

**ETHNOBOTANICAL, ANTIOXIDANT AND TOXICITY STUDY OF
SELECTED MEDICINAL PLANTS USED IN NYAMIRA NORTH SUB-COUNTY,
NYAMIRA COUNTY**

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DECLARATION

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

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DEDICATION

I dedicate this work first to God Almighty for His sustenance throughout the whole process of the study. To my dear wife Margaret and our children Bravy, Amy and Lister for their understanding and the constant support they have continually accorded me.

I am really appreciative.

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LIST OF ABBREVIATIONA AND ACRONYMS

| | |
|------------------|---|
| ATC | Acute Toxic Classic method |
| DPPH | 2, 2- diphenyl-1-picrylhydrazyl |
| DPX | Dibutylphthalate Polystyrene Xylene |
| FBO | Faith Based Organization |
| IC ₅₀ | Median Inhibitory concentration |
| LD ₅₀ | Median lethal dose |
| NADPH | Nicotinamide adenine dinucleotide phosphate |
| NCAPD | National Coordinating Agency for Population and Development |
| NGO | Non-governmental organization |
| NO | Nitric oxide (nitrogen oxide, nitrogen monoxide) |
| OECD | Organization for Economic Co-operation and Development |
| PRA | Participatory rapid appraisal |
| RNA | Ribonucleic acid |
| RNS | Reactive Oxygen Species |
| ROS | Reactive oxygen species |
| RSA | Radical Scavenging Activity |
| SEM | Standard Error of the Mean |
| UV | Ultra violet light |
| WHO | World health organization |

ABSTRACT

Traditional medical practices are common with many Kenyan communities. There is a steady use of herbal remedies to treat diseases despite increased availability of conventional medicines. Therefore it is of paramount significance to document these plants as a means of preserving cultural knowledge of traditional medicine. The study aimed to identify and document all the plants used for oxidative stress-related diseases in the study area and also evaluate the antioxidant and toxic effects of some selected medicinal plants that are used by traditional medical practitioners (TMPs) in Nyamira County.

Nyamira North Sub-county was the study area in which field study was carried out. Thirty six (36) TMPs were selected from the seven sub-county wards namely Bokeira, Magwagwa, Itibo, Ekerenyo, Bomwagamo, Kiabonyoru and Mekenene. The TMPs were interviewed using semi-structured questionnaires. Information of the plants used (scientific name, local name, growth form, habitats, disease treated and the part used) was collected and documented. Four selected plants based on existing literature namely, *Phragmanthera usuiensis*, *Ensete ventricosum*, *Echinops amplexicaulis* and *Rhoicissus tridentata*, were screened for phytochemicals and subsequently subjected to toxicological studies to determine their safety. Frequency tables and data triangulation was used to present the collected data.

Fifty seven (57) medicinal plants species from 36 families and covering 53 genera were identified as being used in management of oxidative stress. Thirty nine plant species were found to have reports of similar use in literature whereas 18 species were being reported for the first time in regard to their use in oxidative stress-related diseases. The most common encountered families were Asteraceae (15.79 %) followed by Solanaceae (7.02%) and then Fabaceae and Rubiaceae each at 5.26%. Majority of the growth forms used were shrubs

(42%), followed by herbs (33%), trees (14%) and climbers (11%). The root/root barks were the parts of the plants that were used in greater percentage (53%) then the leaves (25%), stem bark (12%) and whole plant (8%). Phytochemicals analysis of the selected plants extracted using water and methanol showed presence of various bioactive compounds. The phytochemicals found in aqueous extracts were tannins, glycosides, saponins, flavonoids, phenols, coumarins and free sugars. Phytochemicals found in methanolic extracts include alkaloids, tannins, glycosides, saponins, steroids, flavonoids, terpenoids, phenols, coumarins and free sugars. Radical scavenging activities of the four plant extracts were evaluated using DPPH radical-scavenging method. At concentrations above the IC₅₀, all methanolic extracts except in *E. ventricosum* extracts exhibited higher activity than the aqueous extracts. All groups of test animals did not exhibit any serious toxic or lethal effects even at the administration of the limit dose, 2000 mg/kg body weight.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Plants have since antiquity played a pivotal role in healthcare by providing medicines. Since then, plant parts or plant derived products have proved important in treating various diseases such as aches, swellings, stomach disorders, sores, sexually spread infections, nervous system and mental disorders among others (Mustafa *et al.*, 2017; Wyk & Gericke, 2000). Despite the diverse ethnomedical knowledge, the possibility of various plants to possess medicinal value is yet to be exhaustively studied and documented. entirely. This is due to the fact that many studies on the plant kingdom are often directed to specific ailments, hence revealing only a few active compounds. This is mainly because of unavailability of enough time to do the studies and also due to economic strains. For instance, the screening of over 35,000 extracts from plants that was carried out by the National Cancer Institute (NCI) of the America in the nineteenth century was only directed to phytochemicals of anticancer importance. Compounds without anticancer activity were excluded and therefore not screened for their medicinal value (Queiroz & Wolfender, 2014). Screening of medicinal plants based on ethnomedical information is important in the quest to generate lead compounds for synthesizing new drugs that are cheap, potent, and efficacious and with few adverse side effects (Potier *et al.*, 1996). A review by Fennell *et al* (2004) showed that approximately a hundred and twenty two medicaments could have been discovered through ethnopharmacological study of some species of plants that were screened.

Traditional medicine is too valuable to be unnoticed within the research and development of current drugs. In traditional medicine, a single herb or formula can incorporate many phytochemical materials such as alkaloids, terpenoids, flavonoids and many others. These

chemical substances usually function on their own or in conjunction with each other to provide the desired pharmacological effect (Parasuraman *et al.*, 2014). It is widely known that plenty of plant-originated drugs in medical remedy today were derived from traditional medicine (Li-Weber, 2009). Similarly, it has been demonstrated that these drugs were derived from medicinal plants and their pharmacological effects had been determined through their application in ethnopharmacology (Fabricant & Farnsworth, 2001).

Because of availability of phytochemicals such as flavonoids, terpenoids, alkaloids, diterpenes, tannins, glycosides and other phenols, medicinal plants possess robust antioxidant effects and can be utilized in protecting the human cells against the oxidative damage resulting from free radicals (Krishnaiah *et al.*, 2011). These free radicals like reactive Oxygen Species (ROS), superoxide anion, hydroxyl radical and hydrogen peroxide play a vital role in the development of various illnesses which include arthritis, dementia, cancer, hypertension and diabetes (Devasagayam *et al.*, 2004). The free radicals within the human body are generated through aerobic respiration or from exogenous sources (Halliwell & Gutteridge, 1990). These radicals react with diverse organic molecules particularly fats, proteins and DNA causing lack of balance between the radicals and antioxidants. The presence of antioxidants and numerous phytochemicals in herbal remedies scavenge those reactive species offer safety to human health against diseases associated with oxidative stress (Singh *et al.*, 2016).

This study was therefore carried out in Nyamira North sub-county in Nyamira County, to document the plants used by the herbalist in treatment of various diseases and ailments that are associated with oxidative stress. The study also sought to carry out scientific studies on the safety and antioxidant properties of some selected plants to validate their use.

1.2 Objectives

1.2.1 General objective

To carry out an ethnobotanical survey and investigate pharmacological and safety profiles of medicinal plants used for oxidative stress in Nyamira North Sub-county, Nyamira County

1.2.2 Specific objectives

- a) To determine medicinal plants used by herbalists to treat conditions associated with oxidative stress in Nyamira North sub-county, Nyamira County.
- b) To determine antioxidant activities of selected plant extracts using DPPH radical scavenging method.
- c) To determine acute toxicity of active plant extracts using Acute Oral Toxicity –Acute Toxic Class Method.

1.3 Problem statement and study justification

The world health organization (WHO) estimates that 80% and above of African population depend on plant and animal based medicines to meet their health care needs (WHO, 2002). In Kenya, by the year 2008, the conventional health system provided healthcare only for 30% of Kenyans, thus implying that the remaining 70% (approximately 27 million people) outside the national system had to rely on traditional forms of health care (NCAPD, 2008). The people of Abagusii, whose habitation is Kisii highlands, are rich in their traditions and culture. The traditional medicine practitioners especially herbalists contribute significantly health provision (Omwenga *et al.*, 2015; Ondicho *et al.*, 2015). The documentation of plant diversity and ethnopharmacology information of the Kisii community is scanty and hence the current study aimed to provide knowledge of the medicinal plants used by the community.

1.4. Hypotheses

1.4.1 Null Hypothesis

Extracts of medicinal plants used by herbalists in Nyamira County have no antioxidant and toxic effects

1.4.2 Alternative Hypothesis

Extracts of medicinal plants used by herbalists in Nyamira County have antioxidant and toxic effects

1.5. Assumption

There is uniform distribution of antioxidant activity in the selected parts of the plant.

CHAPTER TWO: LITERATURE REVIEW

2.1 Plant species of ethnomedical importance and ethnobotanical knowledge

There are various therapeutically effective active compounds produced by Medicinal plants which can be used against target diseases (Sasidharan *et al.*, 2011). Various documented data are available with details on use of plants as medicines. An ancient scroll, discovered on a Sumerian clay slab from Nagpur about 5000 years old serves as a proof that herbal remedies have been in use for the longest period of time. It has documented various remedies in form of recipe. The recipes are for herbal drugs comprising of over 250 various herbs such as opium poppy, henbane and mandrake (Holcomb, 1944). Some religious books from Indian like Vedas, point out plant remedies which were used in Ayurvedic medicine. Several spice plants used even today originate from India. They include nutmeg, pepper, clove, etc. (Petrovska, 2012).

The Ebers Papyrus, written around 1550 BC, contained a collection of about 800 drug formulae covering seven hundred plant species used as remedies for various ailments (Shoeb, 2006). Herbal remedies from the Artemisia family, from which antimalarial Artemisinin was derived, had always been used in ancient times to restore and guard health against various ailments (Shoeb, 2006). This explains the origin of the name which was derived from a Greek word the “Artemis”, that means wholesomeness (Petrovska, 2012). The works of Hippocrates that was carried out between 459–370 BC is comprised of three hundred medicinal plants that were classified according to their physiological properties. For examples garlic was used for intestinal parasites, opium and mandrake were used as narcotics. For diuresis, sparrow grass and *Allium sativum* were used and while *Punica granatum* (pomegranate) was employed as an astringent. (Petrovska, 2012)

Whole plants and sections of plants like as roots, backs, flowers, stems, bulbs and twigs have over the years been traditionally used and are still being used these modern times in the management of many diseases. They are used in preparation of enemas, concoctions, inhalants and in various other formulations that are prescribed to patients and administered through different routes administrations (Wyk & Gericke, 2000).

Respiratory infections, diabetes, memory loss, mental and psychiatric disorders, arthritis, sexually transmitted infections, and many other tropical diseases, are some of the conditions that are still being traditionally treated with plants (Uttara *et al.*, 2009). Traditional medicine has become very popular in primary healthcare, and more so in developing countries where 80% of the population, according to WHO estimation, are traditionally used in primary healthcare as the main sources medicine. There is a developing interest from the developed countries in utilizing herbal remedies because they are readily available, have reduced toxicity and are more affordable compared to conventional drugs (Hosseinzadeh *et al.*, 2015).

2.2. Oxidants/ Free radicals

Oxidants such as atoms or chemical species with unpaired electrons are referred to as free radicals. These chemical species play an important role in chemical reactions and are abundant in nature. Many detrimental reactions such as oxidization and food adulteration are all known to progress through reactions by free radicals (Devasagayam *et al.*, 2004; Pham-Huy *et al.*, 2008; Uttara *et al.*, 2009). These reactions by free radicals are also very significant in medicine.

Reactive oxygen species and reactive nitrogen species are crucial for the maturation process of cellular systems and involved in the host's immunity. It is physiologically known that phagocytic cells like macrophages and monocytes and also neutrophils generate free radicals

that attack pathogens as part of the body's immune system against infections (Dröge, 2002; Ginter *et al.*, 2014). The release of free radicals by the host's defense system is vital and is usually noted patients with granulomatous disease (characterized by inflammation and accumulation of macrophages in a tissue). These patients have been seen to have defective membrane-bound NADPH oxidase system which makes them unable to produce the superoxide anion radical. As a result the patient becomes prone to persistent and recurrent infections (Dröge, 2002; Valko *et al.*, 2007). Reactive oxygen species (ROS) and Reactive nitrogen species (RNS) are also necessary for normal functioning of signaling systems (Genestra, 2007; Sies, 1986). They play an important part in the control of cascading signals within the different types of non-phagocytic cells like smooth muscles cells, myocytes and endothelial cells. For instance, nitric oxide (NO), also known as nitrogen monoxide, is an intercellular messenger that is important in dilatation of blood vessels to aid circulation of blood. Nitric oxide also helps to destroy intracellular pathogens and tumors (Genestra, 2007). Nitric oxide is also involved in modulating blood flow.

Environmental pollutants can also generate ROS and RNS especially during irradiation with Ultraviolet rays, by X-rays and gamma rays (Cadenas & Davies, 2000; Inoue *et al.*, 2003). They can also be acquired from exposure to environmental hazards such as cigarette and industrial smoke, pollutants from car exhausts and poor sewerage disposal methods (Pham-Huy *et al.*, 2008)

2.3. Oxidative stress

Oxidative stress is a consequence of imbalance between production and neutralization of free radicals. This can either be due to increased production of reactive metabolites that may overwhelm physiological buffers against the harmful metabolites or defective

antioxidant/neutralizing systems in the human host. The resultant effect of this tilted equilibrium is destruction of important biomolecules and cellular systems and eventually whole organism (Dröge & Schipper, 2007). The damage to the cells and tissues is usually physiologically mitigated against by the action of antioxidants, some of which are enzymes present in the body (Halliwell, 1995). In spite of the availability of antioxidant defense mechanism's in the cells against the reactive species, increased accumulation of the radicals may happen over the years and this has been implicated in aging and in diseases that were associated with old age such as arthritis, dementia, parkinsonism, cardiovascular disease and other chronic ailments (Rahman, 2003)

It is believed that approximately five percent of the oxygen breathed in on daily basis may be transformed to reactive oxygen species (ROS) like superoxide (O_2^-) peroxy ($ROO\cdot$) hydroxyl ($OH\cdot$), and singlet oxygen (1O_2) radicals (Bellion *et al.*, 2010). Inside the human body, these free radicals are rendered harmless through neutralization by various enzyme activities such as catalases, glutathione S-transferase, superoxide dismutase and glutathione peroxidase system (Lee *et al.*, 2007). For instance, superoxide dismutase converts superoxide to hydrogen peroxide and oxygen through oxidation process. Catalase enzymes catalyze the conversion of the potentially noxious hydrogen peroxide (H_2O_2) to water and oxygen (Pham-Huy *et al.*, 2008). This ensures that, the free radicals are neutralized of their harmful effects to cells.

Oxidation processes which are central in energy production of all living systems are always maintained under tight regulation by numerous mechanisms (Uttara *et al.*, 2009). If unchecked, oxidative stress may occur and the equilibrium between production and neutralization of free radicals be shifted to favor overproduction which may lead to cell

damage, commonly referred to as oxidative cell damage. Through unknown mechanism of progressions, the overproduction of these free radicals are thought to exposes cellular components such as nucleic acids, lipids, protein and membranes to attacks. The results of this is lipid oxidation, damage of proteins and nucleic acids and the subsequent onset of the different chronic conditions (Devasagayam *et al.*, 2004)

2.4 Diseases associated with oxidative stress

Oxidative stress plays important role in the pathophysiology of various diseases. It is implicated in the cognitive deterioration associated with normal aging as well as neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Pham-Huy *et al.*, 2008). Cells that line the inner surface of blood and lymphatic system vessels are very important in regulating homeostasis in the vasculature. They do this by producing paracrine proteins that diffuse into the endothelium and regulate the tone of the blood vessels and prevent platelet aggregation. They also hinder white blood cells from adhering to the intima of the blood vessels and also restrict rapid multiplication of vascular smooth muscle. Nitric oxide produced from the endothelium is the major contributor of most of these effects. Impairment of the endothelium can lead to vasoconstriction, thromboembolism, inflammation of the vasculature and uncontrolled growth of intima (Pham-Huy *et al.*, 2008). Excessive generation of the free radicals coupled with oxidative stress in the blood vessels are etiological factors in development of vascular impairment. This has been seen to be a major contributor to many cardiovascular disorders such as arteriosclerosis, high blood pressure, heart attack, hypoxemia in the heart, hypoperfusion and post angioplasty stenosis (Levonen *et al.*, 2008). Some diseases like diabetes mellitus type 1 that are associated with autoimmunity are majorly thought to be as a due to impaired immunological responses against self-antigens of proteins. It has been suggested that the genesis of autoimmunity could probably stem from

alterations in self-antigens (Crowch & Okello, 2009). During periods of cellular stress and impaired oxidative respiration by the mitochondria, there is a notable metabolic demand and susceptibility to viral infections as well as increased physiological factors like interferon, growth factor and interleukins. These agents are likely to escalate generation of reactive species (radicals) to levels that may defeat the physiological defense mechanisms involved in neutralization of these free radicals (Djordjević, 2004). There are some modifications of proteins through oxidation that have been seen in diseases associated with autoimmunity (Scofield *et al.*, 2005). These types of protein modification have been shown to be sources of complexes of glutamic acid (GAD). These complexes usually bind in a stronger manner to serum from type 1 diabetic patients than the auto-antigen of GAD monomer (Trigwell *et al.*, 2001).

Maneesh *et al.*, (2005) showed that excessive damage of cartilages and other joint structures as seen mainly in patients with osteoarthritis is attributable to either the degree of lipid peroxidation or to insufficiently low antioxidants levels. The extent of damage to the joint structures corresponds to increased or raised levels of the reactive species, excessive production of the radicals and insufficiency of enzymes which are responsible for processing oxygen. It is also reported that unsteady oxygen pressure and lack of free radical scavenging molecules could also be a factor in joint degeneration (Devasagayam *et al.*, 2004). Most of the time, treatment of osteoarthritis has mainly been directed in amelioration of symptoms like inflammation and pain and inhibiting agent of inflammation such as eicosanoids. Never the less, this approach has not yielded fruits particular in cases where the condition is chronic and hence there should be another intervention that should be targeted at protecting or impeding destruction of the joint tissues (Ling & Bathon, 1998). Maximum benefits of antioxidants to prevent oxidative stress and subsequent destruction of tissues in patients with

impaired antioxidant defense and high peroxidation of lipids in physiological systems can only be attained by ensuring that the antioxidant remedies are administered in normal healthy individuals or during the early stages of disease illness (Maneesh *et al.*, 2005).

CHAPTER THREE: MATERIAL AND METHODS

3.1 Study design

The study was a cross sectional analytical design where relationship between oxidative stress-related disorders and their management by the TMPs in the region was assessed. Purposive sampling method was employed to identify 36 traditional medical practitioners as participants. Later laboratory experiments for assessing acute toxicity and antioxidant activities of four plants which were selected based on literature were carried out.

3.2 Area of study

Data collected was limited to Nyamira North Sub-county, Nyamira County. The county covers an area of 899.4km² and lies between latitude 00 30° and 00 45° South and between longitude 340 45° and 350 00° East. Nyamira north Sub-county is one of the five sub counties that constitute Nyamira County, the others being Nyamira South, Borabu, Manga and Masaba North. According to the District Health Information System (DHIS2) population estimates for 2018, the Sub-county has a population of 150, 628 people. Males and females constitute 48.9% and 51.1% of the population respectively. The Sub-county has a coverage area of 219.3km² and a population density of 687 people per km². It has a good cool and warm climate and temperatures ranging from 16-23° C and an average rainfall of between 500-2600 mm each year. Due to the favorable climate, the area is good for agriculture where both subsistence farming, producing maize, millets, beans and fruits and commercial farming in cattle, tea and coffee are practiced. The inhabitants of the Nyamira North Sub-county, predominantly comprise the Abagusii who access health services from 28 government facilities (DHIS2), and other Private, FBOs and NGOs owned health facilities.

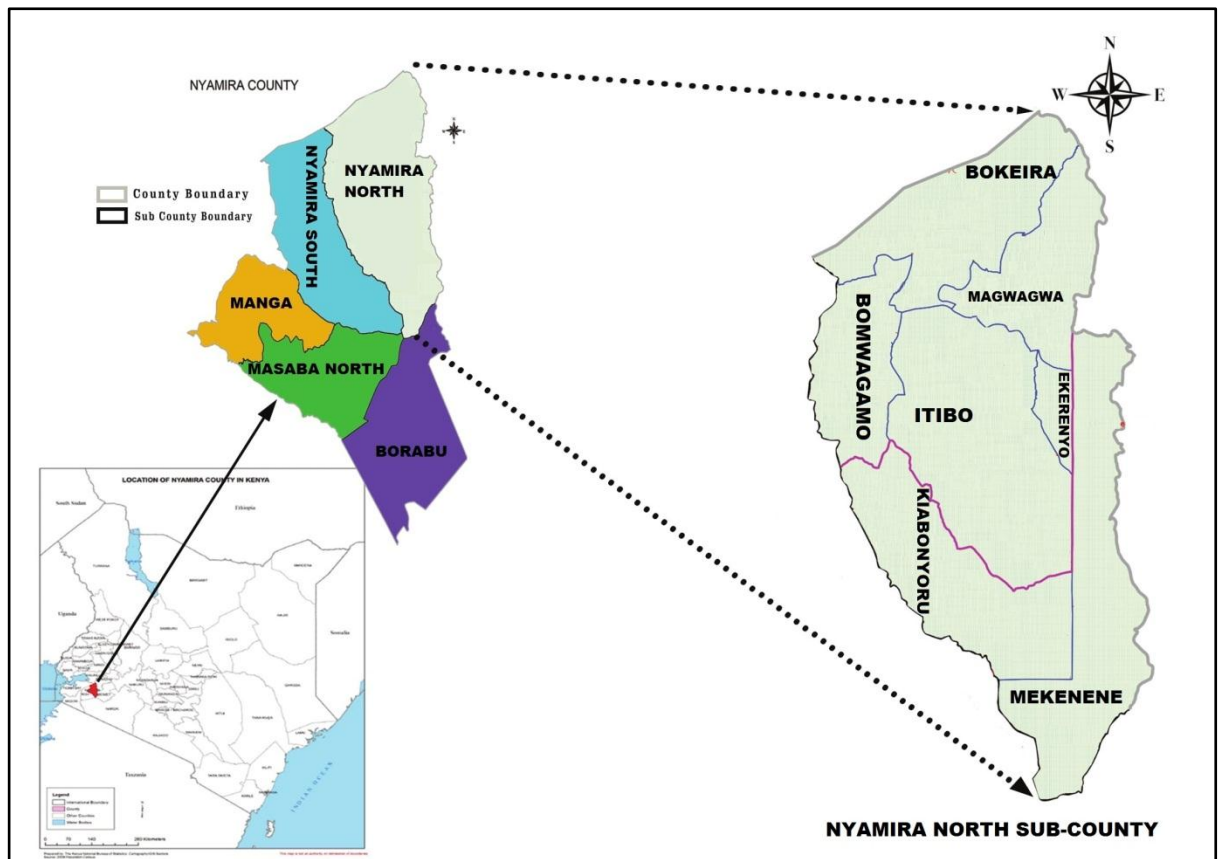


Figure 3.1: Map of the Study area.

3.3 Target population and Sampling

The study targeted traditional practitioners and herbalists within Nyamira North sub county.

Purposive sampling method was used to select key informants.

3.4. Inclusion and Exclusion criteria

3.4.1 Inclusion criteria

The study included herbalists highly respected by the community and have experience of the practice.

3.4.2 Exclusion criteria

The witchdoctors, sorcerers, diviners, rainmakers, spiritualists and young inexperienced herbalists were not recruited in this study.

3.5 Data collection and abstraction

3.5.1. Validation of the data collecting tools

A pilot study was conducted two months before the study to validate the data collection tools. The data collections tools that were validated in the pilot study were the questionnaire, interview guide, informed consent form and the materials for collecting plant specimens. The collection of the raw data was through unstructured form. Digital records, pictures and hand written reports of interviews and observations duly documented. Tracing and measuring of information using printed literatures was used to ensure validation (Jeruto *et al.*, 2008; Johns *et al.*, 1990; Ondicho *et al.*, 2015). The final plant list was forwarded to the East Africa Herbarium for review and update of the names.

3.5.2 Field survey and ethnobotanical documentation

The TMPs were recruited for participation with help of their leader and village heads. A guided structured questionnaire (Appendix VI) and an interview guide were administered by the researcher to selected informants from each of the sub-county divisions. Information on the use of the plants and demographics of the respondents was noted and recorded. Participatory rapid appraisal method (PRA) was applied when gathering indigenous knowledge about the medicinal plants used in management of various medical conditions. This entailed driving to herbalist homes and to traditional medicine practitioners and have direct interview with them. Plant use information was captured in addition to their demographic information. The collection of plant specimen along with their flowering parts was done in the field for the purposes of preparing a herbarium. This was important for identifying plant specimen from the flora. Field notes were taken so as to have information on the plants name, habitat, and the characteristics of the species along with the plant family.

Only those plants which were cited by at least three informants were recorded to ensure good reliability of data (Johns *et al.*, 1990). Photographs of some of the medicinal plants were taken.

3.5.3. Collection and preparation of plant materials

3.5.3.1. Plant material collection

Four plants, *Phragmanthera usuiensis*, *Ensete ventricosum*, *Echinops amplexicaulis* and *Rhoicissus tridentata*, were selected for extraction, phytochemical screening, antioxidant activity and toxicological studies. The selection was based on the fact that, literature search of the plants did not yield this information. The parts of the plants used for phytochemical screening and acute toxicity studies of the selected plants were those parts the herbalist reported to be using. The selected plant materials were collected from Nyamira North Sub-county in Nyamira County, Kenya and identification was carried out with help of a taxonomist from the National Museums of Kenya. At the end of each interview, the plant specimens were collected dried and using specified herbarium techniques before they were identified and preserved. The plant parts were placed between two sheets of newspaper and pressed between two pieces of cardboard. Voucher specimens for all the medicinal plants were deposited at the National Herbarium for future reference. The authentication of the Plant materials collected was carried out by comparing the voucher specimen with those from East African herbarium at the Nairobi national museum. The voucher specimens that were made were then deposited at the museum and the plant materials were transported to Mount Kenya University Herbarium where they were thoroughly washed with running tap water, sliced into small portions and then air dried under shade for fourteen days. Before the extraction, the samples were then ground to coarse powder.

3.5.3.2. Extraction

The extraction was carried out through maceration by use of methanol and distilled water for aqueous extract. Approximately 100 g of each part of the ground material of the plant samples was soaked separately with enough solvent in a 1 litre beaker and then covered with a foil paper for 48 hours with constant shaking using a magnetic stirrer (REMI, 2MLH). Afterwards, the individual extracts were filtered and reduced *in vacuo* at 40 °C and eventually oven-dried at 35 °C. Twenty grams of each of the powdered crude extract was boiled for about 5 minutes to make the aqueous extracts. The dry and lyophilized extracts were weighed and preserved below 20° C for later use in bioassay and phytochemical screening (Richardson & Harborne, 1990).

3.6 Phytochemical screening

Four selected plants based on existing literature were screened for phytochemicals and subsequently subjected to toxicological studies to determine their safety. These plants were *Phragmanthera usiuensis*, *Ensete ventricosum*, *Echinops amplexicauslis* and *Rhoicissus tridentata*.

3.6.1 Screening for alkaloids

Approximately 1g of powder of each sample was mixed with 5ml of 10% H₂SO₄ in a test tube. The test tubes were then dipped in a water bath to warm for a period of 2 minutes and then filtered using a filter paper. To 1ml of the filtrate, 2 drops of Mayer's reagent were added and observation recorded. The remaining filtrate was then reacted with dilute ammonia and extracted with two millilitres of trichloromethane. The trichloromethane was then evaporated off and the resultant residue was then dissolved in 0.2 milliliters of 10% H₂SO₄ and divided into two parts. One drop of Mayer's reagent was added to the first part,

and the second part, one drop of Dragendorff reagent was added. A buff precipitate indicated the presence of alkaloids.

3.6.2 Screening for saponins. (Frothing test)

For saponins, 0.5g of each powdered sample were placed in a test tube and water added. The mixture was shaken and left to stand. Persistent frothing indicated presence of saponins.

3.6.3 Screening for tannins

Exactly 5g of the extracts was boiled with 5ml of distilled water, cooled and filtered. Five drops Ferric chloride reagent was added to the filtrate. A blue green precipitate determined the presence of Tannins.

3.6.4 Screening for Flavonoids

To an aqueous filtrate of the test sample 5mls of dilute ammonia solution were added followed by the addition of concentrated H_2SO_4 . A yellow coloration observation indicated the presence of flavonoids.

3.6.5 Screening for coumarins

Portions weighing 1 gram of powdered sample were put in boiling tubes, mixed with 70% alcohol and then boiled for 5 minutes in a water bath. They were then filtered while hot and then cooled. Four drops of alcoholic Ferric chloride were added to 2ml of the extracts samples. The deep turquoise precipitate which turned yellow on addition of concentrated nitric acid indicated presence of coumarins.

3.6.6 Screening for Steroids

One gram of each sample extract was mixed with 10mls of Trichloromethane ($CHCl_3$) in boiling tubes and boiled for 5 minutes. The extracts were filtered while hot, cooled and to 2ml of the filtrate equal volume of sulphuric acid were added. A yellow middle layer at the separating level of the liquids was formed and after 2 minutes it turned scarlet in color indicating presence of steroids.

3.6.7 Screening for phenols

One gram of each of the extract was boiled with 10ml of 70% EtOH in a water bath using boiling tubes for 5 minutes. The extracts were filtered while hot and cooled. To 2ml of the extract, Five percent Ferric chloride drops were added and a green colored precipitate indicated presence of phenolic compounds.

3.6.8 Screening for Terpenoids (Salkowski test)

For terpenoids, 5 ml of each methanolic and aqueous extract was mixed with 2 ml of chloroform, and 3 ml conc sulphuric acid was slowly added and layer was formed. A reddish brown colored interface in the mixture solutions was formed to show the presence of terpenoids.

3.6.9 Screening for Glycosides (Keller-kilian test)

To 2ml of solutions containing 0.5mg of each extract, 0.4ml of glacial ethanoic acid with ferric chloride was added, gently stirred to dissolution and after which 0.5ml of concentrated H_2SO_4 was added. A blue green colour in the top ethanoic acid layer indicated presence of glycosides.

3.6.10 Screening for Free sugars (Fehling's test)

To 5ml mixture of equal volumes of Fehling's solutions, 2ml of aqueous extract was added in a test tube and then boiled in water bath for about two minutes. A reddish brown colored precipitate indicated presence of reducing sugars.

3.7 Laboratory Bioassays

3.7.1. Antioxidant bioassay

3.7.1.1 DPPH radical scavenging activity

The antioxidant activity of aqueous and methanol extracts against free radicals was studied using the DPPH radical-scavenging method as described by Goze *et al.*, (2009) with slight modifications. By use of a set of 21 test tubes six ten-fold serial dilutions of each extracts (1000 to 0.01µg/ml) were made. Ten milligrams (10mg) of each of the powdered extract was weighed and dissolved in 10ml of methanol to make the stock solution of 1000µg/ml. This stock solution was serial diluted to make 1000µg/ml, 100µg/ml, 10µg/ml, 1µg/ml, 0.1µg/ml and 0.01µg/ml solutions. A solution of 0.3mmol concentration of DPPH was prepared by weighing 5.4mg of DPPH and dissolving it in 45ml of methanol. The reaction mixture constituted of 2.5ml of either the extract or the standard of various concentrations and 1ml of 0.03mMol DPPH. The standard was prepared by weighing 10mg of L-ascorbic acid and dissolving in 10ml of methanol. Three ten -fold dilutions of the standards were made. This served as the positive control, whereas 2.5ml of DPPH solution and 1ml of methanol was used as the negative control. Methanol was used as the blank. The assay mixtures were then kept in a dark chamber for a period of 15 minutes and the test for absorbance carried out using Microprocessor UV-VIS Double beam photometer (AVI-2700) at 517nm for the standard and the extract against the blank. Calculations to get inhibition effects of the extract on DPPH were based on the formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100,$$

where, A_{control} is the absorbance of the control and A_{sample} is the absorbance of the sample. Determination of the half maximal inhibitory concentration (IC_{50}) values was done by plotting a graph of %RSA \pm SEM against the different concentrations of the extracts.

3.7.2 Acute Toxicity Test

3.7.2.1. Acute Toxicity study of the selected extracts

Mature female Swiss albino mice of 20-25g BWT were employed for acute toxicity studies of crude extracts. The mice used were nulliparous and not pregnant. . The animals were acquired from the University of Nairobi, Public Health, Pharmacology and Toxicology Department. The mice were randomly selected and marked on the tails for identification. Their housing was done separately and in polycarbonate cages which measured 35cm (Length) x 25cm (Width) 18 cm (Height) for five days before dosing to enable them adjust and conform to laboratory conditions. The cages were lined with wood shavings which served as beddings for the animals. The housing temperature was kept at $25\pm 3^{\circ}\text{C}$ with relative humidity of $54\pm 4\%$. The animals were fed with rat pellets obtained from a feed supplier called Unga feeds. The provision of water was *ad libitum*

3.7.2.2. Experimental design for acute Toxicity study.

The procedure for main test of Organization for Economic Co-operation and Development (OECD) guidelines of acute oral toxicity –Acute Toxic Classic (ATC) method was followed. Prior to dosing, single animals were fasted for 4 hours and their weight taken before the extracts were administered. Animals in the first eight groups were used to test the extracts while the last group served as the control group. Prior to dosing, single animals were fasted for four hours and weighed before oral administration of the extract. Treatment of the animals was initiated at the starting dose level of 300 mg/kg body weight carried out with six

animals (three animals per step). Treatment of animals at the next dose of 2000mg/kg body weight was delayed until survival of the previously dosed animals was ensured. Physiological saline was administered to the control group. The plant extracts were administered orally by gavage. For restraint, each animal was picked by the base of the tail, pressed down on a table to immobilize it, grabbed by the skin at the back of the neck using the thumb and the index finger and the base of the tail placed between the ring and pinky fingers. The Observations of various physical parameters of wellbeing like fur, state of the eyes, mucosa, salivation, dullness, sleep, tremors, body weight and mortality were noted and recorded after 30minutes, 4 hours, 24 hours, 48 hours, 7 days and 14 days.

After the experiment all animals that survived were humanely euthanized placing the animal individually in an inhalation chamber containing cotton wool soaked in diethyl ether. Gross necropsy was performed on all the animals used in the experiment. The animals were left in the chamber for 5 minutes after cessation of breathing before necropsy was performed. Target organs such as gastrointestinal tract, liver, heart and the kidney were harvested from the test animals and preserved in 10% formalin for histopathological studies.

3.7.2.3 Tissue preparation and histological examination

3.7.2.3.1 Tissue processing and embedding

Each individual organ was first fixed in 10% buffered formalin and then dehydrated using graded alcohol baths of 50%, 70%, 80%, 90% and 100% concentrations. The tissues were then dealcoholized using xylene and infiltration carried out with molten wax, 62 °C, for 30min. Embedding was then done by placing the tissue cases into cassettes and adding molten paraffin wax. The wax was allowed to harden to form tissue blocks and was then

placed into cold water to harden completely. For this purpose a supply of paraffin wax was kept permanently molten at 58 °C in a paraffin wax oven.

3.7.2.3.2 Sectioning and floating out sections

Tissues were sectioned using microtome. The microtome knife was placed into the microtome and clamped securely at an acute angle to allow the block to clear the sharpening bevel. The block was then placed in the microtome and clamped tightly with its face against the microtome knife. Sections were then cut with a deliberate and regular movement to produce a flat ribbon which was being supported with the left hand. The ribbon was then placed on the surface of a bath with warm water (45 °C) by floating it down in a parallel direction to the water surface. A clean glass slide was then slid at a right angle into the water and moved behind the required section approaching from the side. The slide was then moved forward for the section to attach and then lifted vertically and slowly out of the water complete with sections. The slide was placed in a rack to drain and then labeled using a diamond pencil and allowed to dry at 58 °C for at least 2hrs.

3.7.2.3.3 Staining and mounting stained sections.

Before staining, the sections were first hydrated to remove the paraffin wax. Sections were therefore first treated with xylene and then hydrated by draining them with graded alcohol in the order 100%, 90%, 80%, 70% and 50% concentrations and then water. The sections were then stained in Harris' haematoxylin for 4 minutes and then washed in running tap water to remove excess stain. The sections were then counterstained in Putt's eosin for 2 minutes before placing them in running water to differentiate the eosin. Dehydration was then carried out using graded alcohol baths of 50%, 70%, 80%, 90% and 100% concentrations to remove eosin. The slides were then placed in a xylene bath to clear the alcohol.

To mount the stained sections, DPX mounting medium was applied to the center portion of a clean and dry coverslip. The slides were removed from the xylene bath and the xylene wiped

from the back of the slides. The slides were quickly inverted and gently placed on DPX mountant on the coverslip. The slides were then reverted and placed face up on the blotting paper. The slides were then labeled and dried on a warm plate (45 °C) for 1 hour. Histopathological analyses were then carried out with the help of a pathologist by examining the slides under a light microscope. Images of the tissue structures were captured.

3.8 Data management and statistical analysis

Socio-demographic data of the traditional medicine practitioners and ethnobotanical data on vernacular names of the plants, their habitats, the parts used and their medicinal value was analyzed using Microsoft Excel. A simple grid to collate the data provided in the questionnaire was first prepared and then a coding system designed to code closed open questions. The data was then entered into the grid from which proportion of respondents answering for each category of questions was calculated. An excel spread sheet was used to calculate the percentages and to give the results in form of graphs. Data on botanical information was presented in tables, graphs and pictures. Compact disks, soft and hard copies of this information were stored as a data base. The tests were carried out in triple sets and the outcomes are displayed mean \pm standard deviation. Statistical significance was tested using one with ANOVA test using Graph pad prism, version 7.0. A statistical significance of $p < 0.05$ was considered to be significant. Results were reported as the average of three independent experiments where each sample was tested as triplicate. They were expressed as Mean \pm SEM and presented in tables, graphs and histology images. Data from histopathological study was presented in photomicrophic images from a light microscope.

3.9. Ethical Consideration

3.9.1. Disposal of experimental animals

The mice were used and disposed accordingly as per the clearance of the University of Nairobi, ethical committee.

3.9.2. Research approvals

Approval to conduct research was obtained from University of Nairobi graduate school. Biosafety, Animal Welfare and Ethics Committee of the University of Nairobi gave the Ethical clearance (Reference number, FVM BAUEC/2018/168). In the field, written consent was sought from all the herbalists who were willing to participate in the study after the aim of the study was elaborately explained to them. Benefits and potential risks in relation to the study were also communicated to the informants by the researcher prior to the commencement of the study.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Ethnobotanical Survey

4.1.1 Socio-demographics of the respondents

A total of 36 TMPs were interviewed (29 males, 7 females). This is consistent with other studies which showed most of the TMPs being males (Kaingu, *et al.*, 2013; Keter & Mutiso, 2012; Wambugu *et al.*, 2011). However this contrasts Omwenga (2015) who reported 54% of TMPs being females. Majority of the respondents (39%) were between ages 46-55 years (Fig 4.1). This is because the community believes older people have the requisite knowledge in traditional medicine practices from whom it is orally passed down to younger generations. Most of the respondents were considered literate (78%) having completed either primary level, secondary, or tertiary level of education. Only 22% of them were considered illiterate having no formal education (Fig.4.2). This collaborates similar studies that showed education level does not significantly influence ethnomedical practice (Kaingu *et al.*, 2013; Keter & Mutiso, 2012; Omwenga *et al.*, 2015). The study showed that all the TMPs interviewed lived in the rural areas where the majority of them (80.6%) practiced farming as their primary source of livelihood, 5.6% were small scale business people and another 5.6% were in formal employment. Only three (8.3%) of the respondents were exclusive medical practitioners.

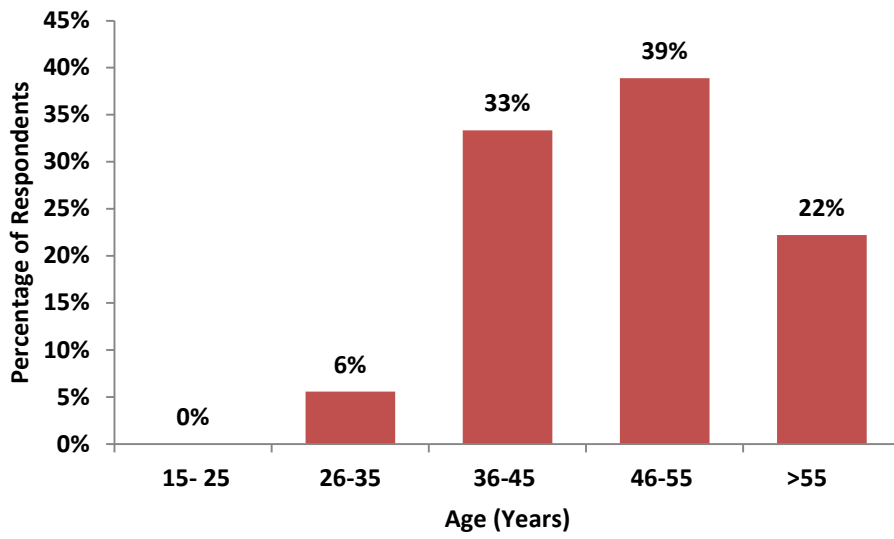


Figure 4.1: Age brackets of the respondents.

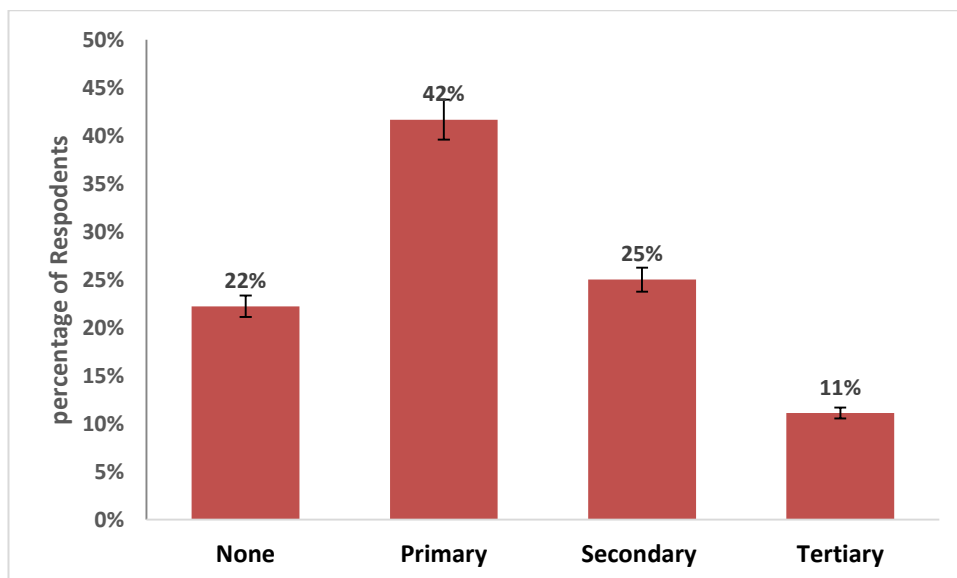


Figure 4.2: Education level of TMPs

4.1.2. Traditional knowledge and practice

Acquisition of knowledge in traditional medicine for most of the interviewees (63.9%) was via inheritance whereby it was mainly passed down from their parents and grandparents. This is supported by Keter & Mutiso (2012) and Kaingu (2013) who reported that the majority of TMPs had acquired knowledge of the trade by inheritance. Seven (19.44%) of the respondents in the study obtained the knowledge through apprenticeship under qualified tutor TMPs and 16.7% through organized or formal training in seminars and workshops. Most of the key informants (33.3%) had 11-19 years of experience in traditional medicine. Six (16.7%) of the interviewed TMPs had practiced for 1-5 years, 25% for 5-10years, 16.7% for 20–40 years and only 8.3% had practiced as traditional medical practitioners for more than 40 years. As with other previous studies, majority of the TMPs had practiced for many years and this is attributable to the fact that experience is key to accumulation of ethnomedical knowledge (Omwenga *et al.*, 2015). All the herbalists interviewed lived in the rural areas and practiced from the comfort of their homes. None of the interviewees had a clinic and only two had acquired a practice license from the necessary authorities. Two (8.3%) TMPs had elaborate botanical gardens where they cultivated most of the medicinal plants as a mitigation measure against the massive deforestation going on in the county as the inhabitant clear land for agricultural use.

4.1.3. Traditional Herbal medicine Practitioners' knowledge of Oxidative stress.

Apart from in arthritis, diagnosis of oxidative stress related disorders was mainly based on hospital tests and evaluations. The TMPs mainly treated patient who had already been diagnosed in the hospitals. Majority (91.67%) TMPs interviewed were not conversant with the various forms of arthritis but they had a fair grasp on the general manifestations of arthritis based on known clinical symptoms such as chronic joint pains, inflammations and

joint stiffness. No difference in terms of management between the various forms of arthritis was noted and hence common remedies were used. In addition to personal diagnosis, all TMPs reported to have sometimes used patient's hospital reports as well as patient's self-evaluations to make a diagnosis. All the TMPs reported that their patients were referred to them by other patients, family members and friends.

4.1.4. Medicinal Plant and the predominant growth forms used to treat oxidative stress.

Fifty seven (57) medicinal plant species covering 53 genera used in management of oxidative stress and related conditions were cited by the key informants in the study. The plants, which belonged to 36 families (Table 4.1), were used by herbalist to make preparations for administration to the patients. Table 4.1 also shows the number of the species cited in each family and their proportions. Table 4.2 shows the plant species, the family, local names, habit, parts used and the mode of preparation. The majority of the herbal remedies utilized in management of diseases associated with oxidative stress as reported by the TMPs in the study belonged to Asteraceae family (15.79 %) followed by Solanaceae (7.02%), Fabaceae and Rubiaceae each at 5.26% and then Asparagaceae, Bignoniaceae, Lamiaceae, Oxalidaceae, Rhamnaceae, and Salicaceae each at 3.57%. The rest had one species each representing 1.75% of the total number of species. It was however hard to get some of the plants mentioned by the herbalist. For example it took three days to obtain *Echinops amplexicaulis* and *Rhamnus prinoides* following the massive clearing of the indigenous habitants in favor of agriculture. Furthermore, despite *E. amplexicaulis* being mentioned by 21 respondents across the study area, it was only obtained from one region. This poses a danger of the medicinal plants going into extinction unless measures are taken to preserve the knowledge and to educate the locals on the importance of preserving natural habitats. Figure.4.3 shows that the

majority of the growth forms encountered were shrubs (42%), followed by herbs (33%), trees (14%) and climbers (11%).

Table 4.1: Families and percentages of medicinal plants species commonly used in Nyamira North Sub-county

| No. | Family | Species | Percentage | No. | Families | Species | Percentage |
|-----|---------------|---------|------------|-----|----------------|---------|------------|
| 1. | Asteraceae | 9 | 15.79% | 19. | Ebenaceae | 1 | 1.75% |
| 2. | Solanaceae | 4 | 7.02% | 20. | Euphorbiaceae | 1 | 1.75% |
| 3. | Fabaceae | 3 | 5.26% | 21. | Hyacinthaceae | 1 | 1.75% |
| 4. | Rubiaceae | 3 | 5.26% | 22. | Musaceae | 1 | 1.75% |
| 5. | Asparagaceae | 2 | 3.57% | 23. | Loranthaceae | 1 | 1.75% |
| 6. | Bignoniaceae | 2 | 3.57% | 24. | Meliaceae | 1 | 1.75% |
| 7. | Lamiaceae | 2 | 3.57% | 25. | Moraceae | 1 | 1.75% |
| 8. | Oxalidaceae | 2 | 3.57% | 26. | Myrtaceae | 1 | 1.75% |
| 9. | Rhamnaceae | 2 | 3.57% | 27. | Papilionaceae | 1 | 1.75% |
| 10. | Salicaceae | 2 | 3.57% | 28. | Peraceae | 1 | 1.75% |
| 11. | Apiaceae | 1 | 1.75% | 29. | Phytolaccaceae | 1 | 1.75% |
| 12. | Anacardiaceae | 1 | 1.75% | 30. | Ranunculaceae | 1 | 1.75% |
| 13. | Hypericaceae | 1 | 1.75% | 31. | Rutaceae | 1 | 1.75% |
| 14. | Apocynaceae | 1 | 1.75% | 32. | Sapindaceae | 1 | 1.75% |
| 15. | Caesalpiaceae | 1 | 1.75% | 33. | Urticaceae | 1 | 1.75% |
| 16. | Celastraceae | 1 | 1.75% | 34. | Verbenaceae | 1 | 1.75% |
| 17. | Crassulaceae | 1 | 1.75% | 35. | Verbenaceae | 1 | 1.75% |
| 18. | Cucurbitaceae | 1 | 1.75% | 36. | Vitaceae | 1 | 1.75% |

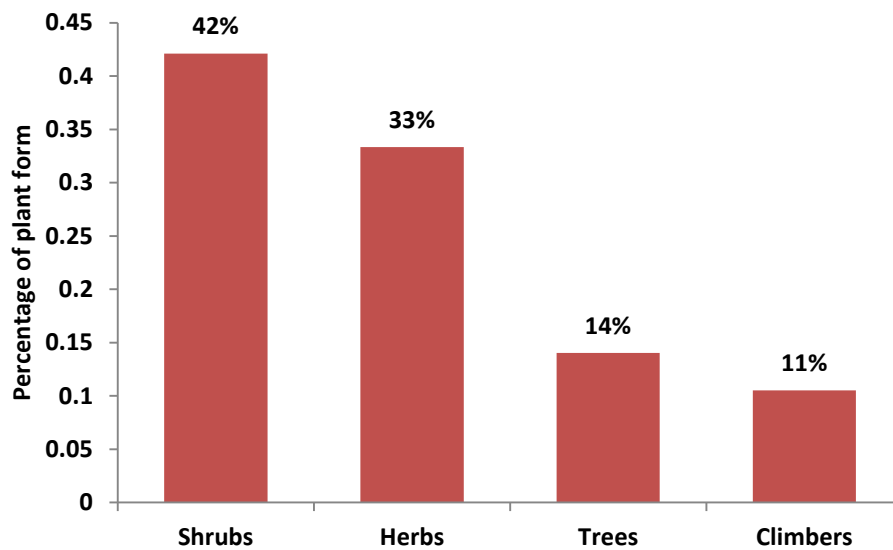


Figure 4.3: Forms of the medicinal plants used by the traditional medical practitioners

4.1.5. Plant parts used, Preparation and Administrations

The root/root barks were the most frequently used plant parts (53%) followed by the leaves (25%), stem bark (12%) and whole plant (8%). The bulb had the lowest number of mentions (2%) while flowers and fruits were not cited by any of the respondents in the study (Fig 4.4). Most of the herbalist cited decoctions as the most common method through which remedies were prepared. This was described as boiling or soaking the fresh or dried plant parts in water. Other methods used include, poultice, ash, powder mixed with water for oral administration and infusion. All the herbal preparations were freshly prepared just before administration. Oral route was cited by all the TMPs as the route of choice through which the medicine was administered in treating arthritis.

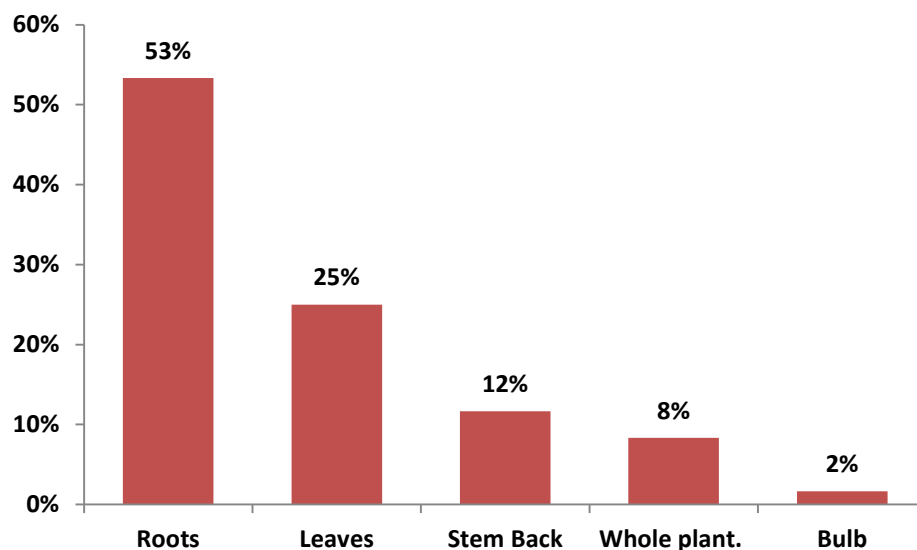


Figure 4.4: Parts of plants used for preparation of medicines.

4.1.6. Diseases encountered by the herbalists

Among the diseases of interest that were managed by the TMPs in the study area, arthritis ranked the highest at 84.21% followed by memory loss and cancer at 5.26% each, diabetes 3.51% and hypertension 1.75% (Fig 4.5). This can be attributed to the fact that Non-communicable diseases like cancer, diabetes and hypertension are mostly diagnosed in the hospitals and are viewed by majority of people as complex and mostly the people affected seek medical attention from conventional medicine. The medicinal plants that were mentioned by TMPs for treating cancer were *Dracaena steudneri* (skin cancer), *Phytolacca dodecandra* (breast cancer) and *Senna didymobotrya* (breast cancer pain). *Centella asiatica*, *Dichrocephala integrifolia* and *Oxalis corniculata* were used to treat loss of memory. *Pappea capensis* and *Dalbergia lactea* were used for diabetes whereas *Lantana trifolia* was used to treat hypertension.

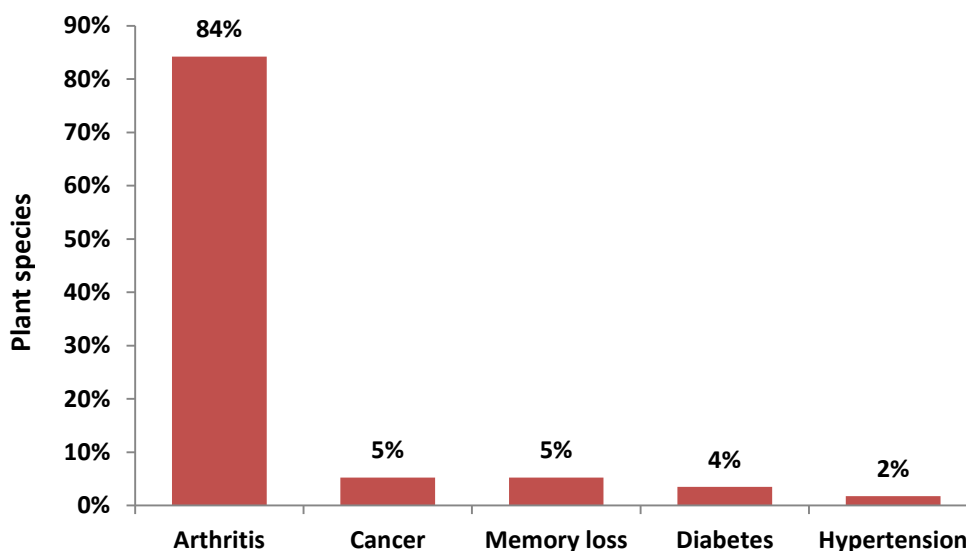


Figure 4.5: Percentages of plants species in oxidative stress disorders as mentioned by Herbalists

4.1.7. Related ethnomedical importance and reported pharmacological activity

Cross referencing of the plant species with documented traditional uses in other communities was conducted with the aim of finding out whether they have been used to treat disorders associated with oxidative stress and related symptoms. Literature search conducted (Table 4.3) showed that among the 48 (84%) medicinal plants used in management of arthritis in the study area, 30 plant species have been reported to be used traditionally to treat arthritis, rheumatism and other joint related conditions and/or possess pharmacological activities relevant to management of arthritis. Among them, eight have been reported to be specifically used for treatment of arthritis. These are *Flacourtia indica* (Lalsare *et al.*, 2011), *Carissa spinarum* (Jeruto *et al.*, 2008), *Clutia abyssinica* (Jeruto *et al.*, 2008), *Phyllanthus amarus* (Patel *et al.*, 2011), *Rhamnus prinoides* (Kimondo *et al.*, 2015; Kiringe, 2006), *Rothea myricoides* (Jeruto *et al.*, 2010), *Trimeria grandifolia* (Some, 2014) and *Withania somnifera* (Mishra *et al.*, 2000).

Plant species that have been reported for use in managing rheumatism include *Acacia* spp (Kipkore et al., 2014), *Albuca abyssinica* (Mesfin et al., 2014), *Asparaguss racemosus* (Hasan et al., 2016), *Carissa spinarum* (Nedi et al., 2004), *Harungana madagascariensis* (Lukwamirwe, 2016), *Momordica foetida* (Yineger et al., 2008) *Markhamia lutea* (Burkill, 1985), *Physalis Peruvian* (Ramadan, 2011; Wu et al., 2004), *Urtica massaica* (PROTA, 2013) and *Withania somnifera* (Mishra et al., 2000). The plants that have been reported to be used in treating joint conditions and symptoms related to arthritis (joint pain and inflammation) include *Asparagus racemosus* (Hasan et al., 2016), *Biophytum umbraculum* (Inngjerdingen et al., 2008), *Cirsium vulgare* (Al-Mustafa & Al-Thunibat, 2008), *Clematis brachiata* (Chhabra et al., 1991), *Eucalyptus* spp (Jun et al., 2013), *Maytenus obscura* (Maina, 2015), *Momordica foetida* (Teklehaymanot, 2009), *Morus nigra* (Nomura & Hano, 1994), *Phyllanthus amarus* (Patel et al., 2011), *Rhamnus prinoides* (Kimondo et al., 2015), *Rhamnus staddo* (Chalo et al., 2016), *Searsia pyroides* (Odongo et al., 2017), *Spathodea campanulata* (Heim et al., 2012) and *Tagetes minuta* (Karimian et al., 2014) and *Vangueria apiculata* (Edna, 2018)

The anti-inflammatory, analgesic and antioxidant activities of some of the plants which were cited in the study have been validated through *in vivo* and *in vitro* model studies. Plants with validated anti-inflammatory activity include *Biophytum umbraculum* (Austarheim et al., 2016), *Carissa spinarum* (Woode, 2007), *Clematis brachiata* (Mostafa et al., 2010), *Eucalyptus* spp (Dixit et al., 2012), *Flacourtia indica* (Gayathri, 2013), *Harungana madagascariensis* (Iwalewa et al., 2008), *Indigofera arrecta* (Madikizel et al., 2014), *Maytenus obscura* (Alajmi & Alam, 2014), *Morus nigra* (Padilha et al., 2010), *Phyllanthus amarus* (Kassuya et al., 2005), *Searsia pyroides* (Odongo et al., 2017), *Spathodea*

campanulata (Ilodigwe & Akah, 2009), *Tagetes minuta* (Karimian *et al.*, 2014) and *Withania somnifera* (Mir *et al.*, 2012).

Eighteen (37.5%) of plants species cited in this study were documented for the first time to be traditionally used in management of arthritis. These species are *Ajuga remota*, *Berkheya spekeana*, *Carduus chamaecephalus*, *Conyza bonariensis*, *Echinops amplexicaulis*, *Ekebergia capensis*, *Ensete ventricosum*, *Erythrina abyssinica*, *Euclea divinorum*, *Kalanchoe densiflora*, *Microglossa pyrifolia*, *Phragmanthera usuiensis*, *Rhoicissus tridentata*, *Rytigynia acuminatissima*, *Solanecio manni*, *Solanum terminale*, *Toddalia asiatica*, and *Vangueria madagascariensis*.

Literature search conducted also revealed reports similar or related use for the plants used for Cancer, memory loss, diabetes and hypertension. All the plants cited for their use in management of loss of memory namely *Centella asiatica*, *Oxalis corniculata* and *Dichrocephala integrifolia* have been reported to possess antioxidant activity (Badwaik *et al.*, 2011; Gohil *et al.*, 2010; Kouémou *et al.*, 2017). Various clinical tests have indicated that oxidative stress is key to loss of neurons as seen in dementia and therefore antioxidants can mitigate against this damage (Christen, 2000). Kouémou *et al.*, (2017) and Li *et al.*, (2006) have reported treatment of memory loss with *Dichrocephala integrifolia* and *Oxalis corniculata* respectively.

Among the three plants that the TMPs in the study used to treat cancer, *Dracaena steudneri* has been reported to possess cytotoxicity effects (Kisangau *et al.*, 2011) and has been used in treatment of cancer in Bukavu, Uganda (Kadima *et al.*, 2016). *Phytolacca dodecandra* ground leaves have been used to treat tumors (PROTA, 2013). However, there are no reports on use of *Senna didymobotrya* in managing pain of breast cancer as claimed by the TMPs but

antioxidant activity has been reported (PROTA, 2013). Various studies on oxidative stress have clearly demonstrated that oxidative DNA damage is a main causative factor in development of cancer (Parthasarathy *et al.*, 1999; Valko *et al.*, 2004).

Regarding plant species cited for their use in diabetes, *Pappea capensis* has been reported to be a Nutraceutical and one that possesses antioxidant activity (Karau *et al.*, 2012; Kiringe, 2006). Lipid oxidation, highly increased in oxidative stress, is a characteristic seen in diabetic patients and is thought to be responsible for insulin resistance (Felber *et al.*, 1987). There is no specific reports on the use of *Dalbergia lactea* in management of diabetes but species of the genus *Dalbergia* L have been shown to possess anti-diabetic activity (Saha *et al.*, 2013). *Lantana trifolia*, the only plant species cited by the TMPs as a useful plant in managing hypertension has been reported in literature to have antioxidant activity (Silva *et al.*, 2005) and to be used for managing angina (Boily & Van Puyvelde, 1986).

Table 4.2: Medicinal plants used to manage Oxidative stress-related disorders in Nyamira North Sub-County

| Family | Plant scientific name (Kisii dialect) | Voucher No. | Form | Part of the plant | Disease treated | Frequency of mentions |
|----------------------|--|-------------|---------|---|-----------------|-----------------------|
| Anacardiaceae | <i>Searsia pyroides</i> (Burch.) Moffett (Obosangora) | SMW/2017/11 | Shrub | Root decoction | Arthritis | 15 |
| Apiaceae | <i>Centella asiatica</i> (L.) Urb. (Enyonyo ya sisigisi) | SMW/2017/56 | Herb | Whole plant infusion | Loss of memory | 14 |
| Apocynaceae | <i>Carissa spinarum</i> L. (Omonyangateti) | SMW/2017/17 | Shrub | Roots decoction | Arthritis | 27 |
| Asparagaceae | <i>Asparagus racemosus</i> Willd. (A.buchananil Bak.) (Ekerebo ekiagarori) | SMW/2017/31 | Climber | Roots decoction inhalation of steam | Arthritis | 28 |
| Asparagaceae | <i>Dracaena steudneri</i> Engl. (Omokubo) | SMW/2017/58 | Tree | Leaves poultice | Skin cancer | 7 |
| Asteraceae | <i>Berkheya spekeana</i> Oliv. (Rigeri) | SMW/2017/02 | Shrub | Roots and leaves decoction and also ash | Arthritis | 7 |
| Asteraceae | <i>Conyza bonariensis</i> (L.) Cronquist. (Omosune) | SMW/2017/35 | Herb | Leaves infusion | Arthritis | 7 |
| Asteraceae | <i>Echinops amplexicaulis</i> Oliv. (Rigeri nyagutwa) | SMW/2017/09 | Herb | Root decoction | Arthritis | 21 |
| Asteraceae | <i>Microglossa pyrifolia</i> (Lam.) Kuntze (Nyomba ya kebaki j or Mote okebaki) | SMW/2017/18 | Shrub | Root and Leaves infusion | Arthritis | 27 |

| | | | | | | |
|-----------------------|--|-------------|-------|---|-----------------------|----|
| Asteraceae | <i>Solanecio manni</i> (Hook.f.) C.Jeffrey (Omotagara) | SMW/2017/13 | Shrub | Leaf poultice | Arthritis | 9 |
| Asteraceae | <i>Tagetes minuta</i> L. (Omotiokia) | SMW/2017/33 | Herb | Root decoction | Arthritis | 6 |
| Asteraceae, | <i>Dichrocephala integrifolia</i> (L.f.) Kuntze (Ekengenta mbori) | SMW/2017/55 | Herb | Leave infusion | Loss of memory | 5 |
| Asteraceaea | <i>Carduus chamaecephalus</i> (Vatke) Olive & Hiern (Egetuke) | SMW/2017/01 | Herb | Whole plant decoction and burnt ash | Arthritis | 5 |
| Asteraceaea | <i>Cirsium vulgare</i> (Savi) Ten. (Rigeri rinene) | SMW/2017/03 | Herb | Whole plant decoction and burnt ash | Arthritis | 6 |
| Bignoniaceae | <i>Markhamia lutea</i> (Benth.) K.Schum, (Omwobo) | SMW/2017/24 | Tree | Root decoction | Arthritis | 5 |
| Bignoniaceae | <i>Spathodea campanulata</i> P.Beauv. (Omonyagasegane) | SMW/2017/32 | Tree | Bark decoction | Arthritis | 21 |
| Caesalpinaceae | <i>Senna didymobotrya</i> (Fresen.) H.S.Irwin & Barneby (Omobeno) | SMW/2017/53 | Shrub | Root decoction | Cancer Breast pain | 3 |
| Celastraceae | <i>Maytenus obscura</i> (A. Rich.) Cufod. (Omosobwa) | SMW/2017/26 | Shrub | Root decoction | Arthritis | 16 |
| Crassulaceae | <i>Kalanchoe densiflora</i> Rolfe (Omoneke) | SMW/2017/40 | Herb | Leave poultice | Arthritis | 17 |

| | | | | | | |
|-----------------------|--|-------------|---------|----------------------------|-----------|----|
| Cucurbitaceae, | <i>Momordica foetida</i> Schumach (Omwatekania) | SMW/2017/25 | Climber | Root decoction | Arthritis | 13 |
| Ebenaceae | <i>Euclea divinorum</i> Hiern (Omomusi) | SMW/2017/30 | Shrub | Roots decoction | Arthritis | 3 |
| Euphorbiaceae | <i>Phyllanthus amarus</i> Schumach. & Thonn. (Egesabisabi) | SMW/2017/06 | Herb | Root decoction. | Arthritis | 3 |
| Fabaceae | <i>Acacia abyssinica</i> Benth. (Omonyenya) | SMW/2017/20 | Shrub | Bark decoction | Arthritis | 18 |
| Fabaceae | <i>Acacia gerradi</i> Benth. (Omokonge) | SMW/2017/19 | Shrub | Bark decoction | Arthritis | 11 |
| Fabaceae | <i>Indigofera arrecta</i> Hochst. ex A.Rich. (Omocheo) | SMW/2017/34 | Shrub | Root and leaf decoction | Arthritis | 9 |
| Hyacinthaceae | <i>Albuca abyssinica</i> Jacq. (Egetunguu ekiagarori) | SMW/2017/08 | Herb | Bulb Poultice | Arthritis | 11 |
| Hypericaceae | <i>Harungana madagascariensis</i> Lam. ex Poir. | SMW/2017/23 | Tree | Back dcoction | Arthritis | 15 |
| Lamiaceae | <i>Ajuga remota</i> Benth. (Omonyantira) | SMW/2017/39 | Herb | Whole plant decoction | Arthritis | 13 |
| Lamiaceae. | <i>Rothea myricoides</i> (Hochst.) Steane & Mabb. (Omonyasese) | SMW/2017/14 | Shrub | Roots decoction | Arthritis | 21 |
| Loranthaceae | <i>Phragmanthera usuiensis</i> (Oliv.) M.G.Gilbert (Egete kienyoni) | SMW/2017/29 | Shrub | Leave infusion | Arthritis | 13 |

| | | | | | | |
|------------------------|---|-------------|---------|----------------------------------|----------------|----|
| Meliaceae | <i>Ekebergia capensis</i> Sparrm (Omoturomesi) | SMW/2017/41 | Tree | Roots decoction | Arthritis | 9 |
| Moraceae | <i>Morus nigra</i> L. (Omokenene) | SMW/2017/43 | Shrub | Leaves infusion | Arthritis | 7 |
| Musaceae | <i>Ensete ventricosum</i> (Welw.) Cheesman | SMW/2017/22 | Herb | Root decoction | Arthritis | 4 |
| Myrtaceae | <i>Eucalyptus spp</i> (Engamu ya bogere) | SMW/2017/38 | Tree | Leaves infusion and warm bath | Arthritis | 5 |
| Oxalidaceae | <i>Biophytum umbraculum</i> Welw. (Ekiebundi) | SMW/2017/12 | Herb | Whole plant decoction | Arthritis | 3 |
| Oxalidaceae, | <i>Oxalis corniculata</i> L. Enyonyo ya gusii | SMW/2017/51 | Herb | Leave infusion | Loss of memory | 8 |
| Papilionaceae | <i>Erythrina abyssinica</i> DC. (Omotembe) | SMW/2017/45 | Tree | Roots and bark decoction | Arthritis | 28 |
| Peraceae | <i>Clutia abyssinica</i> Jaub & Spach (Omosambara igoro) | SMW/2017/04 | Shrub | Root decoction | Arthritis | 4 |
| Phytolaccaceae, | <i>Phytolacca dodecandra</i> L'Hér. Omooko | SMW/2017/57 | Climber | Root poultice plus boric acid | Breast cancer | 4 |
| Ranunculaceae | <i>Clematis brachiata</i> Thunb. (Omonyaigena) | SMW/2017/48 | Climber | Roots decoction | Arthritis | 22 |
| Rhamnaceae | <i>Rhamnus prinoides</i> L 'Hérit. (Omonyakige) | SMW/2017/42 | Shrub | Root decoction | Arthritis | 29 |

| | | | | | | |
|--------------------|--|-------------|-------|-----------------|-----------|----|
| Rhamnaceae | <i>Rhamnus staddo</i> A. Ritch (Omongururo) | SMW/2017/05 | Shrub | Root decoction | Arthritis | 27 |
| Rubiaceae | <i>Rytigynia acuminatissima</i> (K.Schum.) Robyns (Omonyinga) | SMW/2017/10 | Tree | Bark decoction | Arthritis | 4 |
| Rubiaceae | <i>Vangueria apiculata</i> (K. Schum) (Omokomoni) | SMW/2017/07 | Shrub | Root decoction | Arthritis | 24 |
| Rubiaceae | <i>Vangueria madagascariensis</i> J.F.Gmel. (Omwuogi) | SMW/2017/21 | Shrub | Root decoction | Arthritis | 21 |
| Rutaceae | <i>Toddalia asiatica</i> (L.) Lam. (Ekenagwa ekiagarori) | SMW/2017/37 | Shrub | Root decoction | Arthritis | 27 |
| Salicaceae | <i>Flacourtia indica</i> (Burm. f.) Merr. (Rikara) | SMW/2017/16 | Shrub | Back decoction | Arthritis | 5 |
| Salicaceae | <i>Trimeria grandifolia</i> (Hochst.) Warb. (Omoatiuga) | SMW/2017/47 | Shrub | Root decoction | Arthritis | 6 |
| Sapindaceae | <i>Pappea capensis</i> Eckl. & Zeyh. Omonyamanengo | SMW/2017/52 | Herb | Leave decoction | Diabetes | 6 |
| Solanaceae | <i>Physalis peruviana</i> L. (Omosobosobo) | SMW/2017/44 | Herb | Roots | Arthritis | 6 |
| Solanaceae | <i>Solanum incanum</i> L. (Omoratora) | SMW/2017/28 | Herb | Root decoction | Arthritis | 9 |
| Solanaceae | <i>Solanum terminale</i> Forssk. (Omonyainogu) | SMW/2017/46 | Herb | Root decoction | Arthritis | 9 |

| | | | | | | |
|--------------------|---|-------------|---------|------------------------------|---|----|
| Solanaceae | <i>Withania somnifera</i> (L.) Dunal (Omokubinyongo) | SMW/2017/36 | Shrub | Root and leaves decoction | Arthritis | 19 |
| Urticaceae | <i>Urtica massaica</i> Mildbr. (Rise) | SMW/2017/27 | Herb | Root decoction | Arthritis | 13 |
| Verbaceae, | <i>Dalbergia lactea</i> Vatke (Omonyatai) | SMW/2017/54 | Climber | Leave decoction | Diabetes and managing Pain and inflammation | 20 |
| Verbenaceae | <i>Lantana trifolia</i> L. Obori bwe 'nyoni, Ekemba kie 'nyoni | SMW/2015/49 | Shrub | Leaf infusion and ashes | Hypertension | 3 |
| Vitaceae | <i>Rhoicissus tridentata</i> (L.f.) Wild & R.B.Drumm. (Omokorongotwa) | SMW/2017/15 | Climber | Root decoction | Arthritis | 19 |

Table 4.3: Cross-reference of candidate plant species with that are published literature.

| Botanical name an | Relevant biological activity | chemical constituents | Relevant ethnomedical uses |
|-----------------------------|---|---|---|
| <i>Acacia spp</i> | Not reported | Not reported | Rheumatism (Kipkore <i>et al.</i> , 2014) |
| <i>Albuca abyssinica</i> | No reports | No report | Treating and rheumatism (Mesfin <i>et al.</i> , 2013) |
| <i>Asparagus racemosus</i> | Not reported | Flavonoids, isoflavons, phenolic compounds (Hasan <i>et al.</i> , 2016) | Treats inflammation and rheumatism (Hasan <i>et al.</i> , 2016) |
| <i>Biophytum umbraculum</i> | Anti-inflammatory activity (Austarheim <i>et al.</i> , 2016) | Flavone-C-Glycosides (Pham <i>et al.</i> , 2013) Tannins and flavonoids (Pham <i>et al.</i> , 2014) | Treatment of joint pains, inflammations and fever. (Inngjerdingen <i>et al.</i> , 2008) |
| <i>Carissa spinarum</i> | The root extract had antioxidant and anti-inflammatory activities (Woode <i>et al.</i> , 2007) | Sesquiterpenes, Flavonoids, Sterols and triterpenes (Hanan <i>et al.</i> , 2014) | Headache, chest complaints, rheumatism (Nedi <i>et al.</i> , 2004) and arthritis (Jeruto <i>et al.</i> , 2008). |
| <i>Centella asiatica</i> | Cytotoxic and anti-tumour properties (Babu <i>et al.</i> , 1995) Antioxidant and anti-inflammatory (Gohil <i>et al.</i> , 2010) | Glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars (Arumugam <i>et al.</i> , 2011) | Asthma, jaundice, rheumatism, fever, pain and swelling. (Arora <i>et al.</i> , 2002) |
| <i>Cirsium vulgare</i> | Antioxidant (Nazaruk, 2008) | Phenolic acids, Sterols, alkaloids, triterpenes and flavonoids (Nazaruk, 2008) | Used for inflammation (Al-Mustafa & Al-Thunibat, 2008) |
| <i>Clematis brachiata</i> | Antiinflammatory, antinoceptive and | Saponins, flavonoids and terpenoids (Mostafa & | Used to manage pain and swelling (Chhabra <i>et al.</i> , 1991) |

| | | | |
|-----------------------------------|---|--|---|
| | antipyretic activities (Mostafa <i>et al.</i> , 2010) | Afolayan, 2013)) | |
| <i>Clusia abyssinica</i> | Not reported | Flavonoids, phenolic compounds and terpenoids (Jeruto <i>et al.</i> , 2008) | Treatment of arthritis (Jeruto <i>et al.</i> , 2008) |
| <i>Dichrocephala integrifolia</i> | Anti-inflammatory and cytotoxic (Lee <i>et al.</i> , 2015) Neuroprotective antioxidant activities (Kouémou <i>et al.</i> , 2017). | Saponins, alkaloids, phenols, flavonoids, glycosides, terpenoids and tannins(Qin <i>et al.</i> , 2015) | Used as a diuretic, treating venous disorders and also swelling (Siemonsma & Wulijarni-Soetjipto, 1989) Treating dementia (Kouémou <i>et al.</i> , 2017) |
| <i>Dracaena steudneri</i> | Antifungal (Kisangau <i>et al.</i> , 2009) Cytotoxicity effects(D. P. Kisangau <i>et al.</i> , 2011) | Not reported | Used in treatment of cancer(Kadima <i>et al.</i> , 2016) Used in splenomegally, asthma and chest problems(Moshi <i>et al.</i> , 2012) |
| <i>Eucalyptus spp</i> | Antioxidant, anti-inflammatory (Dixit <i>et al.</i> , 2012) and analgesic activities (J. Silva <i>et al.</i> , 2003) | Essential oils, tannins, saponins and steroid (Sani <i>et al.</i> , 2014) | Eucalyptus oil has been reported to be effective in reducing pain, swelling, and inflammation(Jun <i>et al.</i> , 2013) |
| <i>Flacourtia indica</i> | Antioxidant (Ndhlala <i>et al.</i> , 2008) and anti-inflammatory activity (Gayathri, 2013) | Flavonoids, terpenoids, phenols and steroids (Gayathri, 2013) | The bark is believed to be effective in treating arthritis (Lalsare <i>et al.</i> , 2011) |
| <i>Harungana madagascariensis</i> | Stem bark shown high antioxidant (Kouam <i>et al.</i> , 2005) and anti-inflammatory activities | Alkaloids phenolics flavonoids and saponins (Ndam <i>et al.</i> , 2014) | Used for migraine and rheumatism (Lukwamirwe <i>et al.</i> , 2016) |

| | | | |
|----------------------------------|--|--|--|
| | (Iwalewa <i>et al.</i> , 2008) | | |
| <i>Indigofera arrecta</i> | Anti-inflammatory activity (Madikizela <i>et al.</i> , 2014) | Steroids, glycosides and rotenoids (Rahman <i>et al.</i> , 2018) | Leaves used to treat toothache and stomachache (Kipkore <i>et al.</i> , 2014) |
| <i>Lantana trifolia</i> | Anti-inflammatory, antioxidant and analgesic (Noufou <i>et al.</i> , 2012; Silva <i>et al.</i> , 2005) | Sesquiterpenes, monoterpenes (Rwangabo <i>et al.</i> , 1988) | Rheumatism, body pains, indigestion (Ruffo <i>et al.</i> , 2002), angina(Boily & Van Puyvelde, 1986) |
| <i>Markhamia lutea</i> | Anticancer and antioxidant activity (Narendran <i>et al.</i> , 2014) | Flavonoids, phenolic compounds and glycosides (Brindha <i>et al.</i> , 2017) | Treatment of rheumatic pain (Burkill, 1985) |
| <i>Maytenus obscura</i> | Anti-inflammatory (Alajmi & Alam, 2014) | Aqueous and dichloromethane extracts contained flavonoid anthraquinone (Alajmi & Alam, 2014) | Treatment of Inflammation (Maina, 2015) |
| <i>Momordica foetida</i> | Antioxidant activity(Acquaviva <i>et al.</i> , 2013) | Steroids, phenolics and flavonoids and saponins (Ndam <i>et al.</i> , 2014) | Used to treat swelling (Teklehaymanot, 2009) and to manage rheumatism (Yineger <i>et al.</i> , 2008) |
| <i>Oxalis corniculata</i> | Antioxidant, anti-cancer and cardiorelaxant activities (Badwaik <i>et al.</i> , 2011) | flavanoids, tannins, phytosterol, phenol, glycosides, fatty acids and volatile oil(Badwaik <i>et al.</i> , 2011) | Cancer, dementia, convulsion and piles (Li <i>et al.</i> , 2006) |
| <i>Morus nigra</i> | Antioxidant (Mazimba, 2011) and anti-inflammatory activities (Padilha <i>et al.</i> , | Two anthocyanins, cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside in the fruits(Chen <i>et al.</i> , 2016) | Treatment of inflammation disorders (Nomura & Hano, 1994) |

| | | | |
|------------------------------|---|---|---|
| | 2010) | | |
| <i>Pappea capensis</i> | Nutraceuticals and antioxidant (Karau <i>et al.</i> , 2012; Kiringe, 2006) | Flavonoids, steroids, saponins, tannins, phenols, cardiac glycosides, micronutrients and minerals(Karau <i>et al.</i> , 2012) | Strength/nutrient supplement(Kimondo <i>et al.</i> , 2015). Arthritis, Backache (Kiringe, 2006) |
| <i>Phyllanthus amarus</i> | Antioxidant and anti-inflammatory activities (Kassuya <i>et al.</i> , 2005) | Lignans, flavonoids, ellagitannins, alkaloids, triterpenes and sterols(Patel <i>et al.</i> , 2011) | Used traditionally in Brazil for treating joint aches, inflammations and arthritis (Patel <i>et al.</i> , 2011) |
| <i>Physalis peruviana</i> | Antioxidant (Puente <i>et al.</i> , 2011) | Phytosterols, steroid and flavonoid (Author <i>et al.</i> , 2013) | The fruit is reported to be used for rheumatism (Ramadan, 2011; Wu <i>et al.</i> , 2004) |
| <i>Phytolacca dodecandra</i> | anti-inflammatory and diuretic activities (PROTA, 2013) | Saponins, lipids, sugars, pectins and gums.(PROTA, 2013) | Used to treat edema and ground leaves are applied to tumours. Also used to treat epilepsy(PROTA, 2013) |
| <i>Rhamnus prinoides</i> | Antioxidants (Amabye, 2016) | Flavonoids triterpenes and phenols (Onyango <i>et al.</i> , 2014) | Reported use in treating arthritis, back and joint aches (Kimondo <i>et al.</i> , 2015; Kiringe, 2006) |
| <i>Rhamnus staddo</i> | No report | Flavonoids, steroids and sterols | Treatment of headaches, back and joint pain (Chalo <i>et al.</i> , 2016) |
| <i>Rothea myricoides</i> | | Alkaloids, terpernoids and flavonoids (Pascaline <i>et al.</i> , 2011) | Rheumatism and arthritis (Jeruto <i>et al.</i> , 2010) |
| <i>Searsia pyroides</i> | Anti-inflammatory and antioxidant activity (Odongo <i>et al.</i> , 2017) | Biflavonoids (Masesane <i>et al.</i> , 2000) | To treat oxidative stress and inflammatory related conditions (Odongo <i>et al.</i> , 2017) |

| | | | |
|------------------------------|---|---|---|
| <i>Senna didymobotrya</i> | vasorelaxation, antioxidant, antibacterial and antifungal (PROTA, 2013) | anthraquinone derivatives, tannins and flavonoid (PROTA, 2013) | Hypertensions, inflammation and backache (PROTA, 2013) |
| <i>Solanum incanum</i> | antioxidant (Al-Fatimi <i>et al.</i> , 2007), analgesic and antipyretic activities (Mwonjoria <i>et al.</i> , 2014) | The fruit has flavonoids and steroids. (Sambo <i>et al.</i> , 2016) | Kokwaro, (1993) reported treatment of tooth-ache, stomach-ache, fever and chest pains among communities in East Africa. |
| <i>Spathodea campanulata</i> | Antioxidant, anti-inflammatory and analgesic (Ilodigwe & Akah, 2009) | flavones and phenolic compound (Brindha <i>et al.</i> , 2012) | Flowers used to treat inflammation. Leaves used for urethra inflammation (Heim <i>et al.</i> , 2012) |
| <i>Tagetes minuta</i> | Antioxidant and anti-inflammatory (Karimian <i>et al.</i> , 2014) | Essential oils, dihydrotagetone, b-ocimene and tagetenone (Chamorro <i>et al.</i> , 2008) | It has been used in Ayurvedic medicine in treating pain and inflammation (Karimian <i>et al.</i> , 2014) |
| <i>Trimeria grandifolia</i> | Not reported | Contains idesin, lupenone and β -Sitosterol (Kamau <i>et al.</i> , 2017) | In a concoction to treat arthritis and toothache (Some, 2014) |
| <i>Urtica massaica</i> | Not reported | Flavonoids and sterols and (Alphonse <i>et al.</i> , 2008) | Fractures, injuries and rheumatism (PROTA, 2013) Stomach-ache (Kimondo <i>et al.</i> , 2015). |
| <i>Vangueria apiculata</i> | Not reported | Not reported | Used for general body strength, treating arthritis, backache and joint pains. (Edna, 2018) |
| <i>Withania somnifera</i> | Antioxidant and anti-inflammatory activities (Mir <i>et al.</i> , 2012) | Withanoloides, steroidal lactones (Singh <i>et al.</i> , 2010). | Prescribed in Ayurveda for musculoskeletal disorders like arthritis and rheumatism (Mishra <i>et al.</i> , 2000) |

4.2 RESULTS OF BIOASSAY

4.2 Phytochemistry and Pharmacological assays

4.2.1 Preliminary Phytochemical Profile of Selected Plants

Methanolic and aqueous extracts of the selected medicinal plants were screened for phytochemicals and the results indicated presence of various phytochemicals as shown in Tables 4.4 and 4.5. The selected plants screened were, *Phragmanthera usuiensis* (Figure 4.6), *Ensete ventricosum* (Figure 4.7), *Echinops amplexicaulis* (Figure 4.8) and *Rhoicissus tridentata* (Figure 4.8)

Table 4.4: Preliminary phytochemical screening of aqueous extracts

| PHYTOCHEMICALS | AQUEOUS EXTRACTS AND PARTS USED | | | |
|----------------|---------------------------------|----------|-------------|------------|
| | RT (ROOT) | EV(ROOT) | EA(RHIZOME) | PU(LEAVES) |
| Alkaloids | - | - | - | - |
| Tannins | +++ | +++ | +++ | +++ |
| Glycosides | +++ | +++ | + | ++ |
| Saponins | +++ | +++ | + | +++ |
| Steroids | - | - | - | - |
| Flavonoids | +++ | + | - | +++ |
| Terpenoids | - | - | - | - |
| Phenols | + | ++ | + | ++ |
| Coumarins | +++ | +++ | + | +++ |
| Free sugars | +++ | +++ | ++ | +++ |

KEY:

+++Abundantly present
 ++ Moderatly present
 + Fairly present
 - Absent

RT- *Rhoicissus tridentata*
EV- *Ensete ventricosum*
EA- *Echinops amplexicaulis*
PU- *Phragmanthera usuiensis*

Table 4.5: Preliminary phytochemical screening of methanolic extracts

| PHYTOCHEMICALS | METHANOLIC EXTRACTS | | | |
|----------------|---------------------|----------|-------------|------------|
| | RT(ROOT) | EV(ROOT) | EA(RHIZOME) | PU(LEAVES) |
| Alkaloids | +++ | +++ | +++ | +++ |
| Tannins | +++ | - | ++ | +++ |
| Glycosides | +++ | + | +++ | +++ |
| Saponins | +++ | + | - | +++ |
| Steroids | +++ | +++ | - | +++ |
| Flavonoids | -- | +++ | - | - |
| Terpenoids | +++ | +++ | - | +++ |
| Phenols | ++ | +++ | ++ | +++ |
| Coumarins | +++ | +++ | - | +++ |
| Free sugars | +++ | + | +++ | +++ |

KEY:

- | | |
|-----------------------|---|
| +++Abundantly present | RT- <i>Rhoicissus tridentata</i> |
| ++ Moderatly present | EV- <i>Ensete ventricosum</i> |
| + Fairly present | EA- <i>Echinops amplexicaulis</i> |
| - Absent | PU- <i>Phragmanthera usuiensis</i> |



Figure 4.6: *Phragmanthera usuiensis*



Figure 4.7: *Ensete ventricosum*



Figure 4.8: *Echinops amplexicaulis*



Figure 4.9: *Rhoicissus tridentata*

Various studies have been carried out to assess the health benefits of phytochemicals such as the ones that were found in the four plant species evaluated (Table 4.4). For example saponins have been shown to decrease blood lipids, lower cancer risks, and lower blood glucose response (Shi *et al.*, 2004). They also possess anti-inflammatory and antioxidant activity (Sparg *et al.*, 2004). Flavanoids are known to have antioxidant and other biochemical activities that are of significance in diseases like carcinomas, memory loss and atherosclerosis (Borges *et al.*, 2013; Castañeda-Ovando *et al.*, 2009; Jones *et al.*, 2006). In addition, they possess anti-inflammatory, and anti-carcinogenic properties and they are also inhibitors of xanthine oxidase (XO), cyclooxygenase (COX) and lipoxygenase (Iio *et al.*, 1985; Metodiewa *et al.*, 1997; Walker *et al.*, 2000). Many studies have shown that tannins are beneficial in the management of diabetes because they enhance glucose uptake and inhibit adipogenesis (Kumari & Jain, 2012). Glycosides have mostly been employed in managing cardiovascular and renal conditions and have been studied for anticancer properties (Newman *et al.*, 2008; Prassas & Diamandis, 2008)

4.2.2 Percentage radical scavenging activities of plants extracts

The DPPH radical scavenging activity and the IC₅₀ values of different solvents extracts are shown in Tables 4.5 and 4.6. The lower the IC₅₀ value the higher the antioxidant capacity. The 1, 1-diphenyl -2-picrylhydrazyl (DPPH) radical scavenging activity of *P. usuiensis*, *E. ventricosum*, *E. amplexicaulis* and *R. tridentata* methanolic extracts showed higher antioxidant activity compared to the standard (Table 4.5). The highest activity of the methanolic extracts was exhibited by *P. usuiensis* (IC₅₀, 6.65 x 10⁻³ µg/mL) followed by *R. tridentata* (IC₅₀, 7.0 x 10⁻³ µg/mL), *E. ventricosum* (IC₅₀, 7.73 x 10⁻³ µg/mL) and *E. amplexicaulis* (IC₅₀, 7.93 x 10⁻³ µg/mL). Phytochemicals such as flavonoids, terpenoids and tannins might be responsible for the high

powerful antioxidant activity seen with these extracts. Radical scavenging activity of DPPH has also been demonstrated to be related to the nature of phenolics and therefore contribute their ability to transfer electrons or donate hydrogen (Brand-Williams *et al.*, 1995).

The aqueous extracts of *E. amplexicaulis* and *R. tridentata* exhibited higher activities IC_{50} , $7.80 \times 10^{-3} \mu\text{g/mL}$ and IC_{50} , $8.13 \times 10^{-3} \mu\text{g/mL}$ respectively against IC_{50} , $8.40 \times 10^{-3} \mu\text{g/mL}$ of Ascorbic acid standard. However lower activity was exhibited by *P. usuiensis* (IC_{50} , $8.45 \times 10^{-3} \mu\text{g/mL}$), and *E. ventricosum* (IC_{50} , $8.67 \times 10^{-3} \mu\text{g/mL}$). Comparing the IC_{50} values of the extracts indicated in Table 4.5 and Table 4.6, methanolic extracts except *E. amplexicaulis* showed higher activities than the aqueous extracts.

Table 4.6: Antioxidant activity of methanolic extracts compared with L-ASA as standard

| Concentration ($\mu\text{g/mL}$) | %RSA \pm SEM | | | | |
|--|-----------------------------------|-------------------------------|------------------------------------|----------------------------------|-------------------------------|
| | <i>Echinops amplexicaulis</i> | <i>Ensete ventricosum</i> | <i>Phragmanthera usuiensis</i> | <i>Rhoicissus tridentata</i> | L-Ascorbic acid (Standard) |
| 0.01 | 63.12 \pm 1.2441 | 64.02 \pm 1.6224 | 75.92 \pm 1.7820 | 73.61 \pm 0.1867 | 59.62 \pm 3.7330 |
| 0.1 | 67.11 \pm 0.8157 | 65.44 \pm 1.4393 | 76.15 \pm 1.7754 | 74.77 \pm 0.1929 | 65.50 \pm 0.7716 |
| 1 | 69.05 \pm 1.2018 | 72.12 \pm 0.3470 | 76.00 \pm 1.8862 | 79.38 \pm 1.0404 | 79.22 \pm 0.8118 |
| 10 | 71.21 \pm 1.4015 | 73.92 \pm 0.4548 | 81.99 \pm 0.8999 | 86.15 \pm 0.2110 | 82.23 \pm 1.9283 |
| 100 | 77.90 \pm 0.8850 | 74.78 \pm 0.2417 | 83.89 \pm 1.500 | 96.48 \pm 2.2393 | 95.87 \pm 1.4742 |
| 1000 | 94.61 \pm 0.3756 | 78.92 \pm 0.9133 | 87.20 \pm 0.3214 | 98.75 \pm 0.0233 | 98.12 \pm 0.746 |
| IC₅₀ ($\mu\text{g/mL}$) | 7.93 x 10⁻³ | 7.73 x 10⁻³ | 6.65 x 10⁻³ | 7.0 x 10⁻³ | 8.4 x 10⁻³ |

Table 4.7: Antioxidant activity of aqueous extracts compared with L-ASA as standard

| Concentration ($\mu\text{g/mL}$) | %RSA \pm SEM | | | | |
|--|-----------------------------------|-------------------------------|------------------------------------|----------------------------------|-------------------------------|
| | <i>Echinops amplexicaulis</i> | <i>Ensete ventricosum</i> | <i>Phragmanthera usuiensis</i> | <i>Rhoicissus tridentata</i> | L-Ascorbic acid (Standard) |
| 0.01 | 64.26 \pm 1.3778 | 56.95 \pm 9.2171 | 59.10 \pm 0.9693 | 62.02 \pm 4.0373 | 59.62 \pm 3.7330 |
| 0.1 | 66.78 \pm 0.8871 | 66.09 \pm 1.7268 | 63.78 \pm 1.6160 | 61.12 \pm 0.9480 | 65.50 \pm 0.7716 |
| 1 | 68.76 \pm 1.1089 | 66.89 \pm 1.9380 | 66.99 \pm 0.3613 | 62.39 \pm 0.8177 | 79.22 \pm 0.8118 |
| 10 | 70.00 \pm 1.8550 | 69.13 \pm 0.6566 | 70.55 \pm 1.3881 | 65.70 \pm 1.3811 | 82.23 \pm 1.9283 |
| 100 | 73.81 \pm 0.3050 | 70.41 \pm 1.3375 | 76.13 \pm 0.2333 | 83.07 \pm 2.1030 | 95.87 \pm 1.4742 |
| 1000 | 80.90 \pm 1.2527 | 82.74 \pm 2.4169 | 86.08 \pm 0.1466 | 89.36 \pm 0.4168 | 98.12 \pm 0.7460 |
| IC₅₀ ($\mu\text{g/mL}$) | 7.80 x 10⁻³ | 8.67 x 10⁻³ | 8.45 x 10⁻³ | 8.13 x 10⁻³ | 8.40 x 10⁻³ |

As shown by graph plots of the extracts concentrations against their scavenging capabilities, the extracts exhibited concentration dependent effects. At low concentrations, all the extracts demonstrated nearly similar activities which became more distinct as the concentration increased. *P. usuiensis* methanol extract showed a higher activity than the *P. usuiensis* aqueous extract (Fig.4.10) but the activities were almost the same at the 1000 µg/mL. There was no significant variation between the activities of the extracts and the standard ($p < 0.05$) as analyzed through one-way analysis of variance

E. amplexicaulis methanolic extract at 1000µg/mL showed the same activity as the L-ASA standard (Fig.4.11). At 0.01µg/mL doses, the methanolic and the aqueous extract of *E. amplexicaulis* had higher activities than the standard.

At higher doses antioxidant activity of *E. ventricosum* aqueous extract increased and was more than that of the methanolic extract at 1000µg/mL (Fig.4.12). However no statistical difference was found between the methanolic and the aqueous extracts when compared with the standard.

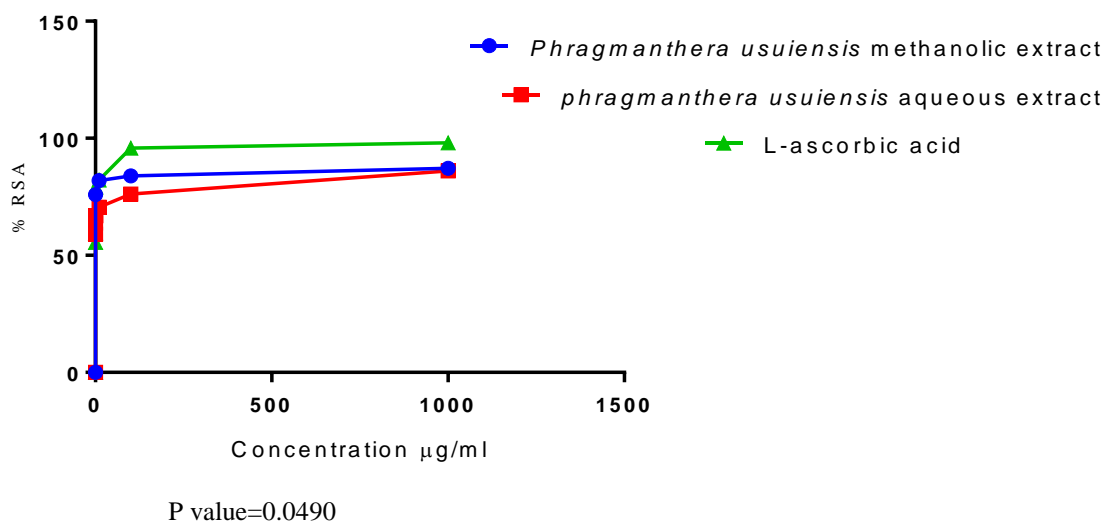


Figure 4.10: DPPH radical scavenging activities of the *P. usuiensis* extracts compared with L-ASA

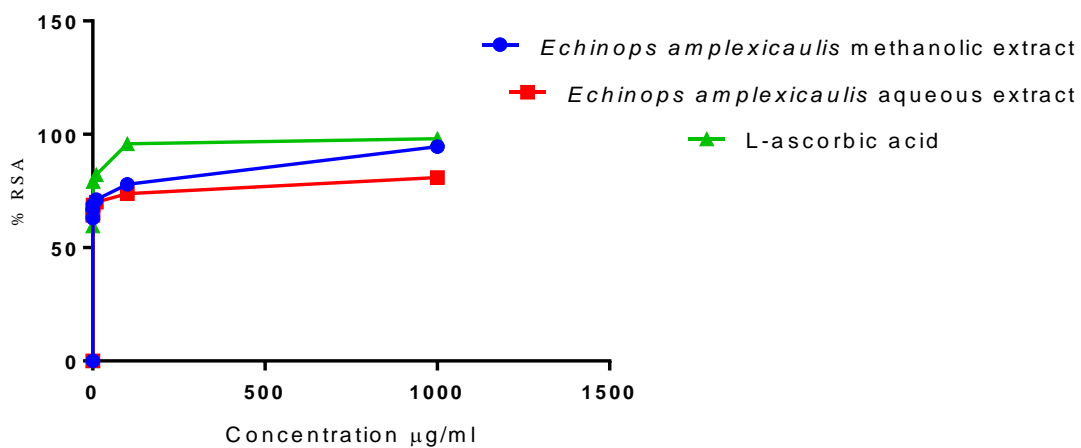
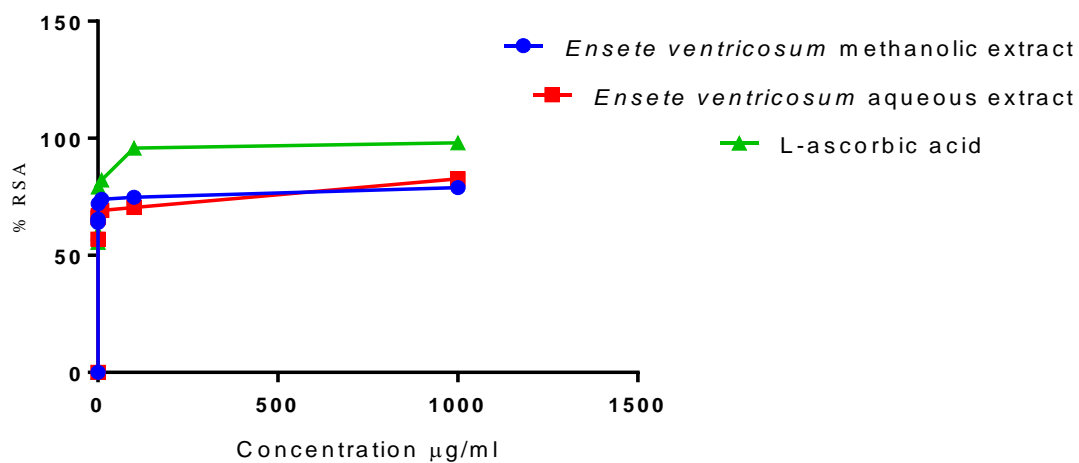
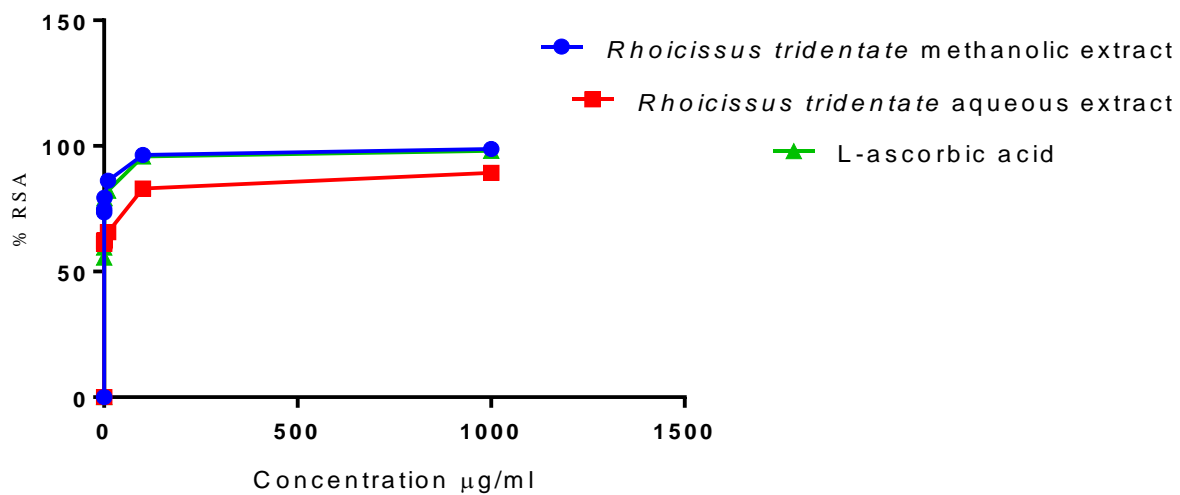


Figure 4.11: DPPH radical scavenging activities of the *E. amplexicaulis* extracts compared with L-ASA



P value =0.0501

Figure 4.12: DPPH radical scavenging activities of the *E. ventricosum* extracts compared with L-ascorbic acid



P value =0.0021

Figure 4.13: DPPH radical scavenging activities of the *Rhoicissus tridentata* extracts compared with L-ascorbic acid

There was a significant variation between the activities of *Rhoicissus tridentata* methanolic and aqueous extracts with that of the standard when analyzed through one-way analysis of variance. The standard (L-Ascorbic acid) showed a higher activity at low concentration but the activity of *R. tridentata* methanolic extract tended to match that of the standard as the concentration increased (Fig.4.13.).

DPPH radical scavenging activity of the four plant extracts evaluated in the present investigation are shown in figures 4.10-4.13. At concentrations above the IC₅₀ all methanolic extracts exhibited higher activities except in *E. ventricosum* extracts where activity of the aqueous extract superseded that of the methanolic extract above 500µg/mL. These outcomes demonstrated that polarity of solvents (water and methanol) may have an influence in the antioxidant capacities of the extracts. Methanol has a polarity index of 5.1 and is mostly used for extraction of various polar compounds. However certain polar compounds are fairly soluble in methanol. Studies have revealed that highly polar solvents, such as methanol, have a high effectiveness as antioxidants. For example methanol proved more successful in extraction of phenols which are usually potent antioxidants (Anokwuru *et al.*, 2011; Koffi *et al.*, 2010; Zhi *et al.*, 2008). The observed high DPPH radical scavenging activity of methanolic extracts studied could therefore be attributed to their high phenolic contents. Contrary, in a study carried out by Yang *et al.*, (2014), higher radical scavenging activity was observed in water extract than in methanolic extract. However in the same study it was methanolic extracts that had higher total antioxidant activity than water extracts.

4.3. Acute toxicity test

All groups of treated animals did not exhibit any toxic or lethal effect. The present study shows that even at the administration of the limit dose 2000 mg/kg, no sign of toxicity was observed except on group two where two animals showed unusual slow movement after five minutes of dose administration. These effects however faded off after 30 minutes. Therefore it was concluded that the LD₅₀ of extracts was above 2000 mg/kg.

4.3.1 Histopathology of the mice liver

Figure 4.14 shows histopathology of a normal liver (control) with hepatocytes that are evenly distributed and with normal architecture separated by hepatic sinusoids. All the animals that were dosed with methanolic and aqueous extracts of *Phragmanthera usuiensis*, *Ensete ventricosum*, *Echinops amplexicaulis* and *Rhoicissus tridentata* did not show any significant histopathological changes in the liver compared to the control group. All the groups that received the aqueous and the methanol extracts at the two dose levels of 300mg/kg and 2000mg/kg body weight showed hepatic portal vein congestion as shown by the liver section of a mouse dosed with *P. usuiensis* aqueous extract (Fig.4.15). This congestion compared with the animals in the control group and therefore was not attributable to the extracts.

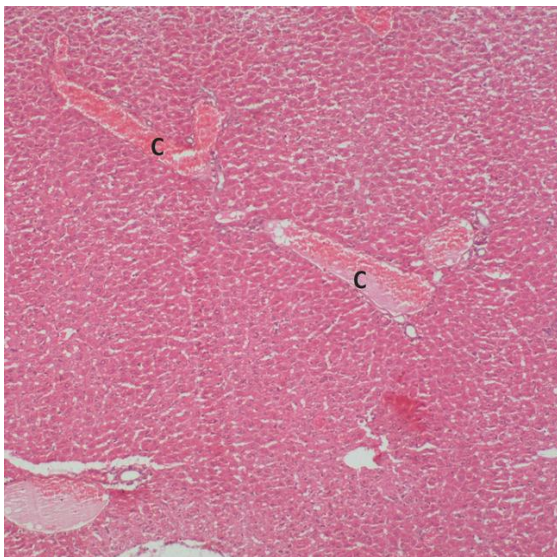


Figure 4.14: Photomicrograph showing a liver section from a control mouse showing hepatocyte structure of a normal mouse liver stained with H&E (400X magnification).

Key: C- congestion in the portal center

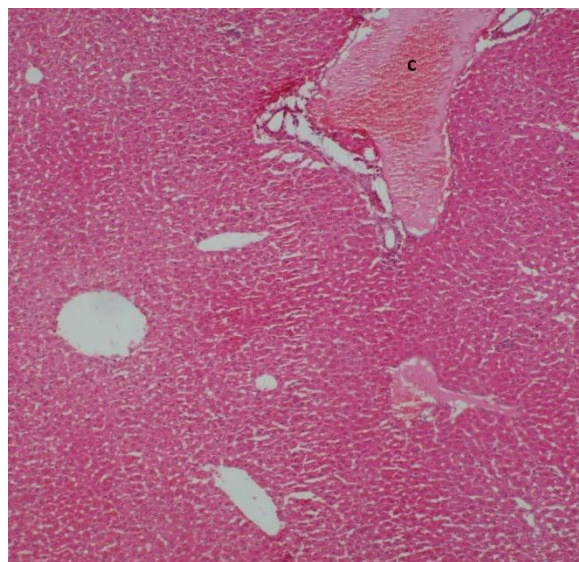


Figure 4.15: Photomicrograph showing a liver section from a mouse dosed with 2000mg/Kg body weight of aqueous *Phragmanthera usuiensis* aqueous extract stained with H&E (400X magnification).

Key: C-Congestion in the portal center

4.3.2 Histopathology of the mice kidneys

Figure 4.16 shows a histological section of a kidney from a control (normal) mouse with regular glomeruli and complete regular renal tubules with clear lamina. There were no significant histopathological changes in the kidneys of the mice that were treated with the four extracts at the two dose levels of 300mg and 2000mg/kg body weight. The kidneys of the mouse number 3 in Group 3B that received 2000mg/kg body weight of *Ensete ventricosum* methanolic extract showed mild congestion of blood vessels and some local infiltration with lymphocytes (Fig.4.17). The kidneys of the mouse number 2 from Group 7B that received 2000mg/kg body weight of *Rhoicissus tridentata* methanolic extract showed mild focal degeneration of tubular epithelium characterized by cell swelling and congestion of blood vessels (Fig.4.18). The kidneys of the mouse number 1 from Group 1B that received 2000mg/kg body weight of *Phragmanthera usuiensis* aqueous extract also showed mild focal degeneration of tubular epithelium characterized by cell swelling and congestion of blood vessels (Fig.4.19).

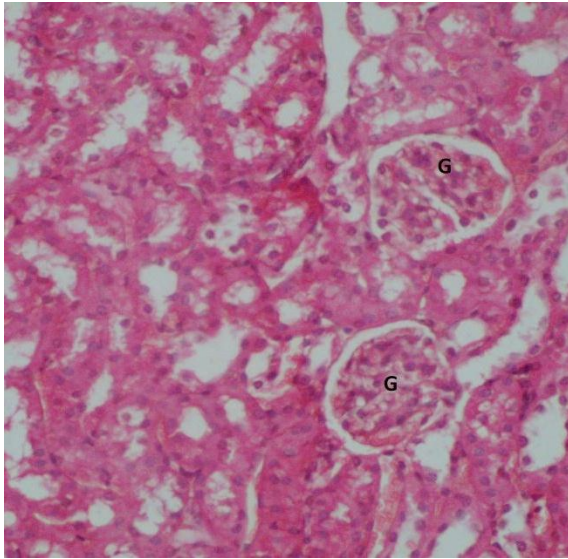


Figure 4.16: Photomicrograph showing a kidney section from a control mouse stained with H&E (400X magnification).

Key: G-glomerulus.

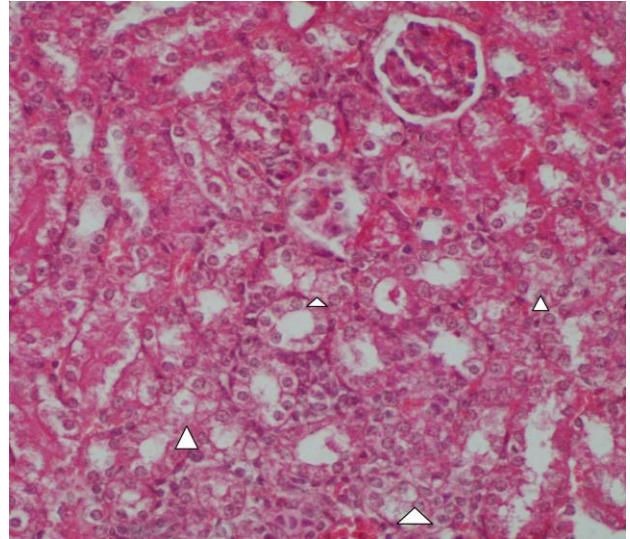


Figure 4.17: Photomicrograph showing a kidney section from a mouse dosed with 2000mg/Kg body weight of *Ensete ventricosum* extract stained with H&E (400X magnification).

Key: White arrow heads: - Local infiltration with lymphocytes.

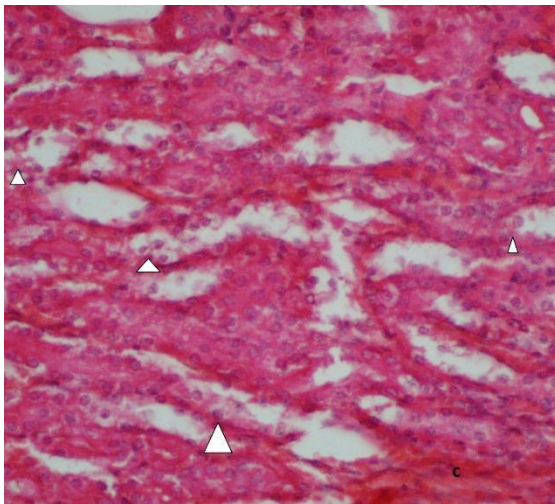


Figure 4.18: Photomicrograph showing a kidney section from a mouse dosed with 2000mg/Kg body weight of *Rhoicissus tridentata* methanolic extract.

Key: Arrow head – focal degeneration of epithelium.

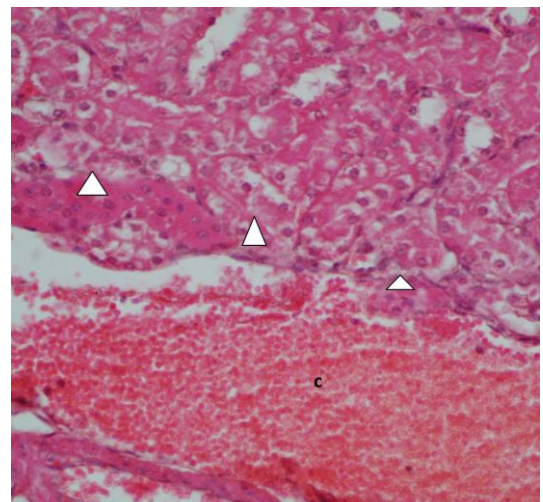


Figure 4.19: Photomicrograph showing a kidney section from a mouse dosed with 2000mg/Kg body weight of *Phragmanthera usuiensis aqueous* methanolic extract

Key: Arrow head –focal degeneration of tubular epithelium

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

The present study was the first to identify and document medicinal plants used to treat arthritis, cancer, memory loss, diabetes and hypertension in the study area. Despite the increased use of conventional medicine, this study shows that the use of traditional herbal remedies continues to be embraced by many people in the study region. However the knowledge of ethnopharmacology is in danger of being extinct considering the fact that it is usually passed verbally from one generation to another. In addition, the natural habitats that house these medicinal plants are slowly disappearing following increased urbanization and massive deforestation as locals clear land for commercial agricultural practices. It is therefore of paramount importance to document these plants as a means of preserving cultural knowledge of traditional medicines.

Literature search conducted to compare claims of the herbalist in the study region with reported similar uses of the plants in other communities serves to justify the use of these plants by the locals in management of these conditions. Documented pharmacological activities of most plants identified in this study also support their use in treating oxidative stress and related conditions. Furthermore, studies have shown that oxidative damage that characterize these ailments are due to free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Dalleau *et al.*, 2013; Pham-Huy, *et al.*, 2008; Valko *et al.*, 2007). Validated antioxidant activities of some of the plants encountered in this study further justifies their use since they are known to neutralize free radicals (Pham-Huy *et al.*, 2008). Compounds from these plants may hopefully provide leads to drug discovery.

Utilization of herbal medicine in primary healthcare especially in the developing countries is gaining popularity day by day and the practice is spreading rapidly across the globe. The active

phytochemicals derived from the medicinal plant are considered to be devoid of side effects on human health, a reason that has escalated their use as nonprescription medicine (Vaghasiya et al., 2011). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. In this study, the oral acute toxicity study of the tested plant extract did not reveal major changes in behavior and mortality of animals in all groups. The extracts seem to be safe at a dose level of 2000 mg/kg, and the LD₅₀ is considered to be >2000 mg/kg. Any pharmaceutical drug or compound with the oral LD₅₀ higher than 1 000 mg/kg could be considered safe and less toxic (Adeneye & Olagunju, 2009). The main target organ for drug or bioactive compound is the liver. The liver is exposed to the foreign substances being absorbed in intestines and metabolized to other compounds which may or may not be hepatotoxic to the mice (Rhiouani et al., 2008). In spite of the congestion of blood vessel observed in the liver of mice that were treated with the four extract, these changes were not considered toxicologically significant, as they were not observed in other animals of the same group and that they compared with the control group. However specific assays of toxicity and more biological studies could provide more information regarding the toxic effects of the extracts on liver.

In histopathological studies of the kidneys, congestion of blood vessels and focal degeneration of tubular epithelium with cell swelling was observed in only two mice and only at a dose level of 2000mg/kg body weight. Since no similar changes were observed on other animals of the same groups, the results were judged to be consistent with a normal tissue with a mild kidney injury. Massive congestion of blood vessels and local infiltration with lymphocytes in the kidney of a mouse that was treated with 2000mg/kg body weight of *Ensete ventricosum* methanolic extracts

was suggestive of nephritis. However, the mechanisms of this toxicity need further investigations.

5.1 Conclusion

- That the present study successfully identified and documented for the first medicinal plants used to treat oxidative stress-related disorders in the study area
- That the plants studied had antioxidant activity
- That the extracts are safe for use as medicinal agents in management of oxidative stress

5.2 Recommendations

- That measures be taken to preserve the traditional knowledge and to educate the locals on the importance of preserving natural habitats
- That further pharmacological and phytochemical investigations be carried out to determine the bioactive compounds responsible for the claims by the TMP
- That more specific toxicity assays and more biological studies be carried out to provide more information regarding the toxic effects of the extracts.
- That more toxicological studies be carried out especially to evaluate the sub-acute and chronic effects of the extracts

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APPENDICES

APPENDIX I: LETTER OF ETHICAL APPROVAL



UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

Dr Samuel M. Wainaina
C/o Dept. of PHPT

REF:FVM BAUEC/2018/168
20/09/2018

Dear Dr Wainaina,

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

Ethnobotanical, antioxidant and toxicity study of selected medicinal plants used in Nyamira North Sub-County, Nyamira County


By Wainaina Samuel Murigi (J56/89125/2016)

We refer to the above MSc proposal that you re-submitted to our committee for review and approval. We have now reviewed the proposal and have noted that you have justified the proposed numbers of animals to be used, that animals will be treated humanely observing early end-points for experiments as necessary and that general good husbandry will be practiced. We require you to work closely with your supervisors, who are Veterinarians, in the care of animals and their treatment.

We hereby approve your work as detailed in your revised proposal.

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

APPENDIX II: APPROVAL OF RESEARCH PROPOSAL



**UNIVERSITY OF NAIROBI
GRADUATE SCHOOL**

Telephone: 3318262
Fax Number: 243626
Telegrams: "Varsity of Nairobi"
E-mail: gs@uonbi.ac.ke
Our Ref: J56/89125/2016

DEPT. OF PUBLIC HEALTH
PHARMACOLOGY & TOXICOLOGY
20 JUN 2018
RECEIVED
C.A.V.S.

P. O. Box 30197 - 00100
NAIROBI, KENYA

18th June, 2018

Dr. Samuel Murigi Wainaina
C/o Dept. of Public Health, Pharmacology and Toxicology
FACULTY OF VETERINARY MEDICINE, CAVS

Dear Dr. Wainaina,

RESEARCH PROPOSAL AND SUPERVISORS

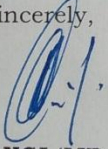
This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled "Ethnobotanical, Antioxidant and Toxicity Study of Selected Medicinal Plants Used in Nyamira North Sub-County, Nyamira County, Kenya."

She has also approved **Prof. James M. Mbaria** and **Dr. Laetitia W. Kanja** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination on or before November, 2018. The Guidelines on Postgraduate Supervision can be accessed on our website (www.gs.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

This letter precedes our earlier letter dated 14th June, 2018.

Yours sincerely,



B. MWANGI (MR.)
FOR: DIRECTOR, GRADUATE SCHOOL

cc Dean – Faculty of Veterinary Medicine
 Chairman – Department of PHPT
 Prof. James M. Mbaria – C/o Department of PHPT
 Dr. Laetitia W. Kanja – C/o Department of PHPT

BM/lwk

*Mfed
File*


APPENDIX III: PLANTS NOTES COLLECTION FORM

| | | |
|-----------------------|-------------------------------|-----------------------|
| Dr Samuel M. Wainaina | MSc Pharmacology & Toxicology | University of Nairobi |
|-----------------------|-------------------------------|-----------------------|

| | |
|---|---|
| <p>Herbarium Plant Notes.</p> <p>1</p> <p>Plant Name:</p> <p>Vern. Name Egetuke/Rigeri rike</p> <p>Locality: Mageri, Mageri SDA Church GPS: 0° 31'35.4"N, 34°56'44.2"E Elevation: 106.7</p> <p>Habitat: Small hill</p> <p>Description: Green Leaves with spines, no stem, purple flowers.</p> <p>Economics: Medicinal</p> <p>Frequency: Rare</p> <p>Date: 18/02/2018</p> | <p>Herbarium Plant Notes.</p> <p>3</p> <p>Plant Name:</p> <p>Vern. Name Rigeri rinene</p> <p>Locality: Mageri, Mageri SDA Church GPS: 0° 31'35.4"N, 34°56'44.2"E Elevation: 106.7</p> <p>Habitat: Small hill</p> <p>Description: Green Leaves with spines, many stems, purple flowers. Grows 0.5-1m high.</p> <p>Economics: Medicinal</p> <p>Frequency: Rare</p> <p>Date: 18/02/18</p> |
|---|---|

| | |
|--|--|
| <p>Herbarium Plant Notes.</p> <p>2</p> <p>Plant Name:</p> <p>Vern. Name Rigeri ria gati</p> <p>Locality: Ng'oina forest/Ng'oina esatate GPS: 0° 31'13.0"N, 35°4'15.5"E Also found in Mageri</p> <p>Habitat: Grassland</p> <p>Description: Grey leaves with spines, yellow flowers. Single stem (0.5-1m long)</p> <p>Economics: Medicinal</p> <p>Frequency: Rare</p> <p>Date: 18/02/18</p> | <p>Herbarium Plant Notes.</p> <p>4</p> <p>Plant Name:</p> <p>Vern. Name Omosamba igoro</p> <p>Locality: Nyagwekoa sub-location, Etono Primary school Etono Dispensary GPS: 0° 30'42.6"N, 34°57'16.9"E</p> <p>Habitat: Hillside</p> <p>Description:</p> <p>Economics: Medicinal</p> <p>Frequency: Rare</p> <p>Date: 18/02/18</p> |
|--|--|

APPENDIX IV: LETTER OF ACKNOWLEDGEMENT OF NOTICE OF INTENT TO SUBMIT THESIS


**UNIVERSITY OF NAIROBI
GRADUATE SCHOOL**

Telephone: 3318262
Fax Number: 243626
Telegrams: "Varsity of Nairobi"
Email: gs@uonbi.ac.ke

P. O. Box 30197,
NAIROBI, KENYA

10th November, 2018

Our Ref: J56/89125/2016

Dr. Samuel Murigi Wainaina
C/o Chairman,
Department of PHPT
CAVS

Dear Dr. Wainaina,

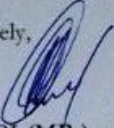
NOTICE OF INTENT TO SUBMIT YOUR MSC. THESIS

We write to acknowledge receipt of your notice of intent to submit your M.Sc thesis dated 29th August, 2018 entitled; **"Ethnobotanical, Antioxidant and Toxicity Studies of Selected Medicinal Plants in Nyamira North Subcounty ."** We also wish to acknowledge receipt of the abstract of the thesis. Please submit four (4) copies of the thesis to the Director, Graduate School

In addition, you should run and submit an anti-plagiarism test on your thesis whose similarity index should be 15% and below.

We look forward to receiving your thesis within three (3) months from the date of this letter.

Yours sincerely,



B. MWANGI (MR.)
FOR: DIRECTOR, GRADUATE SCHOOL

cc. Dean, Faculty of Veterinary Medicine
Chairman, Department of PHPT

Please Note:- Submission of the student's four (4) copies will be subject to submission of the Board of Examiners by the Dean/Director and approval of the same by Graduate School.

BM/twk

APPENDIX V: PLANT IDENTIFICATION REPORT



27/02/2018

REF: NMK/BOT/CTX/1/2

Samuel M. Wainaina

Mount Kenya University

Tel. 0721 -790130

Nairobi - Kenya

Dear Sir,

PLANT IDENTIFICATION

The plant specimens you brought to us for identification from the field have been determined as follows:

- Sample 1 – *Carduus chamaecephalus* (Vatke) Olive & Hiern (**Family: Asteraceae**)
Venacular Egetuke (Kisii)
- Sample 2 – *Berkheya spekeana* Oliv. (**Family: Asteraceae**)
Venacular Rigeri ria gati (Kisii)
- Sample 3 – *Cirsium vulgare* (Savi) Ten. (Family: Asteraceae)
Venacular Rigeri rinene (Kisii)
- Sample 4 – *Clutia abyssinica* Jaub. & Spach (Family: Euphorbiaceae)
Venacular Omosambaxa igoro (Kisii)
- Sample 5 – *Rhamnus staddo* A. Ritch. (**Family: Rhamnaceae**)
Venacular Omonguroro (Kisii)
- Sample 6 – *Tylophora anomala* N.E. Br. (Family: Asclepiadaceae)
Venacular Omochibara (Kisii)

APPENDIX VI: FIELD QUESTIONNAIRE

UNIVERSITY OF NAIROBI.

Personal data:

Name of Traditional medical practitioner (TMP) (Optional)

Phone Number _____

Address _____

Phone Number (Optional) _____

Address (Optional) _____

Nyamira north Sub county wards: Magwagwa [] Bokeira []
Ekerenyo [] Itibo ward [] Bomwagamo [] (Tick appropriately)

1. Age: 15- 25 [] 26-35 [] 36-45 [] 46-55 [] >55 [] (Tick appropriately)

2. Gender: Male [] Female []

3. Literate : Yes [] No []

If yes- Highest qualification (Tick appropriately)

Primary []

Secondary []

Tertiary []

University []

4. Resident Type: (Tick appropriately)

Rural []

Semi urban []

Urban []

5. Occupation

(a) Exclusively medical practitioners []

(b) Farmer []

(c) Government employ []

(d) Others

(specify) _____

6. Number of years of practice: <1 [] 1-5 [] 5-10 [] 11- 19 []

20-40 [] > [] 40 (**Tick appropriately**)

7. How did you acquire the knowledge? []

(a) From family member (Hereditary) []

(b) Apprenticeship (Staying with TMP) []

(c) Training in organized organization (school, church etc.) []

(d) Others

(Specify) _____

8. You started practicing at age

(a) Below 20 years []

(b) Between 20 and 30 years []

- (c) Between 31 and 40 years []
- (d) Between 41 and 50 years []
- (e) Over 51 years []

Disease information

9. Which disease do you treat in order of patient frequency (Tick

| appropriately) | Most frequent | Frequent | Least frequent |
|-----------------|---------------|----------|----------------|
| Cancer | [] | [] | [] |
| Arthritis | [] | [] | [] |
| Epilepsy | [] | [] | [] |
| Diabetes | [] | [] | [] |
| Hypertension | [] | [] | [] |
| Memory loss | [] | [] | [] |
| Others, Specify | | | |

10. What was the type of medication

Plant

Animal material

Minerals

Others,

Specify _____

Disease and Medicine information (Tick appropriately)

11. Prevalence of ailments among patients seen by the herbalists.

| Sno. | Common name | | Signs and Symptoms | Rate (1-3) |
|------|-------------|--------------------|--------------------|------------|
| | Local name | Medical equivalent | | |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |
| 7 | | | | |
| 8 | | | | |

12. What are the traditional medicines and methods that are used to manage the above named ailments?

| S no. | Ailment | Plant/Medicine (s) | Plant Part | How it is prepared | Use | |
|-------|---------|--------------------|------------|--------------------|------------|----------|
| | | | | | Preventive | Curative |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |

Others, Specify _____

13. Where do your patients come from?

(a) Referral from hospitals []

(b) Referral from another Traditional medicine practitioner []

(c) Referral from other patients/relative/friend []

(d) Non referrals []

14. How do you diagnose the disease?

(a) Hospital reports []

(b) Patients self-diagnosis []

(c) Personal diagnosis (TMP) []

APPENDIX VII: INFORMED CONSENT

INFORMED CONSENT FORM

Title of Study: ETHNOBOTANICAL SURVEY OF PLANTS USED FOR MANAGEMENT OF OXIDATIVE STRESS-RELATED DISORDERS IN NYAMIRA NORTH SUB-COUNTY OF NYAMIRA COUNTY, KENYA.

PART A: Background.

We are carrying out a study on the indigenous plants used by the herbalist in Nyamira North sub-county in Nyamira County. This is because over the years, plants have been used by the herbalist on Gusii people to treat many ailments including those which science has proved that they could be as a result of deleterious effects of reactive oxygen species and reactive nitrogen species. Some of those ailments include Joint aches and inflammations, increased high blood pressure, High blood glucose levels, problems of the blood vessels and the related tissues of the circulatory system, loss of memory, asthma and many others. Even though there are many reports by herbalist that these plants have been successful in ameliorating the diseases, there is a need of scientific validation of these claims.

You are therefore humbly invited to take part in this research whose primary aim is to discover statistic information of herbalist and herbal remedies they use for treating ailments which are associated with oxidative stress phenomenon in Nyamira North Sub-
province of Nyamira County.

Kindly peruse this document carefully and pose any inquiries you may have before consenting to take part in the study.

Aim of the Research

To discover statistic information of herbalist in the area of study and the plants species they use as herbal remedies Oxidative stress-associated diseases.

Study Procedure.

Upon your consent of participating in the study, you will be requested to answer questions on personal information, education, occupation and your traditional herbal practice. You will likewise be asked to make reference to some indigenous natural medication you utilize to treat oversee oxidative stress.

Risks of involvement in the research

There are no foreseeable risks associated with this study that can be harmful to you. However, everything about of procedures will be a clearly explained to you by the lead researcher and you will be free to ask for clarification about any aspect of the research that is not clear.

Benefits of Participation

If there be any intellectual property concerns that may arise in the cause of this undertaking, the intellectual property policy of the University will be used to handle it amicably. There shall not be any monetary benefits inform of payment or inducements.

Confidentiality

All the information obtained from you will be treated with utmost confidentiality and that it will not be made available to any other person or entity. Your personal information will not be used in this study nor in any other publications or presentations.

Contacts and Questions

The lead researcher of the study is **Samuel Murigi Wainaina**. Feel free to ask any question of importance to you now or at any the other time of your convenience. You can reach the lead researcher through telephone number: +254721790130.

In case you may prefer to raise any concern regarding the study to another person other than the lead researcher, kindly get in touch with the following:

Supervisor

Prof. James M. Mbaria

University of Nairobi

Chairman Department of Public Health, Pharmacology & Toxicology

Faculty of Veterinary Medicine.

Mobile. +254 722 639 977

Email: jamesmbaria@uonbi.ac.ke

Jared Misonge Onyancha

Mount Kenya University

Research Centre, Research Directorate.

Mobile +254 720 457 987

Email onyancha.jared@ku.ac.ke

PART B: Declaration of the Participant.

I, hereby give consent to assist in the research **ETHNOBOTANICAL SURVEY OF PLANTS USED FOR MANAGEMENT OF ARTHRITIS IN NYAMIRA NORTH SUB-COUNTY OF NYAMIRA COUNTY, KENYA.**

I have read and understood what the study is all about and I agree to the request placed before me to participate in the study. The dangers and advantages have been disclosed to me. Any inquiries I have concerning the research work have been adequately addressed and all explanations are agreeable to me. I have been made aware that I can pull back from the study anytime I wish and without giving any reasons.

Participant's Name.....

Sign..... Date.....

Lead Researcher.....

Sign.....Date.....

APPENDIX VIII; PHOTOS OF SURVEY IN THE FIELD



Dr. Wainaina and TMPs in the field



***Cirsium Vulgare* (Rigeri rinene)**

APPENDIX IX; PHOTOS OF ANTI-OXIDANT AND PHYTOCHEMISTRY



Concentration of methanolic extracts



Phytochemical screening



Preparation of doses for acute toxicity tests