PHYTOCHEMICAL QUANTIFICATION, ANTHELMINTIC, ANTIOXIDANT AND ACUTE TOXICITY PROPERTIES OF DICHLOROMETHANE EXTRACTS OF *MAYTENUS SENEGALENSIS* AND *DALBERGIA MELANOXYLON*

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July, 2022

DECLARATION

I, Boniface Maina Mwangi, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award.

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DEDICATION

I dedicated this work to my beloved wife, Gladys Wangari and our wonderful children, Kellen Mumbi and Sidney Kariuki for their unwavering support, love and encouragement that enabled me to complete this work.

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ABBREVIATIONS AND ACRONYMS

AchE	Acetyl cholinesterase
ADR	Adverse Drug Effects
ALB	Albendazole
ALB	Albumin
ALT	Alanine aminotransprase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BHA	Butyrate hydroxyl anisole
BHT	Butyrate hydroxyl toluene
BZ	Benzimidazoles
CAT	Catalase
Cl^-	Chloride ion
CT	Chorela Toxin
CTB	Cholera Toxin B
D.Bil	Direct bilirubin
DBH	Diameter at breast height
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	Diphenyl-2-picryl hydroxyl
DTD	Dithianediol
EDTA	Ethylene diaminetetra acetic acid
FAMTS	Fatty acidmethylasters
FCR	Folin-Ciocalteau reagent
Fe^{2+}TPTZ	Ferrons tri pyridyltriazine
Fe^{3+}-TPTZ	Fervic tri pyridyltriazine
FRAP	Ferric reducing antioxidant power
FRS	Free radical scavenging
GABA	Gamma Amino butyric acid
GC	Gas chemotography
GC-MS	Gas chromatography – Mass spectrometry
GPS	Global Positioning System
GPX	Giatathine peroxidase
H_2O_2	Hydrogen peroxide
HB	Haemoglobin
IVM	Ivemectin
JNK	Jun N terminalkinsases
K-10	Interleukin 10
KH_2PO_4	Potassium dihydrogen phosphate
KOH	Potassium hydroxide
LEV	Levamizole
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean cell volume

MDA	Malondialdehyde
MDA	Mass Drug Administration
MDA	Malondialdehyde
MEB	Mebendazole
Na^+	Sodium ion
NDGA	Nordihydroguaretic
NF-kB	Nuclear factor kappa B
NIST	National institute standard and technology
NTDs	Neglected tropical diseases
O_2	Oxygen molecule
$OH^{\cdot}$	Hydroxyl radical
OX	Oxantel
NDGA	Nordihydroguaretic
NF-kB	Nuclear factor kappa B
NIST	National institute standard and technology
NTDs	Neglected tropical diseases
PCR	Polymerase chain reaction
PCT	Preventive chemotherapy
PCV	Packed cell volume
PDW	Platelets distribution width
PG	Propyl galate
POX	Peroxide activity
PYR	Pyrantelpamiate
RBC	Red blood cells
RNS	Reactive Nitrogen Species
ROS	Reactive oxygen species
RPM	Revolutions per minute
SEM	Standard error of mean
SOD	Superoxide dismutase
STH	Soil Transmitted Helminthes
T.BIL	Total bilirubin
TBA	Thioberbituric acid
TBARS	Thiobarbithric acid reactive substances
TBHQ	Tertiary butylyhdroquinione
TCA	Trichloro acetic acid
TCM	Traditional Chinese Medicines
TNF	Tumor necrosis factor
UV	Ultra Violet
WBC	White blood cells
WHO	World Health Organisation

ABSTRACT

Maytenus senegalensis was traditionally used as a therapeutic option against chest pains and anthelmintic while *Dalbelgia melanoxylon* was used to treat anthelmintic. However, their anthelmintic potentials, antioxidant potentials and safety have not been scientifically validated. The aim of this study therefore, was to determine *in vitro* anthelminthic properties, quantitative phytochemical properties and *in vivo* sub-acute toxicity of Dicholoromethane plants extracts in mice. Seventy five worms were grouped into 5 groups in each of 3 petri plates. Group I was treated with distilled water (50ml). Group II were treated with albendazole at dose of 25mg/ml. Groups III, IV, V were treated with plants extracts of 12.5, 25 and 50mg/ml, respectively. Results were interpreted as time taken for paralysis and death of earthworms. The extracts manifested paralytic effects on worms after exposure periods ranging between 03.07min and 11.25min for *M. senegalensis* and 02.13min and 07.24min for *D. melanoxylon*. The extracts showed mortality effects on worms after periods ranging between 04.45min and 13.29min for *M. senegalensis* and 03.36min and 08.76min for *D. melanoxylon*. The antioxidant potential of the extracts were assessed against I,I-diphenyl-2-picrylhydrazyl, hydroxyl, hydrogen peroxide, iron chelating and ferric-reducing power. Phytochemical analysis was conducted using gas chromatography linked to mass spectrophotometry. Results revealed the extracts exhibited scavenging activities against all radicals. The extracts exhibited iron chelating and ferric reducing abilities. The extracts indicated a higher half inhibitory concentration value than the standard used. For instance, the extracts of *M. senegalensis* and *D. melanoxylon* exhibited 50% of the formation of 2, 2-diphenyl-i- picrylhydrazine of at concentration of 1.31±0.40 and1.31±0.04µg/ml and standard was 0.50±0.04µg/ml. Similarly, the extract scavenged 50% of hydroxyl radical of *M. senegalensis* at 1.24±0.02 and *D. melanoxylon* at 1.24±0.03 and the standard (citricacid) at 0.04±0.05µg/ml. Further, stem barks of *D. melanoxylon* exhibited significantly higher phenolic content than the extract of *M. senegalensis* at all the tested concentrations. For sub-acute toxicity test, the mice were orally administered with different doses of plants extracts. They were weighed on the first day and after 7 days during treatment with the extracts. After 28 days, the mice were sacrificed and blood samples taken for full hemogram, renal and liver function tests. The extracts had no lethal effects on body organ weights as well as on the haematological and biochemical parameters in normal mice. The study concluded that the plant extracts have phytochemicals safely associated with anthelmintic and antioxidant activities. Further, the plants extracts have *in vitro* anthelmintic activities in earthworms at dose level of 50 mg/ml. The plants extracts studied have no *in vivo* toxicity in normal mice at dose level of 100 and 300 mg/kg body weight. The study recommends the undertaking of *in vivo* antioxidant studies on plants extracts. Also there is need to carry out bioassay guided fractionation and structure elucidation of phytochemicals in the plants extracts. Further there is need for efficient conservation strategies for these medicinal plants in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Backgroung information

Helminthes ailments affect large proportions of mankind in the world population today (Pixyish *et al*., 2013). These infections are responsible for great agricultural losses due to sub-clinical ailments causing reduced growth, milk yield and fertility. Small ruminants are highly affected group especially where extensive grazing is carried out leading to great economic losses (Morgan *et al*., 2013).

Helminthes suck a lot of blood and plasma proteins, cause mortality to young animals, leakages into the gut, reduction of minerals, diarrhea, emaciation, low milk and wool production (Moudgil *et al*., 2017). Farmers are faced with high cost of pest control and other related costs, for instance, high nutritional requirements to attain market desired weight and poor quality of meat usually lead to high indirect economic losses to the affected farmers (Perry and Grace, 2009). Over decades throughout the world, commercial anthelmintic have been used to reduce huge losses caused by helminthes (Morgan *et al*., 2013). Persistent usage of conventional dewormers in livestock industry occasioned widespreadresistance of worms (Wolstenholme *et al*., 2005).

Studies carried in the United Kingdom estimates the cost of parasitic nematodes of sheep at 50 million € annually (Nieuwhof and Bishop, 2005). Similarly, the annual sales of synthetic drugs in European Union (EU) were estimated at 400 million € annually (Selzer *et al*., 2009). It is highly probable that these estimates do not represent the true picture of the livestock helminthiasis within EU (Charlier *et al*., 2015). In accordance with a research conducted in parts of Central Kenya, helminthes prevalence in livestock was at 90% (Waruiru *et al*., 2000). The helminthes infections have been reported to depress growth rates by more than 25% reduce milk production and fertility by thirty percent and increase death rates (Thumbi *et al*., 2015).

According to WHO report, infestations range from 1221 million to 1472 million of ascariasis and between 750 and 1050 million infestation of trichriasis, while hookworm infestation was estimated between 740 and 1300 million cases globally (WHO, 2017).

The annual global infestations from *Ascaris lumbricoides* are 1.5 billion and 65,000 mortalities (Brooker, 2006). Similarly, infections from hookworms account for approximately 1.3 billion cases as well as 65,000 mortalities annually (Pullan *et al*., 2014). Both physical and mental retardation in children has been associated with the infestation of *Trichuris trichiura, Ascaris lumbricoides* and hookworms. Globally, approximately 1.1 billion infestations and 70,000 mortalities result from *T. trichiura* (Pullan *et al*., 2014).

Soil transmitted helminthes have been reported to cause in excess of 80% mortalities in poor states, with children under five years accounting for twenty to thirty percent of the infestations. For instance, a study conducted in Hoima District in Uganda, revealed an overall (STH) prevalence at 26.5%, hookworm infections at 18.5%, *A. lumbricoides* at 9.8% and *T.trichiura* at 0.5% with children aged 5 years recording significantly higher infections (Silvetro *et al*., 2018).

School-aged children as well as immune compromised people are highly susceptible to these worms infestation, where massive infections lead to malnutrition, cognitive impairment, malabsorption, physical retardation and iron-deficiency anemia (Teklemariam *et al*., 2014). Among the adults, worms reduce work productivity negatively affecting economic growth of low-economy countries (Bethony *et al*., 2006).

Ailments associated with STH are classified as Neglected Tropical Diseases (NTDs) which are targeted for elimination (Utzinger *et al*., 2010). Under London 2012 declaration, STHs have been targeted for elimination as a public health concern globally. Elevated prevalence of STH has been associated to poor environmental hygiene, ignorance, poor health services, poverty, and inadequate supply of water and sanitation facilities (Albonico *et al*., 2002).

Albendazole/mebendazole was a preferred medicine especially for mass control in endemic regions. Nevertheless, studies have demonstrated that prevalence of STHs usually revert back to the initial level after six months after treatment (WHO, 2015) because of re-infection. This is common in places where supply of water is a problem, indiscriminate defecation, inadequate sanitary facilities, and poor personal hygiene. This prompted WHO, to recommend provision of water, good hygiene practices, sanitary facilities to complement chemotherapy in order to eradicate STHs (WHO, 2015).

Farmers have used dewormers from herbs extracts in diverse cultures for many years. This is due to the obvious merits they have over the conventional remedies which include availability of plants, arguably affordable and the fact that they are widely and easily incorporated in many cultures in developing countries (Tanner *et al*., 2011). Herbal phytochemicals were employed in the control of different ailments like obesity, inflammation and cancer (Shirin and Zahra, 2013).

Mitochondria are the major area of intracellular oxygen usage as well as major source of reactive oxygen species production (Valko, 2007). Nonmitochondrial origin of reactive oxygen species includes radiation, contaminant in food and environmental pollutants. Most free radicals are produced intracellularly rather than from the environment (Martinet *et al*., 2004). Different studies have suggested several strategies to manage oxidative stress in animals. They include,

body exercises, mitochondrial uncoupling, metal ion sequestration, sleeping and melatonin production, avoiding of physchophysical stress, diet supplements and synthetic antioxidants (Poljsak, 2011).

Helminthes cause inflammation when they implant and penetrate body tissues. Reactions from inflammation are followed with the output of ROS (Bello *et al*., 2000; Egwunyenga *et al*., 2003). Examples of Reactive oxygen species are super oxide radical (O_2), hydroxyl radical ($OH^{\cdot}$) as well as hydrogen peroxide (H_2O_2). The aforesaid ROS cause oxidative destruction of macromolecules like polyunsaturated fatty acids in membrane lipids, essential proteins like nucleic acids and enzymes. Oxidative stressmay cause aging and death of cells (Valko, 2007).

Conversely, cellular antioxidant defense enzymes offer protection against oxidative damage. These comprise superoxide dismutase which turns super oxide radical ($O_2^{\cdot}$) to hydrogen peroxide; catalase and glutathione peroxidase that degenerate H_2O_2 to H_2O, DT-diaphorase which counter lipid decomposition by hydroxyl radical (.OH) and against degeneration of polypeptides and DNA (Gill *et al*., 2010).

Oxidative stress is associated with many human ailments like cardiovascular ailments, diabetes, neurodegeneration disease, rheumatoid arthritis, cancer, gastrointestinal disorder, renal system disorder, pulmonary disorder, eye

disorder, infertility and fertility as well as aging (Ndiamaka *et al*., 2019). Both natural and synthetic antioxidants are used to mitigate oxidative stress. Natural antioxidant sources include grains, fruits, nuts, vegetables, animal tissues, seeds among others. They possess several drawbacks such as undesirable flavor/odour, possible loss during processing, high usage levels as well as low antioxidant efficiency (Andrea *et al*., 2010).

Conventional antioxidants are components which are artificially produced and added to processed foods to prevent browning and rancidity. They include butylated hydroxyl anisole (BHA), octyl gallate, nordihydro guaretic (NDGA), propyl galate (PG), tertiary- butylhydroquinone (TBHQ), butylated hydroxyl toluene (BHT) dodecyl gallate and metal chelating agents such as ethylene diamine tetra acetic acid and polyphosphates (Li, 2011). They are more reliable, easy to use and cost effective compared to natural antioxidants. Drawbacks include low aqueous solubility, high degree of toxicity and subject to radiosensitisation (Li, 2011). Herbal plants are considered as potent and safer substitute to artificial medicine in the management of diseases related to oxidative stress (Solanki, 2010). There is a broad range of antioxidant phytochemicals, for instance, flavonoids, isoflavones, lignans, catechins, anthocyanins, carotenoids, flavones, polyphenols, α-tocophenol, isocatechins, and ascorbic acid to prevent the oxidation of vulnerable biomolecules.

Some of the plants that have been reported as effective antioxidant include

white pepper, rosemary, chili pepper, sage, turmeric and ginger among others (Kaefer and Milner, 2008). There are many conventional anthelmintic currently available against worms. Majority of the world population lack access to, or cannot afford especially in rural areas of low-economic countries (WHO, 2006). Again, continuous use of synthetic anthelmintic medicine poses a risk of drug resistance in many parasite species (Alfredo *et al*., 2010). Therefore, there is an imperative demand for new as well as pocket friendly dewormers that are effective for long before developing resistance. Traditional drugs, based on medicinal herbs provide major and more accessible drugs to low economic countries (WHO, 2002).

Several plants parts such as roots, flowers, fruits, stems, tubers and seeds or the whole herb have been used to evaluate anthelmintic potency. Several studies have attributed some phytochemicals such as isoflavones, quinolines, polyphenols, glycosides, alkaloids, trillin and gracillin, steroidal saponins compounds like dioscin and polyphyllin (Wang *et al*., 2010), alkaloids such as sanguinarine, crytopine, 6-methoxyl-dihydrochelerythrine (Lu *et al*., 2012) to anthelmintic properties.

The continued use of untested ancient herbal drugs continues to the present day. There is demand to differentiate between effectiveness and safety of drugs and inefficacious and/or poisonous drugs to advance their development of health of people in poorcountries (Ekor, 2014). There is need to centre future research on

phytochemical studies of these effective herbs. There is no scientific data to validate the antioxidant efficacy and anthelmintic properties of *Maytenus senegalensis* and *Dalbegia melanoxylon* which are widely used among Aembu people. It is against this backdrop that this work was formulated to examine the active phytochemicals, antioxidant properties and anthelmintic efficacy in addition to the safety of the two plants. The study aims at providing preliminary information on phytochemical profile, anthelminthic and antioxidant and toxicity effects of *D. melanoxylon* and *M. senegalensis* plants.

1.2 Problem Statement and Justification

Conventional anthelmintic are associated with major demerits such as, drug resistance by parasites to drugs, which are major issue for livestock and also an issue for man helminthes diseases (Geerts and Gryseel, 2000). Similarly, anthelminthic drugs are known to be poisonous to man and livestock threatening human well-being (Albonico *et al*., 2004). Treatments of gastrointestinal helminthes parasites in animals have become difficult to poor farmers due to high cost of veterinary drugs and services in Africa (Akanda *et al*., 2014).

Residues from drugs in livestock products and the environment are concern that has lowered the usage of conventional anthelminthic in curing of livestock. In order to inhibit oxidative processes in food substances several artificial antioxidants can be used (Gwaze *et al*., 2009).

Plants remedies in the treatment of worms in humans have been in use for many

centuries (Cock *et al*., 2018). For instance, in India the usage of herbal drugs in the management of worm's related ailments can be traced back to 3500 years BC (Bizhani, 2015). Previous studies exhibited that root oil of *H. spicatum* (*Zingiberaceae*) and *Hedychium coronarium* (*Zingiberaceae*) had greater effects compared to piperazine phosphate against tapeworms (Cock *et al*., 2018). A concoction of boiled betel pepper and pumpkins seeds were used to treat 32 patients successfully against taeniasis in Taiwan (Bizhani, 2015).

Another study investigating the wormicidal effects of plants extracts of *Melia azedarach Linn*. (*Meliaceae*) exhibited better results compared to piperazine phosphate on treatment of *T. solium* (Nathan *et al*., 2006). It is evident that herbal remedies have been used for long and hence there is urgent need to bio screen *M. senegalensis* and *D. melanoxylon* for possible use as anthelmintic and antioxidant to scientifically validate their folklore use.

Herbs are the major source of natural antioxidants (Getoff, 2007). Synthetic antioxidants show adverse side effects. Natural sources of drugs have caused a lot of development in the discovery of antioxidants from herbs. Several phytochemicals such flavones, flavonoids, polyphenols and anthocyanins have been revealed by the recent studies as antioxidants as health supporters with potential to counteract oxidants in the human body (Dorman *et al*., 2003). Majority of the natural antioxidants from plants are safer to health and have greater antioxidant activity (Getoff, 2007). No literature could be found to

provide the information of phytochemicals found in the leaves of *M. senegalensis* and stem bark of *D. melanoxylon* in reference to wormicidal and antioxidant activity. Hence, need to carry out quantitative analysis of phytochemical available in the two plants under study.

Animals as well as man bank on green plants as chief source of curative drugs and for food requirements. Herbal drugs are used against several ailments including helminthes related ones (Zunino *et al*., 2007). However, some food substances have been found to contain some substances which are regarded as poisonous. For instance, cyanogenic glycosides in several fruit seeds, alpha gliadin, thiocyanates of brassica vegetables, lectins of several pulses such as kidney beans and alkaloid of the solanaceae but they are still considered as safe (Dorman *et al*., 2003). Some plants such as comfrey, corynanthe, viscum and dryopteris are known to exhibit hepatotoxicity of pyrrolizidine-alkaloid containing herbs (Sikora *et al*., 2008).

Whereas medicinal plants are assumed to be safe, they have potential to be poisonous through misidentification, improper preparation, administration by poorly trained personnels (Fennell *et al*., 2004). To prevent possible poisoning from medicinal drugs there is need to verify actual identity of plants and detection of harmful compounds (Karimi *et al*., 2015). It is on this backdrop that the plants under review were subjected to sub-chronic toxicity in swiss albino mice.

1.3 Research Questions

i) What is the quantitative phytochemical composition of the DCM extracts of *M. senegalensis* and *D. melanoxylon*?

ii) What are the *in vitro* anthelminthic effects of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* in worms?

iii) What are the *in vitro* antioxidant properties of the DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*?

iv) Are high doses of DCM extracts of *M. senegalensis* and *D. melanoxylon* safe inhealthy Laboratory mice?

1.4 Objectives

1.4.1 General Objective

To determine phytochemical profile, anthelminthic, antioxidant and toxicity effects of DCM extracts of *M. senegalensis* and *D. melanoxylon.*

1.4.2 Specific Objectives

i) To determine quantitative phytochemical profile of DCM extracts of *M.seneg alensis* and *D. melanoxylon* using GC-MS

ii) To determine *in vitro* anthelmintic effects of DCM extracts of *M. senegalensis* and *D. melanoxylon* in worms

iii) To determine *in vitro* antioxidant properties of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*

iv) To determine sub-chronic toxicity of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* in mice

CHAPTER TWO

LITERATURE REVIEW

2.1 Biological description of Helminthes

Helminthes (worms) are invertebrates that have long, round or flat bodies. The helminthes are multicellular organisms whose eggs are microscopic. Their sizes vary from a few millimeters to several metres (Masaku *et al*., 2020). Platyhelminthes are of much importance in medical world. Also known as flatworms' members include tapeworms and flukes. Platy is an expressive word for 'flat' in Greek. Similarly, *Nemato* means 'thread' in Greek. Roundworms are nematodes (Idika *et al*., 2017).

Reproduction through eggs is a general characteristic with all helminthes. Eggs occur in various sizes and shapes based on their genera (Figure 2.1). The eggs sizes ranges from 20-80 μm but schistosomonas have their eggs measuring 180 μm. The eggs density is more than water and structurally gelatinous hence very sticky (Kumar *et al*., 2022).

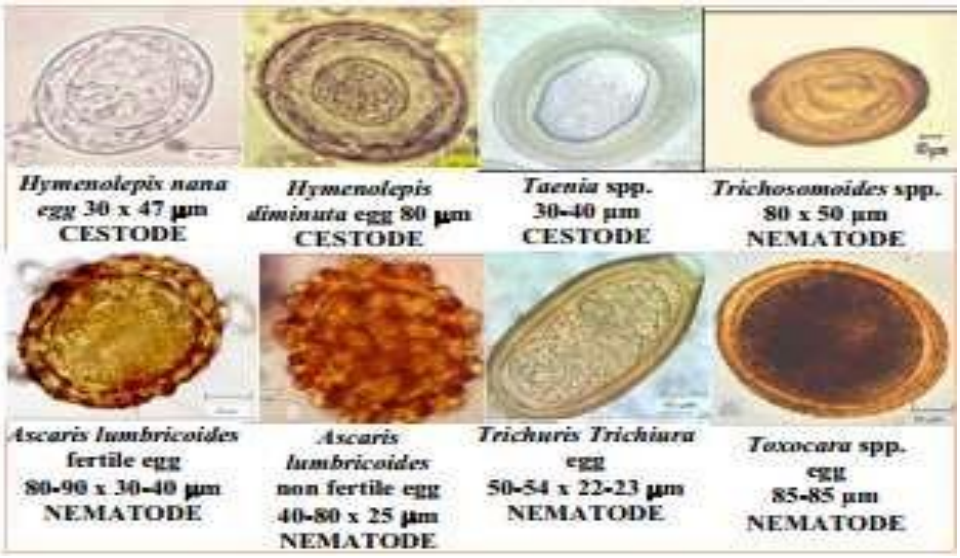

Figure 2.1 **Helminthes eggs seen in sewer water and filth. Courtesy of the Treatment and Reuse Group, UNAM (2000)**

2.1 Classification of Helminthes

As illustrated in Figure 2.2, there exists three various types of helminthes. These include annelids, nemathelminths and Platyhelminthes. Platyhelminthes and nemathelminths infect man through contaminated water. According to their body segmentations, the helminthes are branched into three groups namely: nematodes, cestodes, and Trematodes. Trematodes are simply referred to as flukes, cestodes as tapeworms and nematodes as roundworms. Helminthes are multi-celled organisms with an elaborate lifecycles which is completed in the host mostly cattle (Idika *et al*., 2017). Figure 2.2 illustrates classification of helminthes.

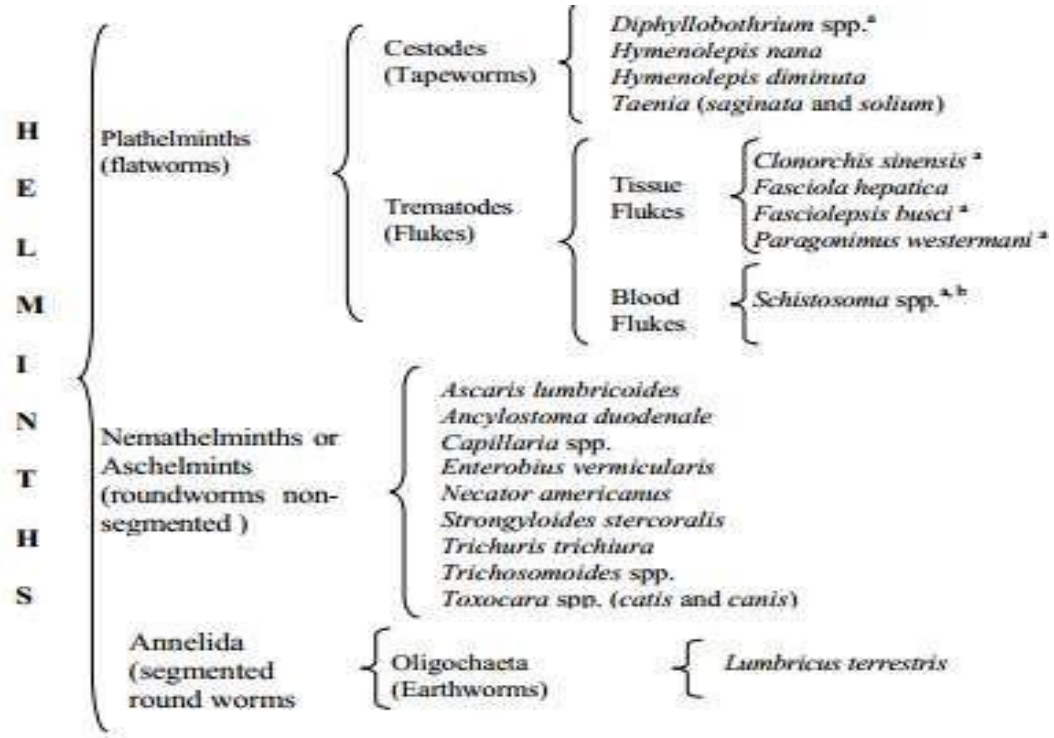

Figure 2.2 Classifications of Helminthes

Key:

[a] Reported in sewer water or filth from some Asian countries.
[b] Reported in sewer water or filth from North Africa and Far East (Chaaoua *et al*., 2018)

2.2 Life Cycle of Helminthes

Helminthes possess varying and elaborate life cycles and perfect surroundings. *Ascaris lumbriocoides* lays non infective eggs. Eggs develop into larva. The development of eggs to larva occurs in optimum temperature and correct soil moisture and takes about 10 days. Once the *Ascaris* eggs are ingested together with contaminated foods or fruits, the eggs move to intestine attaching themselves to the duodenal walls. The larva starts it development by first producing the enzymes that dissolves the shell (Pullan *et al*., 2014).

The eggs hatch to larva and cross the intestine to the bloodstream. *Ascaris* are carried through the blood to lungs, heart and to the bronchial tubes. The larva stays in the lungs tissues for about ten days after which they move to the trachea from where they are ingested back to the gut. In the course of the journey most larva are destroyed and lost in tissues unfit for their survival and development. In certain situations larva forms cysts in organs such as pancreas, appendix or liver necessitating removal through surgery (Martin-Davila *et al*., 2008). *Ascaris* attains adult phase 2-3 months after returning in the intestine. Females lay approximately 27 million eggs. Together with feaces the eggs are passed out in unembryonated state and the life cycle is repeated (Martin-Davila *et al*., 2008).

Schistosoma spp. is another helminthes that has an intermediate host. Schistosoma causes schistomiasis a very widespread ailment in most African and Asian countries. Schistosomiasis is a *Trematoda*, member that infect

humans are generally referred to as blood fluke. The lifecycle of schistosoma starts with discharge of mature eggs with feaces in water bodies. Under optimum temperature and light eggs hatch to miracidia. The miracidia infiltrate various classes of fresh snails as their intermediary host. In a period of four weeks the miracidia evolve through a composite sporocyst to larva cercarial stage making a sole miracidium and several cercarial are produced (Martin-Davila *et al*., 2008).

Cercarie are released into water bodies where they infect humans by penetrating through the skin or through contaminated fish. The man and fish also acts hosts. Cercarie evolve in humans into sexually active adult moving to the lungs within 3-4 days. The adult nematodes are carried through blood stream in the pulmonary capillaries. Mature adults move to mesenteric venules (*Schistosoma japonicum* and *Schistosoma mansoni*) or *Schistosoma haematobium* to vesical plexus. After 70 days *S. haematobium* and thirty five days *S. japonicum* and *S. masoni* release mature eggs with feaces and the lifecycle is repeated (Das *et al*., 2017).

2.3 Infestation and Effects of Worms

Helminthes cause different ailments called helminthiasis across the world. Helminthiasis is common in developing countries especially where sanitation is low. The helminthes eggs are microscopic and highly infective (Masaku *et al*., 2020). Through feaces the helminthes eggs are released in the environment. The

major route of circulation is the oralfaecal of the ailment. Poor waste water management, sewage and sludge contaminate water, crops that when swallowed act as agents of disseminating the ailment. A great number of undeveloped nations lie in tropics and in subtropics (Cisneros, 2011).

The tropics climate provides favorable surroundings for the growth and development of helminthes eggs to larvae. Helminthes have contributed majorly to low production in livestock hence a great loss to farmers. Most grazing animals suffer helminthasis (Thorton, 2010).

Parasitic infections are important economic problem of livestock. They reduce productivity and cause sterility, abortion and death of producing animals (Kaltungo *et al*., 2013). Gastrointestinal (GI) infections of helminthes that infest animals are the major and economically important ailments particularly to grazing animals (Zvinorova *et al*., 2016).

Parasitic ailments leads to low output from livestock products such as skins, meat, hides and milk which negatively affect households of peasant farmers (Zvinorova *et al*., 2016P). *Haemoncus contortus* is a highly infectious nematode mostly infecting small ruminants and capable of causing mass deaths and acute ailments ((Kaltungo *et al*., 2013).). In East Africa haemonchosis has been identified as a leading disease affecting goats and sheep (Zvinorova *et al*., 2016).

Helminthes affect the health of the host negatively since they consume nutrients

causing malnutrition which subsequently leads to retarded growth and physical development. In cases of heavy infections animal suffer reduced cognitive development, anemia, abdominal pains (Stephenson *et al*., 2000). A decline in immunity of the host due to infestation makes the host susceptible to pathogens (Mcsorley *et al*., 2012). Symptoms associated with helminthes infections include; vomiting, weakness, stomach pains, insomnia and gastro obstruction (Stephenson *et al*., 2000). Movement and attachment of helminthes on the ileum walls makes the host animal very uncomfortable (Celiksoz *et al*., 2005) Ascaris larvae move via the lungs causing short-term asthma and other breathing diseases (Mcsorley *et al*., 2012).

In African regions, wild and domestic animals normally share grazing fields which make it possible for cross-infection of ailments, more so feacally-transmitted worms. In various parts of Africa the most common nematodes in cattle includes, *Toxocara* spp., *Trichostrongylus* spp., *Spirurid, Trichurs* spp., *Cooperia* spp., *Oesophagostomum* spp., *Bunostomum* spp., and unidentified *Dictyocaulus* spp. (Ocaido *et al*., 2004).

Fasciola spp., and *Paramphistomum* spp, and *Dicrocoelium* spp, are the trematodes that infest cattle while cestodes include *Stilesia* spp and *Moniezia* spp. (Ocaido *et al*., 2004; Pfukenyi *et al*., 2007). In pastrolism system, cow's prevalence differs in many parts of Africa, although many populations have fewer than 60% (Vanderwaal *et al*., 2017).

In goats, the helminthes of significance are nematodes. They include *Ancylostomatoidea, Trichuroidea, Oxyuroidea, Trichostrongyidea, Strongylodea, Metastrongyloidea, Filarioidea, Rhabditoidea, Spiruroidea and Ascaridoidea* (Zajac, 2006). The commonest and harmful nematodes to goats in Africa includes, *Skrjabinema ovis, Strongyloides* spp., *Haemonchus contortus, Oesophagostomum* spp., *Teladorsagia circumcincta, Cooperia* spp., *Trichuris* spp., *Toxocara* spp. *and Bunostomum* spp.(Bakunzi, 2003; Zajac, 2006; Mekonnen, 2007).

Goats are mainly infected by trematodes the digeneans liver fluke species such as *Dicrocoelium* spp. *Fasciola hepatica, F. gigantic* and rumen fluke *Paramphistomum spp*. (Ocaido *et al*., 2004). Cestodes also cause a lot of harm to goats especially from the family *Taeniidae* of larval stages or cystic stage. These include *Taenia hydatigena* and *Echinococcus granulosus* in addition to *Taenia multiceps* (Mekonnen, 2007). Some adult species of cestode that are also extremely harmful to African goats include *Avitellina, Stilesia, Thysanosoma* and *Moniezia* (Sissay *et al*., 2008).

About 95% of sheep in tropics are affected by helminthes (Mekonnen, 2007) of which *Trichostrongylus* and *Haemonchus* are the worst (Rumosa *et al*., 2009). Sheep and goats have common helminthes such as *Oesophagostomum, Strongyloides, Bunostomum, Trichostrongylus,* and *Haemonchus* (Sissay *et al*., 2008).

2.4 Helminths Control Strategies

2.4.1 Chemical Control

Chemical helminthes control is widely applied all over the world (Hotez *et al*., 2006). Conventional drugs, coupled with proper field hygiene have been the major strategy of controlling helminthes of gastrointestinal diseases, although synthetic. Anthelmintics are currently viewed as the most effective means of helminthes control (Hu *et al*., 2010).

From the WHO List of Essential Drugs, the four approved drugs for the control of helminthes are benzimidazoles (BZ)-mebendazole (MEB) and albendazole (ALB), pyrantel pamoate (PYR) and levamisole (LEV) (Wendelin *et al*., 2019). Oxantel (OX) and ivermectin (IVM) are also effective against helminthes however they are never included in the essential drug list recommending them for the management of helminthes. These drugs are used to cure hookworms, whipworms and ascaris in livestock, pets and horses (Hu *et al*., 2010).

2.4.1.1 Benzimidazoles (Mebendazole and Albendazole)

Mebendazole and ALB are broad-spectrum, oral administered anthelminthic drugs. Both MEB and ALB are available as chewable tablets (100 or 500 mg MEB and 200 or 400 mg ALB) and as an oral suspension (100mg/5ml). The Albendazole sulphoxide is considered as the potent metabolite with the curative potential of ALB but MEB metabolites lack the anthelminthic activity (Wendelin *et al*., 2019).

The two drugs bind selectively to the worm's tubulin and inhibit the enzyme tubulin polymerase, which halts the synthesis of microtubules and hence hindering cellular mitosis. Microtubule polymerization inhibition is achieved chiefly by binding to free β- tubulin (Lu *et al*., 2012). These drugs hinder the glucose uptake, thus elevating glycogen exhaustion, and hamper ATP formation, the source of energy for helminthes (Hatting *et al*., 2018).

Incidences of side effects resulting from use of ALB for treatrment of helminthes are reported to be low with mild gastrointestinal symptoms occurring (Horton, 2000; Urbani and Albonico, 2003). In addition, MEB is highly tolerated with abdominal discomfort and diarrhea occurring in serious infection where patients whine of slight headache, dizziness and fever (Hatting *et al*., 2018).

Studies have demostrated that the BZ medicines are tetratogenic and embryo toxic in pregnant rats and therefore not approved for treatment during gestation (Hashem *et al*., 2019). In spite of the stated findings their safety for young children and expectant mothers has not been fully confirmed following the ongoing widespread use of BZ globally. Nevertheless, this management should not be done in the course of initial stages of gestation (Urbani and Albonico, 2003).

Due to systemic bioavailability of sulphoxide metabolite, it is potent against

cestodes and nematodes dwelling in tissues in animals (Skyler *et al*., 2017). Generally, singledose of albendazole (400 mg) is prescribed to mature persons, but WHO recommends a lower dose of 200 mg in children between one and two years (Macfarlane *et al*., 2019).

Albendazole is highly potent against adult stages of hookworms, *Enterobius* and *Ascaris*. It has also been found to be potent against relocating larval phases of hookworms, larval stage of *Taenia* as well as *Ascaris* (Del Brutto *et al*., 2013). Albendazole has been found to efficacious against eggs of hookworms, *T. trichiura* and *A. lumbricoides* (Skyler *et al*., 2017). When compared with mebendazole, albendazole is outstanding in treating hookworm ailments, although MEB is more potent than ALB for *T. trichiura* ailments in youngones when taken as one dose (Keiser and Utzinger, 2008).

2.4.2 Levamisole (LEV)

Levamisole is a dewormer which belongs to imidazothiazole class. Globally, LEV is available as a chewable tablet (40mg) which is administered at a dosage of 2.5 mg/kg in one execution. Levamisole is usually found in form of hydrochloride salts and sometimes as phosphates (Saemi *et al*., 2021). Levamisole is potent against broad spectrum of animal and human helminthes. Basically, it is highly potent against respiratory roundworms, adult gastro-intestinal ruminants and larvae of different species infecting animals (Saemi *et al*., 2021). Apart from the anthelminthic properties, it also has a revitalizing

activity on cell immune reactions in man (Keiser and Utzinger, 2008).

Levamisole bind to the receptors of acetylcholine of autonomic ganglia where it causes spastic contraction and eventual tonic paralysis of the helminthes (Kaplan *et al*., 2012). It passively eliminates the helminthes (Radfar *et al*., 2012). Levamisole chiefly affects the neuromuscular system of helminthes and also, it alters the mitochondrial fumarate reduction system influencing the mitochondrial energy synthesis which leads vermifuge effectivenesss of levamisole. Further, it alters the carbohydrates metabolism worms (Radfar *et al*., 2012).

Generally, side effects from LEV treatment include; vomiting, abdominal pain, headache, muscle pain, nausea, diarrhea, rash and arthralgia (Radfar *et al*., 2012). Following these complications in several individuals it has been banned in many countries such as Canada (2003) and USA (2000).

2.4.3 Pyrantel (PYR) and Oxantel (OX)

Pyrantel is a narrow range drug which belongs to tetrahydropyrimidines group and generally a pyrimidine derivative. It is mainly used as salts form such as tartrate, citrate and pamoate. Oxantel also belongs to tetrahydropyrimidines group and a metaoxphenyl analogue of PYR (Barda *et al*., 2017). Pyrantel is administered as single dose of 10mg/kg and obtainable in pharmaceutical form as chewable tablets (250mg). Oxantel is usually administered alone at 10mg/kg

or combined with other drugs such as PYR (Palmeirim *et al*., 2021).

Pyrantel is very potent against adult gastrointestinal helminthes of dogs, horses as well as cats. It has been used to treat man against different GI roundworms. It has demonstrated to be very potent against ascariasis in human and hookworm infections (Keiser and Utzinger, 2008). Pyrantel pamoate lack significant efficacy in treatment of *T. trichiura* even though high concentrations reach colon (Barda *et al*., 2017). Oxantel analogue of PYR has shown potent against *Trichuris* species. A combination of the two drugs has demonstrated a wide range of anthelminthic potential (Palmeirim *et al*., 2021).

A mixture of PYR/OX (10mg/kg) as one dose provides a substitute to mebendazole as single dose for management of alimentary helminthes such as *Trichuris* ailments in newborns in Africa (Barda *et al*., 2017). This is due its effectiveness, economical and its appropriateness in small children (Albonico *et al.*, 2002).

Pyrantel being a cholinergic agonist cause spastic paralysis of worm's muscles. It also influences the central nerves system of worms as acetylcholinesterase (AchE) inhibitors, since it is a member of tetrahydropyrimidines group (Lane *et al*., 2006). Acetylcholinesterase inhibition greatly alters the normal movements of the helminthes, which results to depolarization-induced paralysis causing death or expulsion for they cannot attach to the intestinal

walls. On the other hand, OX acts by paralyzing the helminthes (Lane *et al*., 2006) whereby it depolarizes neuromuscular block. It causes paralysis by targeting nicotinic acetylcholine receptor molecules of the helminthes (Barda *et al*., 2017).

Minor and mild adverse effects of PYR and OX have been reported and include; nausea, fever, gastrointestinal irritation and vomiting (Lane *et al*., 2006). Patients with hepatic dysfunction are advised to use them cautiously. Although, no cases of adverse events have been reported, they are not recommended for expectant mothers and children under 12 months old (Albonico *et al*., 2002).

2.4.4 Ivermectin (IVM)

Ivermectin was developed in 1970s as an insecticide from analogue of avermectin B1a (abamectin). Today, it is used in the management of a broad spectrum of ailments from parasitic arthropods and nematodes in animals (Campbell, 2012). It has demonstrated tobe efficacious against worm infestation for instance, ascarisis, strongyloidiasis, trichuriasis and enterobiasis including some skin infections. It is obtainable in form of pills or jabs in pharmaceutical shops provided at a dose of 150 to 200µg/kg one dose. It is an odourless white powder (Laing *et al*., 2017).

Ivermectin is administered orally as single dose (150 to 200µg/kg) after six to twelve months. It is a preferred drug in management of onchocerciasis in young

children above 5 years and above and adults (Crump *et al*., 2011). It is a preferred drug for management of human strongloidiasis at 150 to 200µg/kg at two daily doses (Laing *et al*., 2017).

Ivermectin causes a permanent hyperpolarisation or depolarization of the muscle cell or neuron of the helminthes via Glutamate-gated chloride channels (GluCL-channels). The IVM-activated channels opens at a slow pace but the action is irreversible hence blocking subsequent functions (Wolstenholme and Rogers, 2005). Ivermectin has been confirmed to induce tonic paralysis, affect the GABA receptors and distort feeding behavior of helminthes (Laing *et al*., 2017). Currently the attentiveness has been shifted to GluCL-channels as it is believed to be the likely molecular target for its action. Studies have shown that in onchcerciasis, IVM does not completely eliminate mature helminthes but obstruct the freeing of microfilariae for several weeks after treatment (Foy *et al*., 2019).

Basically the IVM is well accommodated for uninfected humans. Some adverse effects may result from its treatment such as nausea, rashes, vomiting, fatigue, dizziness and fatigue. Furthermore, Ivermectin is not approved for treatment of young ones under 5 years and expectant mothers. WHO, advises against use of IVM in treatment of expectant mothers, lactating mothers in the earliest days after birth, severely sick and babies less than 90cm in height/15 kg body weight (Erber *et al*., 2021).

2.5 Non-chemical Control

Other husbandry measures that aids to control helminthes includes; rotational grazing, avoiding early morning grazing, zero grazing all which should be well planned to maximize production and encourage immunity of animals (Bukhari and Sanyal, 2011). Biological control researches of helminthes have always geared towards predatory fungi like *Arthobotrys oligosporum*, *Duddingtonia flagrans* and *Monacrosporium* species (De and Sanyal, 2009). Nevertheless, nematophagus fungi are only efficacious against larvae in excreta portion but not those that have moved or in the host (Pooda *et al*., 2015).

Efforts to produce vaccines against helminthes in humans and animals are still ongoing with some degree of success (Harris, 2011). A vaccine to protect lambs against *H. contortus* has been successful from six months of age. Similar approach against bovine lungworm using irradiated *Dictyocauulus viviparous* has been successful (Pooda *et al*., 2015). Present vaccine experimentations are geared towards manufacture of conventional vaccines (Newton and Meeusen, 2003).

Particular species and breeds are more resilient than others hence host resistance selection is another method of controlling helminthosis (Harris, 2011). Small East African goat and local red Maasai sheep in Kenya have been found to better resistant compared to dorper and goats (Marshall *et al*., 2013).

Varying pasture management strategies such as intensity of grazing, density, time and age groups of livestock has been found to be effective in prevention of endoparasites in ruminants. This however, requires long term planning (Kumar *et al*., 2013). Sterile male technique, interspecific competition and nutritional management are other non-chemicalmethods (Bamaiyi, 2011). Further, research has demonstrated that plants have anti- parasitic effects (Athanasiadou *et al*., 2007).

2.5.1 Integrated Methods of Helminthes Control

2.5.1.1 Mass Drug Administration

This is carried out either therapeutically or prophylactically. The infected person is treated with anthelmintic drug once. The common medicines are albendazole and mebandazole (Webster *et al*., 2014). Others include pyratel pamoate and levamisole (Blair *et al*., 2015). Protective treatment can also be referred to prophylactic treatmentor preventive chemotherapy (PCT), the anthelmintic drug given to healthy susceptible person periodically. In communities with more than 20% prevalence of worms infestation, WHO recommends PCT once a year for STH ailments and twice per annum where the prevalence is more than 50% (Vercruysse *et al*., 2011). Several reviews have shown the merits of widespread drug administration of medication against helminthes in lowering the frequency of STH ailments (Schmidin *et al*., 2013).

2.5.1.2 Domestic and Peridomestic Animals Treatment

Studies have been carried out on areas that have high prevalence of zoonotic hookworms, human infections (Hotez *et al*., 2004). A study carried out in Cambodia revealed steep infection percentages from dogs infested by canid hookworms using molecular techniques (95.7%) in contrast to microscopic techniques (80.9%) (Inpankaew *et al*., 2015). The research also showed the numbers to be 57.4% by PCR techniques as compared to 26.6% by microscopy. A dog was identified defecating eggs of *Necator americanus* a hookworm that infest humans (Inpankaew *et al*., 2015).The research recommended consistent care of pet dogs to reduce danger of transferral of zoonotic soil transmitted helminthes from infested pet dogs to man.

In Cambodia hookworms are the most common helminthes infection among dogs (Schar *et al*., 2014).The pigs host *Trichuris* spp. and *Ascaris* spp. of STH (Garn *et al*., 2016). There is a necessity to control STH ailments that attack both humans and animals (Stracke *et al*., 2020).

2.5.2 Vaccination

Vaccines against helminthes have been underway for several decades now (Singh *et al*., 2003). A vaccine against *Ancylostoma caninum* has been undergoing development since 1970s targeting hookworm attack in dogs (Gavrea *et al*., 2011). An infective stage of larvae was successfully attenuated using radiation to induce immunity however, high producing and dispersal cost contributed to its stoppage (Megan *et al*., 2016). Effectiveness of a chimeric

protein from *Ascaris suum* (AS16), cholera toxin B subunit (CTB) and mucosal cholera toxin (CT) adjuvant in lowering the levels of larvae in the lung tissues after attack from embryonated eggs in swine is currently under study (Brauer *et al*., 2015).

Ascaris lumbricoides, a human round worm have identical protein to As16 making the vaccine very important candidate in fight against helminthes. Sabin Vaccine Institute Development Partnership plans to develop a Pan Helminthes vaccine against *Ascaris*, hookworms and *Tricharis* ailments. Afew antigens from *Ascaris*, two hookworms'antigens as well as *Stichosome* antigen got from *Tricharis* will be incorporated to develop the vaccine (Zhan *et al*., 2014).

2.5.3 Contaminated Soil Treatment

It is important to eradicate the soil bank of helminthes infectious eggs. Several reviews have shown infectious larvae and ova in topsoil (Zawawi *et al*., 2020). A research carried out in Titagarh City in India demonstrated that 68.6% of specimens collected from sewerwater irrigated farms were efficacious of embryonated ova of *Ascaris lumbricoides* and hookworms (Parija *et al*., 2017). Agents such as sodium borate, calcium cyanamide, methyl bromide, ethylene and dichlor`opene have been tried in treatment of topsoil for eradication of *Ancylostoma caninum* (hookworm) eggs. Although, calcium cyanamide is poisonous to dogs and methyl bromide is toxic to man (Zawawi *et al*., 2020). Proanthocyanidins have been demonstrated to kill the hookworm larvae of felids as well as canids for instance, *Ancylostoma ceylanicum* and *Ancylostoma*

caninum that infects man (Zhan *et al*., 2014). They have been found to cause immotility of larvae of *Ascaris suum in vitro* (Williams *et al*., 2014).

2.5.4 Water Treatment at Source

Water treatment is the most important intervention for prevention of ailments transmitted by fecal matter. Contaminated water in rivers, wells, dams, lakes and ponds occur as a result of open egestion and anal cleaning (Evan, 2014). Infective L3 larvae were found in both brackish water and freshwater (Zawawi *et al*., 2020). Therefore, treatment of water at source as well as point of use by use of UV radiation, reverse osmosis or filters should be done (Abdel-Raouf *et al*., 2012).

2.6 Role of Nematocide Plants in Control of Worms

Although the conventional anthelmintic drugs have been used widely globally, there are several grounds and attentiveness for screening herbal plants. Studies of medicinal plants go beyond anthropological curiosity among the scientific communities. Herbal dewormers have been the focus of this escalating recognition (Abdel-Raouf *et al*., 2012). The aforementioned could be explained by inclusion of appropriate components of ethno veterinary medicine in animal (Tran *et al*., 2013).

Kingdom Plantae is a great source of herbal anthelmintic (Sofowora *et al*., 2013). Several herbs have been in use to manage parasitic infestation in humans and livestock for a long period of time (Jayakumar *et al*., 2018). Usage of herbs

may provide an economical, feasible and a substitute if the compounds are proven to work (Sofowora *et al*., 2013). Aforementioned plant preparations have been in usage for many years by herdsmen and peasant gardeners for the management of their animals against worm's infestation. Research on herbal medicine can help in validation and intensify local usage and provide pointers to medicament with additional prospective (Abel *et al*., 2005).

Many herbs contained anthelminthic properties and were in usage in ancient livestock keeping before conventional drugs were embraced (Sofowora *et al*., 2013). Before the Second World War, Western countries narrowed down their research on animal husbandry on anthelmintic plants. Also, in some Eastern nations as well as India there is dependable data at hand regarding the effects of many medicinal plants or herbal extract on specific parasites (Abel *et al*., 2005). Effective plants anthelmintic include cucurbits, umbelliferae, wormwoods, tansy, and snakeroot. However, tobacco and goose food have significant negative effects that discourage their usage (Evan, 2014).

Allium sativum is a common herbal anthelmintic freely available in most parts of the world. According to Anonymous (2010), *Allium sativum* could be efficacious against *Enterobius* as well as *Ascaris*, and especially in ruminants, against lung worm (Abel *et al*., 2005). Although, *Allium sativum* does not inhibit eggs production but inhibits eggs of some particular worms from advancing into larvae (Evan, 2014).

Different regions of plants like oil, leaves as well as flowers of *Chenopodium ambrosioides* have been used since 1990s as anthelmintic (Gbolade *et al*., 2010). Some of the anthelminthic herbs are *Albizzia abrotanum, Butea frondosa, Embelia schimperi, Artemisia maritime* among others (Liu *et al*., 2003). *Streblus asper* has been tested clinically and proven as anti-filarial either *in vitro* or *in vivo* in India (Rinkal *et al*., 2022).

The present research strived to examine the effectiveness of anthelmintic properties of the two therapeutic herbs extracts of *Maytenus senegalensis* and *Dalbelgia melanoxylon* used by herders and small scale livestock farmers in Kenya against helminthes parasites. Evaluation was carried out *in vitro* using mature earthworms (*Pheretima posthuma*).

2.7 Oxidative Stress and Helmenthiasis

Inflammatory reactions triggered by invasion of worms cause oxidative damage in ileum of ultimate host (Shin *et al*., 2011). Host animal produces ROS during invasion of worms mediate interaction between parasites and worms (Torres *et al*., 2006). Most helminthes live in animal's body for a considerable duration of time owing to their elevated level of antioxidant enzymes activity (Shin *et al*., 2011). For helminthes to survive and grow in the hostile host environment, they are well adapted to live in the intermediate host (Walson *et al*., 2008). The parasites have well developed mechanisms to subvert immunity of the host

(Siracusano *et al*., 2012).

Antioxidant defenses fight ROS and RNS that are churned out during host immune reaction and intracellular oxidative metabolic process. The RNS as well as ROS are destructive to tissue constituents since they destroy proteins, lipids, DNA, and carbohydrates changing their roles (Shin *et al*., 2011). This explains why multicellular as well as unicellular creatures have evolved non-enzymatic in addition to enzymatic systems to manage oxidative stress (Walson *et al*., 2008). When parasites lodge and penetrate the tissues of their host they cause inflammation followed by production of ROS (Shin *et al*., 2011).

Damage caused by oxidative stress is injurious to cell and cause agingand death of the cell (Rinkal *et al*., 2022). A cell has many antioxidant protective enzymes which over defense to cell from oxidative injury unless overwhelmed by production of reactive oxygen species (Walson *et al*., 2008).

Different studies carried out on animals, have demonstrated that, when antioxidants alone or when administered together with other drugs against schistosomiasis and the results were established to be safe with minimum adverse effects (da Costa *et al*., 2021). Majority of antioxidant studies have demonstrated potent against schistosomal either *in vivo* or *in vitro* against immature (Walson *et al*., 2008) and mature (Rojo- Arreola *et al*., 2014) forms of *S. japonicum* and *S. mansonii*. These researches have shown that antioxidants influence *in vitro* the motor activity of helminthes showing likelihood of dysfunction of elements of neuromuscular system (da Costa *et al*., 2021).

The neuromuscular systems control the movement, ventral and oral and suckers which help in the attachment. They also support excretory, reproductive and digestive system whereby they maintain the female inside male's gynecophoral canal (Walson *et al*., 2008). It was found that the antioxidants were able to cause severe alterations of the tegumental an important protective organ against helminthes responses, uptake of nutrients for development of the parasite and plays an important function in host-parasite interaction (da Costa *et al*., 2021).

Further, antioxidants were shown to impair helminthes coupling, an important process to oviposition (Gomes *et al*., 2012). Eggs cause spread of inflammatory granuloma on certain select tissues and imparting of ailments (Walson *et al*., 2008). In addition, the activity of antischistosomal has shown that antioxidants are able to restore the potential of antioxidant liver enzymes almost to the control degree (Gomes *et al*., 2012).

Increased capacity of antioxidant enzymes normally results to reduction on the size of granuloma and number, leading to improved liver architecture and functions (Afshari *et al.*, 2016). Antioxidants are capable of modulating and immunomodulation responses that promote alteration of few cytokines (Gomes *et al*., 2012) which are capable of lowering the amount and size of granulomata.

The gastrointestinal helminthes can alter liver metabolism (Afshari *et al.,* 2016). Koch *et al*. (2016) revealed that invasion of rodents erythrocytes by *Plasmodium vinkei* leads to reduction in GPX activity and other researches in fish models (Gomes *et al*., 2012). Macrophages produce hydrogen peroxide against gastrointestinal helminthes. Hydrogen peroxide being stable crosses freely the plasma membrane and generates the reactive hydroxyl radical after reaction with haeme proteins as per Fenton reaction (Misra *et al*., 2000).

Elevated degrees of MDA have been described in infested animals by helminthes (Hotez *et al*., 2008). Lipidperoxidation is inhibited by DT diaphorase enzyme (Misra *et al*., 2000). The elevated levels of MDA in infested animals show that parasitic infections causes oxidative stress. Several studies have demonstrated that significantly higher degrees of MDA in serum of infected man with *fascioliasis* than controls (Hotez *et al*., 2008) as well as infected man with *falciparum malaria* (Mwangi *et al*., 2006).

2.8 Alternative and Complementary Management

Taking foods rich in antioxidants can manage oxidative stress (Niedowicz *et al*., 2005), these safeguards the body cells from injuries caused by free radicals by contributing electrons to the radicals making them stable. This safeguards the cells damage thatcould have been caused to the cell by radicals (Herrera *et al*., 2001).

Mineral antioxidants include selenium, zinc, iron and copper. Vegetables, nuts, organic green tea and fruits and vitamins B, E and C are required for metabolic processes. Lycopene, beta-carotene, vitamin C, A and E are examples of dietary sources of antioxidants (Matough *et al*., 2012). Enough sleep and lack of stress lowers the danger of raised oxidation activities that create more oxidants (Herrera *et al*., 2001).

2.8.1 Oxidative Stress Management Using Herbal Plants

The World Health Organization recommends determination of plants extracts potential to treat various human diseases in circumstances of no safe conventional drugs (Sofowora *et al*., 2013). In recent times, many herbal plants have been employed in the management oxidative stress globally. Traditional herbal extracts are considered effective, nontoxic, have fewer side effects and explored as novel candidates for oral administration (Herrera *et al*., 2001).

Admittedly, medicinal herbs are extensively spread globally makes them first form of treatment that people seek in case of emergencies especially in the rural areas. Furthermore, most herbal preparations are used in their raw form and do not require complex forms of processing before administration (Ekor, 2014). It is a common knowledge that conventional treatment of DM imposes an economic burden (Ali *et al*., 2018) and this makes it difficult for most patients to access quality healthcare.

Further, herbs and spices have antioxidant activity and are used to lower oxidative stress in various ailments related to oxidative stress including hypertension, cardiovascular disease, and atherosclerosis (Yashin *et al*., 2017). These herbs and spices include turmeric, rosemary, chili pepper, white pepper, sage, thyme, nutmeg, ginger and several Chinese medicinal herbs (Yashin *et al*., 2017).

Some of the most well studied plant antioxidants include vitamin C as well as vitamin Ehave been demonstrated to possess high ability to lower the oxidative damage in model diabetic animals (Ekor, 2014). Other dietary compounds, such as the phytochemicals in herbs, are recognized to have significant antioxidant activities than vitamins as well as minerals (Yashin *et al*., 2017). These are referred to as non-nutrient antioxidants and include phenols, lycopenes in tomatoes, flavonoids and anthocyanins in cranberries (Ekor, 2014). Tocopherol interacts with lipid hydroperoxides to forage them (Ali *et al*., 2018).

The flavonoids, phenolic and tannins antioxidant potential is due to their redox activitythat enable them perform as singlet oxygen quenchers, reducing agents and donor of hydrogen (Yashin *et al*., 2017). Some phytochemicals such as polyphenols shields thebody against oxidative stress (Cao *et al*., 2017). In many incidences, conventional medicines can be used together with plant extracts as complementary medicine to treat and manage oxidative stress. When used

together the patient is expected to finally cease to take the prescribed conventional medicines and continue with herbs (Jing *et al.*, 2010). After all, herbs are natural materials, cheap, more readily available and rarely have side effects (Ali *et al*., 2018).

2.8.2 Toxicity Studies of Medicinal Plants

Toxicities associated with herbal drugs are linked to two factors extrinsic (indirect) and intrinsic (direct) (Ekor, 2014). Availability of active chemicals compounds in medicinal plants like ephedrine-type alkaloids which are herbal toxicity are considered as intrinsic factors. Poisons associated with presence of foreign toxic substances such as contamination, misidentification, adulteration are regarded as extrinsic factors (Quan *et al*., 2020).

2.8.3 Intrinsic Negative Effects

In orthodox medicine, negative effects of herbal drugs can be grouped into four categories (Ekor, 2014). Acute (Type A/augmented) reactions involve interactions with pharmaceuticals and overdose reactions. Mostly concerns with the pharmacological properties of herbal medicines. Over dosage or improper use of herbal drugs could lead to negative effects. Some organs and organ systems may be negatively influenced for instance, kidney, liver, cardiovascular system, digestive and nervous systems (Huang *et al*., 2018).

Bizarre (Type B/idiosyncratic) responses are usually brought about by plant medicines (Uetrecht *et al*., 2013). Responses may vary from dermatitis to the most serious allergic reaction; anaphylactic shock. The third type is the chronic or cumulative reactions which arise from long-term therapy which are normally anticipative. The forth category is the delayed reactions which are usually go unreported due to lack of systemicevaluation of herbal drugs (Ekor, 2014).

2.8.4 Extrinsic Negative Effects

Other than the intrinsic herbal toxicity, presence of other toxic substances could lead to herbs related toxicity. Misidentification, adulteration and contamination of herbal plantsare the most sources of extrinsic adverse effects (Ekor, 2014).

2.9 Plants Used in the Study

2.9.1 *Maytenus senegalensis*

2.9.1.1 Description of *Maytenus senegalensis*

Maytenus senegalensisis (Plate 1) also referred to as *Gymnosporia senegalensis* of the family celastracea (Burrows *et al*., 2005). The common name is "spike thorn" (da Silva *et al*., 2011). *Maytenus senegalensis* is widespread in savannah of Africa, India, Afghanistan and Arabia (da Silva *et al*., 2011).

Maytenus senegalensis is a perennial tree that is extensively spread in Afghanistan, Arabia, India and in Africa. It belongs to *Celastraceae* family (da Silva *et al*., 2011). Herbal decoction from the root and stem bark have been used in many parts of ancient

Africa to cure several ailments and health conditions such as chest pains, rheumatism, fever, malaria, snake bites and wounds (Sanogo *et al*., 2006). Diarrhoea and intestinal worms in calves and dog bites have been cured by the leaves of *M. senegalensis* (Kareru *et al*., 2006) and respiratory ailments (da Silva *et al*., 2011).

Plate 1: **A Photograph of aerial parts of a mature *Maytenus senegalensis* plant taken in December, 2020**

2.9.1.2 Medicinal Usage of *Maytenus senegalensis*

In Sudan, aqueous stem bark extracts of *M. senegalensis* is employed in nursing snakebites, dysentery and tumors in Sudan (Zangueu *et al*., 2018). In some regions in Africa the root and stem bark of *M. senegalensis* were used traditionally to treat various ailments such as snake bites, wounds, rheumatism, chest pain and diarrhea (Prinsloo *et al*., 2018).

Various species of the *genus Maytenus* were recorded in ancient medicine to have been employed as pain reliever agents as well as anti-inflammatory factors when taken orally (Neuwinger, 2000). According to a study by Sanogo *et al*. (2006), a reduction of edema, three hours after injection ofCarrageenan and pain reduction by 72% and anti-inflammatory action was reported respectively. Bukusu community from western Kenya use stems bark, fruit and leaves of *Maytenus senegalensis* to control ticks in cattle (Wanzala *et al*., 2012).

From previous biological research, stem and roots extracts were reported to be effective against plasmodium effects *in vitro* against *P. falciparum* (D10) (Clarkson *et al*., 2004). Further, stem bark extracts of *M. senegalensis* exhibited *in vitro* anti-leishmanial potential against the promastigotes of leishmania main reference vaccine strain (5AKSH) (Sundar *et al*., 2014). Stem bark as well as leaf bark extracts of *M. senegalensis* have demonstrated ability stop an enzyme cyclo-oxygenase-1 which synthesises mediators against inflammation for instance thromboxanes andprostaglandins (Ricciotti *et al*., 2011).

Stem barks extracts of *M. senegalensis* have demonstrated to have ability to inhibit growth of different causative agents of genitor urinary ailments such as *Escherechia coli*, *Pseudomonas aeroginas*, *Proteus vulgaris*, *Klebsiella pneumonia*, and*Staphylococcus aureus* (Mbatchou & Adoum, 2010). Maytenoic acid, a compound identified from root bark extracts of *Maytenus senegalensis* have been previously reported to be effective against *Escherechia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Bacillus subtilis* (Lindsey *et al*., 2006).

Acetone aerial leaves extracts of *M. senegalensis* demonstrated potential to counter the strain H37Rv, 0.5mg/ml of *Mycobacterium tuberculosis* (Ricciotti *et al*., 2011). Inflammation has been successfully suppressed by Aytenoic acid and Pristimerin from *Maytenus* genus (Sosa *et al*., 2007).

Anti-inflammatory potential is attributed to the availability of triterpene and phenol metabolites. Recently, studies have demonstrated anti-inflammatory potential of *M. senegalensis* and *M. heterophyla* ethanolic extracts (70%) by lowering dropsy by 51% and 35% sequentially (Da Silva *et al*., 2003).

2.9.2 *Dalbergia melanoxylon*

2.9.2.1 Description of *D. melanoxylon*

Dalbergia melanoxylon is referred to as African Blackwood in English or mpingo in Swahili (Albano, 2001). *Dalbergia melanoxylon* Gill. & Perr. belong to the family Fabaceae (Washa, 2008). *Dalbergia melanoxylon* is a small shrub or tree, highly branched and multi-stemmed with thorns. Has an irregularly shaped low lying crown (Albano, 2001). The tree occasionally grows to 15m high but the average height is 7.5m. The stem is ridged with narrow fins. The bole length may reach up to 1.2m. It is a naturally growing plant. It has 6 to 22cm long pinnate compound leaves. They have flowers of 6 to 9cm long occurring in dense clusters. They have irregular oblong pods with bluntly pointed tips. The pods range from shapes; annual increases 0.8 to 1.4 cm wide and from 3 to 7cm long (Washa, 2008).

The tree attains timber size at 50 years (Albano, 2001) but most attains this size at 100 years (Albano, 2001). The mean diameter at breast height is 38cm (Washa, 2008). Has white, small scented flowers with 10 stamens joined in tube. Indehiscent fruit containing 1 to 4 seeds (Washa, 2008). The fruiting and flowering seasons are not well defined (Washa, 2008). The roots have nitrogen – fixing nodules (Albano, 2001). The wood is very hard and heavy, oily, fine textured and with indistinct growth rings (Albano, 2001). *D. melanoxylon* has been by the local communities to provide charcoal, pestles, cups, firewood, combs (Washa, 2008). Other uses include bee forage, nitrogen fixation, and

mulch and fodder (Albano, 2001). The tree is locally used to make woodcarvings in Tanzania for tourists as well as making valuable timber (Albano, 2001).

2.9.2.2 Medicinal use of *D. melanoxylon*

Dalbergia melanoxylon has been used to manage abdominal pains, worms, and venereal ailments and wounds (Marshall *et al*., 2002). Various parts of the tree for instance, roots, leaves, as well as barks possess various therapeutic roles including religious rites. Drugs from the roots have been used to cure hernia, gonorrhea, abdominal pain as well as managing complications from abortion (Allen *et al*., 2009). Stem barks have been used to treat loose stool while smoke emanating from glowing roots have been inhaled to cure bronchitis and migraine (Allen *et al*., 2009). Locals smoke dried leaves to cure asthma (Chigora *et al*., 2007), leaves boiled in soup to manage joint pain (Amri and Juma, 2016) and also it has been reported to cure bronchitis and throat inflammation (Panda *et al*., 2010).

Concoctions made from the roots are known to treat hernia, gonorrhea, abdominal pain and abortions complications (Kiondo *et al*., 2014). The roots of *D. melanoxylon* have been employed to relieve abdominal pains, barks used to clean wounds as well as an anthelmintic (Amri and Juma, 2016). Members of genus *Dalberga* where *D. melanoxylon* belong have been shown to possess different therapeutic properties such as anti-inflammatory, analgesic and antipyretic (Kale *et al.*, 2007).

Plate 2: **A Photograph of aerial parts of a mature *Dalbergia melanoxylon* plant taken in December 2020**

CHAPTER THREE

ANALYSIS OF QUANTITATIVE PHYTOCHEMICAL COMPOSITION OF DCM LEAF EXTRACTS OF *M. SENEGALENSIS* AND STEM BARK EXTRACTS OF *D. MELANOXYLON*

3.1 Introduction

Phytochemicals are secondary metabolites or chemicals, which are not immediately required by the plants for survival but enable them to adapt to its environment. The biological activities found in natural products are attributed to phytochemicals, which offer defense against biotic and abiotic strain (Briskin, 2000; Ruba *et al*., 2013). Phyto- compounds possess supportive systems in the human body such as regulation of enzyme activities, anti-bacterial, antioxidant effects, and modulation of hormone metabolism, interference with DNA replication and stimulation of the body defense system (Ngoci *et al*., 2011).

Phytochemicals may be categorized as primary or secondary metabolites. Currently there are over 120 potent compounds which are isolated from herbs and broadly used in conventional medicine. About 80% of these compounds demonstrate affirmative comparison between conventional medicinal usage and ancient use of herbs from which they are derived (Fabricant *et al*., 2001).

Primary metabolites are important for cell functioning and are available in all plants. Secondary phytochemicals have different applications in industries and scientific research. Most secondary metabolites are poisonous and repel herbivores and microorganisms and aid in fighting disease causing microbes.

During plant attack by pathogens or herbivores the synthesis of secondary metabolites increases and, during insect attack, few chemicals are released into the atmosphere (Bennett *et al*., 1994).

Secondary metabolites can be classified into three main categories. Terpenes, which are formed from mevalonic acid and carbon and hydrogen, phenolics, are synthesized from hydrogen and oxygen and simple sugars of benzene rings and nitrogen bearing compounds, which additionally have sulphur (Bidlack *et al*., 2000). When consumed alkaloids inhibit enzymes, interfere with neurotransmission, vomiting, block ion channels, hallucination and death. Phenolics inhibit digestion, block activity of enzymes and inhibition of cell division (Bidlack *et al*., 2000).

A broad range of secondary metabolites are synthesized by herbs to perform essential physiological functions, for instance, attracting plant pollinators and protecting them from biotic stress (Kennedy *et al*., 2011). Majority of secondary substances produced by herbs are used in feeding, cosmetics and pharmaceutical industries for the manufacture of flavors, fragrances, dietary supplements, dyes and drugs. Industrial and scientific interests surrounding herbal secondary compounds are enormous (Ncube *et al*., 2015).

Medicinal potential of herbs could be rated on their antibacterial, anti-oxidant and anti- helminthic outcome of their phytochemical composition (Adesokan *et al*., 2008). Medicinal herbs should be evaluated for their safety, usefulness and their therapeutic properties. World health organization holds that curative herbs are the highestreservoir of a wide range of medicines (Nascimento *et al*., 2000).

A gas chromatography-mass spectrometry (GC-MS) is commonly employed in quantitative phytochemical investigation owing to its high level of efficiency, reproducible and also good analytical platforms (Shrestha *et al*., 2014). This is made possible by vigorous, replicable and high selectiveness of the technique as well as availability of a numerous entrenched libraries both of 'in house' and commercialized databases available (Adusumilli and Mallick, 2017).

In most recent years use of GC-MS techniques has increased as it has demostrated to be very valuable for the investigation of non-polar compounds like fatty acids, alkaloids and essential oils of therapeutic plants (Amirav *et al*., 2008).

In this study, quantitative phytocompounds screening of dichloromethane leaf extracts of *M. senegalensis* as well as stem bark extract of *D. melanoxylon* was undertaken by use of gas chromatography (GC) linked to mass spectrometry. This chapter, therefore, details the ascertainment of quantitative phytocompounds

description of the DCM leaf extract of *M. senegalensis* and extract of *D. melanoxylon*. It was envisaged that the identified and quantified phytochemicals are associated with anthelminthic and antioxidant activities of the two herbs.

3.2 Materials and Methods

3.2. 1 Collection and Preparation of Herbal Specimens

Some freshly cut leaves of *M. senegalensis* and fresh stem barks of *D. melanoxylon* were assembled from Mung'au village, Embu County, Kenya. The GPS co-ordinates of the plant specimen collection are Latitudes 0^0 62' 48S and Longitudes 37^0 70' 13E for *M. senegalensis* and Latitude 0^0 63' 00S and Longitudes 37^0 70' 31E for *D. melanoxylon* respectively. Local traditional medicinal practitioners helped in identification and collection process. The plant samples were identified at National Museums of Kenya, Ref. No: NMK/BOT/CTX/1/2 (Reference IV and V). The plant samples were sorted out, washed properly and carried in khaki containers to the BMB departmental Laboratories of Kenyatta University for research. The acceptable bio-conservation methods were followed accordingly. A recognized taxonomist validated the specimens.

The leaves of *M. senegalensis* and the stem barks of *D. melanoxylon* were chopped into tiny slices using a panga and dehydrated under shade at ambient temperature for four weeks for complete drying. An electric mill was used to separately grind them into fine powders before sieving them through a mesh

sieve. The powders obtained were put into khaki bags awaiting extraction and bioassay in Biochemistry, Microbiology and Biotechnology departmental Laboratories of Kenyatta University.

3.2.2 Extraction

Cold maceration method was used for extraction, where about 200gms of powdered plantparts were weighed separately and extracted with dichloromethane solvent. The powder was soaked in 500ml solvent for 24 hrs. The plant extracts were decanted 500ml solvent added and left for 24 hrs. Filtration was done after 24 hrs, 500ml of the solvent was added and left for another 24 hrs when final extraction was done. Shaking was done occasionally to guarantee complete extraction. Cotton wool and aluminum foil were used to wrap the flask to arrest escape of the solvent. The obtained extracts were filtered by use of muslin cloth in addition to Whatman No.1 filter papers.

Concentration of the extracts was carried out by use of Heidolph rotary evaporator at 40^0C under reduced pressure. The obtained concentrates were left open to evaporate any remaining solvent. The extracts were weighed, held in well labeled specimen bottles, and stored at 4^0C, awaiting usage in quantitative phytochemical screening and other assays.

3.2.3 Specimen Preparation for Phytochemical Analysis

A portion of the dehydrated extracts (1mg) of *M. senegalensis* was disintegrated separately in one millimeter of DCM (Sigma Aldrich gc-grade). They were then swirled for thirty seconds and sonicated in an ultra-bath for fifteen minutes prior centrifugation at 14,000 rpm for five minutes. The supernatant was then dehydrated with Na_2SO_4 (AH). The final stock solution (1mg/ml) was used in preparation of investigational solution where the final concentration was attained at 100 ng/µl. Preparation of samples was done in triplicates. The procedure was repeated with *D. melanoxylon* extracts.

3.2.4 Gas Chromatography-Mass Spectrometry (GC-MS) Screening

Gas Chromatography-Mass Spectrometry (GC-MS) screening of the dichloromethane leaf extracts of *M. senegalensis* as well as stem bark extract of *D. melanoxylon* was conducted as per methods previously described by Dar *et al.* (2012). Sample examination was actualized by usage of GC-MS (78900-5975 Agalent Technology, Inc., Baijing, China) containing of a gas chromatograph connected to mass spectrometer.

Gas Chromatography Mass Spectrometry was furnished with a HP5 MS (about 5% phenylmethyl siloxane) reduced bleed capillary column of 30 mm length, 25 mm diameter and film of 2.5 µm thick. To detect GC-MS, electron ionizing system using ionization energy of 70.00 Ev was employed. Helium (99.99%) was used as carrier gas at a continuous flow rate of 1.25 ml/min in split mode. The

temperature of the injector and mass transfer line were placed at 250°C and 200°C subsequently, as an injection volume of 1 µl was used.

The oven temperature was set from 35°C for 5 min, with an increase of 10°C/min to 280°C for 10.5 min, then 50°C/min to 285°C for 29.9 min with a run time of 70 min. The MS operating parameters included: Ion source temperature; 230°C, Solvent cut time; 3.3 min, interface temperature; 250°C, Ionization energy; 70eV; Scan range 40-550 m/z, and Scan speed 1666 µ/sec.

Reading of mass-spectrum from GC-MS screening was conducted using the main database of the National Institute Standard and Technology (NIST), which have in excessof 62,000 patterns. As for the undetermined compounds, their spectrum was contrasted with those which are established from the NIST library (Wei *et al*., 2014).

3.2.5 Data Management and Statistical Analysis

Investigational data on quantitative phytochemical screening was picked from each oneof the organic extracts of the two herbs. The data obtained was arranged in MS excel program. Identity of phytochemicals was recommended according to basic fragmentation pattern as well as usage of authority spectra reported by library-MS databases (National Institute of Standards and Technology (NIST) 05, 08) together with Adams and Chem ecol-L mass spectral databases. The determination of retention time indices was done by use of C5- C32 hydrocarbons scope. Identification of phytochemicals, the spectra over 60% of the library match

was needed. Phytochemical compound identity, chemical class, structure and molecular weight, of the components of the herbs extracts were also established. The relative amounts of each compound were stated as percentage with peak-area where normalization was calculated. The resultant data of this research was presented in formof tables.

3.3 Results

3.3.2 Phytochemical Screening of the DCM leaf Extract of *M. senegalensis* and stem bark of *D. melanoxylon*

GC-MS screening of dichloromethane leaf extract of *M. senegalensis* identified and quantified 36 constituent compounds as presented in Table 3.1. Based on these results, the phytochemical compounds identified in this extract can be categorized into ten major categories; aromatic hydrocarbons, alkenes, acryclic acids, monoterpenoids, fatty aldehydes, cyclopropylbenzenes, acyclic olefins, triterpenoids, diterpenoids and fatty acids and their derivatives (Table 3.1; Appendix II).

The results revealed four aromatic hydrocarbons present in the DCM leaf extract of *M. senegalensis* where dodecene<1->had the highest relative abundance (1.49%) followed by p-Xylene (0.31%) and finally Naphthalene, 2, 7-dimethyl- with (0.09%) (Table 3.1).

The results further revealed presence of two alkene compounds in the DCM leaf extract of *M. senegalensis* where decene<1-> had the highest percentage relative abundance of 0.33 followed by 5-Undecene, 3-methyl-, (E) - with a percentage relative abundance of 0.04(Table 3.1).

Hexenoic acid<2E-> was the only acrylic acid revealed in the DCM leaf extract of *M. senegalensis* with a relative abundance of (0.30%). Also revealed in this study

was one monoterpenoids, limonene with relative abundance of (0.06%). Nonanal<n-> (0.03%) and benzaldehyde, 3, 4-dimethyl-(0.15%) were the only identified fatty aldehyde and cyclopropylbenzene compounds identified in this plant extract respectively (Table 3.1; Figure 3.1).

Further, the results revealed six sesquiterpenoids present in the DCM leaf extract of *M. senegalensis* where widdrol had the highest relative abundance (1.81%), followed by panasinsene<β>(0.49%), rosifoliol (0.25%), calamenene<trans->(0.22%), humulene<α> (0.19%) and lastly b-Ionone had the least relative abundance of (0.12%) (Table 3.1: Appendix II).

Tetradecene<1-> with a relative abundance 2.75% was the only acyclic olefins identified in these results. Phenol, 2, 4-bis (1, 1-dimethylethyl) - was the solitary octyl phenol revealed in the results with a relative abundance of 5.95%. Hexadecanol<n-> was the only cetyl alcohol with a relative abundance of 3.57% discovered in the DCM leaf extract of *M. senegalensis* (Table 3.1; Appendix II).

Three triterpenoids were revealed in the results, where squalene had the highest relativeabundance of (9.07%), followed by β-Amyrin (3.10%) and friedelan-3-one (0.30%). Five diterpenoids were identified and quantified as follows depending on the percentagerelative abundance from the highest to the lowest, phytol (12.62%), phytol, acetate (6.71%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.23%), 2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(0.55%) and isophytol (0.26%) (Table 3.1).

Ten fatty acids and their derivatives were as well detected in the DCM leaf extract of *M. senegalensis*. N-Hexadecanoic acid had the highest relative abundance of 24.3%. Other fatty acids and their derivatives identified in this extract are 9,12,15- Octadecatrienoic acid, (Z, Z, Z) (17.84%), Octadecanoic acid (2.42%), 9-Octadecenoic acid, methyl ester, (E)-(0.35%), Tridecenol<2E-> (0.35%), Tetradecanoic acid (0.26%),Methyl 8,11,14-heptadecatrienoate (0.18%), Gurjunene<μ> (0.16%), Methyl epi- jasmonate<Z-> (0.07%) and hexanoic acid(0.06%) (Table 3.1; Appendix II).

Table 3.1 Phytochemical Analysis of DCM Leaf Extract of *M. senegalensis*

Class of compound	Compound Name	Molecular formula	Retention Time	Relative Abundance (%)
	Dodecene<1->	$C_{12}H_{24}$	14.24	1.49
Aromatic	p-Xylene	C_8H_{10}	8.08	0.32
Hydrocarbon	Toluene	C_7H_8	5.15	0.15
	Naphthalene, 2,7-dimethyl-	$C_{12}H_{12}$	17.35	0.09
Alkene	Decene<1->	$C_{10}H_{10}$	10.82	0.33
	5-Undecene, 3-methyl-, (E)-	$C_{12}H_{24}$	14.45	0.04
Acrylic acid	Hexenoic acid<2E->	$C_6H_{10}O_2$	11.41	0.30
Monoterpenoids	Limonene	$C_{10}H_{16}$	11.55	0.06
Fatty aldehyde	Nonanal<n->	$C_9H_{18}O$	12.87	0.04
Cyclopropylbenzene	Benzaldehyde, 3,4-dimethyl-	C_9H_{10}	14.68	0.15
Sesquiterpenoids	Widdrol	$C_{15}H_{26}O$	33.39	1.81
	Panasinsene<beta->	$C_{15}H_{24}$	18.54	0.49
	Rosifoliol	$C_{15}H_{26}O$	19.85	0.25
	Calamenene<trans->	$C_{15}H_{22}$	16.72	0.22
	Humulene<alpha->	$C_{15}H_{24}$	18.03	0.20
	b-Ionone	$C_{13}H_{20}O$	18.37	0.12
Acyclic olefins	Tetradecene<1->	$C_{14}H_{28}$	17.07	2.75
Octyl phenols	Phenol, 2,4-bis(1,1-	$C_{14}H_{22}O$	18.62	5.95

	dimethylethyl)-			
Cetyl alcohol	Hexadecanol<n->	$C_{16}H_{34}$	19.56	3.57
Triterpenoids	Squalene	$C_{30}H_{50}$	30.85	9.07
	β-Amyrin	$C_{30}H_{50}O$	38.36	3.10
	Friedelan-3-one	$C_{30}H_{50}O$	42.39	0.30
Diterpenoids	Phytol	$C_{20}H_{40}O$	24.97	12.62
	Phytol, acetate	$C_{22}H_{42}O_2$	22.29	6.71
Diterpenoids	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	22.53	1.23
	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	22.35	0.55
	Isophytol	$C_{20}H_{40}O$	23.38	0.26
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	22.53	1.23
	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	22.35	0.55
Fatty acid and its Derivatives	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	23.57	24.31
	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	25.24	17.84

Octadecanoic acid	$C_{18}H_{36}O_2$	25.38	2.43
9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	24.84	0.35
Tridecenol<2E->	$C_{13}H_{26}0$	20.96	0.35
Tetradecanoic acid	$C_{14}H_{28}O_2$	21.41	0.26
Methyl 8,11,14-Heptadecatrienoate	$C_{18}H_{30}O_2$	22.88	0.18
Gurjunene<gamma->	$C_{15}H_{24}$	20.41	0.16
Methyl epi-jasmonate<Z->	$C_{12}H_{18}O_3$	20.28	0.07
Hexanoic acid	$C_6H_{12}O_2$	10.62	0.06

On the contrary, The GC-MS screening revealed availability of 35 biologically active phytocompounds in the DCM stem bark extract of *D. melanoxylon.* The retention time (Min), molecularformula, chemical class and % RD of compounds are as presented in Table 3.2. From these findings, it was discovered that the DCM stem bark extract of *D. melanoxylon* contained three monoterpenoids; Pinene<β-> had the highest relative abundance (0.75%) followed by limonene (0.17%) while pinene<α> had the least relative abundance (0.11%). It was also observed that intermedeol was the sesquiterpenoid with the highest relative abundance (30.09%) among the twenty discovered sesquitepenoids while bergamotene<α-cis-> had the least relative abundance (0.09%) (Table 3.2; Appendix III).

The findings also revealed that methyl linoleate had the highest percentage relative abundance at 10.96% among the six identified fatty acids and their derivatives in the extract of *D. melanoxylon*. The rest of the identified fatty acids were heptadecanoic acid, Eicosanoic acid, n-Hexadecanoic acid, methyl ester, octadecanoic acid, as well as 8- Octadecenoic acid (Table 3.2).

Only one fatty alcohol, pentadecanol<n->, was identified in the DCM stem bark extract of *D.melanoxylon*. The only alkylbenzene identified was phenol, 2, 4-bis (1, 1-dimethylethyl). Further, the results revealed only one acyclic olefins, tetradecene<1-> was available in the stem bark extract of *Dalbergia melanoxylon* (Table 3.2). Benzene, 1, 4- dimethyl-2, 5-bis (1-methylethyl) - was

the only aromatic hydrocarbon identified in the stem bark extract of *D. melanoxylon*. Dodecene<1->was the only acyclic alkenes identified in the extract of *D. melanoxylon* with a percentage relative abundance of 0.45 (Table 3.2; Appendix III).

Table 3.2 Phytochemical Analysis of DCM Stem Bark Extracts of *D. melanoxylon*

Class of compound	Compound Name	Molecular formula	Retention Time	Relative Abundance (%)
Monoterpenoids	Pinene<beta->	$C_{10}H_{16}$	10.50	0.75
	Limonene	$C_{10}H_{16}$	11.55	0.17
	Pinene<alpha->	$C_{10}H_{16}$	9.59	0.11
Sesquiterpenoids	Intermedeol	$C_{15}H_{26}O$	20.55	30.09
	Gurjunene<beta->	$C_{15}H_{24}$	18.55	16.02
	Rosifoliol	$C_{15}H_{26}0$	19.85	6.07
	Humulene<alpha->	$C_{15}H_{24}$	18.03	5.09
	Gurjunene<gamma->	$C_{15}H_{24}$	20.42	2.95
	Viridiflorene	$C_{15}H_{24}$	20.72	1.85
	Selinene<beta->	$C_{15}H_{24}$	18.45	1.49
	α-Panasinsen	$C_{15}H_{24}$	18.84	1.25
	Farnesene<(E)-beta->	$C_{15}H_{24}$	18.38	1.01
	β-Panasinsene	$C_{15}H_{24}$	20.21	0.95
	δ-Selinene	$C_{15}H_{24}$	38.49	0.73
	Cryptomeridiol	$C_{15}H_{28}O_2$	22.18	0.53
	Selinene<alpha->	$C_{15}H_{24}$	18.29	0.51
	Selin-11-en-4-alpha-ol	$C_{15}H_{26}0$	20.28	0.41
	Guaiol	$C_{15}H_{26}0$	19.77	0.31
	Caryophyllene(E-)	$C_{15}H_{24}$	17.59	0.26
	Kessane	$C_{15}H_{26}O$	19.05	0.22
	Cubebene<alpha->	$C_{15}H_{24}$	16.99	0.12
	Cyclosativene	$C_{15}H_{24}$	16.89	0.12
	Bergamotene<alpha-cis->	$C_{15}H_{24}$	17.74	0.09

Fatty acids and their	Methyl linoleate	$C_{19}H_{34}O_2$	25.15	10.96
Derivatives	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	23.51	7.37
	Octadecanoic acid	$C_{18}H_{36}O_2$	25.37	1.47
	Heptadecanoic acid	$C_{17}H_{34}O_2$	24.43	0.33
Fatty acids and their	Eicosanoic acid	$C_{20}H_{40}O_2$	27.09	0.26
Derivatives	8-Octadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	24.84	0.11
	Eicosanoic acid	$C_{20}H_{40}O_2$	27.09	0.26
Fatty alcohol	Pentadecanol<n->	$C_{15}H_{32}0$	19.55	1.11
Alkylbenzene	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	18.62	2.55
Acyclic olefins	Tetradecene<1->	$C_{14}H_{28}$	17.07	0.83
Metallocene	Ferrocene	$C_{10}H_{10}FE$	16.25	0.07
Aromatic Hydrocarbons	Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	$C_{14}\ H_{22}$	15.23	0.02
Acyclic alkenes	Dodecene<1->	$C_{12}H_{24}$	14.24	0.45

3.4 Discussion

This study evaluated the presence and identity of biologically active compounds of dichloromethane leaves extracts of *M. senegalensis* and root bark extracts of *D. melanoxylon* using GC-MS. The gas chromatogram exhibited relative concentrations of different phytochemicals fractionated at various retention times (Appendix II and III). The heights of the different peaks are suggestive of relative abundance of the compounds exhibited in the herbs extracts (Appendix II and III).

The fragmentation pattern is indicative of dissolution of huge fragments into smaller compounds that produce impression of heights at differing m/z ratios. Mass spectra supplied prototype of phytochemicals of the organic plant extracts, which were identified from the NIST data library (Appendix II and III).

Several ancient health practitioners held the belief that the entire herb extract is more potent than the isolated compounds (Rodriguez-Fragoso *et al*., 2008). However, crude extracts from most herbs may contain, in addition to bioactive compounds, other molecules which are poisonous. For instance, aristolochic acids present in Chinese plant, *Aristolochia fangch* is carcinogenic and nephrotoxic as is directly associated with renal failure (Debelle and Nortier, 2006).

The quantity of the biologically active phytochemical(s) from herbs may differ with season and location in which they were gathered (Altemimi *et al*., 2017). Furthermore, herbs gathered from the wild normally differ in quality, quantity and stability of bioactive components (De Smet, 1999). Again, biologically active compounds of most herbs are highly toxic when taken in plenty, and when herbs extract have low quantity of biologically active compounds than expected they may not be efficacious (Sasidharan *et al*., 2011).

The Gas Chromatography-Mass Spectrometry screening of the two plants extracts unveiled the existence of aromatic hydrocarbons, alkene, monoterpenoids, sesquiterpenoids, triterpenoids, diterpenoids, phenols, fatty acids and its derivatives.

The findings of GC-MS screening of *D. melanoxylon* exhibited the availability of n- hexadecanoic acid. The N-hexadecanoic acid is a very regular fatty acid; present in herbs (Aparna *et al*., 2012). N-Hexadecanoic acid is found to possess anti-inflammatory and antibacterial activities (Aparna *et al*., 2012). It has also been reported to selectively hamper DNA topoisomerase-I, where it averts the multiplication of fibroblast cells of humans (Harada *et al*., 2002). The association of N-hexadecanoic acid with DNA topoisomerase-I was proposed as the mechanism behind cytotoxic activity hence preventing proliferation of cells hence demonstrating

anticancer cytotoxic potential (Lokesh *et al*., 2016).

The results of GC-MS screening of *M. senegalensis* also disclosed the existence of friedelan-3-one. Friedelan-3-one is a triterpene, which has demonstrated antifeedant (Sun *et al*., 2009), hepatoprotective (Dzubak *et al*., 2006), antibacterial, antihypertensive, fungicidal and analgesic activities (Antonisamy *et al*., 2011; Ghosh *et al.*, 2011).

Beta amyrin was also revealed by GC-MS screening of the two plant extracts. Beta- amyrin is a pentacyclic triterpene which belongs to the oleanane group. Oleanane is a free radical captor owing to its sequestration ability of oxygen reactive species (Wang *et al.*, 2010). Preceding researches have reported that α and β-amyrins possess anti-inflammatory, and antitumor activities (Biskup *et al*., 2013), antihyperglycaemic activities (Bindu & Narendhirakannan, 2019) and antimicrobial activities (Jabeen *et al*., 2011). Conforming to a research by Karen *et al.* (2018), the β-amyrin in the leaves extract of *Myrcianthes pungens* are associated with the antioxidant potential of the plant extract through β-carotene bleaching method.

Squalene is a triterpenoid which was also identified in *M. senegalensis* extracts. Earlier studies have demonstrated that squalene contains several properties like antidiabetic and anti-inflammatory (Adekunle and Akinlua,

2007), antimicrobial (Nakuleshwar *et al*., 2012), antihistamine (Kar *et al.*, 2012), antimicrobial, antitumor (Aderibigbe *et al*., 2011), anti-androgenic, anti-proliferative and nematicidal activities (Zayed and Benedict, 2016). According to Chowtivannakule and Tulubmmo, (2012), squalene is also largely associated with antioxidant activities of several medicinal plants.

The GC-MS screening of *M. senegalensis* and *D. melanoxylon* extract also contained different sesquiterpenoids. Sesquiterpenes belong to the class of terpenes with different industrial uses (Scalcinati *et al*., 2012). These compounds contain 3 isoprene units, with 15 carbons and 24 hydrogens per molecule (C15H24) (Davis and Croteau, 2000). Some of the investigated pharmaceutical properties of sesquiterpenes include; antioxidant activities (Abolaji *et al*., 2013), anti-inflammatory (Wang *et al*., 2013), fungicidal (Kundu *et al*., 2013), anticarcinogenic (Afoulous *et al*., 2013) and antimicrobial (Wang *et al*., 2013).

Caryophyllene (E) is widely found among plant oils and extracts. It has been described to contain insecticidal, acaricidal, antifungal and repellent activities (Kim *et al*., 2003), antibacterial activities (Patra, 2012), and anti-inflammatory in addition to anti-allergic activities (Rogerio *et al*., 2009). Antioxidant potential of caryophyllene (E) was also published by Ruberto and Baratta, (2000).

Further, the GC-MS screening of extract of *D. melanoxylon* and leaf extract of *M. senegalensis* unveiled the presence of α-humulene. Work published by Coté *et al*., (2017) attributed the anti-inflammatory properties of *Tanacetum vulgare* to α-humulene. Previous studies have also reported that α-humulene possesses several biological properties such as antioxidant activities (Ruberto and Baratta, 2000), antibacterial and antifungal activities (Schmidt *et al*., 2008). α-humulene has been reported as the active compound which reduced rat paw edema injected with carrageenan (Fernandes *et al.*, 2016). Additionally, it was shown to hinder COX-2 and Inos expression, which prompted the authors to conclude that α-humulene is a crucial compound that can be employed in management of inflammatory ailments.

Limonene is a popular and cheap fragrances mostly used in cosmetics formulation, for instance, shampoos, shower gels, soaps, perfumes and hair conditioners (Filipsson *et al*., 1998) environment friendly pesticides and cleaning products (Nazaroff *et al*., 2004). Another usage of limonene is a flavor as well as fragrance additive in most foods for instance, soft drinks, chewing gums, ice creams, fruit juices and candies owing to its pleasant lemon-like smell (Zulaikha *et al*., 2015). Further, it is regarded as safe food preservative and has been employed as a green solvent extraction of organic products (Chemat *et al*., 2012). A research conducted to study the effect of limonene by Zhang *et al*. (2002) deduced that the compound had anticancerous activities on man's gastric carcinoma cell line MGC803.

A study evaluating the genotoxic, cytotoxicity, and antigenotoxic and antioxidant activities of limonene, Bacanli *et al.* (2015) attributed the reduction in cell viability to limonene. Findings by Squires *et al*. (2010) and Aryal *et al*. (2019) as well associated limonene with the *in vitro* anthelminthic activities of *citrus reticulate* against earthworms in ruminants. Several previous studies on limonene suggested that it has inhibitory effectson plasma membrane pumps, enzymes of parasites, growth of nematodes and alters metabolic pathways of the nematodes (Aryal *et al*., 2019).

GC-MS screening of *D. melanoxylon* plants extracts also identified methyl linoceate. Pinto-Maria *et al.* (2017) demonstrated that methyl linoleate was responsible for antioxidant and fungicidal action of fatty acid methyl esters (FAMEs) form vegetable oils.

Pinene<α> is an organic monotepenoid compound which has been found in eucalyptus, pine trees, camphor and rosemary (Bae *et al*., 2012). Alpha-Pinene was described to have anextensive range of medicinal properties like antioxidant (Dai *et al*., 2010), antimicrobial (Dorman *et al*., 2000), anticancer activities (Matsuo *et al*., 2011), anesthetics (Bae *et al*., 2012) and antipyretic (Bonjardim *et al*., 2012). A study on *Thymus vulgaris* essential oils showed that the main compound was β-pinene which exhibited

antibacterial properties (Imelouane *et al*., 2009). Further, α-pinene and β-pinene from *Croton argyrophyloides* demonstrated greater larvicidal potential against *Aedes aegypti* (Morias *et al*., 2006). The α-pinene has been described previously to impede NF-κB and JNK activation as well as expression of Inos (Schepetkin *et al*., 2016).

Findings of the GC-MS screening of *M. senegalensis* and *D. melanoxylon* exhibited the existence of Phenol-2, 4-bis (1, 1-dimethylethyl). Phenol-2, 4-bis (1, 1-dimethylethyl) is a precursor of several complex compounds and extensively employed as light protection agents, chemical intermediates for production of other chemical intermediates as well as antioxidant.

Phenol-2,4-bis (1, 1- dimethylethyl) is an antimicrobial which can be obtained from plants and animal materials (Janaki *et al*., 2017) as well as microbial metabolites like *Shewanella algae* (Gong *et al*., 2015), *Nocardiopsis* spp. (Sabu *et al*., 2017), *Bacillus velezensis* (Gao *et al*., 2017), *Streptomyces* spp. (Kumar *et al*., 2019), *Bacillus subtilis* (Gao *et al*., 2018), *Vibrio* spp. (Pawar *et al*., 2014), *Vibrio owensii* (Karthick and Mohanraju, 2018), *Monashia flava* (Azman *et al*., 2014) and *Vibrio alginolyticus* (Padmavathi *et al*., 2015) among others.

Previous works have reported that phenol, 2, 4-bis (1, 1-dimethylethyl) has several pharmaceutical properties including antioxidant (Choi and Lee, 2009), antifungal (Malek *et al*., 2012), antiseptic (Abdullah *et al*., 2009) and as antitumor properties (Malek *et al*., 2012).

The GC-MS screening results of stem bark extract of *D. melanoxylon* also unveiledexistence of a metallocene, ferrocene. Ferrocene and its derivatives have been reported in the last decade to possess high potential *in vivo* and *in vitro* against several ailments such as cancer (N'Da *et al*., 2014). Further, some ferrocene derivatives have been shown to havea broad scope of pharmaceutical properties like antitumor, anticonvulsant, analgesic, anti-HIV properties and antioxidant (Santos *et al*., 2008). Preceding researches have described ferrocene derivatives containing nitrogen atoms potential of scavenging in *vitro* free radicals and anti-oxidant properties (Lobo *et al*., 2010). Further, based on Xing *et al*. (2015), ferrcenylhydrazones exhibited antioxidant activities.

Ferrocene was also identified in the studied plant extracts. Ferrocene has been shown to be a stable, nontoxic compound with good redox activities. Owing to its potential to act as an electron donor undergoing oxidation to the ferrocenium ion, ferrocene is used as a model for non-linear optical materials and sensor (Williams *et al*., 2004). Several tests carried out on

ferrocene and its derivatives have shown that it is relatively safe in many mammals (Li *et al*., 2017). Ferrocenyl derivatives have demonstrated antitumour, cytotoxic, antifungal, DNA cleaving and antimalarial activities (Delhaes *et al*., 2001).

The Gas Chromatography-Mass Spectrometry screening of the plant extracts of and *D. melanoxylon* also unveiled the availability of several fatty acids and their derivatives. Fatty acids occur generally in natural fats and diet oils and play a crucial role as nourishing matter and metabolites in organisms (Cakir, 2004). Several fatty acids have been described to exhibit antifungal and antibacterial properties (Chandrasekaran, 2004). A research described by Bohm *et al.* (2016) detailed how the methanolic extract of *M. senegalensis* was linked to a broad scope of chemical classes such as n-hexadecanoic acid, octadecanoic acid and heptadecanoic acid according to the findings of GC-MS. The aforementioned compounds were associated with anthelmintic properties of *M. senegalensis.*

Previous studies have reported the antimicrobial properties of octadecanoic acid (Rahuman *et al*., 2000). A research conducted to investigate the properties of ethyl acetate extract of *Carallocarpus epigaeus* tuber credited the anthelminthic activity to a broad scope of chemical classes such as heptadecanoic acid, octadecanoic acid and n-hexadecanoic acid according to the results of GC-MS (Bohm *et al*., 2016). From previous

studies tetradecanoic acid has been described to contain larvicidal as well as repellant activities (Sivakumar *et al*., 2011).

Phytol is diterpene bio compound also identified in the stem bark extract of *M. senegalensis.* Phytol belongs to a group of branched chain of unsaturated alcohols (Gloerich *et al*., 2007). Phytol is extensively found in the environment since it is a product of chlorophyll metabolism. Phytol has been reported to hinder growth of *Staphylococcus aureus* (Inoue *et al*., 2005) and to block the teratogenic effects of retinol (Arnhold *et al*., 2002). Camila *et al.* (2013) showed that phytol has antihyperalgesic potential in different models and antioxidant activities against free radicals produced *in vitro.*

Phytol reacts with a free radical, give out hydrogen atoms with an unpaired electron(H), turning free radical into less reactive species (Guimaraes *et al*., 2010). In accordance with an *in vitro* research conducted by Santos *et al.* (2012), phytol possesses antioxidant activity since it is capable of scavenging hydroxyl radical and nitric oxide.

Xylene is a volatile aromatic hydrocarbon which is liquid at room temperature, processed through petrochemistry (Le Floch *et al*., 2012). It is usually a mixture of isomers (meta- (m-), para (p) and ortho-(o-) carrying varying amounts of ethylbenzene. Xylene is used as a cleaning agent, a

solvent, leather, rubber and paint factories (Le Floch *et al*., 2012).

Based on a research by Hipolito *et al.* (2018) and Uchida *et al.* (1993) numerous subjective neurological symptoms for instance, dizziness, inability to concentrate, forgetfulness and anxiety were observed among subjects that were chronically exposed toxylene vapors. A survey conducted by Rajan and Malathi, (2014) on animal models described signs of epistaxis, convulsions, hypersalivation and hyperactivity along withelevated aggressiveness in rats provide with mixed xylene for 90 days.

Renal tissues or direct membrane fluidization was attributed to the mechanism of nephrotoxicity of xylene which was associated with reactive metabolite formation (Rajan and Malathi, 2014). A research by Shi *et al*. (2016), unveiled elevated levels of β- glucuronidase in humans exposed to xylene indicative of faster turnover of renal cells because of toxicity of the toxic metabolites of xylene. Liposolubility of xylene in the neuronal membrane has been reported as the cause of toxic symptoms of the central nervous system such as dizziness (Rajan and Malathi, 2014).

Xylene was reported to disturb the activity of the proteins that are crucial for proper neuronal function. Therefore, from the results obtained in this chapter, it is clearly demonstrated that the two plants contain potent and

bioactive phytochemicals which are linked to several therapeutic properties like anti-helminthic and antioxidant properties.

CHAPTER FOUR

IN VITRO ANTHELMINTIC PROPERTIES OF DICHLOROMETHANEEXTRACTS OF *MAYTENUS SENEGALENSIS* AND *DALBERGIA MELANOXYLON*

4.1 Introduction

Helminthiasis is the condition associated with worm infestation. Globally, about 2 billion people have these ailments, translating in excess of the total populace (Hotez *et al*., 2008). More than three hundred million individuals in the world are severely sick due to worms and majority are school going children (Hotez *et al*., 2008). Despite it being preventable and treatable, helminthiasis remains primary cause of mortality globally. In Africa, for instance, the deaths due to worm infestation may be over 200,000 annually (WHO, 2015). Factors such as inadequate sanitary facilities, contaminated water, poverty and illiteracy are associated with widespread nature of these ailments in poor countries ((Hotez *et al*., 2008).

The management of helminthiasis in the 21st century is majorly through the use of conventional synthetic drugs commonly known as anthelmintic (Behnke *et al*., 2008). Anthelmintic eliminate (vermicide) or expel (vermifuge) invading helminthes. High costs, chemical residue in meat and milk of host animals, low supply of drugs, and toxicity have turned majority of global population to rely on traditionalanthelmintic remedies (Behnke *et al*., 2008; Jeyathilakan *et al*., 2010).

From estimates, over 20,000 plant species are used globally for management of different diseases (Jeyathilakan *et al*., 2010). Herbs and fruits were the preferred sources of drugs for ages for curing different ailments particularly those caused by parasites (Behnke *et al*., 2008). The unique chemical compounds in medicinal herbs are responsible for their wide spread use as major sources of drugs (Craker, 2007). The continuous use of conventional drugs may cause dependency whereas there is no possibility of abuse of medicinal plants (Ekor, 2014).

Herbs provide potent and pure compounds that are active against diverse ailments (Gadamsetty *et al*., 2013). These drugs have a lot of potential for they are used as major sources of safer and more efficacious conventional drugs in pharmaceutical industries (Rates, 2001).Based on information sourced from herdsmen, pastoralists and ethno-medicinal survey, *M. senegalensis* and *D. melanoxylon* plants were selected for use in this study (Tariq *et al*., 2016).

Literature review also revealed that the two plants have not been scientifically evaluated for their anthelmintic properties despite their traditional use as anthelminthic. Hence the present study was carried out on dichloromethane extracts on these two plants against the Indian earthworm to confirm their folklore use as well as making scientific validation and

documentation for use of these plants. This will generate preliminary data that will immensely form a basis for development of effective plant-derived anthelmintic drugs from the two medicinal plant extracts.

4.2 Materials and Methods

Specimen collection, preparation and extraction were conducted as earlier reported inchapter three (sections 3.2.1 and 3.2.2).

4.2.1 Collection of Worms

Adult earth worms, *Pheretima posthuma*, were used for this research on account of their anatomical and physiological similarities with human roundworms. *Pheretima posthuma* were obtained from moist, fertile soil in Kagumo-ini village, Kagumoi-in location, Kiru division, Mathioya Sub County, Murang'a County, Kenya. The GPS coordinates of the earthworms collection sites are Latitude 0^{o} 37'16S and Longitudes 36^{0} 58' 45E. The worms were sorted out, washed and carefully ferried in plastic containers to the Kenyatta University Biochemistry, Microbiology and Biotechnology Laboratories where this work was done. Worm were identified and authenticated at the Zoology Department of Kenyatta University. The earthworms were acclimatized for one week to the laboratory conditions before experimentation.

4.2.2 Determination of Anthelmintic Activity

4.2.2.1 Experimental Design

This research adopted an entirely randomized controlled design. Seventy five worms were grouped into 5 groups of 5 worms in each of 3 petri plates. Group I (Normal control) worms were served with distilled water only (50 ml). Group II (Positive control) worms were administered with albendazole (Reference drug) at a dosage of 5 mg/ml. Groups III, IV and V worms were administered with plant extract doses of 12.5, 25 and 50 mg/ml respectively. This design is outlined in Table 4.1

Table 4.1: Experimental Design

Experimental groups	Treatment	N
I (Normal control)	Water (50ml)	15
II (Positive control)	Albendazole (25mg/ml)	15
III	Plant extract (12.5mg/ml)	15
IV	Plant extract (25mg/ml)	15
V	Plant extract (50mg/ml)	15

N= Number of earthworms per treatment

Upon treatment of worms with various treatments as shown in the experimental design (Table 4.1) worms were observed for any signs of paralysis or mortality. Paralysis was considered to occur when worms were unable to move even after vigorous shaking of the plates. Mortality was considered to be loss of motility even after dipping of earthworms in hot water (50^0C). Time taken for paralysis and mortality were recorded in minutes.

4.2.3 Management of Data and Analysis

Experimental data for the period taken for paralysis and death to occur were obtained from all the treatment groups. The data were put down and arranged in a broad sheet using Ms Excel program. The data were scrutinized for normalcy by use of Kolomogorov-Smirnov test. The results were further put through descriptive statistics and manifested as Mean ± SEM. The data were then subjected to inferential statistics using One Way ANOVA followed by Tukey's post hoc test for pairwise separation and comparisons of means. Unpaired student t-test carried out to compare effectiveness of thetwo plant extracts. Statistical significant difference was reported at $p \leq 0.05$. All statistical analyses were executed using Minitab version.17 software (Minitab Inc., Pennsylvania in U.S.A). The derived data of this research was put forward in form of tables and bar graphs.

4.3 Results

4.3.1 Paralytic Effects of DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon* on *P. posthuma*

Generally, the DCM extracts of *M. senegalensis* and extracts of *D. melanoxylon* successfully exhibited anthelmintic activities, which were indicated by the paralysis of earthworms upon exposure to various extract concentrations (Table 4.2; Appendix IV).

Table 4.2: Paralytic Properties of *M. senegalensis* and *D. melanoxylon* on P. posthuma

Treatment groups	Time taken to paralyze *P. posthuma* (Minutes) **(Mean ±SEM)**	
	M. senegalensis	*D. melanoxylon*
I (Normal group)	No paralysis	No paralysis
II (Positive control)	02.82 ± 0.18^{c}	02.82 ± 0.18^{bc}
III (12.5mg/ml Extract)	11.25 ± 0.42^{a}	07.24 ± 0.45^{a}
IV (25.0mg/ml Extract)	06.39 ± 0.36^{b}	03.56 ± 0.24^{b}
V (50.0mg/ml Extract)	03.07 ± 0.16^{c}	02.13 ± 0.04^{c}

Values shown as mean± S.E.M (n=3). Values having dissimilar superscripts along the same column arestatistically different calculated by One Way ANOVA followed by Tukey's post hoc test ($p\leq 0.05$)

The extracts manifested paralytic effects on worms after exposure periods ranging between 3.07 and 11.25 min for *M. senegalensis* and 2.13 and 7.24 min for *D. melanoxylon* (Table 4.2). The time taken by the worms to undergo paralysis due to exposure to the extracts decreased with increasing extract concentrations (Table 4.2). It was noted that the three tested extract concentrations (12.5, 25 and 50 mg/ml) of the two plants, showed paralytic effects that were significantly different from each other ($p<0.05$; Table 4.2). At the extract concentration of 50mg/ml, the

effectiveness of both plant extracts was statistically comparable to properties by Albendazole (Table 4.2). Earthworms exhibited no signs of paralysis upon exposure to distilled water (normal control).

In contrast, the DCM leaf extract of *M. senegalensis* was shown to be more effective in inducing paralysis of *P. posthuma* as opposed to the DCM extract of *D. melanoxylon*. The activity of stem bark extracts at dosages *of* 12.5,25 and 50mg/ml took 7.24min, 3.56 and 2.13min in contrast to 11.25, 6.39 and 3.07 min at the similar dosages ($p \leq 0.05$; Figure 4.1). Nevertheless, the two herbs extract demonstrated comparable time for paralysis to occur in *P. posthuma* at the dosage of 50 mg/ml (Appendix IV).

4.3.1.1 Mortality effects of DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon* on *P. posthuma*

The anthelmintic effects of DCM extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* were also indicated by the short duration of time taken by earthworms to die upon exposure to the extracts (Table 4.3; Appendix V).

The extracts showed mortality (wormicidal) effects on the worms after time periods ranging between 04.45 and 13.29 min for *M. senegalensis* and 03.36 and 08.76 min for *D. melanoxylon* (Table 4.3).Overall, it was noted that exposure of worms to the dichloromethane extracts of *M. senegalensis* and extracts of *D. melanoxylon* resulted in a regular pattern of reduced time taken for death to occur, from the lowest to the highest extract concentrations (Table 4.3). It was evident that the three tested extract concentrations (12.5, 25 and 50mg/ml) of the two plants showed anthelmintic effects that were significantly different from one another. However, at the extract concentration of 50mg/ml, the effectiveness of both plants was comparable to the effects of Albendazole ($p>0.05$; Table 4.3). Exposure of worms to negative control (distilled water) caused no mortality.

Table 4.3: Mortality Properties of *M. senegalensis* and *D. melanoxylon on P. posthuma*

Treatment groups	Time taken for mortality to occur (Minutes) (Mean **±SEM)**	
	M. senegalensis	*D. melanoxylon*
I (Normal group)	No mortality	No mortality
II (Positive control)	03.77 ± 0.16^{c}	03.77 ± 0.16^{c}
III(12.5mg/ml Extract)	13.29 ± 0.32^{a}	08.76 ± 0.46^{a}
IV(25.0mg/ml Extract)	07.92 ± 0.28^{b}	04.79 ± 0.21^{b}
V (50.0mg/ml Extract)	04.45 ± 0.17^{c}	03.36 ± 0.18^{c}

Values indicated as mean ±S.E.M (n =3). Values having dissimilar superscripts along the same column are not significantly different as analyzed by One Way ANOVA followed by Tukey's post hoc test ($p \leq 0.05$)

When compared, the time taken for mortality to occur due to the DCM extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* shown that the *M. senegalensis* extract was significantly more efficacious compared ($p \leq 0.05$) to the *D. melanoxylon* extract of all the tested extract concentrations (Appendix V).

4.4 Discussion

Generally the DCM extracts of the two plants exhibited significant anthelminthic activities against earthworms. Several studies have reported that medicinal plants possess anthelmintic activities globally (Molla, 2016). In central Ethiopia, concoctions from *E. capensis* have been used for treatment of helminthiasis (Hailu *et al*., 2018).

Terminalia schimperiana has been used to treat heminthiasis in Cameroon (Toyang *et al*.,2005). *Terminalia avicenniodes* has also been described to be efficacious against helminthes in sheep (Nsekuye, 1994). Further, *Chenopodium ambrosioides* has been reported as an effective herb against *Haemonchus contortus* infestation in goats (Ketzis *et al*., 2002). The seeds of cucumber and pumpkin (*Cucubitaceae*) have been utilized by tropical American farmers for a long time as treatments of tapeworm ailments (Waller *et al*., 2001).

According to Waller *et al*. (2001) leaves from tobacco (*Nicotiana rustica*) or their synthetic analogues such as nicotine sulphate have been used for centuries in the management of nematodes in ruminant animals. Some forage plants, especially legumes with high content of proanthocyanidins, have been used for the management of worms in grazing lambs with great success (Hoste *et al*., 2015). Most of evidence on anthelmintic property of medicinal herbs is according to anecdotal observations. This has led to a growing number of controlled experiments geared towards scientifically validating and quantifying such bioactivities (Athanasiadou *et al*., 2007).

The present study used *in vitro* techniques, which are preferred because they are economical and simple to use (Markus *et al*., 2005). According to Asase *et al*. (2005), *in vitro* studies using Indian earthworms provide a means of evaluating the anthelmintic properties of medicinal herbs under study (Asase *et al*., 2005).

Several parts of *M. senegalensis* have been evaluated for nematocidal activity demonstrating diverse results. For instance, a study carried out on the ethanolic unrefined extract of *Maytenus senegalensis* did not exhibit remarkable property on egg-laying as well as larval growth of *H. conrottus* (Kone *et al*., 2002). In another work, acetone/water leaf extract of *M. senegalensis* exhibited remarkable antinematocidal anthelmintic properties using larval exsheathment inhibition assay (LEIA) (Mengistu *et al*., 2017).

The current research agrees with previous studies carried on the same plant albeit using different solvents. Most traditional practioners use water for extraction as it is readily available as well as it is non-toxic (Zang *et al*., 2018). Dichloromethane is a low polar solvent that extracts non-polar compounds especially terpenoids or most methoxylated phenolics (Stalikas, 2007). Different researchers have used dichloromethane while extracting components from plants extracts (Cosa *et al*., 2006).

The current study employed dosages of 12.5, 25 and 50 mg/ml which were in agreement with earlier similar studies. For instance, when studying the anthelmintic properties of extracts from *artemisis* plants against nematodes, Khan *et al*. (2014) used dosages of 50, 25, 12.5 and 6.25 mg/ml. Further, a study by

Kalpesh and Priya (2020) used 25, 50, 75 and 100 mg/ml doses when evaluating *in vitro* anthelmintic activity of *Corallocarpus epigaeus* using ethyl acetate extracts and albendazole at 20 mg/ml. The dosages used are also comparable to those in the review done by Sabir *et al*. (2007) when evaluating *in vitro* vermifuge effects of aqueous and methanolic extracts of *Murraya koenigii* on *H. contortus*

However, this is in contrary to a review by Sushmita *et al*. (2018) which used doses of 1.56, 3.125, 6.25, 12.50, 25 and 50mg/ml when evaluating the *in vivo* and *in vitro* effectiveness of *Eucalyptus citriodora* leaf extracts in gastro intestinal roundworms in goats.

The current study used *Pheretima posthuma* to evaluate anthelmintic activity. The worms were selected because they share physiological and anatomical similarities with human intestinal roundworms. This is comparable to the study by Mitesh *et al*. (2015), Amit and Sangh (2015), Arshad *et al*. (2012) and Bairagi *etal*. (2011). On the contrast, Das *et al*. (2017) used aquarium worm, *Tubifex tubifex*, to determine vermifuge properties of the stem bark and leaf extract of *Tamarindus indica* Linn.

The crude extracts of *M. senegalensis* and *D. melanoxylon* on earthworm exhibited dose dependent anthelminthic activities. The DCM leaf extracts of *M. senegalensis* in concentration 12.5, 25 and 50 mg/ml showed paralysis of worms within 11.25 to 3.07 min. depending on concentration. This trend was similar to the observations published by Yadav & Temjenmongla, (2012), who evaluated the effects of different aerial extracts of *Lasia spinosa* Thwaites on Indian earthworms. Bendgude *et al*. (2012) also reported dose dependent vermifuge properties of

leaves of *Mimosa pundica*. Further, another study established the potential of ethanolic and aqueous extracts of *A. fistulosum* to paralyze and cause mortality of worms in a dose dependent manner (Husori *et al*., 2016).

Presence of enough concentration of potent compounds in the dose level of 50 ml/ml than lower dose levels of the extracts can be a possible explanation to the trend. Further, rapid metabolism and removal of the potent phytocompound(s) found insufficient concentrations in the lesser dose levels of the extracts, is another possibleexplanation (Li, 2011).

When exposed to the body surfaces of worms, the compounds in the extracts may have interfered with basic biochemical, physiological, metabolic and behavioral functions of worms. Therefore, the extracts may have mediated the anthelmintic activities in earthworms in different ways. The muscles of worms are notable to contain excitatory neuromuscular junctions with different receptors where acetyl-choline is their neurotransmitter (Neal, 2001). Phytochemicals performing as ganglion stimulant may have activated these neuromuscular junctions leading to paralysis in the worms andeventual mortality. Further, the death could also be as a result of flaccid paralysis.

Several phytochemicals have very potent functional groups in their composition such as epoxides, SH-groups, aldehyde and triple bonds (Van *et al*., 2015), which form covalent bonds with peptides and proteins (Wink and Wie, 2005). Epoxides readily react with free amino groups of polypeptide and DNA bases or SH-groups of polypeptides. Exocyclic methylene groups in the phytocompounds could have bound to SH-groups in glutathione and proteins hence, interfering with the normal

biological, biochemical and physiological functions of the earthworms and causing paralysis and eventual death (Butterfield *et al*., 2006).

Phytochemicals may also have invaded several of proteins for instance enzymes, ion channels, cytoskeletal proteins, receptors, transporters as well as transcription factors of worms in a non-selective manner. They may have acted by a way of non-covalent modification of protein forming many hydrogen bonds with electronegative atoms (O, N) in peptides and polypeptides (Van and Wink, 2015). Further, helminthes have bio membranes which contain several proteins such as receptors, transporters, and ion channels, which allow communication with neighboring cells and tissues. Hence, if a bio membrane is lysed then death occurs. Most phytochemicals have affinity for bio membranes (Van *et al*., 2015).

Lipophilic phytochemicals can regulate the activity of ion channels. When they get into contact with worm cells, they stick to the lipophilic inner membrane bilayer (Van *et al*., 2015). For instance, mint oil transforms calcium channels and movement of smooth muscular cells in the ileum (Van *et al*., 2004). Therefore, phytochemicals in the extracts could have affected the worms' protein membranes activity, which could have altered their interaction with phospholipids leading to paralysis and death of the worms.

Anthelmintic also act by interacting with nucleic acids. A few anthelmintic phytochemicals intercalate or even alkylate DNA, which leads to mutation or cancer (Van *et al*., 2004). The potent components in the extracts used in the current study could have been lipophilic and aromatic, which could

have made them interlate DNA of the worms (Wink and Schimmer, 2010). Therefore, observed anthelmintic activity in the current work could be credited to the existence of phytochemical compounds in the extracts as exhibited by GC-MS screening. The compounds are n-hexadecanoic acid, heptadecanoic acid, tetradecanoic acid and octadecanoic acid among others, which are toxic to helminthes (Bohm *et al*., 2016).

A few fatty acids identified in the organic extracts of *M. senegalensis* and *D. melanoxylon* have been demonstrated to contain anthelmintic potency (Bohm *et al*., 2016). Dubal *et al*. (2013) associated n-hexadecanoic acid (Palmitic acid), contained in methanolic extract of rhizome of *Tectaria coadunate,* to the nematicidal efficacy of the plant extract. Hexadecanoic together with octadecanoic fatty acids were shown to possess nematicidal activities (Sabithira *et al*., 2017: Ishnava and Konar, 2020). The anthelmintic effects of ethyl acetate of *Carollacarpus epigaeus* tuber was also credited to a broad spectrum of fatty acids like n-hexadecanoic acid, octadecanoic acid together with heptadecanoic acid (Ishnava and Konar, 2020). Furthermore, according to Dubal *et al*. (2013), these fatty acids have been associated with nematocidal properties. It is therefore, possible that fatty acids paralyzed and killed the worms in the current work.

The abundance of phenolic substances in the leaf extracts of *M. senegalensis* as well as *D. melanoxylon* might be behind the anthelmintic

properties observed in this study.

Phenolics contain single or more phenolic OH-groups, which can easily dissociate to negatively charged phenolate ions under physiological environment (Wink and Wie,2005). Positively charged amino groups easily establish ionic bonds with negatively charged groups. Where polyphenolics forms many hydrogen and ionic bonds with apolypeptides or catalytic or binding site, the functional together with structural flexibility of protein get impaired.

Phytochemicals form many ionic and hydrogen bonds thereby affecting proteins. Phenolic components are usually gylcosylated with single or more sugar molecules (Tapas *et al*., 2008). Many hydroxyl groups of carbohydrates may also aid the phenolic- proteins interaction by strengthening hydrogen bond. Some polypeptides are transcription factors, which moderate differential gene expression in an animal (Morais *et al*., 2006). Phenolics can regulate transcription factors which form covalent bonds. Modulation of genes could be affected accidentally (El-Readi *et al*., 2013).

The anthelmintic activities of the extracts could also be associated to the existence of pinene-type monoterpene (beta-pinene, limonene). Alpha-pinene and β-pinene extracted from *Croton argyophyloides* demonstrated significant larvicidal property against *Aedes aegypti* (Morais *et al*., 2006).

Further, the anthelmintic activities were credited to the existence of triterpenoids in the *M. senegalensis* extracts. A research by Avani *et al*. (2010), when examining the anthelmintic properties of leaf extract of *Tephrosea purpurea* (Linn), credited the anthelmintic properties to the existence of triterpenoids in the extracts.

The phytochemicals with anthelmintic properties in these extracts could have acted individually or collectively thus potentiating their toxicity effects on worms (Aggarwal *et al*., 2016). It is also highly possible that the potential of the extract was directly to the synergistic association or antagonistically of many phytochemicals existing, which cannot be identifiedwhen one compound is assessed alone (Eid *et al*., 2012).

The current research employed usage of albendazole as the standard anthelmintic drug. Like many other synthetic anthelmintic, it causes hyperpolarization of intestinal worms' muscles by GABA agonistic action. This occurs by opening of chlorine channels thatleads to relaxation and inhibition of contractile action responsiveness of acetylcholine byelevating chloride ion conductance of helminthes muscular membrane (Eid *et al*., 2012).

Therefore, theproduced hyperpolarization and lowered excitability causes muscular relaxation as well as flaccid paralysis (Mali *et al*., 2008).

Albendazole has also been revealed to block glucose uptake by the worms, which results in exhaustion of glycogen reserves and laterlowering of ATP thereby causing paralysis and eventual mortality of the helminthes (Eid *et al*., 2012). Studies have also shown that albendazole causes mortality of adult intestinal helminthes, kills ova and cercaria (Chai *et al*., 2021).

Experimental evidence has shown that helminthes treated with Albendazole suffer from metabolic disruption at various sites, mostly energy synthesis sites in worms (Horton, 2000). In contrary, non-benzimidazole drugs, acts on worms' neuromuscular pathways to cause paralysis (Horton, 2000). Benzimidazoles cause metabolic disruption by inhibiting beta-tubulin polymerase, where they disrupt cytoplasmic microtubules formation (Horton, 2000).

Therefore, the organic extracts of *M. senegalensis* and *D. melanoxylon* were found to possess anthelmintic properties by inducing paralysis and eventual death of worms. The extracts of the two plants have ability indicative of their potential development as anthelmintic agents by destroying the cytoskeletal structure of helminthes.

CHAPTER FIVE

IN VITRO ANTIOXIDANT PROPERTIES OF DICHLOROMETHANEEXTRACTS OF *MAYTENUS SENEGALENSIS* AND *DALBELGIA MELANOXYLON*

5.1 Introduction

Helminthiasis ailments in animals are extensively spread globally, and their effects closely resemble nematode infections in humans. Helminthiasis and associated influx of eosinophilis have been reported as the main sources of tissue injury, maybe due their reactive oxygen species output (Spencer *et al*., 2010). Invasion of helminthes into the bodies of animals is associated with uncontrolled output of free radicals, leads to cellular destruction (Rosenberg, 2013).

Previous researches have demonstrated existence of oxidative stress in animals as well as humans infested with helminthes (Upcroft and Upcroft, 2001). Further, studies have explored antioxidant defense mechanisms that occur in human or animal hosts (Gersch *et al*., 2009). Cells have different antioxidant processes that take major part to counter ROS and RNS (Rosenberg, 2013).

Constituents of antioxidant protective mechanisms are either external or internal in originand can act harmoniously and independently to counteract RNS and ROS (Kurutas, 2016). These antioxidant compounds comprise metal binding proteins like ceruloplasmin and albumin which sequester free

Zn^{2+} and Cu^{2+} ions in protoplasm or Mn^{2+} ions in mitochondria matrix that work as stimulant compounds in redox responses (Kaliora *et al*., 2006). Elevated oxidative stress in red blood cells of cows infested with *Theileria annulata* has been recorded, and it was attributed to elevated erythrocyte fragility following membrane lysis (Grewal *et al.,* 2005), probably due to the accompanying helminthiasis as a result of oxidative stress.

Conventional antioxidants like butylated hydroxytoluene, butylated hydroxyanisole as well as tert-butylhydroxyquinone have been linked to negative effects in animals (Hung *et al.,* 2019). Increase in anthelmintic drug resistance, high cost, residues in animal products and unavailability, calls for the need to explore novel integrated approaches to management of helminthiasis. Usage of plants as supplements in cattle nutritional and helminthes management is a substitute to conventional dewormers (Makkar *et al*., 2007). Usage of substitute anthelmintic containing natural antioxidants constitutes a new management tool that is ethical, environmentally friendly and green and (Martin, 2006) and user friendly.

A previous study on the stem barks extract of *D. melanoxylon* using *in vitro* methods showed that the plant extract has antioxidant activities (Olugbami *et al*., 2014). However, the study does not report the link between antioxidant activity and anthelmintic activity. Moreover, Cansian *et al*. (2015) published *in vitro* antioxidant activities of members of *Maytenus* family namely, *M. aquifolium, M. ilicifolia*, *M. dasyclada*. There is

however, poracity of data regarding antioxidant activities of *Maytenus senegalensis*.

Understanding antioxidant effects of anthelmintic herbal extracts is important in the knowledge of the correct plant extracts and concentrations to use for adequate control of oxidative stress- related helminthes infections. New plant antioxidants with anthelmintic properties should be identified and characterized for development of safe antioxidants.

Maytenus senegalensis and *Dalbelgia melanoxylon* were used traditionally by the Mbeerecommunity in Embu County, Kenya for the management of helminthiasis, in particular young children, and livestock. In addition to the fact that this treatment use has not been scientifically validated, it is known whether their anthelmintic potential is due to their antioxidant activities. This study envisages that the two plants treat helminthiasis by, among other activities, mounting antioxidant defense precipitated by helminithiasis.

5.2 Materials and Methods

5.2.1 Sample Collection, Preparation and Extraction

Sample gathering, preparation and extraction were conducted as previously reported inchapter three (sections 3.2.1 and 3.2.2).

5.2.2 Determination of *in vitro* Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

The potential of *M. senegalensis* and *D. melanoxylon* DCM extracts to scavenge DPPH radical *in vitro* was resolved in reference to the protocol described by Kuo *et al*. (2002). As per this protocol, 3 ml of methanol was put into 0.5 ml of methanolic solution of DPPH. Different dilutions namely 0.05, 0.1, 0.5, 1, 2, and 5µm of the herb extracts in addition to ascorbic acid were formulated. One ml of each plant sample was put into the mixture. The mixture was whirled thoroughly for 5 mins and left to stay for 30 mins in dark. The preparation of assays was done in triplicates. The mixture absorbance was read at 517 nm using a spectrometer. Absorbance decrease was measured against the control. A blank solution with all the reagents minus plant extracts acted as the negative control. Per-cent DPPH scavenging potential of the herbs extracts was then determined by the formula by Kadare *et al*. (2011)

$$\%\,\text{DPPH Scavenging activities} = \frac{\text{Absorbance of control} - \text{Absorbance of sample/standard}}{\text{Absorbance of control}} \text{ x100}$$

Linear regression analysis in MS excel was used to analyze the IC_{50} of the DCM extracts and the standard. The determination of IC_{50} was carried out from a graph of percentage scavenging activity in relation to sample concentration.

5.2.3 Determination of Hydroxyl Radical (-OH) Scavenging Activity

The hydroxyl radical ($OH^{\cdot}$) scavenging action was determined by a protocol reported by Harsha and Latha (2012). In brief, 100 µL of 28 mM 2-deoxy-2-ribose, 20 mMKH_2, PO_4- KO.H buffer of 7.04. 200 µM of FeCl 3 (1: 1), 200 µL E DTA (1.04 mM), 100µL ascorbic acid (1.0mM) and plants extracts (100-500µg/mL) were mixed to give an eventual capacity of 1ml. The incubation of the resultant mixture was done for one hour at 37^0C. After one hour of incubation, 1.0 ml of 1% of TBA and 1.0 ml of 2.8% TCA wereput into the mixture. The resultant mixture was then incubated at 100^0C for 20 minutes. Pink colour was observed. The mixture was cooled, after which the optical density was read at 532 nm. The blank had all the reagents minus the plants extracts. Gallic acid was employed as the positive control (standard). All tests were carried in triplicates. The hydroxyl radical scavenging potential was determined using the formula by Hsu *et al.*, 2012).

$$\%\ \text{Hydroxyl radical scavenging} = \frac{\text{Control absorbance} - \text{Extract or Standard}}{\text{Control absorbance}} \times 100$$

5.2.4 Ferric Reducing Ability of Plasma Assay (FRAP)

The *in vitro* ferric reduction potential of the DCM extracts and Vitamin C (positive control) was done according to a formula by Benzie and Strain (1996) with some adjustments. Concisely, varying concentrations (0.2-1mg/ml) of 1ml of the herbs extracts as well as vitamin C were put into 2.5 ml of 0.2 M phosphate buffer at pH7. The resultant solution was added to

2.5 ml of potassium ferricyanide and incubation was done at 50^{o}C for 20 mins. Thereafter, 2.5ml of trichloro acetic acid (10%) was put into the mixture after which centrifugation was done for 10 minutes at 3000 rpm. Then, 2.5 ml of the filtrate was put into distilled water (2.5 ml). Addition of 0.5ml freshly prepared ferric chloride solution $FeCl_3$ (1%) was done. The assays were carried out in triplicates. The blank included all the reagents minus the plant extracts. Absorbance of the DCM extracts as well as ascorbic acid was read at 700 nm by use of a spectrophotometer.

5.2.5 Iron (Fe^{2+}) Chelating Activity Assay

The iron ion chelating activity of the extracts was measured by repression of the evolution of iron (ii)-ferrozine compound after treatment of different concentration and plants extracts with Fe^{2+} as described by Decker and Welch (1990). In brief, a volume of1 ml of ferrozine (0.3125 mM), 1 ml of 0.125 mM ferrous sulphate and 1 ml plant extract sample were vortexed rigorously. Incubation of the mixture was done for 10 mins at 25^{0}C. All the reaction reagents were put in the control except the plants extracts. Citric acid was employed as the standard. The assays were conducted in triplicates. The absorbance of the Fe^{2+} ferrozine complex was read at 562 nm using spectrophotometer against a blank. The potential of the plant specimen in chelating Fe^{2+} was determined in relation to the control according to a formulary described by Zhao *et al*. (2006).

$$\% \text{ Iron chelating activity} = \frac{\text{Control absorbance} - \text{Extract or standard}}{\text{Control absorbance(blank)}} \times 100$$

5.2.6 Determination of Total Phenolic Contents

Determination of the total phenolic levels of the herbs extracts was carried out conducted the Folin-ciocalteu reagent as reported by Hatami *et al*., (2014). Concisely, 2ml of each of the herb extracts, 2.5 ml of 10% dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3(7.5% w/v) were whirlwind for 15 mins and then incubated at 40^0C for 30 min. Determination of absorbance was read at by use of a spectrophotometer. Calobration curve was derived by use of garlic acid which was employed as a reference. The total phenolic content was resolved by use of linear equation according to the calibration curve and contents indicated as milligrams of garlic acid equivalent per gram of dry weight (mgGAE/g dw) (Medini *et al*., 2014).

5.3 Data Management and Statistical Analysis

Data on absorbance measures were fed in Microsoft Excel spreadsheet program. Data were organized and then exported to Minitab spreadsheet for analysis. Before data analysis was conducted, the data were scrutinized for normalcy by use of Kolomogorov-Smirnov test. Inferential statistics were carried out by use of One-Way Analysis of Variance followed by Tukey's post hoc tests for pair-wise separation and comparison of means at 5% level of significance. Total phenolics data was exposed to unpaired student's t-test analysis for comparison of the contents in the two plants extracts at

varying concentrations. All statistical interpretation was done using Minitab Version 17(Minitab Inc. Pennyslvania, U.S.A). The results were presented in tables and bar graphs.

5.4 Results

5.4.1 *In Vitro* DPPH Radical Scavenging Properties of DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon*

The DCM extracts of *M. senegalensis* and *D. melanoxylon* demonstrated concentration- dependent rise in DPPH radical scavenging properties (Figure 5.1). The elevated concentration demonstrated the highest DPPH radical scavenging properties while as the lowest concentration demonstrated the least DPPH radical scavenging property (Figure 5.1). As Figure 5.1 demonstrates, all extract concentrations of the two plants significantly lower DPPH radical scavenging activities compared with the standard compound, ascorbic acid ($p<0.05$; Figure 5.1). Moreover, DPPH radical scavenging activities of the herbs extracts were comparable to all the tested extract concentrations ($p>0.05$; Figure 5.1).

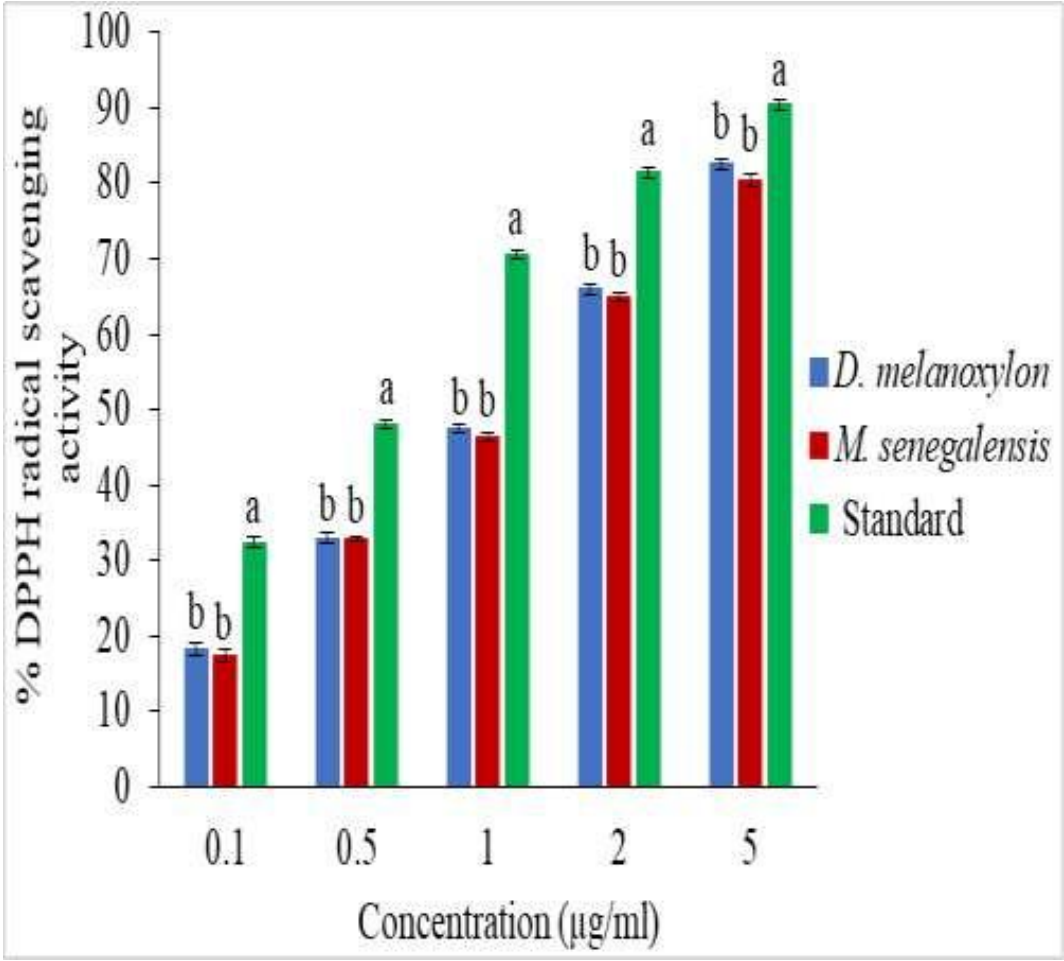

Figure 5.1: ***In vitro*** **DPPH radical scavenging activity of dichloromethanolic (DCM) of *M. senegalensis* and of *D. melanoxylon* activity.**
Bars with the same letter at the same concentration are not significantly different by one-way ANOVA followed by Tukey's post hoc test ($p>0.05$).

Further analysis showed that the concentrations of DCM extracts of *M. senegalensis* and *D. melanoxylon* required scavenging the DPPH radical by fifty percent (IC_{50} value) was 1.31± 0.40 and 1.31± 0.03µg/ml respectively whereas IC_{50} value of the standard compound was 0.50± 0.04µg/ml (Table 5.1). The results of IC_{50} presented in figure 5.1 corroborate with the data tabulated (Table 5.1).

Table 5.1: The concentration of dichloromethanolic (DCM) extract of *M. senegalensis* and *D. melanoxylon* needed to inhibit fifty percent of theradical

Type of radical formed	Sample	IC_{50} of the sample (g/ml)
DPPH radical	*M. senegalensis*	1.31 ± 0.04^{a}
	D. melanoxylon	1.33 ± 0.03^{a}
	Ascorbic acid	0.50 ± 0.04^{b}

Results are indicated as mean ± SEM for replicate measurements n=3. Values with the alike superscript arenot remarkably different from each other at (p>0.05)

5.4.2 *In vitro* Activity of Hydroxyl Radical Scavenging of *M. senegalensis* and *D. melanoxylon*

The DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* demonstrated potent efficacy against hydroxyl radical at each and every tested extract concentrations (Figure 5.2). The potential of the two plant extracts to scavenge hydroxyl radicals was concentration-dependent (Figure 5.2). As indicated in Figure 5.2, the hydroxyl radical scavenging activity of garlic acid was significantly higher than those of *M. senegalensis* and *D. melanoxylon* extracts at each and every tested concentration (p> 0.05).

The hydroxyl radical scavenging properties of the two herbs extracts were statistically comparable at concentrations of 0.05, 0.5 and 1µg/ml (p>0.05) but remarkably different at concentrations of 0.1 and 5µg/ml (p<0.05; Figure 5.2).

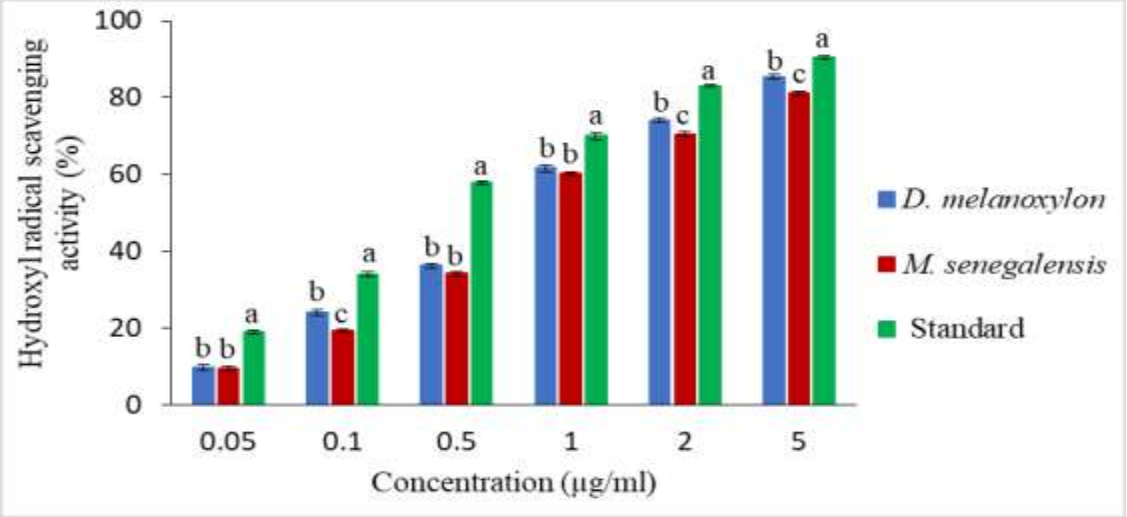

Figure 5.2: ***In vitro*** **hydroxyl radical scavenging activity of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*.**
Bars with the identical letter are not statistically different by one-way ANOVA followed by Tukey's post hoc test ($p > 0.05$).

Results from the current research also demonstrated that DCM leaf extract of *M. senegalensis* and stem bark extracts of *D. melanoxylon* had lesser IC_{50} values of 1.04 ±0.02µg/ml as well as 0.03µg/ml subsequently, compared to the standard compound, whose IC_{50} value was 0. 28±0.03µg/ml (Table 5.2). The results of IC_{50} presented in figure 5.2 also corroborate with the data tabulated in the table 5.2.

Table 5.2: The concentration of the DCM leaf extract of *M. senegalensis* and stem bark extract of *D. melanoxylon* needed to limit fifty cent of the radical

Type of radical	Sample	IC_{50} of the sample (µg/ml)
Hydroxyl radical	*M. senegalensis*	1.04 ± 0.02^{a}
	D. melanoxylon	1.01 ± 0.03^{a}
	Ascorbic acid	0.28 ± 0.03^{b}

Results are expressed as mean ±SEM for replicate measurements n= 3. Values with the identicalsuperscript are not statistically different from each other at p> 0.05

5.4.3 *In vitro* Activity of Ferric reducing Anti-oxidant of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*

The DCM extracts of *M. senegalensis* and *D. melanoxylon* also exhibited a concentration dependent ferric reduction power activity as displayed in Figure 5.3. The activity of ascorbic acid (Standard) was statistically higher compared to the two plants extracts at allthe assayed concentrations (Figure 5.3). The reduction power was exhibited by a hike in absorbance with a hike in extract concentration at 700 nm (Figure 5.3).

Ferric-reducing activities of the two plants were statistically comparable at concentrationsof 0.1.0.5, 1 and 2µg/ml ($p> 0.05$) but statistically different at concentrations of 0.05 and 5µg/ml ($p< 0.05$; Figure 5.3).

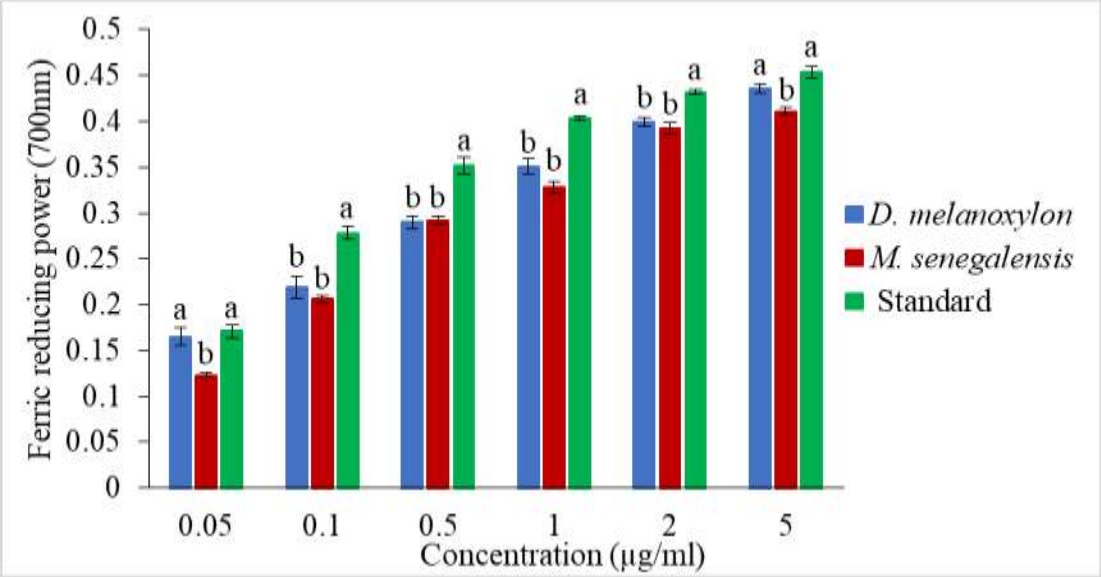

Figure 5.3: **In vitro potential of ferric reducing antioxidant DCM of *M. senegalensis* and *D. melanoxylon*.**

Bars with the identical letter are statistically comparable using one-way ANOVA and Tukey's post hoc test ($p > 0.05$).

5.4.4 *In vitro* Iron Chelating Potential of DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon*

The potentiality to impede the formation of iron (II)-ferrozine compound by the two plant extracts were statistically similar amid the tested extract concentrations ($p > 0.05$) except at concentrations of 1µg/ml where the activity of *D. melanoxylon* extract was statistically higher compared to *M. senegalensis* ($p < 0.05$; Figure 5.4).

Further, the action of garlic acid (standard) was remarkably higher compared to those of the assayed concentrations (Figure 5.4). The highest plants extract concentration exhibited statistically higher activity compared to lesser plants extract concentration ($p < 0.05$).

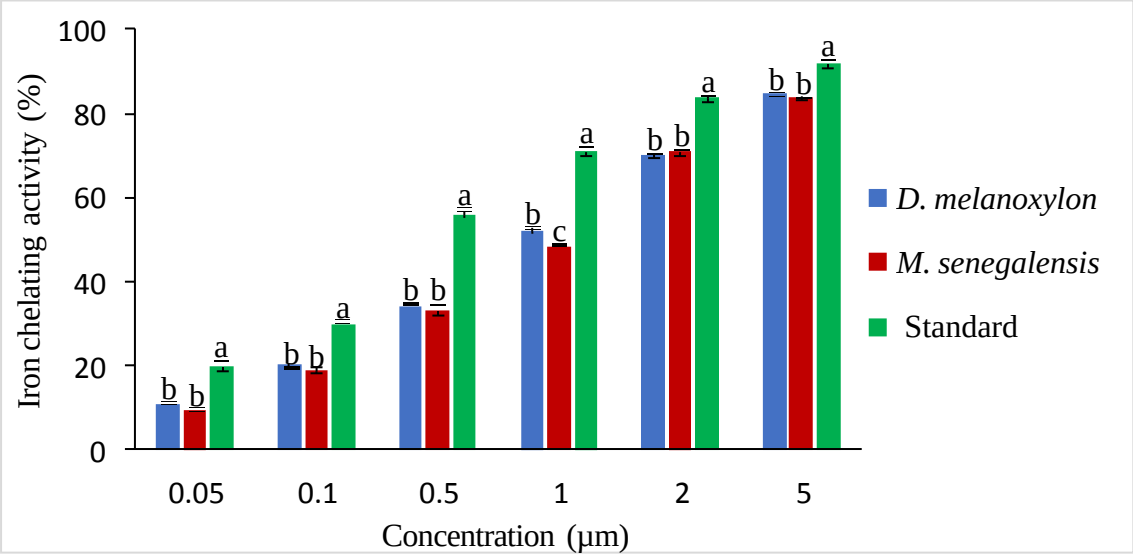

Figure 5.4: ***In vitro* ferrous chelating potential of DCM leaf extract of *M. senegalensis* and stem bark extract of *D. melanoxylon***

Bars with the identical letter are statistically comparable by one way ANOVA followed byTukey's post hoc test ($p > 0.05$).

Moreover, the IC_{50} values of the dichloromethane extract of *M. senegalensis* and *D. melanoxylon* were established to be 1.29 ± 0.02 and 1.24 ± 0.03 respectively, whereas theIC_{50} of the standard was 0.04 ± 0.05 (Table 5.3). The results of IC_{50} presented in Figure agree with the data tabulated in the table 5.3.

Table 5.3: The concentration of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* needed to inhibit 50% of the radical

Iron chelating activity (µg/ml)	Sample	IC_{50} of the sample
	M. senegalensis	1.29±0.02 [a]
	D. melanoxylon	1.24±0.03 [a]
	Citric acid	0.04±0.05 [b]

Results are indicated as mean ±SEM for replicate measurements n= 3. Values with the identical superscriptare not statistically dissimilar from each other at p> 0.05

5.4.5: *In Vitro* Total Phenolics Content of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*

The total phenolics content of DCM extracts of *M. senegalensis* and *D. melanoxylon* were computed from the garlic acid calibration curve (y = 0.0106x +0.0542, R^2=0.9995) (Figure 5.5).

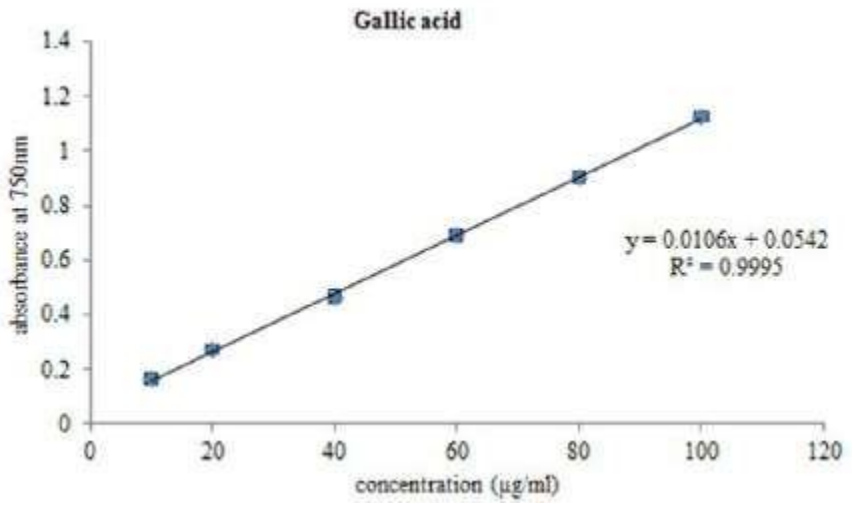

Figure 5.5: The mean value of total phenolic content.

Values for total phenoliccontent (R^2= 0.9995) was observed at 95% confidence level.

The stem bark extracts of *D. melanoxylon* exhibited significantly higher phenolic content than the leaf extract of *M. senegalensis* of each and every tested concentration ($p < 0.05$; Figure 5.6).

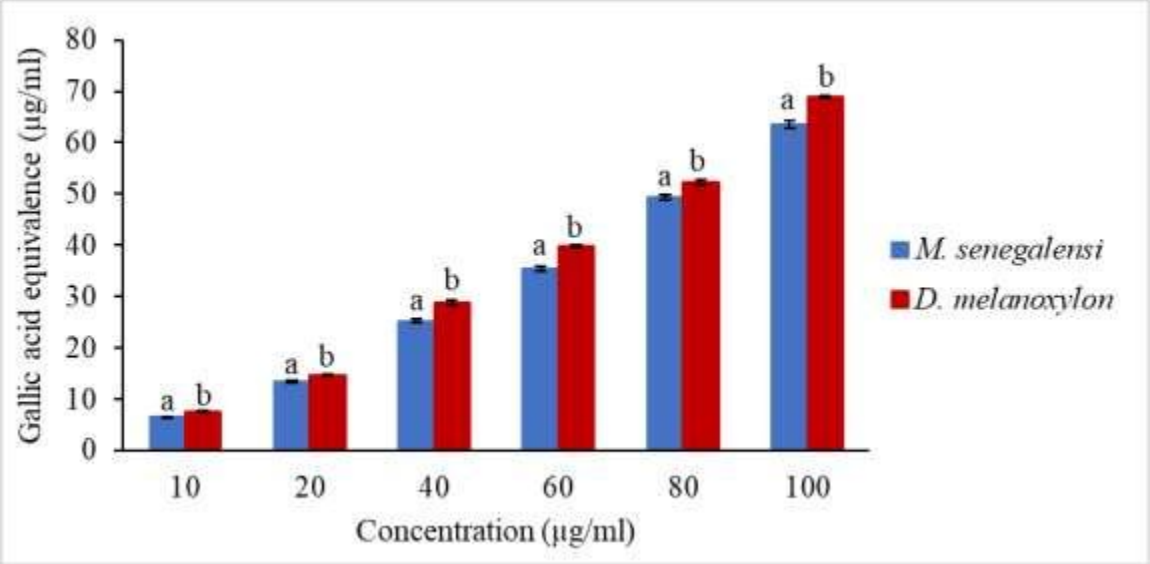

Figure 5.6: **In vitro total phenolics content of DCM leaf extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon*.**
Bars with the similar lowercase letters are statistically comparable at the same concentration ($p < 0.05$)

5.4 Discussion

Oxidative stress refers to variation of antioxidants and oxidants with potential to cause damage (Hansen *et al*., 2006). RNS and ROS are harmful to natural body systems (Rudolf, 2001). Antioxidants inhibit the effect of free radicals by countering, competing for or reacting with compounds whose end electron acceptor is molecular oxygen (O_2) (Lallianranwna, 2013).

Various commercial synthetic antioxidant agents have been reported to be carcinogenic as well as toxic; therefore, natural antioxidants are better alternatives to counter the consequences of oxidative stress (Wojdylo *et al*., 2007). Herbal agents provide useful medicinal components in the avoidance and the control of oxidative stress and associated ailments (Sofowora *et al*., 2013). In the present work, DCM leaf extract of *M. senegalensis* and stem bark extract of *D. melanoxylon* exhibited remarkable *in vitro* free radical scavenging and antioxidant activities.

Medicinal plants contain varying bioactive components with antioxidant potential such asphenolics, sterols, flavonoids, glucosinolates, alkaloids and carotenoids (Saranya *et al*., 2017). Provision of foods containing antioxidants is needed to lower the damage by oxidation (Halliwell and Gutteridge, 2003). Synthetic antioxidants are considered unstable at high temperatures, highly volatile and carcinogenic. Therefore, natural

antioxidants are favoured over artificial antioxidants (Valko *et al*., 2007). Natural antioxidants are commonly derived from medicinal plants for human use (Dolai *et al*., 2012). Numerous herbal plants such as *Zingiber officinale* (Tohma *et al*., 2017), *Crocus sativus* (Kakouri *et al*., 2017), *Anacardium occidentale* (Chan *et al*., 2014),*Allium sativum* (Elosta *et al.,* 2017), *Caesalpinia bonducella* (Gupta *et al*., 2014), *Datura fastuosa (*Dhiman *et al.,* 2012) were reported to demonstrate antioxidant properties.

Several members of the genus *Maytenus* have previously been investigated either *in vivo* or *in vitro* for antioxidant activities. For example, a research conducted by Cansina *et al*. (2015) on *M. dasyclada, M. ilicifolia* and *M. aquifolium* exhibited *in vitro* antioxidant properties and pro-oxidant effects *in vivo*. An earlier study by Melo *et al*. (2001) published an *in vivo* antioxidant activity of *M. ilicifolia.* Several other researches conducted on members of this genus namely *M. ilicifolia* and *M. aquifolium* da Silva *et al*., (2011) which were announced antioxidant activities, attributable to presence of flavonoids and polyphenols as the free radicals scavengers. Another *Maytenus* species (*M. senegalensis*) was evaluated in this work for its *in vitro* antioxidant activities.

The radical scavenging potential in addition to antioxidant activities of medicinal herbs is associated with their phytochemical compounds (Alabri *et al*., 2014). In this work, the antioxidant potential of *M. senegalensis* and

D. melanoxylon was measured using DPPH and Hydroxyl radical scavenging potential; FRAP, Iron chelating property and Total phenolic content. Using one test to evaluate antioxidant activities of a specimen would be insufficient to accurately determine the antioxidant potential due to several factors (Iqbal, 2015).

The evaluation of *in vitro* DPPH reduction is anchored on hydrogen endowment potential to lower DPPH radical in methanol forming the non-radical DPPH-H. The present study on *M. senegalensis* and *D. melanoxylon* demonstrated significant concentration-dependent DPPH radical scavenging properties. The reaction between the plants extracts and DPPH may have taken place through shifting of electrons and hydrogen ions to 2, 2-diphenyl-1- picrylhydrazyl radical to form a steady 2, 2-diphenyl-1-picrylhydrazine molecule (DPPH) (Matthaus, 2002). Normally, the strongest absorbance of DPPH radical is at wavelength of 517 nm. DPPH is a stable free radical, which can receive hydrogen radical or an electron to be a steady diamagnetic molecule hydrazine. Suitable reducing agents react with DPPH radical pairing off the electrons and the solution colour changes from violet to yellow stoichiometric ally, where several electrons are absorbed as is shown by the reduction in absorbance at 517 nm (Rajani and Ashok, 2009; Bajpai *et al*.,2014).

Previous studies have again shown DPPH radical scavenging properties of various herbal extracts. A study conducted on methanol extract of *C. mathewii* demonstrated remarkable antioxidant activities (Fatma *et al*., 2016). More work by Basker *et al.* (2012) on methanol extracts of *Moticilla maderaspatensis,* hexane extracts of *M. emarginata*, ethyl-acetate extract of *Asclepias curassavica*, ethyl acetate as well as methanolic extract of *A. marmelos* and as ethyl-acetate and methanol extracts of *O. mungos* demonstrated the ability to scavenge DPPH *in vitro*.

The IC_{50} of the two plants extracts in DPPH test hydroxyl radical scavenging evaluation and iron chelating properties assay were higher than vitamin C. Elevated IC_{50} is indicative of low antioxidant activity (Arika *et al*., 2019). This was expected since the study used crude extracts without purification. Results for purified extracts could produce results closer to those of ascorbic acid (Kayini *et al*., 2016). This, however, contradicts work published by Anna *et al*. (2017) which announced higher IC_{50} than the extract of *Cerrena unicolor*.

The current study also evaluated the ability of the two plants extracts to scavenge hydroxyl radicals. Hydroxyl radicals are greatly reactive free radicals that are made in natural processes and are associated with high cell damaging activity in free radical pathology. In presence of ferrous ions, these radicals can be made from hydrogen peroxide and superoxide anion.

In humans, there is no a specific enzyme to neutralize this reaction. It is imperative, therefore, to find a natural compound with good antioxidant activities (Bajpai *et al*., 2014).

The capability of the DCM extracts of the two plants to impede hydroxyl radical mediated de-oxyribose injury was investigated by the response of Fenton and by use of iron (II)- dependent DNA injury test as described by (Kar and Chattopadhyaya, 2017). The hydroxyl radicals formed by the Fenton's process break down DNA deoxyribose sugar, where Fe^{2+} salts are used as a catalytic compound (Balu *et al*., 2005). Both *M. senegalensis* and *D. melanoxylon* demonstrated the potential to quench hydroxyl radicals in a concentration dependent manner by creating a pale pink chromophore as the extracts concentrations increased.

Hydroxyl radical is reported as a very active reactive oxygen species in free radical pathology of body organ processes with ability to destroy all cell constituents (Lobo *et al*., 2010). The hydroxyl radicals are majorly created from hydrogen peroxide in addition to superoxide anion where Cu^{2+} and Fe^{2+} are present. Hydroxyl radicals are known to cause damage to unsaturated fatty acids moieties of the phospholipids of the plasma membrane and initiate lipid peroxidation. Further, hydroxyl radicals have been reported to damage nucleic acids by breaking polynucleotide strand thereby causing cell death and cell mutation (Balu *et al*., 2005).

Hydroxyl radical is one of strongest free radicals highly associated with irreversible injury formed by oxidative stress (Lobo *et al*., 2010). Hydroxyl radicals generally cause mutagenesis, aging and carcinogenesis (Lobo *et al*., 2010). In present study, the incubation of ferric-EDTA with hydrogen peroxide and vitamin C at pH 7.4 caused synthesis of hydroxyl radicals. The presence of HO radicals was demonstrated by their capability to break down 2-deoxy-D-ribose into fragments, on heating with TBA at low PH causing a pink chromogen (Lobo *et al*., 2010).

FRAP test evaluates the reducing ability of an antioxidant by reacting with Fe^{3+}-ligand complex to form Fe^{2+}(Perl's Prussian blue ferric ferrocyanide) Free radical chain breaking occurs through donating a hydrogen atom(Gomes *et al*., 2008). At low pH of 3.6, reducing Fe^{3+}- ligand to blue coloured Fe^{2+}-(Perl's Prussian blue ferric ferrocyanide) occurs at an absorbance of 700 nm. The two herbs extracts used in this research exhibited a concentration dependent reducing antioxidant potential. The results are concomitant to reports by a study conducted on methanolic extracts of *Myxopyrum serratulum,* which demonstrated a concentration-dependent activity (Alam *et al*., 2006).

The reducing ability of the extracts demonstrates that they can act as electron donors, which reduce the oxidized intermediates of lipid peroxidation processes, where they are used as primary and secondary

antioxidants (Reza *et al*., 2021). Antioxidant properties may be attributed to different mechanisms including decomposition of peroxides, prevention of chain initiation, radical scavenging and reducing ability (Arika *et al.*, 2019). The outcomes demonstrated that the extracts possess good reducing ability.

Phytochemicals present in the plants directly bind to metal ions or accidentally inhibit their chelating reactions by taking over coordination sites (Njogu and Obi, 2009). Phenolic components are crucial plant compounds with good antioxidant potential (Soobratte *et al*., 2005).

The total phenolic content value of the plants extracts confirms that the studied extracts are rich in phenolics. Phenolics are extensively spread secondary phytochemicals in the kingdom Plantae and are well known sources of potent natural antioxidants because of their potential in acting as effective metal chelators and radical scavengers (Lim *et al*., 2007). Fukumoto *et al*. (2000), after studying the antioxidant activities of phenolic components deduced that an elevation in scavenging potential is squarely associated to an increase in hydroxyl groups in polyphenols as well as reduction in glycosylated groups. So, phenolic contents of herbal extracts directly promote their antioxidant potential. Further, Kennedy *et al*. (2011) proposed that the antioxidant potential demonstrated by herbal extracts is due to the synergism between phytochemicals in herbs.

A related study showed a dose dependent limitation of the creation of the ferrozine-Fe^{2+} compound by the activity of *Typha domingensis* (Chai *et al*., 2014). Further, Mehdi *et al*. (2013) described chelating potential of the crude extracts of *Chlorophytum borivilianum* in a concentration-dependent mode.

The antioxidant properties demonstrated by the medicinal plants extracts are attributable to the existence of different phytocomponents that are believed to act collaboratively and antagonistically to counteract RNS and ROS (Ishino *et al*., 2010). The ability of phytochemicals are primarily due to their redox activities (Zhang and Wang, 2001), which are crucial in scavenging free radicals (Adedapo *et al*., 2008).

Previous studies by Ananta *et al*. (2016) on methanolic extracts of *M. koenigii, L. javanica* and *E. foetidum* reported that these extract have substantial quantities of carbohydrates, flavonoids, vitamin C, alkaloids, proteins and phenolics. The *in vitro* antioxidant tests disclosed considerable free radical scavenging properties of all the four studied herbs extracts. Further, *C. viscosum* extract demonstrated better antioxidant ability with IC_{50} values of 29.74 ± 3.63 and 148.77 ± 18.38µg for DPPH as well as TBA reactive species subsequently (Nandi and Lyndem, 2016).

An earlier study by Akkari *et al*. (2016) on flower and fresh leaves bud aqueous extracts of *Caesalpinia spinosa* demonstrated that flavonoid derivatives kaempferol, rutin and quercetin were the most plentiful polyphenol components. The aforesaid flavonoids were announced to have extensive therapeutic properties like antioxidant, antibacterial and anti-allergic properties (Shaik *et al.*, 2006). Further, quercetin in *Bridelia ferruginea* stem barks have demonstrated anti-nematocidal potential where kaempferol, rutin and quercetin are the likely potent molecules (Lasisi and Kareem, 2011).

According to Ferreira (2011), antioxidant flavonoids have been suggested to have potent anthelmintic potential. Further, a research by Akkari *et al*. (2016) on crude aqueous extracts of *Caesalpinia spinosa* exhibited potential *in vitro* dose dependent antioxidant as well as anthelmintic activity against *H. controus.*

It can, therefore, be postulated that phytochemical compounds present in the DCMextracts of *M. senegalensis* and *D. melanoxylon* might be behind their antioxidant properties. The GC-MS screening of the two plants extracts under study disclosed the existence of bioactive compounds among which are terpenoids (sesquiterpenoids, monoterpenoids and diterpenoids), lipids (fatty acid esters and their derivatives) and metallocene. The aforesaid bioactive components were demonstrated to manage the redox homeostasis

by a series of processes of antioxidant responses which entails free radicals' commencement, generation and subdivision (Ishinon, 2010).

Terpenoids are biologically active compounds with antiparasitic, antioxidant, antimicrobial, anti-inflammatory, antifungal and antihyperglycemic (Priyanka *et al*., 2013). Terpenoids were shown to quench free radicals where they act as reduction factors and are required in stoppage of the free radical series response (Pham-Huy *et al*., 2008). Beta-amyrin, a terpenoid, was revealed by the GC-MS in this study. The beta-amyrin isomer is a pentacyclic triterpene belonging to oleanane group. It has been reported as a free radical captor because of integration ability of the oxygen reactive species (Wang *et al*., 2010). It is postulated to have contributed to oxidative activity of *M. senegalensis* extract.

Other compounds quantified by GC-MS in this study include squalene, limonene and phytol from the *M. senegalensis* extract while caryophyllene, α-humulene, methyl linoceate, α-pinene and ferocene were quantified in the *D. melanoxylon* extract. Antioxidant potential of squalene has been suspected to provide defense against helminthes-related inflammation (Zhang *et al*., 2015). Squalene elevates the generation of IL-10, which is an anti-inflammatory cytokine that hinders antigen presentation and proinflammatory cytokines (Cristina *et al*., 2018).

Phytol, a diterpenoid, was quantified from the leaf extracts of *M. senegalensis*. The antioxidant potential of phytol is attributable to the existence of hydroxyl group (OH) in the molecule. Phytol was shown to exhibit strong antioxidant ability *in vitro* by reducing hydroxyl radicals as well as nitric oxide and prevents the production of TBARS (Camila *et al*., 2013). Maybe, phytol reacts with a free radical, to give away hydrogen atoms with unpaired electron (H^+), and thereby changes free radicals into lesser reactive species (Guimaraes *et al*., 2010).

Alpha-Pinene exists as an organic monoterpene component which was found in the leaf extracts of *M. senegalensis*. It was demonstrated to show weak antioxidant activities (Elanur *et al*., 2013). Alpha-Pinene were shown to act as an uncoupler of oxidative stress and disrupting the electron transport chain thereby disrupting energy metabolism in bio membranes of helminthes (Abraham *et al*., 2003). Further, Perry *et al*. (2001) announced that α-Pinene exerts spasmolytic and myorelaxant activity on the smooth muscles of the intestines (Camare *et al*., 2003). This effect may explain the role of α- pinene role as anthelmintic agent in expulsion of helminthes from the gut.

Caryophyllene exists as a natural bicyclic sesquiterpene found richly in essential oils of most therapeutic oils. In the current work caryophyllene (E) was quantified in the plant extracts of *D. melanoxylon*. Rodriguez *et al*.

(2012) demonstrated a strong antioxidant potential by essential oil of *Murraya paniculata,* where caryophyllene (E) was reported as major compound.

In a different research by Fernandes *et al*. (2007), α-humulene was shown to lower rat paw induced edema, which was treated with carrageenan. The researchers concluded that α-humulene was the active compound. It was suggested that the compound may be useful in cure and control of inflammation associated to the helminthes diseases (Fernandes *et al*., 2007).

The free radical-scavenging and antioxidant property of *M. senegalensis* and *D. melanoxylon* can be credited to the existence of phytocompounds that are associated with antioxidant activities. Consequently, the synergistic effect of these biologically active components elevates their bioavailability on many molecular targets hence remedying imbalance caused by oxidative stress. The antioxidant activity exhibited by the extracts of *M. senegalensis* and *D. melanoxylon* could have been due to the individual or collective effects of the phytochemicals present in the extracts (Bhat *et al*., 2015).

Generally, findings from this study indicate that *M. senegalensis* and *D. melanoxylon* can be useful therapeutic agents in the promotion of antioxidant defense mechanism in the affected animals. This means that the two plants extracts can promote anthelmintic activity through amelioration of helminthes- driven oxidative stress.

CHAPTER SIX

SUB-ACUTE TOXICITY STUDIES ON DICHLOROMETHANE EXTRACTS OF *MAYTENUS SENEGALENSIS* AND *DALBELGIA MELANOXYLON* IN MICE

6.1 Introduction

Toxicity is the degree to which a given substance can harm an organism. This is asa result of interaction between a cell and the toxicant. The degree of toxicity depends on chemical properties of poison and plasma membrane. Toxicity may take place either on the surface of the cell, inside the cell or at the extracellular tissue level. Exposure to chemicals can result in negative effects on man hence the need to evaluate toxic properties of substances. Generally, evaluation of toxicity entails evaluating a substance for its carcinogenicity, acute toxicity, chronic toxicity and effects on reproduction (Asante-Duah, 2002).

The toxicant or its metabolites can accumulate, be excreted from the body or respond with DNA leading to DNA adducts, whereas mutagenic toxins may be cancerous (Romano *et al*., 2015). Protein adducts can lead to abnormal immune reaction that can cause cell damage. Furthermore, toxicants may affect oxidative preventive mechanisms, thereby causing cellular death through necrosis or apoptosis (Sinha *et al*., 2013).

Toxicity can be measured by the effect on the target cell, tissue or organ. The effects can range from minor changes, for instance change in the level of hormones, reduced weight, and weight gain or in extreme cases death of an organism. Varying levels of toxicity can lead to death (Home office, 2004). Toxicological studies have grown fast in pharmaceutical field prompted by discovery of toxicity of some medicinal plants, exposure of chemicals to the environment from industries and as a good manufacturing practice (Traina, 2006).

Several cases of herbal toxicity have been reported in several instances, which have necessitated the study of toxicity of medicinal plants (Komlaga *et al*., 2015). Flavreau *et al*. (2000) reported a study where patients were subjected to a dietary supplement, which contained yohimbine, norephendrine, 3, 5- diodothyronine and sodium usiniate. All the patients developed hepatotoxicity in three months, only to recover after the withdrawal of the supplement. Inaddition, a study carried out in Zimbabwe reported increase in herbal toxicity incidences (Mudzviti *et al*., 2012). These facts show the need to have toxicity tests of all medicinal plants.

In vitro toxicity assessment provides information of the medicinal plants potential to cause toxicity in animals and man (Amit *et al*., 2015). *In vivo* tests in animals are usually conducted to determine the organs affected by various dose levels, examine the nature of poisonous dose under more actual environment. This shows the highest dose level that can exhibit

symptoms of toxicity without killing the animals. Further, low dose level that can induce no poisonous effect and lastly show the intermediate dose level of the drug under investigation is determined (Agrawal and Paridhari, 2007).

Previous biological studies have demonstrated that *M. senegalensis* has many health benefits. Despite primary evidence of medicinal use and ancient benefits of *M. senegalensis* and *D. melanoxylon* from Embu County, their *in vivo* safety has not been established. The current study is, therefore, meant to examine the likely sub-acute toxicity of orally administered dichloromethane leaf extract of *M. senegalensis* as well as DCM stem bark extract of *D. melanoxylon* in normal mice.

6.2 Materials and Methods

Specimen collection, preparation and extraction were conducted as earlier reported in chapter three (sections 3.21 and 3.2.2).

6.2.1 Sub-acute Toxicity Testing

A total of twenty (20) Wistar mice were used for sub-acute toxicity evaluation. The mice were randomly separated into four (4) groups of five (5) male animals each. Group 1 (control), was orally administered 10% Dimethyl Sulfoxide (DMSO) every day for 28 days. Mice in groups 2, 3 and 4 were orally given with the plants extract doses of 100 mg/kg, 300 mg/kg, and 1000 mg/kg body weight, respectively, suspended in 1% DMSO

every day for 28 days. Calculation of doses was done by progression factor of 3.2 starting from 1000mg/kg body weight descending, which is the standard recognized limit (OECD, 2001). All animals were provided standard pellet diet and water *ad libitum* throughout the treatment period. Mice were observed every day for clinical toxicity signs and death for the 28 days. The mice were weighed prior to administration of various dosages, and after every seven days, and prior to sacrifice on the 28^{th} day. Weight of every mouse was noted independently.

After 28 days of treatment, all the mice were anaesthetized in airtight dissection jar carrying cotton soaked in chloroform. Each anaesthetized mouse was laid on a dissecting board and a pair of scissors used to dissect the mice by making a cut through vertical mid-line from neck to peritoneum (Osano *et al*., 2016). Liver, Brain, spleen,heart, kidneys and pancreas were extracted and weights taken after cleaning them in normal saline. The organs were put in plastic containers with 10% buffered formalin solution to preserve them.

6.2.2. Blood Sampling

Blood samples were collected through cardiac puncture. After common anesthesia with chloroform, a 22-gauge needle connected to a 3 ml syringe was introduced to the notch atthe caudal aspect of the sternum and guided to the heart. The location of the heart was resolved by palpating for the

heartbeat. The plunger was pulled rearwards smoothly in order to draw out blood. The collected sample was divided into two portions; one for haematological examination, which was collected in tubes carrying anticoagulant Ethylenediaminetetraacetic Acid (EDTA) and the other for biochemical examination, which was collected in tubes lacking anticoagulant. Blood sample for biochemical analysis was retained for one hour at ambient temperature to allow for sufficient coagulation. Centrifugation followed at 3000 revolutions per minute (rpm) for ten minutes to achieve serum. The serum achieved was put in Eppendorf tubes and kept at - 20ºC awaiting biochemical examination (Musila *et al*., 2017).

6.2.3. Haematological Assays

Hematological parameters assayed in this study include red blood cells (RBC) counts, mean cell volume (MCV), red blood cells distribution width (RDW), white blood cells differential counts (WBC), platelets counts, haemoglobin concentration (HB), mean corpuscular haemoglobin concentration (MCHC), platelet distribution width (PDW) and packed cell volume (PCV) They were determined by use of Cell-tac alpha automated haematology analyzer (Nihon Kohden Corporation, Tokyo, Japan) conforming to the manufacturer's directions.

6.2.4. Determination of Biochemical Parameters

Biochemical parameters explored in this study include Total proteins, alanine aminotransferase (ALT), bilirubin, albumin, Total bilirubin, aspartate aminotransferase (AST), direct bilirubin, creatinine, urea, and electrolytes. These parameters were determined by use of automated BS-200 biochemistry auto analyzer (Shenzhen Mindray Bio-medicals Co., Ltd, Hamburg, Germany) conforming to the manufacturer's guidelines.

6.3 Experimental Data Management, and Analysis

The measurements on organ weight, body weight, hematology test and biochemistry were fed in Microsoft Excel spreadsheet program. Data were arranged and then put through Minitab spreadsheet for audit. Before analysis was done, data were checked for normality using Kolomogorov-Smirnov test. Data were put through descriptive statistics and results demonstrated as mean± standard error of mean (SEM). Inferential statistics were carried out by use of One-Way Analysis of Variance (ANOVA). The Means were separated using Tukey's Post hoc test and $p < 0.05$ was regarded statistically significant. Results were presented in tables.

6.4. Results

6.4.1. Effects of Orally Administered DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon* on Body Weight of Mice

In general, it was observed that the extracts of *M. senegalensis* and *D. melanoxylon* manifested remarkable changes in body weight of mice (Table 6.1 and Table 6.2; Appendix VI and VII).Overall, the effects of the *M. senegalensis* and *D. melanoxylon* extracts were mainly dose dependent, as they created a uniform sequence of change in body weight of mice, from the lowest to the highest studied dosages.

Table 6.1: Effect of orally administered leaf extracts of *M. senegalensis* on body weight.

Treatment	Percentage change in body weight				
	Day 7	Day 14	Day 21	Day 28	Total change
10% DMSO	10.10±0.39[a]	21.44±0.78[a]	30.91±0.70[a]	36.08±0.82[a]	24.63±0.57[a]
100 mg/kg bw	09.96±0.33[a]	21.38±0.87[a]	28.50±0.66[a]	34.64±0.67[a]	23.62±0.52[a]
300 mg/kg bw	09.89±0.31[a]	18.95±1.19[ab]	28.08±1.05[a]	34.30±0.94[a]	22.80±0.61[a]
1000 mg/kg bw	09.02±0.33[a]	16.44±0.84[b]	23.94±0.47[b]	27.22±0.34[b]	19.16±0.10[b]

Values indicated as mean±S.E.M (n=5).

Comparison was done across the columns and values followed by the identical superscripts through the columns are statistically comparable by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

It was observed that, at the seventh day of experiment period, the effects of the *M. senegalensis* extract at all dosages were statistically comparable to each other and to the effects observed in the normal control group ($p > 0.05$; Table 6.1; Appendix VI) After 14 days of the test period, it was apparent that the DCM leaf extract *M. senegalensis* at the dose level of 1000 mg/kg had the lowest change in body weight of mice which was significantly similarto the effects of 300 mg/kg ($p > 0.05$; Table 6.1; Appendix VI).

After 21 and 28 days of the test period, the DCM extract of *M. senegalensis* at the dose level of 1000mg/kg continued to show the least change in body weight of mice. This efficacy was statistically different from the effects observed in the other extract concentration groups as well as in the control grouping ($p < 0.05$; Table 6.1; Appendix VI).

It was also observed that, on average, the total variation in body weight of animals treated with the least tested dosages of DCM leaf extract *M. senegalensis* (100 and 300 mg/ kg) were comparable ($p > 0.05$) to each other as well as to DMSO treated group (Table 6.1; Appendix i). However, the efficacy of the plants at dosage of 1000 mg/kg was significantly different from the effects demonstrated by the rest of the treatment groups (Table 6.1; Appendix VI).

On the contrary, the DCM stem bark extract of *D. melanoxylon* remarkably increased the body weight of mice by an average of between 09 and 35.67% (Table 6.2; Appendix VII) within the 28 days duration of the experiment. The *D. melanoxylon* stem bark extract concentration of 100 mg/kg, achieved the highest change in body weight of 35.67% at 28 days after treatment. The extract concentration of 1000 mg/kg produced the lowest change in body weight of 16.9% at 7 days after treatment.

After 7 days of the test duration, the effectiveness of all the three studied DCM stem bark extract dosages (100, 300 and 1000 mg/kg) of *D. melanoxylon* on body weight of mice were statistically comparable to the effects observed in the DMSO treated grouping ($p>0.05$; Table 6.2; Appendix VII).

At fourteenth and 21st days of the test period, the effect of DCM stem bark extract of *D. melanoxylon* at dosage of 1000 mg/kg was statistically different from what was seen in DMSO administered grouping ($p<0.05$; Table 6.2; Appendix VII). During the 14 and 21 days of the experiment time, the effects of *D. melanoxylon* at dosages of 100 and 300mg/kg were statistically comparable ($p<0.05$; Table 6.2; Appendix VII).

It was also noted that on 28 days after treatment, the effect of DCM extract of *D. melanoxylon* at the dosage level of 1000 mg/kg was statistically

different from the effects in the other extract dose levels (100 and 300 mg/kg) and in the DMSO treated grouping ($p<0.05$; Table 6.2; Appendix VII). Similarly, the average total variation in body weight of the mice were dose dependent with there being no statistical difference in the effectiveness of *D. melanoxylon* at extract dosage levels of 100 and 300mg/kg from the control groups ($p>0.05$; Table 6.2; Appendix VII).

Table 6.2*:* **Effects of orally administered DCM stem bark extracts of *D. melanoxylon* on body weight of mice**

Treatment	Percentage change in body weight				
	Day 7	Day 14	Day 21	Day 28	Total change
10% DMSO	10.10±0.39[a]	21.44±0.78[a]	30.91±0.70[a]	36.08±0.82[a]	24.63±0.57[a]
100 mg/kg bw	09.91±0.45[a]	18.98±0.91[ab]	29.95±1.18[ab]	35.67±1.24[a]	23.63±0.56[a]
300 mg/kg bw	09.54±0.38[a]	19.49±0.74[ab]	29.31±0.87[ab]	33.40±0.97[a]	22.93±0.69[a]
1000 mg/kg bw	09.37±0.45[a]	16.90±0.49[b]	26.67±0.53[b]	27.39±0.93[b]	20.8±0.51[b]

Values indicated as mean ±S.EM (n=5).

Comparison was done across the columns and values followed by identical superscripts along the columns are statistically comparable by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

6.4.2. Effects of Orally Administered DCM Leaf Extracts of *M. senegalensis* and Stem Bark *D. melanoxylon* on Organ Weight of Mice

Over all, the DCM extracts of *M. senegalensis* and *D. melanoxylon* at all the tested dose levels caused changes in weight of organs in mice which were similar to the effects observed in control group (Tables 6.3 and 6.4; Appendix VIII and IX).

The orally administered DCM leaf extracts of *M. senegalensis* into mice elicited statistically similar changes in weight of the liver, lungs, heart, kidneys and brain (Table 6.3; Appendix IX). Further, the activities of the plants extract at different dosages on mice organ weights were also significantly comparable to the effects seen in the control grouping ($p>0.05$; Table 6.3; Appendix VIII).

Nevertheless, it was observed that at the highest extract concentration (1000 mg/kg) the dichloromethane extracts of *M. senegalensis* evoked significantly reduced organ weightof spleen in mice (0.56g) which was only comparable to DMSO treated grouping ($p>0.05$; Table 6.3; Appendix VIII).

Table 6.3: Effects of orally administered DCM leaf extracts of *M. senegalensis* on organ weight of mice

Organ	Relative organ weight			
	DMSO (10%)	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
Liver	5.33±0.22[a]	5.58±0.20[a]	5.30±0.14[a]	5.56±0.09[a]
Lungs	0.62±0.02[a]	0.61±0.02[a]	0.65±0.01[a]	0.60±0.01[a]
Spleen	0.55±0.01[b]	0.73±0.02[a]	0.73±0.01[a]	0.56±0.01[b]
Heart	0.45±0.01[a]	0.44±0.02[a]	0.46±0.01[a]	0.44±0.02[a]
Kidneys	1.38±0.02[a]	1.32±0.03[a]	1.37±0.03[a]	1.40±0.03[a]
Brain	1.51±0.03[a]	1.46±0.03[a]	1.46±0.04[a]	1.47±0.02[a]

Values expressed as mean ±S.EM (n=5). Comparison was made across a row and values followed by the same superscripts along the row are significantly comparable by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

Similarly, the DCM stem bark extract of *D. melanoxylon* did not bring about statistically different adjustments in weight of major organs of mice as compared to the changes noted in the DMSO treated group ($p>0.05$; Tables 6.4; Appendix IX). However, the extract of *D. melanoxylon* at dose of 1000 mg/kg, as well as DMSO treatment caused significant similar decrease in the weight of the spleen of treated mice ($p> 0.05$; Tables 6.4; Appendix IX) but dissimilar from the effect of the rest extract doses (Tables 6.4; Appendix IX)

Table 6.4: Effect of orally administered DCM stem bark extracts of *D. melanoxylon* on organ weight of mice

Organ	Relative organ weight			
	DMSO (10%)	100 mg/kg bw	300mg/kg bw	1000mg/kg bw
Liver	5.33±0.22[a]	5.38±0.08[a]	5.41±0.04[a]	5.44±0.05[a]
Lungs	0.62±0.02[a]	0.65±0.01[a]	0.66±0.02[a]	0.67±0.01[a]
Spleen	0.55±0.01[b]	0.64±0.01[a]	0.64±0.02[a]	0.58±0.02[b]
Heart	0.45±0.01[a]	0.48±0.01[a]	0.48±0.02[a]	0.46±0.01[a]
Kidneys	1.38±0.02[a]	1.52±0.03[a]	1.61±0.19[a]	1.53±0.03[a]
Brain	1.52±0.03[a]	1.52±0.06[a]	1.52±0.03[a]	1.54±0.02[a]

Values expressed as mean ± SEM for five replicates per group (n=5).
Statistical comparison was made along a row and values followed by the same superscripts along the row are not significantly different by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

6.4.3. Effects of Orally Administered of DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon* on Haematological Profiles of Mice

Generally, it was observed that the DCM extracts of *M. senegalensis* and *D. melanoxylon* caused a reduction on the levels of different haematological parameters in mice (Tables 6.5 and 6.6). It was found that overall haematological effects observed in all the groups treated with the herbs extracts were largely comparable to the effects manifested in the sample treated with DMSO (control group) ($p> 0.05$; Tables 6.5 and 6.6).

The levels of granulocytes, platelets, monocytes, RDW, MCHC, MCV and HCT in the mice treated with DCM leaf extract of *M. senegalensis* increased with a rise in the extract concentration (Table 6.5). However, the change in the levels of the rest of haematological parameters was not found to follow a specific pattern of extracts concentrations (Table 6.5).

Table 6.5: Properties of orally administered of DCM leaf extracts of *M. senegalensis* on haematological profiles of mice

Parameter	Treatment (Extract Concentration)			
	10% DMSO	100 mg/kg bw	300mg/kg bw	1000mg/kg bw
RBC	007.32±0.17^{a}	007.10±0.23^{a}	007.33±0.37^{a}	007.28±0.25^{a}
HB	013.74±0.34^{a}	013.42±0.34^{a}	014.44±0.23^{a}	014.40±0.35^{a}
HCT	043.14±0.64^{ab}	041.00±0.90^{b}	042.84±0.67^{ab}	044.36±0.39^{a}
MCV	056.95±0.45^{ab}	056.10±0.33^{b}	058.34±0.41^{a}	058.38±0.50^{a}
MCHC	034.96±0.47^{a}	034.68±0.57^{a}	036.08±0.46^{a}	036.50±0.24^{a}
RDW	014.60±0.35^{a}	015.28±0.51^{a}	015.28±0.21^{a}	015.80±0.27^{a}
Lymphocytes	081.42±0.73^{a}	082.18±0.55^{a}	083.48±0.60^{a}	083.14±0.51^{a}
Monocytes	005.12±0.29^{a}	005.04±0.25^{a}	005.34±0.27^{a}	006.02±0.18^{a}
Granulocytes	014.40±0.35^{a}	014.18±0.27^{a}	015.26±0.30^{a}	015.32±0.39^{a}
Platelets	367.60±3.97^{a}	369.40±3.70^{a}	378.40±3.70^{a}	381.40±4.27^{a}
PDW	008.56±0.38^{a}	08.88±0.410^{a}	008.66±0.49^{a}	008.62±0.44^{a}
PCT	00.48±0.02^{a}	00.51±0.030^{a}	000.53±0.01^{a}	000.56±0.01^{a}

Values expressed as mean ± SEM for five replicates per group (n=5).
Statistical comparison was made along a row and values followed by the same superscripts along the row are not significantly different by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

The DCM leaf extract of *M. senegalensis* at the least dosage (100 mg/kg) exhibited a remarkable reduction in the levels of HCT (41.0) which was statistically different fromthe effects noted in the grouping served with the highest dose of 1000 mg/kg (44.36) ($p<0.05$; Table 6.5). Nevertheless, this effect was similar to the properties of control grouping (Table 6.5).

It was further apparent that *M. senegalensis* at the extract dose of 100mg/kg shown remarkable reduction in the mean cell volume (MCV) than the effect of the higher dose levels of 300 and 1000mg/kg ($p<0.05$; Table 6.5). However, the observed effect was also comparable to the mean cell

volume (MCV) demonstrated in the DMSO treatedmice (Table 6.5).
The results of the rest of studied haematological parameters of mice (RBC, HB, MCHC, RDW, lymphocytes, monocytes, granulocytes, platelets, PDW and PCT) after treatment with *M. senegalensis* at all the various dosages (100, 300 and 1000 mg/kg bw), were significantly comparable to each other as well as the observation in the DMSO treated group ($p > 0.05$; Table 6.5).

On the contrary, the DCM stem bark extract of *D. melanoxylon* caused varied changes in the haematological parameters in mice (Table 6.6). The levels of HCT, MCV, lymphocytes, PDW and PCT marginally increased with rise in concentration of DCM stem bark extract of *D. melanoxylon* (Table 6.6).

It was found that upon treatment of mice with *D. melanoxylon* at all dose levels (100, 300 and 1000 mg/kg), the change in the levels of RBC, MCV, MCHC, RDW, lymphocytes, monocytes, granulocytes, platelets, PDW and PCT were statistically comparable to each other in addition to the DMSO treated group ($p>0.05$; Table 6.6). The DCM stem bark extracts of *melanoxylon* at the least tested dose level of 100 mg/kg manifested the highest reduction in the level of heamoglobin (Hb) than the rest of the extract dosages (300 and 1000 mg/kg) ($p>0.05$; Table 6.6). However, this effect was also comparable to DMSO treated grouping ($p > 0.05$; Table 6.6).

Haemoglobin (Hb) level in *D. melanoxylon* treated mice was comparably higher at doses of 300 and 1000 mg/kg though still statistically similar to the Hb level in the DMSO treated mice (control group) ($p < 0.05$; Table 6.6). Moreover, it was established that the extract of *D. melanoxylon* at the dose level of 100mg/kg lowered the HCT levels significantly as than to the levels noted in the animals treated with extract at 1000 mg/kg as well as DMSO treated grouping (Table 6.6).

Table 6.6: Effects of orally administered DCM stem bark extracts of *D. melanoxylon* on haematological profiles of mice

Parameters	Treatment (Extract Concentration)			
	10% DMSO	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
RBC	007.32±0.17^{a}	007.13±0.30^{a}	006.84±0.31^{a}	007.65±0.20^{a}
HB	013.74±0.34^{ab}	012.90±0.33^{b}	014.50±0.28^{a}	014.34±0.42^{a}
HCT	043.14±0.64^{ab}	041.96±0.59^{b}	043.40±0.52^{ab}	044.43±0.41^{a}
MCV	056.95±0.45^{a}	056.62±0.36^{a}	057.44±0.47^{a}	057.72±0.44^{a}
MCHC	034.96±0.47^{a}	035.58±0.23^{a}	035.08±0.67^{a}	034.92±0.28^{a}
RDW	014.60±0.35^{a}	014.86±0.49^{a}	015.04±0.32^{a}	014.98±0.32^{a}
Lymphocytes	081.42±0.73^{a}	080.80±0.79^{a}	082.04±0.55^{a}	082.22±0.62^{a}
Monocytes	005.12±0.29^{a}	005.28±0.30^{a}	005.22±0.16^{a}	005.42±0.22^{a}
Granulocytes	014.40±0.35^{a}	014.86±0.26^{a}	015.14±0.29^{a}	015.52±0.22^{a}
Platelets	367.60±3.97^{a}	377.20±5.19^{a}	381.60±4.43^{a}	384.60±7.76^{a}
PDW	008.56±0.38^{a}	008.84±0.52^{a}	009.28±0.44^{a}	009.64±0.26^{a}
PCT	000.48±0.02^{a}	000.51±0.02^{a}	000.52±0.02^{a}	000.53±0.02^{a}

Values expressed as mean ± SEM for five replicates per group (n=5).
Statistical comparison was made along a row and values followed by the same superscripts along the row are significantly similar by one- way ANOVA ($p>0.05$) followed by Tukey's post hoc test

6.4.4 Effects of Orally Administered DCM Leaf Extracts of *M. senegalensis* and Stem Bark Extracts of *D. melanoxylon* on Biochemistry of Mice

Generally, it was observed that the orally administered DCM extracts of *M. senegalensis* and *D. melanoxylon* for twenty days led to diverse biochemical effects on renal and liver functions of the mice (Table 6.7 and 6.8). It was observed that the studied biochemical effects largely did not follow a specific pattern in relation to the extract concentrations (Table 6.7 and 6.8).

Table 6.7: Effects of orally administered of DCM leaf extracts of *M. senegalensis* of biochemistry of mice

Parameters	Treatment (Extract Concentration)			
	10% DMSO	**100 mg/kg bw**	**300 mg/kg bw**	**1000 mg/kg bw**
T. protein(g/l)	005.82±0.09^{a}	005.37±0.18^{a}	005.46±0.25^{a}	005.27±0.15^{a}
ALB(g/l)	004.64±0.16^{a}	004.34±0.23^{a}	004.32±0.17^{a}	004.21±0.09^{a}
T. Bilirubin(µm)	003.78±0.14^{a}	004.09±0.28^{a}	003.77±0.14^{a}	003.39±0.34^{a}
D. Bilirubin(µm)	001.59±0.19^{a}	001.96±0.08^{a}	001.60±0.18^{a}	001.39±0.14^{a}
AST(IU/L)	001.23±0.06^{b}	001.63±0.08^{a}	001.49±0.07^{a}	001.68±0.02^{a}
ALT(IU/L)	109.70±0.30^{b}	111.62±0.58^{ab}	111.92±0.59^{a}	112.12±0.46^{a}
Urea(mmol)	010.68±0.32^{b}	013.14±0.07^{a}	013.80±0.30^{a}	013.91±0.25^{a}
Creatinine(µm)	038.36±0.35^{a}	038.43±0.40^{a}	040.76±0.93^{a}	040.09±0.59^{a}
Na^{+}(mmol)	150.06±0.63^{a}	149.98±0.44^{a}	149.60±0.38^{a}	149.62±0.51^{a}
Cl^{-}(mmol)	153.72±0.45^{a}	152.39±0.72^{a}	151.56±0.63^{a}	151.51±0.70^{a}

Values expressed as mean ± SEM for five replicates per group (n=5).
Statistical comparison was made along a row and values followed by the same superscripts along the row are not significantly different by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

Table 6.8: Effects of orally administered of DCM stem bark extracts of *D. melanoxylon* of biochemistry of mice

Parameters	10%DMSO	100 mg/kg bw	300 mg/kg bw	1000 mg/kg bw
T. protein (g/l)	005.82±0.09^{a}	005.55±0.19^{ab}	005.35±0.18^{ab}	005.06±0.14^{b}
ALB (g/l)	004.64±0.16^{a}	003.91±0.23^{a}	003.93±0.32^{a}	004.19±0.24^{a}
T. Bilirubin (µm)	003.78±0.14^{a}	004.05±0.08^{a}	004.13±0.06^{a}	004.10±0.05^{a}
D. Bilirubin (µm)	001.59±0.19^{a}	001.62±0.06^{a}	001.80±0.09^{a}	001.96±0.08^{a}
AST (IU/L)	001.23±0.06^{b}	001.77±0.08^{a}	001.94±0.08^{a}	001.94±0.08^{a}
ALT (IU/L)	109.70±0.30^{b}	110.04±0.41^{b}	111.24±0.59^{ab}	112.38±0.26^{a}
Urea (mmol)	010.68±0.32^{b}	012.66±0.34^{a}	014.31±0.53^{a}	013.62±0.44^{a}
Creatinine (µm)	038.36±0.35^{b}	039.03±0.20^{b}	039.18±0.50^{b}	151.20±0.74^{a}
Na^{+} (mmol)	150.06±0.63^{a}	150.15±0.39^{a}	150.93±0.43^{a}	152.59±0.74^{a}
Cl^{-} (mmol)	153.72±0.45^{a}	152.31±0.64^{ab}	150.48±0.54^{b}	152.59±0.70^{ab}

Values expressed as mean ± SEM for five replicates per group (n=5).
Statistical comparison was made along a row and values followed by the same superscripts along the row are not significantly different by one-way ANOVA ($p>0.05$) followed by Tukey's post hoc test

The findings of this research demonstrated that the DCM extract of *M. senegalensis* did not result to a remarkable effect on the total protein, albumin, total bilirubin, double bilirubin, creatinine, sodium and chlorine than the levels observed in the DMSO treated group mice ($p>0.05$; Table 6.7). In addition, it is apparent that the DCM extract of *M. senegalensis* at all dosage levels (100, 300 and 1000 mg/kg) resulted to increase in AST levels which was significantly comparable to each other ($p<0.05$; Table 6.7) but comparableto the levels manifested in the control grouping (Table 6.7).

Comparably, the potency of DCM extract of *M. senegalensis* at the higher dosages (300 and 1000 mg/kg) on ALT levels was statistically different

from the normal control grouping ($p<0.05$; Table 6.7). However, the increase in ALT levels caused by *M. senegalensis* at the dose of 100 mg/kg bw was significantly comparable to the levels seen in the DMSO treated mice ($p>0.05$; Table 6.7).

On the contrary, orally administered DCM stem bark extract of *D. melanoxylon* for 28 days also caused significant effects on renal and liver functions (Table 6.8). However, it was noted that the DCM stem bark extract of *D. melanoxylon* did not cause any remarkable variation in the levels of albumin, total bilirubin, double bilirubin and sodium when compared to the changes manifested in the DMSO treated mice (control grouping) ($p >0.05$; Table 6.8).

The DCM extract of *D. melanoxylon* extract at the entire tested dose levels (100, 300 and 1000 mg/kg) exhibited remarkable increase in AST levels in mice which weresignificantly similar to each other ($p >0.05$; Table 6.9) but statistically different from the effects observed in the DMSO treated mice ($p<0.05$; Table 6.9).

It was further established that the DCM stem bark extracts of *D. melanoxylon* at the highest studied concentration (1000 mg/kg) caused an increased ALT levels which was not comparable to both the least extract dose (100 mg/kg) and the control groups ($p<0.05$;Table 6.8). However, the

elevation in ALT levels as a result of treatment with 100 and 300 mg/kg was not statistically different to the DMSO treated grouping ($p>0.05$; Table 6.8).

The analyzed results also found that the DCM extracts of *D. melanoxylon* at the dosage levels of 100 and 300mg/kg caused remarkable effects on total protein levels which was comparable to each other as well as to the effects of the control group ($p>0.05$; Table 6.8). However, the effects elicited by *D. melanoxylon* extract at the dose levels of1000 mg/kg on total protein levels was notably different from the effects observed in the rest of treatment groups ($p<0.05$; Table 6.8).

It was observed that after the 28 days of the treatment period, the urea levels rose remarkably at all the dose levels of *D. melanoxylon* extract as compared to the effects seen in the DMSO treated group ($p<0.05$; Table 6.8). The levels of creatinine were also found to be significantly different in the group of rodents administered with *D. melanoxylon* extract at dose level of 1000 mg/kg than to the effects observed in the groups treated with other extract dosages as well as DMSO ($p<0.05$; Table 6.8).

It was established that the effects of *D. melanoxylon* stem bark extract on the chloride levels was significantly comparable among groups of varied

extract concentrations $p>0.05$; Table 6.8). Nevertheless, it was established that only the group treated with 300mg/kg dose of *D. melanoxylon* extract was statistically different from the observations seen on the DMSO treated group ($p<0.05$; Table 6.8).

6.4 Discussion

The present study used Wistar mice as the model organisms because they are mammals with their development, physiology, body plan, diseases and behavior being much similarto those of human beings. In fact, 99% of mice genes have homologs in man (Austin *et al*., 2004; Foster *et al*., 2006; Sellers and Ward, 2012). Mice are also small size, considerably docile, easy and inexpensive to maintain and have short reproductive time (Foster *et al*., 2006). It has also been reported that studies on mice provide clinical toxicity predictions that are highly comparable or even superior to predictions from monkey or dog studies (Sellers and Ward, 2012).

The current study was designed to evaluate likely sub-acute toxicity of orally administered DCM leaf extracts of *M. senegalensis* in addition to DCM stem bark extractsof *D. melanoxylon* in normal mice. It was apparent that the two plant extracts were generally nontoxic in mice. The herbs extracts lacked pronounced toxic effects on the organ and body weights and on the haematological as well as biochemical parameters in normal mice. The modest changes noted in any parameter studied were within the normal biological range since none of the parameter increased or decreased remarkably in relation to the control at all dosage levels.

There are extensive scientific publications on noxious effects of both crude extracts of herbs and isolated phytocompounds (Altemimi *et al*., 2017).

Many reviews have indicated no significant effects of herbs extracts on the body weight, organ weight as well as haematological and biochemical parameters of model animals and hence regarded safe (Ashafa *et al*., 2015). The nontoxic status of DCM leaf extracts of *M. senegalensis* and DCM stem bark extracts *D. melanoxylon* reported in the current research correlates with other previous works by Chen *et al*. (2011) and Gurley *et al*. (2005).

As stated by Merlin *et al*. (2019), plant species such as *Musanga cropioides, Aspalathus lineous, Xylopia aethiopica, Vernomia amygdalina, Sena alata, Calatropis procera* and *Artemisis afra* have shown outstanding *in vivo* safety in mammals. The extracts of*Gingko biloba, Allium sativum, Silybum marianum* and *Hypericum perforatum* are also safe in animal models. Other plant species such as *Afrostyrax lepidophyllus, Drypetesgossweileri*, *Napoleona vogelii* and *Tectoma grandis* have also been published to have negligible toxicity in animals (Merlin *et al*., 2019).

The extract concentration ranges used for sub-acute toxicity assessments in the current reported work were inside the dose scope employed by Osano *et al.* (2016), Saiga *et al*. (2017), Prabhat *et al.* (2019) and Osafanme *et al*. (2020). When evaluating sub-acute virulent profile of methanolic leaf extract of *Geophila obvallata* in Wistar rats Osafanme *et al*. (2020) used extract concentrations of 100, 500 and 1000 mg/kg. Osano *et al.,* (2016)

used dosages of 100, 350 and 1000 mg/kg in evaluating the *in vivo* virulence of DCM: Meth. extracts of *P. juliflora* in laboratory rodents. Similar laboratory based researches by Prabhat *et al.* (2019) to examine the toxicity of alcoholic leaf extracts of *Reinwardtia indica* using extract concentrations levels of 50, 250, 500 and 1000 mg/kg. Further, Saiga *et al*. (2017) also used extract dosages of 50,100 and 200 mg/kg when evaluating the sub-acute toxicity of a Pakistan polyherbal formulation, which was within the range of dosages used in this study.

The current study was *in vivo* in set up and execution. This was informed by the fact that some extracts lacking toxicity initially may become toxic metabolites after being bio transformed by liver enzymes (Jaishankar *et al*., 2013). Secondly, ability of the extractto penetrate the tissues, and clearance and excretion of the metabolites and metabolic products cannot be accounted for using cellular models. Further, use of organic and aqueous extracts makes it difficult to relate the toxicity of the plant to safety in traditionaluse (Jaishankar *et al*., 2013).

The usage of *in vivo* analysis in the current work was in agreement with earlier work conducted by Kimani *et al*. (2014), Arika *et al*. (2016) and Musila *et al*. (2017). Kimani *et al*. (2014) used a similar assay when working on safety of *Prosopis juliflora* and *Entada leptostachya* extracts in Wistar rats. Likewise, Musila *et al*. (2017) conducted a similar *in vivo*

assessment when evaluating oral toxicity of methanoilc extract of *Caesalpina volkensii*. Further, Arika *et al*. (2016) evaluated safeness of aqueous leaf extract of *Lippa javanica* in animal models being *in vivo* analysis. Although, the current work contrasted with a research by Nalbantsoy *et al*. (2012), who employed *in vivo* method when evaluating toxicity of toxin from the Cypriot blunt-nosed viper *Macrovipera lebetina* as well as antivenom production.

That there was no remarkable effect on body weight of mice following gradual administration of these herbs extracts is a pointer that the extracts are apparently safe for medicinal use. Body weight change is a primary factor to consider when evaluating toxicity of substances (Hayes *et al*., 2000). The observed insignificant weight gain in mice during the study period shows that the dichloromethane leaf extracts of *M. senegalensis* as well as DCM stem bark extracts of *D. melanoxylon* did not negatively affect the growth of mice (Ezeja *et al*., 2014).

The variations in weight are pointers of harmful effects of chemical and drugs and whenever the body weight loss observed is higher than 10%, it is regarded as statistically significant (Raza *et al*., 2002 and Teo *et al*., 2002). The noticed insignificant rise in body weight concurs with prior work by Anofi *et al*. (2014), who established a negligible rise in body weight of Wistar rats administered with hydro ethanol extract of *Dianthus basuticus*.

Organ weight as well is a crucial marker of pathological and physiological condition of animals. That there was no remarkable organ weight change in the mice treated with dichloromethane extracts of *M. senegalensis* and DCM extracts of *D. melanoxylon* is an indication that the organs were not exposed to any form of injury or toxicity from the extracts. The liver, lungs, spleen, heart, kidneys and brain are the main organs strained by metabolic responses brought about by toxicants (Dybing and Eagles, 2010). However, this did not happen in this research. The work agrees with the previous research by Kelly *et al*. (2008), who noted that treatment of mice with ethanolic leaf extract of *Maytenus obtusifolia* did not induce changes in the body organs.

The variation in spleen weight seen in the current work could be credited to its chief function it performs in elimination of exotic substances from the body (plants extracts) but not to virulence of the extracts (Cesta, 2006; Agbaje *et al*., 2009). Spleen weight in mice can be a sensitive marker of hypo- or hyper cellularity of blood cells (Keller *et al*.,2006). Thus, decrease in spleen weight at high doses might not be a marker of toxic potential of the extract on spleen. Mebratu *et al*. (2014) reported similar findings with significant change in spleen weight in animal administered with different concentrations of methanolic leaf extract of *Vernonia bipontini*.

The current study showed no remarkable variations in haematological parameters in the different groups of treated mice, demonstrating that *M. senegalensis* and *D. melanoxylon* extracts do not interfere with normal erythropoiesis. Studies have demonstrated that ingestion of herbal substances or medicines can change the standard range of haematological variables (Oloruuisola *et al*., 2012).

That the plants extracts showed no significant effects against the normal production of RBC, MCV and MCHC in the treated mice indicates that the herbal extracts have no poisonous effects on leucopoiesis and haemopoiesis in general. This contrasted the results described by Muriithi *et al*. (2015), where the methanolic extract of *Solanum incanum* induced remarkable rise in levels of RBCs and hematocrit. A decrease in blood hemoglobin concentration could have caused anaemia, while decrease in lymphocytes and granulocytes could have suggested immunosuppression and eventual decline in immune systems reaction to infection or antigens (Yamthe *et al*., 2012).

The insignificant changes in the levels of platelets as well as platelet width (PDW) observed in the current work are attributable to normal physiological readjustments in mice (Vagdatli *et al*., 2010). The steady increase in plateletcrit (PCT), albeit negligible could be credited to synthesis of thrombopoetin. Perhaps thrombopoietin yield was triggered by phytocompounds in the extracts of the two plants under study by creating

conducive environs for hematopoiesis (Maina *et al*., 2010). The findings agree with a study by Osano, (2016), who reported similar findings when evaluating *in vivo* virulent of *P. juliflora* in rat models.

Further, it is supposed that a rise or reduction in the blood parameter profiles is as a reaction of defense response to counter the administered plants extracts, which occurs through erythropoiesis stimulation. Competition between phytochemical compounds present in the extracts caused stimulation of erythropoiesis, which acts as promising scavengers, with RBC for oxygen, leading to hypoxia. This causes stimulation of the kidneys leading to direct production of erythropoietin (Corzo- Martinez *et al*., 2007).

That the plants extracts elicited no significant effects on lymphocytes, monocytes and granulocytes, suggests that the plant extracts have no deleterious effects on WBC. Insignificant increase in profiles of WBC after DCM extracts of *M. senegalensis* and *D. melanoxylon* were orally administered could be credited to different phytocompounds that have antibacterial effects, which leads to rapid increase of leucocytes that guard against invading agents (Wang *et al*., 2017). Elevation in monocytes may have resulted from the anthelmintic and anti-oxidant and anti-microbial effects of the two plant extracts under study (Lindsey *et al*., 2005).

Monocytes can be active directly by attacking microbial products, which causes production of pro-inflammatory cytokines. Different cytokines synthesis from monocytes includes interleukin-1, interleukin-2 and TNF (Swirski *et al*., 2009).

The current work found that the extract of the two plants under study elicited significant elevation of AST and ALT, which are usually associated with hepatocellular injuryindicative of hepatic injury. Elevated volume of liver enzymes indicate hepatocellular poisoning (Brautbar and Williams, 2002), while a reduction may suggest enzyme impediment (Akanji *et al*., 2013). It may be suggested that the plants extracts are little toxic because they cause increase of liver enzymes. However, no one or two liver profilescan be regarded as a marker of hepatotoxicity in isolation of all the others such as total and conjugated bilirubin levels (Reuben, 2004). The findings in the current research is in agreement with the findings described by Chineye *et al*. (2019), when studying sub-acute virulent of aqueous extract of *Carallum dalzielii* in animal models.

That the plants extract elicited significant elevation of urea levels in the treated mice is indicative that the renal excretion was reduced which led to accumulation of urea in blood. Elevated urea levels suggest that the kidneys may not be functioning well, or the mice were dehydrated (Wang *et al*.,

2017). Blood urea levels could rise due to high protein diet, dehydration, shock, severe hemorrhage, intestinal hemorrhage among others (Feinfeld *et al*., 2002). Decrease in the levels of urea could be as a result of low protein diet, diabetes insipidus, anabolic steroids and liver failure (Wang *et al*., 2017). The results concurs with the finding published by Chineye *et al*. (2019) who described elevated urea levels when studying toxicity of *Carallum dalzielli* in mice and rats. However, the results of this work are contrary to results by Debjit *et al*. (2019), who described a significant a reduction of blood urea in rats when working on acute and sub-acute virulent of *Aegialitisrotundifolia* leaves extracts in Wistar rats.

That the plants extracts elicited no significant effects on the levels of albumin, total bilirubin, double bilirubin and sodium is an indication that the plants extracts have no negative reaction to the liver. It was apparent that the plant extracts did not cause any liver injuries such as mitochondrial injury, canalicular and cholestatic damage, calcium homeostasis, stimulation of apoptosis, calcium homeostasis or cell membrane injury (Annonymous *et al*., 2009).

Therefore, the two studied plant extracts did not cause profound *in vivo* toxicity in mice.It is not surprising therefore, that Hamisi *et al*. (2015) never observed any *in vivo* toxicity reactions of Tanzanian *M. senegalensis* extracts in animal models. Similarly, Sanogo *et al*. (2006) of Mali did not find any toxicity effects of *M. senegalensis* extracts in mice. It is only Da Silva *et al*. (2010) in their trial toxicity studies who observed signs of virulent of *M. senegalensis* in animal models. It is therefore, not surprising that the Kenyan *M. senegalenis* extracts did not show any toxicity effects in mice.

Reduced toxicity or safety of the studied of the two plants extracts can also be attributed to oral route of the administration. This is because the phytochemicals may be subjected to detoxification by the alimentary canal microorganisms, biotransformation responses of the liver, binding and sequestration (Hodges *et al*., 2015). Further, it could be linked to unrecognized interactions between different phytocompounds in the extracts and drying of samples prior to extraction (Heinrich *et al*., 2012).

The extracts studied may therefore be considered safe when administered orally as potential anthelmintic and antioxidants. No wonder the Mbere people of Embu County, Kenya traditionally used the two studied plants to treat helminthiasis in cattle and humans. This study therefore, reveals the

possible potency of *M. senegalensis* and *D. melanoxylon* extracts can be used as an effective anthelmintic agent as well as an effective antioxidant.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECCOMMENDATIONS

7.1 General Discussion

The current study evaluated the presence and identity of biologically active compounds of DCM leaf extracts of *M. senegalensis* and stem bark of *D. melanoxylon* using GC-MS. The gas chromatogram revealed relative abundance of different phytochemicals eluted at different retention times (Appendices VI and VII). The fragmentation pattern indicated disintegration of large fragments into smaller compounds that gave rise to appearance of peaks at different m/z ratios. Mass spectra provided a blueprint of phytochemicals of the organic leaf extracts of *M. senegalensis* and stem bark of *D. melanoxylon*, which were identified from the National Institute of Standards and Technology (NIST) data library (Appendices VI and VII).

This study identified 36 and 35 biologically active phytochemicals in the DCM leaf extracts of *M. senegalensis* and stem bark of *D. melanoxylon* respectively (Tables 3.1 and 3.2). The GC-MS results showed the presence of active anthelmintic compounds in *M. senegalensis* and stem bark of *D. melanoxylon.* Compounds which contained anthelmintic activities include squalene, fatty acids such as n-hexadecanoic acid, octadecanoic acid, heptadecanoic acid while those containing antioxidant activities includes caryophyllene, α-humulene, α-pinene, squalene. This is in agreement to findings of previous studies using the plant extracts albeit using

different solvents (Silva *et al*., 2010; Zangueu *et al*., 2018; Jaiwal *et al*., 2015).

This study established that the plants extracts of *M. senegalensis* and *D. melanoxylon* had anthelmintic potential, which were attributed to constituent phytochemicals that apparently interfered with basic biochemical, physiological, metabolic and behavioural functions of the worms.

Phytochemicals may also have invaded a great number of proteins like enzymes, ion channels, cytoskeletal proteins, receptors, transporters in addition to transcription factors of worms in a non-selective manner. They may have acted by a way of non-covalent modification of protein forming many hydrogen bonds with electronegative atoms (O, N)in peptides and polypeptides (Jovan *et al*., 2016).

This study also established that the DCM extracts of *M. senegalensis* and *D. melanoxylon* had potential to quest hydroxyl radicals in a concentration dependent manner by creating a fail pink chromophore as the extracts concentrations increased. The FRAP test revealed that the studied extracts exhibited concentration dependent reducing potential, demonstrating that they can be employed as electron donors, which lower the oxidized intermediates of lipid peroxidation processes, where they perform as primary and secondary antioxidants. Antioxidant properties may be attributed to different mechanisms including decomposition of peroxides, prevention of chain initiation, radical scavenging and reducing capacity (Biswas *et al*., 2010).

This research associated the antioxidant and free radical-scavenging properties of DCM extracts of *M. senegalensis* and *D. melanoxylon* to the existence of phytocompounds that have been linked to antioxidant activities. Consequently, the synergistic effect of these biologically active components elevates their bioavailability and activity on many molecular targets hence remedying instability brought about by oxidative stress.

This study also established that the DCM extracts of *M. senegalensis* and *D. melanoxylon* caused insignificant effect on body weight of mice suggesting that the extracts did not hinder the growth of mice. It further revealed that the organic extracts of the selected plants caused insignificant organ weight indicating that the organs were not exposed to any form of injury or toxicity.

The current study also established that the two plant extracts studied did not significant changes in haematological parameters. This is a demonstration that the extracts did not interfere with normal production of erythrocytes. Further, the study revealed that the biochemical profiles in mice were not adversely affected by the extracts of the twostudied plants, indicating that the extracts were not poisonous to mice.

As the results of the current research indicate, all the tested plant extracts demonstrated potential anthelmintic and antioxidant properties. The activities were attributed to secondary metabolites in the extracts. The observed medicinal

properties of the two studied herbs extracts are associated to individual and possible collective effects of the constituent phytochemicals. This confirms and documents, in a systematic way, the anthelmintic properties of *M. senegalensis* and *D. melanoxylon* used for many years by people of Embu County, Kenya. This work also presents a platform on which to more research on the DCM extracts of *M. senegalensis* and *D. melanoxylon* as plant derived sources of drugs that could serve as novel agents for anthelmintic against helminthes and as antioxidants.

7.2 Conclusions

In conclusion, the current work demonstrated that;

i. The DCM extracts of *M. senegalensis* and *D. melanoxylon* have phytochemicalsthat are safely associated with anthelmintic and antioxidant properties.

ii. The DCM extracts of *M. senegalensis* and *D. melanoxylon* have *in vitro* anthelmintic activities in earthworms at the dose level of 50mg/ml.

iii. The DCM extracts of *M. senegalensis* and *D. melanoxylon* have *in vitro* antioxidant activities.

iv. The DCM extracts of *M. senegalensis* and *D. melanoxylon* have no *in vivo* toxicity in normal mice at dose levels of 100 and 300mg/kg body weight.

Consequently, the research questions hypothesized in current work were answeredin affirmative.

7.3 Recommendations

7.3.1 Recommendations from this Work

From the findings of the following recommendations can be made from this work.

i. There is need to undertake *in vivo* antioxidant studies on the extracts of *M. senegalensis* and *D. melanoxylon* to confirm the observation obtained from *invitro* studies.

ii. There is demand to carry out bioassay guided fractionation and structure elucidation of anthelmintic and antioxidant components in the extracts of the two studied medicinal plants

iii. There is need for efficient conservation strategies for Kenyan population of *M. senegalensis* and *D. melanoxylon* due to their high bioactivity and abundance in phytochemicals.

7.3.2 Recommendations for further work

i. There is need to undertake the study using aqueous extraction of *M. senegalensis* and *D. melanoxylon* to establish how its anthelmintic activities can be compared with the observations made from the selected organic extracts.

ii. Further research should be done to elucidate possible mechanisms of anthelmintic activities of *M. senegalensis and D. melanoxylon* extracts on worms.

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APPENDICES

NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON

The East African Herbarium
P.O Box 45166 00100 Nairobi, Kenya
Telephone: 3743513, 3742131/4 ext. 2274

Fax: 3741424

E-MAIL:botany@musuems.or.ke

4th December, 2020

REF: NMK/BOT/CTX/1/2
Boniface Mwangi
P.O Box 73 – 10200
Murang'a

Dear Boniface,

PLANT IDENTIFICATION

The plant specimens that you deposited at East African Herbarium for identification were
Determined as follows;

1. *Dalbergia melanoxylon* Guill. & Perr. (Family: Fabaceae)
2. *Maytenus senegalensis* (Lam.) Exell (Famiy: Celastraceae)

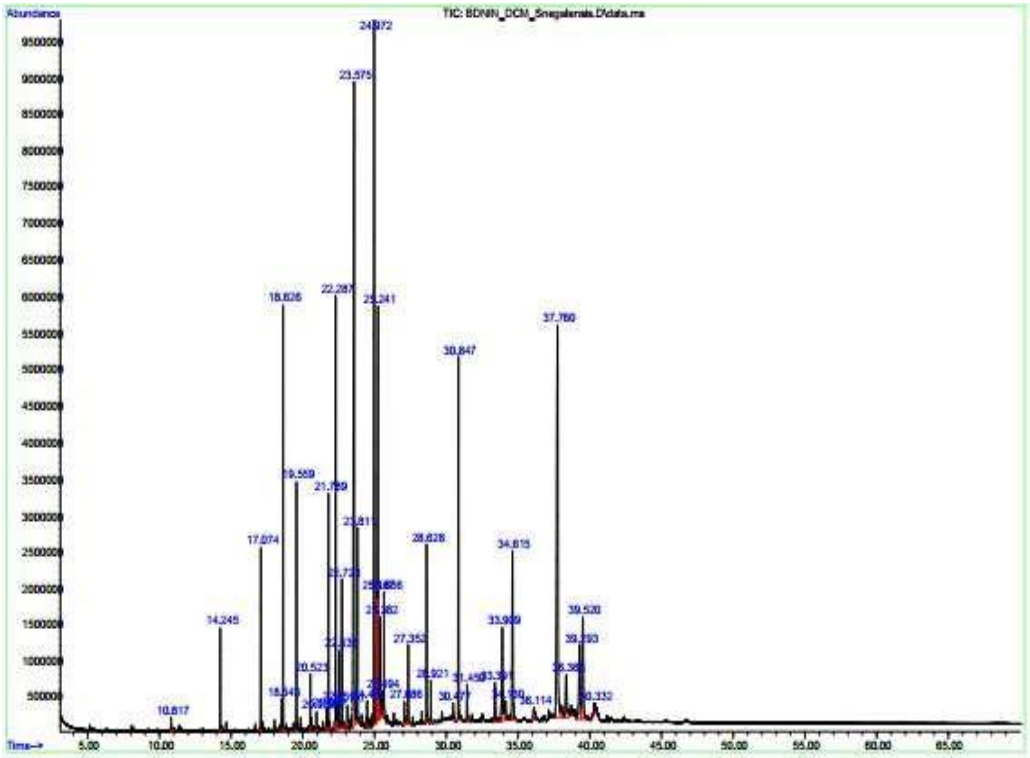
Thank you for vising EA Herbarium.

Yours Faithfully,

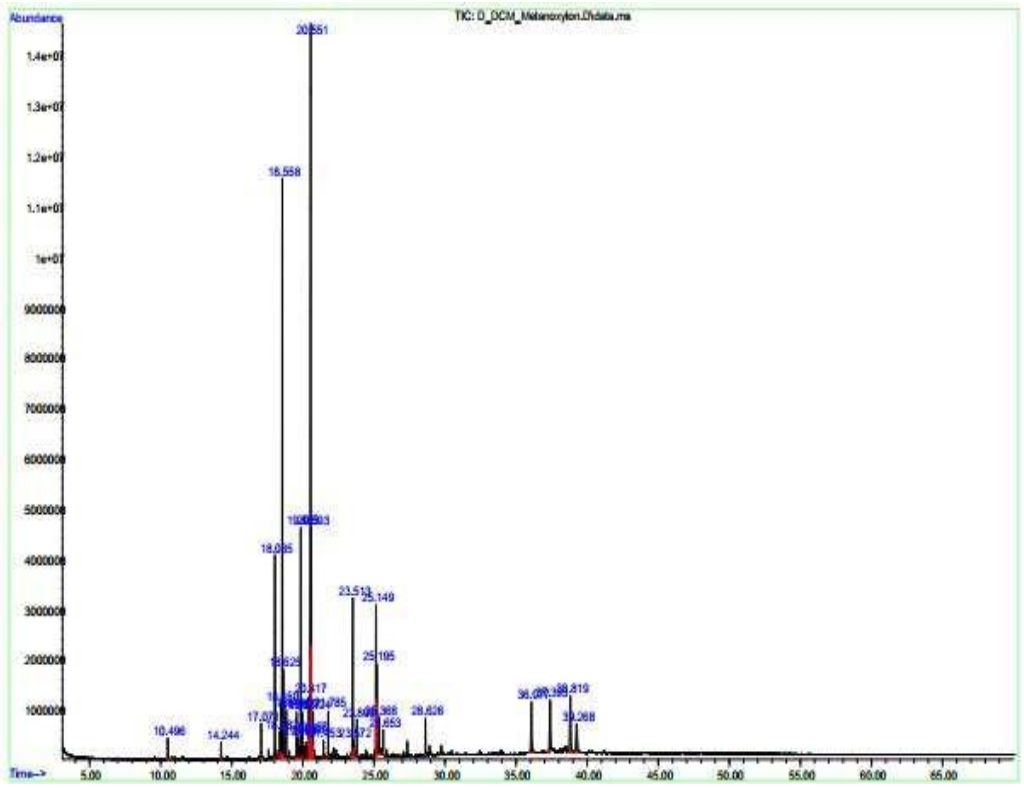
NATIONAL MUSEUMS OF KENYA
04 DEC 2020
HERBARIUM NAIROBI

Kennedy Mathenge
For
Head, Botany Department

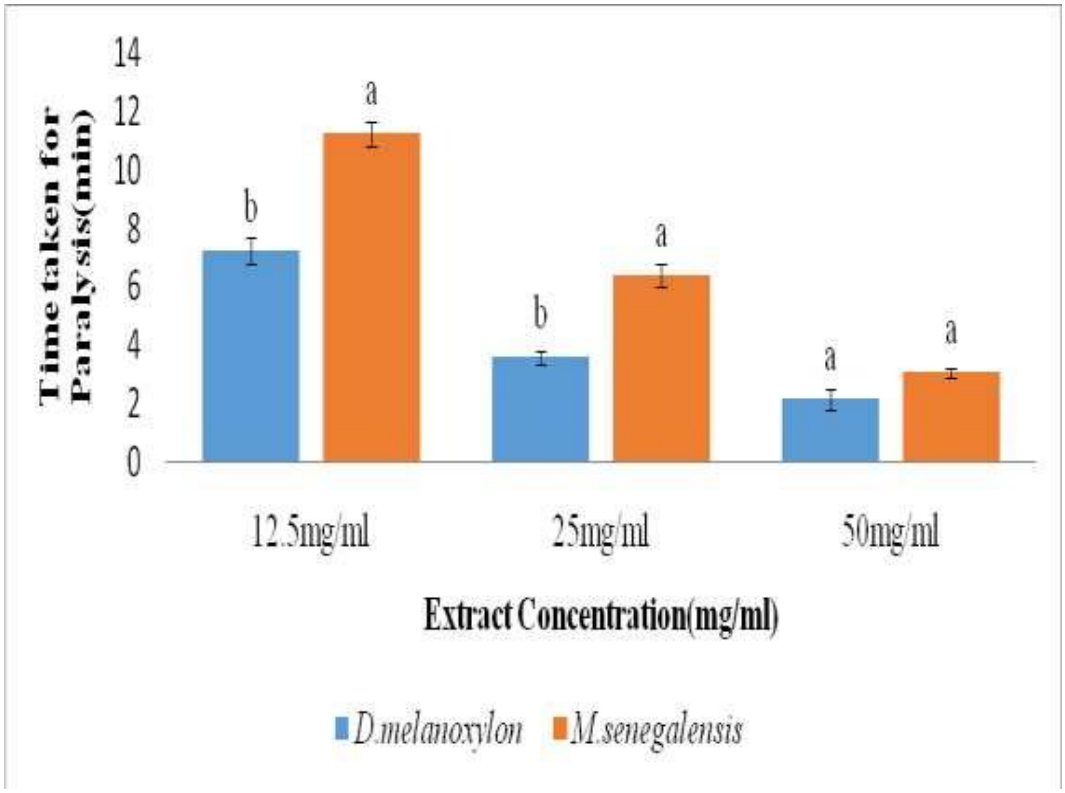
Appendix I: Letter of Plants Identification



Appendix.II: The GC-MS total ion chromatogram of DCM leaf extract of *M. senegalensis* with retention time for phytochemicals concentration determination

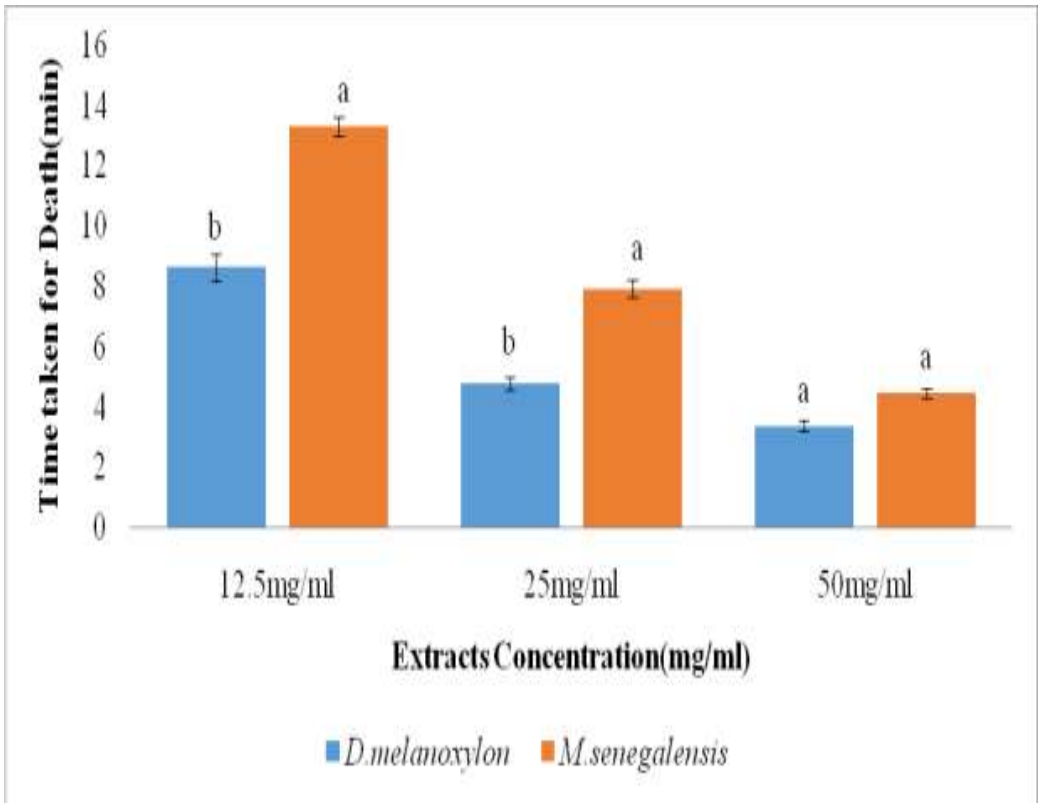


Appendix: III **The GC-MS total ion chromatogram of DCM of *D. melanoxylon* with retention time for phytochemicals concentration determination**

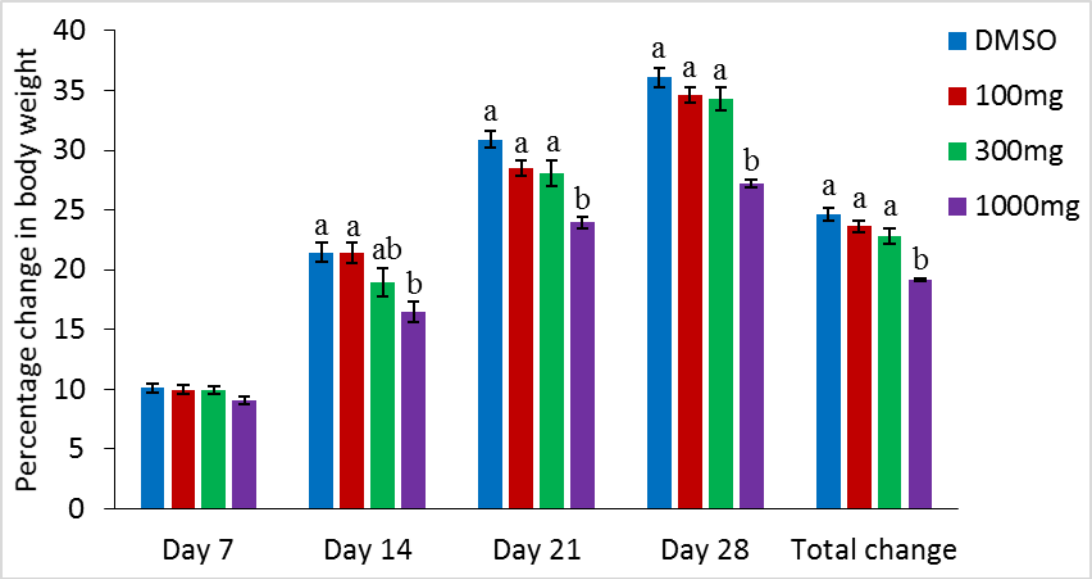


Appendix IV: **Comparison between Paralysis Effects of DCM Extracts of *M. senegalensis* and of *D. melanoxylon* on *P. posthuma*.**

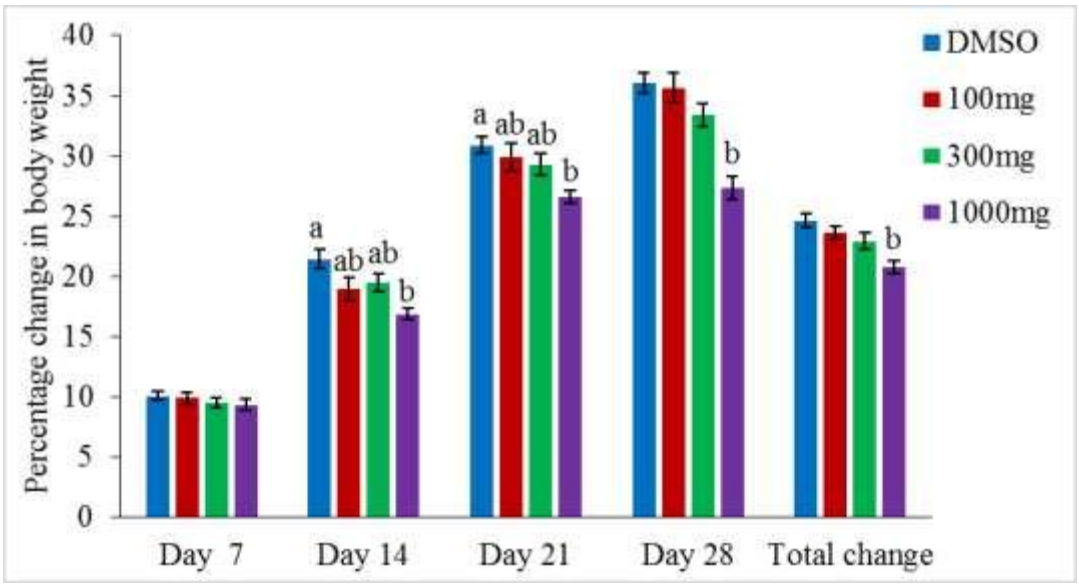
Bar graphs with similar superscripts within the same extract concentration are not significantly different as analyzed by unpaired t- tests ($p>0.05$)



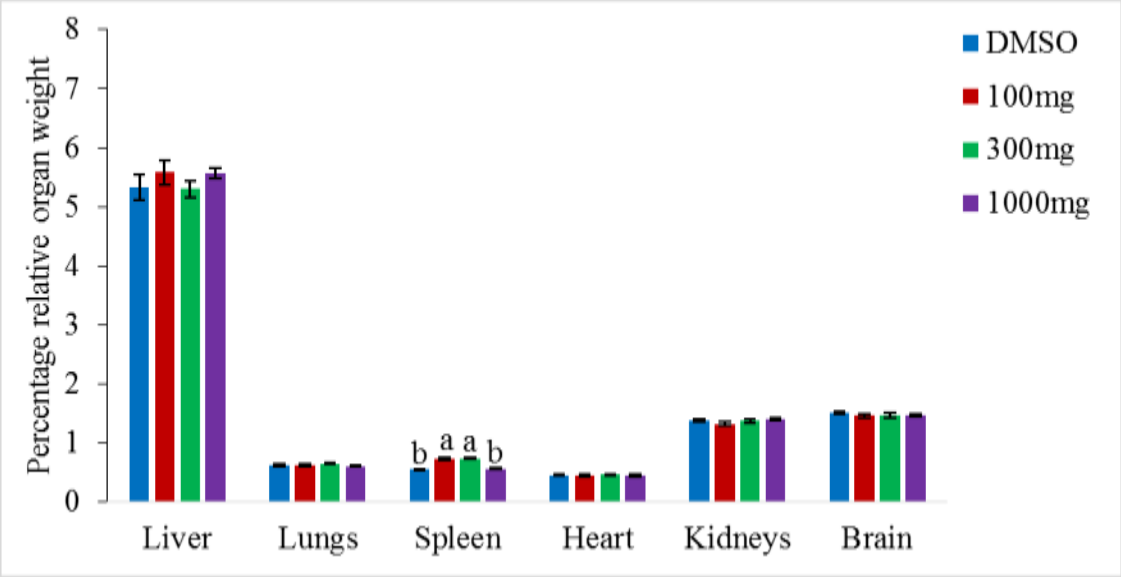
Appendix V: **Comparison of Mortality Effects of DCM Extracts of *M. senegalensis* and stem bark extracts of *D. melanoxylon* on *P. posthuma*.**
Bar graphs with similar superscripts within the same extract concentration are not significantly different as analyzed by unpaired t- tests($p > 0.05$)



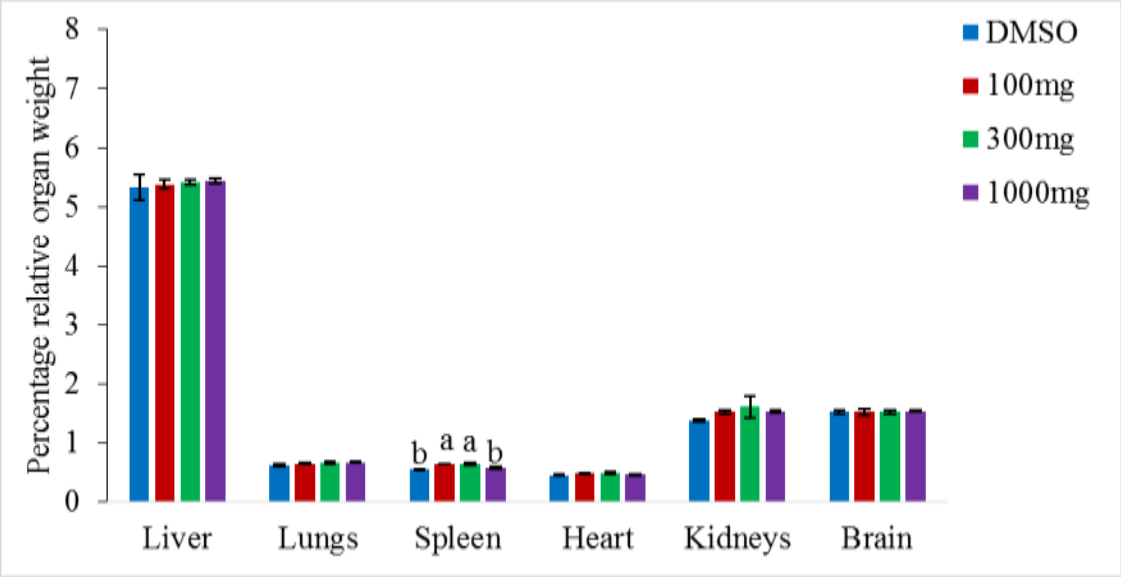
Appendix VI: Effects of extract *of M. senegalensis* on body weight of mice.
Bars without superscript are statistically comparable. Bars with the different lower case letter are significantlydifferent at the same concentration ($p<0.05$)



Appendix VII: Effects of extract of *D. melanoxylon* on body weight of mice.
Bars without superscript are statistically comparable. Values followed by the same superscripts are not significantly different ($p>0.05$)



Appendix VIII: **Effects of *M. senegalensis* on organ weight of mice.**
Bars without superscript are statistically comparable. Values followed by the same superscripts are not significantlydifferent ($p>0.05$)



Appendix IX: **Effects of extract of *D. melanoxylon* on organ weight of mice.**
Bars without superscript are statistically comparable. Values followed by the same superscripts are not significantly different ($p>0.05$)