EVALUATION OF THE ANTHELMINTIC PROPERTIES OF *AFZELIA AFRICANA* 'SM' AND *COMBRETUM MOLLE* 'R.BD/GON'.

BY

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DECLARATION

I declare that the work in this thesis entitled **'Evaluation of the anthelmintic properties of *Afzelia africana 'SM'* and C*ombretum molle 'R.Br/G.Don'*;** was carried out by me in the Department of Veterinary Parasitology and Entomology under the supervision of **Prof. O.J Ajanusi, Dr. M.S Abubakar** and **Prof. B.D George**.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented else where for a degree or diploma.

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CERTIFICATION

This thesis entitled EVALUATION OF THE ANTHELMINTIC PROPERTIES *AFZELIA AFRICANA* 'SM' AND *COMBRETUM MOLLE* R.BD/GON' BY **SIMON, MALANG KAWE** has met the regulations governing the award of the degree of Master's of Veterinary Parasitology of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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I dedicate this work to my lovely children Daniel and Daniela.

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ABSTRACT

The anthelmintic activity of partitioned fractions of crude methanolic extracts of *Afzelia africana* and *Combretum molle* stem bark were evaluated *in-vivo* in rats experimentally infected with *Nippostrongylus braziliensis.* The crude methanolic extract of the plants were obtained after extraction with absolute methanol. These were further partitioned in three solvents to obtain four fractions (i.e. petroleum ether, chloroform, N-butanol and the aqueous methanol). Phytochemical screening conducted on the extracts of both plants revealed constituents such as alkaloids, steroids, saponins, tannins, flavonoids, cardiac glycoside and carbohydrates. The extracts fractions (with the exception of petroleum ether) were tested for anthelmintic activity against *Nippostrongylus braziliensis* in rats. The anthelmintic activity was assessed by comparing the number of worms recovered from rats treated with the fractions to those from non-treated infected controls rats. This study considered deparasitization rate of 70 % or greater as biologically significant. Out of the eight fractions of both plants tested, the aqueous methanol of *C. molle* had the highest significance ($P<0.05$) anthelmintic activity with 86.98 % deparasitization; followed by chloroform fraction of *A. africana*, chloroform fraction of *C. molle*, N-butanol of both plants with deparasitization of 79.20 %, 75.0 %, 72.72 % respectively when the maximum tolerated dose of 1,000 $mgkg^{-1}$ was administered. The aqueous methanol fraction of *A. africana* and the crude methanolic extracts of both plants produced induced a non-significant ($P>0.05$) deparasitization rates of 53.33 %, 62.50 % and 48.61 % respectively. The maximum tolerated dose studies on the crude methanolic extracts of both plants were conducted to determine the therapeutic dose. A dose

range 10 to 1,000 $mgkg^{-1}$ were considered practically non-toxic while 1,600 to 5,000 $mgkg^{-1}$ were considered slightly toxic and unsafe even though failed to produce gross and histological lesions on the internal organs. Thus, this study recommends further investigations to validate their efficacy in natural or experimental infection in ruminant.

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LIST OF ABBREVIATION

ANOVA- ANALYSIS OF VARIANCE ……...89

CME - CRUDE METHANOLIC EXTRACT ……107

COWP- COPPER OXIDE WIRE PARTICLES ……32

CTS - CONDENSED TANNINS…….35

DPZ - DEPARASITIZATION ……..……..113

EVM - ETHNOVETERINARY MEDICINE ……42

FAO - FOOD AND AGRICULTURAL ORGANIZATION…… .9

FEC - FAECAL EGG COUNT ……..…3

GABA - GAMMA ANIMO BUTYRIC ACID ……5

GI - GASTROINTESTINAL ……22

GIT - GASTROINTESTINAL TRACT ……..18

MCV - MAXIMUM CONVENIENT VOLUME ……107

MTD - MAXIMUM TOLERATED DOSE ……..65

PCR - POLYMERASE CHAIN REACTION …….24

PCV - PACKED CELL VOLUME ……..17

TLC - THIN LAYER CHROMATOGRAPHY ……..57

TWC - TOTAL WORM COUNT ……..…….……121

W - WEIGHT ……..107

WAAP- WORLD ASSOCIATION FOR THE ADVANCEMENT OF VETERINARY PATHOLOGY…. ……84

CHAPTER ONE

1:0 INTRODUCTION

1.1. Chemical control of helminths and other gastrointestinal parasites.

1.1.1. Prospect and draw backs

For decades, the growth of the animal health industry has been fueled by the discovery, development and sales of anti-parasitic drugs which account for more than 20 % of the total animal health market (Wesley, 2002). The treatment and prevention of parasitism in livestock and companion animals form part of the expenditure encured in any livestock outfit (Zajae *et al*., 2000). Over the past forty years, the industry provided stable and useful new products that revolutionized the practice of veterinary medicine. Among these anthelmintics available in the market today are the broad and the narrow spectrum anthelmintics. Livestock producers have generally derived substantial benefits from the use of conventional anthelmintic drugs in controlling livestock parasitosis. In Africa, however, declining funding for veterinary services and the rising costs (occasioned by depreciating value of local currencies) of these services has made it difficult for resource-poor farmers to have access to such services. These have led to the increasing demand for effective and low cost anthelmintic drugs by African smallholder livestock producers and pastoralists in order to reduce expenditure on the costly imported drugs. This will also boost their economic self-reliance (Abdu *et al*., 2000; Ademola *et al.*, 2004; Uza *et al*., 1996; Olayiwola, 1993).

In recent times, the developed world is increasingly placing more interest in ethnomedical and ethnoveterinary practices especially as it relates to the use of medicinal plants in treating various diseases. This move is in response to the production of animals free from industrial chemical inputs (Gasbarre *et al.*, 2001), the

public concern over perceivable drug residue in animal products (Athanasiadou *et al.*, 2005; McKellar, 1997), the possible tendencies of environmental pollution (Vierra *et al.*, 1999); as well as the problem of the rapid escalation in anthelmintic resistance worldwide (Jackson and Coop, 2000; Sangster, 1999). There is also an augent need to discover new therapeutic substances of natural origin with low toxicity to man and animals (Guarrera, 1999). In Africa, this practice was borne out of neccesity-lack of affordability (Schillhorn van Veen, 1997), poor efficacy of adulterated anthelmintics sold in the market and the lack of veterinary services, especially in isolated pastoral areas (Monterio *et al.*, *1998*; Wanyangu *et al.*, 1996). In addition, there is also the awareness that increase in the production of ruminants through effective and cheaper means of treating helminthiasis can only be achieved through development of effective drugs from reasonably inexpensive and available raw materials (Ibrahim *et al.*, 1984; Nwude, 1988).

1.2. Alternative methods for control of helminths and other gastrointestinal parasites

Other alternative methods of parasite control being developed include good hygienic practices in animal herds, pasture rotation (Harrison *et al.*, 1996; Michel, 1985), targeted drenching (Robert and Swan, 1982), the use of fungi (mixed with feed) to kill parasite larvae in the host gastrointestinal tract and faeces (Waller and Larsen; 1993; Larsen, 1999, 2000), the use of copper wire oxide to reduce establishment and fecundity, especially of *H contortus* (Knox, 2002; Chartier *et al.*, 2000), vaccination using gut-expressed antigens of the parasite (Knox and Smith, 2001; Gray, 1997; Bain, 1999; Barnes *et al.*, 1995), and planting of plants in animal grazing areas that not only affect

the nutritional status of the animals, but also have antiparasitic effects (Waghorn and McNabb, 2003).

1.2.1. Use of natural remedies in the control of helminth diseases

A lot of efforts are directed at screening herbal medicinal plants that are used locally by traditionalist and pastoralist as remedies for helminthiasis in *in-vitro* and *in-vivo* (Hordegen *et al.,* 2003, Githiori *et al.,* 2003b; Alawa *et al.,* 2003; Youn *et al.,* 2003; Ademola *et al.,* 2004). Brander *et al* (1991) and Brown and Tailor (1996) reported that *Arecas catechu* (betelnut) produced a paralyzing effect on nerves and muscles of *Taenia spp* and *Hymenolepis nana* leading to evacuation of the worms by peristaltic contraction of the intestine. In Nigeria, *Ocimum gratissimum* (mosquitor plant) was shown to yield a volatile oil that has both antimicrobial and anthelmintic properties (Sofowora, 1982). Asuzu and Njoku (1996) reported the anthelmintic effects of bark aqueous extract of *Alstonia boonei* and aqueous leaf extracts of *Nauclea latifolia* against infective larvae of *Trichostrongylus axei*. *Carica papaya* (pawpaw) seeds were also shown to possess activity both *in-vitro* and *in-vivo* against *H nana* and *Nippostrongylus brasiliensis* in rats (Paninga, 1985).

Githiori *et al* (2003a) reported reduction in faecal egg count (FEC) when mice infected with *Heligmosomoidis polygyrus* were treated with the extracts of *Rapanea melanophloeos* and *Azadirachta indica*. *Vernonia amygdalina,* a plant widely used in Nigeria for human consumption as well as for ethnomedical and ethnoveterinary practices, has been reported to possess both endoparasitic and ectoparasitc effects (Huffman *et al.,* 1993; Regassa, 2000; Jisaka *et al.,* 1993). Suleiman *et al.* (2005) reported that *Xylopia aethiopica* has activity against *N. braziliensis* in rats. Other

plants such as *Annona senegalensis* (bark), *Khaya senegalensis* (bark) and *Anoigeissus leoicarpus* (bark) were equally found to demonstrate anthelmintic properties both in *in -vitro* and *in-vivo* studies (Alawa *et al.,* 2003, Ademola *et al.*, 2004; Ibrahim *et al.,* 1984).

1.2.2. Ethnoveterinary practices in the control of helminth diseases

Herdsmen are generally very knowledgeable about the effectiveness of a lot of plants against common diseases and ailments affecting their livestock, like gastrointestinal parasites of cattle. Even though Fulani pastoralists have a number of folklore remedies for a very long time (and they strongly believe in their efficacy), there were no scientific evidence to confirm such beliefs. Hence, studies are now carried out not only to determine their efficacy scientifically (Ibrahim *et al.,* 1984; Nfi *et al.*, 2001), isolate the active principles as well as to determine their mode of action (Vercruysse *et al.,* 2001; Ibrahim *et al.,* 1984; Abdul Ghani, 1990) and their toxicity, but also to standardize their dosage (Nwude and Ibrahim, 1980). The use of alternative drugs has been recommended as a measure to avoid the development of resistant strains of helminth parasites, reduce drug residue in animal products, and reduce environmental pollution and to explore the possibility of reducing cost of controlling helminthiasis (Athanasiaduo *et al.,* 2005; McKellar, 1997; Schillhorn van Veen, 1997).

1.2.3. Chemicals use in the control of helminth parasites

Benzimidazole (comprising of anthelmintics such as albendazole, cambendazole, febantel, fenbendazole, flubendazole, luxabendazole, mebendazole, netobimin, oxfendazole, oxibendazole, pabendazole, thiabendazole and thiophanate) have been shown to inhibit the enzyme fumarate reductase and inhibit glucose uptake, causing

depletion of the parasite glycogen reserves *in-vitro*. This has the ability to polymerize tubulin in the microtubules of cells, which affects the worms' ability to digest and absorb nutrients. In addition, benzimidazole also acts by increasing the permeability of cell membrane to protons thereby killing the parasite (McCracken *et al*., 1982).

Drugs like disophenol, niclosamide, rafoxanide (flukicides) and dichlorophen (anticestodal) affect the energy-generating metabolism of worms by inhibiting mitochondrial phosphorylation; piperazine causes paralysis of the parasite by causing hyperpolarization of the parasite smooth muscle cells; pyrantel and morantel achieve similar effect as that of piperazine by depolarizing the muscle cells, while levamisole is a ganglion stimulant which also depolarizes the parasite muscle cells (Faulkner *et al.*, 1972; Le Jambre, 1976).

The mechanism of the macrolide avermectin is still not known except that it increases the permeability of the parasite membranes to chloride ions. It has also been shown to block neuromuscular transmission by stimulating release of the neurotransmitter γ-aminobutyric acid (GABA) which keeps the chloride channels open. This prevent the all-important changes in polarity along the motor neuron, essential for transferring signals to and from the muscle cells from occurring. Thus, the worms become paralyzed (Campbell and Benz, 1984).

Organophosphates (dichlovos, coumaphous, trichlorfom and naphthalophos) are a group of compounds which paralyzes both nematodes and insects by inhibiting the enzyme acetylcholenesterase, whose function is to stop the action of acetylcholine (Andrew *et al.*, 1983). Praziquantel (a tape worm remedy) causes vacuolation of the tegument of the

worms, and also produces paralysis by increasing the permeability of cell membrane to positively charged ions (Na^+ and Ca^{2+}) (Andrew *et al.*, 1983).

Diamphenethide an active metabolite and a flukicide remedy act in a similar way with praziquantel (Harfenist, 1973).

1.3. Importance of parasitic nematodes in livestock industries

Parasitic nematodes are among the most common and economically important agents of infectious disease of grazing livestock, especially in small ruminants; in the tropics, subtropics and other parts of the world (Perry *et al.*, 2002; Prichard, 1994). The parasitic diseases of production animals are widely distributed, but have different impart in different parts of the world (Perry and Randolph, 1999). Livestock production in tropical climates suffers heavy economic losses due to gastrointestinal parasites (Copeman, 1980; Al-Quaisy *et al.*, 1987). The loss due to helminthiasis in small ruminant in Nigeria is serious and of major economic importance (Schillhorn van Veem, 1973) and account for an estimated 144 million dollars annually (Akerejola *et al*., 1979). The loss is characterized by lower output of animal products (meat, milk, hides and skins), manure, traction, carcass and organ condemnation, death and medication costs, which all impart negatively on the livelihood of small holder farmers (Perry and Randolph, 1999; Chiezey *et al.*, 2000). The greatest losses associated with nematode parasitic infection are sub-clinical and economic assessment show that financial costs of internal parasitism are enormous (Preston and Allonby, 1979; McLeod, 1995).

1.4. Hypotheses.

Extracts from *Afzelia africana* and *Combretum molle* have direct anthelmintic effects on larval and/or adults of *N. braziliensis.*

1.5. Research aims and objectives.

1.5.1. General objectives

In view of the public concern over perceivable drug residues in animal products, coupled with the increasing prevalence of anthelmintic-resistant strains of nematodes, as well as the rising cost of such organic substances, there is an urgent need for the development of sustainable alternatives to conventional anthelmintics in ruminant production system. This can be achieved through research into newer anthelmintics from natural plants and plant products.

1.5.2. Specific objectives

The aim of this study was to investigate the claims by pastoralist and traditionalist that *A. africana* and *C. molle* plants posses' anthelmintic effects when ruminant are drendge with concoctions from both the leaves and stem bark. Therefore, the investigation was done by;

1 Asses the *in vivo* anthelmintic effects of the methanol extract of the stem bark of *Afzelia africana* and *Combretum molle* against adult *Nippostrongylus braziliensis* in experimentally infected rats.

2 Identify the active fractions from the crude methanolic extracts of these plants, with the view of providing scientific basis for their use in ethno-veterinary practices.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. LIVESTOCK PRODUCTION IN NIGERIA

The major cattle production systems in Nigeria are the pastoralist, agropastoralist and the sedentary/settled systems. However, specialized production system such as ranches and smallholder cattle fattening have emerged in significant numbers especially in economically and ecologically viable urban and peri-urban areas where the demand for meat and livestock products are high due to population increase (Alawa *et al*., 2000). Sheep and goat breeding are, recently becoming more developed in Nigeria because of their capacity to resist adverse conditions. The interest in small ruminant exploitation comes from the fact that sheep and goats are the major sources of protein (meat) to the rural populace and a source of income through the sale of some by products such as skin for export (Assis *et al*., 2003; Molokwu, 1982; Anon, 1978). Geary (2002) wondered whether in the near future the livestock industries will migrate from more developed to less developed countries. This migration will be made possible by some motivating factors which include lower regulatory oversight, fewer complaints from new urban neighbours about the smell associated with animal agriculture, and lower land and labor costs (Geary and Thompson, 2003).

2.2. Economic significance of livestock

2.2.1. Draught power

Animal traction is the major source of farm power in many developing countries (Smith, 1990), providing 75-90% draught power for agriculture and transportation in large rural areas of developing countries (Gefu and Otchere, 1994). Almost half of the

world's populations (in about 50 developing countries) depend heavily on draft animals as sources of energy for both agricultural operations and haulage purposes (Ramaswamy, 1998). In Northern part of Nigeria, bulls are used to draw ploughs while donkeys, horses and camel are employed as beasts of burden (Okagbare and Akpodiete, 1999).

2.2.2. Source of food

Animals are a significant source of food, particularly high quality protein, minerals, vitamins and micronutrients. Meat, milk and eggs provide 17-18% of the dietary protein in African diets (FAO, 1977).

2.2.3. Source of manure

Manure is the principal soil nutrient replacement available to large number of farmers. Manure is the most important contribution that livestock provides to agricultural growth. Animal wastes are used as a source of nitrogenous fertilizer for soil improvement (Okagbare and Akpodiete, 1999). Farmyard manure and compost is derived from the mixture of animal beddings, straw and waste products (faeces and urine) of the animals (Okagbare and Akpodiete, 1999).

2.2.4. Source of income

The livestock industry contributes immensely to the national income. The sales of animals and their products generate income for both farmers and the nation. Adu (1997) stated that the US Department of Agriculture in 1990 reported that livestock commodity output (meat, milk, eggs, hides and skin) in Nigeria was worth $1.749 billion (#139.92 billion), which constituted about 5 percent of the then total Gross Domestic Products or 26 percent of the agricultural domestic products.

2.2.5. Source of raw materials for industries.

Products from livestock serve as raw material for many industries. These industries convert animal by-products into a vast number of commodities useful to man. For example, horns and hooves are used in the production of gelatin, glues, combs, buttons, handles of spoons, knives and other kitchen and house hold utensils. Wools and hairs and bristles are used in the production of fabrics used as clothes, tent, covers etc. Hides and skin are used in the production of leather used for making shoes, bags, belts and house hold furniture. Animal fat is used in the manufacture of seals and adhesives, candles, protective clothing's, soap, lubricating grease, linoleum and synthetic rubbers. It is also used in the manufacture of certain drugs. Blood and glands serve as source of hormones like insulin and protein substances like albumin, which are used by the pharmaceutical industries for the manufacture of drugs (Okagbare and Akpodiete, 1999).

2.2.6. Source of employment.

In addition to its economic values, the livestock industry also plays an important role as it offers employment to millions of Nigerians in the areas of rearing, extension services, trading and transportation. Workers in agro-based industries use livestock as raw materials (Okagbare and Akpodiete, 1999).

2.2.7. Socio-cultural roles.

The social role of livestock is often overlooked in the complex socio-cultural relationship in rural communities in developing countries such as Nigeria. Livestock are often used as source of social ties through exchange of gifts and services

(Scoones, 1992). Livestock also play an important role in marriage contracts and ceremonial festivities in many societies that is not quantifiable (Barrett, 1992).

2.3. Problems militating against livestock production

Factors limiting livestock production in Nigeria and Africa includes, poor nutrition, disease, poor management, harsh climate, social and economic factors, policy changes as well as institutional problems (Alawa *et al*., 2001; Okagbare and Akpodiete, 1999).

2.3.1. Poor nutrition

Inadequate supply of feed is one of the major problems facing livestock production in Nigeria. An excess supply of feed during the rainy season is usually followed by a grave deficit in the following dry season. The scarcity of high-quality forage results generally in animal's not being able to meet up to their protein and energy requirements. Consequently, not only do they experience marked weight loss but lower disease resistance, high death rate, reduced fertility, low milk yield as well as low growth rates of calves, lamb and kids (Okagbare and Akpodiete, 1999). It is therefore imperative that during the dry season, livestock feeding has to be supplemented with concentrate or readily available sources of energy. Deficiencies in the quality and quantity of feed resource base which exist makes it difficult to meet the feed requirements for maintaining existing levels of production, let alone for any increases (Plaizier, 1993).

2.3.2. Poor management

A large proportion of the cattle, sheep and goats are in the hands of nomadic pastoralists who move their herds about in search of feed and water. Under this system of management, productivity of the animals is reduced as the animals are

exposed to diverse health hazards and a lot of energy is spent on movement. It also makes meaningful planning for the introduction of improvements or the adoption of new technologies to boost production difficult (Alawa *et al*., 2001; Okagbare and Akpodiete, 1999).

.2.3.3. Disease

Disease is also an impediment to livestock productivity across all agro-ecological zones of the African continent. Losses from preventable diseases such as gastrointestinal parasites are enormous. Associated with these is the inability of relevant Government Agencies to maintain effective surveillance and control measures (Alawa *et al*., 2001).

2.3.4. Climate

The most important climatic factors that limit livestock productivity are ambient temperature, effective rainfall, length of day light and the intensity of solar radiation. There are two main seasons in Nigeria, the wet and the dry seasons. The wet season is characterized by high rainfall, low temperature and high relative humidity; while little or no rainfall, low humidity and high temperature are experienced during the dry season. In the northern part, ambient temperatures are above 26ºC (especially in the dry season) for most part of the year. This result in low feed intake, increased water consumption and reduced grazing time, thus reducing the productivity of the animals. The low temperature and humidity observed during the rainy season, provides a favorable breeding environment for internal and external parasites, as well as pathogens such as bacteria, fungi and viruses. For example, in the southern part of the country, the climate supports dense vegetation, which supports a high tsetse

population and makes ruminant production difficult. In the dry season, grasses dry up and there is dehydration with a high degree of loss of water, low energy and nutritive value. The savannah region is worse hit and animals are moved down to the south in search of food and water. The long trek, coupled with the consumption of low quality forage and exposure to tsetse fly greatly reduces productivity of these animals (Okagbare and Akpodiete, 1999).

2.3.5. Social and economic factors

The total welfare of the traditional herdsmen has not been well catered for. Incentive of loans, subsidies, insurance, medication and security guarantees for them and their animals are negligible. In the past, grazing reserves were established by government to settle the Fulani herdmen because the traditional extensive system of managing their animals is inadequate, but this was not very successful because they lacked the basic amenities such as markets for disposal of milk and beef, health centers and refrigerating facilities for the storage of milk and meat (Mohammed, 1989).

2.3.6 Policy changes

The livestock sector in Africa, especially in Nigeria has been subjected to a wide variety of government policies which change with changes in government. These inconsistencies do not allow for the development of efficient livestock industry (Alawa *et al*., 2001).

2.3.7. Helminthiasis

The parasitic helminths of grazing animals belong to the Phyla Platyhelminthes and Nemathelminthes (Soulsby, 1982). Platyhelminthes, which are otherwise referred to as flatworms, have two classes of parasites, cestodes (tapeworms) and trematodes

(flukes). These parasites are flattened dorsoventrally and are hermaphroditic. The most common and abundant cestode parasite in grazing small ruminants is *Moniezia spp* (Soulsby, 1982). Nemathelminthes is the phylum to which Class Nematoda (roundworms) belongs. Some of the superfamilies of veterinary importance in the phylum include; ancylostomatoidea, ascaridoidea, oxyuroidea, rhabditoidea, strongyloidea and trichostrongyloidea (Anderson, 1992).

Helminths are mostly endoparasites though some live externally. Helminths occur in all geographic regions of the world and have varying definitive hosts as well as predilection sites in the host. Some helminths require vectors/intermediate hosts to complete their life cycle. Helminthoses refers to a complex of condition caused by parasite of classes' nematoda, trematoda and cestoda (Githiori, 2004).

The deleterious effect of helminths on an animal or group of animals varies. These harmful effects depend on the type of helminths, location within the host, degree of parasitism, nutritional status of the host and other factors (Alawa *et al.*, 2002). The absence of obvious clinical signs that is associated with low levels of gastrointestinal parasitism makes early diagnosis and intervention difficult. By the time clinical signs become obvious, parasitization is advanced with consequent production losses. Direct and indirect losses due to nematode infections are estimated to be high (Preston and Allonby, 1979), and control of these parasites is therefore considered important. In most areas in the tropics, animals continuously graze on pasture all year round. This exposes the animals to continuous parasite pressure when climatic conditions are favourable for the development and survival of free living stages (Dinnik and Dinnik, 1958).

Helmintic disease though may not be associated with heavier mortalities in large numbers (except in young animal), cause serious economic losses through reduced productivity and cost of preventive or curative measures (Alawa *et al.*, 2001).

In addition to continuing economic impart of subclinical parasitism in conventional livestock systems; helminths continue to be a problem for those producers wishing to raise livestock without the use of chemical inputs such as anthelmintics. These organic producers are often forced to employ reduced stocking and/or suboptimal pasture utilization to minimize impart of parasites (Gasbarre *et al*., 2001).

2.4. Impart of helminthoses in livestock production

2.4.1. Feed intake and utilization

Heavy gastrointestinal parasite load is known to reduce feed intake and utilization by animals, which can in turn influence the pathogenesis of the parasitic infection. It is generally accepted that malnourished animals are poor at withstanding parasitism (Gibson, 1965). Parkins and Holmes (1989) reported a reduction in feed intake of up to 20 % in moderate to severe gastrointestinal parasite infection. This reduction in feed intake is thought to be due to decreased gastrointestinal tract motility and ingesta flow or inherent abdominal pain as evidenced by depression and teeth grinding in heavily parasitized animals. Some gastrointestinal parasites utilize digested nutrients meant for host animal and in so doing, reduce the amount of nutrients available to the host animal (Sykes and Coop, 2001).

2.4. 2. Injuries to organs

Parasites also inflict pathophysiological damage on host's organs or cell. It is known that the fourth stage larvae of *Trichostrongylus* produce injury to the gastric glands of

the abomasum, while adult *Teladorsagia* also invade the abomasum creating lession commonly referred to as “Morocco leather”. This damage can be quite extensive in heavily parasitized animals leading to mucosal ulceration and sloughing (Bowman *et al*., 2003; Parkins and Holmes, 1989). Little *et al* (1990) showed that PCV levels in heavily parasitized animals declined more rapidly than those less heavily parasitized and on the same plane of nutrition. Between one-fifth and one- tenth of circulating erythrocytes can be removed daily from host circulation in heavy parasitization by *Haemonchus contortus* (Georgi, 1964; Georgi and Whitlock, 1965). Thus gastrointestinal parasites can produce significant pathology in livestock, especially in the young. The ability of animals to perform work is affected by heavy gastrointestinal parasitism. Arene and Ja’afar (1998), in their study in Adamawa State, showed that traction animals infected with fascioliasis spent shorter hours at work compared with healthy animals. Mortality rates between 10-30 %, weight losses of 15-80 % and up to 20 % decrease in milk production have been attributed to gastrointestinal parasites (Symons *et al*., 1982). Dipeolu (1996) indicated that 17 % of mortality in sheep around Ibadan area of Nigeria was caused by helminths infections and Ajanusi *et al.* (1988) reported deaths in Yankasa sheep resulting from *Fasciola gigantica* infection

2.5. Factors influencing the establishment of helminthoses caused by nematode in ruminant

The factors that influence gastrointestinal helminths infection and the degree of parasitization are varied. The magnitude of these factors also varies depending on the livestock species, the species and the predilection site of the helminth.

2.5.1. The number of animals

The risk and ease of parasite transmission increase with increased number of animals in pens or confining large number of animals in the same place, thus increasing contact between them and consequently easing parasite transmission. For gastrointestinal parasites, greater number of animals in the same area means that there will be accumulation of large quantities of faeces with eggs and larvae; this increases the possibility of contact with infective larvae in the faeces by individual animals. However, large numbers of animals in the same place is some times beneficial. For example, where insecticidal ear tags are used, the greater the number of animals the better the contact between individual animals and another's ear tag; this in turn increases the insecticidal activity of the ear tags (Alawa *et al*., 2002).

2.5.2. Introduction of new animals

As with diseases generally, introduction of new animals carries with it the risk of introducing new parasites into a herd. This is of particular importance for locals and smallholder livestock farmers who are in the habit of obtaining replacement animals from the open market or neighbors stock (Alawa *et al*., 2002). It is important that all new animals for addition to an existing stock be quarantined and treated appropriately before introduction.

2.5.3. Hygiene

The importance of hygienic practices as a means of reducing disease risk within a herd cannot be overemphasized. Simple procedures such as changing beddings/flooring can help reduce the degree of parasitsism. Cleaning removes faeces and thus disturbs the normal environment of most gastrointestinal parasites by

preventing them from completing their life cycle. Where possible, routine use of disinfectants such as calcium oxide (CaO) which when dissolved in water (at 10%), produces a larvicidal calcium hydroxide which kills the larval stages of helminths in livestock faeces (Alawa *et al*., 2002).

2.5.4. Nutrition

Malnourished and/or undernourished animals have reduced resistance to infection (and infection agents) and are therefore unable to cope with parasitization (Parkins and Holmes, 1989; Little *et al.*, 1990). Rural livestock producers in developing countries have a poor feed resource base, characterized by seasonal feed shortage both in quality and quantity. This feed shortage when superimposed on gastrointestinal parasitism further exacerbates the condition (Akinbamijo *et al*., 1994). Adequate feeding of all classes of livestock is important in order to increase resistance and achieve maximum production. Feed supplementation goes a long way to make up for this in time of feed shortage as experienced during certain seasons in the tropics.

2.5.5. Health status

The state of well being of any animal or group of animals to a great extent determines how it reacts to the presence of an infectious agent. Healthy animals withstand or offer some resistance to infectious agent. Stress, whether physical, nutritional or otherwise decreases the ability of animals to resist parasites and other pathogenic organisms (Alawa *et al*., 2002). It is important to ensure that animals are in relatively good healthy state at all times. Maintaining a healthy stock does not only ensure

absence of diseases but enhances maximum economic returns on production (Alawa *et al*., 2002).

2.5.6. Climate and or seasons

The climate of an area and the season of the year have a bearing on the parasites that predominate at any particular time. In Nigeria, gastrointestinal parasites are known to pose serious problems in livestock production during the wet season (Fabiyi, 1973). Mbab and Chiroma (1998) reported that the highest helminth count in three Nigerian breeds of sheep (Yankasa, Uda and Balami) occurred during late raining season, while Okon and Enyenihi (1977) indicated that prolonged evenly distributed rainfall patterns increases longevity of *Haemonchus contortus* contamination of pasture. A survey of gastrointestinal parasitism in small ruminants across all seasons in South-eastern Nigeria revealed that *H. contortus* accounted for 73 % of all gastrointestinal parasites in the wet season and 49.2 % in the dry season (Alawa *et al*., 2002). Gasbarre *et al*. (2001) reported that the most common time of deworming by farmers in the U.S.A was spring followed by fall and in Nigeria, Nwosu *et al.*, 1996 reported that the rainy season was the ideal time. Adverse environmental conditions (fall and winter in temperate countries and dry season in the tropics) account for hypobiosis that occurs in *H. contortus* (Bowman *et al*., 2003). Temperature and moisture availability to a large extent determines the ability of a parasite to survive outside the host (Alawa *et al*., 2002).

2.5.7. Ecology/pest and wildlife

Most parasites utilize specific intermediate hosts to complete their life/cycles. This intermediate hosts can be pests or other wildlife. It is therefore important that

livestock are not unduly exposed to pests like mosquitoes and wildlife such as lions and wild cats (Macpherson, 1994). Wild animals are found in abundance and diversity in Africa, particularly in areas inhabited by nomadic pastoralists. Such close contact facilitates exchange of parasites. Grazing livestock especially ruminants in the hands of pastoralists experience this problem most. Roepstorff *et al* (2001) in their study determined the distribution and transmission rate of *Ascaris suum* eggs and *Oesophagostomum dentatum* larvae in a pasture/pig house facility, which had been contaminated during the preceeding summer, showed that there was ecological influence on the transmission rates of *Ascaris suum* in pig pastures. Their result demonstrated that yearly rotations may not be sufficient in the control of parasites with long-lived eggs such as *Ascaris suum* and that pasture rotation must include all areas, including housing.

2.5.8. Management system

Animals are raised by various methods by different categories of people and for different purposes depending on the species of livestock. The system of management that is adopted can influence the rate and severity of parasitic infection. Animals that are housed or kept in feedlots systems where pasture growth is suppressed (due to animal concentration in confined area) and fed from feeders and waterers that are kept above the floor are at lower risk of gastrointestinal parasitism. In this kind of system the absence of vegetation contributes to making the environment not conducive for parasite multiplication. However, this type of system can increase the risk of spreading ectoparasites. The level of pasture contamination with parasites depends on factors such as concentration of animals, duration of time spent by the animals on the

pasture, climate/weather condition and frequency of anthelmintic treatment. It is therefore important that management practices be such that decreases parasitic infection (Alawa *et al.*, 2002). Alternate or mixed grazing of different livestock species is a well recognized principle of non-medical control of parasitic helminthes (Thamsborg *et al.*, 1999; Macpherson, 1994; Roepstorff *et al.*, 2001). Steenhard *et al*. (2001) in their study reported extremely poor rate of development and survival of *Ostertagia ostertagi* and *Cooperia oncophora* from cattle when passaged in pigs.

2.5.9. Gastrointestinal parasitism

Gastrointestinal parasitism, a common condition encountered in livestock production in all regions of the world, remains an impediment to the efficient raising of livestock on pasture (Gasbarre *et al.*, 2001). The effect of gastrointestinal parasitism on livestock varies with the animal species, system of management, environment and season among others.

In Nigeria, several reports (Ugochukwu and Nwaneri, 1984; Voh *et al.*, 1993; Alawa *et al.*, 2001) indicate a high prevalence for gastrointestinal parasites in small ruminant especially during the wet season. Tekdek and Ogunsusi (1987) reported a high prevalence (74 %) of gastrointestinal parasitism in calves around Zaria. A five-year study by Onwuliri *et al.* (1993) indicated that over 68.9 % of all samples from abattoirs in the survey were positive for various parasitic nematodes. Fakae (1990) reported a high incidence of *H. contortus* infection in West African Dwarf sheep and goats in the Nigerian derived Savannah with no definite seasonal distinction. Nwosu *et al.* (1996) also reported that 90.3 % of the Red Sokoto goats that were moved from Sokoto area to Ibadan for slaughter over a six-month period had *H. contortus*

infection. Other reports of high prevalence rates of gastrointestinal parasites in Nigeria include that of Ogunrinade (1985) and Nwosu and Srivastava (1993). Ndamkoung (1983) at the institute of Animal and Veterinary Research Makon Station Cameroon identified *H. contortus, T. axei*, and *T. columbriformis* as the main parasites responsible for death and poor live weight gains in sheep and goats in Cameroon. In Kenya, studies conducted by Lutu (1983) and Githiorri (2004) showed that eggs per gram of faeces (epg) could be as high as 50,000 during the rainy season and necropsy findings revealed that *Haemonchus* species was higher in number than Nematodirus, Trichuris, Oesophagustomum and Strongyloides. Haemonchus has also been identified as one of the ten constraints to sheep and goats production in East Africa (Perry *et al.*, 2002). Fabiyi (1987) identified *Haemonchus* infection as a major problem in livestock production in all regions of the world.

2.6. Diagnosis of helminth infection

Depending on the helminth type, samples usually collected for analysis may include faeces, blood, sputum, urine, lachrymal fluid, skin snips and muscle biopsy. Several procedures can be adopted for laboratory analysis, but the common ones are those carried out on faecal samples such as direct smears, flotation, sedimentation and baermannization (Soulsby, 1982). Recent techniques developed for detection, differentiation and diagnoses of parasites that have been used for helminths includes Polymerase Chain Reaction (PCR) technique and other state of the art biochemical, immunological and molecular assays (Zarlenga and Higgins, 2001).

2.7. Control strategies against helminths infection

Control of gastrointestinal parasite is not only necessary for maintaining healthy animals but also for achieving maximum economic returns on livestock production.

Effective control measures require a comprehensive knowledge of the epidemiology and ecological factors that govern pasture larval populations and the role of host resistance to infection (The Merck Veterinary Manual, 1998). The control measure applied depends on the species, resources available and the purpose for which the livestock are kept. Generally control of gastrointestinal parasites can be achieved through managerial, chemotherapeutic and biological methods.

2.7.1. Use of synthetic anthelmintic drugs:

The use of anthelmintics at the same time is limited by their high cost, uncertain availability and poor quality (Monterio *et al.*, 1998). Anthelmintics can be classified as follows:

2.7.1.1. The broad spectrum anthelmintics:

The broad-spectrum anthelmintics, which remove parasites in different stages of development within the host species, are the cornerstone of parasite control in GI nematode infection. The major classes of synthetic anthelmintics used for GI nematode parasites of ruminant livestock are:

I. The benzimidazole/probenzimidazoles group. The mode of action of this group is by interference with polymerization of microtubules (Harder, 2002). These drugs bind to the protein tubulin of the parasite, thereby causing death by starvation (Roos, 1997).

II. The tetrahydropyrimidines group (levamisole/pyrantel-morantel). These Drugs affect acetylcholine neuro-transmission by interacting with nicotinic acetylcholine receptors (Roos, 1997: Harder, 2002).

III. The macrocyclic lactones or ivermectin/milbemycin group. These groups are thought to interact with chloride channels on the gamma-aminobutyric acid (GABA) receptor complexes of helminths, and also inhibit pharyngeal pumping (and hence feeding), motility and fecundity in susceptible nematodes, resulting in paralysis and ultimately, elimination from the host (Harder, 2002; Yates *et al*., 2003).

2.7.1.2. The narrow spectrum anthelmintics:

These are group of compounds which have activity against fewer species of parasites and/or lack high levels of efficacy against some or all stages of the parasites (Bowman *et al*., 2003). Examples of these anthelmintics include napthalopos, salicylanilide and substituted phenols (closantel, oxyclozanide and nitroxynil), and tricalbendazole.

2.7.2. Factors influencing the efficacy of anthelmintics

2.7.2.1. Route of administration

Most anthelmintics are given orally or parenterally (subcutaneously or intramuscularly). When full dose of an anthelmintic is given correctly ether orally or parenterally, the efficacy is similar (Niezen *et al*., 1983). However, giving anthelmintics in feed or water may result in variable efficacy, particularly when the drug is unpalatable or the water is not completely consumed (Alawa *et al*., 2002). Efficacy with the oral route when animals are fed in groups can never be as good as individual treatment because of variation in intake. Nielsen *et al*. (1983) showed that

great variation occurred in the plasma concentration of levamisole given to cattle in drinking water when compared to intramuscular administration.

2.7.2.2. Particle size

The rate of anthelmintic solubilization is faster when the surface area to gut fluids is increased (Alawa *et al*., 2002). Arundel (1985) stated that micronisation gave greater surface area, led to higher concentration in the gastrointestinal fluids, more rapid absorption, and higher blood and tissue fluid levels and took longer period before excretion is completed. Achieving very high concentration in the gastrointestinal fluid and blood coupled with persistent dose lead to higher efficacy with possible increase in spectrum of activity. However, this may also lead to toxicity.

2.7.2.3. Plasma protein binding

Anthelmintics that strongly bind to plasma protein are known to be efficient against blood sucking parasites such as *Haemonchus contortus*, *Fasciola hepatica* and hookworm. This is demonstrated by the fact that disophenol is more efficient against adult *Ancylostoma caninum* than the immature stages which are not affected until they commence blood ingestion (Arundel, 1985). It is known that the adult stage of the liver fluke (*Fasciola hepatica*) is particularly affected by compounds like bromosalans, oxyclozanide, closantel and rafoxanide in the blood as these compounds and/or their molecules are in the bile and binds to plasma protein. The longer the period the drug binds to the plasma protein, the longer the period of activity of the anthelmintic. Arundel (1985) observed that disophenol removed *H, contortus* for up to eight weeks post treatment. Prolonged plasma binding period entails longer extended drug withdrawal periods especially for food animals (Arundel, 1985).

2.7.2.4. Anthelmintic resistance

Parasite resistance to anthelmintics is increasingly becoming a serious problem in livestock production in all regions of the world. Resistance of parasites to medications increases costs of production, reduces the efficiency of production, depletes the stock of effective control tools and increases the risk of environmental contamination (Donald, 1994). Resistance is said to be present when there is a greater frequency of individuals within a population that is able to tolerate doses of a compound than in normal population of the same species, and is heritable (Prichard *et al.*, 1980).

Intensive use of anthelmintics selects for those nematodes that can survive the treatment (Harder, 2002). These individual nematodes that become genetically and physiologically resistant will reproduce and their progeny will over time under the same anthelmintic regimen progressively select for anthelmintic resistance in their worm populations. This process usually continues with each successive treatment until it reaches a high level when the anthelmintic becomes ineffective in suppressing worm burdens (Prichard, 1994).

According to Geerts and Dorny (1995), factors responsible for anthelmintic resistance include mass treatment, frequent use of same class of drug over prolong periods and underdosing. The relative high cost of anthelmintic in the developing countries has resulted in the tendency of farmers to under-dose animals in an attempt to spread drugs over many animals (Fielding, 2000). The availability of fake and/or adulterated anthelmintics in the drug market at the local level in many of the less developed countries is another contributing factor (Monteiro *et al.*, 1998). Arundel (1985) suggested that the most common cause of failure of parasitized livestock to thrive

following treatment is the rapid re-infection that occurs when stock is put in heavily infected paddock soon after treatment. The emergence of hypobiotic larvae in some species such as *H. contortus* after treatment may also lead to re-infection (Alawa *et al.*, 2002).

Sometimes incorrect diagnosis of nutritional or other disease conditions may be confused with anthelmintic resistance. The use of ineffective anthelmintic against certain species of parasites may also contribute towards resistance

The earliest reports of anthelmintic resistance were made shortly after the introduction of thialbendazole in the 1960's (Conway, 1964; Drudge *et al.*, 1964). Since then, there have been several other reports of anthelmintic resistance in cattle, sheep, goats and equine from several countries.

Beveridge *et al*. (1990) found high level of benzimidazole and levamisole resistance in *H. contortus*, *Nematodirus* spp, *Ostertagia ostertagi, Trichostrogylus axei, T. columbriformis* in Southern Australia. Borgsteede *et al* (1991) reported increasing incidence of anthelmintic resistance in sheep in the Netherlands. Uhlinger *et al*. (1992) also reported that there was benzimidazole resistance in *H. contortus* in sheep in North Carolina. In the U.S.A, Ivermectin resistance has been reported in *H. contortus* (Egerton *et al.*, 1988) and *T. columbriformis* in sheep (Giordano *et al*., 1988) in experimental infections. Under field conditions, ivermectin resistance was noticed in *H. contortus* in sheep and goats in South Africa (CarMichael *et al.*, 1987; van Wyk *et al.,* 1987; van Wyk and Malan, 1988), in Brazil (Echevarria and Trindale, 1989; Echevarria *et al*., 1991) and in the U.S.A (Craig and miller, 1990; DeVaney *et al.,* 1992). In Nigeria, Obemeasor *et al.* (1997) reported anthelmintic resistance

resulting from incorrect dosage and improper regimen of anthelmintic administration by farmers. Conventionally, anthelmintic resistance is diagnosed by comparism of egg count and post mortem worm count of treated and untreated animals, *in vitro* egg hatch test and *in vivo* motility assays (Prichard, 1994).

Once anthelmintic resistance is confirmed in any stock, it is usually better to change the drug to a different class of compound with a different mode of action.

2.7.3. Strategic deworming

The aim of strategic deworming is to reduce worm burdens at critical periods. Knowledge of the seasonal changes in infection and the regional epidemiologies of various helminthoses are vital to the timing of administration of anthelmintics in strategic deworming (The Merck Veterinary manual, 1998). In Nigeria, early, mid and the end of the raining season are specific times that require prophylactic treatment to suppress the normal increase of gastrointestinal parasites (Alawa *et al*., 2002). Strategically timed treatments have proven successful in preventing serious parasitic diseases (Taylor *et al*., 1985). Taylor *et al*. (1995) showed that cattle subjected to strategic deworming over a two year period were protected from gastroenteritis and had significant better weight gains than untreated groups.

2.7.4. Grazing management:

Pasture management is designed to prevent infection of ruminant with internal parasites, and requires long-term planning. Grazing management such as rapid rotational grazing systems applied in the humid tropics (Banks *et al.*, 1990), tethering and zero grazing, which separate the host from infected larvae on the pasture, are advocated as means of control (Semenye *et al.*, 1992).

2.7.5. Targeted drenching

A general feature of parasitic infections in animal flocks/herds is that the distribution of the parasites is skewed (Roberts and Swan, 1982; Wilson and Grenfell, 1997). That is, a few individuals in a flock/herd have high parasite burdens; while majority have few or no parasites (Shaw and Dobson, 1995). Targeted drenching is based on selected treatment of those individual animals that are diagnosed as heavily infected, and with clinical symptoms of the disease. The system utilizes clinical manifestation of anaemia in infected animals (Malan and van Wyk, 1992). Thus, instead of treating the whole flock, only those animals with infections inducing anaemia are treated with an effective anthelmintic (van Wyk *et al.*, 1998; van Wyk and Bath, 2002; Vatta *et al.*, 2002). This system is only suitable in situations where anaemia is attributable to infection by *H. Contortus*.

2.7.6. Biological control:

Biological control of parasitic nematodes in livestock aims at establishing a situation where grazing animals are exposed to low levels of infective larvae, such that it will secure the development of naturally acquired immunity in the same animal (Thamsborg *et al.,* 1999).

Current research has been focused almost exclusively on *Duddingtonia flagrans* in sheep, goats, cattle horses and pigs in a range of geoclimatic conditions worldwide (Chandrawathani *et al.*, 2003; Dimander *et al.*, 2003). This is because the fungus is able to survive the gut passage of ruminant as a resistant stage (chlamydospores) when administered per os with grains. Also, its ability to grow rapidly in fresh animal dung and its voracious nematophagous ability has made the fungus the new focus of

research in nematode control (Larsen, 1999; 2002). Its only limitations are that, the nematode-trapping fungi are only effective against larvae in faecal pats but not against those that have migrated to vegetations or against those in the host animals (Githiagi *et al.*, 1997).

2.7.7. Copper oxide wire particle (COWP):

In studies conducted in sheep in New Zealand (Bang *et al.*, 1990) and Australia (Knox, 2002), and in goats in France (Chartier *et al.*, 2000), COWP administered as a capsule was shown to be effective in reducing the establishment and fecundity of *H. contortus*. The mechanism of action is assumed to be based on the lethal effects of ionic copper on the parasite, the ionic cooper being librated from the COWP by the acid secretion of mucosa of the abomasal. However, the concentration necessary for an anthelmintic effect and the potentials for toxicity in copper sufficient animals, or those exposed to copper-accumulating plants, are yet to be established.

2.7.8. Utilization of immune system:

Immunity, or host resistance, as it applies to nematode parasites is the ability of an animal to prevent infection with parasites, and/or reject established parasites in the animals, by utilizing both innate and acquired immune responses (McClure, 2000). Innate immunity is inherent, whereas acquired immunity gradually develops in the animals after exposure to parasites. Most adult ruminants' exhibit naturally acquired protective immunity to gastrointestinal nematodes (McClure, 2000). However unlike in bacterial infections, where vaccination and natural boosting often produces high levels of acquired immunity, young sheep infected by GI nematodes are unable to respond as early or as profoundly (McClure, 2000). In addition, this protection is only

partial (incomplete) as parasites are also present after immunity has developed. The speed with which immunity develops is influenced by factors such as the dose of larvae ingested and the species of the parasites (Dobson *et al.*, 1990). The protective effect is finally expressed by the ability of an animal to reject incoming larvae, to depress worm fecundity and/or to expel adult parasites (McClure, 2000). Immunity is also subject to physiological and external factors such as age, pregnancy and lactation, health, sex, genotype, vaccine, nutrition and stress of the animal. The use of vaccines, nutrition and breeding for resistance are all influenced by immunity, and their use as a control strategy against nematodes is discussed below.

2.7.9. Vaccines:

Current mathematical models on worm control have shown that a vaccine yielding 60% protection in 80% of herd, or flock (i.e 54 % of the herd), would be a highly valuable control tool (Barnes *et al*., 1995). Following the success of the irradiated larval vaccine against bovine lungworm, *Dictyocaulus viviparous*, a similar approach using irradiated *H. contortus* L_3 was found to consistently offer good protection in sheep older than six months (Gray, 1997). However, hope for commercial production of an irradiated *H. contortus* vaccine disappeared when it became clear that high levels of protection could only develop if sheep were worm-free prior to vaccination (Gray, 1997; Bain, 1999). Currently research on helminths vaccines has generally concentrated on the production of synthetic or recombinant vaccines using either natural or hidden antigen (Schalling *et al.*, 1997; Smith *et al.*, 2003). A number of reviews have been published on possible recombinant vaccines developed against nematode parasites of ruminants (Smith, 1999; Knox and Smith, 2001; Claerebout *et*

al., 2003; Dalton *et al.*, 2003; Meeusen and Piedrafita, 2003; Newton and Meeusen, 2003). Irrespective of the progress that has been made, and the promise for the future, it will take a long time before these vaccines have a place in control of *H, contortus* in the flocks of pastoralists and smallholder livestock farmers.

2.7.10. Resistant and resilient breeds:

Resistance has been described as the ability of the host to prevent or limit the establishment or development of infection. Resilience is the ability of an animal to maintain reasonable levels of production when subjected to parasitic challenge (van Houtert and Sykes, 1996). Considerable research over decades have been undertaken in many sheep rearing countries to identify breeds that have natural resistance to gastrointestinal nematode parasites infection; such breed are the merino sheep of Australia (Woolaston and Baker, 1996; Raadsma *et al*., 1998).

2.7.11. Strategic nutritional supplementation:

GI nematodes impair animal productivity through reduction in voluntary food intake and/or reduction in the efficiency of food use, especially inefficient use of absorbed nutrients (Coop and Kyriazakis, 2001).

Disturbances in protein metabolism and reduced absorption and/or retention of minerals are significant during parasite infection (Coop and Kyriazakis, 2001). The magnitude of these effects is influenced by the size of the larval challenge and the increased loss of endogenous proteins into the GI tract (Coop and Kyriazakis, 2001). Considerable research has shown that nutritional supplementation, especially improved dietary protein supply, reduced production loses and mortality from GI

nematodes parasites (van Houtert & Sykes, 1996; Coop and Kyriazakis, 2001; Sykes and Coop, 2001).

2.8. Plants with antiparasitic properties

Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects (Waghorn & McNabb, 2003). For example, plants that contain condensed tannins, a class of phenolic secondary metabolites, have these effects. Tannins are mainly subdivided into two groups; Hydrolysable tannins: these are polymers esterified to a core molecule commonly glucose or polyphenol such as catechin. Hydrolysable tannins are potentially toxic to ruminants (Reed, 1995).

Proanthocyanidins (condensed tannins); these are relatively stable in the digestive tract of the animal, and rarely have toxic effects (Reed, 1995). Condensed tannins (CTs) are powerful anti-metabolites, and in amounts exceeding 5% of rument contents (which are commonly attained with tropical forages) can impair microbial functioning of ruminants (Singh & Bhat, 2003). At high concentrations, the CTs act as anti-feedants because they bind in tight chemical complexes with proteins, as well as microbial enzymes, thereby reducing fermentation and degradation of fibrous tissues in the rumen (Reed, 1995; Singh and Bhat, 2003). Additionally, they may also bind to digestive enzymes, thus reducing their activity, and also have astringent taste (D'Mello, 1992; Reed, 1995; Min and Hart, 2003). The nutritive values of plants containing CTs become reduced when the latter bind to proteins. Consequently, research in the tropics is now being directed towards overcoming these undesirable qualities of tanniferous plant in grazing animals (Butler, 1992; Norton, 2000).

2.8.1. Plant constituents with known anthelmintic activity.

Successes of use of herbal remedies in China, India and Russia have been recorded. For instance; a compound derived from a herb in China is now a drug of choice against cerebral malaria and benzolthiocyanate derived from the seed of C*arica papaya* were intensively used for the treatment of ascariasis in children, dog and bird and *oxyuriasis* in mice (Sofowora, 1982). Benzolthiocyanate was also found to be active against *Hymenolopis nana*, both *in-vitro* and *in-vivo* (Ampofo, 1979). Tannins are secondary plant metabolites, which have been closely associated with plant defence mechanisms towards insects (Schultz, 1989) and mammalian herbivores (Hagerman and Butler, 1991). Tannins are usually divided according to their chemical structure and properties into two groups; hydrolysable and condensed tannins. The later is the most widespread group of tannins in nature and has been considered responsible for causing a number of detrimental effects towards monogastric (Vernon, 1999) and ruminant herbivores (Aerts *et al.*, 1999). However, ruminants can benefit from the presence of condensed tannins in their diets; the consumption of average concentration of condensed tannins can result in increase weight gain, wool growth, milk secretion (Barry and McNabb, 1999) and decrease the detrimental effects of gastrointestinal parasitism (Aerts *et al.*, 1999). Recent studies in ruminants suggest that parasitized sheep and red deer grazing on forages high in condensed tannins had lower faecal egg counts and worm burdens compared to those grazing on forages low in condensed tannins (Hoskin *et al.*, 1999; Niezen *et al.*, 1995). Recently, a direct anthelmintic effect of a plant extract high in condensed tannins (Quebracho) towards *T. colubriformis* population has been demonstrated (Athanasiadou *et al.*, 2000). The

in-vitro anthelmintic effects of embelin an extract from *Embelin schimperi*, was evaluated in mice/ rats infected with the cestodes *Hymenolepis microstoma* and *H. diminuta* and in mice infected with the trematodes *Echinostoma caproni,* and the nematode *Heligmosomoides polygyrus*. The extract had activity only against the cestodes (Bogh *et al*., 1996). Atanine, a quinolone alkaloid extracted from dried fruits of *Evodia rutaecarp*, inhibited *in-vitro,* the motility of free-living stages of the trematode *Schistosoma mansoni*, and also had activity against the nematodes *C. elegans* adult as well as the larval stages of *Teladorsargia circumcinta* (Perrett and Whitfield, 1995). B- sitosterol (from leaves of *Mentha cordifolia*), has been tested in an *in vitro* assay against the adult of the porcine roundworm *Ascaris suum,* and found to have similar activity to mebendazole a synthetic anthelmintic (Villasenor *et al.*, 2002). Mangiferin is a major polyphenol in the aqueous extract (vimang) acquired from *Mangifera indica. In-vitro* test showed that the polyphenol and the aqueous extract were effective against L_2 and L_3 stages of *Trichinella spiralis* in a mouse model. Moderate effects were reported against the L_4 in the muscles but not against the adult parasites (Garcia *et al.*, 2003). Flavan-3-ols (the monomer units of condensed tannin), and their galloyl derivatives were evaluated *in vitro* on the viability of eggs, the development and the viability of the free-living stages of *Trichostrongylus colubriformi;* and was found to inhibit egg hatch in addition to impairing the development of larvae (Molan *et al.*, 2003).

2.9. Ethnoveterinary

Ethnoveteriary medicine according to McCorkle (1986) and Warren (1991), refers to people's beliefs, knowledge, skills and practices relating to care of their animals,

whereas Ndi (1990) described pastoralist knowledge of ethnoveterinary medicine as encompassing surgery, pharmacology and toxicology. The traditional systems of veterinary services are useful where modern techniques are either nonexistent, or are available but too expensive and beyong reach (Wanyama, 1997a, b; Dano and Bogh, 1999).

Ethnoveterinary knowledge is a preserve of a few people and the knowledge is more or less a guarded secret, this is passed down the lineage (Wanyama, 2000). From the beginning of animal husbandry practice, animal health and management have relied on empirically derived practices. The origin of modern veterinary medicine however, has been traced to prehistoric traditional medicine as practiced in China, India and the Middle East. King Hammurabi of Babylon made laws concerning fees chargeable for cattle and donkey treatment as far back as 1800 BC (Schillhorn van Veen, 1997). It was believed that animal hospitals were created during the reign of King Ashoka (269-232 BC) in Buddist India (Smith, 1924, quoted by Lordrick, 1981). This was followed by the development of Centers of veterinary and other medical practice knowledge in China, Egypt and later Arabia (Alawa *et al*., 2002). Schwabe (1978), in comparing the beliefs and practices of past and present herdsmen in the Nile valley suggested that there is a long tradition behind contemporary ethnoveterinary practice. In history, medical knowledge was for a long time subordinate to religious certainties as empirical art rather than a science until the 20th century after the second World War when chemotherapeutic control of disease became predominant and disease could be explained and treated based on pathophysiology and immunology. This was dominated by western world and rarely reached other parts of the world with the

exception of mass vaccination and the use of insecticides (Schillhorn van Veen, 1997). Livestock owners in poorer developing countries did not receive these western developments in animal disease treatment and control, and continued to rely on their age-long methods. They often saw western animal health care practices as expensive and not embedded in local customs and beliefs (Alawa *et al.*, 2002).

Recent interest in traditional veterinary practices is not unconnected with renewed western interest in traditional human practice as; disciplines involving herbal and holistic medicines previously ignored have become increasingly acceptable worldwide (Alawa *et al.*, 2002). In 1996, the American Veterinary Medical Association at her annual convention recognized veterinary acupuncture and acultherapy, veterinary chiropractic, naturaceutical and holistic veterinary medicine as modalities to be offered in the context of a valid client/patient relationship (Schillhorn van Veen, 1997).

In the past, traditional veterinary practices though used in many parts of the world were rarely recorded in mainstream literature except in anthropology (Lodrick, 1981; McCorkle, 1986). Presently there are several numbers of books written on ethno-veterinary Medicine (EVM) (Mathias-Mundy and McCorkle, 1989; Anonymous, 1996; Bizimana, 1994; McCorkle *et al.*, 1996; Kohler-Rollefson *et al.*, 2001; Martin *et al.*, 2001) and a few databases and web sites on the subject exist (Ethnovetweb, 2003). However, in most of these sources, there is only a brief description of the plants used, and the purported conditions that they treat, and often no validity of the effect against these conditions are provided. In Kenya, a manual was recently published that identified a number of plants used against all major groups of

helminths (i.e. cestodes, trematodes and nematodes) (Anonymous, 1996). In this book, an attempt was made to identify the method of preparation of the plants listed. Similarly, Kokwaro (1993) listed 21 plants used against hookworms (*Ancylostoma spp.*) in humans in East Africa, six against roundworms, (without specifying whether nematodes or ascarids), 22 against tapeworms (cestodes), and one against thread worm (S*trongyloides spp) as well as* a list of 79 plants used as general anthelmintics in humans in East Africa. Elsewhere in Africa, Ibrahim *et al.* (1984) screened 18 plants species used in West Africa for their anthelmintic activity, while Kasonia *et al.* (1991) reported 11 plants used for the same purpose in Zaire.

From Nigeria, 92 plant species were identified to be used in traditional veterinary practice, with 15 reported to be used against general worm infestation and three against fascioliasis in cattle (Nwude and Ibrahim, 1980). A study in the Madikwe area of South Africa identified eight plants that were used as dewormers of cattle, although cattle owners in that area had poor understanding of helminths infestations of cattle (van der Merwe, 2000). In the Sanaag area in Somalia, six plants were reported to be used for treatment of helminths in livestock (Catley and Mohammed, 1996). Tagboto and Townson (2001) in their review listed 39 plants used against cestodes, 16 against trematodes and 45 against nematodes in humans worldwide. There are also recent reports in Africa of plants that had anthelmintic activity when fed to livestock due to their condense tannins content (Kabasa *et al*., 2000; Kahiya *et al*., 2003). In Tanzania, Minja (1994) classified seven plants as having anthelmintic properties. In Nigeria, 18 plants were identified to have anthelmintic effects, although no target animal host species was identified (Ibrahim *et al.,* 1984). Recently in Northern Nigeria, four

plants were identified as being active against helminths infection of livestock (Alawa *et al*., 2002).

A number of studies and reviews in recent years have enhanced interest in traditional veterinary practices (Schwabe, 1978; McCorkle, 1986; McCorkle and Mathias-Mundy, 1992; Stem, 1996, Bizimana, 1994). These developments have coincided with the emergence of a consensus on the validity and importance of indigenous knowledge and traditional ecological knowledge and the awareness that these knowledge including traditional veterinary practices could be lost (Inglis, 1995). `

Like many of their counterparts in the world, pastoralists and other local/smallholder livestock farmers in Africa relied on locally available plants and herbal preparations for treatment of themselves and their animals before the advent of western (orthodox) medicine. About 80 % of the world's population is known to use herbal treatment for various purposes (FAO, 1986). According to Brandt *et al.* (1995), this practice is still very much in use in developing countries and being an integral part of the people's culture; it is not likely to change significantly in years to come.

Most of the research on testing of EVM preparations has so far been carried out in Asia (Akhtar *et al.*, 2000)

Though the developments of synthetic drugs have decreased the use of medicinal plants in the past, there is presently a worldwide movement towards the use of natural products and an increasing demand for herbal preparations. In recent times, the developed world is increasingly placing more interest in ethnomedical and ethnoveterinary practices especially as it relates to the use of medicinal plants in treating various diseases. This move is in response to the production of animals free

from industrial chemical residues, and the need to discover newer therapeutic substances of natural origin with low toxicity to man and animals and to avoid the rapidly escalating anthelmintic resistance (Gasbarre *et al.*, 2001; Herd, 1996; McKellar, 1997; Athanasiadou *et al.*, 2005; Vierra *et al.*, 1999; Melo *et al.*, 2003; Guarrera, 1999; Jackson and Coop, 2000; Sangster, 1999).

2.9.1. Limitation of Ethno-Veterinary Medicine (EVM)

The use of EVM is limited by the seasonal availability of certain plants, the ineffectiveness of some treatments, the existing harmful practices and the often inadequate ethno-diagnosis (Mathias-Mundy and McCorkle, 1989; Martin *et al.*, 2001). There is normally a lack of pathophysiological understanding of disease, which results in poor diagnosis (Schillhorn van Veen, 1997). The understanding of the cause of disease is poorly developed, and therefore treatment and prevention may be inappropriate. In many cases, disease classification by pastoralist and small holder livestock farmers is based on observed signs and abnormalities in the animal, and treatment is usually offered to alleviate these symptoms (Ohta, 1984; Iles, 1993; Catley and Mohammed, 1996; Hammond *et al.*, 1997; Wanyama, 1997a; Namanda, 1998; Tamboura *et al.*, 2000; Nfi *et al.*, 2001; Alawa *et al.*, 2002; Ole-Miaron, 2003).

Although EVM is widely used in many parts of Africa, it lack acceptance among scientists and veterinarians because of the superstitious belief associated with it, and the belief that it is the domain of “quacks” (Mesfin and Obsa, 1994; Dano and Bogh, 1999). This is mainly because EVM does not follow the paradigms of scientific evidence-based demonstration of efficacy (Githiori, 2004).

2.10. Afzelia africana.

Afzelia africana SM (Plate 2.1) is a wide spread species with a rather opened crown and massive branches most readily recognized by a conspicuously hard blackish fruits, the opened pods persisting for a long time on the tree). It is mostly found in savanna fringing forest and drier parts of forest regions. It is commonly referred to as, kawo, gayohi, gayo, bachi, yasa, arinyan, akpasi, kemkwa-epe, apa and akpalata in Hausa, Fula, Kanuri, Nupe, Tiv, Edo, Etsako, Boki Yoruba and Igbo respectively. The tree grows up to 30 m high, but usually about 12 m in the dried situations, with a girth that is up to 3 m with a widely spreading crown and large irregular branches. The bole rarely exceeds 15 m, with a pale to brown bark that is slightly fissured flaking off in large patches leaving conspiciuosly paler areas; slash pinkish and granular. The leaves has a common stalk that is 10-20 cm long, swollen at the base with 3-8 pairs of leaflet that are 5-15 cm long by 3.5-8.5 cm broad, widely spaced, broadly elliptic, blunt or shortly acuminate at the apex, rounded or broadly cuneate at the base with one margin joining the leaflet-stalk slightly lower than the other, dark green and glossy above, with the midrib and nerves markedly paler, stalk of leaflet stout, wrinkled, only slightly twisted, 6-10 mm. It flowers around February to April and June of each year. The flowers are inconspicuous but strongly scented, green except for the creamy white petal streaked with red at the base; in open panicles 7-20 cm long upright at the base of the leaf terminating the curren's year shoot; individual flowers about 3.5 cm long from the base of the stalk to the apex of the petal, sepals about 10 mm long, petal about 12 mm across, notched at the apex, 7 stamens equaling the petal in length and slightly longer style with a pin-head stigma. It fruit twice every year (April-June and

December-January) on the stouts more or less at right angle to the pod which projects at the base slightly beyond the stalk, up to 18 cm long by 9 cm broad, and 3.5 cm thick, very hard, glabrous with a minutely pitted surface; rounded at both ends, with a short beak at the apex; splitting open explosively and the two valves remaing flat. The seed (8 per pod) is ellipsoid, 18-30 mm long, glossy black, with waxy orange aril forming a cup at the base; arranged transversely side by side in grooves in the middle of the pod. The wood is hard, tough and heavy, coarsely grained with lighter streaks, bandeddark and light brown, eventually turning into a uniform dark brown (Keay, 1989). The seed is widely used for medicinal purposes, for industrial use as in soap, margarine, and candle making and as diets such as condiments and thickening in soup (Ajah and Madubuike, 1997). The leave is mostly utilized as foder by herdsmen during dry season when there is little or no green pasture. The herdsmen claims that their animals usually gain weight, increase milk yield in cattle, initiate and enhance horn growth and improve body conformation when fed with the leaves of the plant (Onyeanusi, 1986). As a result of these claims, the plant is reported to have the highest percentage lopping resulting to its near extinction. Atawodi (2000) identified the use of herbal preparations involving *Afzelia africana* in the treatment of helminthiasis. In an earlier *in vitro* study, the extracts of *Afzelia africana* (bark) was found to be effective in reducing nematodes eggs hatch (up to 90%) in a faecal egg hatch assay. This was found to be comparable to those of albendazole used as control in the experiment (Simon, 1997).

Plate 2.1 *Afzelia africana* tree with fruits on top in its natural habitat

2.11 Combretum molle

Combretum molle R.Br/G.Don (Plate 2.2) (also called *C. leonense, C. sokodense or C velutinum*) is a tree with large, straighter bole than most species of *Combretum*, distinquished by its rough bark and dense crown. It is commonly referred to as wuyan damo (Hausa), damoruhi (Fulani) and aragba (Yoruba); the common name (English) is obscured. The tree often grows high, up to 30 ft or more. The bole is usually straight, with black bark that is deeply fissured into rectangular bosses; slash yellow, exuding gum. Branches are drooping, with slender twigs; young shoots and foliage is usually densely hairy. The leaves are 2.5-7 inches long by 1.5-4 inches; usually broad; mostly opposite; elliptic to ovate, acute, mostly rounded at the base; dark green, often softly and densely hairy; with 10-12 pairs of thick lateral nerves, very prominent beneath, the veins more or less parallel and at right angles to the nerves; stalk 0.25 inches long (Keay, 1989). There was no reported medicinal used of this apart from the purported claims of the nomads and the pastoralist that their stem bark possessed anthelmintic activity in animals when given as concoction (Nwude and Ibrahim, 1980; Atawodi *et al.*, 2000); also an earlier study indicated that the crude methanol extract of the stem bark showed activity in an *in vitro* egg hatch assay with 90% egg hatch inhibition (Simon, 1997)

However other species of *Combretum* (*C glutinosum* and *C padicalatum*) have being reported to have antimicrobial activity against gastroenteritis in humans' especially those caused by *Salmonella* species (Farmsworth *et al.*, 1985). Atawodi (2000) in an assay showed that the *C glutinosum* and *C padicalatum* methanolic extract had better anti-Salmonella activity especially on *S. pollurum* and *S. gallinarum* in chicken.

Plate 2.2 *Combretum molle* tree branch in its natural habitat

2.12. Screening methods for evaluating plant for anthelmintic activity

2.12.1. *In vitro* method

In vitro assay is an evaluation technique for the assessment of the pharmacological activity of either a newly synthesized conventional drug or that of crude extract from a plant intended for use on either animal or man; usually carried outside a biologic system (i.e. in test tubes or other medium) on the target organism, in order to validate their efficacy or otherwise harmful effect before introduction into a biologic system (Le Jambre, 1976). Some *in vitro* evaluations of plant preparations have utilized the nematode parasites *H. contortus*, or in other instances mixed gastrointestinal nematodes have been tested in these assays (Asuzu and Onu, 1993). Non-parasitic nematodes have been used in many *in vitro* assays for determination of anthelmintic efficacy. For example, in several trials, *in vitro* effects of plants extracts on the free-living nematodes C*aenorhabditis elegans* and *Rhabditis pseudoelongata* (Okpekon *et al.*, 2004) were evaluated.

2.12.2. Advantages of *in vitro* techniques;

In-vitro tests for the long-term safety evaluation of drugs offer certain advantages;

1. To replace animal procedures with non-animal techniques wherever possible.

2. Specific properties of drugs can be identified including mutagenic and carcinogenic effects. Also, the mechanisms leading to toxicity can be assessed.

3. Tissue from several species, including man, can be examined.

4. These tests should reduce the number of test animal required for screening new drugs,

5 Because the experiments are carried outside of the animal, they will not suffer any

consequence (Pearson**,** 1986).

2.12.3. In vivo method

As in *In-vitro, in vivo* assay is usually conducted within a living organism. *In vivo* research is more suited to observe an overall effect than in *in vitro* research, which is better suited to deduce mechanisms of action. *In vitro* research aims to describe and understand the effect of an experimental variable on a subset of an organism's components. *In vitro* research has the advantage over *in vivo* research in that there are fewer variables which can confound an experiment, and that if an experimental effect is subtle the result will be more clearly visible (Pearson**,** 1986). Several investigations have assessed *in vivo* plant efficacy against different helminths parasites, in animal and human hosts. In a few studies, the anthelmintic properties of plants in naturally or artificially infected sheep, goats and cattle, have been determined against the nematodes *H. contortus*, as well as against mixed trichostrongyle nematode infections in ruminants. Similarly, *in vivo* efficacy of plant preparations was investigated against cestode-infected hosts such as humans, rodents and ruminants (Desta, 1995; Galal *et al.*, 1991a, b; Ghost *et al.*, 1996; de Amorin *et al.*, 2001; Molgaard *et al.*, 2001 Akhtar and Riffat, 1986).

2.12.4. Advantages of *in vivo* techniques

In vivo research has the following advantages;

1. Whether the aim is to discover drugs or to gain knowledge of biological systems, the nature and properties of a chemical tool cannot be considered independently of the system it is to be tested in.

2. Compounds that bind to isolated recombinant proteins are one thing; chemical tools that can perturb cell function another; and pharmacological agents that can be tolerated by a live organism and perturb its systems are yet another (Lipinski and Hopkins, 2004).

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1. Plants sample collection and identification

The stem-barks of *Combretum molle,* '*R.BR/G.DON*' (Keay, 1989) and that of *Afzelia Africana,* '*SM*' *(*Keay, 1989) were collected in April, 2005 from New Bussa in Niger State and Zaria in Kaduna State Nigeria respectively. The plants were identified at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria and the following voucher numbers were given-V/No 2276 for *Afzelia africana* and V/No 297 for *Combretum molle.*

3.2. Preparation of plants sample

3.2.1. Drying and pounding of plants sample

Five kilogrammes of the samples were air-dried for 72 hours in the laboratory, pulverized into powdered form by pounding it using morta and pistle and sieved as described by Onyeyili *et al.* (2001). The fine powered particles of the plants sample were stored in air-tight plastic containers (Table 3.1).

Table 3.1: Plant collection, location and powder output.

Plant part collected	Time of collection (Month)	Location	Output on pulverizing 5 kg	Colour
A. africana Bark	May	Shika-Zaria	2 kg	Brown
C.molle Bark	May	New-Bussa	2 Kg	Brown

3.2.2. Extraction of plant materials

The absolute methanol (2.5 litres) was poured into a round bottom flash which was then placed on a heating mantle and a soxhlet apparatus (Quick fit corning Ltd. A division of Stafford England) (Plate 3.1) was mounted on the flash. Five hundred grammes (500g) of the powdered plants material was placed into a timble and subsequently pushed into the soxhler extractor. The extractor was fitted to a condenser connected to a water inlet (tap water). When the mantle (at 60 ºC) heats the flash, the methanol vapourarized with the vapour passing through the extractor; the vapour was then condensed by the cooled tap water passing round the condenser and the condensed methanol then drop back into the flash through the timble. As the timble get soaked, the active component together with the methanol drops into the flash. The vapourization circle continiues until the timble becomes clear and the circle discontinued. The extracts were then concentrated by evaporating to dryness in a vacuum using a rotary evaporator. The dried extracts were then stored at 4 ºC in a refrigirator until required. The the flow chart of extraction is shown in Figure3.1

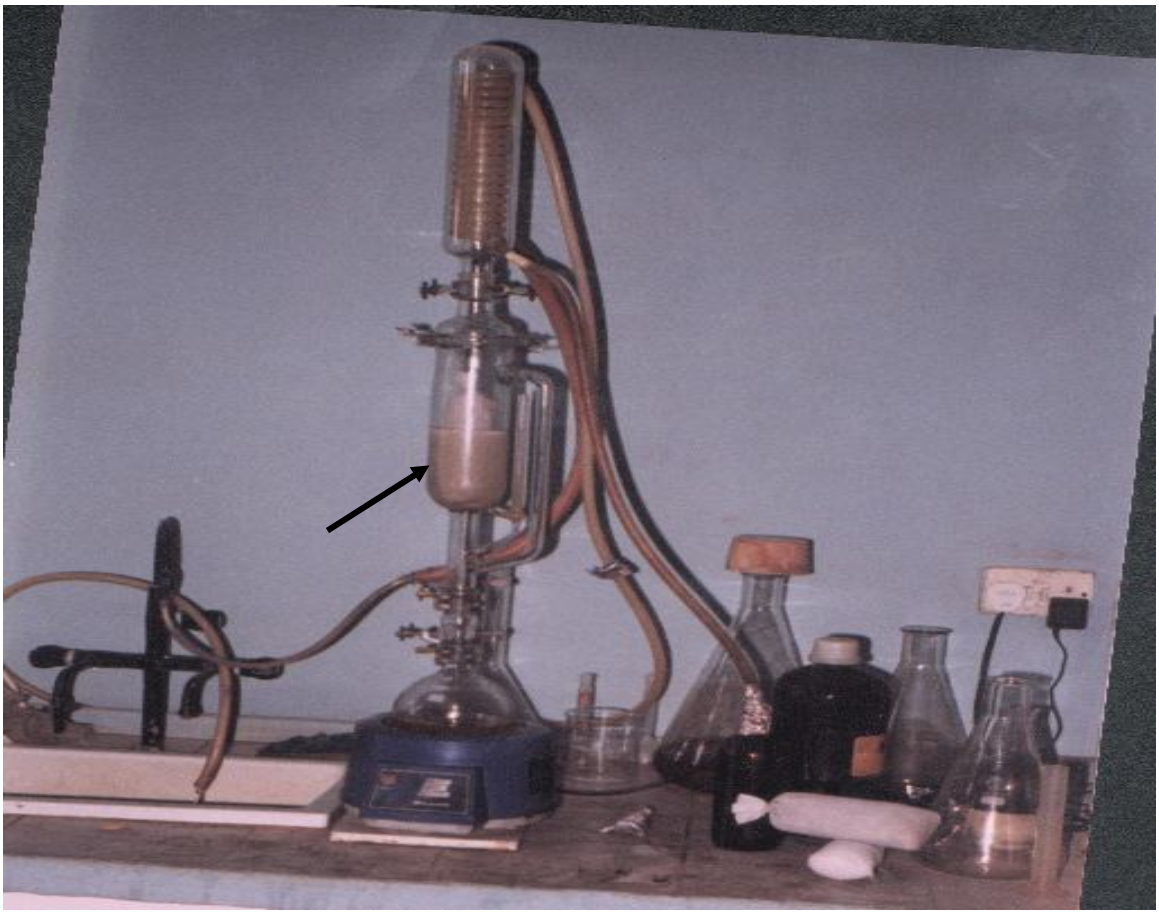
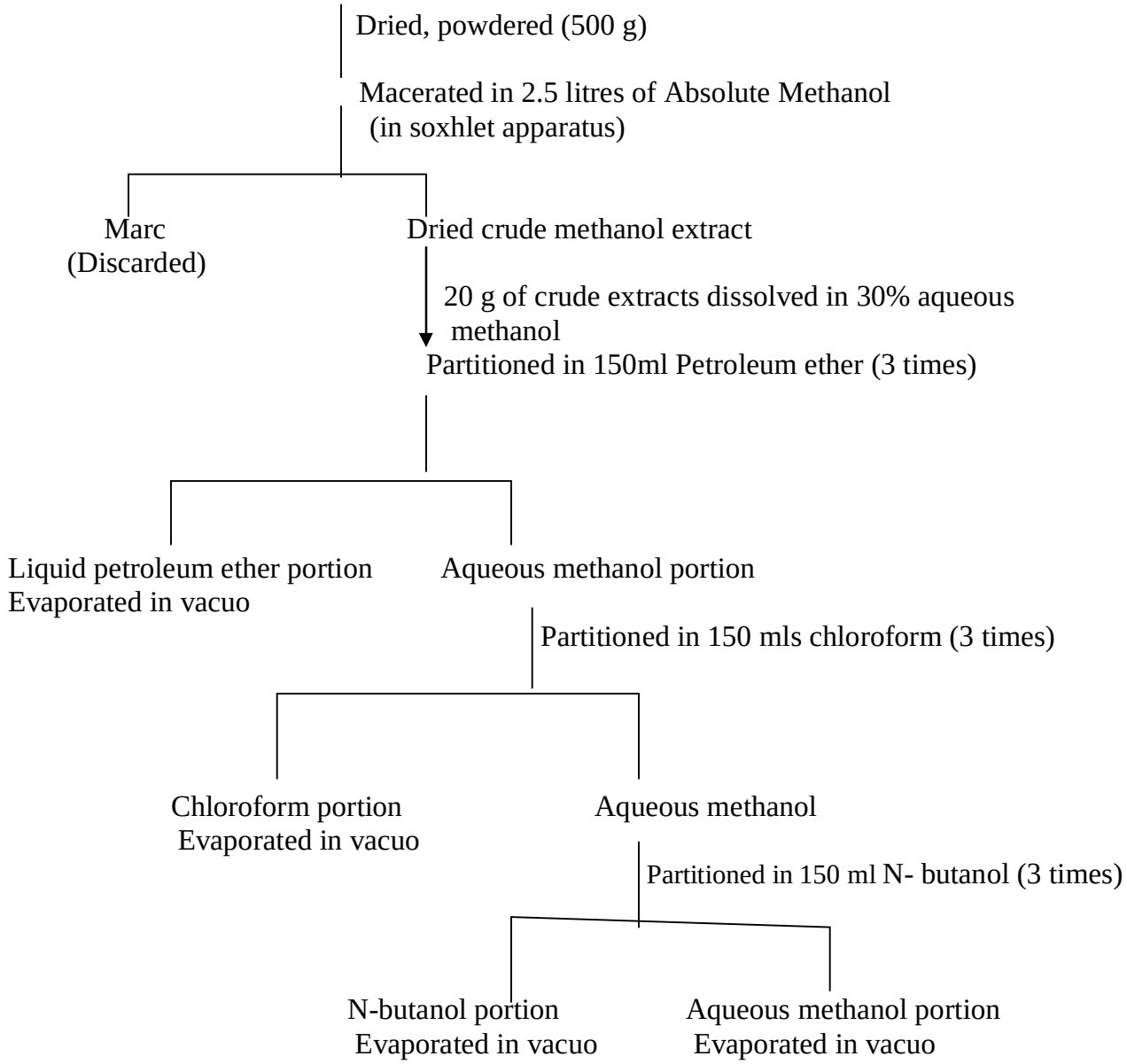


Plate 3.1 Soxhlet apparatus (Quick fit corning Ltd. A division of Stafford England) used for the extraction of plant material

Figure 3.1 Flowchart depicting extraction and solvent partitioning of the bark of *Afzelia africana* and *Combretum molle*



3.2.3. Partitioning of crude methanol extract

The crude methanol extract of the plants (20 g each) were suspended in 200 ml of 30 % v/v aqueous methanol (i.e. 30 ml absolute methanol and 70 ml distil water). This was then partitioned with three portions each of 150 ml petroleum ether using separating funnel (Plate 3.2). The petroleum ether portion was carefully separated into a clean beaker. Subsequently, the aqueous methanol portion was partitioned with three (3) portions (each 150 ml) of chloroform; followed by three portions of N-butanol; each 150 ml (Fig. 3.1). The portions were subsequently referred to as petroleum ether, chloroform and N-butanol portion respectively. The separated fractions of the extract were evaluated by thin layer chromatography (TLC) according to the method of Brain and Turner (1975). After evaporating the solvents, the fractions were tested for anthelmintic activity on rats infected with *Nippostrongylus braziliensis* using the method described by Ibrahim *et al.* (1984) and Suleiman *et al*, (2005) to get the most active fractions.

Plate 3.2. Separating funnel for partitioning of plant extract

3.2.4. Thin layer chromatography (TLC) of extract.

One gramme (1g) of the crude methanolic extract was placed into a test tube containing 5 ml of distilled water. The mixture was shake and allowed to stand for 2 hrs, after which it was filtered into a test tube using cotton wool to quicken the filration process. The resultant filtrate was then used for chromatographic analysis using petroleum ether, acetic acid and distil water (4:1:5) as the solvent and silica gel (0.25mm) as the absorbent. The ascending technique was used and visualized by spraying with ninhydrin (Brain and Turner, 1975).

3.2.5. Phytochemical screening of the crude methanol extracts and the various fractions of *Afzelia africana* and *Combretum molle*

The crude methanolic extract, petroleum ether, chloroform, N-butanol and the aqueous methanol fractions of *A. africana* and *Combretum molle* were subjected to phytochemical tests using standard techniques as described by Cuilei (1981); Trease and Evans (1984); Geissman (1962); Sofowara (1982) Brain and Turner (1975) as state below. The extract was tested for the presence of steroids/triterpenes, saponins, tannins, alkaloids, cardiac glycoside, flavanoids and carbohydrates.

3.2.5.1. Test for Flavanoids

I. Shinoda test

The extract (0.5 g) was dissolved in 5 ml of water; 2 ml of the mixture was transfered into a test tube; followed by addition of 500 mg of magnesium chips and 4 drops of concentrated hydrochloric acid. The mixture was observed for colour change for 2 minutes. A pink or red colour indicates the presence of flavanoids (Geissman, 1962; Cuilei 1981).

II. Sodium hydroxide test

The extract (1 g) was mixed with 5 ml of water in a test tube and 5 ml of 10 % sodium hydroxide was added; a yellow colour indicated the presence of flavanoids. If addition of one drop of concentrated hydrochloric acid (HCl) caused the solution to become colourless, it was confirmatory for the presence of flavanoids according to the method of Trease and Evans (1984)

3.2.5.2. Test for Tannins.

This was carried out as described by Brain and Turner (1975) and Trease and Evans (1984).

I. Lead sub-acetate test

The extract (2 g) was placed in a test tube and 10 ml of 50 % ethanol was added, after which the mixture was filtered through a filter paper into a test tube. To 3 ml of the filtrate, 3 drops of concentrated lead sub-acetate solution was added. The resultant colour change was observed. A change in colour from white to buff-colour precipitate was confirmatory for the presence of tannins

II. Ferric chloride test.

To another 3 ml of the filtrate in (1) above in a test tube, 3 drops of concentrated ferric chloride solution was added. The reaction was observed for colour change. A bluish-black colour change was confirmatory of the presence of tannins.

3.2.5.3. Test for Saponins: Frothing test.

The extract (1 g) was dissolved in 10 ml of distilled water in a test tube and shake vigorously for 1 min. The tube was allowed to stand in a vertical position for 1 min after which observation for colour change was made as per Brain and Turner (1975);

Sofowora (1982) procedure. A formation of froth which last for more than 15 minutes was confirmatory of the presence of saponins

3.2.5.4. Test for carbohydrate.

I. molisch's test

The extract (0.5 g) was dissolved in 3 ml of water and heated over a Bunsen burner for 30 second after which 5 drops of molisch's reagent was added. 3 drops volume of concentrated H_2SO_4 was added from the side of the test tube to form a lower layer. The colour of the interface was observed (Trease and Evans, 1984).

II. Fehling's test

The extract (0.1 g) was dissolved in 5 ml of water and 5 ml, (i.e a mixture of 2.5 ml each of A and B) Fehling's solution was added. The mixture was then boiled for 5 min and observed for formation of precipitate (Trease and Evans, 1984).

3.2.5.5. Test for Cardiac glycoside: Keller-Kiliani test

The extract (1 g) was dissolved in 10 ml of 70 % methanol in a test tube and placed over a water bath for 2 min after which it was filtered through a filter paper into another tes tube. The filtrate was diluted by adding water that was twice its volume. 1 ml of 10 % lead sub-acetate solution was then added to the filtrate. The suspension was filtered, and equal volume of chloroform was added to the filtrate. The chloroform mixture was stirred for 1 min and allowed to stand for 5 min until a layer of separation was observed. The chloroform layer was then pipetted and evaporated to dryness in a dish over a water-bath. The residue was dissolved in 3 ml of 3.5 % ferric chloride in glacial acetic acid and allowed to stand for 1 min. The solution was transferred into a test tube. 1.5 ml of H_2SO_4 acid was carefully added with a pipette by

the side of the tube so that a separation layer was formed at the bottom of the tube. A purple-brown interface formation was confirmatory of the presence of cardiac glycoside (Brain and Turner, 1975).

3.2.5.6. Test for sterols/triterpenes: **Lieberman-Buchard reaction**

The extract (1 g) was dissolved in 10 ml 70 % methanol in a test tube and placed over a water bath for 2 min, after which it was filtered. 2 ml of the filtrate from the extract was placed in a test tube and 1 ml of concentrated acetic anhydride was added, followed by 0.5 ml of chloroform. 2 drops of concentrated H_2SO_4 acid was added at the bottom of the tube using a pipette. The mixture was observed for colour change for 5 min (Cuilei, 1981).

3.2.5.7. Test for Alkaloids

10g of the extract was placed into a beaker and strong ammonia solution was added in sufficient amount to moisten it and the mixture was allowed to stand for about 10 minutes. 5 ml of a 1:1 mixture of chloroform and ethanol was added just to soak and suspend the powder; after which the mixture was allowed to stand for 20 minutes with occasional stirring. The mixture was then filtered through a plug of cotton wool in a filter funnel. The residue on the cotton wool plug was washed twice with 2 ml of chloroform and the washing was added to the filtrate. The filtrate was concentrated to dryness on a 60 °C water bath, taking care to avoid over heating. The chloroform extract was transferred to a separating funnel and was shaken with 3 ml H_2SO_4. The two layers were allowed to separate; the chloroform was drained off and kept aside. About 3 ml fresh chloroform was further added to the acid layer and the procedure repeated until the acid layer was colourless. The drained off chloroform were pooled

together. The acid layer was made alkaline by adding 0.5 ml of 10 % ammonia and extracted with 3 ml of chloroform. The extract was retained and evaporated to dryness. The residue was dissolved in 3 ml of ethanol and the following tests were carried out after neutralizing with diluted H_2SO_4 (Brain and Turner, 1975; Trease and Evans, 1984).

3.2.5.8. General Tests for alkaloids

To 0.5 ml of the ethanolic extract residue obtained from above extraction was added 2-3 drops Wagmer's and Dragendorff's reagent in separate test tube to detect the presence of alkaloids. The corresponding color change shows the presence or absence of alkaloids.

3.3. Experimental animals

One hundred and two (102) six to seven weeks old albino Wistar rats of both sexes weighing between 100 to 160 g were purchased from the Department of Physiology and Pharmacology, Faculty of Veterinary Medicine Ahmadu Bello University, Zaria.The rats were acclimatized for two weeks in the laboratory and fed on commercially prepared feed; water was given *ad lib*. All the rats for the anthelmintic study were dewormed using albendazole at 200 $mgkg^{-1}$ (being the maximum convenient dose recommended by the manufacturer) two weeks before infection (Suleiman *et al.*, 2005).

3.4. Evaluation of maximum tolerated dose (MTD) of the crude methanol extracts

Due to lack of information on the precise dosage of the plants preparations as used by the traditional herdsmen and pastoralists, a maximum tolerated dose (MTD)

experiment as described by Loomis (1978) and Lorke (1983) was carried out on the crude methanolic extracts. Various doses ranging from 10, 100, 1,000, 1,600, 2,900 and 5,000 $mgkg^{-1}$ body weight were each administered orally to three (3) rats in a successive manner beginning with 10 to 1,000 $mgkg^{-1}$ body weight and then later 1,600 to 5,000 $mgkg^{-1}$ body weight. The dose that did not produce any sign of toxicity was considered as the MTD. The established MTD was then used as the basis for the administration of the crude methanolic extract and the various fractions of the plants extracts in the anthelmintic activity studies. The demerit of method is that, large number of animals are required and these animals are exposed to substances whose toxicity are not determined; thereby, subjecting them to suffer from such intoxication.

3.5. Post-mortem findings

Six rats (i.e one from each of the dose levels of the preliminary MTD trial) were randomly selected and salvaged for gross and histological changes on the visceral organs (stomach, intestine, liver, kidney, spleen, lungs and heart) resulting from the administration of the crude methanolic extract of theser plants. The same procedure was similarly carried out on rats randomly selected from each of the partitioned fraction of the extract after the experimental treatment. This to observe possible pathological changes (both gross and histological) that may occur as a result of ingessting the plant extracts (Suleiman, 2002).

3.6. Helminth parasite (*Nippostrongylus braziliensis*).

A rat-adapted strain of *Nippostrongylus braziliensis* were obtained from Helminthology Laboratory of the Scottish Agricultural Institute Scotland U.K in May, 2006; and were

acclimatized and maintained in the Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology Ahmadu Bello University Zaria for use in the studies.

3.6.1. Recovery and enumeration of larvae of *N braziliensis*

Faeces from rats that had been previously infected with *N braziliensis* were cultured in petri dishes (plate 3.5) and maintained at 27 °C in an incubator (Plate 3.4). The infective third stage larvae (L_3) were recovered from 7-9 day old vermiculite faecal cultures using a modified Baermann Apparatus (Plate 3.6) according to the method of Suleiman (2002).

Sample bottles half-filled with cultures transferred from Petri dishes, were filled with tap water and inverted onto clean petri dishes. About 10 ml of water was then placed around the bottle in the petri dishes. It was allowed to stand for at least 5 hrs to allow the larvae migrate out of the culture materials in the inverted sample bottles. The water in the petri dishes were then transferred into clean 250 ml beakers and allowed to stand for one hour to allow the parasites settle at the bottom of the beaker. Thereafter, the water in the beaker was decanted in order to concentrate the parasites. About 0.1 ml of the concentrated parasite was pipetted and spread onto a glass slide; a drop of Lugos iodine was applied to immobilize the L_3 larvae. The slide was mounted on a microscope and the L_3 observed and counted at x40 magnification. The process was repeated five times and the mean average numbers of larvae was recorded. Water was added where necessary and the volume adjusted such that 0.2 ml of the solution would contain about 200 infective larvae (L_3).

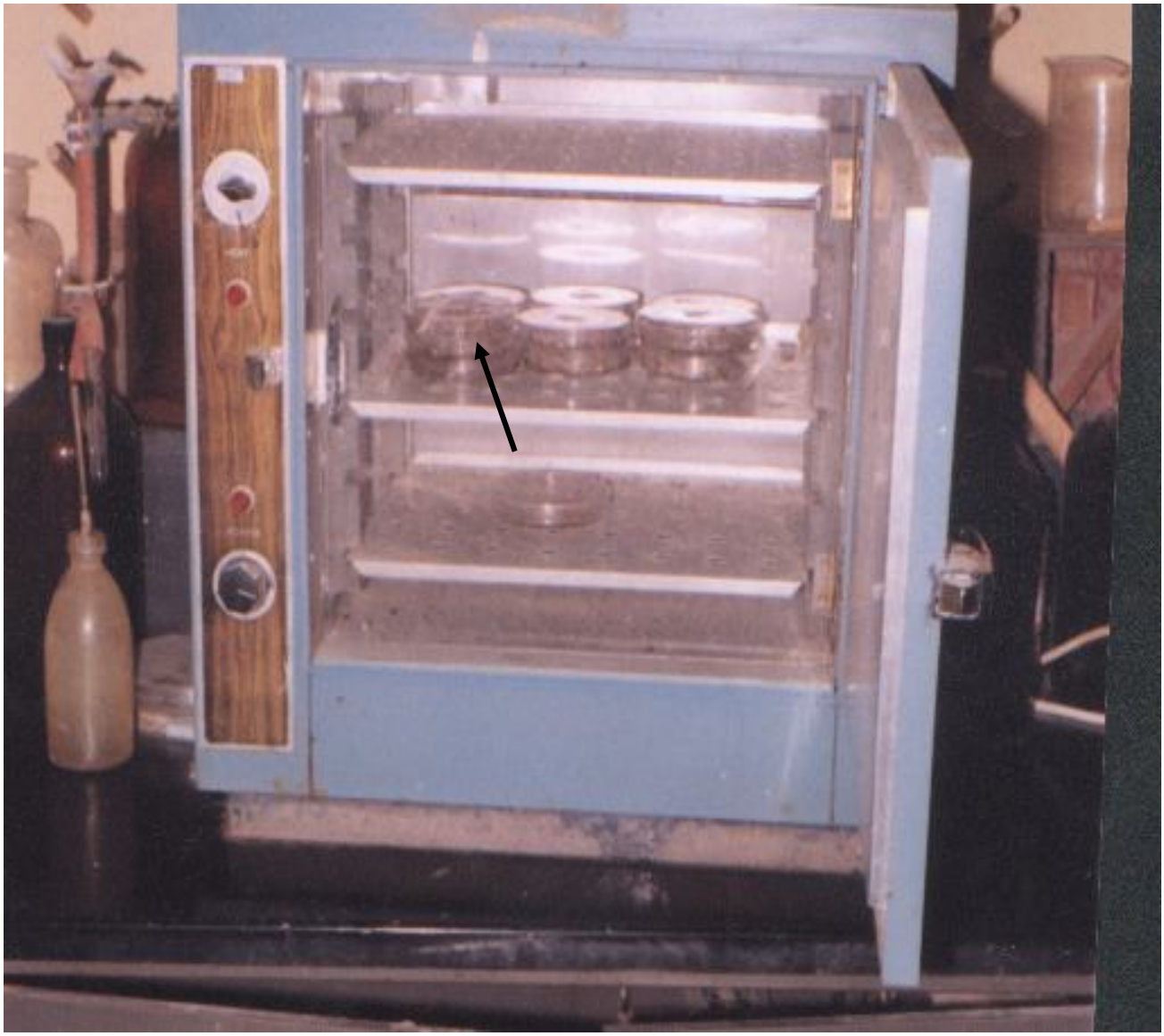


Plate 3.3. Culture plates inside Oven (incubator) used for culturing the egg of *N. braziliensis*

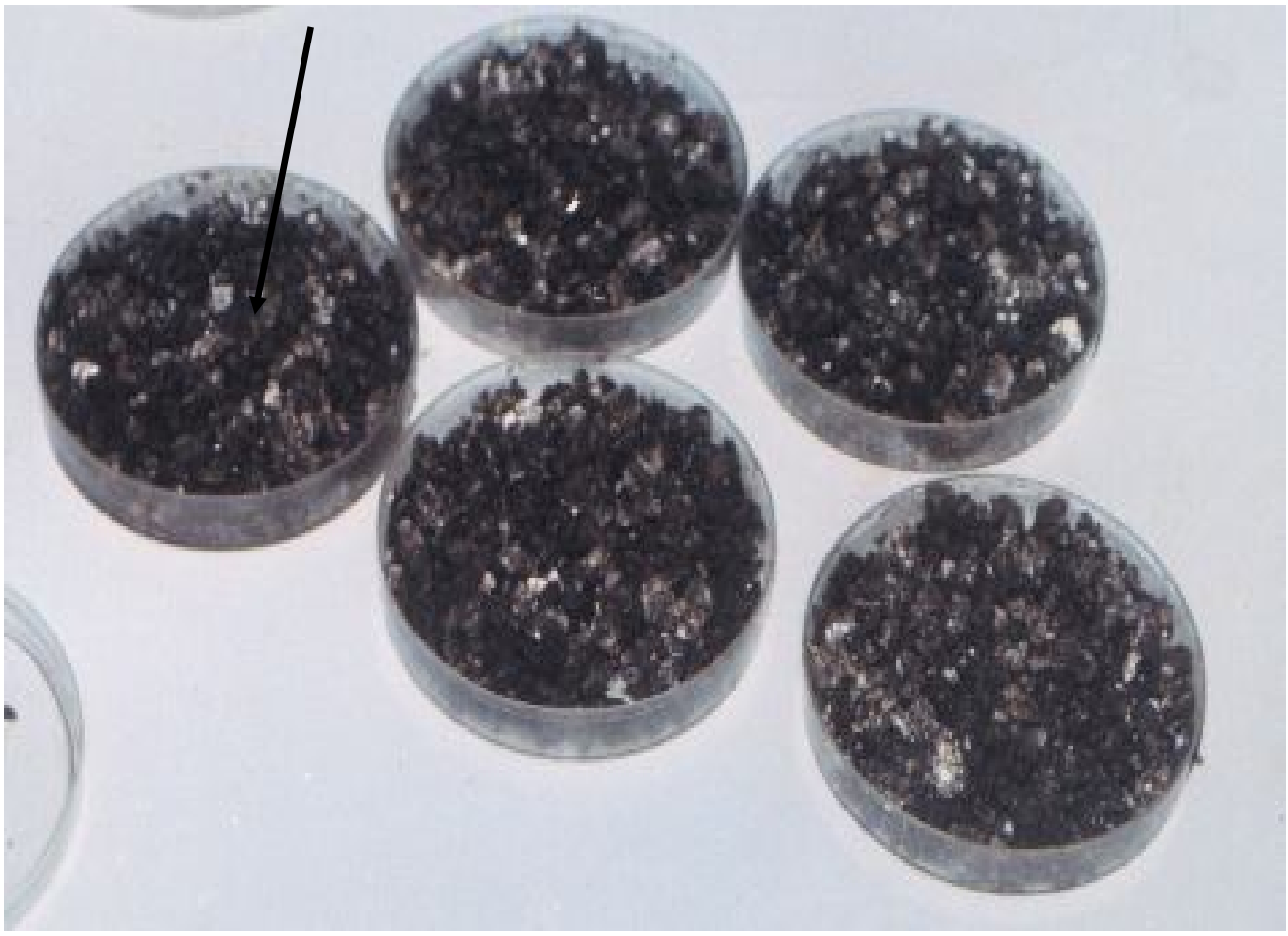


Plate 3.4. Petri dishes containing culture materials.

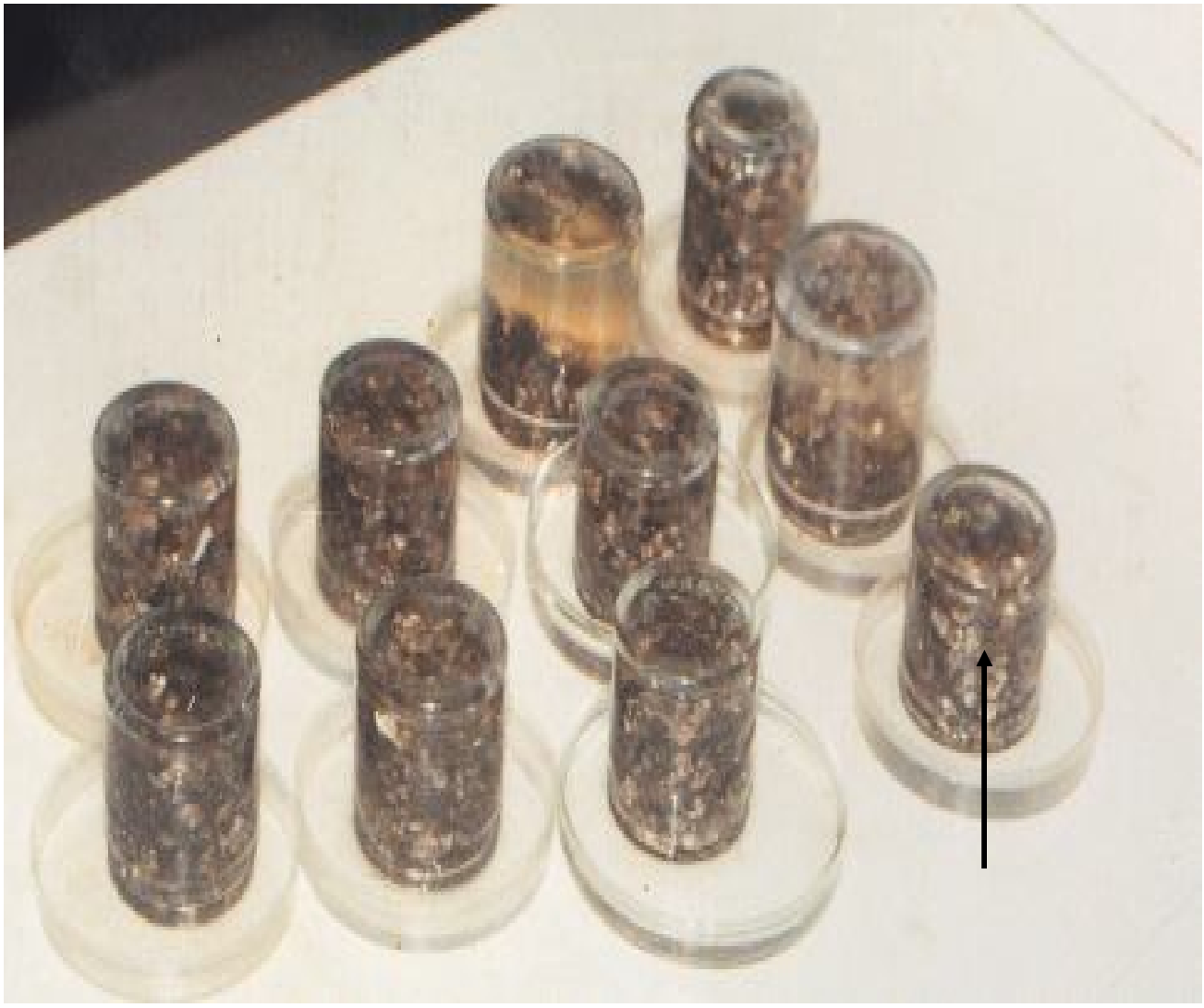


Plate 3.5. Modified Baermann apparatus for harvesting infective L_3 larvae of *N. braziliensis*

3.7 Experimental infetion/design

Each of the sixty six (66) worm-free rats were infected subcutaneously in the cervical region with 200 viable L_3 of *N braziliensis* in 0.2 ml of water using an 18-gauge needle attached to an insulin syringe (Suleiman *et al.*, 2005). After five days post infection; fresh faecal samples from each infected rat were collected by squeezing it out of the rectum. It was examined quantitatively for *N braziliensis* egg using the simple floatation method (Soulsby, 1982) (using 5 ml of flotation medium 42 % w/v sodium chloride and 72 % w/v sucrose in the ratio of 2:1, and specific gravity of 1.18). Rats not shedding ova of *N braziliensis* were excluded from the experiment.

The infected rats were randomly allocated to three (3) experimental groups (A-C) for each of the plant extract. Group A (positive control group) having six rats, were treated with albendazole at 200 $mgkg^{-1}$ body weight (Suleiman *et al.*, 2005). Group B divided into four sub-groups of six rats each for evaluation of the crude methanolic, chloroform, N-butanol and aqueous methanol fractions of the extract based on the MTD (Vercruysse *et al.*, 2001); whereas group C (negative control) were subdivided into two groups of six rats each and given water and propylene glycol as placebo based on the maximum convenient volume (MCV) of 5 $mlkg^{-1}$ (Ibrahim, 1984)

3.8. Treatment with crude methanolic extract and the fractionated layers

Oral treatment with the crude methanol extract and the various fractionated layers of the plants extract was carried out on day seven (7) post infection. Before the treatment all rats were weighed to determine the appropriate dose and the maximum convenient volume (MCV) for individual rats as shown in Tables 3.2 to 3.10**.** Observation was

made daily for three days for abnormal behavioural signs as a result of ingesting the extract (Githiori *et al.*, 2003b).

3.9. Worm counts

On Day 2-post treatment, all the rats were fasted for 24 hrs, and salvaged for adult worm count using the WAAVP guides (Powers *et al.,* 1982). The first 15 cm of the small intestine was removed, cut longitudinally and placed between two clean 20 cm glass slides (Plate 3.6). The section was examined at x40 magnification of a dissecting microscope (Plate 3.7). Visible worms were counted and recorded (Suleiman *et al.,* 2005). The fraction that caused the highest reduction in worm count without producing any behavioral changes in the rats was considered to be the most active fraction.

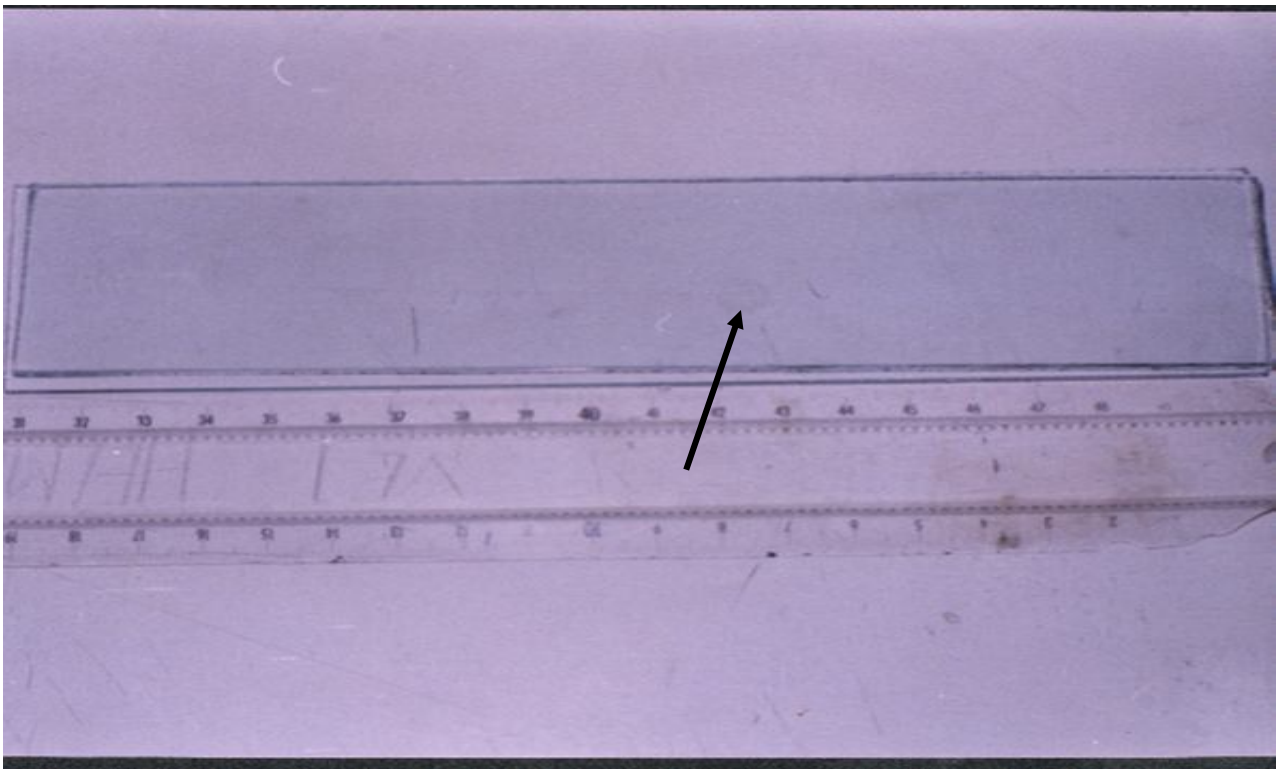


Plate 3.7. Longitudinal section of the first 15 cm portion of rat small intestine pressed between two glass slides

Plate 3.8.Dissection microscope for counting infective L3 larvae of *N. braziliensis*

3.10. Percentage efficacy (Deparasitization)

Percentage efficacy (deparasitization) of the various fractions of each plant was calculated according to the method of Cavier (1973) using the formular:

$$\% \text{ Efficacy} = \frac{N-n}{N} \times 100\%$$

N= number of worms counted in the placebo-treated rats

n= number of worms counted in the plant extracts and or albendazole-treated rats.

It was considered 'a priori' that the efficacy of the plant extracts would be biologically significant if a reduction in total worm count (TWC) above 70 % occurred (Githiorei *et al.*, 2003b).

3.11. Statistical analysis

Means of data obtained from the experimental treatment were analyzed using the software package for GraphPad prism (version 4.0-----2003). Statistical significance for the anthelmintic effect of crude methanolic extract, chloroform, N-butanol portion and aqueous methanol portion was assessed by ANOVA. Subsequently Borferroni's multiple comparison tests was used with P value of < 0.05 considered as significant.

CHAPTER FOUR

4.0. RESULTS

4.1. Methanolic extraction of plant material and solvent fractionation of crude methanolic extracts

Following subjection of 500 g of plant material to extraction with 70 % absolute methanol as solvent, *A. africana* yielded 80 g (16 %), while *C. molle* yielded 150 g (30 %). The colours of the extract were dark brown and light brown respectively. Using petroleum ether, chloroform and N-butanol as solvents, the respective yields following fractionation of 20 g crude methanolic extract of *C. molle* was 0 g (0 %), 5 g (25 %), 7 g (35 %), leaving 8 g (40 %) as the aqueous methanol fraction. The petroleum ether fraction was colourless while the chloroform, N-butanol and aqueous methanol fractions had yellow, reddish brown and brown colours respectively.

For *A. africana,* the respective yields from the fractionation of 20 g of the crude methanol extract with the three solvents were 0 g (0 %), 3 g (15 %), 7 g (35 %) and leaving 10 g (50 %) as the aqueous methanol fraction. The petroleum ether fraction was colourless while the chloroform, N-butanol and aqueous methanol fractions had yellow, reddish brown and brown colours respectively.

4.2. Phytochemical screening of the extracts

The results obtained from the various phytochemical tests indicated that both *A. africana* and *C. molle* either have all or some of these as constituents: alkaloids, steroids, saponins, carbohydrates, flavonoids, tannins and cardiac glycosides.

4.2.1. Phytochemical screening of crude methanol extract of *A. africana*

Crude methanolic extract of the plant had alkaloids, steroids, saponins, carbohydrated, flavonoids, tannins and cardiac glycosides as constituents with cardiac glycoside being more prominent (Table 4.1).

Table 4.1 Observations of phytochemical screening of crude methanolic extract of *A. africana*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	no colour change	-
	Mayer's	orange precipitate	+
	Wagner's	no colour change	-
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	no colour change	-
Flavonoids	Shinoda's	no colour change	-
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	++

key

(+) indicate presence

(-) indicate absence

4.2.2. Phytochemical screening petroleum ether portion of *A. africana*

Phytochemical screening of the petroleum ether portion revealed the presence of alkaloids and steroids (Table 4.2),

Table 4.2 Observations of phytochemical screening of petroleum ether fraction of *A. africana*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	orange precipitate	+ +
	Mayer's	"	+
	Wagner's	"	+
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	no colour change	-
Carbohydrate	Molish's	no colour change	-
	Fehling's	no colour change	-
Flavonoids	Shinoda's	no colour change	-
	Sodium hydroxide	no colour change	-
Tannins	Lead sub acetate	no colour change	-
	Ferric-chloride	no colour change	-
Cardiac glycoside	Keller-Killiani	no colour change	-

key

(+) indicate presence

(-) indicate absence

4.2.3. Phytochemical screening of the chloroform extract of *A. africana*

The phytochemical screening of the chloroform extract, showed the presence of alkaloids, steroids, flavonoids, tannins and cardiac glycosides, with the alkaloids being more prominent (Table 4.3).

Table 4.3 Observations of phytochemical screening of chloroform fracrtion of *A. africana*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	orange precipitate	+++
	Mayer's	"	+
	Wagner's	"	+
Steroids	Lieberman-Buchard	violet ring colour	+ +
Triterpenes	"	no colour change	-
Saponins	Frothing	no colour change	-
Carbohydrate	Molish's	no colour change	-
	Fehling's	no colour change	-
Flavonoids	Shinoda's	pink colour	+
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish- black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	+++

key

(+) indicate presence

(-) indicate absence

4.2.4. Phytochemical screening of N-butanol fraction of *A. africana*

The screening of the N-butanol fraction revealed the presence of carbohydrate, alkaloids, steroids, flavonoids, tannins and cardiac glycosides. The alkaloids and cardiac glycosides were more prominent amongst the constituents (Table 4.4).

Table 4.4 Observations of phytochemical screening of N-butanol fraction of *A. africana*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	orange precipitate	+
	Mayer's	"	+ +
	Wagner's	"	+
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	no colour change	-
Flavonoids	Shinoda's	pink colour	+
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish- black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	+++

key

(+) indicate presence

(-) indicate absence

4.2.5. Phytochemical screenig of the aqueous methanol fraction of *A. africana*

Screening of the aqueous methanol fraction revealed the presence of steroids, flavonoids, tannins and carbohydrate and cardiac glycosides; with cardiac glycoside being more prominent (Table 4.5).

Table 4.5 Observations of phytochemical screening of aqueous methanol fraction of *A. africana*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	no colour change	-
	Mayer's	"	-
	Wagner's	"	-
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	no colour change	-
Flavonoids	Shinoda's	no colour change	-
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish- black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	+

key

(+) indicate presence

(-) indicate absence

4.2.6. Phytochemical screening of crude methanolic extract *C. molle*

The crude methanolic extract of this plant had steroids, saponins carbohydrate, flavonoids, tannins and cardiac glycosides as constituents (Table 4.6).

Table 4.6 Observations of phytochemical screening of crude methanolic extract of *C. molle*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	no colour change	-
	Mayer's	"	-
	Wagner's	"	-
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	red precipitate	+
Flavonoids	Shinoda's	pink colour	+
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	+

key

(+) indicate presence

(-) indicate absence

4.2.7. Phytochemical screening of petroleum ether fraction of *C. molle*

The petroleum ether fraction had only alkaloids and steroids as the partitioned constituents (Table 4.7).

Table 4.7 Observations of phytochemical screening of petroleum ether fraction of *C. molle*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	orange precipitate	+ +
	Mayer's	"	+
	Wagner's	"	+
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	no colour change	-
Carbohydrate	Molish's	no colour change	-
	Fehling's	no colour change	-
Flavonoids	Shinoda's	no colour change	-
	Sodium hydroxide	no colour change	-
Tannins	Lead sub acetate	no colour change	-
	Ferric-chloride	no colour change	-
Cardiac glycoside	Keller-Killiani	no colour change	-

key

(+) indicate presence

(-) indicate absence

4.2.8. Phytochemical screening of chloroform fraction of *C. molle*

The chloroform fraction had alkaloids, steroids, flavonoids, tannins and cardiac glycosides as the partitioned constituent with alkaloids being more prominent (Table 4.8).

Table 4.8 Observations of phytochemical screening of chloroform fraction of *C. molle*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	orange precipitate	+++
	Mayer's	"	+
	Wagner's	no colour change	-
Steroids	Lieberman-Buchard	violet ring colour	+ +
Triterpenes	"	no colour change	-
Saponins	Frothing	no colour change	-
Carbohydrate	Molish's	no colour change	-
	Fehling's	no colour change	-
Flavonoids	Shinoda's	pink colour	+
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	no colour change	-
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	++

key

(+) indicate presence

(-) indicate absence

4.2.9. Phytochemical screening of N-butanol fraction of *C. molle*

The N-butanol fraction had steroids, saponins, carbohydrate flavonoids and tannins as constituent (Table 4.9).

Table 4.9 Observations of phytochemical screening of N-butanol fraction of *C. molle*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	no colour change	-
	Mayer's	"	-
	Wagner's	"	-
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	red precipitate	+
Flavonoids	Shinoda's	pink colour	+
	Sodium hydroxide	solution turns yellow	+
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish- black colour	+
Cardiac glycoside	Keller-Killiani	no colour change	-

key

(+) indicate presence

(-) indicate absence

4.2.10. Phytochemical screening of aqueous methanol fraction of *C. molle*

The constituents observed in the aqueous methanolic fraction are cardiac glycosides, steroids, saponins, carbohydrate, flavonoids and tannins (Table 4.10).

Table 4.10 Observations of phytochemical screening of aqueous methanol fraction of *C. molle*

Compound tested for	Test carried out	Observation	Inference
Alkaloids	Dragendorff's	no colour change	-
	Mayer's	"	-
	Wagner's	"	-
Steroids	Lieberman-Buchard	violet ring colour	+
Triterpenes	"	no colour change	-
Saponins	Frothing	copious froth persisting for 15 min	+
Carbohydrate	Molish's	a reddish brown ring interface	+
	Fehling's	red precipitate	+
Flavonoids	Shinoda's	no colour change	-
	Sodium hydroxide	"	-
Tannins	Lead sub acetate	buff- colour precipitate	+
	Ferric-chloride	bluish- black colour	+
Cardiac glycoside	Keller-Killiani	purple-brown ring interface	+

key

(+) indicate presence

(-) indicate absence

4.3. Toxicity studies and determination of maximum tolerated dose (MTD).

At a dose range of 10 to 1,000 $mgkg^{-1}$, the crude methanolic extract of *Afzelia africana* did not cause any visible toxic effect on rats as they were active 6 hrs after recovering from the stress due to administration procedure. On the other hand, from the dose range of 1,600 to 5,000 $mgkg^{-1}$, the rats demonstrated varying degrees of signs of toxicity which manifested as visible body weakness, inability to move and reduced appetite from 24 hrs to 7 days post administration (Table 4.11). Therefore the doses of 1,600 to 5,000 $mgkg^{-1}$ were considered unsafe for the rats; and the dose of 1,000 $mgkg^{-1}$ body weight was chosen as the experimental treatment dose for both the crude methanolic extracts and the various fractions.

Table 4.3.1. Maximum tolerated dose of crude methanolic extract of *Afzelia africana*

Dose($_{mg/kg^{-1}}$)	10	100	1000	1600	2900	5000
Initial number of rat	3	3	3	3	3	3
Mortality	0	0	0	0	0	0
Observation	a	a	a	b	c	d
Inference	-	-	-	+	++	+++

Key

a = rats active 6-24 hrs and beyong

b = rats showed weakness for more than 24 hrs

c = rats showed weakness for more than 48 hrs

d = rats showed weakness for more than 7 days

- = no sign of toxicity

+ = slightly toxic

++ = toxic

+++ = more toxic

Similarly, at a dose range of 10 to 1,000 $mgkg^{-1}$, the crudee methanolic extract of *C. molle* did not cause any visible toxic effect in the rats- the rats were active 6 to 12 hrs after recovering from the stress of the administration procedure. On the other hand, at a dose range of 1,600 to 5,000 $mgkg^{-1}$, there were indications of toxicity – weakness after 24 hrs of administration, a case of mortality at 24 hrs in the group given 2,900 $mgkg^{-1}$, and two cases of mortality at 12 hrs in the group given 5,000 $mgkg^{-1}$ (Table 4.12). Therefore the 1,000 $mgkg^{-1}$ was choosen as the experimental dose for both the crude methanol and the various fractions for both plants.

Table 4.3.2. Maximum tolerated dose of crude methanolic extract of *C. molle*

Dose($_{mg/kg^{-1}}$)	10	100	1000	1600	2900	5000
Initial number of rat	3	3	3	3	3	3
Mortality	0	0	0	0	1	2
Observation	a	a	a	b	c	d
nference	-	-	-	+	++	+++

Key

a = rats active 6-24 hrs and beyong

b = rats showed weakness for more than 24 hrs

c = rats showed weakness for more than 48 hrs

d = rats showed weakness for more than 7 days

- = no sign of toxicity

+ = slightly toxic

++ = toxic

+++ = more toxic

4.4. Post-mortem findings

There were no gross pathological and histological lesions on the visceral organs (stomach, intestine, liver, kidney, spleen, lungs and heart) nor that of the reproductive organs (testis, uterus and ovaries) resulting from the administration of the tested doses of the crude methanolic extracts and the various fractions of both plants.

4.5. Experimental treatment with crude methanol extracts, the various fractions, albendazole and water/ propylene glycol.

The variuos doses, individual weight of each rat, the maximum convinient volume and observation on each rat 7 days post extrasct administration are presented on Tables 4.5.1 to Tables 4.5.11.

Table 4.5.1. Rat weight (W), dose and maximum convenient volume (MCV) of crude methanolic extract of *C molle* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	103	103	0.52	active
2	118	118	0.59	“
3	125	125	0.63	“
4	108	108	0.54	“
5	103	103	0.52	“
6	112	112	0.57	“

Table 4.5.2.Rat weight, dose and MCV of chloroform fraction of *C molle* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	93	93	0.93	active
2	106	106	1.06	“
3	132	132	1.32	“
4	128	128	1.28	“
5	139	139	1.39	“
6	120	120	1.2	“

Table 4.5.3. Rat weight (W), dose and MCV of N-butanol fraction of *C molle* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	126	126	0.63	active
2	100	100	0.5	“
3	112	112	0.56	“
4	148	148	0.74	“
5	153	153	0.77	“
6	158	158	0.79	“

Table 4.5.4. Rat weight (W), dose and MCV of aqueous methanol fraction of *C molle* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	130	130	0.65	active
2	112	112	0.56	“
3	100	100	0.5	“
4	114	114	0.57	“
5	129	129	0.65	“
6	117	117	0.59	“

Table 4.5.5. Rat weight (W), dose and MCV of crude methanolic extract of *A. africana* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	112	112	0.56	active
2	104	104	0.52	“
3	102	102	0.51	“
4	108	108	0.54	“
5	138	138	0.69	“
6	103	103	0.52	“

Table 4.5.6. Rat weight (W), dose and MCV of chloroform fraction of *A. africana* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 mgm^{-1} / rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	108	108	0.55	active
2	118	118	0.59	“
3	102	102	0.51	“
4	140	140	0.7	“
5	103	103	0.52	“
6	103	103	0.52	“

Table 4.5.7. Rat weight (W), dose and MCV of N-butanol fraction of *A. africana* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$ / rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	140	140	0.7	active
2	127	127	0.64	“
3	108	108	0.54	“
4	120	120	0.6	“
5	160	160	0.8	“
6	112	112	0.57	“

Table 4.5.8. Rat weight (W), dose and MCV of aqueous methanol fraction of *A. africana* at a dose rate of 1000 $mgkg^{-1}$ and at a concentration of 200 $mgml^{-1}$/ rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	102	102	0.51	active
2	118	118	0.59	"
3	108	108	0.55	"
4	140	140	0.7	"
5	104	104	0.52	"
6	110	110	0.55	"

Table 4.5.9. Rat weight (W), dose and MCV of albendazole at a dose rate of 200 $mgkg^{-1}$ and at a concentration of 30 $mgml^{-1}$ / rat.

Rat ID	W (g)	dose (mg)	MCV (ml)	Observation
1	102	10.2	0.51	active
2	116	11.6	0.58	“
3	132	13.2	0.66	“
4	148	14.8	0.74	“
5	151	15.1	0.76	“
6	103	10.3	0.52	“

Table 4.5.10.Rat weight, dose and MCV of control (water) at a dose rate of 5 $mlkg^{-1}$ / rat.

Rat ID	W (g)	dose ($mlkg^{-1}$)	MCV (ml)	Observation
1	102	5	0.51	active
2	116	“	0.58	“
3	110	“	0.55	“
4	158	“	0.79	“
5	106	“	0.53	“
6	120	“	0.6	“

Table 4.5.11.Rat weight (W), dose and MCV) of control (propylene glycol) at 5 $mlkg^{-1}$ / rat.

Rat ID	W (g)	dose ($mlkg^{-1}$)	MCV (ml)	Observation
1	115	5	0.58	active
2	109	“	0.55	“
3	123	“	0.62	“
4	100	“	0.5	“
5	121	“	0.61	“
6	101	“	0.51	“

4.6. Anthelmintic effect of extracts on *N. braziliensis*

4.6.1. Anthelmintic effect of extracts of *A. africana*

The anthelmintic effect of the crude methanolic extract and the various fractions of *A. africana* are shown in Tables 4.6.1.

Rats that were infected orally with 200 L_3 followed by treatment with crude methanolic extract at 1,000 mg/kg^{-1} had a mean worm count of 4.5; while those infected and treated with chloroform (1,000 mg/kg^{-1}), N-butanol (1,000 mg/kg^{-1}), aqueous methanol (1,000 mg/kg^{-1}) fractions and albendazole (200 mg/kg^{-1}) had respective mean worm count of 2.5, 3.5, 6.0 and 0; compared to the mean worm count (12.83 and or 12.0) from the negative controls rats. The extract and the respective fractions produced percentage deperasitization of 62.50 % (crude methanolic extract), 79.20 % (chloroform), 72.72 % (N-butanol) and 53.24 % (aqueous methanol). Albendazole (positive control) a purified conventional anthelmintic, gave 100 % deparasitization compared to the crude methanolic extract and the respective fractions whereas the negative controls gave 0 % deparasitization.

The deparasitization produced by the chloroform and N-butanol were significant ($P<0.05$) when compared to that produced by the placebo-treated negative control rats, while the deparasitization produced by the albendazole was highly-significant ($P<0.001$). The depaasitization produce by the crude methanolic extract was significant ($P<0.05$) (but not biologically significant) whereas the aqueous methanol fractions did not produce significant reduction in worm count.

Table 4.6.1 Worm count and percentage deparasitization of the *N. braziliensis* 7 days after treatment with crude methanol extract and fractions of *A. africana*

	Worm cout after treatment with						
Rat	CME (1000mgkg⁻¹)	Chloroform (1000mgkg⁻¹)	N-butanol (1000 mgkg⁻¹)	Aqueous (1000 mgkg⁻¹)	Albendazole (200 mgkg⁻¹)	Placebo1 (5 ml-water)	Placebo2 (5 ml-p. glycol)
1	4	2	4	14	0	4	6
2	2	3	3	4	0	17	16
3	7	4	2	8	0	18	15
4	5	1	5	3	0	12	10
5	5	3	1	5	0	14	11
6	4	2	6	2	0	12	14
Mean ± SD	4.5 ± 1.64*b	2.5 ± 1.05a*	3.5 ± 1.87a*	6.0 ± 4.43b	0.0 ± 0.0a*	12.83± 5.0 b	12.0 ± 3.74 b
% DPZ	62.50	79.20	72.72	53.24	100	0	0

Mean with * within the column are significantly different at $p<0.001$, while those with the letter ª and ъ show no significant difference between their means at $p>0.05$ as determined by Borferroni's multiple comparison test.

%DPZ= percentage deparasitization

Fig 4.1 shows that chloroform fraction of *A. africana* demonstrated simillar anthelmintic activitity comparable to albendazole (positive control). This was closely followed by N-butanol fraction while aqueous methanol fraction has a much lower activitity compaired to albendazle. The crude methanol and the aqueous methanol demonstrated poor anthelmintic activity and followed the pattern of the negative control (water and P. glycol).

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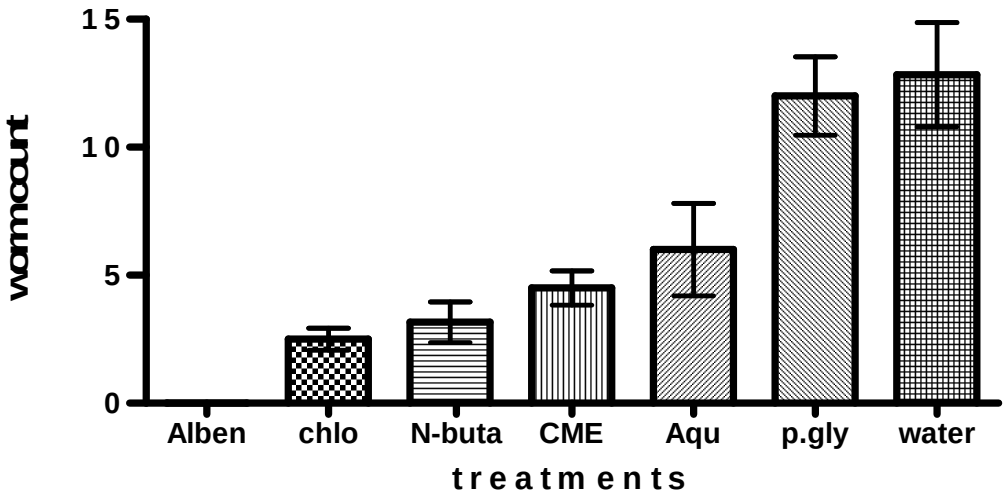

Fig 4.1 Mean ± SD of worm count after treatment with the various fractions of *A. africana* extracts, albendazole and the placebos (see Table 4.13)

Key:
Alben- albenazole;
Chlo- chlorofoprm fraction;
N-but- N-butanol fraction;
Aqu- aqueous methanol fraction;
P.gly- propylene glycol

4.6.2. Anthelmintic effect of extracts of *C. molle*

The anthelmintic effect of the crude methanol extract and the various fractions of *C. molle* are shown in Tables 4.26. Similarly, rats that were infected with 200 L_3 followed by treatment with crude methanolic extract at 1,000 mg/kg^{-1} had a mean worm count of 6.17; while those infected and treated with chloroform (1,000 mg/kg^{-1}), N-butanol (1,000 mg/kg^{-1}), aqueous methanol (1,000 mg/kg^{-1}) of the fractions and albendazole (200 mg/kg^{-1}) had respective mean worm count of 3.0, 3.5, 1.67 and 0; compared to the mean worm count (12.83 and or 12.0) from the negative controls rats. The extracts and the respective fractions produced percentage deperasitization of 48.61 % for the crude methanolic, 75.0 % for the chloroform, 72.72 % for the N-butanol and 86.98 % for the aqueous methanol. The positive control group albendazole gave 100 % deparasitization compared to the crude methanolic extract and the respective fractions whereas the negative controls that had either extract or drug gave 0 % deparasitization.

The deparasitization produced by the chloroform, N-butanol and aqueous methanol fractions were significant ($P<0.05$) when compared to that produced by the placebo-treated negative control rats, while the deparasitization produced by the crude metanolic extract was non-significant ($P>0.05$).

Table 4.6.2. Worm count and percentage deparasitization of the N. braziliensis 7 days after treatment with crude methanolic extract and the various fractions of *C. molle*

	Worm cout after treatment with						
Rat	CME (1000mgkg⁻¹)	Chloroform (1000mgkg⁻¹)	N-butanol (1000 mgkg⁻¹)	Aqueous (1000 mgkg⁻¹)	Albendazole (200 mgkg⁻¹)	Placebo1 (5 ml-water)	Placebo2 (5 ml-p. glycol)
1	7	2	6	2	0	4	6
2	2	3	0	0	0	17	16
3	7	3	5	0	0	18	15
4	8	4	3	4	0	12	10
5	7	3	3	2	0	14	11
6	6	3	4	2	0	12	14
Mean ± SD	6.17 ± 2.11b	3.0 ± 0.63a*	3.5 ± 2.09a*	1.67 ± 3.37a*	0.0 ± 0.0a*	12.83± 5.0b	12.0 ± 3.74b
% DPZ	48.61	75.0	72.72	86.98	100	0	0

Mean with * within the column are significantly different at $p<0.001$, while those with the letter ª and ъ show no significant difference between their means at $p>0.05$ as determined by Borferroni's multiple comparison test.

%DPZ= percentage deparasitization

Fig 4.2 shows that the aqueous methanol fraction of *C. molle* demonstrated anthelmintic activity comparable to albendazole; this was followed by chloroform and N-butanol fractions respectively. Crude methanol extract had little antiparasitic activitity on the parasites as do the negative controls

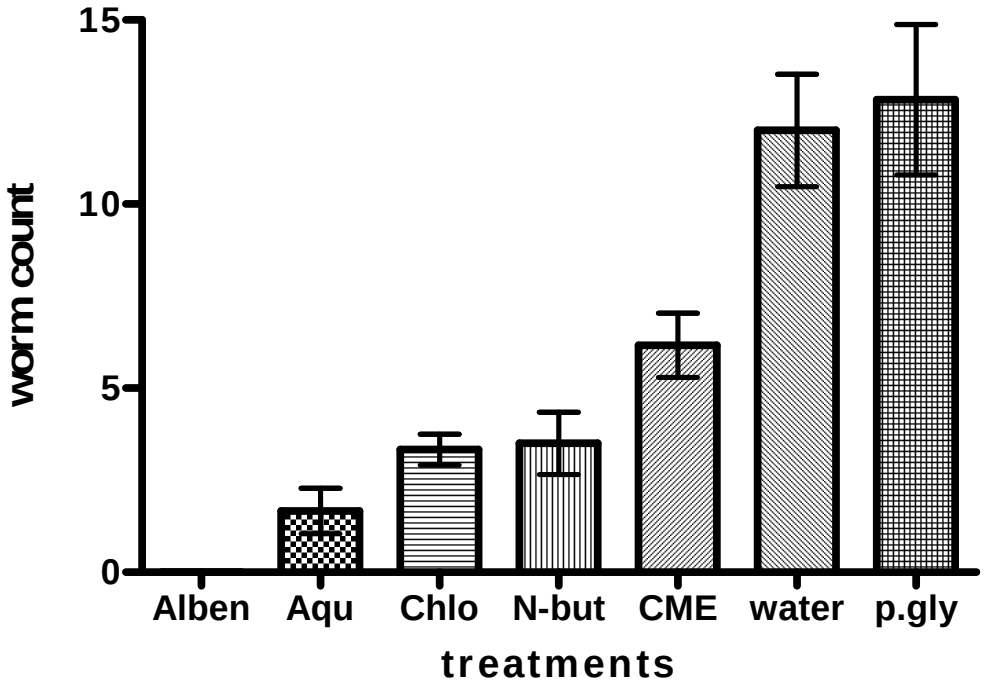

Fig 4.2 Mean ± SD of worm count after treatment with the various fractions of *C. molle* extracts, albendazole and the placebos (see Table 4.14)

Key:
Alben- albenazole;
Chlo- chlorofoprm fraction;
N-but- N-butanol fraction;
Aqu- aqueous methanol fraction;
P.gly- propylene glycol

CHAPTER FIVE

5.0 DISCUSSION

Different methods exist for the extraction and separation of plant materials for pharmacological and medicinal uses. In this study, exhaustive extraction of the dried powdered materials from the stem-bark of the two plants with methanol, a polar solvent and the stepwise partitioning in various solvents (petroleum ether, chloroform and N-butanol) was used. The crude methanolic extract (CME) yield of *C. molle* was relatively higher (30 %) per gram of plant material than that of *A. africana* (16 %). Recent harmonizations on anthelmintic efficacy guidelines in ruminants have indicated that for a drug to be considered efficacious, a 90 % reduction in total worm count (TWC) should be achieved (Vercruysse *et al*., 2001). However, the *in-vivo* anthelmintic effect of *A. africana* and *C. molle* are unknown. Thus, the efficacy of the plant extracts would be biologically significant if a reduction in total worm count (TWC) above 70 % occurred (Githiorei *et al*., 2003b). Treatment of rats with solvent-partitioned extracts (i.e chloroform, N- butanol, and aqueous methanol fractions) of both plants showed anthelmintic activity. The aqueous methanol fraction of *C. molle* showed the highest reduction in TWC of 86.98 %, by day 3 post treatment in comparison to the untreated control groups. This was followed by chloroform fraction of *A. africana* with reduction in TWC of 79.20 %. The chloroform and N-butanol fractions of *C. molle* also had the required significant biological reduction in TWC of 75.0 % and 72.20 % respectively. Also the N-butanol fraction of *A. africana* had the required significant biological reduction in TWC of 72.20 % compared to the untreated control groups. The crude methanolic extract of both plants and the aqueous methanol fraction of *A.*

africana did not produce the required significant biological reduction in TWC. However, the crude methanol extract of *A. africana* was statistically significant in comparison with the untreated control groups. That of *C. molle* on the other hand was not statistically significant in comparison with untreated control groups. Although the crude methanolic extract of both plants showed reduced activity against *N. braziliensis* in rats in this study; their significant nematocidal activity has been demonstrated against *H. contortus* in *in-vitro* egg hatch assay (Simon, 1997). The results of this study equally demonstrated that the parasite *N. braziliensis* was highly sensitive to albendazole with complete deparasitzation at a dose rate of 200 mg/kg^{-1} body weight (Suleiman *et al.*, 2005).

Partitioning plants extracts is a method of seperating plant components based on their solubilities in the solvents used (Harbone, 1973).

The N-butanol and aqueous methanol fractions were soluble in water and other polar solvents like alcohol, suggesting that the constitiuent of these fractions are mainly polar compounds. However, the crude methanol extract and chloroform fractions were only soluble in propylene glycol (a non polar solvent). This informed the inclusion of propylene glycol as one of the placebo.

The outcome of the phytochemical screening revealed that both plants have constitiuents including tannins, alkaloids, flavonoids, cardiac glycosides and steroids which may have anthelminthic activities (Athanasiadou *et al.*, 2000, 2001, 2005; Gamenara *et al.*, 2001; Niezen *et al.*, 1998; Lahlou., 2002; Onyeyili *et al.*, 2001; Lateef *et al.*, 2003; Prasharth *et al.*, 2001)

Tannins are complex substances consisting mainly of polyphenolic acids (Bate-Smith, 1962). They occur in several plants and are more common in woody rather than herbacious plants (Mehansho *et al.*, 1987) and have been shown to have anthelmintic activities (Niezen *et al.*, 1995; Kahn and Diaz-Hernandez, 2000; Athanasiadou *et al.*, 2001; Waller *et al.*, 2001). However, the anthelmintic effect of plants containing tannins depends on the type and content of tannins in the plant (Niezen *et al.*, 1998; Athanasiadou *et al.*, 2001). For instance, *in-vitro* studies Kahiya *et al.* (2003) revealed that condensed tannins from the leave extract of *Acarcia nitotica* inhibited the development of *H. contortus* larvae from goat.

In another similar but seperate study Gamenara *et al.* (2001) demonstrated that, tannins polyphenols from bryophytes exhibited anthelmintic activity against *N braziliensis.* Athanasiadou *et al.* (2001) in *in-vitro* and *in-vivo* studies reported the anthelmintic activity of condensed tannins extracted from Quebracho on the larvae of *H. contortus, Teladorsagia circumcinta* and *Trichostrongylus vitrinus.* The observed anthelmintic activity of Quebracho extracts *in-vitro* could be attributed to the capacity of tannins to bind to proteins, and could operate via several mechanisms (Athanasiadou *et al.*, 2001). Tannins could also bind to the free proteins available in the GIT of the host, reducing nutrients available to the parasites, and resulting into starvation of the parasite and subsequent death (Athanasiadou *et al.*, 2001; Schultz, 1989). Futher mode of action of tannins is that they are capable of binding with the glycoproteins on the cuticle of the parasites, leading to death of the parasite (Thompson and Geary, 1995). Tannins have vasoconstriction effect and could be advantageous in preventing worm implantation onto the mucosa of the GIT making it

easier for them to be expelled from the GIT (Aguwa and Nwako, 1988). Tannins-containing plants increase the supply and absorption of digestible protein by animals (Wang *et al*., 1994; Waller *et al*., 2001; Madibela and Jansen, 2003). This is achieved by formation of protein complexes in the rumen by tannins, which later dissociate at low PH in the abomasum to release more proteins for metabolism in the small intestine of ruminant animals (Waller *et al*., 2001). The increase in protein supply due to tannins is suggested to improve immunity against gastrointestinal parasites (Coop and Kyriazakis, 1999; Coop and Holmes, 1996; van Houtert and Sykes, 1996; Donadson *et al*., 1997). A study by Athanasiadou *et al.* (2000), showed that reduction in FEC occurred within 2 days of administration of condensed tannins, a period similar to that after treatment with conventional anthelmintic drugs. In addition, tannins or their metabolites have a direct effect on the viability of the pre-parasitic stages of helminths (Duncan, 1996). Some synthetic phenolic anthelmintic (e.g niclosamide, oxyclozanide, bithionol, nitroxynil etc) are shown to interfere with energy generation in helminths parasites by uncoupling oxidative phosphorylation (Martin, 1997). Tannins contain mixtures of phenols and these are uncouplers of oxidative phosphorylation. Phenols readily combine with plasma proteins rendering them resistant to proteolytic enzymes secreted by the worms (Mitcell *et al*., 1983). Phenols are also known to have high efficacy against blood-ingesting parasites and those that live close to the intestinal mucosa; presumably they are released to poison the parasites that ingest the blood (Chiezey *et al.,* 2000). It is therefore reasonable to assume that the herdsmen/pastoralist claims may be right since the plants used in this study contain tannins and could have had similar anthelmintic effect with the ones

earlier described. Further more, tannin-containing foods taken freely might be another way with which to control the detrimental effect of gastrointestinal parasitism (Suleiman *et al*., 2005). This is to say that herdsmen's drenching of their animals with concoction of the plants could bring about deparasitization since the plants contain tannins.

Flavonoids (e.g. Hesperidin), occur both in the free-state and as glycoside. They are widely distributed in nature and are frequently found in their highest concentrations in association with essential oils. They are generally soluble in water and insoluble in organic solvent (Brain and Turner, 1975). The aglycones of flavanoids are only sparingly soluble in water but soluble in ether. Hesperidin is one of the most important flavonoids and is found in bitter orange peel in association with isohesperidin, hesperic acid and essential oil (Brain and Turner, 1975). Hashizume *et al.* (1978) reported that flavonoids offer some protection in ulcer development by increasing capillary resistance and through improved microcirculation which renders the cell less injurious to tissues penetrating worms. Flavonoids are also believed to stimulate intestinal motility similar to that produced by acetylcholine (Akendenque, 1992), thereby causing rapid worm expultion from the GIT. Lahlou (2002) reported that flavonoid is one of the phytochemicals that have anthelmintic effect. Having identified flavonoids in almost all the fractions used in this study, it is possible that it contributed significantly to the observed anthelmintic effect on the *N. braziliensis*.

Alkaloids are nitrogeneous compounds which occur in plants. Many are optically active and they are basic in nature and form salts with plant acids (Brain and Turner, 1975). Their solubility show considerable variation and depend upon whether they are

present as the salt or as the free base. Their salts are soluble in water and insoluble in organic solvents (Brain and Turner, 1975). In *in-vitro* and *in-vivo* studies, Al-qarawi *et al.* (2001) reported that alkaloids extracted from both the latex and leaves of C*alotropis procera*, was effective in inhibiting the exsheatment of L_3 of *H. contortus* to L_4 in sheep. Lateef *et al.* (2003) also reported that alkaloids and their glycosides extracted from the root of *Adhatoda vestica* was effective against mixed gastrointestinal infections in sheep. Also, Onyeyili *et al.* (2001) reported that tannins and alkaloids, the active principles of *Nauclea latifolia* stem bark, were effective against mixed infections of nematode in sheep. The present study has shown that alkaloids are present in all the fractions except the crude methanolic extract of *C. molle.* It is possible that the presence of alkaloids contributed to the significant deparasitization observed. Conversely, the absence of alkaloids in the crude methanolic extract of *C. molle* may have accounted for the very poor and insignificant deparasitizatioin observed with this fraction

Glycosides are non-reducing substances which on hydrolysis yield a non-sugar moiety and one or more sugars. The non-sugar part of the molecule is called aglycone or genin. It is this portion (aglycone) that is responsible for the fundamental differences in the pharmacological responses which are produced by the different glycosides (Brain and Turner, 1975). Cardio-active principles spiganthine isolated from a plant *Spigelia anthelmia* (Achenbach *et al*., 1995) was reported to have similar anthelminthic activity in reducing motility in the free living nematode, *Caenohabditis elegan* (Maryon *et al*., 1998). Glycoside extracted from the root of *Adhatoda vestica* was found to be effective against mixed gastrointestinal nematode infection in sheep

(Lateef *et al*., 2003). Cardiac glycoside was identified in the plants extracts used in this study. This compound has been shown to be responsible in inducing tonic contraction that resulted in the expulsion of the worms from the rats GIT (Kim *et al*., 1992; Hong, 2000).

Steroids were identified as the active principles of *Butea monosferma* seed and reported to have *in-vitro* anthelmintic activity against adult *Caenorhabditis elegans*, a free-living nematode (Prasharth *et al*., 2001). However, it is unknown whether the steroids identified in all the fractions of both plants could have had the same effect *in-vivo*.

A scientific valid investigation into acute toxicity is necessary and from many points of view of great value (Lorke, 1983). Thus, a maximum tolerated dose (MTD) trial, otherwise referred to as dose determination studies (Vercruysse *et al*., 2001), was carried out on the premise that the plant extracts under investigation had no alternative data to support any intended dosage. Therefore it was as stated by Lorke (1983), desirable to have a test to determine the toxicity or otherwise of the plant extracts. With this objective, a figure which expresses the toxic effect was sought. This figure was intended to indicate the amount of the substance which is injurious after a specific mode of intake. Since the term injuries always involves a high degree of subjectivility, an objective criterion was selected; namely death (Lorke, 1983).

In this work, the injurious dose and the MTD were determined. However, there were differences in toxicity between the two plants; as *A. africana* could not produce death even when the highest dose (5,000 $mgkg^{-1}$) was given. *C. molle* on the other hand

produced death effect at the dose of 1,600 to 5,000 $mgkg^{-1}$. Nevertheless the two plants had a common MTD of 1,000 $mgkg^{-1}$.

CHAPTER SIX

6.0 CONCLUSION

Result from this study demonstrated that the aqueous methanol of C. molle as well as the chloroform and N-butanol fractions of both plants is effective against experimental *N. braziliensis* infection in rats at a non-toxic dose of 1,000 $mgkg^{-1}$. The chemicals believed to constitute the active principles in *A. africana* and *C. molle* have significant anthelmintic efficacy whereas albendazole was found to be highly efficacious. However, the efficacies of these fractions were comparable to that of albendazole (a conventional anthelmintic) at a dose rate of 200 $mgkg^{-1}$. The *in-vivo* model was also found to be a useful tool for rapid screening of anthelmintic activity of plant preparations against nematode parasites. This parasite-host model has been exploited by the pharmaceutical industries in anthelmintic screening procedure for many years because it is relatively easy to maintain (Wahid *et al*., 1989)

The investigation of chemical compounds from natural products is fundamentally important for the development of new anthelmintic drugs, especially in view of the vast worldwide flora. Thus, a quality controlled extraction of *A. africana* and *C. molle* and the isolation of their bioactive compounds could be a promising alternative to conventional anthelmintic for the treatment of gastrointestinal helminths of ruminant in the future. Such a treatment could be used in control strategies against gastrointestinal nematodes in organic and conventional production systems.

One problem associated with the use of these plants in traditional medicine is lack of consistency of the dose. However, this was circumvented by evaluating different

doses of the crude methanolic extract in a maximum tolerated dose study inorder to reveal the appropriately non-toxic dose that was used in this study.

Factors unknown to us might have influenced the anthelmintic activity of these plant extracts. For instance, the rats might have generated a strong T. cell-depedent immune response that brought about expulsion of the worms from the intestine (Mitcell *et al.*, 1983)

Since a link has been established between *N. braziliensis* and *Trichostrongylus* of sheep and hook worm of dog and man; the result obtained in this study justify further investigation into anthelmintic effects of the various fractions of the plants in other animal species. Also since the life cycle of *N. braziliensis* is similar to that of parasitic nematodes of veterinary importance; it is likely that this model can be used to draw inference to related parasites of other monogastric animals such as dog, pig, horse and man.

In conclusion, most of the plants fractions tested in this study had the hypothesized reduction in TWC in rats, and thus recommended for further investigations in ruminants to validate their acclaimed efficacy.

6.1 RECOMMENDATIONS

More detailed studies are needed to isolate, characterize and evaluate the active components and the mechanism of action of the identified active principles. Also, studies on the toxicity, evaluation of the *in-vivo* effect of the aqueous fraction of *C. molle* (the most effective fraction in this study) against economically important gastrointestinal nematode species and the establishment of adequate doses for sheep, goats and cattle are needed.

Definitive proof of anthelmintic efficacy of any Ethno-Veterinary medicine preparations against gastro-intestinal parasites of ruminant livestock must be based on *in vivo* testing of such products against parasites species in the normal host (e.g. *H. contortus* in sheep or goats).

Involvement of traditional healers, herdmen and pastoralist is critical in future validation processes, especially when accurate information needs to be obtained from them.

To enhance free flow of information, building confidence with the traditional healer is vital. This most include the safeguarding of their interlectual property, as well as established a platform for reciprocal exchange of information and results from any research carried out.

The Forestry Research Institute should identify all plants claimed to have medicinal value by traditional healers and propagate them for re-planting to avoid possible extinction due high demand for such usage.

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