ETHNOBOTANICAL, BIOACTIVITY AND PHYTOCHEMICAL EVALUATION OF ANTHELMINTIC HERBAL REMEDIES OF MIGORI COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University or Institution and that all sources of materials used for the thesis have been duly acknowledged.

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To my father Jackton Sirama, my mother Cyprine Sirama and my friend David Onyisi for their invaluable support throughout the study.

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ACRONYMS AND ABBREVIATIONS

ANOVA	: Analysis of Variance
DMSO	: Dimethylsulfoxide
DNA	: Deoxyribonucleic acid
IPR	: Intellectual Property Rights
IR	: Independent Reports
MI	: Mortality Index
NAI	: Nairobi University Herbarium
NBC	: National Boundaries Commission
PBS	: Phosphate buffered saline
PHC	: Primary Health Care
PPB	: Pharmacy and Poisons Board
SD	: Standard deviation
TM	: Traditional Medicine
TMPs	: Traditional Medicine Practitioners
WHO	: World Health Organization

ABSTRACT

Intestinal worms affect a host of individuals resulting in malnutrition, stunted growth, intellectual retardation and cognitive deficits. The aim of this study was to investigate the indigenous plants of Migori County with anthelmintic activity using adult *Haemonchus contortus* worm as a model and determining the presence of active principles. Twenty six (26) herbalists between the ages 20-69 years (10 men and 16 women) were interviewed on plants used as anthelmintics. Local name, parts used, mode of preparation and administration were documented. There was a high correlation (r=0.96) between age of informants and number of medicinal plant citations. The study identified 21 anthelmintic plants distributed among 21 genera and 13 families. The plant families most commonly used includes Asteraceae (27.72%), Leguminosae (15.79%) and Lamiaceae (10.53%). The three most frequently used plants: *Eclipta alba* (Asteraceae), *Vernonia amygdalina* (Asteraceae) and *Plectranthus barbatus* (Lamiaceae) were collected, extracted in methanol, acetone and distilled water for phytochemical studies. The phytochemical screening focussed on saponins, tannins and cardiac glycosides using standard procedures. *In-vitro* anthelmintic activities study of crude extracts of these selected plants were also done using adult *Haemonchus contortus* worms to test their potency. The anthelmintic activity of 6.25 mg/ml, 12.5 mg/ml and 25 mg/ml concentrations of aqueous, acetone and methanol crude extracts of *Eclipta alba* (whole plant), leaf and root extracts of *Vernonia amygdalina* and leaf extract of *Plectranthus barbatus* were compared with the effect produced by the standard reference drug albendazole. Extracts at 25 mg/ml concentrations were most active. Phosphate Buffered Saline (PBS) was used as a negative control. Death was determined within a period of 24 hrs. Extracts tested were active against *Haemonchus contortus* except 6.25 mg/ml concentration of acetone extract of *P. barbatus*. *Eclipa alba* (whole plant) extract was the most potent followed by *Vernonia amygdalina* root extract. *Plectranthus barbatus* leaf extract

was the least potent. Albendazole had an overall average MI of 1.00 which translates to 100% potency. Analysis of variance revealed that there were significant differences in the anthelmintic activity of the plants' extracts of all the concentrations (6.25, 12.5 and 25 mg/ml) used at $p<0.05$. All the three plant extracts exhibited anthelmintic activity therefore could be a cheap and readily available alternative source of anthelmintic treatment.

Key words: *Eclipta alba*, *Vernonia amygdalina*, *Plectranthus barbatus*, *Haemonchus contortus*, Phytochemicals, *In-vitro* anthelmintic activity, Albendazole, Migori County, Kenya.

CHAPTER ONE

1.0 Introduction

Disease has been an integral part of man from the beginning of his existence. The subject of drugs and the search for remedies to combat disease is perhaps equally old. In Asian countries, especially in China, Japan and Korea, herbal medicine has been extensively used, safely and effectively to alleviate various symptoms of disease for more than a millennium (Muthu *et al.*, 2011).

Worldwide, traditional herbal medicine has preoccupied mankind in his evolution (Mwangi *et al.,* 2005). The World Health Organization (WHO) estimates that 70-90 % of Africa's rural population relies on traditional medicine to meet its health needs, and thus recognizes herbal medicine as an essential component of primary health care (PHC) (Mwangi *et al.,* 2005).

A large population of people in the developing countries depend on traditional medicine for PHC and it is important that these herbal remedies are investigated for their efficacy by determining their chemical composition and safety, since adulterated, poor quality or poisonous herbal preparations are serious threats to public health (Awodele *et al.*, 2011; Kokwaro, 2009).

Studies of wild animals show that they instinctively eat certain plants to treat themselves from certain illnesses (Oreagba *et al.,* 2011). Similarly since time immemorial man has used plants to treat various human and livestock ailments. The whole plant or sometimes plant parts have been used to prepare the drugs. Different methods such as boiling, soaking, burning, pounding, chewing, heating or roasting have been used to prepare drugs (Kokwaro, 2009). Much of the traditional medicine research has centered on the medicinal value and efficacy of herbs and other pharmacopoeia (Kokwaro, 1976).

Plants existing in different geographical regions exhibit varying concentrations of secondary metabolites. In addition, plant secondary metabolites differ from plant to plant and also within the same plant they differ from one part to the other (Ali *et al*., 2003). Plant secondary metabolites offer the plants protection from animals, diseases, pests and adverse environmental conditions making them grow well in their environment. From the pharmacological point of view, plant secondary metabolites have various bioactivities responsible for toxicity and therapeutic value (Ali *et al*., 2003).

Helminth infections (helminthiasis) are the most common infections in man that affect large proportions of the world's population (Piyush and Rupali, 2011). Most diseases caused by helminths are chronic and debilitating in nature. They probably cause more morbidity, greater economic and social deprivation among humans and animals than any other parasites. Helminthiasis is endemic in regions with poor sanitation, poor family hygiene, malnutrition and crowded living condition. It has been estimated that about half of the world's population suffers from helminthiasis and the number is increasing. In the treatment of helminthiasis, anthelmintic drugs are used irrationally and recently anthelmintics use has been found to produce toxicity in human beings (Piyush and Rupali, 2011). However the high costs of conventional anthelmintics has limited effective control of the parasites. In some cases, wide spread use of low quality anthelmintics has enhanced development of resistance (Piyush and Rupali, 2011). Hence the discovery of new plants containing bioactive substances that act as anthelmintics is considered a breakthrough in managing this disease (Piyush and Rupali, 2011). The use of medicinal plants for the prevention and treatment of gastro-intestinal parasitism has its origin in ethnoveterinary medicine (Athanasiadou *et al.*, 2007).

Currently, pharmaceutical industries invest millions of dollars seeking promising medicinal herbs with novel chemical compounds which could be used as disease therapies (WHO, 2002). Helminths pose a large threat to public health and contribute to the prevalence of anaemia, eosinophilia, pneumonia and malnutrition (Ravindra and Anita, 2007). Various other opportunistic infections can also stem from helminthic infections. Because of the prevalence and impact of these parasitic worms, anthelmintic drug discovery is a priority in the pharmaceutical industry (Piyush and Rupali, 2011).

A large number of medicinal plants are claimed to possess anthelmintic property in traditional systems of medicines and are also utilized by ethnic groups (Ravindra and Anita, 2007). Information on chemical composition of these plants can be generated for further advanced research work, which may include the isolation of these chemicals and their subsequent use in the development of more efficient and safe anthelmintic drugs.

1.1 Literature review

1.1.1 Ethnobotany

Ethnobotany is the study of classification, use and management of plants by people (Gary, 2004). It is a multidisciplinary science, may involve ecology, economics, public health or whatever is needed to better understand the plants-people relationship (Alfredo, 2014). Major approaches to ethnobotanical research includes ethnobotany of all plants used by one group or several groups of people, ethnobotany of one plant species, ethnobotany of economically related plants, ethnobotany of plants with specific uses which was the focus of this research study, ethnobotany of plants by morphorlogical character and qualitative ethnobotany (Alfredo, 2014). Ethnobotanists explore how plants are used for such things as food, shelter, clothing, hunting and religious ceremonies (Connie and Steven, 1996). Ethnobotany has its roots in botany, the study of plants (Connie and Steven, 1996).

1.1.2 Traditional medicine (TM) and its significance

This is the sum total of all the knowledge and practices, whether applicable or not, used in the diagnosis, prevention and elimination of physical, mental or social disequilibrium and relying exclusively on practical experience and observation handed down verbally or in writing from generation to generation (WHO, 1978; Kokwaro, 2009). Traditional medicine utilizes plants/herbs, animal and mineral substances.

Many people in Kenya are already taking herbal medicines as self medication; these are usually prepared at home, or obtained from herbalists, pharmacies and supermarkets. In Kenya the registration of herbal medicines is done by the Pharmacy and Poisons Board (PPB); the national drug regulatory authority (Mwangi *et al.*, 2005).

In Kenya unlike in other countries like Germany and France, medical doctors do not receive any training in herbal medicine while in Asia medical doctors acquire postgraduate degrees or diplomas in herbal medicine (Mwangi *et al.*, 2005).

It is estimated that in Kenya, the doctor: patient ratio is 1:7142. However, complementary medicine practitioner: patient ratio is much better for example 1:987 in Mathare (an urban area) and 1:378 Kilungu (a rural area) are not very different from those in other developing countries (Mwangi *et al.*, 2005).

Kenya faces several challenges in the integration of traditional herbal medicine in national health care. These include: lack of a national policy and regulatory framework; issues pertaining to safety, efficacy, quality, access and rational use of traditional herbal medicine; lack of healthy cooperation and communication between complementary medicine providers and medical practitioners; lack of a clear policy on Intellectual Property Rights (IPR) and equitable benefit sharing relating to herbal medicine; traditional/indigenous knowledge; biopiracy and unsustainable use of medicinal plants (Mwangi *et al.*, 2005).

Since the 1960s forest cover in Kenya has greatly reduced from 12.5% to 2.5%. This is attributed to rapidly expanding population, illegal logging and acquisition of land for cultivation (FSK, 2008). This has led to a major impact on medicinal plants, and loss of some species (Shanley, 2003). It is therefore important that plant biodiversity is conserved in order to protect our natural habitat in the long run (Ugulu, 2011). This calls for systematic cultivation of medicinal plants in order to conserve biodiversity and protect endangered species (Joy *et al.,* 1998).

In the recent years, the importance of herbal drugs in medicine has tremendously increased because of their association with fewer side effects (Piyush and Rupali, 2011). Consequently, the demand for herbal formulations is increasing. The phytochemical constituents and their standardization have been accelerated with the development of analytical tools which makes this field important and new for investigation (Piyush and Rupali, 2011).

Traditional medicine has gained popularity as a source of primary health care (PHC) worldwide because of its affordability and social acceptance. In very remote areas where it's very difficult to access modern medicine services, the inhabitants mainly resort to herbal medicine for their health care needs. Many diseases in the rural areas can be prevented or treated by simple means; by traditional healers, who while not scientifically qualified, are within easy reach (Kokwaro, 2009). The technical inadequacies of modern medicine and the ever rising cost of modern drugs means that with the possible discovery of new medicaments and broader utilization of the resources of the African pharmacopoeia, the treatment of certain diseases might become more effective and less costly. TM can also be used in the prevention of certain diseases through measures against vectors and parasites (Kokwaro, 2009). Through scientific

investigations of the medicinal plants, the discovery and development of new drugs can be achieved.

Although TM has been accepted worldwide, some of its aspects require attention if it is to be mainstreamed in health services. These include its imprecise nature of diagnosis, lack of precision in dosage, possible misuse of non-material aspects and practice of quackery (Kokwaro, 2009).

1.1.3 Traditional Medicine practitioners (TMPs)

A traditional medical practitioner also called traditional healer or simply medicine man is described as a person who is recognized by the community in which he/she lives as competent to provide health care by using plant, animal and mineral substances and certain other methods based on the social, cultural and religious background as well as on the knowledge, attitudes and beliefs that are prevalent in the community regarding physical, mental and social well-being and the causation of disease and disability (Kokwaro, 2009).

Lack of appropriate training, proper qualification and licensing schemes for TMPs makes it difficult for national authorities and consumers to identify qualified TMPs. There is also lack of organized networks of TMPs (WHO, 2003).

1.1.4 Anthelmintic plants

These are plants that are used traditionally in expelling the worms that are parasitic in nature from the body by either stunning or killing them. They are also known as vermifuges or vermicides (Kokwaro, 2009; Piyush and Rupali, 2011). A review perfomed by Ravindra and Anita, 2007 on plants with anthelmintic activity indicated several plants which include *Piliostigma thonningii*, *Butea monosperma*, *Cucurbita maxima, Punica granatum, Capparis decidua*, *Capparis spinosa, Anacardium occidentale*, *Carica papaya*, *Piper longum, Nigella*

sativa, Trachyspermum ammi, Ficus insipida, Nicotiana tabacum, Trifolium repens, Cannabis sativa, Melia azedarach, Embelia ribes, Ananas comosus, Strobilanthes discolor, Calotropis procera, Gynandra gynandra, Centratherum anthelminticum, Evolvulus alsonoides, Sapindus trofoliatus, Momordica charantia, Quercus petraea, Moghania vestita, Xylopia aethiopia (Ravindra and Anita, 2007). Several research studies both *in-vivo* and *in-vitro* on plants with anthelmintic potential have been conducted, a majority being *in-vitro* using worm samples like indian earthworm *Pheretima posthuma, Ascaris galli, Ascaris lumbricoides, Haemonchus contortus* e.t.c (Ravindra and Anita, 2007) therefore more plants of anthelmintic potential have been revealed.

1.1.5 Helminth parasites

Helminths are macroscopic worms causing a wide variety of diseases globally called helminthiases. There are three different kinds of helminthes: Platyhelminths (flat worms), Nemathelminths (non-segmented roundworms) and Annelida (segmented round worms). Those infecting human belong to the first two groups. Helminths are multi-cellular worms with sizes varying from 1mm to several metres in length. They develop through egg, larval (juvenile), and adult stages. Depending on the species, the infective stage is egg or larva (Jimenez-Cisneros and Maya-Rendon, 2007).

This study used *Haemonchus contortus* adult worm (fig 1), commonly known as 'barber's pole worm' found in the abomasums of sheep and goats (Wahab and Suhaila, 2007). It is a nematode belonging to the order Strongylida and is responsible for anaemia, bottle jaw (fluid accumulation in sub-mandibular tissues) and death of infected sheep and goats mainly during summer months in warm, humid climates (Joan, 2005). *Haemonchus contortus* is cylindrically shaped, tapered at both ends, and has a complete digestive system (Maria, 2006). It is a worldwide threat, but is

more prevalent in sub-temperate and temperate regions under warm and wet conditions (Maria, 2006). This parasite has a short life cycle of approximately three weeks. Grazing animals pick up the infective larvae on forages that are relatively short. Once in the rumen it continues to develop, move to the abomasum and become adults (20-30 mm). An adult female can lay thousands of eggs daily and can consume 200 microlitres of blood daily. An average of 10000 adults is enough to kill a sheep or a goat. The eggs (thin-shelled) are deposited in faeces, which then hatch to begin the life cycle again. During drought or very cold conditions a majority of larvae become dormant or die thus the transmission to the animal is very low (Joan, 2005).

Figure 1: *Haemonchus contortus* adult worm. Photo by Jim E.M

1.1.6 Bioactivity studies and its relevance

Bioactivity study is one of the novel areas of research. Bioactive molecules are derived from natural (biological) resources, recombinant DNA technology or synthetic chemicals. Their bioactivity may be beneficial or of adverse effects on the living cell (Wan-Loy and Ammu, 2008). In bioactivity studies, bioactive molecules are screened for their bioactivities which include: cytotoxicity, anticancer, antioxidative, antimicrobial, immuno-modulating, anti-inflammatory and anthelmintic effects. Bioactive molecules from biological resources are a potential source of pharmaceuticals and nutraceuticals (Wan-Loy and Ammu, 2008). It is

therefore important to assess the bioactivity of plant extracts (Subbaraju *et al*., 2005). This is the salvation of the natural product chemist, and as such it must be performed with all useful bioactive botanicals if these products are to be accepted and incorporated into legitimate long term, health practices (McLaughlin *et al*., 1998).

Helminthiasis causes threat both to human and domestic animals world-wide. Study of anthelmintic medicinal plants could help in discovering alternative sources of medicine for the eradication of helminth parasites.

1.2 Taxonomic description, ethnomedicine and phytochemistry of selected plants

1.2.1 *Eclipta alba* L. (Asteraceae)

Commonly known as false daisy. The specific epithet *alba* means white which refers to the color of the flowers. It is an erect or prostrate, small much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile, lanceolate, subentire, acute or subacute sparsely strigose with appressed hairs on both sides and with a tapering base, 2.5-7.5cm long, brown stem and small white flowers on a long stalk. It grows 3" tall (Mithun *et al.*, 2011; Muthu *et al.*, 2011).

Figure 2: *Eclipta alba* L. (Asteraceae). Photo by Sirama V.O

The herb has been known for its curative properties and has been utilized as antimytotoxic, analgesic, antibacterial, antiviral, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, anti-inflammatory, immunomodulatory, a good rejuvenator and as an anti-aging (Mithun *et al.,* 2011). It is used as a tonic and diuretic in spleen enlargement, treatment of memory disorder, edema, rheumatic joint pains, fever and improving digestion (Mukesh and Smita, 2010). It is also used in catarrhal jaundice and for skin diseases. The fresh juice of leaves has been used for jaundice, anemia, dysentery, eye diseases, asthma (Muthu *et al.,* 2011), increasing appetite, and as a mild bowel regulator (Mithun *et al.*, 2011)

The root has been reported to possess emetic and purgative property. The tincture of the plant is used for liver and kidney problem and it is also reported to have therapeutic potential against cardiovascular disorders. (Muthu *et al.,* 2011).

A wide range of chemical compounds including coumestans which is the main active principle, alkaloids, thiophenes, flavonoids, polyacetylenes, triterpenes, carbohydrates, saponins, tannins, phenols and their glycosides have been isolated from this species (Mithun *et al.,* 2011; Mohd *et al.,* 2013; Mukesh and Smita, 2010).

The leaves contain stigmasterol, β-terthienylmethanol, wedelolactone, dimethylwedelolactone and demethylwedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol; the aerial part is reported to contain a phytosterol, β-amyrin in the n-hexane extract and luteolin-7-glucoside, β-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant (Mithun *et al.,* 2011).

1.2.2 *Vernonia amygdalina* Del. (Asteraceae)

This is a shrub or a small tree (fig 3). Leaves lanceolate to obovate-lanceolate, up to 15 cm long, 5 cm broad, finely glandular and pubescent beneath. Flower heads are white, sweet scented, 8 mm in diameter. Pappus tawny, bristles equal or some shorter (Kokwaro and Johns, 1998).

Figure 3: *Vernonia amygdalina* Del. (Asteraceae). Photo by Sirama V.O

Extracts from leaves have been found to possess antimalarial activity against plasmodium (Masaba, 2000; Abosi and Roseroka, 2003) and possess activity against sexually transmitted diseases. Chewing a stick of *V. amygdalina* has been found to have antibacterial activity (Taiwo *et al.*, 1999) and water soluble anti cancer agents have also been discovered from the plant (Izevbigia, 2003).

A concoction made form *V. amygdalina* is prescribed treatment for schistosomiasis, amoebic dysentery, and several other intestinal parasites. It has also been reported that *V.*

amygalina causes a marked reduction in blood pressure. Extracts from *V. amygdalina* have also been suggested to have cell growth inhibitory effects in prostate cancer cell line (Salah, 2006). Leaf ash or pounded paste has been rubbed onto scarification around the snake bite as an antidote; leaf juice has been used as an ear drop (Kokwaro, 2009).

Previous researches conducted by Akinjogunla *et al.,* (2011) and Anowi *et al.,* (2011) revealed the presence of flavonoids, tannins, saponins, cardiac-glycosides, anthraquinones, alkaloids, cyanogens and terpenoids.

1.2.3 *Plectranthus barbatus* Andr. (Lamiaceae)

This is a shrub up to 4 m high. Leaves are up to 10 cm long, margin crenate, softly hairy, velvety. Flowers bright purple-blue, up to 2.5 cm long (fig 4) (Kokwaro and Johns, 1998).

Figure 4: *Plectrathus barbatus* Andr. (Lamiaceae). Photo by Sirama V.O

P. barbatus have been used in the treatment of gastritis , teeth and gum disorders, burns, wounds, sores, insect bites, allergies, ringworms and measles (Kokwaro, 1993; Lukhoba *et al.,*

2006). It relieves colds, cough, bronchitis, pneumonia and for general respiratory ailments, throat and mouth infections, tonsillitis, gastro-intestinal and genitourinary infections. The plant is said to have antibacterial, antiviral, antifungal and antiprotozoal activity (Lukhoba *et al.*, 2006).

Organic extracts of *P. barbatus* have been reported to possess anti-inflammatory, antioxidant, cytotoxic, hypotensive, spasmolytic, hepatoprotective, antibacterial and antitumor activities (Alasbahi and Melzig, 2010).

A phytochemical study done by Kisangau *et al.*, (2007) indicated the presence of triterpenes, tannins, anthraquinones, flavonols, flavones and chalcones.

1.3 Problem statement

Half of the world's human population is suffering from helminth infections; the source of infection is due to poor sanitation, poor family hygiene, malnutrition, and crowded living conditions (Piyush and Rupali, 2011). Similarly millions of livestock are also affected by helminths resulting in considerable economic losses in farmyard and domestic animals (Ravindra and Anita, 2007). The development of anthelmintic resistance and the high cost of conventional anthelmintic drugs have led to the evaluation of medicinal plants as an alternative source of anthelmintics (Eguale and Giday, 2009).

1.4 Justification of the study

There is limited availability and affordability of modern medicines thus most of the world's population depends to greater extent on traditional medical remedies (Ravindra and Anita, 2007). Recently the use of anthelmintic drugs have been found to produce toxicity in human beings, so there is a need to develop safe anthelmintic drugs from plant sources (Piyush and Rupali, 2011). There is a need to search for new therapeutic agents due to the increasing instances of drug resistance by helminthic agents of human and animal diseases.

The number of higher plant species on earth are estimated at 250,000-500,000, of these, only about 6% have been screened for biological activity and a reported 15% have been evaluated phytochemically (Daniel and Norman, 2001). There are a great number of plants with purported antiparasitic properties, which have not been reproduced under experimental conditions (Athanasiadou *et al.,* 2007). This study opens opportunity for new discoveries that would be beneficial in managing helminthic infections.

1.5 Hypothesis

Anthelmintic plants used traditionally in a more repetitive fashion are more potent in the destruction and elimination of helminths than the less frequently used ones.

1.6 Research objectives

1.6.1 General objective

To study the ethnobotany, phytochemistry and bioactivity of anthelmintic plants of the Migori County, Kenya.

1.6.2 Specific objectives

1. To identify and record the major anthelmintic medicinal plants of the Luo of Migori County.
2. To determine anthelmintic plants cited with respect to the age of the informants.
3. To determine the presence of tannins, saponins and cardiac glycosides in selected anthelmintic plants.
4. To determine *in-vitro* activity of the selected anthelmintic plants on adult *Haemonchus contortus*.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1. Area of study

Migori County is located in the western part of Kenya in Nyanza Province between latitude 0°.24' South and 0°.40' South and longitude 34° East and 34°.50' East. It covers an area of 2,597 km^2 and borders Kisii, Homabay and Narok counties (see figure 5).

According to 2009 census, Migori County has a population of approximately 917,170 of which 34% of the population lives in the urban areas. The proposed County capital is Migori which is a cosmopolitan town. The Luo ethnic group is demographically dominant. Other ethnic groups include the Kuria, Luhya and Kisii. Migori County has four district hospitals. There are also clinics and dispensaries distributed within the County.

Districts that are mapped in this County according to the National Boundaries Commission (NBC) are Kehancha, Ntimaru, Migori, Nyatike and Rongo which have been divided further into a total of thirteen Divisions namely Karungu, Nyatike, Muhuru, Suba East, Suba West, Uriri, Awendo, Rongo, Mabera, Masaba, Kehancha, Kegonga and Ntimaru. The County has vibrant commercial centres which include Migori, Awendo, Rongo, Sori Karungu, Muhuru, Kehancha and Isibania (see figure 6).

Migori County experiences high temperatures of 21 degrees Celsius during the cold season and 35 degrees Celsius during the hot season. Rainfall is received in two seasons (March-May; October-December) with an annual average of 1200 mm. The County has an altitude of 1000 metres.

The major economic activity undertaken by most of the residents of Migori County is agriculture with the main commercial crops being sugarcane and tobacco. Other economic activities include fishing, mining and entrepreneurship.

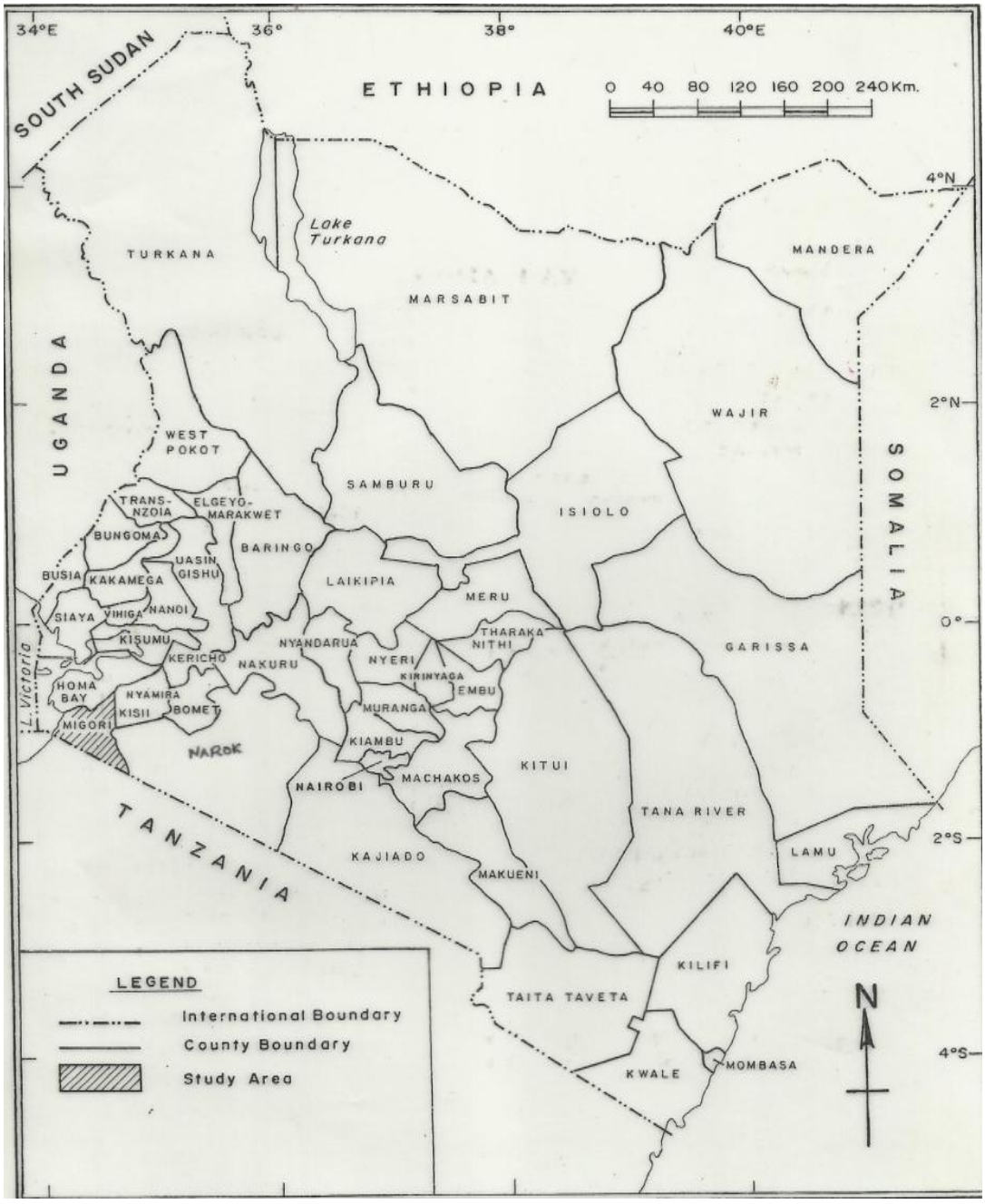

Figure 5: Map of Kenya showing the location of Migori County

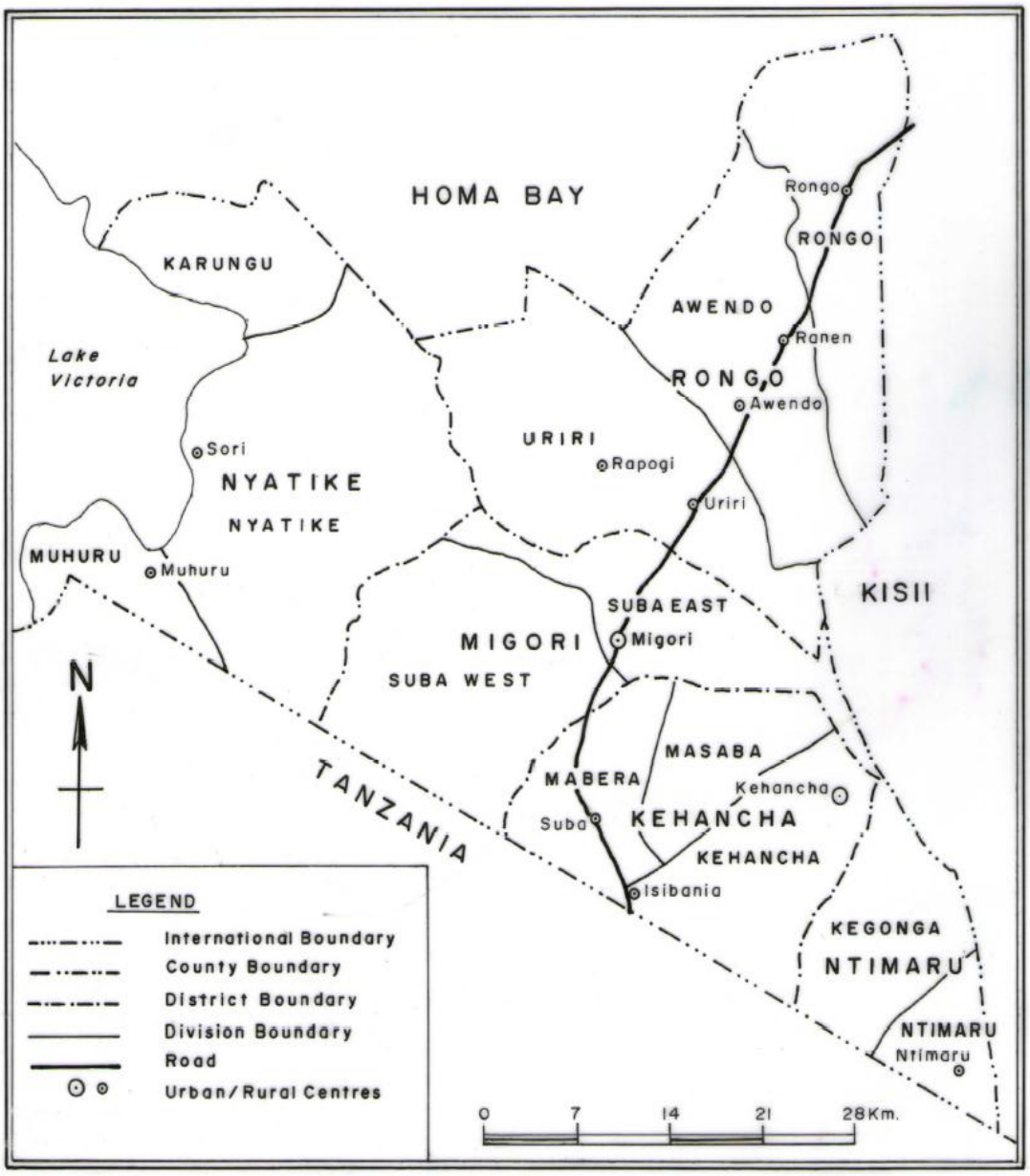

Figure 6: Map of Migori County showing thirteen Divisions

2.2 Collection of ethnobotanical data and plant specimens

A field survey was done prior to data collection, during which, a list of herbalists was prepared with the assistance of rural dwellers and the local authorities (chiefs, assistant chiefs) of Migori County. Thereafter, information on the anthelmintic plants was collected for two months (August 2013 and September 2013). During this period, identified herbalists were visited in their homes and interviewed on their knowledge of anthelmintic plants. As such, the sampling was intentionally non-random under the assumption that herbalists would provide more specific and higher quality information concerning anthelmintic plants (Luiz *et al.,* 2005).

Ethnobotanical data was collected in all the thirteen Divisions in the County. Data collection was based on open ended interviews of the herbalists (medical practitioners). A questionnaire was used and for any additional information, complementary questions were asked (Owuor, 1999). Twenty six (26) herbalists between the ages 20-69 years (10 men and 16 women) were interviewed on plants used as anthelmintics. For every plant cited, vernacular name, parts used, mode of preparation and administration was recorded. Guided tours to observe and collect the plants mentioned for identification and laboratory studies were done with the help of respondents. Ethnobotanical data was compiled from field notes and herbarium sheets.

Plant specimens were collected in duplicate; one specimen was used for preliminary identification in the field with the help of floras (Agnew, 2013; Beentje, 1994) while the other was pressed and transported to the University of Nairobi herbarium (NAI) for authentic identification by comparing with the permanently prepared herbarium collections at the NAI herbarium.

2.3 Selection of priority plants

Priority plants were selected based on a survey carried out between August 2013 and September 2013 in Migori County. The frequency report as an anthelmintic agent by the respondents was prepared, the plants were ranked according to the number of times they were mentioned as being anthelmintic from the highest to the lowest. Three plants that had the highest frequency were selected and the parts used as medicine were collected and subsequently subjected to chemical and bioactivity tests.

2.4 Collection of *Haemonchus contortus* worms

H. contortus worms were collected from the abomasums of freshly slaughtered sheep at Burma abattoir in Nairobi. The worms were washed with distilled water (1 litre) then suspended in 500 ml of phosphate buffer saline (PBS) which was prepared by dissolving 0.85g of sodium chloride and 1g glucose in 1 litre of distilled water. They were then transported to the Zoology laboratory at School of Biological Sciences, Chiromo campus, University of Nairobi in an air tight can. They were then left for 2 hrs to acclimatize before beginning tests (Ombasa *et al.*, 2012).

2.5 Preparation of crude extracts.

Eclipta alba (whole plant) was washed with water, dried and then chopped into small pieces; this was then dried under a shade for three weeks and then ground into a powder using an electric mill (Egwaikhide *et al.*, 2007). It was then packed in a labeled packet. 50 g of this powder was soaked separately in 300 ml of methanol, 300 ml of acetone, and 300 ml of water in 500 ml conical flasks, covered with aluminium foil for 72 hrs and then filtered using the Whatman filter paper. The methanol and acetone extracts were each evaporated on a rotary evaporator at 60°C to obtain crude extracts which were transferred to separate marked vials which were then placed in an oven at 40°C for 2 hrs to dry the plant extracts into powder.

Methanol and acetone extracts gave 3.53 grams and 4.19 grams respectively. 4.02 grams of water extract was realized. Water extract was deep frozen, freeze dried into powder then placed in a separate marked vial. The sample vials were kept at 4ºC for further use (Ombasa *et al*., 2012).

The above procedure was repeated for the other plant materials.

2.6 Phytochemical screening

Chemical tests were carried out on various extracts using standard procedures to identify the constituents (Ombasa *et al*., 2012).

2.6.1 Test for tannins:

The dried powdered extract sample was weighed into 0.5 mg, boiled in 10 ml of distilled water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration (Mariita *et al.*, 2011).

Procedure was repeated for the other extracts.

2.6.2 Test for saponins:

The dried powdered extract sample was weghed into 0.5 mg, added to 5 ml of distilled water and shaken vigorously for a stable persistent froth to occur. The froth was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Mariita *et al.*, 2011).

Procedure was repeated for the other plant extracts.

2.6.3 Test for cardiac glycosides (Keller-Killani test):

The dried powdered extract sample was weighed into 0.5 mg, boiled in 10 ml of distilled water then 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of 0.1% ferric chloride solution. This was then underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of cardiac glycosides. A violet ring may

appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Ombasa *et al*., 2012).

Procedure was repeated for the other extracts.

2.7 *In-vitro* anthelmintic activity

This was carried out as described by Ombasa *et al.,* (2012) with minor modification in the extract concentrations used. Each powdered extract was weighed into 0.625 gm, 1.25 gm and 2.5 gm, dissolved in 5 ml of dimethylsulfoxide (DMSO) and made to 100 ml mark using distilled water to make 6.25 mg/ml, 12.5 mg/ml and 25mg/ml solutions (Chinnaperumal *et al.,* 2011). Filter paper discs, 6 mm in diameter each impregnated with the above extract solutions were dried at room temperature to evaporate the DMSO. The discs for water extracts were not dried. Ten (10) adult *Haemonchus contortus* worms were placed into a sterile Petri dish containing 10 ml of phosphate buffered saline (PBS). The filter paper discs containing the aqueous extract was added and agitated; the same was done with the other filter discs impregnated with the other solvent extracts.

After 24 hours, the worms were removed from the Petri dish and then suspended in PBS for 30 minutes for possible recovery of their motility. Death was concluded when the worm lost their motility coupled with fading away of their body colour (Kumar *et al.,* 2009). The number of motile (alive) and immotile (dead) worms were counted using a hand lens and recorded. The above procedure was repeated for all the other plants' extracts. Triplicates were performed for each treatment. Albendazole (0.55mg/ml) was used as a reference drug (positive control). PBS was used as a negative control. Worm motility and mortality was used as the rationale for anthelmintic activity.

2.8 Statistical analysis

Descriptive statistics were used to analyse the data whereby applications in MS Excel® 2007 spread sheet were utilized to make simple calculations, determine proportions of plant families, habit, preparation methods and to draw graphs. Pearson correlation coefficient was used to determine the correlation of plant knowledge versus age range of informants or medicinal practitioners (Best and Kahn, 2011). The results obtained for anthelmintic activity were given as mean value ± standard deviation and the data were subjected to statistical analysis using analysis of variance (ANOVA) to determine whether there were significant differences in activity of the plant extracts at different concentrations used.

CHAPTER THREE

3.0 RESULTS

3.1 Ethnobotany of the identified anthelmintic plants

The study identified twenty one (21) anthelmintic plants distributed among thirteen (13) families and 21 genera. The plants botanical, local names, description and their mode of preparation are given below:

***Kigelia africana* (Lam.) Benth (Bignoniaceae) – Yago -**VOO 001/2013

A wide-low-spreading savannah tree, up to 16m high; bark grey to pale-brown usually rough. Leaves in threes up to 45 cm long, pinnate, leaflets usually 7-9, 6-12 cm long, scabrous, entire or serrate margin and rounded or acuminate apex. Corolla pale with reddish lines outside, reddish-purple, up to 10 cm long. Fruit grey-green, sausage-shaped, 30-90 cm long, 7-10 cm broad, usually round at both ends, fibrous, slightly rough on both surfaces. (Beentje, 1994; Kokwaro and Johns, 1998)

The root bark or leaves are used. The leaves are dried and burnt, concocted and then given to the patient using a spoon. The root bark are decocted and then given orally. Used for patient above one year.

***Rotheca myricoides* (Hochst.) Vatke (Verbenaceae) – Okwero -** VOO 002/2013

An erect shrub, 1.3 m high, with pubescent or glabrous branchlets. Leaves opposite or in whorls of 3-4, shortly petiolate or subsessile, ovate or slightly obovate, base cuneate or attenuate, apex acute, margin toothed or rarely entire, 5-15 cm long, glabrous above. Flowers blue or purple occasionally greenish with one lobe blue or blue with two lobes white; in lax panicles; corolla-tube 5-10 mm, lobes 8-17 mm long; upper lobes white or pale-blue, lower lobes violet-

blue. Fruit black 8mm long (Beentje, 1994; Kokwaro and Johns, 1998; Kokwaro and Johns, 2013).

Roots are pounded, infused and given orally. Normally used for children above one year.

***Vernonia amygdalina* Del. (Asteraceae) – Olusia -** VOO 003/2013

A shrub or a small tree 2-8 m; bark pale grey; twigs tomentose. Leaves lanceolate to obovate-lanceolate, up to 15 cm long, 5 cm broad, finely glandular and pubescent beneath. Flower heads white, sweet scented, 8mm in diameter, phyllaries 3-4 mm long with dark tips. (Beentje, 1994; Kokwaro and Johns, 1998).

Either the leaves or the roots are made into an infusion and drunk.

***Solanecio mannii* (Hook. F) C. Jeffrey. (Asteraceae) - Marowo/Maroo -** VOO 004/2013

A tree or a shrub, up to 7 m high, subsucculent, much branched and soft wooded, stem green with prominent leaf scars, leaves serrate, oblong-elliptic, acuminate, up to 45 m long, 10 cm broad. Flowers yellow or orange, scented, in terminal inflorescences, up to 60 cm long (Beentje, 1994; Kokwaro and Johns, 1998).

Leaves infused and given orally.

***Leonotis nepetifolia* (L.) R. Br. (Lamiaceae) – Nyanyodhi -** VOO 005/2013

A finely hairy, woody, annual or perennial herb, stems pubescent with soft white hairs. Leaves long-stalked, petioled, ovate, green, thinly hairy to densely clothed with soft white hairs beneath. Whorls numerous, many-flowered. Calyx densely hairy. Corolla white, yellow or orange, not much longer than the calyx (Agnew and Agnew, 1994; Kokwaro and Johns, 1998).

Leaf decoction drunk.

***Albizia coriaria* Oliv. (Leguminosae subfam. Mimosoideae) – Ober -** VOO 006/2013

A tree 6-36 m high, with flat spreading crown; bark rough and flaking. Leaves 3-6 pairs of pinnae; leaflets 6-11 pairs, 13-33 mm long, 5-14 mm broad. Flowers on pedicels 0.5-2 mm long; corolla 8-13.5 mm long, white. Fruit glossy brown or reddish, 10-21 mm by 3-4 cm, glabrous or nearly so. (Beentje, 1994; Kokwaro and Johns, 1998).

An infusion prepared by mixing pounded leaves with the pounded roots of *Euclea divinorum and Harrisonia abyssinica*, is taken orally.

***Carica papaya* L. (Caricaceae) - Poipoi -** VOO 007/2013

A tree up to 7 m high; trunk is about 20 cm in diameter with no lateral branches but sometimes dividing into several erect stems bearing heads and leaves. Bark pale-grey; smooth, well marked with leaf scars. Leaves simple, sometimes 60 cm across, usually palmate and deeply 7-lobed. Staminate flowers sessile in slender racemes; pistillate flowers solitary, about 2.5 cm long, corolla yellow. Fruit large, to 50 cm long, oblong or nearly spherical, green at first but turning yellow or orange when ripe, seeds black (Kokwaro and Johns 1998; Dharani 2002).

Root decoction drunk.

***Ximenia americana* L. (Olacaceae) – Olemo -** VOO 008/2013

A tree up to 7 m high, deciduous, bark dark brown to black, with rectangular small scales; spines slender, 6-12 mm long. Leaves alternate or clusterd on spur shoots, elliptic or obovate 4-8 cm long, 2-4 cm broad, base cuneate, apex obtuse to emerginate. Flowers white,

about 1 cm in diameter, in auxillary cymes. Fruits orange, ellipsoid and edible (Beentje, 1994; Kokwaro and Johns, 1998).

Roots mixed with that of *Euclea divinorum*, decocted and drunk.

***Erythrina abyssinica* Lam. Ex DC. (Leguminosae subfam. Papilionoideae) - Orembe -** VOO 009/2013

A tree up to 8 m high with a short trunk and thick spreading branches; bark yellow brown; thick and corky, deeply fissured, with blunt woody prickles, branchlets with recurved prickles. Leaflets broadly ovate to rhomboid, pubescent or grey-tomentose up to 20 cm broad. Inflorescences 5-15 cm long. Flowers with brilliant orange-red heads. Pod 10-12 cm long, velvet pubescent and woody. Fruit: woody, straight or curved, furry brown pods up to 10 cm long (Kokwaro and Johns, 1998; Najama 2002).

The bark decoction drunk.

***Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae) - Ng'ong'o -** VOO 010/2013

A tree up to 11m high, bark pale grey, cracked; branchlets thick. Leaves 15-30 cm long, tufted at the ends of the branchlets; leaflets 11-19, elliptic or ovate opposite, 2-5 cm broad, margins entire or serrate-dentate. Flowers sessile, in erect terminal spikes 5-6 cm long; female flowers 2-3 together at the twig ends, petals green with purple-red tips. Fruits pale yellow and edible (Beentje, 1994; Kokwaro and Johns, 1998).

Bark decoction drunk.

***Plectranthus barbatus* Andr. (Lamiaceae) – Okita -** VOO 011/2013

A shrub up to 4 m high. Leaves up to 10 cm long, elliptic or ovate, base attenuate to truncate, apex obtuse to acute, edge crenate, softly hairy, velvety. Flowers bright purple-blue, up to 2.5 cm long (Beentje, 1994; Kokwaro and Johns, 1998).

Leaf decoction is taken orally. Usually suitable for patients who have stomach ache.

***Euclea divinorum* Hiern. (Ebenaceae) – Ochol -** VOO 012/2013

A much branched evergreen small tree up to 15 m. Bark dark grey, fissured. Leaves opposite, elliptic, 5-10 cm long, 2-4 cm broad, glabrous except for some reddish or pale scales beneath, base long-cuneate, apex obtuse. Flowers fragrant, cream, males 10 cm or more together in lax racemes, up to 4 cm long, females in stouter and short racemes up to 2 cm long; calyx lobes short. Fruit green, round, 6-7 mm (Beentje, 1994; Kokwaro and Johns, 1998).

Decoction of its roots mixed with concoction of the roots of *Harissonia abyssinica*, *Ximenia americana* and *Albizia coriaria* is drunk.

***Harrisonia abyssinica* Oliv. (Simaroubaceae) – Pedo -** VOO 013/2013

A much branched prickly shrub or small tree up to 6 m high, with grey bark. Leaves variable, imparipinnate, up top 12 cm long, often with pairs of prickles at the base, rachis winged. Flowers small and yellow in auxillary or terminal inflorescences. Fruit a small, black, 4-5 lobed berry (Beentje 1994; Kokwaro and Johns 1998).

Decoction of the roots either alone or in combination with *Euclea divinorum*, *Ximenia americana* and *Albizia coriaria* is drunk.

***Tamarindus indica* L. (Caesalpinioideae) – Chwaa -** VOO 014/2013

An evergreen tree, up to 16 m high with compact rounded crown and drooping branches. Bark rough, grey-brown. Leaves up to 15 cm long; leaflets in 10-15 pairs, oblong, base unequal, apex rounded, opposite, 1-2.5 cm long. Flowers in slender racemes about 8 cm long: sepals 4, yellow inside, reddish outside; petals 3, yellow streaked with red or orange. Pod pale-brown, about 10 cm long, usually curved, Fruit rusty-brown, straight or curved, edible (Beentje, 1994; Kokwaro and Johns, 1998).

Bark or leaf decoction drunk.

***Combretum collinum* Fres. (Combretaceae) – Keyo -** VOO 015/2013

A shrub or a tree up to 10 m high; crown flat or rounded, bark dark grey, usually smooth. Leaves alternate, opposite or verticillate, glossy above, ovate or (ob)ovate, base cuneate or rounded, apex obtuse to cuminate, paired or in whorls of 3-4, 10-12 cm long, about 4 cm broad, dark-green; petiole slender. Flowers white, in axillary spikes 5-10 cm long. Fruit 2.5-4 cm long and broad, dark brown (Beentje, 1994; Kokwaro and Johns, 1998).

Decoction of the roots used.

***Searsia natalensis* (Beernh. ex C. Krauss) F.A. Barkley (Anacardiaceae) – Sangla –**

VOO 016/2013

A small tree up to 6.5 m high, branchlets grey-brown. Leaves pale, trifoliate; petiole 1-4 cm long; leaflets sessile, obovate to oblanceolate, glabrous beneath, entire crenulate. Panicles slender, up to 15 cm long; flowers greenish-yellow and very small. Fruit orange, globose, about 8mm in diameter, edible (Beentje, 1994; Kokwaro and Johns, 1998).

Root decoction drunk.

***Bidens pilosa* L. (Asteraceae) – Anyiego -** VOO 017/2013

An erect annual herb, 60-100 cm high, glabrous or nearly so. Leaves 3-5 partite or pinnatifid; segments very sharply serrated, more or less ovate, 45-60 cm long, glabrous or slightly setulose on the upper surface. Flower-heads 7-15 cm in diameter, pedunculate in lax open corymbose cymes, ray flowers usually, sometimes yellow. Achenes black, ribbed; bristles usually 2 or 3, with reflexed barbs. A disturbing weed with achenes sticking on clothes and livestock skins (Agnew and Agnew, 1994; Kokwaro and Johns, 1998; Agnew, 2013).

The whole plant concoction taken orally.

***Cucumis aculeatus* Cogn. (Cucurbitaceae) – Otangle -** VOO 018/2013

A perennial herb with spiny yellow-hooked hairs on stem ridges and major veins underneath the leaves; leaves ovate not circular, deeply or shallowly 3-lobed; male flowers solitary, yellow-green, about 11 mm long; fruit green to yellow to 7 cm long with scattered bristle-tipped projections (Agnew and Agnew, 1994).

The fruit is cut then decocted, ½ a glass given to adults, 1 spoonful given to children below the age of 5 years while 2 spoonful given to children over 5 years. Insertion can also be made through the anus by the use of a syringe.

***Aloe secundiflora* Engl. (Aloaceae) – Ogaka -** VOO 019/2013

Fleshy herb with a rosette or opposite leaves, leaves very spiny at the edge, green or reddish. Flowers red, orange or yellow, in simple or branched inflorescences arising from the centre of rosette. Perianth tubular with 6 lobes. Fruit a capsule (Kokwaro and Johns, 1998).

The outer covering of the leaves are scrubbed off then the leaf is decocted and given orally.

***Eclipta alba* L. (Asteraceae) – Osieko -** VOO 020/2013

A low trailing bristly-hairy annual herb with stalkless elliptic leaves and shortly stalked heads with white florets; rays numerous no longer than phyllaries: phyllaries broadly ovate, 6 mm long: achenes 3 mm long, with corky warts (Agnew and Agnew, 1994).

The whole plant infusion is warmed in hot sun, kept overnight then given to the patient in the morning who is then not allowed to eat for a period of 5 hours.

***Hypitis suaveolens* Poit. (Lamiaceae) - Oluwo ndara -** VOO 021/2013

A strongly aromatic hairy annual herb to 1.3 m tall, with stalked ovate to heart-shaped leaves 18 by 13 mm; flowers purple in a loose inflorescence; sepal tube 6 by 4 mm, with 5 curved bristle-like teeth 3 mm long (Agnew and Agnew, 1994).

Whole plant decoction administered orally.

The frequency of usage of the plants by the herbalists was used to rank and the first three top-ranked were picked to be used for bioassay as given in table 1.

Table 1: Anthelmintic plants identified during the study.

Botanical name	Vernacular name	Family	Habit	Parts used	Mode of preparation	Number of Independent Reports (IR)	Frequency reporting Ranking
Eclipta alba VOO 020/2013	Osieko	Asteraceae	Herb	Whole plant	Infusion	26	1
Vernonia amygdalina VOO 003/2013	Oluswa	Asteraceae	Tree	Leaves, roots	Infusion	25	2
Plectranthus barbatus VOO 011/2013	Okita	Lamiaceae	Shrub	Leaves	Decoction	24	3
Cucumis aculeatus VOO 018/2013	Otangle	Cucurbitaceae	Herb	Fruits	Decoction	23	4
Solanecio mannii VOO 004/2013	Maroo	Asteraceae	Shrub	Leaves	Infusion	21	5
Albizia coriaria VOO 006/2013	Ober	Leguminosae subfam. Mimosoideae	Tree	Leaves	Infusion	20	6
Carica papaya VOO 007/2013	Poipoi	Caricaceae	Tree	Roots	Decoction	18	7
Aloe secundiflora VOO 019/2013	Ogaka	Aloaceae	Herb	Leaves, roots	Decoction	17	8
Rotheca myricoides VOO OO2/2013	Okwero	Verbenaceae	Herb	Roots	Infusion	16	9
Tamarindus indica VOO 014/2013	Chwaa	Leguminosae subfam. Ceasalpinioideae	Tree	Bark	Concoction	15	10
Kigelia africana VOO 001/2013	Yago	Bignoniaceae	Tree	Bark	Concoction	14	11
Ximenia americana VOO 008/2013	Olemo	Olacaceae	Tree	Roots	Decoction	12	12
Sclerocarya birrea VOO 010/2013	Ng'ong'o	Anacardiaceae	Tree	Bark	Decoction	11	13

Erythrina abyssinica VOO 009/2013	Orembe	Leguminosae subfam. Papilionoideae	Tree	Bark	Decoction	10	14
Euclea divinorum VOO 012/2013	Ochol	Ebenaceae	Tree	Roots	Decoction	8	15
Bidens pilosa VOO 017/2013	Anyiego	Asteraceae	Herb	Whole plant	Decoction	7	16
Combretum collinum VOO 015/2013	Keyo	Combretaceae	Tree	Roots	Decoction	6	17
Leonotis nepetifolia VOO 005/2013	Nyanyodhi	Lamiaceae	Herb	Leaves	Decoction	5	18
Harrisonia abyssinica VOO 013/2013	Pedo	Simaroubaceae	Tree	Roots	Infusion	4	19
Searsia natalensis VOO 016/2013	Sangla	Anacardiaceae	Tree	Roots	Decoction	2	20
Hypitis suaveolens VOO 021/2013	Oluwo ndara	Lamiaceae	Herb	Whole	Decoction	1	21

Twenty six (26) herbalists; ten (10) men and sixteen (16) women between the ages 20-69 years were interviewed during the study and the age range and average age calculated (table 2).

Table 2: Average age of the herbalists of Migori County.

Age class	**No. of herbalists (f)**	**Mid age class (x)**	**fx**
20-29	3	24.5	73.5
30-39	5	34.5	172.5
40-49	5	44.5	222.5
50-59	7	54.5	381.5
60-69	6	64.5	387.0
∑	**26**		**1237.0**

Number of herbalists = 26; Age range 69-20 = 49 years; Average age (∑fx/∑f) = 47.58 years.

Usage of anthelmintic plant families of Migori County is shown in table 3. The table indicates that Asteraceae is the mostly used with the highest percentage (27.72%) of the total number of independent reports (IR) followed by Leguminosae (15.79%). The least used is Simaroubaceae (1.40%).

Table 3: Usage of plant families

Plant families	Total no. of IR	% of total IR of family mentioned as anthelmintic
Aloaceae	17	5.96
Anacardiaceae	13	4.56
Asteraceae	79	27.72
Bignoniaceae	14	4.91
Caricaceae	18	6.32
Combrataceae	6	2.11
Cucurbitaceae	23	8.07
Ebenaceae	8	2.81
Lamiaceae	30	10.53
Leguminosae	45	15.79
Olacaceae	12	4.21
Simaroubaceae	4	1.40
Verbenaceae	16	5.61
Total	285	100

Differences in plants' usage depending on their habit was also recognised in which the trees had the highest representation (50.88%) followed by herbs (33.33%) and lastly shrubs (15.79%) as represented in pie chart (fig 7).

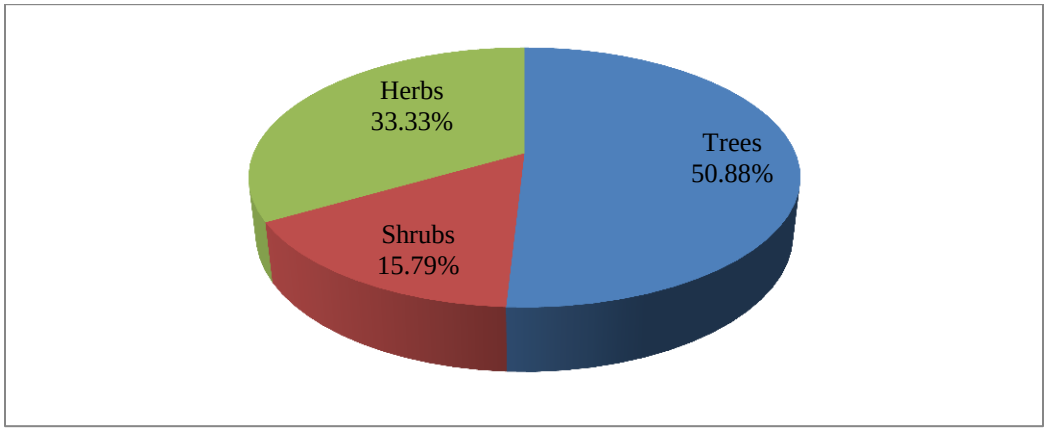

Figure 7: Distribution of anthelmintics in plant habit

Different plant parts were used with the leaf having the highest percentage (34.25%) followed by root (33.03%), bark (15.29%), whole plant (10.40%) and fruits (7.03%).

The various methods of preparation of medicine were applied with the decoction having the highest percentage of application (50.53%), infusion (39.30%) and concoction (10.18%). Pounding was universal as it appeared to be the preliminary method for every medicinal preparation.

Three plants *Eclipta alba* (whole plant), *Vernonia amygdalina* (roots) and *Plectranthus barbatus* (leaves) were selected for chemical and bioactivity tests based on their ranking as the top three plants most frequently used (table 4).

Table 4: Selected priority plants

Plant species	**Part collected**	**Rank**
Eclipta alba	Whole	1
Vernonia amygdalina	Leaves, Roots	2
Plectranthus barbatus	Leaves	3

50 grams of each powdered plant extract was soaked separately in acetone, methanol and distilled water to extract the plant compounds. Each of the crude plant extract obtained was weighed to determine their yield. Percentage yield was then calculated as follows:

Percentage yield=Quantity of Extract /Quantity of plant material X 100

The results are given in table 5.

Table 5: Yield and percentage yield of crude plant extracts

Plant species	**Methanol extract**		**Acetone extract**		**Water extract**		
	Yield (grams)	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**	**Average yield (grams)**
E. alba (whole)	3.53	7.06	4.19	8.38	4.02	8.04	3.91
V. amygdalina (leaves)	3.29	6.58	3.92	7.84	3.11	6.22	3.44
V. amygdalina (roots)	4.34	8.68	4.67	9.34	4.20	8.40	4.40
P. barbatus (leaves)	3.32	6.64	3.98	7.96	3.36	6.72	3.55
Average	3.62	7.24	4.19	8.38	3.67	7.35	3.83

Extraction efficiency was high in *V. amygdalina* (roots) with the highest yield averagely 4.40 grams and *E. alba* (whole) (3.91grams). *V. amygdalina* (leaves) was the least efficient (3.44 grams).

3.2 Knowledge on anthelmintic plants

From the twenty six interviews done on herbalists between the ages 20-69 years, the age group 60-69 years had the highest number of plants cited (15); the age group 20-29 years had the least number (4). The older the informants the more the plants cited as shown in fig 8.

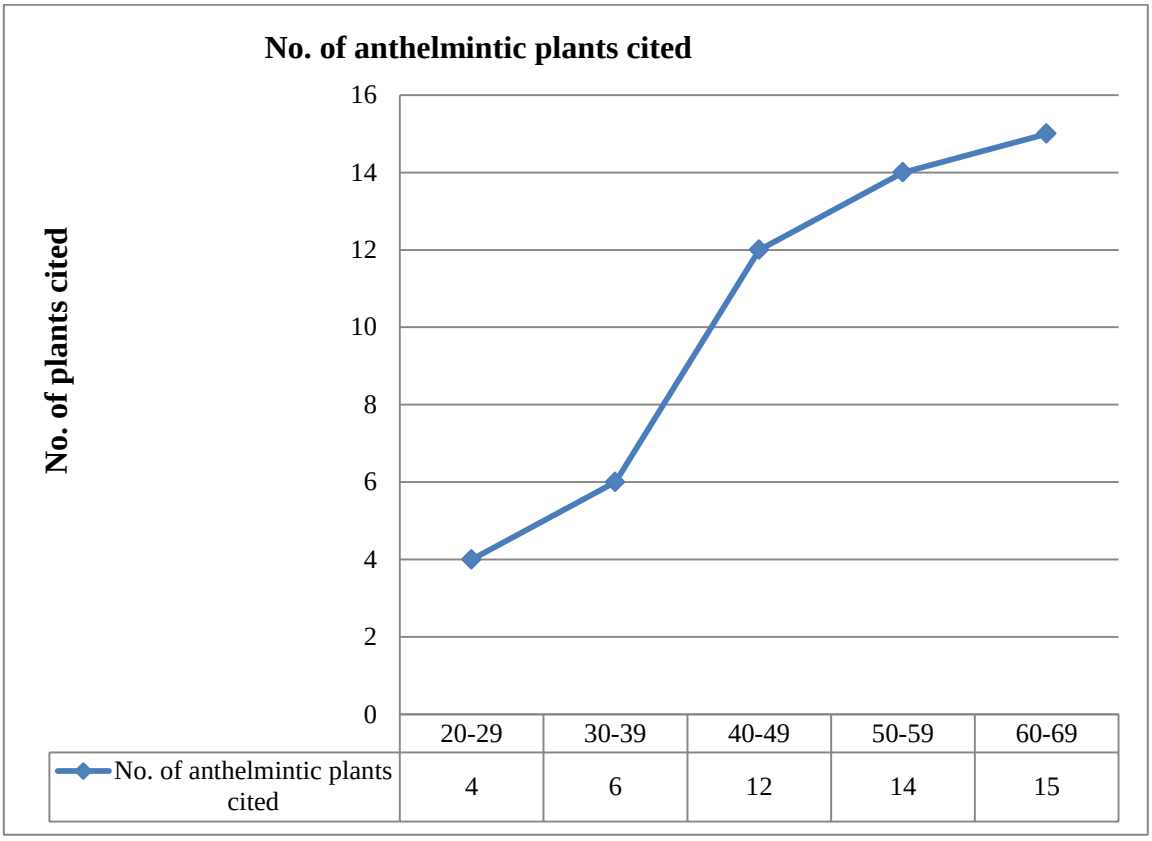

Figure 8: Age class of informants and the no. of anthelmintic plants

From this study the Pearson correlation coefficient (r =0.96) test, calculated from the line graph (fig 8) showed existence of high degree of positive correlation between age and the number of anthelmintic plant citations**.**

3.3 Phytochemical analysis of crude plant extracts for secondary metabolites

Extracts of each priority species was screened for tannins, saponins and cardiac glycosides using standard procedures (Ombasa *et al.*, 2012). The results are given in table 6.

Table 6: Phytochemical screening for each crude extracts for secondary metabolites.

Solvent / 2^{o} metabolites screened / Plant species	Methanol			Acetone			Distilled water		
	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides	Tannins	Saponins	Cardiac glycosides
E. alba (whole)	+	-	+	+	-	+	+	-	+
V. amygdalina (leaves)	+	+	+	+	+	+	+	+	+
V. amygdalina (roots)	+	+	+	+	+	+	+	+	+
P. barbatus (leaves)	+	-	-	+	-	-	+	-	-

Key: + = Present, - = Absent

3.4 *In-vitro* anthelmintic activity of crude plant extracts

Each of the solvent crude plant extract at concentrations of 6.25 mg/ml, 12.5 mg/ml and 25mg/ml was tested in triplicate for anthelmintic potential. Mean mortality at various concentrations were calculated as represented in table 7.

Table 7: Mean mortality ± SD of the extract concentrations used.

Plant species	**Extract**	**Mean mortality ± SD**		
		6.25 mg/ml	**12.5 mg/ml**	**25 mg/ml**
Eclipta alba (whole)	Acetone	2.67±0.577	3.33±0.577	3.67±0.577
	Methanol	4.00±1.000	5.33±0.577	7.67±0.577
	Aqueous	3.00±0.000	3.67±0.577	5.33±0.577
Vernonia amygdalina (roots)	Acetone	2.00±0.000	2.33±0.577	2.67±0.577
	Methanol	3.33±0.577	4.67±0.577	5.67±0.577
	Aqueous	2.00±0.000	2.67±0.577	3.33±0.577
Vernonia amygdalina (leaves)	Acetone	1.00±0.000	1.33±0.577	1.67±0.577
	Methanol	1.67±0.577	2.33±0.577	3.67±0.577
	Aqueous	1.00±0.000	1.67±0.577	2.33±0.577
Plectranthus barbatus (leaves)	Acetone	0.00±0.000	0.33±0.577	0.67±0.577
	Methanol	1.67±0.577	2.33±0.577	3.33±0.577
	Aqueous	0.33±0.577	0.67±0.577	1.33±0.577
Albendazole	0.55mg/ml	10.00±0.000	10.00±0.000	10.00±0.000
PBS	10 ml	0.00±0.000	0.00±0.000	0.00±0.000

Mortality index (MI) of the *Haemonchus contortus* was then calculated for the different crude extract concentrations (6.25, 12.5 and 25 mg/ml) by dividing the number of dead worms with the total number of worms per Petri dish and then overall mean mortality calculated; see tables 8, 9 and 10.

Table 8: Mortality index of *Haemonchus contortus* worms at 6.25 mg/ml.

Plant species	**Extract**	**1st trial MI**	**2nd trial MI**	**3rd trial MI**	**Mean MI for plant species in each solvent extract**	**Overall mean MI for each plant species±SD**
E. alba (whole)	Acetone	0.2	0.3	0.3	0.27	0.32±0.068
	Methanol	0.4	0.3	0.5	0.40	
	Aqueous	0.3	0.3	0.3	0.30	
V. amygdalina (roots)	Acetone	0.2	0.2	0.2	0.20	0.24±0.075
	Methanol	0.4	0.3	0.3	0.33	
	Aqueous	0.2	0.2	0.2	0.20	
V. amygdalina (leaves)	Acetone	0.1	0.1	0.1	0.10	0.12±0.0.040
	Methanol	0.1	0.2	0.2	0.17	
	Aqueous	0.1	0.1	0.1	0.10	
P. barbatus (leaves)	Acetone	0.0	0.0	0.0	0.00	0.07±0.091
	Methanol	0.1	0.2	0.2	0.17	
	Aqueous	0.1	0.0	0.0	0.03	
Albendazole		1.0	1.0	1.0	1.00	1.00±0.000
PBS		0.0	0.0	0.0	0.00	0.00±0.000

Table 9: Mortality index of *Haemonchus contortus* worms at 12.5 mg/ml.

Plant species	**Extract**	**1st trial MI**	**2nd trial MI**	**3rd trial MI**	**Mean MI for plant species in each solvent extract**	**Overall mean MI for each plant species±SD**
E. alba (whole)	Acetone	0.3	0.3	0.4	0.33	0.41±0.109
	Methanol	0.5	0.5	0.6	0.53	
	Aqueous	0.4	0.3	0.4	0.36	
V. amygdalina (roots)	Acetone	0.3	0.2	0.2	0.23	0.32±0.129
	Methanol	0.4	0.5	0.5	0.47	
	Aqueous	0.3	0.2	0.3	0.27	
V. amygdalina (leaves)	Acetone	0.2	0.1	0.1	0.13	0.18±0.050
	Methanol	0.2	0.2	0.3	0.23	
	Aqueous	0.2	0.2	0.1	0.17	
P. barbatus (leaves)	Acetone	0.0	0.0	0.1	0.03	0.11±0.106
	Methanol	0.3	0.2	0.2	0.23	
	Aqueous	0.1	0.0	0.1	0.07	
Albendazole		1.0	1.0	1.0	1.00	1.00±0.000
PBS		0.0	0.0	0.0	0.00	0.00±0.000

Table 10: Mortality index of *Haemonchus contortus* worms at 25 mg/ml.

Plant species	**Extract**	**1st trial MI**	**2nd trial MI**	**3rd trial MI**	**Mean MI for plant species in each solvent extract**	**Overall mean MI for each plant species±SD**
E. alba (whole)	Acetone	0.4	0.3	0.4	0.37	0.56±0.201
	Methanol	0.8	0.7	0.8	0.77	
	Aqueous	0.6	0.5	0.5	0.53	
V. amygdalina (roots)	Acetone	0.3	0.2	0.3	0.27	0.39±0.159
	Methanol	0.6	0.6	0.5	0.57	
	Aqueous	0.4	0.3	0.3	0.33	
V. amygdalina (leaves)	Acetone	0.2	0.2	0.1	0.17	0.26±0.103
	Methanol	0.3	0.4	0.4	0.37	
	Aqueous	0.3	0.2	0.2	0.23	
P. barbatus (leaves)	Acetone	0.1	0.0	0.1	0.07	0.18±0.136
	Methanol	0.3	0.4	0.3	0.33	
	Aqueous	0.1	0.2	0.1	0.13	
Albendazole		1.0	1.0	1.0	1.00	1.00±0.000
PBS		0.0	0.0	0.0	0.00	0.00±0.000

Overall mean mortality index for plant species at various crude extract concentrations are given in table 11.

Table 11: Overall mean mortality index for each plant species crude extract concentrations used (6.25, 12.5, 25 mg/ml).

Plant species	**6.25 mg/ml**	**12.5 mg/ml**	**25 mg/ml**
Eclipta alba (whole)	0.32±0.068	0.41±0.109	0.56±0.201
Vernonia amygdalina (roots)	0.24±0.075	0.32±0.129	0.39±0.159
Vernonia amygdalina (leaves)	0.12±0.0.040	0.18±0.050	0.26±0.103
Plectranthus barbatus (leaves)	0.07±0.091	0.11±0.106	0.18±0.136
Albendazole	1.00±0.000	1.00±0.000	1.00±0.000
PBS	0.00±0.000	0.00±0.000	0.00±0.000

Average MI of *Haemonchus contortus* at 6.25, 12.5 and 25 mg/ml crude extracts concentrations were calculated and have been represented in figures 9, 10 and 11 respectively.

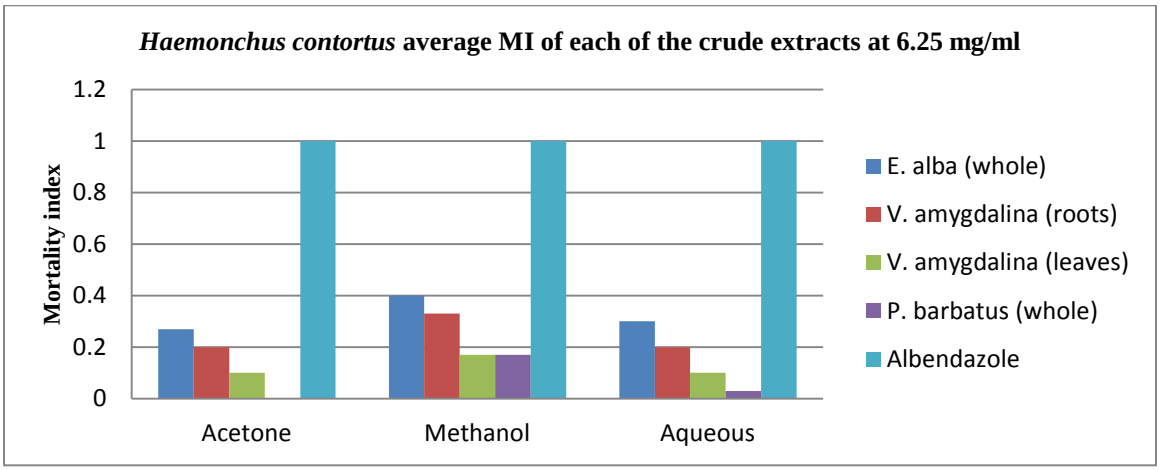

Figure 9: Average mortality index of *Haemonchus contortus* worms of the solvent extracts at 6.25 mg/ml.

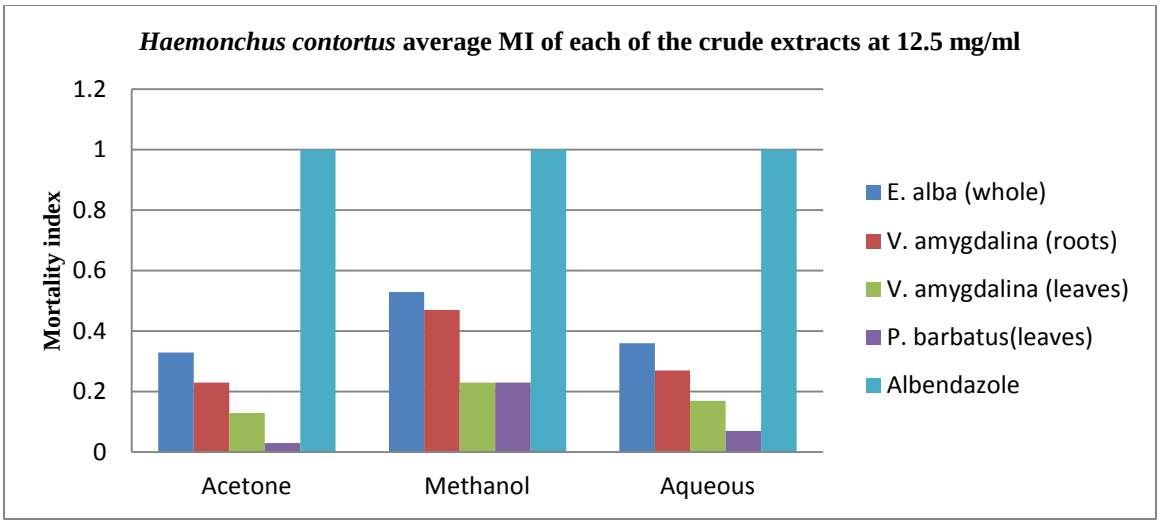

Figure 10: Average mortality index of *Haemonchus contortus* worms of the crude extracts at 12.5 mg/ml.

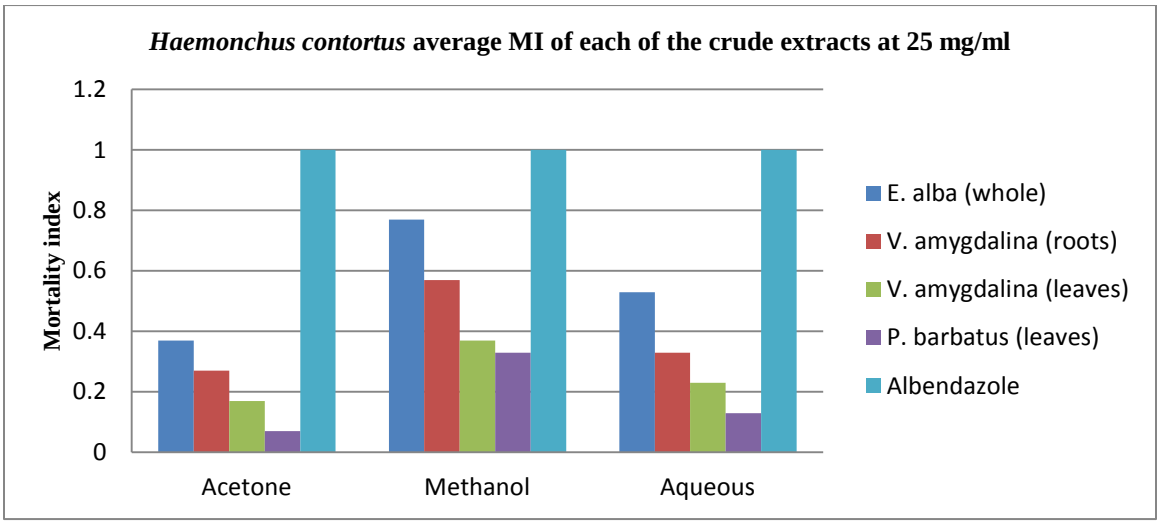

Figure 11: Average mortality index of *Haemonchus contortus* worms of the crude extracts at 25 mg/ml.

Overall average MI for each plant species for all the crude extracts at 6.25, 12.5 and 25 mg/ml concentrations was also calculated and represented in figure 12.

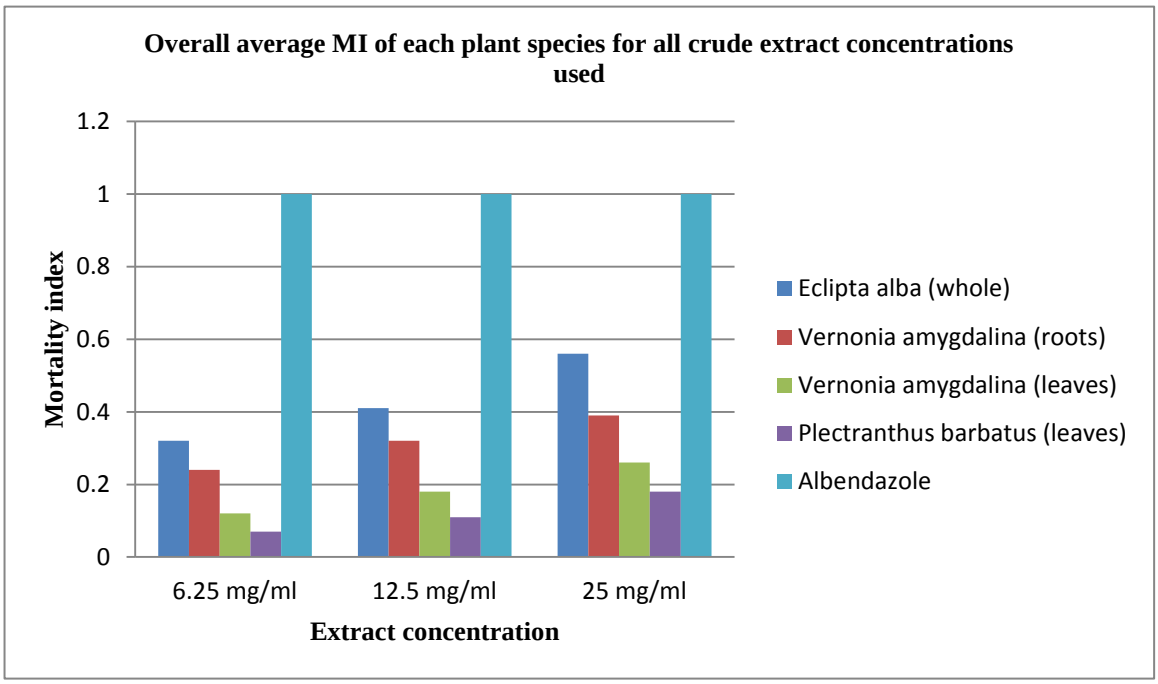

Figure 12: Overall average mortality index of each plant species for all crude extracts concentrations (6.25, 12.5 And 25 mg/ml).

CHAPTER FOUR

4.0 DISCUSSION

The knowledge on plant use is as old as the human race; it has been passed from generation to generation and was based mainly on the trial and error of a plant to a particular disease, and 'Doctrine of signatures' where a plant's ethnomedical usage was attributed to its characteristics (shape or colour).

Migori County traditional healers have rich and invaluable ethnomedicinal knowledge of indigenous and exotic plants which are found within the County. Most of these traditional practitioners are old men and women between the ages 20-69 years. Traditional medicine in Migori County faces a challenge of loss because these old people do not want to share the knowledge with the young people because they fear revelation which may compromise the effectiveness of the herbal medicines. Most of these traditional practitioners therefore prefer passing the knowledge to trustworthy people who can either be their children or relatives, or if need be, to other people in exchange with valuables. Some of the herbalists die without revealing their knowledge to other people leading to distortion or loss of valuable information (Kokwaro, 2009). Most of the herbalists interviewed in this study in Migori County are women between the ages 26-69 years; this is attributed to the fact that women nurses babies who are still vulnerable to various disease infections because of their underdeveloped immune systems and, they also give the post natal care to fellow women who still need a lot of medical attention.

During the study it was realised that traditional medicine men (magicians) use plants for treatment of some ailments and for most of their magical arts. Their knowledge of these plants are through ''*juogi*'' (magical powers or simply spirits) that illuminate, inspire and inform them on plants to use, where to collect them, parts to use and their mode of preparation.

The study identified 21 anthelmintic plants (table 1). The value of plant families as sources of anthelmintics were estimated by the total number of their independent reports (Table 3). The three most commonly used plant families are Asteraceae (27.72%), Leguminosae (15.79%) and Lamiaceae (10.53%). In Migori County, Asteraceae had the highest number of plants used as anthelmintic; this can be explained by the fact that this family contains a wide range of biologically active compounds (Heinrich *et al*., 1998). Some of the phytochemicals of pharmacological importance in Asteraceae are flavonoids and sesquiterpene lactones which have been studied and antimalarial therapies have been developed from them. Indeed the presence of specific sesquiterpene lactones in various genera within the family has made it possible for them to be used as chemosystematic markers (Christian, 2008). The wide usage of Asteraceae may also be attributed to the fact that the family is the most diverse of all Angiosperms. It includes 1,700 genera and approximately 24,000-30,000 species globally distributed in almost all terrestrial ecosystems except for Antarctica (Funk *et al*., 2005).

There was a high correlation (r=0.96) between age and number of medicinal plant citations due to increase in knowledge with increasing age of the herbalists which is in agreement with a previous study (Seid and Tsegay 2011).

A study done by Raj *et al.,* (2012) which used a larger set of plant species indicated Leguminosae to be the mostly used plant family. Different environmental conditions/stress in different geographical locations in which the studies are conducted can also bring differences on the results. Plants species in different geographical locations produce different chemical constituents to enable them survive the conditions. The various plants mentioned fall under three plant habits; the trees, shrubs and herbs. Trees had the highest number of plants (12) mentioned and represented 50.88% of the total independent reports, herbs had six plants (33.33%) while

shrubs had the least number 3 (15.79%) (fig. 7).The same tree can be used by several herbalists preferring different parts. Most commonly used anthelmintic plants of Migori County are mainly trees.

Different plant parts which were used to prepare medicine included roots, leaves, bark, fruits and whole plant. The leaf was most used 34.25%. The other commonly used part was root (33.03%). The fruit was least used (7.03%). This was consistent with the study done by (Okpekon *et al.,* 2004). In some plants, e.g *Vernonia amygdalina,* more than one part (leaves and roots) was used; either can be prepared and administered. Some plants (*Harrisonia abyssinica, Euclea divinorum* and *Albizia coriara*) parts (roots) were prepared separately and mixed or mixed and prepared and then administered. The argument was that this enhances their anthelmintic activity. It is thought that different plant parts from different plants have various medicinal properties. When administered at the same time they will act together in synergy and reinforce each other to bring about cure of various diseases (Flatie *et al*., 2009).

Drugs were first prepared in order to extract the active principles which have medicinal properties before administration. Methods of preparation included; pounding (universal for every plant), infusion, concoction and decoction. In most cases pounding acted as the preliminary method followed by infusion, concoction or decoction. All the plant extracts were drunk except for *Cucumis aculeatus* fruits decoction which could either be drunk or inserted in the anal region using a syringe. Most plants were prepared by decoction represented by 50.53% of the total independent reports. Decoction method is widely used because it is simple, convenient and carried out at low cost (Mohammed *et al*., 2011).

Among the three solvents, acetone gave the highest percentage (8.38%) of the extract as compared to methanol (7.24%) and water (7.35%). On average roots of *V. amygdalina* gave

more of the crude extract (4.40 grams) when compared to its leaves (3.44 grams) as well as the whole plant of *E. alba* (3.91 grams) and the leaves of *P. barbatus* (3.55 grams) (table 5).

There is a high risk of depletion of some anthelmintic plants of Migori County due to their unsustainable use. Field observations and interviews indicated that *Sclerocarya birrea, Kigelia africana* and *Albizia coriaria* are preferred by charcoal burners and tobacco growers. *Solanecio mannii* and *Rotheca myricoides* are very rare and thus most of the herbalists grow them in their homes. Others like *Eclipta alba* which is the most used, grow naturally on cultivated lands majorly during rainy seasons. Some are cut down in the event of clearing land for agriculture, settlement, or any other form of economic activity as need be. From interviews and plant usage reports helminthiasis appears significant in all the 13 Divisions of Migori County.

Phytochemicals have received increasing attention because of interesting new discoveries considering their biological activities (Mohd *et al.,* 2013) and the three mostly used anthelmintic plants were considered for phytochemical screening to assess the presence of tannins, saponins and cardiac glycosides with possible anthelmintic effect. The mode of action of some secondary metabolites has been documented. Tannins are known to produce anthelmintic activity by binding to glycoprotein on the cuticle of the parasite. They hinder energy production in helminth parasites by uncoupling oxidative phosphorylation, which can cause death (Danquah *et al.,* 2012). Though the exact mechanism of saponins against gastrointestinal nematodes is not very well known (Ombasa *et al.,* 2012), they are known to produce inhibitory effect on inflammation (Mohd *et al.,* 2013), an activity which prevents inflammatory effects normally caused by the GI worms to the host. Tannins have also been reported to be useful in the treatment of inflamed or ulcerated tissues (Mohd *et al.,* 2013). Albendazole works by interference with the polymerization

of microtubule (Harder, 2002). The drug binds to the protein tubulin of the *H. contortus* hence causing death by starvation (Ombasa *et al.,* 2012). Cases of cardiac glycosides human poisoning have been reported (Eddleston *et al.,* 2000); therefore in its low concentrations in plant materials, when ingested by human, it can contribute to the killing of the gastrointestinal worms through its toxic effect.

Preliminary phytochemical screening of the top three anthelmintic plants for saponins, tannins and cardiac glycosides of the various solvent extracts gave out similar results. Tannins were present in all the plants; saponins were present only in *V. amygdalina* while cardiac glycosides were absent only in *P. barbatus. E. alba* had tannins and cardiac glycosides. The phytochemical result for *V. amygdalina* is in agreement with the result obtained from the study done by Nalule *et al.*, 2013. Tannins, saponins and glycosides have been isolated from *E. alba* (Mithun *et al.,* 2011; Mohd *et al.,* 2013; Mukesh and Smita, 2010), this study indicated the absence of saponins in *E. alba*. A phytochemical study done by Kisangau *et al.,* (2007) on *P. barbatus* indicated the presence of tannins but absence of glycosides and saponins which agrees with this study.

In-vitro anthelmintic activity of three selected plant species from Migori County based on their wide usage was tested using adult *H. contortus* worms. The criteria for *in-vitro* anthelmintic activity was based on (Ombasa *et al.,* 2012) in which worm mortality was established by lack of motility for an observation period of 5-6 seconds after exposure to PBS. As shown on table 7, all methanol extracts of all plant species were most potent followed by water extracts. Acetone extract was the least potent. The potency of the plant extracts was highest for *E. alba* (whole), then *V. amygdalina* (roots), *V. amygdalina* (leaves), and least was *P. barbatus* (leaves) (tables 8, 9 and 10). All were weaker than albendazole which was 100% potent. Albendazole works by

either causing paralysis or death of worms such that they are expelled in the faeces of man and animals (Ajaiyeoba *et al.,* 2001). These properties were demonstrated by the extracts at 6.25, 12.5 and 25 mg/ml concentrations. The potency of extracts increased with increase in extract concentration. *Haemonchus contortus* mortality was high at 25 mg/ml and least in 6.25 mg/ml.

There were significant differences in the worm mortality caused by acetone, methanol and aqueous extracts of the three selected plants. At extract concentrations of 6.25 and 12.5 mg/ml (tables 8 and 9) there was no significant difference in worm mortality caused by methanol extracts of *V. amygdalina* (leaves) and *P. barbatus* (leaves). Acetone extract of *V. amygdalina* (roots) and aqueous extract of *V. amygdalina* (roots) and, acetone extract of *V. amygdalina* (leaves) and aqueous extract of *V. amygdalina* (leaves) exhibited no significant difference in worm mortality at 6.25 mg/ml (table 8). At extract concentration of 12.5 mg/ml (table 9) there was no significant difference in worm mortality caused by acetone extracts of *V. amygdalina* (roots) and methanol extracts of *V. amygdalina* (leaves) and *P. barbatus* (leaves). Acetone extract of *E. alba* and methanol extract of *V. amygdalina* (leaves) and, aqueous extract of *V. amygdalina* and methanol extract of *P. barbatus* indicated no significant differences in worm mortality at 25 mg/ml extract concentration (table 10). Analysis of variance (ANOVA) revealed that there were significant differences in the anthelmintic activity of the plant extracts at all the concentrations (6.25, 12.5 and 25 mg/ml) used at $p<0.05$. This research study revealed that *V. amygdalina, E. alba* and *P. barbatus* has anthelmintic activity. The study conducted by Nalule *et al.,* 2013 on the ethanolic extract of *V. amygdalina*, and that of Sijat *et al.*, 2010 on *in-vitro* anthelmintic potential methanolic extract of *E. alba* whole plant using *Posthuma pheretima* as a model, revealed that these two plants have anthelminic activity which is in a greement with this study. There has not been any anthelmintic activity documented for *P. Barbatus.*

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study aimed at identifying, documenting and evaluating the biological activity of medicinal plants used as anthelmintics by the Luo of Migori County. The main research questions were: which plants are used in managing anthelmintic infections? And do they exhibit biological activity?

Herbal medicine is important in the management of helminthic infections. The knowledge of anthelmintic plants is directly proportional to the age of the herbalist. Preliminary phytochemical screening of *Eclipta alba*, *Vernonia amygdalina* and *Plectranthus barbatus* indicated that they have three important secondary metabolites associated with anthelmintic activity, therefore, these plants, particularly *Eclipta alba*, can be used in seeking new/alternative medicine for the treatment of helminth parasites.

5.2 Recommendations

Based on the results obtained from the study it can be therefore recommended that other alternative solvents other than methanol, acetone and water need to be studied in the extraction of anthelmintic plants in Migori County. The identified potent plant species should be cultivated for further studies. Policies to regulate the utilization of these plant species should be formulated.

For future research studies it is recommended that; isolation, purification and structure elucidation of compounds in the crude extracts of *E. alba, V. amygdalina* and *P. barbatus* should be undertaken, pharmacological actions of isolated and purified compounds of the three most potent plants should be determined and bioassay study of purified compounds should also be done.

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APPENDICES A-D

Appendix A: *In-vitro* anthelmintic activity at 6.25 mg/ml

6.25 mg/ml						
Eclipta alba (whole)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	8	2	6	4	7	3
2nd	7	3	7	3	7	3
3rd	7	3	5	5	7	3
Average mortality		2.67		4.00		3.00
Vernonia amygdalina (roots)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	8	2	6	4	8	2
2nd	8	2	7	3	8	2
3rd	8	2	7	3	8	2
Average mortality		2.00		3.33		2.00
Vernonia amygdalina (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	9	1	9	1	9	1
2nd	9	1	8	2	9	1
3rd	9	1	8	2	9	1
Average mortality		1.00		1.67		1.00
Plectranthus barbatus (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	10	0	9	1	9	1
2nd	10	0	8	2	10	0
3rd	10	0	8	2	10	0
Average mortality		0.00		1.67		0.33

Appendix B: *In-vitro* anthelmintic activity at 12.5 mg/ml

12.5 mg/ml						
Eclipta alba (whole)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	7	3	5	5	6	4
2nd	7	3	5	5	7	3
3rd	6	4	4	6	6	4
Average mortality		3.33		5.33		3.67
Vernonia amygdalina (roots)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	7	3	6	4	7	3
2nd	8	2	5	5	8	2
3rd	8	2	5	5	7	3
Average mortality		2.33		4.67		2.67
Vernonia amygdalina (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	8	2	8	2	8	2
2nd	9	1	8	2	8	2
3rd	9	1	7	3	9	1
Average mortality		1.33		2.33		1.67
Plectranthus barbatus (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	10	0	7	3	9	1
2nd	10	0	8	2	10	0
3rd	9	1	8	2	9	1
Average mortality		0.33		2.33		0.67

Appendix C: *In-vitro* anthelmintic activity at 25 mg/ml

25 mg/ml						
Eclipta alba (whole)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	6	4	2	8	4	6
2nd	7	3	3	7	5	5
3rd	6	4	2	8	5	5
Average mortality		3.67		7.67		5.33
Vernonia amygdalina (roots)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	7	3	4	6	6	4
2nd	8	2	4	6	7	3
3rd	7	3	5	5	7	3
Average mortality		2.67		5.67		3.33
Vernonia amygdalina (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	8	2	7	3	7	3
2nd	8	2	6	4	8	2
3rd	9	1	6	4	8	2
Average mortality		1.67		3.67		2.33
Plectranthus barbatus (leaves)						
	Acetone		Methanol		Aqueous	
Trials	Alive	Dead	Alive	Dead	Alive	Dead
1st	9	1	7	3	9	1
2nd	10	0	6	4	8	2
3rd	9	1	7	3	9	1
Average mortality		0.67		3.33		1.33

Appendix D: Interview guide

1. Name a plant used to treat intestinal worms.
2. State the parts of the plant used.
3. What is the mode of preparation of the plant?
4. Is the plant used singly or in combination with another?
5. Name the source of the plant.
6. What is the habit of the plant?
7. Is the plant easily available?