **Murch, S. J. (2007).** Medicinal biotechnology in the genus *Scutellaria*. *In Vitro Cellular & Developmental Biology-Plant, **43*(4)**, 318-327.

Coopoosamy, R. M., and **Naidoo, K. K. (2012)**. An ethnobotanical study of medicinal plants used by traditional healers in Durban, South Africa. *African Journal of Pharmacy and Pharmacology,* ***6*****(11)**, 818-823.

Corson, T. W., and **Crews, C. M. (2007)**. Molecular understanding and modern application of traditional medicines: triumphs and trials. *Cell,* ***130*****(5)**, 769-774.

Cos, P., Hermans, N., De Bruyne, T., Apers, S., Sindambiwe, J.B., Berghe, D.V., Pieters, L. and **Vlietinck, A.J., (2002)**. Further evaluation of Rwandan medicinal plant extracts for their antimicrobial and antiviral activities. *Journal of Ethnopharmacology*, ***79*****(2)**, 155-163.

County Government of Kisumu. (2013). *County Government of Kisumu*. Retrieved 4 5, 2020, from https://www.kisumu.go.ke/about-us/sub-counties/kisumu-east/

David, B., Wolfender, J. L., and **Dias, D. A. (2015)**. The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Reviews,* ***14*****(2)**, 299-315.

De Castro, R. D., de Souza, T. P., Bezerra, L. M., Ferreira, G. L., de Brito Costa, E. M., and **Cavalcanti, A. L. (2015).** Antifungal activity and mode of action of thymol and its synergism with nystatin against *Candida* species involved with infections in the oral cavity: an in vitro study. *BMC Complementary and Alternative Medicine*, **15(1)**, 1-7.

Delorenzi, J.C., Attias, M., Gattass, C.R., Andrade, M., Rezende, C., da Cunha Pinto, Â., Henriques, A.T., Bou-Habib, D.C. and Saraiva, E.M., (2001). Antileishmanial activity of an indole alkaloid from *Peschiera australis*. *Antimicrobial Agents and Chemotherapy*, ***45*****(5)**, 1349-1354.

Demilew, W., Adinew, G. M., and **Asrade, S. (2018).** Evaluation of the Wound Healing Activity of the Crude Extract of Leaves of *Acanthus polystachius* Delile (Acanthaceae). *Evidence-based Complementary and Alternative Medicine: eCAM, 2018*.

Derebe, D., and **Wubetu, M. (2019).** Antimalarial Activity of Hydroalcoholic Root Extract of *Acanthus polystachyus* Delile (Acanthaceae) Against *Plasmodium berghei*–Infected Mice. *Journal of Evidence-Based Integrative Medicine*, *24*, 2515690X19885322.

Deressa, T., Mekonnen, Y., and **Animut, A. (2010).** In Vivo anti-malarial activities of *Clerodendrum myricoides*, *Dodonea angustifolia* and *Aloe debrana* against *Plasmodium berghei*. *Ethiopian Journal of Health Development, 24*(1).

Dhanani, T., Shah, S., Gajbhiye, N. A., and **Kumar, S. (2017)**. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry, **10***, S1193-S1199.

Djeussi, D.E., Noumedem, J.A., Seukep, J.A., Fankam, A.G., Voukeng, I.K., Tankeo, S.B., Nkuete, A.H. and Kuete, V. (2013). Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. *BMC Complementary and Alternative Medicine*, **13(1)**, 164.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., and **Ju, Y. H. (2014)**. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of Food and Drug Analysis*, **22(3)**, 296-302.

Doughari, J. H. (2006). Antimicrobial activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research*, ***5*(2)**, 597-603.

Doughari, J. H., Elmahmmod, A. M., and **Manzara, S. (2007).** Studies on the antibacterial activity of root extracts of *Carica papaya* L. *African Journal of Microbiology Research*, ***1*(3)**, 037-041.

Dua, V.K., Verma, G., Singh, B., Rajan, A., Bagai, U., Agarwal, D.D., Gupta, N.C., Kumar, S. and **Rastogi, A. (2013).** Anti-malarial property of steroidal alkaloid conessine isolated from the bark of *Holarrhena antidysenterica*. *Malaria Journal*, **12(1)**, 194.

Duraipandiyan, V., Ayyanar, M., and **Ignacimuthu, S. (2006).** Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine*, ***6*(1)**, 35.

Edeoga, H. O., Okwu, D. E., and **Mbaebie, B. O. (2005).** Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, ***4*(7)**, 685-688.

Elufioye, T. O., and **Agbedahunsi, J. M. (2004).** Antimalarial activities of *Tithonia diversifolia* (Asteraceae) and *Crossopteryx febrifuga* (Rubiaceae) on mice in vivo. *Journal of Ethnopharmacology*, ***93*(2-3)**, 167-171.

Emukule, G. O., Paget, J., van der Velden, K., and **Mott, J. A. (2015).** Influenza-associated disease burden in Kenya: a systematic review of literature. *PloS One*, ***10*(9)**, e0138708.

Estrada, A., Katselis, G. S., Laarveld, B., and **Barl, B. (2000)**. Isolation and evaluation of immunological adjuvant activities of saponins from *Polygala senega* L. *Comparative Immunology, Microbiology and Infectious Diseases, **23*(1)**, 27-43.

Fabry, W., Okemo, P., and **Ansorg, R. (1996)**. Fungistatic and fungicidal activity of East African medicinal plants. *Mycoses, **39*(1-2)**, 67-70.

Fadeyi, S. A., Fadeyi, O. O., Adejumo, A. A., Okoro, C., and **Myles, E. L. (2013)**. In vitro anticancer screening of 24 locally used Nigerian medicinal plants. *BMC Complementary and Alternative Medicine, **13*(1)**, 79.

Feikin, D.R., Njenga, M.K., Bigogo, G., Aura, B., Audi, A., Jagero, G., Muluare, P.O., Gikunju, S., Nderitu, L., Balish, A. and **Winchell, J., (2012).** Etiology and Incidence of viral and bacterial acute respiratory illness among older children and adults in rural western Kenya, 2007–2010. *PloS One, **7*(8)**.

Feng, L., Li, Z., Zhao, S., Nair, H., Lai, S., Xu, W., Li, M., Wu, J., Ren, L., Liu, W. and **Yuan, Z., (2014).** Viral etiologies of hospitalized acute lower respiratory infection patients in China, 2009-2013. *PloS One, **9*(6)**, e99419.

Ferkol, T., and **Schraufnagel, D. (2014)**. The global burden of respiratory disease. *Annals of the American Thoracic Society, **11*(3)**, 404-406.

Figueiredo, A. C., Barroso, J. G., Pedro, L. G., and **Scheffer, J. J. (2008).** Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour and Fragrance Journal, **23*(4)**, 213-226.

Figueiredo, M. S., and **Grelle, C. V. (2009).** Predicting global abundance of a threatened species from its occurrence: implications for conservation planning. *Diversity and Distributions,* ***15*****(1)**, 117-121.

Fuller, J.A., Summers, A., Katz, M.A., Lindblade, K.A., Njuguna, H., Arvelo, W., Khagayi, S., Emukule, G., Linares-Perez, N., McCracken, J. and **Nokes, D.J., (2013).** Estimation of the national disease burden of influenza-associated severe acute respiratory illness in Kenya and Guatemala: a novel methodology. *PloS One, 8*(2).

Gaya, C. H., Kawaka, J. F., Muchugi, A., and **Ngeranwa, J. J. (2013)**. Variation of alkaloids in the Kenyan *Zanthoxylum gilletii* (De Wild Waterman). *African Journal of . Plant Science,* ***7*****(9)**, 438-444.

Gbadamosi, I. T. (2012). Evaluation of antibacterial activity of six ethnobotanicals used in the treatment of infectious diseases in Nigeria. *Botany Research International,* ***5*****(4)**, 83-89.

Gemechu, A., Giday, M., Worku, A., and **Ameni, G. (2013)**. In vitro Anti-mycobacterial activity of selected medicinal plants against *Mycobacterium tuberculosis* and *Mycobacterium bovis* Strains. *BMC Complementary and Alternative Medicine,* ***13*****(1)**, 291.

Ghalem, B. R., and **Mohamed, B. (2008)**. Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and Pharmacology,* ***2*****(10)**, 211-215.

Ghorbani, A., Naghibi, F., and **Mosadegh, M. (2006)**. Ethnobotany, ethnopharmacology and drug discovery. *Iranian Journal of Pharmaceutical Sciences ,* ***2*****(2)**, 109-118.

Ghosh, D. (2015). Tannins from foods to combat diseases. *International Journal of Pharmaceutical Sciences Review and Research, **4*****(5)**, 40-44.

Goffin, E., Ziemons, E., De Mol, P., de Madureira, M.D.C., Martins, A.P., da Cunha, A.P., Philippe, G., Tits, M., Angenot, L. and **Frederich, M., (2002).** In vitro antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin C. *Planta Medica, **68*****(06)**, 543-545.

Govindarajan, M., Rajeswary, M., Hoti, S. L., Bhattacharyya, A., and **Benelli, G. (2016)**. Eugenol, α-pinene and β-caryophyllene from *Plectranthus barbatus* essential oil as eco-friendly larvicides against malaria, dengue and Japanese encephalitis mosquito vectors. *Parasitology Research, **115*****(2)**, 807-815.

Grace, O. M., Light, M. E., Lindsey, K. L., Mulholland, D. A., van Staden, J., and **Ja`ger, A. K. (2002).** Antibacterial activity and isolation of active compounds from fruit of the traditional African medicinal tree *Kigelia africana*. *South African Journal of Botany, **68*****(2)**, 220-222.

Griffin, S. G., Wyllie, S. G., Markham, J. L., and **Leach, D. N. (1999)**. The role of structure and molecular properties of terpenoids in determining their antimicrobial activity. *Flavour and Fragrance Journal, **14*****(5)**, 322-332.

Guédé, N. N., Dibié, T. E., and **Grellier, P. (2010)**. Ethnopharmacological study of plants used to treat malaria, in traditional medicine, by Bete Populations of Issia (Côte d'Ivoire). *Journal of Pharmacy Science Research, **2*****(4)**, 216-227.

Gülçin, İ., Topal, F., Sarikaya, S. O., Bursal, E., Bilsel, G., and **Gören, A. C. (2011).** Polyphenol Contents and Antioxidant Properties of Medlar (Mespilus germanica L. *Records of Natural Products*, ***5*(3)**, 158.

Guo, N., Liu, J., Wu, X., Bi, X., Meng, R., Wang, X., Xiang, H., Deng, X. and **Yu, L., (2009).** Antifungal activity of thymol against clinical isolates of fluconazole-sensitive and-resistant Candida albicans. *Journal of Medical Microbiology*, ***58*(8)**, 1074-1079.

Hamidi, M. R., Jovanova, B., and **Panovska, T. K. (2014).** Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. *Macedonian Pharmaceutical Bulletin*, ***60*(1)**, 9-18.

Hamilton , A. C. (2008). Medicinal plants in conservation and development case studies and lessons learnt. Salisbury (United Kingdom): *Plantlife International.*

Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. *Biodiversity & Conservation*, ***13*(8)**, 1477-1517.

Hamza, O.J., van den Bout-van, C.J., Matee, M.I., Moshi, M.J., Mikx, F.H., Selemani, H.O., Mbwambo, Z.H., Van der Ven, A.J. and **Verweij, P.E., (2006).** Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. *Journal of Ethnopharmacology*, ***108*(1)**, 124-132.

Hazen, K. C. (1998). Fungicidal versus fungistatic activity of terbinafine and itraconazole: an in vitro comparison. *Journal of the American Academy of Dermatology*, **38(5)**, S37-S41.

Henrich, C. J., and **Beutler, J. A. (2013).** Matching the power of high throughput screening to the chemical diversity of natural products. *Natural Product Reports, **30*(10)**, 1284-1298.

Heywood, V. H., and **Iriondo, J. M. (2003).** Plant conservation: old problems, new perspectives. *Biological Conservation, **113*(3)**, 321-335.

Hirota, H., Tomono, Y., and **Fusetani, N. (1996)**. Terpenoids with antifouling activity against Barnacle larvae from the marine sponge *Acanthella cavernosa*. *Tetrahedron, **52*(7)**, 2359-2368.

Hossain, M. A., AL-Raqmi, K. S., AL-Mijizy, Z. H., Weli, A. M., and **Al-Riyami, Q. (2013).** Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine, **3*(9)**, 705-710.

Houghton, P. J., Woldemariam, T. Z., Watanabe, Y., and **Yates, M. (1999).** Activity against Mycobacterium tuberculosis of alkaloid constituents of Angostura bark, *Galipea officinalis*. *Planta Medica, **65*(03)**, 250-254.

Hussein, S., and **Dhabe, A. (2018)**. Ethnobotanical study of folk medicinal plants used by villagers in the Hajjah district-Republic of Yemen. *Journal of Medicinal Plants Studies, **6*(5)**, 24-30.

Ibrahim, M. A., Koorbanally, N. A., and **Islam, M. S. (2014).** Antioxidative activity and inhibition of key enzymes linked to type-2 diabetes (α-glucosidase and α-amylase) by *Khaya senegalensis*. *Acta Pharmaceutica, **64*(3)**, 311-324.

Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., and **Haines, D. D. (2015)**. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and Phoradendron californicum. *BMC Research Notes, **8*(1),** 396.

Iqbal, E., Salim, K. A., and **Lim, L. B. (2015).** Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science,* **27(3)**, 224-232.

Iqbal, S., Younas, U., Chan, K. W., Zia-Ul-Haq, M., and **Ismail, M. (2012)**. Chemical composition of *Artemisia annua* L. leaves and antioxidant potential of extracts as a function of extraction solvents. *Molecules, **17*(5)**, 6020-6032.

Jaberian, H., Piri, K., and **Nazari, J. (2013)**. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. *Food Chemistry, **136*(1)**, 237-244.

James, D. B., Abu, E. A., Wurochekke, A. U., and **Orji, G. N. (2007).** Phytochemical and antimicrobial investigation of the aqueous and methanolic extracts of *Ximenia americana*. *Journal of Medical Sciences, **2**,* 284-288.

Jaradat, N., Hussen, F., Al Ali, A., Alniss, H., and **Dweikat, M. (2015).** Phytoconstituents, Free Radical Scavenging Potential, Total Phenols and Total Flavonoids Assessments for Violet Horned Poppy from Jerusalem Mountains. *Journal of Materials and Enviromental Science **6*(10)**, 2958-2966.

Jati, S. P., and **Ginandjar, P. (2017).** Potential impact of climate variability on respiratory diseases in infant and children in Semarang. *In IOP Conference Series: Earth and Environmental Science*, *55*(1), 012049. IOP Publishing.

Ji, S., Fattahi, A., Raffel, N., Hoffmann, I., Beckmann, M. W., Dittrich, R., and **Schrauder, M. (2017)**. Antioxidant effect of aqueous extract of four plants with therapeutic potential on gynecological diseases; *Semen persicae*, *Leonurus cardiaca*, *Hedyotis diffusa*, and *Curcuma zedoaria*. *European Journal of Medical Research, **22*(1)**, 50.

Kaigongi, M. M., and **Musila, F. M. (2015).** Ethnobotanical study of medicinal plants used by Tharaka people of Kenya. *International Journal Ethnobiology and Ethnomedicine, **1*(1)**, 1-8.

Kaingu, C. K., Oduma, J. A., and **Kanui, T. (2012)**. Preliminary investigation of contractile activity of *Ricinus communis* and *Euclea divinorum* extracts on isolated rabbit uterine strips. *Journal of Ethnopharmacology*, ***142*(2)**, 496-502.

Kaingu, C. O., Kaingu, C. K., Oduma, J. A., Mbaria, J. M., and **Kiama, S. G. (2013)**. Medicinal plants traditionally used for the management of female reproductive health dysfunction in Tana River County, Kenya. *Tang [Humanitas Medicine]*, ***32***, 17-1.

Kaingua, C. K., Mbaria, J., Oduma, J. A., and **Kiama, S. G. (2014)**. Ethnobotanical study of medicinal plants traditionally used in Tana River County for management of illnesses. *Asian Journal of Complementary and Alternative Medicine, **2*(02)**, 01-05.

Kamau, L. N., Mbaabu, P. M., Mbaria, J. M., Gathumbi, P. K., and **Kiama, S. G. (2016).** Ethnobotanical survey and threats to medicinal plants traditionally used for the management of human diseases in Nyeri County, Kenya. *Tang,* ***6*****(3)**, 23-37.

Kamita, M. K., Matu, E. N., Njenga, E. W., Wanga, J., Amalemba, G., and **Kigondu, E. v. (2014).** In vivo antifertility activity and phytochemical screening of selected Kenyan medicinal plants. *African Journal of Pharmacology and Therapeutics,* ***3*****(3)**.

Kankara, S. S., Ibrahim, M. H., Mustafa, M., and **Go, R. (2015).** Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. *South African Journal of Botany,* ***97***, 165-175.

Kareru, P. G., Gachanja, A. N., Keriko, J. M., and **Kenji, G. M. (2008).** Antimicrobial activity of some medicinal plants used by herbalists in eastern province, Kenya. *African Journal of Traditional, Complementary and Alternative Medicines,* ***5*****(1)**, 51-55.

Kariuki, A. C., and **Njoroge, G. N. (2011).** Ethnobotanical and antimicrobial studies of some plants used in Kibwezi (Kenya) for management of lower respiratory tract infections. *African Journal of Traditional, Complementary and Alternative Medicines, 82*.

Kariuki, H. N., Kanui, T. I., Yenesew, A., Mbugua, P. M., and **Patel, N. B. (2012)**. Antinocieptive activity of the root extracts of *Rhus natalensis* Kraus and *Senna singueana*. *Phytopharmacology,* ***2***, 312-317.

Kariuki, H. N., Kanui, T. I., Yenesew, A., Patel, N., and **Mbugua, P. M. (2013).** Antinocieptive and anti-inflammatory effects of *Toddalia asiatica* (L) Lam.(Rutaceae) root extract in Swiss albino mice. *The Pan African Medical Journal, 14*.

Karou, S.D., Tchacondo, T., Djikpo Tchibozo, M.A., Abdoul-Rahaman, S., Anani, K., Koudouvo, K., Batawila, K., Agbonon, A., Simpore, J. and **de Souza, C. (2011).** Ethnobotanical study of medicinal plants used in the management of diabetes mellitus and hypertension in the Central Region of Togo. *Pharmaceutical Biology, **49*(12)**, 1286-1297.

Kasali, F. M., Mahano, A. O., Kadima, N. J., Mpiana, P. T., Ngbolua, K. N., and **Tshibangu, T. S. (2014)**. Ethnopharmacological survey of medicinal plants used against malaria in Butembo City (DR Congo). *Journal of Advanced Botany and Zoology, **1*(1)**.

Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., and **Ogwal-Okeng, J. W. (2010)**. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *Journal of Medicinal Plants Research, **4*(9)**.

Katz, M.A., Muthoka, P., Emukule, G.O., Kalani, R., Njuguna, H., Waiboci, L.W., Ahmed, J.A., Bigogo, G., Feikin, D.R., Njenga, M.K. and **Breiman, R.F., (2014).** Results from the first six years of national sentinel surveillance for influenza in Kenya, July 2007–June 2013. *PloS One, **9*(6)**, e98615.

Kengni, F., Tala, D. S., Djimeli, M. N., Fodouop, S. P., Kodjio, N., Magnifouet, H. N., and **Gatsing, D. (2013)**. In vitro antimicrobial activity of *Harungana madagascriensis* and *Euphorbia prostrata* extracts against some pathogenic *Salmonella* sp. *International Journal of Biological and Chemical Sciences, **7*(3)**, 1106-1118.

Kenya National Bureau of Statistics (2013). *Kenya National Bureau of Statistics* . Retrieved 8 8 2018, from https://www.knbs.or.ke/economic-survey-2013

Kenya National Bureau of Statistics. (2019). *Kenya National Bureau of Statistics*. Retrieved 4 5, 2020, from https://www.knbs.or.ke/?wpdmpro=2019-kenya-population-and-housing-census-volume-i-population-by-county-and-sub-county

Kenya National Bureau of Statistics (2020). *Kenya National Bureau of Statistics* . Retrieved 29 4 2020, from https://www.knbs.or.ke/?wpdmpro=economic-survey-2020

Kerntopf, M. R., de Albuquerque, R. L., Machado, M., Matos, F. J., and **Craveiro, A. A. (2002).** Essential oils from leaves, stems and roots of *Plectranthus barbatus* Andr.(Labiatae) grown in Brazil. *Journal of Essential Oil Research*, ***14*****(2)**, 101-102.

Khalid, H., Abdalla, W. E., Abdelgadir, H., Opatz, T., and **Efferth, T. (2012)**. Gems from traditional north-African medicine: medicinal and aromatic plants from Sudan. *Natural products and Bioprospecting*, ***2*****(3)**, 92-103.

Khan, S. M., Page, S., Ahmad, H., and **Harper, D. (2013)**. Identifying plant species and communities across environmental gradients in the Western Himalayas: Method development and conservation use. *Ecological Informatics*, **14**, 99-103.

Kidane, B., van Andel, T., van der Maesen, L. J., and **Asfaw, Z. (2014)**. Use and management of traditional medicinal plants by Maale and Ari ethnic communities in southern Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, ***10*****(1)**, 46.

Kidane, B., Van Der Maesen, L. G., van Andel, T., and **Asfaw, Z. (2014)**. Ethnoveterinary medicinal plants used by the Maale and Ari ethnic communities in southern Ethiopia. *Journal of Ethnopharmacology*, ***153*****(1)**, 274-282.

Kigen, G., Kamuren, Z., Njiru, E., Wanjohi, B., and **Kipkore, W. (2019)**. Ethnomedical Survey of the Plants Used by Traditional Healers in Narok County, Kenya. *Evidence-Based Complementary and Alternative Medicine, 2019*.

Kigen, G., Maritim, A., Some, F., Kibosia, J., Rono, H., Chepkwony, S., Kipkore, W. and **Wanjoh, B., (2016)**. Ethnopharmacological survey of the medicinal plants used in Tindiret, Nandi County, Kenya. *African Journal of Traditional, Complementary and Alternative Medicines,* ***13*****(3)**, 156-168.

Kigen, G., Rono, H. K., Kipkore, W. K., and **Rotich, J. K. (2013)**. Current trends of traditional herbal medicine practice in Kenya: a review. *African Journal of Pharmacology and Therapeutics*, ***2*****(1)**, 32-37.

Kigondu, E.V., Rukunga, G.M., Keriko, J.M., Tonui, W.K., Gathirwa, J.W., Kirira, P.G., Irungu, B., Ingonga, J.M. and **Ndiege, I.O. (2009).** Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. *Journal of Ethnopharmacology*, ***123*****(3).**

Kilani, A. M. (2006). Antibacterial assessment of whole stem bark of Vitex doniana against some enterobactriaceae. *African Journal of Biotechnology*, ***5*****(10)**.

Kim, S. K., Ryu, S. Y., No, J., Choi, S. U., and **Kim, Y. S. (2001).** Cytotoxic alkaloids from Houttuynia cordate. *Archives of pharmacal research, **24*****(6)**, 518-521.

Kimani, C. N., Mbaria, J. M., Suleiman, M., Gakuya, D., and **Kiama, S. G. (2015)**. Antihyperglycemic activity of *Zanthoxylum chalybeum* stem bark extract in diabetic rats. *Journal of phytopharmacology, **4*****(3)**, 183-189.

Kimondo, J., Miaron, J., Mutai, P., and **Njogu, P. (2015)**. Ethnobotanical survey of food and medicinal plants of the Ilkisonko Maasai community in Kenya. *Journal of Ethnopharmacology*, ***175***, 463-469.

Kipkore, W., Wanjohi, B., Rono, H., and **Kigen, G. (2014)**. A study of the medicinal plants used by the Marakwet Community in Kenya. *Journal of Ethnobiology and Ethnomedicine, **10*****(1)**, 24.

Kiraithe, M. N., Nguta, J. M., Mbaria, J. M., and **Kiama, S. G. (2016)**. Evaluation of the use of *Ocimum suave* Willd.(Lamiaceae), *Plectranthus barbatus* Andrews (Lamiaceae) and *Zanthoxylum chalybeum* Engl.(Rutaceae) as antimalarial remedies in Kenyan folk medicine. *Journal of Ethnopharmacology*, ***178***, 266-271.

Kisangau, D. P., Hosea, K. M., Joseph, C. C., and **Lyaruu, H. (2007)**. In vitro antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. *African Journal of Traditional, Complementary and Alternative Medicines*, ***4*****(4)**, 5.

Kiswii, T. M., Monda, E. O., Okemo, P. O., Bii, C., and **Alakonya, A. E. (2014).** Efficacy of selected medicinal plants from Eastern Kenya against *Aspergillus flavus*. *Journal of Plant Sciences*, ***2*****(5)**, 226-231.

Kokila, K., Priyadharshini, S. D., and **Sujatha, V. (2013).** Phytopharmacological properties of *Albizia* species: a review. *International Journal of Pharmacy and Pharmaceutical Sciences*, ***5*****(3)**, 70-3.

Kokwaro, J. O., and **Johns, T. (1998)**. *Luo Biological Dictionary* (First ed.). Nairobi: East African Educational Publishers Ltd.

Kuglerova, M., Tesarova, H., Grade, J. T., Halamova, K., Wanyama-Maganyi, O., Van Damme, P., and **Kokoska, L. (2011)**. Antimicrobial and antioxidative effects of Ugandan medicinal barks. *African Journal of Biotechnology*, ***10*****(18)**, 3628-3632.

Lage, H., Duarte, N., Coburger, C., Hilgeroth, A., and **Ferreira, M. J. U. (2010)**. Antitumor activity of terpenoids against classical and atypical multidrug resistant cancer cells. *Phytomedicine*, ***17*****(6)**, 441-448.

Lagnika, L., Amoussa, M., Adjovi, Y., and **Sanni, A. (2012)**. Antifungal, antibacterial and antioxidant properties of *Adansonia digitata* and *Vitex doniana* from Bénin pharmacopeia. *Journal of Pharmacognosy and Phytotherapy*, ***4*****(4)**, 44-52.

Lambert, J., Leonard, K., Mungai, G., Omindi-Ogaja, E., Gatheru, G., Mirangi, M., Owara, J., Herbst, C.H., Ramana, G.N.V. and **Lemiere, C. (2011).** The contribution of traditional herbal medicine practitioners to Kenyan health care delivery: results from

community health-seeking behavior vignettes and a traditional herbal medicine practitioner survey.

Lamchouri, F., Settaf, A., Cherrah, Y., Hassar, M., Zemzami, M., Atif, N., Nadori, E.B., Zaid, A. and **Lyoussi, B. (2000).** In vitro cell-toxicity of *Peganum harmala* alkaloids on cancerous cell-lines. *Fitoterapia*, ***71*(1)**, 50-54.

Lata, H., Chandra, S., and **Arora, R. (2010).** Biotechnological characterization of different populations of an endangered medicinal herb-*Podophyllum hexandrum* Royle. In A. R. (Ed.), *Medicinal Plant Biotechnology* (p. 36). UK: CABI.

Le, N. H., Malterud, K. E., Daillo, D., Paulsen, B. S., Nergard, C. S., and **Wangensteen, H. (2012)**. Bioactive polyphenols in *Ximenia americana* and the traditional use among Malian healers. *Journal of Ethnopharmacology*, ***139*(3)**, 858-862.

Lei, C., and **Sun, X. (2018)**. Comparing lethal dose ratios using probit regression with arbitrary slopes. *BMC Pharmacology and Toxicology*, **19(1)**, 61.

Li, H., Wang, Z., and **Liu, Y. (2003)**. Review in the studies on tannins activity of cancer prevention and anticancer. *Zhong yao cai= Zhongyaocai= Journal of Chinese medicinal materials*, **26(6)**, 444-448.

Liasu, M. O., and **Ayandele, A. A. (2008)**. Antimicrobial activity of aqueous and ethanolic extracts from *Tithonia diversifolia* and *Bryum coronatum* collected from Ogbomoso, Oyo state, Nigeria. *Advances in Natural and Applied Sciences*, ***2*(1)**, 31-34.

Lino, A., and **Deogracious, O. (2006).** The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea*-Ugandan medicinal plants. *African Health Sciences, **6*(1)**, 31-35.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y. and **AlMazroa, M.A.,** (2010). Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet, **380*(9859)**, 2095-212.

Ma, Z., Hano, Y., Nomura, T., and **Chen, Y. (2004)**. Novel quinazoline–quinoline alkaloids with cytotoxic and DNA topoisomerase II inhibitory activities. *Bioorganic & Medicinal Chemistry Letters, **14*(5),** 1193-1196.

Madikizela, B., Ndhlala, A. R., Finnie, J. F., and **Staden, J. V. (2013)**. In vitro antimicrobial activity of extracts from plants used traditionally in South Africa to treat tuberculosis and related symptoms. *Evidence-Based Complementary and Alternative Medicine, 2013*.

Maikai, V. A., Kobo, P. I., and **Maikai, B. O. (2010)**. Antioxidant properties of *Ximenia americana*. *African Journal of Biotechnology, **9*(45)**, 7744-7746.

Maiyo, Z. C., Ngure, R. M., Matasyoh, J. C., and **Chepkorir, R. (2010)**. Phytochemical constituents and antimicrobial activity of leaf extracts of three *Amaranthus* plant species. *African Journal of Biotechnology, **9*(21)**, 3178-3182.

Mandal, P., Babu, S. S., and **Mandal, N. C. (2005).** Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia*, **76(5)**, 462-465.

Mander, L., and **Liu, H. W. (2010).** Comprehensive natural products II: *Chemistry and Biology* (First ed.). UK: Elsevier.

Manjunatha, B. K. (2006). Antibacterial activity of *Pterocarpus santalinus*. *Indian Journal of Pharmaceutical Sciences, **68*(1)**,* 115.

Maobe, M.A., Gitu, L., Gatebe, E., Rotich, H., Karanja, P.N., Votha, D.M., Nderitu, I.W. and **Kungu, W. (2013).** Antifungal activity of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii Region, Southwest Kenya. *World Journal of Medical Sciences, **8*(1)**,* 74-78.

Maroyi, A. (2013). Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of Ethnobiology and Ethnomedicine, **9*(1)**,* 31.

Maroyi, A. (2013). Warburgia salutaris (Bertol. f.) Chiov.: A multi-use ethnomedicinal plant species. *Journal of Medicinal Plants Research, **7*(2)**,* 53-60.

Mascolo, N., Pinto, A., Capasso, F., Yenesew, A., and **Dagne, E. (1988)**. Antipyretic and analgesic studies of the ethanalic extract of *Teclea nobilis* delile. *Phytotherapy Research, **2*(3)**,* 154-156.

Mastelic, J., Politeo, O., Jerkovic, I., and **Radosevic, N. (2005)**. Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions. *Chemistry of Natural Compounds*, **41(1)**, 35-40.

Matu, E. N., and **van Staden, J. (2003)**. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *Journal of Ethnopharmacology*, ***87*(1)**, 35-41.

Mazid, M., Khan, T. A., and **Mohammad, F. (2011)**. Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine*, ***3*(2)**, 232-249.

McGaw, L. J., and **Eloff, J. N. (2008)**. Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. *Journal of Ethnopharmacology*, ***119*(3)**, 559-574.

McKay, D. L., and **Blumberg, J. B. (2006).** A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). Phytotherapy Research:. *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, ***20*(8)**, 619-633.

Mclaughlin, J. L., Rogers, L. L., and **Anderson, J. E. (1998).** The use of biological assays to evaluate botanicals. *Drug Information Journal*, ***32*(2)**, 513-524.

McMorrow, M.L., Emukule, G.O., Njuguna, H.N., Bigogo, G., Montgomery, J.M., Nyawanda, B., Audi, A., Breiman, R.F., Katz, M.A., Cosmas, L. and **Waiboci, L.W. (2015)**. The unrecognized burden of Influenza in young Kenyan children, 2008-2012. *PloS One*, ***10*(9)**.

Meragiaw, M. (2016). Wild useful plants with emphasis on traditional use of medicinal and edible plants by the people of Aba'ala, North-eastern Ethiopia. *Journal of Medicinal Plant and Herbal Therapy Research*, **4(1)**, 1-16.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. j., and **McLaughlin, J. L. (1982).** Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, **45**:31–4.

Mohammed, H. A., Alshalmani, S. K., and **Abdellatif, A. G. (2013)**. Antioxidant and quantitative estimation of phenolic and flavonoids of three halophytic plants growing in Libya. *Journal of Pharmacognosy and Phytochemistry*, **2(3)**, 89-94.

Moshi, M. J., Otieno, D. F., and **Weisheit, A. (2012)**. Ethnomedicine of the Kagera Region, north western Tanzania. Part 3: plants used in traditional medicine in Kikuku village, Muleba District. *Journal of Ethnobiology and Ethnomedicine*, ***8*(1)**, 14.

Moshi, M. J., Otieno, D. F., Mbabazi, P. K., and **Weisheit, A. (2010).** Ethnomedicine of the Kagera Region, north western Tanzania. Part 2: The medicinal plants used in Katoro Ward, Bukoba district. *Journal of Ethnobiology and Ethnomedicine*, ***6*(1)**, 19.

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., and **Bakri, M. M. (2018).** Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences*, **25(2)**, 361-366.

Moulari, B., Pellequer, Y., Lboutounne, H., Girard, C., Chaumont, J. -P., Millet, J., and **Muyard, F. (2006).** Isolation and in vitro antibacterial activity of astilbin, the bioactive

flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir.(Hypericaceae). *Journal of Ethnopharmacology*, ***106*****(2)**, 272-278.

Muanda, F., Koné, D., Dicko, A., Soulimani, R., and **Younos, C. (2011).** Phytochemical composition and antioxidant capacity of three malian medicinal plant parts. *Evidence-Based Complementary and Alternative Medicine, 2011*.

Muhammad, I. U., Alhassan, A. J., Sule, M. S., Idi, A., Mohammed, A., El-ta'alu, A. B., and **Abdulmumin, Y. (2016).** Anti-hyperglycemic activity of solvents extract of *Khaya senegalensis* stem bark in alloxan induced diabetic rats. *Journal of Advances in Biology & Biotechnology*, ***6*****(2)**, 1-8.

Muhammad, I. U., Alhassan, A. J., Wudil, A. M., & Jarumi, I. K. (2015). Toxicological and protective effect of aqueous stem bark extract of *Khaya senegalensis* (ASBEKS) on liver of experimental rat. *British Journal of Applied Science Technology*, ***9*****(6)**, 600-605.

Mukungu, N., Abuga, K., Okalebo, F., Ingwela, R., & Mwangi, J. (2016). Medicinal plants used for management of malaria among the Luhya community of Kakamega East sub-County, Kenya. *Journal of Ethnopharmacology*, ***194***, 98-107.

Murray, C.J., Ezzati, M., Flaxman, A.D., Lim, S., Lozano, R., Michaud, C., Naghavi, M., Salomon, J.A., Shibuya, K., Vos, T. and **Lopez, A.D. (2012).** GBD 2010: a multi-investigator collaboration for global comparative descriptive epidemiology. *The Lancet*, ***380*****(9859)**, 2055-2058.

Muruganandan, S., Srinivasan, K., Chandra, S., Tandan, S. K., Lal, J., and **Raviprakash, V. (2001).** Anti-inflammatory activity of *Syzygium cumini* bark. *Fitoterapia*, ***72*(4)**, 369-375.

Mwanauta, R. W., Mtei, K. A., & Ndakidemi, P. A. (2014). Prospective bioactive compounds from *Vernonia amygdalina*, *Lippia javanica*, *Dysphania ambrosioides* and *Tithonia diversifolia* in controlling legume insect pests. *Agricultural Sciences*, ***5*(12)**, 1129.

Nadembega, P., Boussim, J. B., Poli, F., & Antognoni, F. (2011). Medicinal plants in baskoure, kourittenga province, Burkina Faso: an ethnobotanical study. *Journal of Ethnopharmacology*, ***133*(2)**, 378-395.

Nagesh, K. S., and **Shanthamma, C. (2011).** Micropropagation and antioxidant activity of *Mollugo nudicaulis* Lam. *Journal of Medicinal Plants Research*, ***5*(6)**, 895-902.

Naidu, J. R., Ismail, R., and **Sasidharan, S. (2014).** Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha spicata* L (Lamiaceae). *Tropical Journal of Pharmaceutical Research*, ***13*(1)**, 101-107.

Nanyingi, M.O., Mbaria, J.M., Lanyasunya, A.L., Wagate, C.G., Koros, K.B., Kaburia, H.F., Munenge, R.W. and **Ogara, W.O. (2008)**. Ethnopharmacological survey of Samburu district, Kenya. *Journal of Ethnobiology and Ethnomedicine*, ***4*(1)**, 14.

Naz, R., Ayub, H., Nawaz, S., Islam, Z.U., Yasmin, T., Bano, A., Wakeel, A., Zia, S. and **Roberts, T.H. (2017).** Antimicrobial activity, toxicity and anti-inflammatory potential of methanolic extracts of four ethnomedicinal plant species from Punjab, Pakistan. *BMC Complementary and Alternative medicine*, **17(1)**, 302.

Nedi, T., Mekonnen, N., and **Urga, K. (2004)**. Diuretic effect of the crude extracts of *Carissa edulis* in rats. *Journal of Ethnopharmacology*, ***95*****(1),** 57-61.

Ngarivhume, T., van't Klooster, C. I., de Jong, J. T., and **Van der Westhuizen, J. H. (2015).** Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. *Journal of Ethnopharmacology*, ***159***, 224-237.

Ngo, T. V., Scarlett, C. J., Bowyer, M. C., Ngo, P. D., and **Vuong, Q. V. (2017)**. Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of *Salacia chinensis* L. *Journal of Food Quality, 2017*.

Ngoungoure, V. N., Muñoz, P., Tizabi, Y., Valdes, R., Moundipa, P. F., and **Segura-Aguilar, J. (2019)**. Protective Effects of Crude Plant Extracts against Aminochrome-induced toxicity in Human Astrocytoma Cells: Implications for Parkinson's Disease. *Clinical Pharmacology and Translational Medicine*, ***3*****(1)**, 124.

Ngoupaye, G. T., Bum, E. N., Taiwe, G. S., Moto, F., and **Talla, E. (2014).** Antidepressant properties of aqueous acerate from Gladiolus Dalenii corms. *African Journal of Traditional, Complementary and Alternative Medicines*, ***11*****(1)**, 53-61.

Nguta, J. M., and **Kiraithe, M. N. (2019)**. In vitro antimicrobial activity of aqueous extracts of *Ocimum suave* Willd., *Plectranthus barbatus* andrews and *Zanthoxylum chalybeum* Engl. against selected pathogenic bacteria. *Biomedical and Biotechnology Research Journal (BBRJ)*, ***3*****(1)**, 30.

Nguta, J. M., Appiah-Opong, R., Nyarko, A. K., Yeboah-Manu, D., Addo, P. G., Otchere, I., and **Kissi-Twum, A. (2016).** Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. *Journal of Ethnopharmacology*, ***182*,** 10-15.

Nguta, J. M., Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., Kabasa, J. D., and **Kiama, S. G. (2011)**. Biological screening of Kenyan medicinal plants using *Artemia salina* (Artemiidae). *Pharmacologyonline*, ***2***, 458-478.

Nguta, J. M., Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., and **Kiama, S. G. (2010)**. Antimalarial herbal remedies of Msambweni, Kenya. *Journal of Ethnopharmacology*, ***128*(2)**, 424-432.

Nguta, J. M., Mbaria, J. M., Gakuya, D. W., Gathumbi, P. K., Kabasa, J. D., and **Kiama, S. G. (2012)**. Cytotoxicity of antimalarial plant extracts from Kenyan biodiversity to the brine shrimp, *Artemia salina* L.(Artemiidae). *Drugs and Therapy Studies*, **2(1)**, e12-e12.

Nguta, J. M., Opong, R. A., Nyarko, A. K., Manu, Y. D., and **Addo, P. G. (2015)**. Current perspectives in drug discovery against tuberculosis from natural products. *International Journal of Mycobacteriology*, ***4*(3)**, 165 - 183.

Njeru, S. N., Obonyo, M., Nyambati, S., Ngari, S., Mwakubambanya, R., and **Mavura, H. (2016).** Antimicrobial and cytotoxicity properties of the organic solvent fractions of *Clerodendrum myricoides* (Hochst.) R. Br. ex Vatke: Kenyan traditional medicinal plant. *Journal of Intercultural Ethnopharmacology*, ***5*(3)**, 226.

Njoku, V. O., Obi, C., and **Onyema, O. M. (2011)**. Phytochemical constituents of some selected medicinal plants. *African Journal of Biotechnology*, ***10*(66)**, 15020-15024.

Njoroge, G. N., and **Bussmann, R. W. (2006).** Diversity and utilization of antimalarial ethnophytotherapeutic remedies among the Kikuyus (Central Kenya). *Journal of Ethnobiology and Ethnomedicine, **2*(1)**, 8.

Njume, C., Afolayan, A. J., and **Ndip, R. N. (2009).** An overview of antimicrobial resistance and the future of medicinal plants in the treatment of *Helicobacter pylori* infections. *African Journal of Pharmacy and Pharmacology **3*(13)**, 685-699.

Nóbrega, R. D. O., Teixeira, A. P. D. C., Oliveira, W. A. D., Lima, E. D. O., and **Lima, I. O. (2016).** Investigation of the antifungal activity of carvacrol against strains of *Cryptococcus neoformans*. *Pharmaceutical Biology*, ***54*(11)**, 2591-2596.

Nureye, D., Assefa, S., Nedi, T., and **Engidawork, E. (2018)**. In vivo antimalarial activity of the 80 Methanolic root bark extract and solvent fractions of *Gardenia ternifolia* Schumach. & Thonn.(Rubiaceae) against *Plasmodium berghei*. *Evidence-Based Complementary and Alternative Medicine, 2018*.

Nurmagambetov, T., Kuwahara, R., and **Garbe, P. (2018)**. The economic burden of asthma in the United States, 2008–2013. *Annals of the American Thoracic Society*, ***15*(3)**, 348-356.

Nyong, B. E., Edem, C. A., Obochi, G. O., and **Malu, S. P. (2009)**. Effect of methods of extraction on phytochemical constituents and antibacterial properties of *Tetracarpidium conophorum* seeds. *Global Journal of Pure and Applied Sciences*, ***15*(3-4)**.

Ochanga, O., and **Chacha, M. (2016).** Antifungal and cytotoxicity activity of plants used as herbal teas in Tanzania. *European Journal of Medicinal Plants,* 1-8.

Odhiambo, J. A., Siboe, G. M., Lukhoba, C. W., and **Dossaji, S. F. (2010)**. Antifungal activity of crude extracts of *Gladiolus dalenii* Van Geel (Iridaceae). *African Journal of Traditional, Complementary and Alternative Medicines, **7(1)***.

Odongo, D. O., Wakhungu, W. J., and **Stanley, O. (2017)**. Causes of variability in prevalence rates of communicable diseases among secondary school Students in Kisumu County, Kenya. *Journal of Public Health*, ***25*(2),** 161-166.

Ogunleye, D. S., and **Ibitoye, S. F. (2003).** Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. *Tropical Journal of Pharmaceutical Research, **2*(2)**, 239-241.

Okoli, A. S., Okeke, M. I., Iroegbu, C. U., and **Ebo , P. U. (2002)**. Antibacterial activity of *Harungana madagascariensis* leaf extracts. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, **16*(2)**, 174-179.

Okpo, S. O., Igwealor, C. O., and **Eze, G. I. (2016).** Sub-acute toxicity study on the aqueous extract of *Albizia zygia* stem bark. *Journal of Pharmacy & Bioresources, **13*(1)**, 32-41.

Olajuyigbe, O. O., and **Afolayan, A. J. (2011)**. Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* Willd. subsp. *mucronata* Willd. *BMC Complementary and Alternative Medicine, **11*(1)**, 130.

Olaleye, M. T. (2007). Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *Journal of Medicinal Plants Research, **1**(**1**)*, 9-13.

Olarbi, A. A., Bekoe, E. O., Agyare, C., Osafo, N., and **Boamah, V. E. (2016)**. In vivo anti-inflammatory and antioxidant properties of *Albizia zygia* DC Macbr. *Planta Medica, **82**(**S 01**)*, 198.

Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., and **Pereira, J. A. (2008).** Total phenols, antioxidant potential and antimicrobial activity of walnut (J*uglans regia* L.) green husks. *Food and Chemical Toxicology*, ***46*(7)**, 2326-2331.

Olowa, L. F., and **Nuñeza, O. M. (2013)**. Brine shrimp lethality assay of the ethanolic extracts of three selected species of medicinal plants from Iligan City, Philippines. *Mortality, 1*(T2), T3.

Oloyede, G. K., and **Oguniade, A. O. (2013).** Phytochemical screening, antioxidant, antimicrobial and toxicity activities of polar and non-polar extracts of *Albizia zygia* (DC) stem-bark. *Annual Research & Review in Biology*, 1020-1031.

Omoruyi, B. E., Bradley, G., and **Afolayan, A. J. (2012).** Ethnomedicinal survey of medicinalplants used for the management of HIV/AIDS infection among local communities of Nkonkobe Municipality, Eastern Cape, South Africa. *Journal of Medicinal Plants Research, **6**(**19**)*, 3603-3608.

Omosa, L. K., and **Okemwa, E. K. (2017)**. Antiplasmodial Activities of the Stem bark Extract and Compounds of *Zanthoxylum gilletii* (De wild) PG Waterman. *Pharmacognosy Communications, 7*(1).

Onyango, A. O. (2016). An Ethnobotanical, phytochemical, toxicity and efficacy study of selected AntiBlue-tick (*Boophilus decoloratus*) herbal remedies for cattle of Suba Sub-County, Kenya (Doctoral dissertation, UNIVERSITY OF NAIROBI).

Orlowski, P., Tomaszewska, E., Gniadek, M., Baska, P., Nowakowska, J., Sokolowska, J., Nowak, Z., Donten, M., Celichowski, G., Grobelny, J. and **Krzyzowska, M. (2014).** Tannic acid modified silver nanoparticles show antiviral activity in H*erpes simplex* virus type 2 infection. *PloS One*, **9(8)**, e104113.

Orwa, J. A., Jondiko, I. J., Minja, R. A., and **Bekunda, M. (2008).** The use of *Toddalia asiatica* (L) Lam.(Rutaceae) in traditional medicine practice in East Africa. *Journal of Ethnopharmacology*, ***115*(2)**, 257-262.

Orwa, J. A., Mwitari, P. G., Matu, E. N., and **Rukunga, G. M. (2007)**. Traditional healers and the managment of malaria in Kisumu District, Kenya. *East African Medical Journal*, ***84*(2)**, 51-55.

Orwa, J. A., Ngeny, L., Mwikwabe, N. M., Ondicho, J., and **Jondiko, I. O. (2013).** Antimalarial and safety evaluation of extracts from *Toddalia asiatica* (L) Lam.(Rutaceae). *Journal of Ethnopharmacology*, ***145*(2)**, 587-590.

Osuagwu, G. G., and **Eme, C. (2013)**. The phytochemical composition and antimicrobial activity of *Dialium guineense, Vitex doniana* and *Dennettia tripetala* leaves. *Asian Journal of Natural and Applied Sciences, **2**(**3**)*, 69-81.

Otang, W. M., Grierson, D. S., and **Ndip, R. N. (2012).** Ethnobotanical survey of medicinal plants used in the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa. *Journal of Medicinal Plants Research, **6**(**11**)*, 2071-2080.

Otieno, J. N., Magadula, J. J., Kakudidi, J. J., 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.

Owolabi, O. J., Omogbai, E. K., and **Obasuyi, O. (2007)**. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. *African Journal of Biotechnology*, *6*(14).

Oyekunle, M. A., Aiyelaagbe, O. O., and **Fafunso, M. A. (2006)**. Evaluation of the antimicrobial activity of saponins extract of *Sorghum bicolor* L. Moench. *African Journal of Biotechnology*, 5(23).

Pandey, G., Verma, K. K., and **Singh, M. (2014)**. Evaluation of phytochemical, antibacterial and free radical scavenging properties of *Azadirachta indica* (neem) leaves. *International Journal of Pharmacy and Pharmaceutical Sciences, **6**(**2**)*, 444-7.

Pandey, S. (2015). Preliminary phytochemical screening and in vitro antibacterial activity of *Bauhinia variegata* Linn. against human pathogens. *Asian Pacific Journal of Tropical Disease*, **5**:123–9.

Pankey, G. A., and **Sabath, L. D. (2004).** Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clinical Infectious Diseases*, **38(6)**, 864-870.

Parasuraman, S. (2011). Toxicological screening. *Journal of Pharmacology & Pharmacotherapeutics*, ***2*(2)**, 74.

Parthiban, R., Vijayakumar, S., Prabhu, S., and **Yabesh, J. (2016)**. Quantitative traditional knowledge of medicinal plants used to treat livestock diseases from Kudavasal taluk of Thiruvarur district, Tamil Nadu, India. *Revista Brasileira de Farmacognosia*, ***26*(1)**, 109-121.

Pascaline, J., Charles, M., George, O., and **Lukhoba, C. (2011)**. An inventory of medicinal plants that the people of Nandi use to treat malaria. *Journal of Animal and Plant Sciences*, ***9***, 1192-1200.

Pascaline, J., Charles, M., Lukhoba, C., and **George, O. (2011)**. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. *Journal of Animal & Plant Sciences*, ***9*(3)**, 1201-1210.

Passoni, F. D., Oliveira, R. B., Chagas-Paula, D. A., Gobbo- Neto, L., and **Da Costa, F. B. (2013).** Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A. gray and identification of the toxic compounds. *Journal of Ethnopharmacology*, ***147*****(2)**, 3.

Pathmanathan, M. K., Uthayarasa, K., Jeyadevan, J. P., and **Jeyaseelan, E. C. (2010).** In vitro antibacterial activity and phytochemical analysis of some selected medicinal plants. *International Journal of Pharmaceutcial and Biological Archive* ***1*****(3)**, 291-299.

Peasah, S.K., Purakayastha, D.R., Koul, P.A., Dawood, F.S., Saha, S., Amarchand, R., Broor, S., Rastogi, V., Assad, R., Kaul, K.A. and **Widdowson, M.A. (2015).** The cost of acute respiratory infections in Northern India: a multi-site study. *BMC Public Health*, ***15*****(1)**, 330.

Phillips, O. L. (1996). Some quantitative methods for analyzing ethnobotanical knowledge. *Advances in Economic Botany*, ***10***, 171-197.

Pierre, S., Toua, V., Tschobsala, Fernand-N, T. F., Alexandre- Michel, N. N., and **Jean, M. (2011).** Medicinal plants used in traditional treatment of malaria in Cameroon. *Journal of Ecology and the Natural Environment*, ***3*****(3)**, 104-117.

Prassas, I., and **Diamandis, E. P. (2008).** Novel therapeutic applications of cardiac glycosides. *Nature Reviews Drug Discovery*, ***7*****(11)**, 926-935.

Qwarse, M., Mihale, M. J., Joseph, S., Mugoyela, V., Henry, L., and **Sunghwa, F. (2018)**. Ethnobotanical Survey of Medicinal and Pesticidal Plants used by Agro-pastoral

Communities in Mbulu District, Tanzania. *Tanzania Journal of Science and Technology*, ***1*(1)**, 22-35.

Rabe, T., and **Van Staden, J. (2000).** Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. *Journal of Ethnopharmacology*, ***73*(1-2)**, 171-174.

Rameshkumar, A., and **Sivasudha, T. (2012).** In vitro antioxidant and antibacterial activity of aqueous and methanolic extract of *Mollugo nudicaulis* Lam. leaves. *Asian Pacific Journal of Tropical Biomedicine*, ***2*(2)**, S895-S900.

Rasoanaivo, P., Wright, C. W., Willcox, M. L., and **Gilbert, B. (2011).** Whole plant extracts versus single compounds for the treatment of malaria: synergy and positive interactions. *Malaria Journal*, ***10*(1)**, S4.

Riaz, B., Zahoor, M.K., Zahoor, M.A., Majeed, H.N., Javed, I., Ahmad, A., Jabeen, F., Zulhussnain, M. and **Sultana, K. (2018).** Toxicity, phytochemical composition, and enzyme inhibitory activities of some indigenous weed plant extracts in fruit fly, *Drosophila melanogaster*. *Evidence-Based Complementary and Alternative Medicine, 2018*.

Richardson, A., Williams, V., Rath, J., Villanti, A. C., and **Vallone, D. (2014).** The next generation of users: prevalence and longitudinal patterns of tobacco use among US young adults. *American Journal of Public Health*, ***104*(8)**, 1429-1436.

Rodrigues, C. G., Ferreira, P. R., Oliveira, M., Júnior, R. R., Valerio, H. M., Bramdi, I. V., and **Oliveira, D. A. (2014)**. Antibacterial activity of tannins from *Psidium guineense* Sw.(Myrtaceae). *Journal of Medicinal Plant Research*, **8**, 1-5.

Ross, I. A. (2007). Medicinal plants of the world, volume 3: Chemical constituents, traditional and modern medicinal uses (Vol. 3 ed.). Totowa, New Jersey: Springer Science & Business Media.

Ruhsam, M., and **Hollingsworth, P. M. (2018)**. Authentication of *Eleutherococcus* and *Rhodiola* herbal supplement products in the United Kingdom. *Journal of Pharmaceutical and Biomedical Analysis, **149***, 403 - 409.

Salih, E.Y.A., Kanninen, M., Sipi, M., Luukkanen, O., Hiltunen, R., Vuorela, H., Julkunen-Tiitto, R. and **Fyhrquist, P. (2017).** Tannins, flavonoids and stilbenes in extracts of African savanna woodland trees *Terminalia brownii*, *Terminalia laxiflora* and *Anogeissus leiocarpus* showing promising antibacterial potential. *South African Journal of Botany, **108***, 370-386.

Samie, A., and **Mashau, F. (2013)**. Antifungal activities of fifteen Southern African medicinal plants against five *Fusarium* species. *Journal of Medicinal Plants Research, **7***, 1839-1848.

Saravanan, G., and **Pari, L. (2008)**. Hypoglycaemic and antihyperglycaemic effect of *Syzygium cumini* bark in streptozotocin-induced diabetic rats. *Journal of Pharmacology and Toxicology, **3*****(1)**, 1-10.

Sautron, C., and **Cock, I. E. (2014).** Antimicrobial activity and toxicity of *Syzygium australe* and *Syzygium leuhmannii* fruit extracts. *Pharmacognosy Communications, 4*(1).

Scortichini, M., and **Rossi, M. P. (1991)**. Preliminary in vitro evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) Winslow et al. *Journal of Applied Bacteriology, **71*(2)**,* 109-112.

Scott, J. A., Cunningham, D. G., and **Marles, R. J. (2010)**. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. *Toxicology and Applied Pharmacology, **243*(2)**,* 198-216.

Sharma, P., Jha, A. B., Dubey, R. S., & Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany, 2012*.

Silva, O., Barbosa, S., Diniz, A., Valdeira, M. L., and **Gomes, E. (1997)**. Plant extracts antiviral activity against *Herpes simplex* virus type 1 and African swine fever virus. *International Journal of Pharmacognosy, **35*(1)**,* 12-16.

Silva, O., Duarte, A., Cabrita, J., Pimentel, M., Diniz, A., and **Gomes, E. (1996).** Antimicrobial activity of Guinea-Bissau traditional remedies. *Journal of Ethnopharmacology, **50*(1)**,* 55-59.

Sindhu, T., Rajamanikandan, S., Ragavendran, P., Sophia, D., Meenakshi, P., and **Durga, D. (2010)**. Antidiabetic activity of *Mollugo nudicaulis* against alloxan induced diabetic rats. *International Journal of Applied Biology and Pharmaceutical Technology, **1*(3)**,* 511-519.

Singh, A. G., Kumar, A., and **Tewari, D. D. (2012)**. An ethnobotanical survey of medicinal plants used in Terai forest of western Nepal. *Journal of Ethnobiology and Ethnomedicine, **8*(1)**, 19.

Singh, B., and **Singh, S. (2003)**. Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. *Phytotherapy Research, **17*(7)**, 814-816.

Singh, R., Singh, S., Jeyabalan, G., and **Ali, A. (2012)**. An overview on traditional medicinal plants as aphrodisiac agent. *Journal of Pharmacognosy and Phytochemistry, **1*(4)**, 43-56.

Stærk, D., Lykkeberg, A. K., Christensen, J., Budnik, B. A., Abe, F., and **Jaroszewski, J. W. (2002).** In Vitro Cytotoxic Activity of Phenanthroindolizidine Alkaloids from *Cynanchum vincetoxicum* and *Tylophora tanakae* against Drug-Sensitive and Multidrug-Resistant Cancer Cells. *Journal of Natural Products, **65*(9)**, 1299-1302.

Staniek, A., Bouwmeester, H., Fraser, P.D., Kayser, O., Martens, S., Tissier, A., van der Krol, S., Wessjohann, L. and **Warzecha1, H.** (2014). Natural products–learning chemistry from plants. *Biotechnology Journal, **9*(3)**, 326-336.

Subramanian, S., Gakunga, R., Kibachio, J., Gathecha, G., Edwards, P., Ogola, E., Yonga, G., Busakhala, N., Munyoro, E., Chakaya, J. and **Ngugi, N (2018).** Cost and affordability of non-communicable disease screening, diagnosis and treatment in Kenya: Patient payments in the private and public sectors. *PloS One, **13*(1)**, e0190113.

Suliman, S., Van Vuuren, S. F., and **Viljoen, A. M. (2010)**. Validating the in vitro antimicrobial activity of *Artemisia afra* in polyherbal combinations to treat respiratory infections. *South African Journal of Botany, **76*(4)**, 655-661.

Sultana, S., Khan, A., Safhi, M. M., and **Alhazmi, H. A. (2016).** Cough suppressant herbal drugs: A review. *International Journal of Pharmaceutical Science Invention, **5*(5)**, 15-28.

Swami, S. B., Thakor, N. S., Patil, M. M., and **Haldankar, P. M. (2012)**. Jamun (*Syzygium cumini* (L.)): a review of its food and medicinal uses. *Food and Nutrition Sciences, **3*(08)**, 1100.

Taddese, S., Asres, K., and **Gebre-Mariam, T. (2003)**. In vitro antimicrobial activities of some selected topically applied medicinal plants of Ethiopia. *Ethiopian Pharmaceutical Journal, **21***, 39-46.

Tadesse, E., Engidawork, E., Nedi, R., and **Mengistu, G. (2017)**. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BMC Complementary and Alternative Medicine,* **17(1)**, 190.

Tajehmiri, A., Issapour, F., Moslem, M. N., Lakeh, M. T., and **Kolavani, M. H. (2014)**. In vitro antimicrobial activity of *Artemisia annua* leaf extracts against pathogenic bacteria. *Advanced Studies in Biology, **6*(3)**, 93-97.

Tan, M. C., Tan, C. P., and **Ho, C. W. (2013)**. Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. *International Food Research Journal, **20*(6)**, 3117.

Tardio, J., and **Pardo-de- Santayana, M. (2008)**. Cultural importance indices: a comparative analysis based on the useful wild plants of Southern Cantabria (Northern Spain). *Economic Botany*, ***62*****(1)**, 24-39.

Teke, G. N., Elisée, K. N., and **Roger, K. J. (2013).** Chemical composition, antimicrobial properties and toxicity evaluation of the essential oil of *Cupressus lusitanica* Mill. leaves from Cameroon. *BMC Complementary and Alternative Medicine*, **13(1)**, 130.

Teklay, A., Abera, B., and **Giday, M. (2013)**. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, ***9*****(1)**, 65.

Thoithi, G. N., Ndwigah, S. N., and **Maima, A. O. (2014).** Antimicrobial properties of some medicinal plants of the Luo community of Kenya. *African Journal of Pharmacology and Therapeutics*, ***3*****(4)**.

Tornheim, J. A., Manya, A. S., Oyando, N., Kabaka, S., Breiman, R. F., and **Feikin, D. R. (2007)**. The epidemiology of hospitalized pneumonia in rural Kenya: the potential of surveillance data in setting public health priorities. *International Journal of Infectious Diseases*, ***11*****(6)**, 536-543.

Tugume, P., Kakudidi, E. K., Buyinza, M., Namaalwa, J., Kamatenesi, M., Mucunguzi, P., and **Kalema, J. (2016).** Ethnobotanical survey of medicinal plant species used by communities around Mabira Central Forest Reserve, Uganda. *Journal of Ethnobiology and Ethnomedicine*, ***12*****(1)**, 5.

Ugoh, S. C., Agarry, O. O., and **Garba, S. A. (2014)**. Studies on the antibacterial activity of *Khaya senegalensis* [(Desr.) A. Juss)] stem bark extract on *Salmonella enterica* subsp. *enterica serovar* Typhi [(ex Kauffmann and Edwards) Le Minor and Popoff]. *Asian Pacific Journal of Tropical Biomedicine, **4***, S279-s283.

Usman, H., Abdulrahman, F. I., and **Usman, A. (2009)**. Qualitative phytochemical screening and in vitro antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional, Complementary and Alternative Medicines, **6*(3)**.

Van Andel, T., Ruysschaert, S., Van de Putte, K., and **Groenendijk, S. (2013)**. What makes a plant magical? Symbolism and sacred herbs in Afro-Surinamese winti rituals. *In African Ethnobotany in the Americas*, 247-284.

Vasconcelos, L. C., Sampaio, F. C., Albuquerque, A. D., and **Vasconcelos, L. D. (2014)**. Cell viability of *Candida albicans* against the antifungal activity of thymol. *Brazilian Dental Journal*, **25(4)**, 277-281.

Wabe, N. T., Mohammed, M. A., and **Raju, N. J. (2011).** An ethnobotanical survey of medicinal plants in the Southeast Ethiopia used in traditional medicine. *Spatula DD, **1*(3)**, 153-158.

Wang, H., Naghavi, M., Allen, C., Barber, R. M., Bhutta, Z. A., Carter, A. and **Coggeshall, M. (2016).** Global, regional, and national life expectancy, all-cause mortality, and cause-specifi c mortality for 249 causes of death, 1980-2015: a systematic analysis for the global burden of disease study 2015. *Lancet, **388*(10053)**, 1459 - 1544.

Wanjiku Ngari, E. (2010). Ethnomedicne of Ogiek of River Njoro watershed, Nakuru, Kenya. *Ethnobotany Research and Applications, **8**,* 135-152.

Willis, K. J. (2017). State of the world's plants report-2017. State of the world's plants report-2017. UK: Royal Botanic Gardens.

Wintola, O. A., and **Afolayan, A. J. (2015).** The antibacterial, phytochemicals and antioxidants evaluation of the root extracts of *Hydnora africana* Thunb. used as antidysenteric in Eastern Cape Province, South Africa. *BMC Complementary and Alternative Medicine*, **15(1)**, 307.

World Health Organization. (2018). *Global Heath Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region 2000-2016.* Geneva.

World Health Organization. (2019). Global Health Observatory data repository. Retrieved from 29 4 2020 from https://apps.who.int/gho/data/node.main.1317?lang=en

Yang, Y., Xiu, J., Liu, J., Zhang, L., Li, X., Xu, Y., Qin, C. and **Zhang, L. (2013).** Chebulagic acid, a hydrolyzable tannin, exhibited antiviral activity in vitro and in vivo against human enterovirus 71. *International Journal of Molecular Sciences*, **14(5)**, 9618-9627.

Yashaswini, S., and **Vasundhara, M. (2011).** Coleus (*Plectranthus barbatus*)-A multipurpose medicinal herb. *International Research Journal of Pharmacy, **2**(**3**)*, 47-58.

Yong, Y. K., Zakaria, Z. A., Kadir, A. A., Somchit, M. N., Lian, G. E., and **Ahmad, Z. (2013).** Chemical constituents and antihistamine activity of *Bixa orellana* leaf extract. *BMC Complementary and Alternative Medicine*, **13(1)**, 1-7.

York, T., De Wet, H., and **Van Vuuren, S. (2011).** Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa. *Journal of Ethnopharmacology*, ***135*****(3)**, 696 - 710.

Younis, W., Asif, H., Sharif, A., Riaz, H., Bukhari, I. A., and **Assiri, A. M. (2018).** Traditional medicinal plants used for respiratory disorders in Pakistan: a review of the ethno-medicinal and pharmacological evidence. *Chinese Medicine*, ***13*****(1),** 48.

Zar, H. J., and **Ferkol, T. W. (2014).** The global burden of respiratory disease—impact on child health. *Pediatric Pulmonology*, ***49*****(5)**, 430-434.

Zerabruk, S., and **Yirga, G. (2012).** Traditional knowledge of medicinal plants in Gindeberet district, Western Ethiopia. *South African Journal of Botany*, ***78***, 165-169.

APPENDICES

Appendix I: Ethical approval letter from University of Nairobi- Faculty of Veterinary Medicine Biosafety, Animal Care and Use Committee (FVM-BACUC)

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE

REF: FVM BAUEC/2019/210

Dr. James Kiamba Mailu
University of Nairobi
Dept of PHP & T
15/04/2019

Dear Dr. Mailu,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Ethnopharmacological and Toxicological Study of Medicinal plants Used Against Respiratory Infections in Kisumu East Sub-County.

By Dr. James Kiamba Mailu J56/8202 /2017.

We refer to your MSc proposal submitted to our committee for review and your application letter dated 1st April 2019.

We have reviewed your proposal and are satisfied that the proposed Ethnopharmacological and Toxicological study meets acceptable minimum standards of the ethical regulation guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine

Appendix II: Approval from National Commission for Science, Technology & Innovation (NACOSTI)

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **224284**

Date of Issue: **09/January/2020**

RESEARCH LICENSE

This is to Certify that Dr.. James Mailu of University of Nairobi, has been licensed to conduct research in Kisumu on the topic: ETHNOPHARMACOLOGICAL AND TOXICOLOGICAL STUDY OF MEDICINAL PLANTS USED AGAINST RESPIRATORY INFECTIONS IN KISUMU EAST SUB-COUNTY for the period ending : 09/January/2021.

License No: **NACOSTI/P/20/3004**

224284

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document. Scan the QR Code using QR scanner application.

Appendix III: Consent to participate in a study of medicinal plants used against respiratory infections in Kisumu East Sub-County

INVESTIGATOR

Name: James Mailu

Department: Public Health, Pharmacology and Toxicology, University of Nairobi

Phone No: 0726 340 608

INTRODUCTION

You are humbly requested to take part in a research study to look at the ethnomedicinal practice of locals within Kisumu East- Sub County. You have been selected as a possible participant as you are residents of the Sub County, above 18 years of age and identified as a key resource person with good knowledge on medicinal plants.

I urge you read the form and I will happily address any of your concerns.

PURPOSE OF THE STUDY

To conduct an ethnopharmacological and toxicological study of plants of medicinal value used against respiratory infections in Kisumu East Sub-County. This work will possible be published in a peer review journal.

STUDY PROCEDURE

Upon agreeing to participate in the study, you will be asked questions regarding basic demographics and plants used in management of respiratory diseases. You will highlight type of plant species, part of plant material used, how it is prepared, route of administration, dosage, contraindications and possible side effects. In addition, you will assist the researcher in

identifying the plant/plants and aid in collection of the same for documentation and subjection to further analysis.

RISKS IN PARTICIPATING IN THE STUDY

There are no foreseeable risks expected in the study.

BENEFITS OF PARTICIPATING IN THE STUDY

Benefits of being in the study include helping in sharing of vital information for posterity, learning from likeminded persons and possible identification of lead compounds that may alleviate human suffering.

CONFIDENTIALITY

Information shared will be treated confidential. Your identity will be kept anonymous. Electronic information will be coded and secured using a password.

RIGHT TO REFUSE OR WITHDRAW

You retain the right to refuse or withdraw your participation in the study.

RIGHT TO MAKE ENQUIRIES

You can ask any question to the investigator at any time during the study. The investigator will address any concerns raised at any point in the study.

CONSENT

I have clearly read and understood the information provided and hereby willingly participate in the study.

Informant's name ……

Informants signature…….

Date……

Investigators Signature…… Date……

Appendix IV: A questionnaire to collect ethnomedicinal data of herbal medicinal plants used against respiratory infections in Kisumu East Sub -County

Kindly respond to the questions below. This information is confidential and will be used for research purpose only

1. Name._____

2. Gender Male ☐ Female ☐
3. Age _____
4. Level of education

No formal education ☐ Primary education ☐

Post-secondary education ☐ Secondary education ☐

5. Ward/Village _____
6. Have you heard of herbal medicinal practice? Yes ☐ No ☐
7. Do you practice herbal medicine? Yes ☐ No ☐
8. Are you a herbal practitioner? Yes ☐ No ☐
9. If yes, for how many years have you been practicing? …….....
10. Which illnesses are prevalent in this area? ……
11. Which methods do you use to treat them? .....

12. Are respiratory infections a problem in this locality?……

13. How do you treat respiratory infections here? …….……..

14. Do you use medicinal plants to respiratory infections? .....

15. If yes, which plants do you use?.....

16. How can you prioritize these plants in the management of respiratory infections? ……

17. What is the local name of the plant? ……

18. Which parts of this plant do you use to treat respiratory infections? ……

19. How do you prepare it? ……

20. How is it administered and for how long? ……

21. Do you use the plant fresh?.....

22. If NO, how do you store the plant for future use? ……

23. Does it have side effects? ……..

24. If yes, which ones? ……

Plant (local name)	Condition treated	Part of plant used	Preventive/curative	Method of preparation and form of plant extract administered	Amount of plant extract consumed, frequency and route of plant extract administration	Possible side effects/precaution while administering or during use of the plant extract	Other uses of the plant and plant extract	Comments/ Notes

Appendix V: Photos taken in Kajulu hills during collection of plant specimens

Appendix VI: Sample of plant specimens collected and deposited in University of Nairobi Herbarium

Family: Acanthaceae

Botanical name: *Acanthus polystachius*

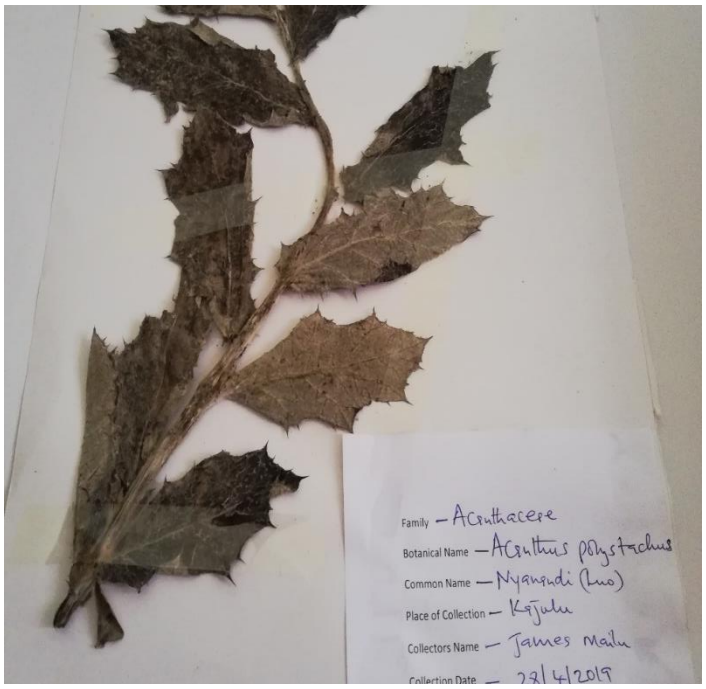
Common name: Nyanandi

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 28.4.2019

Voucher no: JM2019/284/003



Family: Apocynaceae

Botanical name: *Carissa edulis*

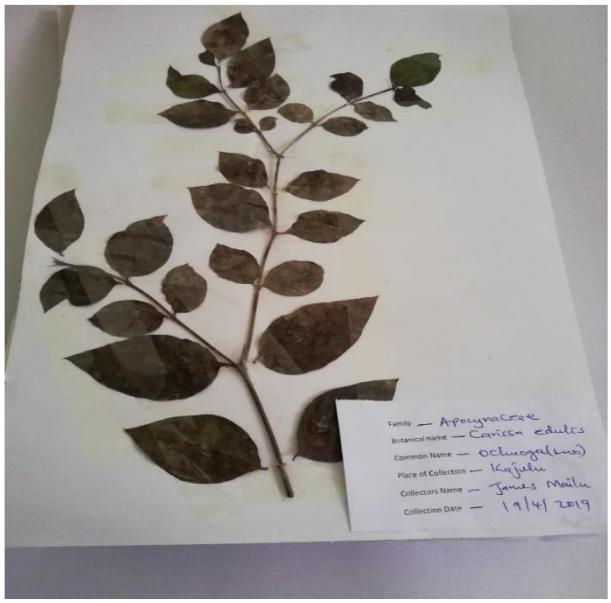
Common name: Ochuoga

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 19.4.2019

Voucher no: JM2019/194/022



Family: Fabaceae

Botanical name: *Tylosemma fassonglesse*

Common name: Ombasa

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 19.4.2019

Voucher no: JM2019/194/016

Family: Meliaeae

Botanical name: *Khaya senegalensis*

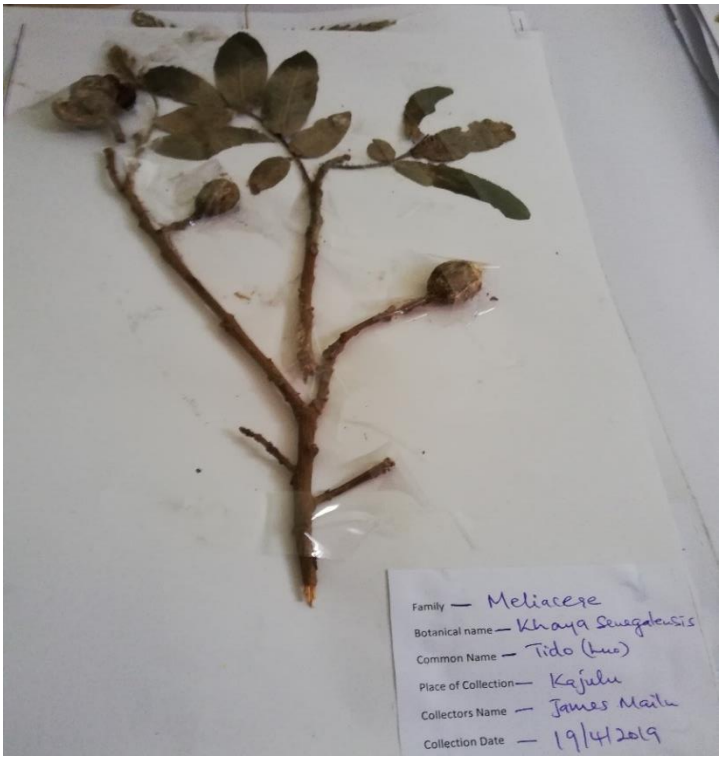
Common name: Tido

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 19.4.2019

Voucher no: JM2019/194/016



Family: Ebenaceae

Botanical name: *Euclea divinorum*

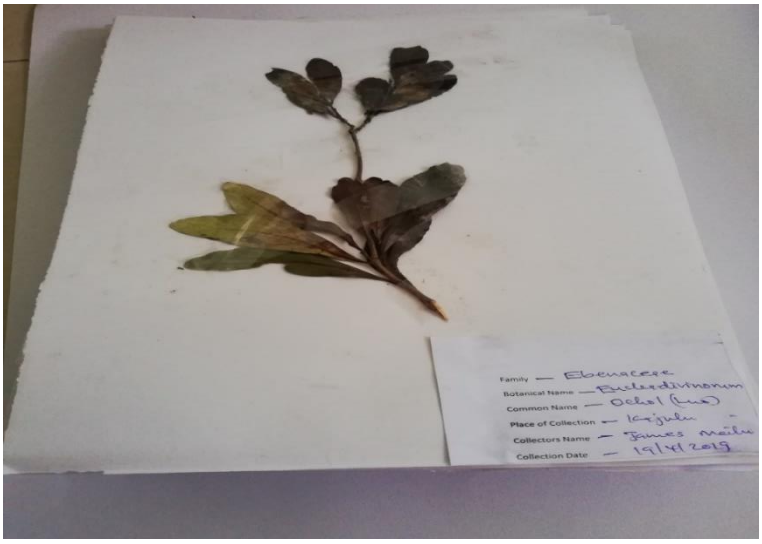
Common name: Ochol

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 19.4.2019

Voucher no: JM2019/194/023



Family: Rubiaceae

Botanical name: *Keetia gueinzii*

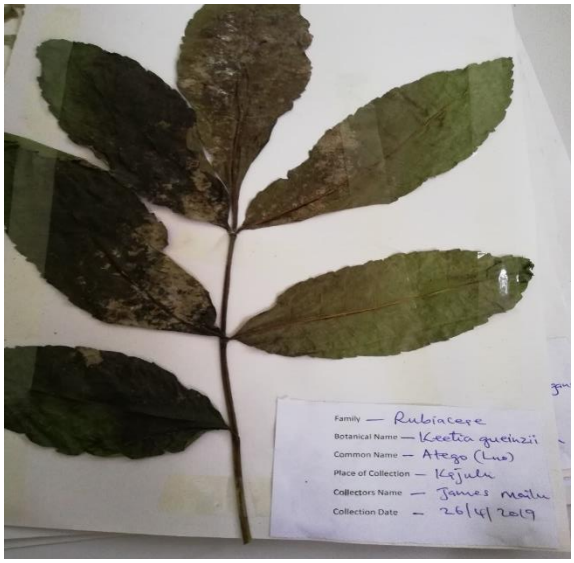
Common name: Atego

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 19.4.2019

Voucher no: JM2019/194/001



Family: Verbenaceae

Botanical name: *Clerodendrum myricoides*

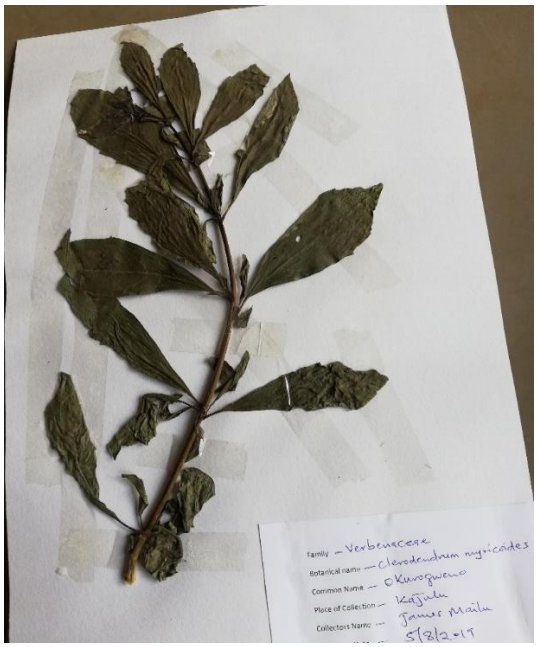
Common name: Okwerogweno

Place of collection: Kajulu hill

Collectors name: James Mailu

Collection date: 5.8.2019

Voucher no: JM2019/058/021



Family: Euphorbiceae

Botanical name: *Croton megalocarpus*

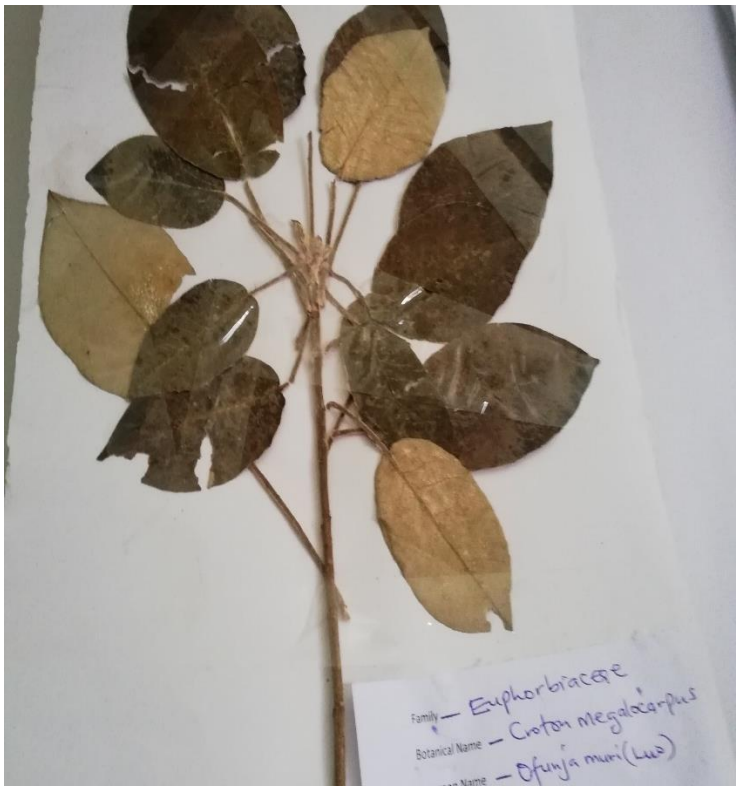
Common name: Ofunjamuri

Place of collection: Kajulu hill

Collectors name: James Mailu

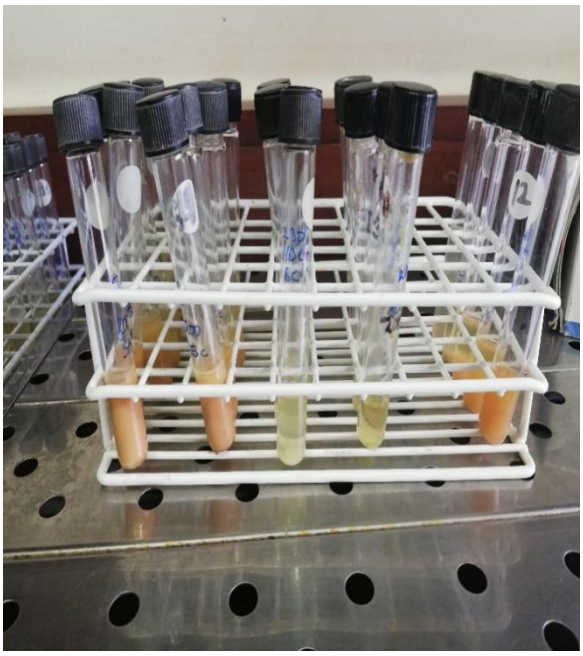
Collection date: 5.8.2019

Voucher no: JM2019/058/021

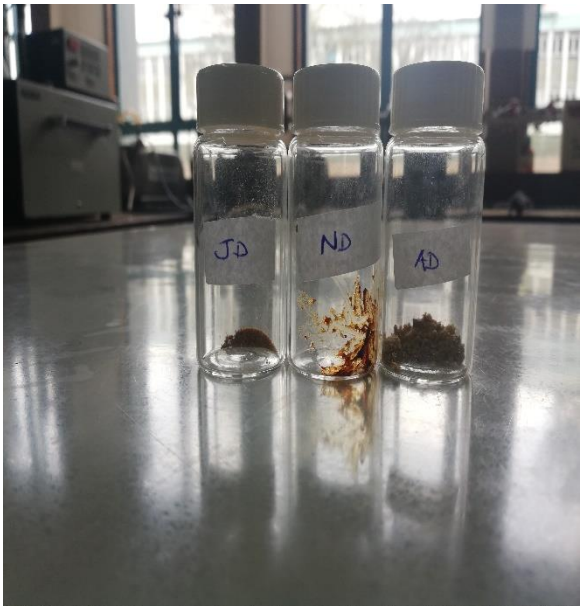


Appendix VII: Photos of extraction process

Appendix VIII: Photos of determination of Minimum Inhibitory Concentration (MIC) values



Appendix IX: Photos of cytotoxic evaluation (Brine shrimp lethality assay)



Appendix X: Percentage mortality of the crude extracts of *Acanthus polystachius*, *Keetia gueinzii* and *Rhynchosia elegans*

Table 1: Percentage mortality of aqueous extract of *Acanthus polystachius* Delile.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	0
2	10	1	0	0
3	10	1	0	0
4	10	1	0	0
5	10	1	0	0
1	100	2	0	0
2	100	2	0	0
3	100	2	0	0
4	100	2	0	0
5	100	2	0	0
1	1000	3	0	0
2	1000	3	0	0
3	1000	3	0	0
4	1000	3	0	0
5	1000	3	0	0

Table 2: Percentage mortality of aqueous extract of *Keetia gueinzii* (Sond.) Bridson.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	0	1.0334
3	100	2	0	1.0334
4	100	2	0	1.0334
5	100	2	0	1.0334
1	1000	3	0	1.0334
2	1000	3	0	1.0334
3	1000	3	20	4.1584
4	1000	3	10	3.7184
5	1000	3	0	1.0334

Table 3: Percentage mortality of aqueous extract of *Rhynchosia elegans* A. Rich.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	0	1.0334
3	100	2	0	1.0334
4	100	2	0	1.0334
5	100	2	0	1.0334
1	1000	3	100	8.9538
2	1000	3	100	8.9538
3	1000	3	80	5.8416
4	1000	3	90	6.2816
5	1000	3	80	5.8416

Table 4: Percentage mortality of acetonic extract of *Acanthus polystachius* Delile.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	10	3.7184
3	100	2	0	1.0334
4	100	2	0	1.0334
5	100	2	0	1.0334
1	1000	3	100	8.9538
2	1000	3	100	8.9538
3	1000	3	100	8.9538
4	1000	3	100	8.9538
5	1000	3	100	8.9538

Table 5: Percentage mortality of acetonic extract of *Keetia gueinzii* (sond.) Bridson.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	0
2	10	1	0	0
3	10	1	0	0
4	10	1	0	0
5	10	1	0	0
1	100	2	0	0
2	100	2	0	0
3	100	2	0	0
4	100	2	0	0
5	100	2	0	0
1	1000	3	0	0
2	1000	3	0	0
3	1000	3	0	0
4	1000	3	0	0
5	1000	3	0	0

Table 6: Percentage mortality of acetonic extract of *Rhynchosia elegans* A. Rich.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	0	1.0334
3	100	2	10	3.7184
4	100	2	0	1.0334
5	100	2	10	3.7184
1	1000	3	100	8.9538
2	1000	3	100	8.9538
3	1000	3	100	8.9538
4	1000	3	100	8.9538
5	1000	3	100	8.9538

Table 7: Percentage mortality of methanolic extract of *Acanthus polystachius* Delile.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probits
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	20	4.1584
2	100	2	30	4.4756
3	100	2	0	1.0334
4	100	2	0	1.0334
5	100	2	10	3.7184
1	1000	3	100	8.9538
2	1000	3	90	6.2816
3	1000	3	100	8.9538
4	1000	3	100	8.9538
5	1000	3	100	8.9538

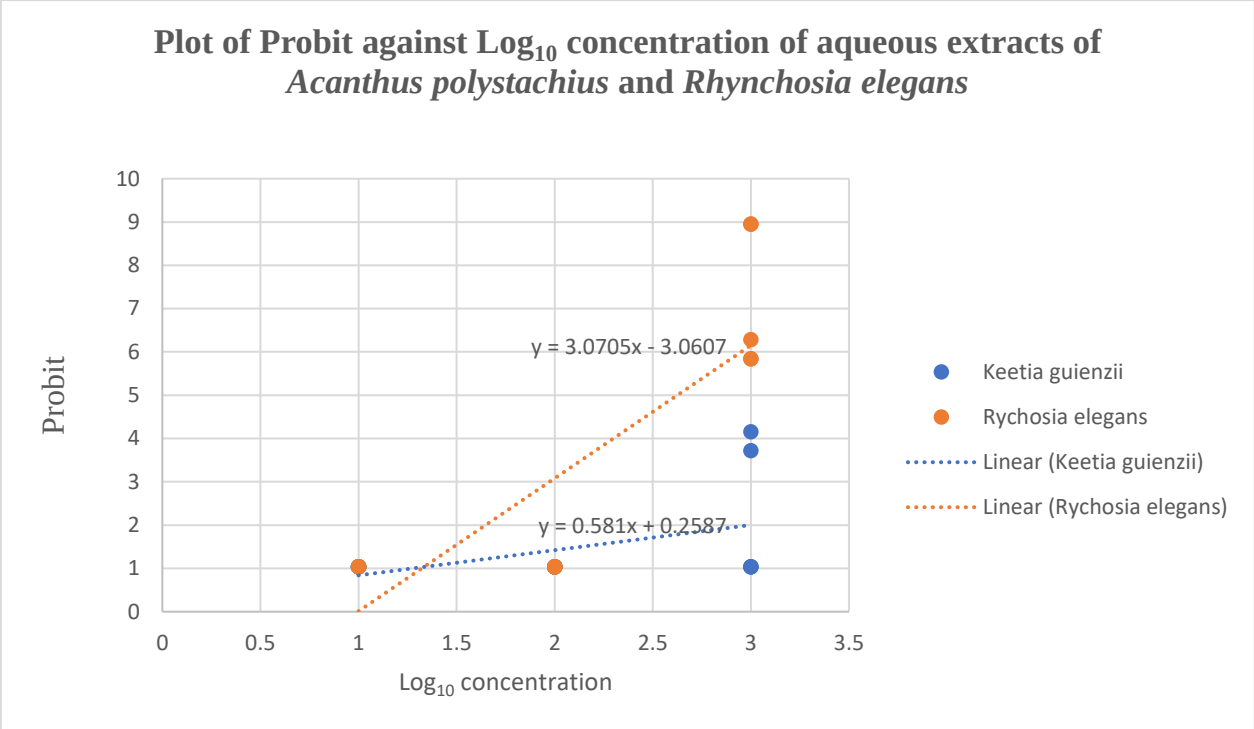
Table 8: Percentage mortality of methanolic extract of *Keetia gueinzii* (Sond.) Bridson.

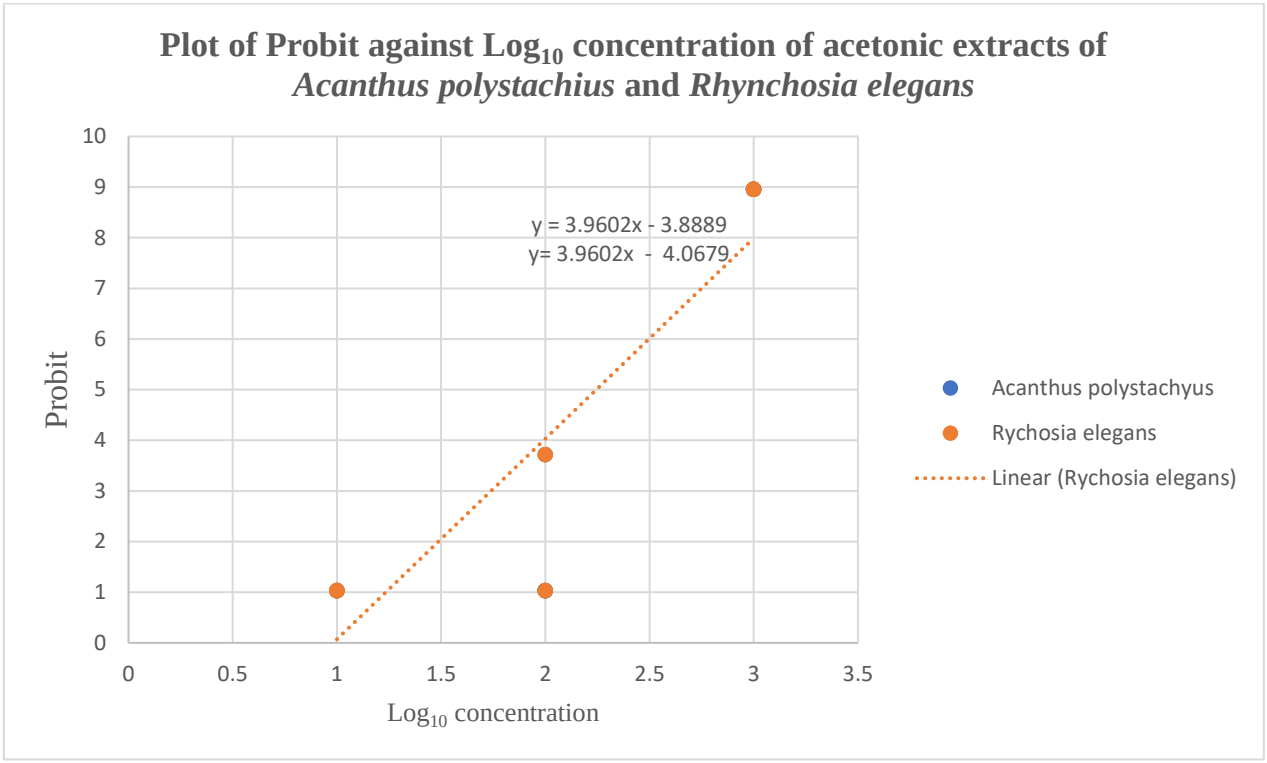
Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probits
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	0	1.0334
3	100	2	0	1.0334
4	100	2	0	1.0334
5	100	2	0	1.0334
1	1000	3	0	1.0334
2	1000	3	10	3.7184
3	1000	3	0	1.0334
4	1000	3	0	1.0334
5	1000	3	0	1.0334

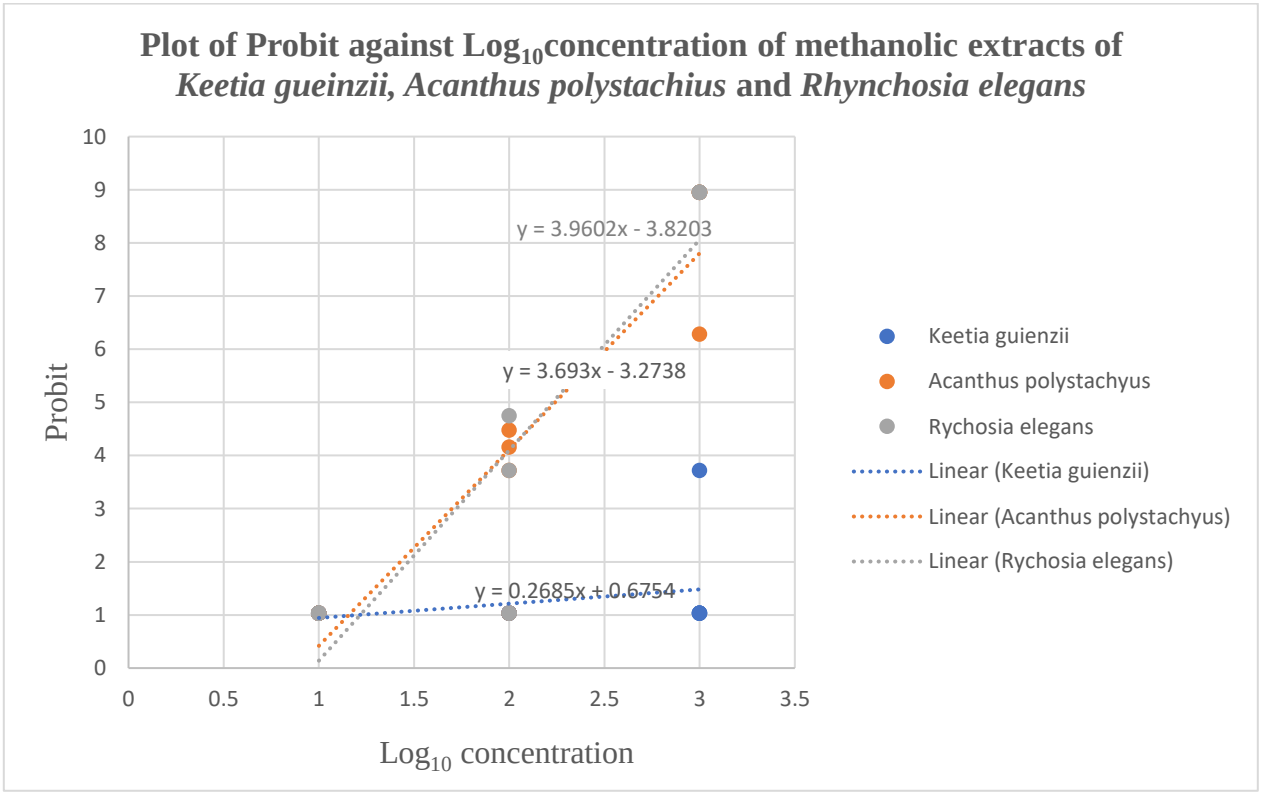
Table 9: Percentage mortality of methanolic extract of *Rhynchosia elegans* A. Rich.

Tube Number	Concentration µg/ml	Log_{10} concentration	% Mortality	Probit
1	10	1	0	1.0334
2	10	1	0	1.0334
3	10	1	0	1.0334
4	10	1	0	1.0334
5	10	1	0	1.0334
1	100	2	0	1.0334
2	100	2	10	3.7184
3	100	2	40	4.7467
4	100	2	0	1.0334
5	100	2	0	1.0334
1	1000	3	100	8.9538
2	1000	3	100	8.9538
3	1000	3	100	8.9538
4	1000	3	100	8.9538
5	1000	3	100	8.9538

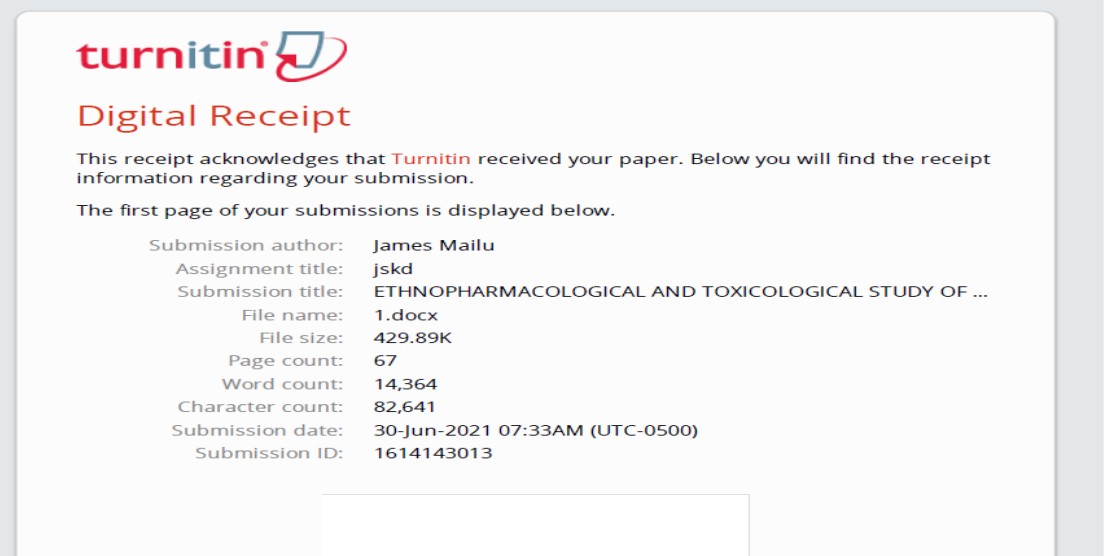
Appendix XI: Probit analysis and determination of LC_{50} values for *Acanthus polystachius*, *Keetia gueinzii*, and *Rhynchosia elegans* using Microsoft Excel 2019




Plot of Probit against Log10 concentration of acetonic extracts of Acanthus polystachius and Rhynchosia elegans
Probit
Log10 concentration
y = 3.9602x - 3.8889
y= 3.9602x - 4.0679
Acanthus polystachyus
Rychosia elegans
Linear (Rychosia elegans)


Plot of Probit against Log10concentration of methanolic extracts of Keetia gueinzii, Acanthus polystachius and Rhynchosia elegans
Probit
Log10 concentration
y = 3.9602x - 3.8203
y = 3.693x - 3.2738
y = 0.2685x + 0.6754
Keetia guienzii
Acanthus polystachyus
Rychosia elegans
Linear (Keetia guienzii)
Linear (Acanthus polystachyus)
Linear (Rychosia elegans)

Appendix XII: Turnitin report



turnitin

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	James Mailu
Assignment title:	jskd
Submission title:	ETHNOPHARMACOLOGICAL AND TOXICOLOGICAL STUDY OF ...
File name:	1.docx
File size:	429.89K
Page count:	67
Word count:	14,364
Character count:	82,641
Submission date:	30-Jun-2021 07:33AM (UTC-0500)
Submission ID:	1614143013

ETHNOPHARMACOLOGICAL AND TOXICOLOGICAL STUDY OF MEDICINAL PLANTS USED AGAINST RESPIRATORY INFECTIONS IN KISUMU EAST SUB-`COUNTY

ORIGINALITY REPORT

7%	7%	8%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.researchsquare.com Internet Source	6%
2	cmjournal.biomedcentral.com Internet Source	1%
3	T. K. Lim. "Edible Medicinal And Non Medicinal Plants", Springer Science and Business Media LLC, 2012 Publication	1%

Appendix XIII: Published work associated with the thesis

Published in BMC CHINESE MEDICINE JOURNAL

Mailu et al. Chin Med (2020) 15:95
https://doi.org/10.1186/s13020-020-00374-2

Chinese Medicine

RESEARCH Open Access

Medicinal plants used in managing diseases of the respiratory system among the Luo community: an appraisal of Kisumu East Sub-County, Kenya

James Kiamba Mailu[1,2*], Joseph Mwaruia Nguta[1], James Mucunu Mbaria[1] and Mitchel Otieno Okumu[1,3]

Abstract

Background: Poor access to healthcare in rural communities causes many people to seek herbalists who use medicinal plants for the treatment of various disease conditions. Most knowledge of traditional herbal medicine makes use of indigenous remedies which are often undocumented and are at risk of being lost. The preservation of this knowledge may facilitate scientific inquiry into promising new therapeutic molecules.

Methods: Semi-structured questionnaires were used to collect the sociodemographic information of 30 herbalists in Kisumu East Sub County. The local names of medicinal plants used in managing illnesses of the respiratory system, their habit, active parts, indications, methods of preparation, routes of administration, scientific identity, and conservation status were also recorded. Other reported traditional uses, pharmacological activities, and toxicological data were identified via a literature search.

Results: Most herbalists were female (86.7%), aged between 61 and 70 years (43.3%) with no formal education (56.7%), and had 21–30 years of practice (30%). 44 plant species, belonging to 43 genera and 28 families were identified. Leguminosae and Rutaceae plant families were predominant, leaves were frequently used (33%), and trees were the most common habit (44.4%). Most plants were collected in the wild (79.2%), preparation was mainly by decoction (68.8%), and the administration was mainly orally. The main indication was cough and 79.5% of all documented plant species had previously been reported to have a pharmacological activity relevant to the mitigation of respiratory illnesses. Toxicological data was available for 84.1% of the plant species identified.

Conclusions: The predominant use of roots, root barks, and root tubers by herbalists in Kisumu East Sub County threatens to negatively impact the ecological survival of some plant species. The preservation of herbalists' knowledge of medicinal plants in the study area is a pressing concern considering their advanced age and little formal education. There is a need to conserve some of the medicinal plants documented in this study. The medicinal claims made by herbalists also warrant scientific scrutiny.

Keywords: Ethnopharmacology, Medicinal plants, Kisumu East, Luo, Ethnomedicinal, Ethnobotanical, Respiratory diseases, Cough

*Correspondence: jkmailu@gmail.com
[1] Department of Public Health, Pharmacology, and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, PO Box 29053-00625, Nairobi, Kenya
Full list of author information is available at the end of the article

Background

The global burden of respiratory diseases makes for daunting reading. Lower respiratory tract infections (LRTI) and chronic obstructive pulmonary disease (COPD) reportedly claimed 6 million human lives in