ETHNOBOTANICAL STUDY OF PLANTS USED IN TRADITIONAL MEDICINE AND AS BIOPESTICIDES IN MERU CENTRAL, KENYA AND PRELIMINARY TOXICOLOGICAL EVALUATION OF *TEPHROSIA VOGELII*

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DECLARATION

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

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DEDICATION

This work is dedicated to my wife Zipporah Karea Mwebia

and

My Children Maureen Gatwiri, June Gacheri and Ignatius Kirimi

and

My parents Mr.Martin M'Itonga and Mrs Rosaria M'Itonga

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LIST OF ACRONYMS AND ABBREVIATIONS

AIDS -Acquired Immuno Deficiency Syndrome

BSLT- Brine Shrimp Lethality Test

CNS-Central Nervous System

E.C.F-East Coast Fever

EM- Ethnomedicine

EVM- Ethnoveterinary Medicine

GIT- Gastrointestinal Tract

gms-Grammes

GOK – Government of Kenya

HIV- Human Immuno Deficiency Virus

LARMAT- Land Resource Management and Agricultural Technology

LD_{50}- Median Lethal Dose

mg-Milligrammes

mls-millilitres

NGO- Non Governmental Organization

PHC-Primary Health Care

TK- Traditional Knowledge

UON- University of Nairobi

WHO-World Health Organization

ABSTRACT

An ethnobotanical survey was carried in July 2009 at Igane and Gatuune sub locations, Abothugushi East division of Meru Central district, Kenya. This was done using semi-structured questionnaire and focused group discussion to obtain information from traditional healers and farmers. The focused group discussion also involved the research team and local administrators. The data obtained was on identity of plants, their uses, availability, part used and methods of application. The healers and farmers identified the plants in the area by their local names and the samples were collected for botanical identification at the University of Nairobi and National Museum of Kenya.

Among the plants mentioned by the herbalists during the interview, *Tephrosia vogelii* was ranked number one and this formed the bases for its selection for both Cytotoxicity and acute toxicity tests.

The results of the ethnobotanical survey revealed that herbalist belong to both genders with majority being males (82.6%) and the rest females (17.4%). Their ages ranged from 28-82 years. Eighty five (85) plant species belonging to 37 families were identified as being used as medicinal or biopesticides. The families encountered were Euphorbiaceae (10.59 %), Papilionaceae (8.24%), Compositae (8.24%), Labiteae (7.06%), Rubiaceae (7.06%), Caesalpiniaceae (5.88%), Rutaceae (4.70%), Apocynaceae (4.70%), Liliaceae (3.52%), Verbenaceae (2.35%), Flacourtiaceae (2.35%), Moraceae (2.35%), Myrtaceae (2.35%), Bignoniaceae (2.35%), Graminae (2.35%) and the rest of the families (22) each had 1.18%. The medicinal plants were used to treat and manage parasitic, microbial, anthelmintic, protozoal as well as metabolic diseases.

Bioactivity of *Tephrosia vogelii* extract was tested using brine shrimp lethality test. Acute toxicity of the same plant extract was studied in inbred albino weaned female rats by determination of Median Lethal Dose (LD50), symptoms of toxicity and complete histopathology. The median lethal dose was determined using the method described by Reed and Muench (1938). A total of 58 rats were used. Routine standard pathological procedure was used in the pathological studies.

The important use of plants as biopesticides was found to be the control of rodent moles by use of *Tephrosia vogelii*. It is planted in the farms to control invasion of moles. Pharmacological and toxicological study of the plant showed that it is lethal to rats with an LD_{50} of 3163mg/kg bodyweight. The clinical signs of toxicity were starry coat, anorexia, arched back, restlessness, dyspnoea, salivation, initial excitation, convulsions, incoordination of gait and paralysis of hind limbs prior to death.

Gross pathology of the dead and sacrificed experimental animals showed lesions in the lungs, kidney, liver and spleen where some areas were dark red in colors than normal and were highly congested. Histopathology, showed that some neurons were affected and appeared abnormally enlarged, with shrunken or no nucleus and in various stages of necrosis and degenerative changes. The LC_{50} of *Tephrosia vogelii* in Brine Shrimp Lethality Test was found to be 123µg/ml with 95% confidence interval of 46-262 µg/ml. The study shows that Meru Central district has a rich biodiversity with numerous medicinal plants and plants with biopesticidal activity. The results of the current study support the use *Tephrosia vogelii* to control moles in the farm since it was shown to cause paralysis of the rodents and damages the brain. This indicates that the plant obtained from

Meru has ingredient responsible for rodenticidal effects. In conclusion, Meru Central district is rich in biodiversity of medicinal plants and it is in the custody of older generation. In addition, medicinal plants are faced with threats of extinction.

This study recommends the need to conduct country wide ethnobotanical survey and document all the useful plants used as medicinal and biopesticides and come up with the national policy on their sustainability and conservation strategies. Herbalist need to form a registered herbalist association. Farmers should be encouraged to use *Tephrosia vogelii* to control invasion of rodents in their farms since the plant has been shown to have rodenticide activity in the current study. Further research is needed to scientifically validate the herbalists' claims on efficacy and safety of ethnobotanicals documented. This should also include their Phytochemistry.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Traditional medicine has been in existence from the time immemorial as major African socio-cultural heritage (Okigbo and Mmeka, 2006). The Traditional Medicine has been the focus for wider coverage of primary health care delivery in Africa and the rest of the world (Elujoba *et al.,* 2005). The World Health Organization (WHO) estimates that around 80% of the population in Africa use traditional medicines and about 85% of traditional medicine involves use of plant extracts (Farnsworth and Soejarto, 1985a; Hack-Seang. 2005).

The Traditional Medicine Programme of WHO defines Traditional medicine (TM) as the sum total of all knowledge and practice, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing (WHO. 2002b). Inclusion of TM in national reproductive health and AIDS management programmes has been advocated by WHO (UNAIDS, 2006).

Many communities in Kenya rely on a wide range of indigenous practice to manage human and animal diseases and conditions (Gathuma *et al.,* 2004; Githiori, 2004; Kokwaro, 1993; Miaron *et al.,* 2004). Plants have been used in traditional medicine for several thousand years (Abu-Rabia, 2005). In Africa, traditional healers (TH) using remedies made from plants play an important role in health of millions of people (Kala *et al.*, 2004).

Medicinal plants form the major source of traditional medicine. Sumerians used herbs or plants such as laurel and thyme for medical uses for over 5000 years. Ancient Egyptians in 1000 BC are known to have used garlic, opium, castor oil, coriander, mint indigo and other herbs (Falodun, 2010). Herbalism is the Traditional Medicine (TM) or folk medicine practice based on the use of plant and plants extracts (WHO, 1977). The herbs produce a wide range of chemical substances that act on the body and produce physiological responses forming the basis of their use. Since new drugs are often not affordable, about 80% of the population uses medicinal plants as remedies (Kirby, 1996; Hostettmann and Marston, 2002).

Many plants synthesize substances that are useful for maintenance of health in human and animals. Drugs derived from natural products are important to modern pharmaceutical industry e.g. aspirin, morphine, digoxin, quinine, ergometrine, reserpine and atropine (Samuelsson, 2004). The first ever antimalarial, quinine, was derived from the bark of the South American Cinchona tree. The morphine and codeine were derived from poppy and digoxin from the foxglove plants (Kutchan, 1995). Medicinal plants and drugs derived from them are of economic and strategic value for African continent.

Increase in Africa's population and higher demand for TM has led to depletion of natural resources due to loss of habitat. Increased commercialization of medicinal plants has resulted in increased demand, over harvesting and in some cases, near extinction of some valued indigenous species. Other factors that have stimulated the rise in demand includes rapidly and urbanizing population, the affordability, accessibility and acceptability of TM

over the western (conventional) medicine and the high rate of unemployment and low level of formal education especially in rural areas. Harvesting and provision of medicinal plants to meet the demand has thus become an environmentally destructive activity (Cunningham, 1997; 1988). This may result in many medicinal plants and other genetic materials becoming extinct before they are even documented (Rukangira, 2001). Use of traditional medical knowledge of medicinal plants and their use by indigenous cultures are useful for conservation of cultural traditions and biodiversity and also for community healthcare and drug development in the present and future (Pei, 2001).

Kenya is endowed with diverse range of plants, many of which are used by indigenous people to make infusions, decoctions and other traditional dosage forms for treatment of various ailments. The use of herbal remedies is mainly in Ethnoveterinary Medicine (EVM) and Ethnomedicine (EM) practiced in the Traditional Medicine systems (Bussmann, 2006; Miaron, 2003; Wanyama, 1997, 2000). In Kenya only a few medicinal plants have been evaluated for biological activities but many of medicinal plants are being destroyed or cleared to give way for human settlement and agricultural activities. Therefore, there is urgent need to evaluate these plant species biologically before they become extinct (Schweitzer *et al.*, 1991). Herbal remedies are available for treatment and control of helminthiasis, bacterial, protozoan, viral and other diseases caused by microorganisms (Chopra *et al.*, 1982; Koko *et al.*, 2000; Kokwaro, 1993).

In Meru Central region of Kenya, traditional use of medicinal plants is very important. However there is scanty information and documentation. *Tephrosia vogelii Hook f* plant is traditionally used as rodenticide and insecticides. These plant species are planted one metre apart round the farms to keep off the moles (*Tachyoryctes splendens*) invading the

farms. The current study is aimed at documenting the use of ethnobotanicals in Meru region and generating scientific data on bioactivity and toxicological aspects of *Tephrosia vogelii* since inadequate information has been reported of its toxicity. However, its use needs scientific validation. This study was intended to generate information on acute toxicity including symptomatology and LD_{50} determination. The study also investigated the bioactivity of *Tephrosia vogelii* by using Brine Shrimp Lethality Test (Meyer *et al.*, 1982).

1.2. Research hypothesis

It was hypothesized that Meru Central district is rich in biodiversity of plants used as medicinal and biopestides. It was also hypothesized that the *Tephrosia vogelii* extract had significant toxicity to rodents and has the potential for use in control of moles in the farms.

1.3. Objectives of the study

1.3.1 General objective:

To document the use of ethnobotanicals in Meru regions of Kenya and generate toxicological data on *Tephrosia vogelii*.

1.3.2 Specific objectives:

1. To document of plants used in ethnoveterinary and ethnomedical practices in Meru region of Kenya and the traditional dosage forms used.
2. To determine the cytotoxicity of methanol extracts of *Tephrosia vogelii* leaves using brine shrimps.

3. To determine the acute toxicity of methanol extracts of *Tephrosia vogelii* leaves in rats.

1.4. Justification

Meru Central District has slightly over 160 health facilities that are spread all over the district. There is a problem of accessibility of health facilities since the average distance to the nearest health facility is 7 km. There is only 1 doctor for every 33,259 patients (Meru Central District Strategic Plan 2005 - 2010). This implies, that majority of people get their Primary Health Care (PMC) from traditional herbal medicine. The medicinal plants in the area are being depleted at a very fast rate and therefore there is need to collect, document and scientifically validate the biopesticidal and medicinal plants used in the area before they become extinct.

Rodent pests are considered a major problem in agriculture and public health (Makundi *et al.*, 1999) because they cause considerable economic losses in staple crops, mainly tuber crops and cereals. This economic loss to the food crops leads to decreased food security. Traditionally farmers have used *Tephrosia vogelii* to control moles. Compared to traditional biopesticides, conventional pesticides are expensive and toxic to humans, livestock and environment. Biopesticides are cheaper, safer, biodegradable and readily available and will offer alternative to conventional pesticides. Herbal remedies used for hundreds of years can be put to commercial use, but scientists are demanding that traditional knowledge should be validated, to verify the safety and efficacy of the treatments (Mathias and Mccorkle, 2004).

CHAPTER TWO

LITERATURE REVIEW

2.1 Background information on medicinal and biopesticidal plants

Traditional knowledge has been used to cater for health problems involving humans and animals worldwide (Dery *et al.*, 1999; Rukangira, 2001). Medicinal plants in form of fresh or dried part, whole, chopped, powdered, aqueous extract or organic solvent plant extract constitute major area of traditional medicine (Mukherjee, 2002).

Research on the chemical constituent of the plants and pharmaceutical screening may lead to development of new drugs derived from plants (Olatunji and Atolanii, 2009). Thousands of pure chemical substances extracted from higher plants are used in medicine throughout the world (Farnsworth and Soejarto, 1985). Some of the lives saving drugs in modern medicine have originated from these plants (Mohammad, 2010). More than 60,000 plant species are used for various purposes all over the world according to F.A.O. (1991).

Advantages associated with usage of ethnobotanical remedies include low cost, ease of accessibility and ability to treat and cure certain diseases which would not be cured by conventional medicine (Sindiga *et al.,* 1995). The disadvantages of the ethnobotanicals are that plant medicines are hard to standardize, cumbersome to prepare, large doses may be needed and preparation and dosages for the same remedy often vary greatly. The

required natural material may also not be available throughout the year and the pharmacological active ingredients vary with season, harvest time, maturity and other factors (Sindiga *et al.,* 1995).

Worldwide, 24,000 (6%) amongst plant species have been investigated for bioactivity while 60,000 (15%) have been studied for Phytochemistry (Cragg *et al.,* 1997). This therefore calls for pharmacological and toxicological evaluation and biological activities of herbal medicine (Olatunji and Atolani, 2009). In the developed countries, 33% of drugs are produced from higher plants (Mwangi, 2004). Plant materials contain thousand of chemicals which act against diseases and infections of humans and animals when properly used (Gulfraz *et al.,* 2006). Many of conventional medicines have been developed on the basis of traditional medicines though some of their modes of action have only been identified recently (Verpoorte and choi, 2006).

Developing countries worldwide continue to rely heavily on the use of traditional medicines as their primary source of healthcare. Ethnobotanical studies carried out throughout Africa confirm that native plants are the main constituent of traditional African medicines (Hedberg, *et al.*, 1983a; 1983b; 1982, Kokwaro, 1976; Oliver, 1987). Medicinal plants are now being given serious attention, as is evidenced by the recommendation given by the World Health Organization in 1970 that proven traditional remedies should be incorporated within national drug policies in a move towards greater professionalism within African medicine (Last and Chavunduka, 1986).

World Health Organization (WHO) estimates that about 80% of the world population depends on medicinal plants for their health care needs, and more than 30% of the pharmaceutical preparations are based on plants (Gulfraz *et al.,* 2006). In Tanzania

medicinal plants are widely used and they constitute a potentially useful resource for new and safe drugs for treatment of opportunistic infections (Mainen *et al.*, 2007). Five out of every six HIV patients receive their medical attention from traditional healer rather than from a hospital or primary health care facility (AIDS Analysis Africa, 1996).

Kenyan plants used in traditional herbal medicine are showing promising medicinal properties in their ability to treat diseases such as herpes and malaria (Ochieng, 2004). This may be due to the facts that the traditional healers are less expensive than medical doctors and are reliable. There are also few medical doctors in the rural areas where most of the population lives. In Kenya, the national policy and regulation of herbal medicine is yet to be finalized. Moreover product packaging, dosage determination and standard prescription intended to protect the public health are yet to be practiced in traditional medicine. Healers need to learn on how to prepare medicine hygienically and package them conveniently for proper storage (Ehsan, 2005).

In Kenya, medicinal plants are becoming harder to find in their natural habitants. Kenya's rapid decline in its medicinal plant resources is not only affecting the health of millions of people, it is also reducing incomes and diminishing age old customs. The advent of HIV/AIDS has led to more people turning to plants for their curative properties and for nutrition since some of them are rich in micronutrients. Indiscriminate and over harvesting of medicinal plant species has made these plants rare and sparse in many parts of the country (Hassan *et al.*, 2010). Sustainable approaches including proper harvesting methods, cultivation and capacity building for community based conservation of medicinal plants are very important.

Some plants are used both as biopesticides and herbal medicine depending on the dosage and part of the plant used among other factors. Some plants have also been used as antidote of some poisoning or as antivenin in case of venomous snake bites (Owuor and Kisangau 2005; Kisangau, 1999).

2.2. Importance of Traditional Medicine.

Traditional knowledge on the health of humans and animals has existed in all countries (Rukangira, 2001). TM has been described as one of the surest means to achieve total health care coverage of the world's population (Adenike *et al.*, 2007; WHO, 1978a). The interest in traditional knowledge is more and more widely recognized in development policies, the media and scientific literature. Despite the marginalization of traditional medicine practiced in the past, the attention currently given by governments to widespread health care application has given a new drive to research, investments and design of programmes in this field in several developing countries.

The important role played by traditional healers and remedies is revealed by the relative ratios of Traditional Practitioners and University trained doctors. In Ghana, for example, in Kwahu district, Traditional practitioner to people ratio is 1:224 against University trained doctor to people ratio is 1:21,000 (WHO, 2002a). In Meru Central district, Kenya doctor to patient ratio is 1:33,259 and there is a problem of accessibility of health facilities since the average distance to the nearest health facility is 7 km (Meru District Strategic Plan 2005 – 2010). Traditional herbalists come in handy to offer health services

2.3 Evaluation of traditional medicine

Lack of scientific evidence regarding effectiveness of traditional medicine limits its use (Rukangira, 2001). Evaluation of traditional medicine should be preceded and guided by information-gathering on purported efficacy and safety. Observational studies should be conducted to generate further information on safety and assess preliminary indicative efficacy (WHO, 2002b). New practical and acceptable research tools need to be developed in accordance with the minimum regulatory requirements that WHO has developed, for registration and use of traditional medicines in Africa with respect to quality, safety, and efficacy (WHO, 2002b). Results should be shared with the primary beneficiaries of the studies as well as disseminated to the community and other stakeholders. Accessibility to the traditional treatment should be ensured by using local ingredients. Their processing and packaging should be in a low-cost form suitable for ease of administration and distribution (Jaco *et al.*, 2004).

2.4. Status of the medicinal plants base resource

Vast resources of medicinal and aromatic plants are found in most developing countries (Jacob *et al*., 2008). In developing countries majority of people live in rural areas where they continue to use these plants for their welfare (Cunningham, 1997). Despite ethnobotanical literature being well documented, there is very little scientific information such as efficacy and Phytochemistry on indigenous medicinally used plants (Van Vuuren, 2008).

The demand of the majority of the people in developing countries for medicinal plants has led to indiscriminate harvesting (Augustino and Gillah, 2005). This has resulted in many plant species becoming extinct and some endangered (Chandra, 1999). Numerous medicines have been derived from the knowledge of tropical forest people. There is therefore the need to conserve biodiversity and protect threatened species by introducing systematic cultivation of medicinal plants necessitated by continuous demand for the raw materials (FAO, 1997).

In Africa 1%, that is 2.2 million hectares of closed forest are lost annually due to deforestation. Africa has one of the highest rates of deforestation in the world (Achard *et al.*, 2002). This threatens the loss of plant resources, traditional community life, cultural diversity and the accompanying knowledge of the medicinal value of several endemic species (Ragunathan and Abay, 2009; UNESCO, 1994). Thus many of the medicinal plants and other genetic materials will become extinct before they are even documented (Moran, 2000). In order to save the world's plant resources, more protection and management, research, and an increasing level of public awareness about our vanishing heritage need to be established (Balick, *et al.*, 1996).

2.5. Active ingredients in the plants

The rationale for use of plant in medicine and in pest control is because plants synthesize chemicals with bioactivity. Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavors, fragrances, proteins, amino acids, bioactive peptides, phyto hormones, sugar, flavonoids and biopesticides (Gulfraz *et al.*, 2006). Most plants contain Oils (fats), Alkaloids, Glycosides, Benzoquinones, Toxalbumins and Anthraquinones cathartics (Kokwaro, 1993). Gulfraz *et al.*, (2006) extracted and purified

various organic compounds in selected medicinal plants of Kotli Sattian, District Rawalpindi, Pakistan. Natural products perform various functions and many of them have interesting and useful biological activities (Galal *et al.*, 1991; Koshy *et al.*, 2009).

2.5.1. Oils

Oils are divided into fixed oils, essential oils and sulphur oils. Fixed oils, fats, waxes, phosphatides and lecithins are members of the lipid group. Lipids are often a main constituent in drugs, separated by expression from the crude vegetable (plant) matter and presented as drugs in the refined state. Plant seeds are the largest source of lipids (Kokwaro 1993).

2.5.1.1. Fixed oils

Fixed oils consist of molecules of fatty acids which form some kind of salt or ester with one molecule of glycerine. Some of the plant species containing fixed oils are Annona, Balanites, Trichilia, and Euphorbiaceae like Castor oil plant *(Ricinus communis)* and Croton species. Balanites and Trichilia species are used as ointment bases and emollients while *Ricinus communis* and are used as purgatives. Resin oils are extremely irritating and Croton species some of them cause vomiting to purging if taken in large doses (Evans, 2005). Some are poorly absorbed from gut and are used as vermifuge for round worms and hookworms. Purgative is a drug/substance that causes increased intestinal motility resulting in expulsion of intestinal contents (Kokwaro, 1993).

2.5.1.2 Volatile/essential oils

Volatile (essential) oils regulate intestinal movement, prevent or control violent contraction of gastrointestinal tract (GIT) and assist in flow of food through the bowel

and are therefore used for gastrointestinal disorders. They also inhibit bacterial growth and can therefore be used for treatment of infections. Less absorbed essential oils like those contained in Chenopodium species of plants have anthelmintics activity. Volatile oils are usually responsible for the odor of a plant. They can contain hundreds of constituents, the highest of which are terpenes, hydrocarbons, alcohols and aldehydes. Therapeutically, volatile oils have many uses. They can serve as a mode of transportation, to distribute medicine equally throughout the body. They can act as antiseptics. Volatile oils tend to stimulate tissues they come in contact with; hence they can be rubefacients, counter-irritants and/or vasodilators. Internally, volatile oils may cause an increase in saliva, perspiration, peristalsis, and/or stimulate the heart muscle (Abena *et al.*, 2007; Cimanga *et al.,* 2002).

2.5.1.3 Sulphur oils

The sulphur oils contain sulphur and are very irritating and cause reddening to vesication of the skin. They are carminatives in small doses and emetics in high doses. Carminatives are drugs that cause expulsion of gases from stomach when administered orally. Plants containing such oils include Capsicum species, Salvadora species, Cruciferae species, Capparis species and Cleome species (Kokwaro, 1993).

2.5.2 Alkaloids

Alkaloids are natural, organic substances that are predominantly found in plants and normally contain at least one nitrogen atom in their chemical structure. The first alkaloid, morphine, from the opium poppy (*Papaver somniferum*) was identified in 1806, and more than ten thousand alkaloids have been isolated from plants (Kutchan, 1995).

From the time immemorial, alkaloid-containing plant extracts have been used in all cultures as potions, medicines, and poisons (Kutchan, 1995). In modern times, the stimulants caffeine in coffee, tea, and cacao and nicotine in cigarettes are consumed worldwide (Falodun, 2010). Alkaloids with hallucinogenic, narcotic, or analgesic properties have found medical application as pure compounds (e.g., morphine, atropine, and quinine) or served as model compounds for modern synthetic drugs, while several are abused as illicit drugs (e.g., cocaine). Other alkaloids are too toxic for any therapeutic use (e.g., coniine and strychnine), but plant constituents are still screened for new, biologically active compounds. Gulfraz *et al.*, (2006) found that *Berberis lyceum* Royle have alkaloids present in the leaves and fruits whereas they accumulated in roots and leaves of *Justica adhatoda* L. Initial studies on alkaloids from Lombok medicinal plants, representing 49 families and 80 genera; revealed that 23% of the medicinal plants investigated tested positively for alkaloids (Surya and Bremner, 2001).

2.5.3. Glycosides (Tannins glycoside and cardiac glycosides)

Glycosides consist of glucose sugars eg glucose or rhamnose attached to an aglycone moiety and an unsaturated lactone ring which is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes. Cyanoglycoside a toxin in cherry pits is released only when bitten by herbivores. Glycosides have vast medicinal applications as they are found in almost every therapeutic class. Alcohol glycosides have been used as antirheumatics and analgesics. Salicin, from Salix species, is the glycosidic precursor that is transformed to salicylic acid in our bodies. Salicylic acid was synthesized to be aspirin (Bolan and Steele, 1968).

Anthraquinone glycosides have laxative actions. Flavonoid glycosides are yellow pigments in flowers and plants which have demonstrated anti-inflammatory, anti-allergic effects, anti-thrombotic and vasoprotective properties. Medicinally, coumarin glycosides have been shown to have hemorrhagic, antifungicidal, and antitumor activities (Abena *et al.,* 2007; Cimanga *et al.,* 2002). Cardiac glycosides exert a specific action on the myocardial muscle and allay myocardial infarction. Digitalis, from the foxglove plant, is an allopathic prescription. Cyanogenic glycosides, which contain hydrogen cyanide (HCN) compounds, are toxic to unadapted farm animals and humans. Several plants such as cassava (*Manihot escaluta*), *Adenia volkensii, Phaseolus lunatus,* Sorghum contain cyanogenic glycosides in one or more parts (Maitai and Mungai, 2005). Plant species containing glycosides are Acacia species, Apocynacea species, Kigelia species and Pterocarpus species (Kokwaro, 1993).

2.5.4 Benzoquinones

Benzoquinones are yellow crystalline organic compounds with an unpleasant odor. Some plants containing benzoquinones have anthelmintic activity. Some of these reported to have anthelmintic activity includes *Embelia Schimperi, Maesa lanceolata, Myrsine africana* and *Rapanea melonophloeos* (Gathuma *et al.*, 2004).

2.5.5 Toxalbumins

Toxalbumins are protein phytotoxins that are capable of inhibiting protein synthesis. They are ribosome inactivating protein (RIP). Toxalbumins are poisonous proteins called albumins and are usually irritants in nature and mainly found in seeds of some plant species.

They can irritate mucous membranes such as those of the nose or eye and can cause violent vomiting and purging when swallowed. Immunity to the toxalbumins poison may be established by the repeated administration of small doses. The most important known toxalbumins are Ricin, Abrin and Amanita toxin. Plant species containing Toxalbumins are *Euphorbiaceae, Cassia* and *Croton species* (Pengelly, 2004).

2.5.6 Anthraquinones cathartics

These are yellow crystalline chemicals used in the manufacture of dyes. Anthraquinones cathartics may be found in plants uncombined or as glycosides. Cassia species, Aloe species, Rhamnus species and Rumex species are the most important genera containing these types of compounds. Anthraquinones are more likely to be present in the plants as glycosides owing to the variety of sugar contents and this enhances the range of the compound. Anthraquinone is used as a laxative. However prolonged use and abuse leads to melanosis (Kokwaro, 1993; Pengelly, 2004).

2.6. Development of phytomedicine

2.6.1 The first, second and third generations of plant medicine

The first generations of plant medicine were simple botanical materials employed in more or less crude form. These medicines such as Cinchona, Opium, Belladonna and Aloe were selected based on empirical evidence as gathered by traditional practitioners (Iwu, *et al.*, 1999). The second-generation phytopharmaceutical agents were pure molecules whose compounds differ from the synthetic therapeutic agent only in their origin, for example taxol from Taxus species, quinine from Cinchona and reserpine from Rauvolfia species (Iwu, *et al.*, 1999). In the development of third generation of plant medicine, the formulation is based on well-controlled double-blind clinical and toxicological studies

with phytomedicine to improve the quality, efficacy, stability and the safety of the preparations (Akerele, 1993; Petrovick *et al.*, 1999).

2.7. Methods used in pharmacological and toxicological evaluation of plants

2.7.1 Brine shrimp lethality test. (BSLT)

BSLT is used to screen medicinal plants for their biological activities (Meyer *et al.*, 1982). Most active plant constituents are toxic in high doses and therefore, evaluating their toxicity to zoological systems is an indicator of their bioactivity. Brine shrimp lethality test uses the larvae of the brine shrimp, Artemia *species*. The brine shrimp eggs hatch within 48 hours and these larvae are used to detect lethality of many plant constituents. Brine shrimp larvae have been used as a bioassay for a variety of toxic substances.

Mwangi *et al.*, (1999) used brine shrimp lethality test to screen 78 plant samples for medicinal purposes in Kenya and found that 36 of 78 samples showed toxicity to the brine shrimp (LC_{50} < 1000 µg/ml). The aim of this bioassay is to provide a frontline screen that can be backed up by more specific and more expensive bioassays once the active compound have been isolated. BSLT is predictive of cytotoxicity and pesticidal activity (Ghisalberti, 1993).

Since its introduction this *in vivo* lethality test has been successively employed for bioassay guide fractionation of active cytotoxic and antitumour agents such as trilobacin from bark of *Asimina triloba*, cis-annonacin from *Annona muricata* (Rieser *et al.*, 1996) and ent-Kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (Mongelli *et al.*, 2002). However lack of lethality using BSLT does not mean absence of biological activity. For example *Prunus africana* shows no BST lethality, but has clearly been demonstrated to

counteract benign prostate hyperplasia. Some well known drugs like atropine and phentolamine also showed no BSLT activity (Serrano *et al.*, 1996).

2.7.2. Determination of Median lethal dose (LD_{50})

This test method uses laboratory animals to determine the order of lethality of plant extract of known doses administered to the laboratory animals after which those animals are observed for 24 hours for any death and symptomatology of the poisoning. Median Lethal Dose (LD_{50}) is based on the number of laboratory animals that die in a group administered with a given dose of a drug at different dose levels. The percentage of deaths at each dose level is used to calculate the LD_{50} (Reed and Muench, 1938).

2.8. Uses of plants in pests control

Biopesticides are certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals. A screening for pesticidal activity of plant extracts with some known medicinal attributes could lead to the discovery of new agents for pest control (Sertkaya *et al.*, 2009). Globally there has been search for alternatives to chemical pesticides with the aim of testing the use and efficacy of natural products for pest control and crop protection (Balasubramanian *et al.*, 2008, 1997).

Larvacidal activities of five Meliaceae plants species are documented to act against *Anopheles gambiae* (Ndung'u *et al.*, 2004b). Crude methanol extracts of *Turraea wakefieldii* and *Turraea floribunda* were found to be more potent than Azadiractin against *Anopheles gambiae* (Ndung'u *et al.*, 2004a). Larvacidal effects of Neem extracts have been reported (Vatandoost and Vaziri, 2004). In Kenya. the acaricidal effects of some plants and the traditional knowledge in tick control were also documented

(Wanzala *et al.*, 2006). In Saudi Arabia, acaricidal effects of some plants extracts are also documented against *Hyalomma dromedarii* (Al-Rajhy *et al.*, 2003). Balasubramanian *et al.*, (2008) found that Neem seed extract is effective against leaf folder, aphids, jassids, fruit borer and stem borer for 1month in all crops. *Trichilia emetica* and *Azadirachta indica* have been shown to have mosquito larvacidal activity (Ngassapa *et. al.*, 1999).

2.8.1 Advantages of using biopesticides

Biopesticides are usually less toxic than conventional pesticides (Joshi, 2006). This is because they affect only the target pest and closely related organisms as opposed to the broad spectrum, conventional pesticides. They are often effective in very small quantities and are biodegradable therefore less pollution as opposed to the conventional pesticides.

2.8.2. Insecticides

Plant products have been successfully used as insecticides, insect repellent, and insect antifeedants. Pyrethroids are the most successful used plant product insecticide. The plant *Chrysanthemum cinerariaefolium* produces a class of insecticides called Pyrethrins (Wallis, 1967). The insecticidal properties of the several Chrysanthemum species were known for centuries in Asia and even today, powders of the dried flowers of these plants are sold as insecticides and anthelmintic (Mbaria *et al.*, 1998). Six terpenoid esters (pyrethrins) are responsible for the insecticidal activity of these plants.

Nicotine and nornicotine, components of several members of the genus Nicotiana, have been used commercially as insecticides. *Nicotiana rustica* is the chief commercial of this product. Other natural analogues of nicotine have also been shown to have significant

insecticidal properties. For example, anabasine or neonicotine is an insecticide from *Anabasis aphylla* plant (Krieger, 2001).

Ryanodine, an alkaloid from, *Ryania speciosa* plant has been used as a commercial insecticide against European corn borer (Krieger, 2001). Physostigmine, an alkaloid from *Physostigma venenosum* was the compound upon which Carbamates insecticides and Acaricides were developed. Preparations of roots from the genera Derris, Lonchocarpus and Tephrosia, containing rotenone were commercial insecticides in the 1930s (Clark, 1930). Larvae of several insect species, when fed these compounds and exposed to light are rapidly killed. Other photodynamic compounds such as polyacetylenes from plants are acutely toxic to insects. However, their general toxicity would probably preclude them from commercial use (Duke, 1990). Plants produce many compounds that are insect repellents or act to alter insect feeding behaviour, growth and development of ecdysis (moulting), and behavior during mating and oviposition. Most insect repellents are volatile terpenoids such as terpenen-4-ol. *Ocimum sauve* has been used as mosquito repellant (Perez – Alonzo, *et al.*, 1995).

2.8.3. Molluscicide and Nematicides

Control of the intermediate snail host is the most important means of control of schistosomiasis. The molluscicidal and antischistosomal activity of *Zingiber officinale* L. was tested using *Biomphalaria glabrata* and *Schistosoma mansoni* respectively. It was found that the two fractions containing the pure compounds gingerol and shogaol resulted in 80% mortality of *Biomphalaria glabrata* snails at a concentration of 25 mg/l. Gingerol (5 mg/l) arrested the ability of *Schistosoma mansoni* miracidia and cercariae to infect

both snails and mice (Adewunmi *et al.*, 1990). Many plant species are known to be highly resistant to nematodes. The most well-documented of these include marigolds (*Tagetes species*.), rattlebox (*Crotalaria spectabilis*), chrysanthemum (Chrysanthemum species), castor bean (*Ricinus communis*), margosa (*Azadirachta indica*), and many members of the family Asteraceae (Germani and Plenchette 2004; Sharma and Trivedi 2002; Wang *et al.*, 2007).

2.8.4. Plants with Rodenticidal activity

Some plants produce compounds that are poisonous to mammals. Some of these, such as Strychnine are used as commercial rodenticides. Rotenone is produced by extraction from the roots and stems of several tropical and subtropical plant species, especially those belonging to the genus Lonchocarpus or Derris. Some of the plants containing rotenone are *Tephrosia virginiana*, *Lonchocarpus utilis*, *Lonchocarpus urucu*, *Derris elliptica*, *Duboisia* and *Verbascum thapsus* (Fang, 1999).

2.8.5. Current scenario and future of biopesticides from plants

Biological control agents would provide a viable commercial option since some conventional chemical control does not give sufficient control and in other cases there is development of insecticide resistance. Biopesticides are a suitable alternative where conventional chemicals are too expensive; or where statutory restriction to application of chemicals is in place. Global search for alternatives to chemical pesticides is going on and various efforts are being made to test the use and efficacy of natural products for pest control and crop protection (Balasubramanian *et al.*, 2008).

Plants contain a virtually untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides (Jitendra. 2009). Advances in chemical and biotechnology are increasing the speed and ease with which man can discover and develop secondary compounds of plants as pesticides. These advances, combined with increasing need and environmental pressure, are greatly increasing the interest in plant products as biopesticides. Various communities have used herbal plants to control pests on livestock (Nanyingi, 2008). The study carried out in Zimbabwe on Utilisation of *Tephrosia vogelii* in controlling ticks in dairy cows by small-scale commercial farmer indicated that *T. vogelii* could reduce tick numbers effectively on dairy cows just as Triatix® (Coopers ltd) did. *Tephrosia vogelii* was therefore recommended to the smallholder dairy production farmers (Gadzirayi *et al.*, 2009).

2.9 Commonly used biopesticide plant in Meru Central District

2.9.1 Tephrosia species

The plant belongs to the class Magnoliopsida (dicotyledons), subclass Rosidae order Fabales, family Fabaceae (Papilionoideae), genus Tephrosia Pers. and species vogelii Hook. (Gaskins *et al.*, 1972). The flower of *T. vogelii* is typically papilionaceous, about 2.5 cm across and purple with white markings or white. The flowers are borne on compact racemes that bloom over a 3 to 6 week period. There may be 20 to 30 flowers per raceme with up to 200 flowers per plant. Pods usually contain 8 to 16 seeds. The flowers have a faint but definite pleasant aroma, and bees visit them freely for both nectar and pollen.

All parts of *Tephrosia vogelii* are used in tropical Africa for numerous ethnomedical and traditional veterinary practices. The leaves have been used as an insecticide, rodenticide

and anthelmintic. Extracts from the root have been used as a molluscicide (Dzenda *et al.*, 2008). The biological activities are due mainly to rotenoids isolated from the plant. Tephrosia species have been used by many indigenous cultures as fish poison (Watt and Breyer-brandwijk, 1962). Active ingredients contained in *T. vogelii* are the rotenoids which includes rotenolone, tephrosin, rotenone and deguelin. (Lambert, 1993).

A preliminary study on assessment of the availability and extent use of *T. vogelli* as an acaricide in Iringa and Mbeya regions of Tanzania was undertaken in 2002.

The research findings revealed that *T. vogelli* contains four insecticidal and pesticidal compounds namely rotenone, deguelin, tephrosin and *6a, 12a- d*ehyrodeguelin (Mwambane, 2000). *Tephrosia* has been cultivated for use as insecticides, fish and arrow poison obtained from leaves (Gaskin *et al.*, 1972). Dry and crushed leaves are used as insecticide against lice, fleas and ticks, and as a molluscicide. *Tephrosia* has also been used as abortifacient, emetic, bactericide, purgative, skin diseases, schistosomiasis, ringworm and parasitic infections. Root decoctions are used to treat constipation (Beentje, 1994). According to Ayurveda system of medicine, *Tephrosia* is digestible, anthelmintics, alexiteric, antipyretic, and cures disease of the liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma and poisoning among others (Dzenda et *al.*, 2008).

Siamba *et al.* (2007) studied the anthelminitic activity of *Tephrosia vogelii* in poultry. The plant leaf aqueous extract had an efficacy of 77.4% against *Ascaridia galli* in indigenous chicken. *Tephrosia vogelii* extracts have been shown to have activity against insects and to protect stored legume and grain seeds against damage by weevils (Koona and Dorn, 2005) justifying its continued use as a biopesticide. *Tephrosia vogelii* has been shown to have toxic and repellent effects against certain insect pests of stored grains (Ogendo *et al.*, 2004; Koona and Dorn, 2005; Pandey *et al.*, 1986; Sharma, *et al.*, 1992,

Smith and Baudoin, 2000) supporting the widespread use of the plant by local farmers as a grain protectant. *Tephrosia vogelii* has been used as a rat poison by compounding with ground nut (Aliu, 1996; Dalziel, 1937; Nwude 1997).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Meru Central District is one of the thirteen districts in Eastern province in Kenya as shown in figure 1. The district lies to the East of Mt. Kenya whose peak cuts through the Southwest border of the district. To the West it borders Laikipia district, to the South it borders Nyeri, Kirinyaga and Meru South districts to the South, Tharaka district to the East and Meru North and Isiolo districts to the North. The district straddles the equator, lying within latitude 0^{0} 3'45" North about 0^{0} 2'30" South and within longitudes 37^{0} and 38^{0} East. The district covers a total area of 2,982 Km^{2} of which Mt. Kenya and Imenti forests cover 1,030 Km^{2} leaving only 1,952 Km^{2} for human settlement. Meru Central District is about 258 km from Nairobi.

Meru Central district is divided into ten (10) administrative divisions as shown in table-1. Timau division is the largest while Mirigamieru West is the smallest division. The district has a total of fifty (50) locations and one hundred and forty four (144) sub locations. Mirigamieru West is the most densely populated division with 1,306 persons per Km^{2} while Timau, which is the largest division in the district, is the least densely populated with 72 persons per Km^{2} as at 1999 (Meru Central District Strategic Plan 2005-2010).

Administrative units by division	Area (Km2)	Population	Density	Locations
Igoji	113.1	45,173	399	5
Abogeta	148.5	56,575	381	6
Nkuene	131.4	54,554	415	3
Abothuguchi West	147.1	59,823	407	1
Abothuguchi Central	57.8	29,673	513	3
Abothuguchi East	213.1	37,020	174	3
Mirigamieru West	53.2	69,469	1,306	5
Mirigamieru East	168.7	56,958	338	6
Buuri	238.4	40,517	170	4
Timau	680.7	49,118	72	4
Forest	1,030.0	------	------	------
TOTAL	**2,982.0**	**498,880**	**167**	**50**

Source: District's Office, Meru Central, 2001

Table 1. The various administrative units in the District and their size

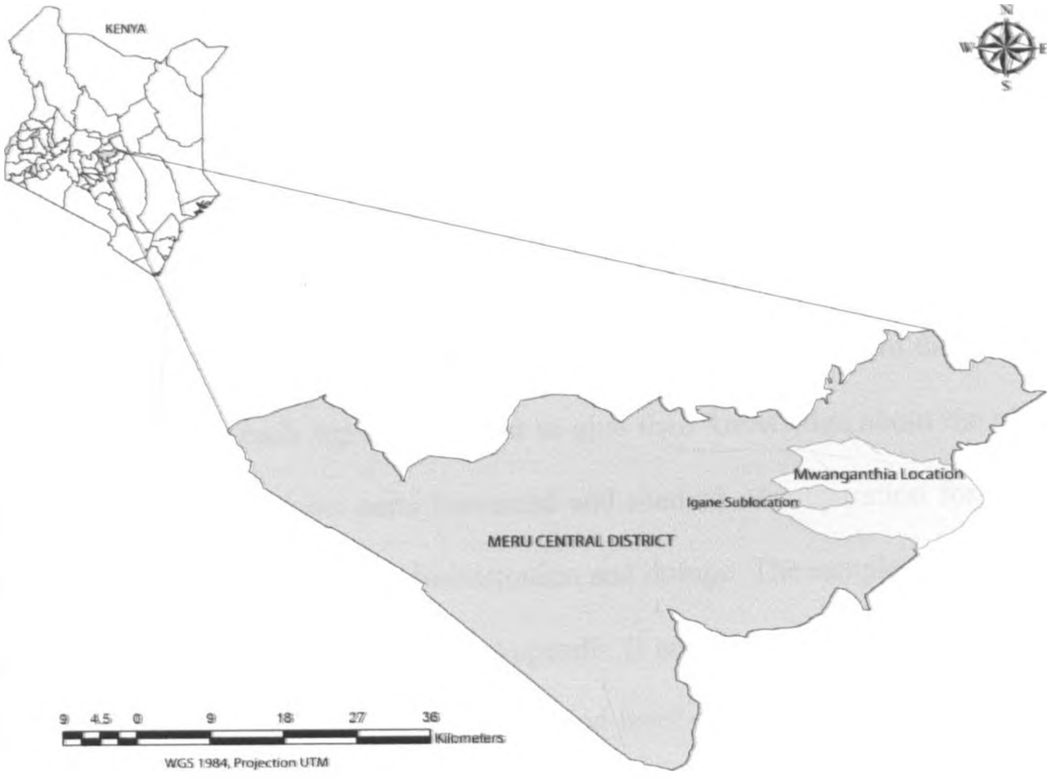

Figure 1. Map of Kenya illustrating the geographical location of Meru district

3.2: Ethnobotanical Survey

Sensitization workshop was held between 17th and 19th July 2009 at Igane sublocation, Abothugushi East division. This was followed by an ethnobotanical survey using semi structured questionnaire and interviews conducted in Kimeru through interpreters. The administration of the questionnaire and discussion with 23 informants (traditional herbalists) were conducted by the investigator and the supervisors from the University of Nairobi. They were each separately asked to give their knowledge about the plants they use to treat diseases, plants parts harvested and method of preparation for the remedy including the details of route of administration and dosage. The sample questionnaire and the list of informants are shown in the Appendix II and III which included the biodata of the respondent like name, age, sex, location and level of education. Details of medicinal, toxic and biopesticides plants were asked which included name of plant, method of method, form and route of administration, dosage and part of plant used and species and conditions treated.

3.3: Collection and botanical identification of Plant specimen.

The plants were colloquially identified by traditional herbalist followed by harvesting of the identified plants using sharp Knives. The specimens of the identified medicinal plants and biopesticidal plants were collected during regular systematical walk in the field and transported to Nairobi for botanical identification at University of Nairobi Land Resource Management and Agricultural Technology (LARMAT) herbarium and also at National Museum of Kenya. The plant specimens were deposited both at LARMAT herbarium of

the University of Nairobi and National Museum of Kenya. Photographs of some important plants were also taken (see plates 1-4).

Tephrosia vogelii leaves were specifically collected in large quantity for evaluation of its toxicological properties since it was ranked by traditional herbalist as the most commonly used biopesticides in the study area.

3.4: Preparation of *Tephrosia vogelii* leaves extracts

The leaves were air dried under the shade for three weeks after which they were ground into powder using an electrical mill. The powder was then stored in airtight plastic containers at room temperature for one week to be used later for extraction. Cold percolation method using 70% v/v methanol was used for extraction of active ingredient from plant materials as described by Gakuya (2001). Six hundred grams of the leaves powder were put in conical flask and 70% v/v methanol added until the powder was submerged. The conical flask was corked with a stopper and shaken thoroughly. The process took 4 days at room temperature during which proper shaking was regularly done to allow proper percolation and extraction. On the 5^{th} day, the extracts were decanted and filtered using Whatman No.1 filter papers.

The methanol in the filtered extracts was evaporated using a rotary evaporator under vacuum at a temperature $40^{o}C$. The resultant viscous substance was stored in a refrigerator at + $4^{o}C$ pending freeze drying using Edwards' lyophilizer (Edwards High Vacuum, Model M6B). After freeze drying the dried samples in form of powder were stored in airtight containers in cool dry place pending acute toxicity and brine shrimp lethality tests.

3.5. Cytotoxicity studies

3.5.1. Preparation of serial concentration of test extracts for bioassay

Thirty three (33) gram of commercial marine salt mixture was weighed using electric weighing machine (Mettler PM 4600, Delta Range) and this was transferred in 1 liter conical flask where 1 liter of distilled water was added gradually while stirring to dissolve the marine salt. Distilled water was added to 1 liter mark after ensuring that all the marine salt was dissolved to make the marine salt solution (Meyer, *et al.*, 1982).

One tenth (0.1) grams *of Tephrosia vogelii* methanol extract was weighed using electric weighing machine (Mettler PM 4600, Delta Range) and this was transferred into a universal bottle. Ten (10) mls of the marine salt solution was added to the universal bottle containing the extract and made to dissolve by stirring using electrical mixture (Voltex Reamix 2789) at 2800 rpm to make a final stock solution of 10,000 µg/ml. Serial dilutions for bioassay were prepared from this stock solution (Gakuya, 2001; Wagate, 2008).

3.5.2. Hatching the brine shrimps

300 mls of the marine salt solution was transferred into a rectangular plastic box which was divided into two unequal compartments by a partition which had several 2 mm holes. Approximately 50 mg of brine shrimp eggs (procured from JBL Novo Termaand Gm BH and Co. Germany) was sprinkled to the smaller compartment and about 1 mg of dry yeast added and then this compartment was covered. The larger compartment was left open and illuminated by a 40 watts electric bulb. After 48 hours the brine shrimp eggs hatched into nauplii (larvae) which were attracted to illuminated compartment by the light. The hatched shrimps were collected by use of Pasteur pipette and subjected to brine shrimp lethality test (Meyer *et al.*, 1982).

3.5 3. Testing of bioactivity using brine shrimp lethality test

Three dilutions were prepared by transferring 500µl, 50µl and 5µl of the stock solution to a set of five (5) graduated tubes for each concentration. Ten (10) brine shrimps were transferred to each vial in each set of tubes using Pasteur pipette and marine salt solution was added to 5 ml mark to make dilutions of 1000 µg//ml, 100 µg/ml, 10 µg/ml A further five graduated vials were set as control with only marine salt mixture and 10 brine shrimps transferred accordingly. After 24 hours, the numbers of live nauplii were counted and percentage mortality calculated for the various plant extract concentrations and controls. Where the deaths in control vials occurred within 24 hours, the data was corrected using Abbots formula (Abbotts, 1925). Percentage was calculated using the formula: % death= ((test-control)/control)) x100. The results were interpreted using probit method of Finney computer program to determine the lethal concentration (LC_{50}) at 95% confidence interval (Finney, 1971).

3.6: Acute Toxicological studies

3.6.1 Experimental animals

Fifty eight (58) inbred albino weaned female rats aged 8 weeks were procured from Department of Veterinary Anatomy and Physiology, University of Nairobi. The animals were fed on mice pencils (from Unga Feeds Ltd) and water supplied *ad libitum.* All the animals were acclimatized for 2 weeks before use and they were maintained in hygienic environment in cages in the animal house at the Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kabete, University of Nairobi.

Preparation of the concentration of extracts was done by dissolving 5 grams in 25mls of distilled water to make a stock solution of 200 mg/ml. To calculate the volume of *Tephrosia vogelii* extracts to be administered to each rat, the following formula was used

Volume (ml) = (Dosage **(mg/kg) x** Body weight **(kg))/**Concentration **(mg/ml)**

3.6.2 Determination of median lethal dose (LD_{50}) and symptoms of toxicity

Before the experiment began, food and water was withheld for 24 hours and all the rats were weighed and labeled and the weight of each rat recorded. The rats were randomly allocated groups 1, 2, 3, 4, 5, 6, 7, 8 and 9 with N= 5, 5, 5, 5, 8, 7, 9, 7 and 7 respectively. Group 1 (N=5) was the control group. Each rat in groups 2 to 9 was orally drenched with the *T. vogelii* methanolic extract dosages of 175, 550, 1500, 1750, 2000, 2500, 3000, and 5000 mg/kg respectively. The control group was treated with normal saline solution. The animals were then observed for clinical manifestations and any mortality for 24 hours and data recorded. The results of the mortality were used to compute the mortality percentage for the group and in determination of LD_{50}. The dose of the test compound killing 50% of the test population (LD_{50}) was determined by the method described by Reed-Muench (1938). The animals that survived were observed for 14 days and sacrificed for gross and histopathological examinations.

3.6.3 Determination of pathological changes

Symptoms of toxicity and number of dead rats were recorded. The dead rats were subjected to a complete postmortem examination. The rats were dissected and the organs examined systematically for gross pathological lesions which were recorded and photographed. Animals that survived after 24 hours were observed for 2 weeks after which they were all sacrificed and tissues/organs were obtained and fixed in 10% formalin for at least 48 hours and then processed for histopathological examination. The following samples were taken for histological examination: liver, kidneys, spleen, stomach, duodenum, intestines, thymus, skeletal muscles, heart, thigh bones, brain, spinal cord and lungs.

3.6.4. Histopathological examination of tissues

Tissues were processed for routine histopathological examination using standard procedures (Cormack, 2001) and then examined under light microscope at magnification of x 400.

CHAPTER FOUR

RESULTS

4.1 Results of ethnobotanical survey

4.1.1 Age of the respondents

Of 23 traditional herbalists interviewed 82.6% were males and 17.4% were females. The female informants' ages ranged from 48 years to 82 years with a mean age being 59.8 years and the male informants' ages ranged from 28 years to 80 years and the mean age was 59.4 years.

4.1.2 Identity of medicinal and biopesticidal plants and their uses

The number of ethnobotanically important plant species documented in this study was 85 belonging to 37 families. The most frequently utilized family of plant species are shown on table-2

S/No	Family of Plant	No. of Spp	Percentage (%)
1	Euphorbiaceae	9	10.59
2	Papilionaceae	7	8.24
3	Compositae	7	8.24
4	Labiteae	6	7.06
5	Rubiaceae	6	7.06
6	Caesalpiniaceae	5	5.88
7	Apocynaceae	4	4.71
8	Rutaceae	4	4.71
9	Liliaceae	3	3.53
10	Graminae	2	2.35
11	Myrtaceae	2	2.35
12	Verbenaceae	2	2.35
13	Flacourtiaceae	2	2.35
14	Moraceae	2	2.35

15	Bignoniaceae	2	2.35
16	Hypericaceae	1	1.18
17	Convolvulaceae	1	1.18
18	Commelinaceae	1	1.18
19	Mimosaceae	1	1.18
20	Capparidaceae	1	1.18
21	Plumbagiaceae	1	1.18
22	Caricaceae	1	1.18
23	Cyperaceae	1	1.18
24	Olecaceae	1	1.18
25	Ebenaceae	1	1.18
26	Simaroubaceae	1	1.18
27	Menispermaceae	1	1.18
28	Boraginaceae	1	1.18
29	Celastraceae	1	1.18
30	Rosaceae	1	1.18
31	Melanthaceae	1	1.18
32	Meliaceae	1	1.18
33	Bursalaceae	1	1.18
34	Combretaceae	1	1.18
35	Solanaceae	1	1.18
36	Acanthaceae	1	1.18
37	Cannelaceae	1	1.18
		85	100

Table 2: Results on ethnobotanical survey carried out in Meru

Among the medicinal and biopesticide plants used, shrubs were the commonest with 38.8%. Trees accounted for 35.3% while Herbs and liana had 24.7% and 1.2% respectively as shown in figure 2.

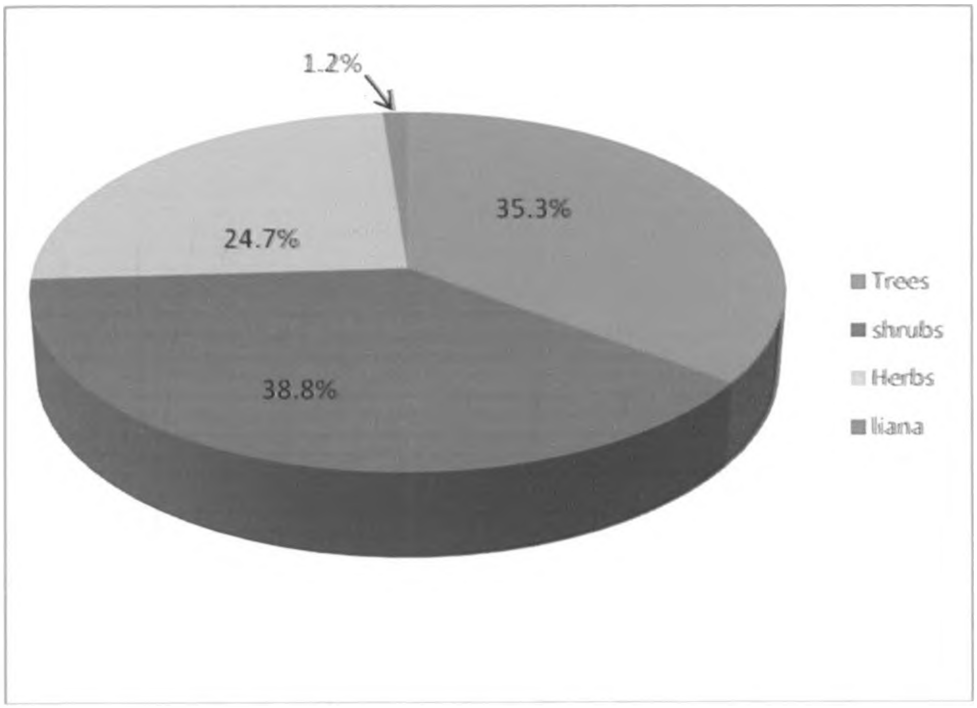

Figure .2: The percentages of different life forms of medicinal/biopesticides plants used in Meru Central District, Kenya

The reported ailments and the plants used were grouped into 15 categories based on the information gathered from the interviewees namely: Medicinal plants used for treatment of reproductive problems, endoparasites (helminthosis), malaria (used as antimalarials), gastrointestinal disorders, respiratory disorders, Pests (biopesticides), eye infections, wounds, cuts and fractures, teeth infections, ear infections, general body sickness, skin conditions, East Coast Fever (E.C.F) and anaplasmosis in cattle and allergic conditions. The medicinal and toxic plants found in the area are also recorded in (Tables 3-17). Some of the most important plants collected from the area are also shown in Plate 1-4.

Table 3-17: Ethnobotanical data of Medicinal, Biopesticides and Toxic plants use in Meru Central District

Spp No.	Plant species	Meru name	Specific problems/ conditions	Part of plant used
1	ACANTHACEAE *Anisotes ukambesis*	Mugituri	Control of bleeding in pregnant women	Bark
2	CAESALPINIACEAE *Pilliostigma thoningii*	Mukuura	Prolonged menstruation Gonorrhea and other venereal diseases	Leaves/stem bark
3	CAPPARIDACEAE *Capparis sepiaria*	Mutanda mbogo	Retained placenta in cattle	Root
4	COMPOSITAE *Vernonia lasiopus*	Mwatha	Decreased libido	Leaves/roots
5	LABIATEAE *Fuerstia Africana*	Mutaciune	Gonorrhea and other venereal diseases	Leaves/roots
6	CAESALPINIACEAE *Cassia didymobotrya*	Kirao	Gonorrhea and other venereal diseases	Leaves
7	PAPILIONACEAE *Securandica longependiculata*	Muguruka	Quicken delayed delivery	Roots
8	HYPERRICACEAE *Harungana madagascariensis*	Munyamwe	Hasten development of breast in young women	Bark
9	VERBENACEAE *Clerodendrum eriophyllum*	Muringa iria	Swollen testicles	Roots/Leaves

Table 3 Medicinal plants used for treatment of reproductive disorders

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	BORAGINACEAE			
	Cordia abyssinica	Muringa	Anthelmintics	Bark
2	**CAESALPINIACEAE**			
	Caesalpinia volkensii	Munjuthi	Anthelmintics	Leaves/Seed
3	**CARICACEAE**			
	Carica papaya	Mubabai	Anthelmintics	Seed/Roots/Leaves
4	**COMBRETACEAE**			
	Terminalia brownie	Muruuku	Anthelmintics (when mixed with *Erythrina abyssinica & Trichilia roka)*	Bark
5	**COMPOSITAE**			
	Aspilia mosambiescensis	Muuti	Anthelmintics	Leaves
6	**COMPOSITAE**			
	Crassocephalum manii	Matoroboro	Anthelmintics	Fruit/Leaves
7	**COMPOSITAE**			
	Vernonia colorata	Mwatha jwa njau	Anthelmintics	Roots
8	**EBENACEAE**			
	Euclea divinorum	Mumanku	Anthelmintics	Roots
9	**EUPHORBIACEAE**			
	Croton megalocarpus	Mukinduri	Anthelmintics	Leaves
10	**LABIATEAE**			
	Leucas mollis	Majara	Anthelmintics	Leaves
11	**MELIACEAE**			Bark
	Trichilia roka	Mutwati	Anthelmintics	

Table .4 continued

12	**OLECACEAE** *Ximemia caffra*	Muroroma	Anthelmintics	Leaves/Roots
13	**PAPILIONACEAE** *Erythrina abyssinica*	Muhuti	Anthelmintics	Roots/Bark
14	**PAPILIONACEAE** *Indigofera lupatana*	Muthara	Anthelmintics	Roots
15	**PAPILIONACEAE** *Melletia dura*	Mwangua	Anthelmintics	Roots
16	**PLUMBAGIACEAE** *Plumbago zeylanica*	Karocho	Anthelmintics	Roots
17	**RUBIACEAE** *Petodon rentadrus*	Mukurwa	Anthelmintics	Roots
18	**RUBIACEAE** *Vanguera rotundata*	Mukuura	Anthelmintics	Roots
19	**RUTACEAE** *Zanthoxylum chalybeum*	Mugucwa	Anthelmintics	Leaves/Bark/ Roots
20	**VERBENACEAE** *Clerodendrum eriophyllum*	Muringa iria	Anthelmintics	Leaves

Table .4 Medicinal plants used for treatment of livestock and human endoparasites (anthelmintics)

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use
1	**APOCYNACEAE**		
	Conopharyngia holstii	Murigurigu	Anti-Malarial
2	**CAESALPINIACEAE**		
	Caesalpinia volkensii	Munjuthi	Anti-Malarial
3	**CAESALPINIACEAE**		
	Cassia didymobotrya	Kirao	Anti-Malarial
4	**CANNELACEAE**		
	Warbugia ugandensis	Muthiga	Anti-Malarial
5	**CONVOLVULACEAE**		
	Astripomoea malvaceae	Mukua na nthi	Anti-Malarial
6	**FLACOURTIACEAE**		
	Flacourtia indica	Muroo	Anti-Malarial
7	**HYPERICACEAE**		
	Harungana madagascariensis	Munyamwe	Anti-Malarial
8	**LABIATEAE**		
	Ajuga remota	Kirurage	Anti-Malarial
9	**LABIATEAE**		
	Ocinum sauve	Makuri	Anti-Malarial
10	**LILIACEAE**		
	Aloe vera	Gicukurui	Anti-Malarial
11	**LILIACEAE**		
	Aloe secundiflora	Gicukurui/gikunu	Anti-Malarial
12	**MIMOSACEAE**		
	Albizia gummifera	Muchambi	Anti-Malarial
13	**PAPILIONACEAE**		
	Indigofera lupatana	Muthara	Anti-Malarial
14	**PAPILIONACEAE**		
	Tephrosia purpuera	Kagundugundu	Anti-Malarial

Table 5. continued

Part of plant used
Roots
Seed/Leaves
Leaves
Leaves
Roots
Roots
Bark
Leaves
Leaves
Leaves
Leaves
Bark
Roots
Roots

15	**RUBIACEAE**		
	Adina microcephala	Mugoti	Anti-Malarial
16	**RUTACEAE**		
	Fagaropsis angolensis	Mukuria mpungu	Anti-Malarial
17	**RUTACEAE**		
	Zanthoxylum chalybeum	Mugucwa	Anti-Malarial
18	**SIMAROUBACEAE**		
	Harrisona abyssinica	Mutagata	Anti-Malarial

Table 5. Medicinal plants used for treatment of malaria (Antimalarials)

Roots /Bark

Bark/ Roots

Bark

Leaves/ Roots /Bark

Spp No.	Plant species	Meru name	Specific problems/ conditions treated	Part of plant used
1	**APOCYNACEAE**			
	Conopharyngia holstii	Murigurigu	typhoid, anti-amoeba	Bark/Roots
2	**APOCYNACEAE**			
	Carrisa edulis	Mukaawa	Stomach-ache	Roots/Bark
3	**CAESALPINIACEAE**			
	Cassia didymobotrya	Kirao	emetics and purgatives	Leaves
4	**CAESALPINIACEAE**			
	Cassia occidentalis	Mukura werune	Stomach ache, Anti-diarrhea	Whole Plant
5	**CANNELACEAE**			
	Warbugia ugandensis	Muthiga	Stomach ache,	Leaves
6	**CAPPARIDACEAE**			
	Capparis sepiaria	Mutanda mbogo	emetic and purgatives	Roots
7	**CARICACEAE**			
	Carica papaya	Mubabai	anti- amoeba	Seed/Roots
8	**COMPOSITAE**			
	Conyza floribunda	Mweru	Laxatives-(leaves) in children	Roots
9	**COMPOSITAE**			
	Crassocephalum manii	Matoroboro	purgatives, indigestion & dysentery	Roots /Leaves
10	**COMPOSITAE**			
	Vernonia lasiopus	Mwatha	Stomach ache-	leaves boiled or chewed
11	**CONVOLVULACEAE**			
	Astripomoea malvaceae	Mukua na nthi	Stomach ache, Dysentery, Relieves constipation	Roots
12	**EBENACEAE**			
	Euclea divinorum	Mumanku	Relieves constipation & purgatives	Roots

Table 6 continued

13	**EUPHORBIACEAE**			
	Phillanthus sepialis	Mukuuru	Relieves constipation	roots boiled & decoction taken -1 cup twice daily
14	**FLACOURTIACEAE**			
	Flacourtia indica	Muroo	Stomach ache, Indigestion	Roots
15	**LABIATEAE**			
	Ajuga remota	Kirurage	dysentery & stomach ache	Leaves
16	**LABIATEAE**			
	Ocinum sauve	Makuri	Stomach ache (combined with *Vernonia colorata, Lantana tryphilla & Adina microcephala)*	Leaves
17	**LABIATEAE**			
	Rosimarinus officinalis	Kamucubu	Appetizer when boiled with soup	Leaves boiled with soup
18	**MENISPERMACEAE**			
	Stephania abyssinica	Kamutuma/ciontu	colic in infants, stomach ache	Roots
19	**MIMOSACEAE**			
	Albizia gummifera	Muchambi	Relieves stomach pains	crushed pods
20	**MORACEAE**			
	Ficus natalansis	Mugumo	Anti-diarrheal for sheep & goats when combined with *Milletia oblata*	Roots /B/L
21	**OLECACEAE**			
	Ximemia caffra	Muroroma	Anti-diarrhoeal in calves	Leaves
22	**PAPILIONACEAE**			
	Erythrina abyssinica	Muhuti	Anti-diarrheal	Bark Roots decoction
23	**PAPILIONACEAE**			
	Securandica longepenculata	Muguruka	Purgatives,	
24	**RUTACEAE**			
	Clausenia anisata	Mukiibia	Anti-diarrhoeal	Leaves

Table 6 continued

25	**VERBENACEAE**			
	Clerodendrum eriophyllum	Muringa iria	Anti-amoeba and stomach-ache	Roots
26	**VERBENACEAE**			
	Lantana tryphilla	Mugumbau	Constipation and stomach ache	Roots
27	**COMPOSITAE**			
	Lactuca capensis	Muthunka	Heart burn	Whole Plant
28	**PAPILIONACEAE**			
	Tephrosia vogelii	Mumpuko	Roots relieve constipation.	Roots

Table.6 Medicinal plants used for treatment of gastrointestinal disorders

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**APOCYNACEAE**			
	Conopharyngia holstii	Murigurigu	Pneumonia	Bark
2	**BIGNONIACEAE**			
	Markhamia hildebrandtii	Mugwani	Pneumonia (when combined. with mango bark & *Terminalia brownii*)	Bark
3	**BORAGINACEAE**			
	Cordia abyssinica	Muringa	Pneumonia (H)	Bark
4	**BURSERACEAE**			
	Comiphora zimmermanii	Mutunguu	Pneumonia (when combined. with *Cyperus articulatus*, *Conophagia holstii*, *Terminalia brownie* and *Carica papaya*	Bark
5	**CAESALPINIACEAE**			
	Pilliostigma thoningii	Mukuura	Cough-	Bark /Leaves
6	**CANNELACEAE**			
	Warbugia ugandensis	Muthiga	Pneumonia and flu.	Leaves

Table 7 continued

7	**CAPPARIDACEAE**			
	Capparis sepiaria	Mutanda mbogo	Chest pains	Roots /Leaves
8	**CARICACEAE**			
	Carica papaya	Mubabai	Pneumonia when combined with other herbs	Seed/ Roots /Leaves
9	**CELESTRACEAE**			
	Cartha edulis	Muraa	Pneumonia (when combined. with *Erythrina abyssinica, milletia oblata,Aloe,Conopharygia holstii and Terminalia brownie*	Leaves
10	**COMBRETACEAE**			
	Terminalia brownie	Muruuku	Pneumonia, fever in children,	Bark
11	**COMPOSITAE**			
	Vernonia colorata	Mwatha jwa njau	Cough	Roots
12	**CYPERACEAE**			
	Cyperus papyrus (articulatus)	Ndago	Pneumonia –(when combined with onions, lemons & *Commiphora zimmernaanii)*	Roots
13	**EUPHORBIACEAE**			
	Bridellia micrantha	Mukwego	Pneumonia. Combined with other plants for ECF	Bark
14	**LABIATEAE**			
	Ajuga remota	Kirurage	Pneumonia (when combined with others)	Leaves
15	**LABIATEAE**			
	Ocinum sauve	Makuri	Pneumonia	Leaves
16	**LABIATEAE**			
	Plectranthus barbatus	Mwaraaka	Pneumonia	Leaves
17	**LILIACEAE**			
	Allium cepa	Gitunguru	Pneumonia (combined with *cyperus articulatus & commiphora zimmernaanii)*	Bulb
18	**LILIACEAE**			
	Aloe secundiflora	Gicukurui/gikunu	Chest pains	Leaves
19	**MELIACEAE**			
	Trichilia roka	Mutwati	Pneumonia in cattle	Bark

Table 7 continued

20	**MIMOSACEAE**			
	Albizia gummifera	Muchambi	Pneumonia	Bark
21	**MYRTACEAE**			
	Eucalyptus saligna	Mubamauta	Fever, cold, flu	Leaves
22	**PAPILIONACEAE**			
	Melletia dura	Mwangua	Pneumonia (combined with *Ajuga remota, Conopharygia holstii* and *C. zimmernaanii*	Bark
23	**RUBIACEAE**			
	Pavetta teitana	Murema muthwa	Pneumonia (combined with other *plant)*	Leaves/ Roots
24	**RUBIACEAE**			
	Petodon rentadrus	Mukurwa	Pneumonia, Cough	Roots
25	**RUBIACEAE**			
	Vanguera tomentosa	Mwarakware	Shipping fever when combined with *Ximenia caffra*	Leaves
26	**RUTACEAE**			
	Citrus limon	Ndimu	Pneumonia (combined with *onion,Cyperus articulatus & Commiphora zimmernaanii*	Fruit
27	**RUTACEAE**			
	Clausenia anisata	Mukiibia	Pneumonia	Roots/bark
28	**VERBENACEAE**			
	Lantana tryphilla		Pneumonia	Leaves
29	**ROSACEAE**			
	Pygeum africanum	Mwiria	Cough, flu, congested chest,	Bark

Table 7 Medicinal plants used for treatment of respiratory disorders

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**APOCYNACEAE** *Thevetia peruviana*	Karwego	Mosquito repellant	Flower/Leaves
2	**COMPOSITAE** *Vernonia lasiopus*	Mwatha	Biopesticide for crop pests Paste kills maggots	Leaves
3	**FLACOURTIACEAE** *Oncoba routledgei*	Mwege	Anti-jigger-fruit juice applied on the infested areas Also can be combined with Sodom apple;	Fruits
4	**LABIATEAE** *Ocinum sauve*	Makuri	Mosquito repellant (biopesticides) Bees attractant	Leaves
5	**LABIATEAE** *Plectranthus barbatus*	Mwaraaka	Biopesticides (weevils, fleas, cockroaches)	Leaves
6	**PAPILIONACEAE** *Tephrosia vogelii*	Mumpuko	Piscicide, Abortifacient, Biopesticide, Rodenticide Ectoparasiticide Biopesticides to control crop pests	Whole Plant

Table 8 Plants used as biopesticides

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**BORAGINACEAE**			
	Cordia abyssinica	Muringa	Eye infections	Bark
2	**COMMELINACEAE**	Mukenkeiya	Eye infections	
	Commelina benghalensis			Flower sap
3	**COMPOSITAE**			
	Bidens pilosa	Munyugunyugu	Conjunctivitis	Leaves juice
4	**LILIACEAE**			
	Aloe secundiflora	Gicukurui/gikunu	Conjunctivitis	Leaves juice
5	**MORACEAE**			
	Morus alba	Mutaratare	Blindness	Leaves

Table 9 Plants used for treatment of eye infections

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**BORAGINACEAE**			
	Cordia abyssinica	Muringa	Fresh juicy bark used to tie fractures. Powdered roots applied on wounds	Fresh juicy bark Powdered roots applied on wounds
2	**CAPPARIDACEAE**			
	Capparis sepiaria	Mutanda mbogo	Wounds	Roots
3	**COMPOSITAE**			
	Aspilia mosambiescensis	Muuti	Wounds-leaves pounded & applied on fresh wounds	Leaves
4	**EUPHORBIACEAE**			
	Croton megalocarpus	Mukinduri	Bark used for control of, bleeding	Bark
5	**EUPHORBIACEAE**			
	Jatropha curcas	Kiariki gia chomba	Arrests bleeding on cut or wounds (Coagulant)	Leaves/S
6	**EUPHORBIACEAE**			
	Ricinus communis	Mwariki	Arrests bleeding on cut or wounds-it's a coagulant (Coagulant)	Bark/Seed
7	**MYRTACEAE**			
	Eucalyptus saligna	Mubamauta	Wounds (when combined with *Aloe)*	Leaves/Bark
8	**LILIACEAE**			
	Aloe vera	Gicukurui	Treatment of wounds	Leaves

Table 10 Plants used for treatment of wounds, cuts and fractures

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**BIGNONIACEAE**			
	Markhamia hildebrandtii	Mugwani	Bark chewed for tooth-ache	Bark
2	**CANNELACEAE**			
	Warbugia ugandensis	Muthiga	Tooth ache,	Leaves/Stem
3	**CELESTRACEAE**			
	Cartha edulis	Muraa	Tooth ache	Leaves
4	**COMPOSITAE**			
	Conyza floribunda	Mweru	Tooth ache	Roots
5	**LABIATEAE**			
	Ajuga remota	Kirurage	Tooth ache	Leaves
6	**RUTACEAE**			
	Clausenia anisata	Mukiibia	Used as Toothbrush.	Stem
7	**RUTACEAE**			
	Zanthoxylum chalybeum	Mugucwa	Tooth ache	Leaves/Bark/ Roots
8	**PLUMBAGIACEAE**			
	Plumbago zeylanica	Karocho	Removal of pseudo teeth in children	Roots

Table 11 Plants used for treatment of tooth ache and teeth infections

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**APOCYNACEAE**			
	Carrisa edulis	Mukaawa	Dizziness (H Dizziness (H	Bark/Root
2	**CAESALPINIACEAE**			
	Caesalpinia volkensii	Munjuthi	Shivering when combined with *Psidia guajava*)	Seed/Leaves
3	**CANNELACEAE**			
	Warbugia ugandensis	Muthiga	Rheumatism	Leaves
4	**COMPOSITAE**			
	Bidens pilosa	Munyugunyugu	Dizziness	Leaves
5	**CYPERACEAE**			
	Cyperus papyrus (articulatus)	Ndago	Head ache-(when bulb chewed)	Roots
6	**EUPHORBIACEAE**			
	Phillanthus sepialis	Mukuuru	General body illness	Roots boiled & decoction taken -1 cup twice daily
7	**PAPILIONACEAE**			
	Indigofera lupatana	Muthara	Back-ache	Roots
8	**PAPILIONACEAE**			
	Securandica longepenculata	Muguruka	Dizziness, head ache	Roots
9	**RUBIACEAE**			
	Adina microcephala	Mugoti	Head ache	Bark
10	**ROSACEAE**			
	Pygeum africanum	Mwiria	Prostate cancer, back ache, , joint ache, Anthrax,	Bark
11	**RUBIACEAE**			
	Vanguera tomentosa	Mwarakware	Shipping fever when combined with *Ximenia caffra*	Leaves

Table .12 Plants used for treatment of general body sickness

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**BIGNONIACEAE**			
	Kigelia aethiopica	Murantina	ECF &Anaplasmosis (when mixed with other herbs	Fruit
2	**BORAGINACEAE**			
	Cordia abyssinica	Muringa	ECF &Anaplasmosis (when mixed with other herbs	Bark
3	**COMBRETACEAE**			
	Terminalia brownie	Muruuku	ECF(when mixed with other herbs)	Bark
4	**EUPHORBIACEAE**			
	Bridellia micrantha	Mukwego	Combined with other plants for ECF in livestock.	Bark
5	**EUPHORBIACEAE**			
	Croton megalocarpus	Mukinduri	ECF (when combined with other herbs)	Leaves
6	**EUPHORBIACEAE**			
	Synadenium compactum	Muthuri	Applied on swollen lymph node in case of ECF	Sap
7	**HYPERICACEAE**			Bark
	Harungana madagascariensis	Munyamwe	ECF	
8	**ROSACEAE**			Bark
	Pygeum africanum	Mwiria	ECF	
9	**LABIATEAE**			
	Plectranthus barbatus	Mwaraaka	ECF (when combined with other herbs)	Leaves
10	**MYRTACEAE**			
	Eucalyptus saligna	Mubamauta	ECF (when combined with others	Leaves/Bark
11	**MYRTACEAE**			
	Psidium guajava	Mupera	ECF (when combined with others	Leaves
12	**PAPILIONACEAE**			
	Erythrina abyssinica	Muhuti	ECF (when combined with others ECF (when comb with others	Bark
13	**COMPOSITAE**			
	Crassocephalum manii	Matoroboro	Anaplasmosis when mixed with *Ricinus communis*	Leaves

Table .13 Plants used for treatment of East Coast Fever (E.C.F) and Anaplasmosis in Cattle

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**APOCYNACEAE**			
	Conopharyngia johnstonii	Mutuu / Gituu	Roots, stem &fruits are toxic (AH)	Fruit/Stem
2	**APOCYNACEAE**			
	Thevetia peruviana	Karwego	Mosquito repellant	Leaves
3	**CAESALPINIACEAE**			
	Cassia didymobotrya	Kirao	Medicinal but toxic when over dosed	Leaves
4	**EUPHORBIACEAE**			
	Croton megalocarpus	Mukinduri	Seeds toxic when taken orally	Seed
5	**EUPHORBIACEAE**			
	Euphorbia milii	Jerusalem	Moderately toxic mucus membranes & skin	Leaves
6	**EUPHORBIACEAE**			
	Jatropha curcas	Kiariki gia chomba	Seeds very toxic	Seed
7	**EUPHORBIACEAE**			
	Manihot esculatum	Mukwaci	Roots and leaves very toxic to animals and humans	Leaves/ Roots
8	**EUPHORBIACEAE**		Highly toxic to mucus membranes and skin- causes	
	Synadenium compactum	Muthuri	local necrosis.	Stem/Leaves latex
9	**GRAMINAE**			
	Sorghum versicolor	Muiya	Young plant is toxic to livestock	Leaves
10	**MELANTHACEAE**			
	Bersama abyssinica	Muthandathande	Highly toxic to both humans and animals when ingested.	Leaves
11	**PAPILIONACEAE**			
	Tephrosia vogelii	Mumpuko	Highly toxic to fish, causes abortion in women and animals, Used as biopesticides Controls moles, Kills ectoparasites Used to control crop pests Roots relieve constipation.	Leaves/ Roots

Table .14 Toxic plants found in the area of study.

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**COMMELINACEAE**			
	Commelina benghalensis	Mukenkeiya	Ear-ache (ear infections)	sap from stem
2	**LABIATEAE**			
	Leucas mollis	Kijara /Majara	Otitis media(ear infections)	Leaves

Table .15 Plants used for treatment of ear infections or ear ache

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**VERBENACEAE**			
	Clerodendrum eriophyllum	Muringa iria	Treatment of snake bites	Roots /Leaves
2	**PLUMBAGIACEAE**			
	Plumbago zeylanica	Karocho	Skin blisters, and swollen legs.	Root decoction Root decoction
3	**EUPHORBIACEAE**			
	Euphorbia milii	Jerusalem	Removal of warts	milky sap from the flower or the leaves

Table .16 Plants used for treatment of other conditions including skin conditions

Spp No.	Plant species	Meru name	Specific problems/ conditions/ use	Part of plant used
1	**SOLANACEAE**			Leaves/ Roots
	Withania somnifera	Mugumbau	Allergic conditions	

Table .17 Plants used for treatment of allergic conditions

Micrographs of some important medicinal and biopesticide plants

Plate .1: Micrograph of *Tephrosia vogelii* (Kim: Mumpuko)

Plate .2: Micrograph of *Ajuga remota* (Kim: Kirurage)

Plate .3: Micrograph of *Warbugia ugandensis* (Kim: Muthiga)

Plate .4: Micrograph of *Ocimum sauve* (Kim: Makuri)

4.1.3. Dosage levels in treatment using the medicinal plants

Generally the dosage of medicinal plants administered to children was less than adults and the same case applied to young and adult livestock. Adults were given up to one glass (about 200 ml) while adult cattle was given up to one bottle (about 700 mls) for most of the plant extracts. Children were given between 20-50 mls while young ones of livestock ranged between 50-200 mls depending on their sizes. The quantity of plant parts used was measured by number of leaves, seeds and fruits and the length of the root or at times handful of the plant parts. The dosage depended on age and the physical appearance of the patient, social cultural explanation of the illness, diagnosis and the experience of the herbalist. The most common route of drug administration was the oral route.

4.2: Brine Shrimp Lethality Test (BSLT).

The observation indicated that the serial dilutions of *Tephrosia vogelii* extract were toxic to brine shrimp at <1000 µg /ml (see Table 18) The LC_{50} was found to be 123 µg/ml with 95% confidence interval of 45-261.

Plant Name (Botanical & Kimeru)	Part of Plant used	Percentage deaths at 24 hours			LC_{50} (µg/ml)
		10 µg/ml	100 µg/ml	1000 µg/ml	
Tephrosia vogelii (Mumpuko)	Leaves	12	26	100	123 (45-261)

Table .18. Brine shrimp lethality test LC_{50} (confidence interval) of *Tephrosia vogelii* leaves methanol extracts.

4.3 Acute toxicity

4.3.1. Symptomatology

Upon oral administration of the plant extract to rats some manifested starry coat, arched back, anorexia, initial excitation, restlessness, salivation, dyspnoea, incoordination of the gait and clonic convulsions. All the rats had hind limbs paralysis prior to death.

4.3.2 Gross pathological lesions

Grossly, lesions were seen in lungs where some areas were dark red in colour. Kidneys, liver and spleen were dark red due to marked congestion. The stomach mucosa appeared dark reddish than normal and this spread downwards to the duodenum up to 1/3 portion of the ileum. Distal portion of the small intestines down to the colon and rectum were normal in appearance. The stomach contents resembled the color of the plant extract which was yellowish in color. The dark red color of the stomach mucosa fades downward toward the intestines. Some of gross lesions are demonstrated in plate 5

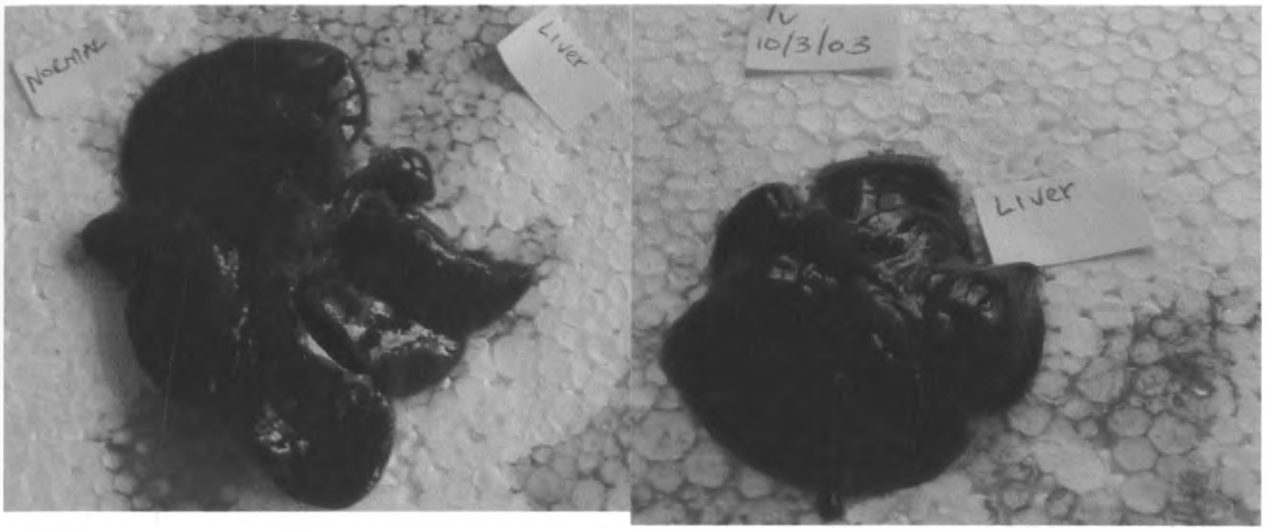

Plate .5: Comparative gross pathology of livers from control and treatment groups.

Dark red liver due congestion (on the right) from rat administered orally with 3000 mg/kg of *Tephrosia vogelii* extract. On the left is the liver (appears red) from the control group

(H.E. X 400)

4.3.3. Histopathological examination of tissues

The most significant histological changes occurred in the brain tissue. Some neurons were enlarged while in others the cells exhibited varying degree of Chromatolysis in which the nissil substance was dispersed, frequently scattered leaving a light staining perinuclear zone (Central Chromatolysis). Plate-6 shows varying degrees of neuronal degeneration and necrosis occurred in some neurons. The most remarkable lesions in the brain were a combination of neuronal degeneration bordering necrosis. Some neurons had no nucleus. These were the ghost cells (silhouette) or shadow of previously normal cells. Some nucleus of the neurons had shrunken cytoplasm and the nucleus was pushed towards the periphery (margination of nucleus). Cytoplasm of some degenerated neurons was condensed with dark basophilic cytoplasm and the cells had become smaller in size than normal and Chromatolysis was also evident.

Tissues of other organs like the lungs, liver, spleen and the heart were highly congested with distended blood vessels in the parenchyma. Some epithelial cells of the intestines were eroded. The common histopathological manifestations are in the brain are illustrated in plate-6.

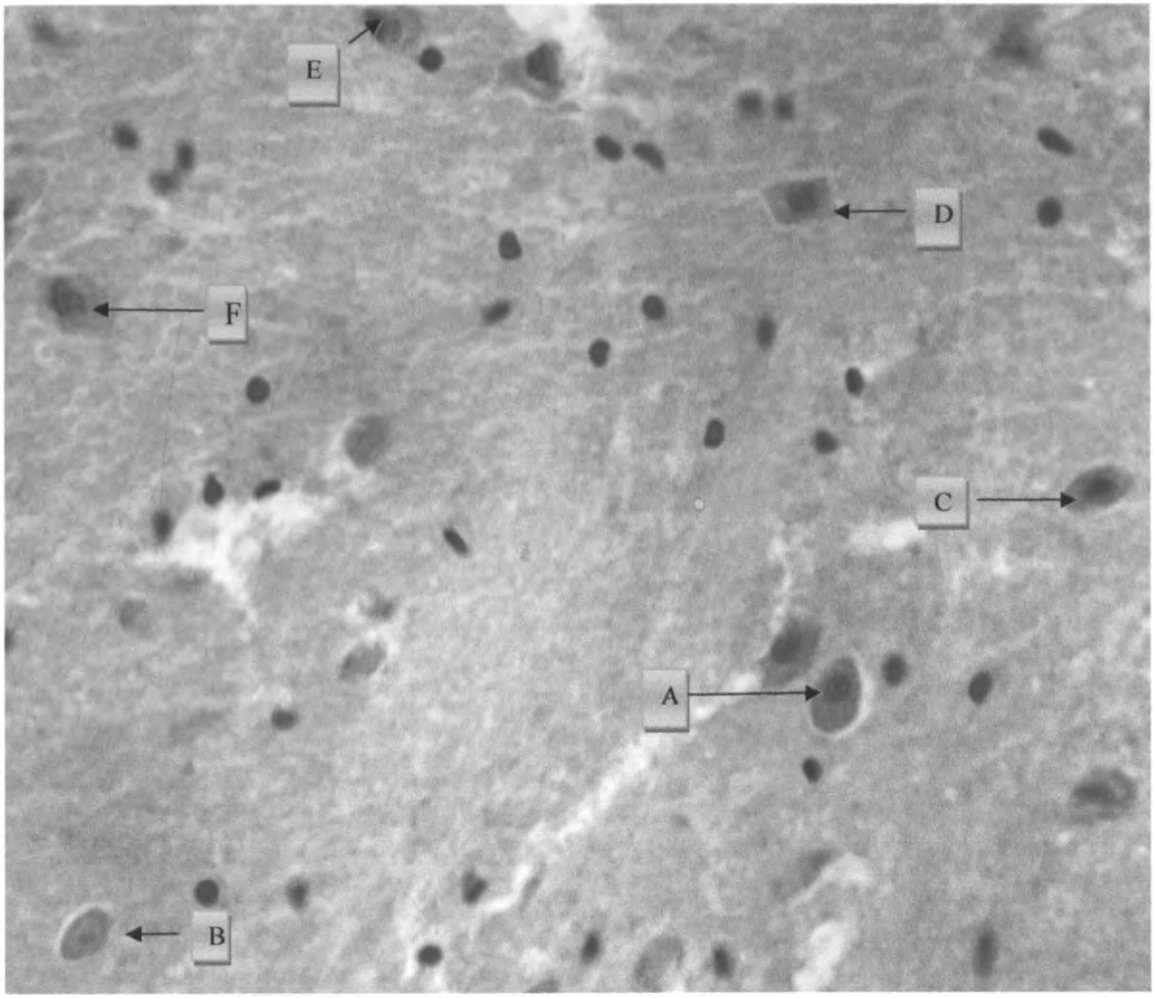

Plate .6 Neurons of brain tissue in Rats govaged with a single dose of 3000 mg/kg orally of methanolic *Tephrosia vogelii* extract. Postmortem done on day 2 after 1 day of treatment. (H.E. X 400)

A - Condensed basophilic staining neurons
B. - Silhouette or ghost cell (Neurons with no nucleus)
D- Condensed basophilic neuron with central Chromatolysis. Astrogliosis is seen in the background
E-neuron undergoing necrosis as it is invaded by astrogliocytes.
F – A neuron showing margination of nucleus indicating neuronal degeneration

4.3.4. Median Lethal dose (LD_{50})

In acute toxicity tests, the median lethal dose (LD_{50}) was found to be 3163 mg/kg body weight. The survival, deaths and percentage mortality at various dose of *T. vogelii* methanolic are displayed in table-19.

No. of Rats (N)	Dose mg/kg	Log dose	Died	Survived	Accumulated			% Mortality
					Died	Survived	Total	
5	0		0	5	0	47	47	0
5	175	2.24	0	5	0	42	42	0
5	550	2.74	0	5	0	37	37	0
5	1500	3.18	0	5	0	32	32	0
8	1750	3.24	3	5	3	27	30	10.0
7	2000	3.30	1	6	4	22	26	15.4
9	2500	3.40	1	8	5	16	21	23.8
7	3000	3.48	2	5	7	8	15	46.7
7	5000	3.70	4	3	11	3	14	78.6

Table .19: Data on Lethal Median Dose (LD_{50}).

CHAPTER FIVE

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussions

The average age of the respondents in the study area was approaching 60 years. This information on the age implies that this vital indigenous/traditional knowledge is not being passed to younger generation as evidenced from the average age of herbalist which was approaching 60 years. This in essence means that with time this knowledge will be lost unless efforts are made to reverse this situation.

On the level of treatments, the herbalist have no standardization of dosages and preparation of plants based medicines and this calls for further research to have standardized methods of preparations and dosages, efficacy including pharmacological and toxicological properties.

Study carried out elsewhere, indicated that methanolic extract of *Ajuga remota* has been shown to have some antimicrobial activity (Wagate, 2008; Musau, 2011). Coll and Tandrón, (2005) isolated compounds with anti-feedant activities against *Spodoptera littoralis* from *Ajuga remota. Ajuga remota* was among the plants identified in the study area. Therefore the ethnobotanical survey carried out in Meru central district justifies it use by traditional healers.

Mbaria *et al.,* 2006 carried out brine shrimp lethality test on *Chrysanthemum cinerariaefolium* (pyrethrum) which is used as commercial biopesticide. In that study the LC_{50} of *Chrysanthemum cinerariaefolium* was reported to be 107µg/ml. This LC_{50} was in the same range as that of the current study using *Tephrosia vogelii* which was found to be

123 µg/ml with 95% confidence interval of 45-261. *Tephrosia vogelii* which is used traditionally as a rodenticide has therefore a high potential for development as a commercial biopesticides and further research is therefore suggested.

A similar study has been done on the plant *Tephrosia purpurea* which belongs to the same genus as *Tephrosia vogelii* (Adoum *et al.*, 1997). The LC_{50} of methanol extract of leaves and stem of *Tephrosia purpurea* (L) Pers BSLT was found to be 135 (80-229). This LC_{50} is within the same range as *Tephrosia vogelii*. *Tephrosia purpurea* (L) Pers is used as a fish poison, anthelmintic and as a purgative (Watt and Breyr-Brandwijk, 1962). Brine shrimp lethality test done by Wagate (2008) showed LC_{50} of *Warbugia ugandensis* to be 397.4 and classified as toxic in brine shrimp assay. This supports it continued use traditionally in the current study area. *Erythrina abyssinica is* one of the medicinal plants used in Meru Central district. The ethyl acetate extract of the stem bark of *Erythrina abyssinica* showed anti-plasmodial activity against the chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* (Yenesew *et al.*, 2004). Ocimum species have compounds that are antifungal, antibacterial, insecticidal and insect repellant activities (Musau, 2011; Perez – Alonzo, *et al.*, 1995). Its traditional use as mosquito repellant by Meru herbalists is therefore in agreement by study done by Yenesew *et al.* (2004).

Significant clinical findings involved the Central nervous system which was the most affected body system. The clinical signs were as a result of the central nervous system being affected. Perhaps the same clinical manifestations in other animals including livestock would be manifested in acute toxicity by *Tephrosia vogelii.* The anthelmintic activity of *Tephrosia vogelii* aqueous leaf extract against *Ascaridia galli* reported by

Siamba *et al.*, (2007) may perhaps be due to paralysis of the intestinal worms and thus expulsion by peristaltic movements of the gastrointestinal tract.

The data on Table 19 was used to calculate median lethal dose (LD_{50}) of the plant extract using the method described by Reed and Muench (1938). Based on classification of toxicities of substances by Loomis (1974), *Tephrosia vogelii* is classified as being slightly toxic since its LD_{50} was found to be between 500-5000 mg/kg of body weight. The plant *Tephrosia vogelii* is used traditionally as rodenticide to control moles in the farms. The current study has shown that the plant causes paralysis of the hind limbs in rats. The paralysis of the hind limbs may be as a result of damage of the neurons in the brain which was seen in histopathological sections in the current study. This plant therefore is classified as a neurotoxic, although the exact mechanisms of action are currently unknown. Literature indicates that the plant contain rotenone and related compounds which are known to be neurotoxic.

The clinical manifestations for acute toxicity exhibited by rats in this study may perhaps be used to tentatively diagnose cases of suspected poisoning in other animals by this plant. Moreover, *Tephrosia vogelii* has also been used as fish poison and traditionally its pounded leaves are put in water to paralyze the fish and make them float in water and are then collected. Further studies should be carried out on the toxic effects on humans consuming fish poisoned with this plant. In addition, Dzenda, *et al.*, 2008 described this plant as abortifacient and therefore consumption of this plant by pregnant animals requires further investigation. Consumption of poisoned fish by T. vogelii should be discouraged since this plant is abortifacient.

5.2. Conclusions.

1) Meru Central district is rich in biodiversity of medicinal plants and at least 85 species of both medicinal and biopesticides plants are in this area.

2) Traditional medicines in Meru are in the custody of old people and young generation are less involved in the herbal practice.

3) Ethnodiagnosis and traditional management of diseases are adequate in Meru Central District because traditional practitioners could identify various diseases and their clinical manifestations.

4) Medicinal plants are faced with the threat of extinction since the herbalists claimed to travel long distance in search of medicinal plants that used to be common in the area.

5) Policy concerning use of herbal medicines is weak since herbalists were working individually and were not registered or regulated.

6) *Tephrosia vogelii* is highly toxic to brine shrimps since its LC_{50} of 123 µg/ml and it toxicity is similar to that of pyrethrum (*Chrysanthemum cinerariaefolium)* which is a commercial biopesticide whose LC_{50} was found to be 107 µg/ml in an earlier study

7) *Tephrosia vogelii* is toxic to rats with the LD_{50} of 3163mg/kg body weight and its use by farmers as a botanical rodenticide against moles is justified.

8) The clinical manifestations in acute toxicity, post mortem lesions and the histopathological lesions show that *Tephrosia vogelii* is a neurotoxicant.

5.3. Recommendations

1. There is a strong need to conduct country wide ethnobotanical survey and document all the useful plants used as medicinal and biopesticides and come up with the national policy on their sustainability and conservation strategies.
2. Herbalist in the study area should be sensitized on the need of forming a registered herbalist association with relevant authority with the aim of exchange of knowledge, commercialization and conservation. This will allow the engagement of the various stakeholders in all areas of the value chain of medicinal plants. Some herbalists in some other areas of Kenya has already initiated this process.
3. Further research is needed to scientifically validate the herbalists' claims. This will include efficacy and safety.
4. Further research on the Phytochemistry of the important plants identified and collected in the study areas is also required. This research will bring together the herbalists, community members, researchers from government institutions and other stakeholders in the industry.
5. Farmers should therefore be encouraged to use *Tephrosia vogelii* to control invasion of rodents in their farms since the plant has been shown to have rodenticide activity in the current study.
6. Farmers should be advised to conserve this plant by cultivation for future sustainability.
7. Screening of bioactivity and toxicity of plants identified from the study area is recommended as a step towards their value addition for commercialization. Proper

use of these medicinal plants can be a source of income to the community and other stakeholders where these plants are naturally growing.

8. There is a need to educate and sensitize young generation on the importance of medicinal plants as a livelihood support enterprise. The areas that need to be emphasized on include; conservation, harvesting, marketing and value addition and utilization. The paucity of knowledge about medicinal plants in the young generation is evidenced by the results of the current study where the average age of the respondents interviewed was approaching 60 years.

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APPENDICES

Appendix I: Botanical plant identification

NATIONAL MUSEUMS OF KENYA

NATIONAL MUSEUMS OF KENYA

E. A. HERBARIUM
P. O. Box 45166
NAIROBI, KENYA.
Tel 743513

Ref No.Our ref..KN,UoN/Herb/2008

Date: 28th April 2008

Dr Itonga Stanley
University of Nairobi
Dept. of Public Health and Pharmacology
P.O. Box 29053
Nairobi

Dear Sir,

REF: PLANT IDENTIFICATION

The plant specimen which you submitted to the Herbarium for identification has now been determined as Tephrosia vogelii Hook.f.in the family Papilionoideae.
This plant is cultivated as a fish poison and is probably not indigenous in Kenya
The root decoction is said to treat scabies and yaws, while the decoction of the root is used in the treatment of constipation. (Kokwaro, 1993. Medicinal plants of East Africa.

There is a good account of this plant given in the Wealth of India. Vol. X Sp-W pg. 155-157.

Yours sincerely.

Geoffrey M Mungai
FOR: BOTANIST IN CHARGE

Appendix II: Data acquisition and survey questionnaire

Project Title: Documentation on use and evaluation of ethno botanicals in Meru, Kenya.

Serial number of the questionnaire.....

Name of the interviewer..... Date.....

Part One: Consent

A .Researcher's declaration

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary rights of the herbal practitioners/Meru community.

2. We will at no time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.

3. We will be under no obligation to edit or tamper with the information provided by the respondents.

4. The respondents will be informed of the intended project elaborately prior to questionnaire administration and in confidence to eliminate any degree of conspiracy.

5. The information collected will be used for the described purpose and not any undisclosed intentions.

Researchers:

Dr Itonga S. M. (BVM).....Date.....

B: Respondents consent agreement

I.....hereby agree to participate in this study with my full consent and conscience and declare that to best of my knowledge the information that I have provided is true, accurate and complete.

Signature/Thumb print.....Date.....

Part Two

A: Biodata (Respondent details)

Name.....age (yrs).....sex.....

Location- division.....location.....sub location.....village.....

Occupation.....

Level of education (none; primary; secondary; college; other.....

Contact address.....

B: Medicinal Plants

Type of plant (vernacular name)	Preparation method	Adm. Form and route of adm.	Part of plant used	Approx dosage	Conditions treated and humans/animal species

C: Toxic Plants

Type of plant (vernacular name)	Preparation method	Adm. Form and route of adm.	Part of plant used	Approx. dosage	Conditions treated and humans/animal species

D: List of plants used as biopesticides

Type of plant (vernacular name	Part of plant used	Preparation, route of adm. and dosage	Pest(s) controlled.	Duration of response after application.

Appendix III: List of informants and researchers

No.	Name	Sex	Age	Remarks
1	Dr Stanley M. Itonga	M	-	Investigator (University of Nairobi)
2	Dr. James M. Mbaria	M	-	Researcher (University of Nairobi
3	Dr Daniel W. Gakuya	M	-	Researcher (University of Nairobi)
4	Jeniffer K. Marangu	F	49	Chief Mwanganthia Location
5	Fabian M. Murira	M	-	Assistant Chief Gatuune Sub location
6	Geoffrey K. Mwitia	M	-	Assistant Chief Igane Sub location
7	William M'Kiambati	M	60	Herbalist
8	Zaverio Nkonge Muthamia	M	63	Herbalist
9	Paskasio Gitonga	M	62	Herbalist
10	Romano M'Muga	M	73	Herbalist
11	Peter Mutwiri	M	54	Herbalist
12	Henry Gikamati	M	60	Herbalist
13	Edward Kabui	M	55	Herbalist
14	Alfano Njogu	M	56	Herbalist
15	Joseph Mbwiri	M	60	Herbalist
16	Silas M'Mwamba Kobia	M	71	Herbalist
17	Joseph Mbau Muthamia	M	52	Herbalist
18	Isabella Nderi	F	48	Herbalist
19	Zachariah Mwaki	M	39	Herbalist
20	M'Mbugi M'mukiri	M	79	Herbalist
21	John Mbaabu Mwobobia	M	55	Herbalist
22	Juliana M'Mbugi	F	60	Herbalist
23	Fredrick Kiraithe	M	28	Herbalist
24	Mutiga M'Muthuri	M	65	Herbalist
25	Lewis Kirimi	M	58	Herbalist
26	Domenic Mutwiri Rukunga	M	68	Herbalist
27	General Muchori (Ex- mau mau fighter)	M	80	Herbalist
28	Evangiline M'Mwamba	F	82	Herbalist

Appendix IV: Data on Brine Shrimp Lethality Test Record Sheet

Date before exposure...4/3/10.....Name of drug/plant extract Tephrosia..... **Date after exposure.....**										
	Tube 1		**Tube 2**		**Tube 3**		**Tube 4**		**Tube 5**	
	No. shrimps		**No. shrimps**		**No. shrimps**		**No. shrimps**		**No. shrimps**	
Conc. µg/ml	B/4	Dead after 24 hrs	B/4	Dead after 24 hrs	B/4	Dead after 24 hrs	B/4	Dead after 24 hrs	B/4	Dead after 24 hrs
Control	10	0	10	0	10	0	10	0	10	0
10	10	2	10	1	10	1	10	0	10	2
100	10	3	10	2	10	1	10	4	10	3
1000	10	10	10	10	10	10	10	10	10	7

Key B/4- Before

Appendix V: Life forms of plants in ethnobotanical in Meru Central District

	Life Form	**Number**	**Percentage**
1	Tree	30	35.3
2	Shrub	33	38.8
3	Herb	21	24.7
4	Liana	1	1.2
	Total	**85**	**100**