AN EVALUATION OF THE ANTI-COCCIDIAL POTENCY OF SOME ETHNOMEDICINAL PLANTS USED IN THE TREATMENT OF POULTRY DISEASES IN ZARIA, NIGERIA

BY

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OCTOBER, 2016

DECLARATION

I declare that the work in this Thesis entitled "An Evaluation of the Anti-coccidial Potency of some Ethnomedicinal plants used in the Treatment of Poultry Diseases in Zaria, Nigeria," was carried out by me in the Department of Zoology. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

| --- | --- | --- |
| Bashirat Ibrahim | Signature | Date |

CERTIFICATION

This Thesis entitled AN EVALUATION OF THE ANTI-COCCIDIAL POTENCY OF SOME ETHNOMEDICINAL PLANTS USED IN THE TREATMENT OF POULTRY DISEASES IN ZARIA, NIGERIA, by BASHIRAT IBRAHIM meets the regulations governing the award of the degree of Doctor of Philosophy of Science of the Ahmadu Bello University, Zaria and is approved for its' contribution to knowledge and literary presentation.

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ABSTRACT

The anti-coccidial potentials of crude extracts of some folkloric ethno medicinal plants used in the treatment of poultry diseases were evaluated andsome anti-coccidial constituents were isolated and characterized from them. A total of 19 plants belonging to 14 families associated with the treatmentof various poultry diseases were identified and tested for their anti-coccidial effects. *In vitro* anti-coccidial efficacies of the methanolic extracts of ten ethno-veterinary plants that are frequently used in Zaria were carried out on sporulated oocysts of *Eimeria tenella*; viability and population structure of the oocysts over a 96 hr period was observed. Five different dilutions of the respective crude extracts of ten ethnomedicinal plants and Amprolium® (1:25, 1:50, 1:100, 1:200, and 1:400) in distilled water were placed in separate Petri dishes labelled appropriately. 0.01ml of a suspension of freshly sporulated oocysts of *E. tenella* containing 500 oocysts was added to each Petri dish. The set up was kept at room temperature, semi-covered with periodic homogenization. 0.1ml suspension of each dilution was examined microscopically after 24, 48, 72 and 96 hours. Damaged oocysts and structure of parasite population were registered. Extracts of the different plants had anti-coccidial activities and there was a significant difference ($P< 0.05$) in the efficacies of the plant extracts. *Azadirachta indica*,*Nauclea diderichii*and*Solanum dasyphyllum* extracts exerted the greatest percentage (363) (72.50%), (466) (93.2%) and (453) (90.6%) oocyst lethality. The observed efficacy was concentration dependent with significant difference ($P< 0.05$) between concentration 1:25; 1:50;1:100; 1:200 as well as 1:400 concentrations. Phytochemical screening of these plantmethanolic extracts showed that they all contain alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, terpenoids and tannins with steroids found only in *S. dasyphyllum*. Acute toxicity study showed that the suspension of these extracts was not toxic when administered orally route in experimental birds at 5000mg/kg body weight. The effects of methanolic extract of *S. dasyphyllum*,*N. diderichii* and *A. indica* carried out in 180 coccidia-infected chickens revealed that all the infected birds in groups A1 to E1A2 to E2 as well as A3 to E3 showed clinical signs of depression, weakness, ruffled feathers and bloody diarrhoea, oocysts were detected in their faeces but absent in the non-infected birds (F1 to F3). (A1, A2 and A3 are*E. tenella*- infected birds and treated with Amprolium; B1, C1 and D1 are*E. tenella*-infected birds and treated with 1000mg/kg, 500mg/kg and 250mg/kg of *S. dasyphyllum* extract respectively; B2, C2 and D2*E. tenella*-infected birds and treated with 1000mg/kg, 500mg/kg and 250mg/kg of *N. diderichii* extract respectively; and B3, C3 and D3 *E. tenella*-infected birds and treated with 1000mg/kg, 500mg/kg and 250mg/kg of *A. indica* extract respectively;E1 –E3 are infected and untreated birds; F1-F3 are uninfected and untreated birds.After 7 day post treatment, no coccidia oocysts were found in the birds that had been treated with *S. dasyphyllum* and *N. diderichii* but scanty oocysts found in Amprolium and *A. indica*-treated groups. The birds in the infected, untreated groups were discharging oocysts of *E. tenella* up to the 14th day post infection. The fractions of *S. dasyphyllum* and *N.diderichii* as well as isolated substances, substance form Fractions from *S. dasyphyllum* and *N. diderichii* methanolic extracts respectively (BS1 and BS2) were tested for their *in vitro* anti-coccidial efficacies in comparison with Amprolium (the universal drug). Fractions 7 and 8 of the six *N. diderichii* fractions showed the highest anti-coccidial activity against sporulated oocysts of *E. tenella*. Fractions 6 and 9 of the six fractions of *S. dasyphyllum* were more efficient against sporulated oocysts of coccidia. BS2 was more potent against sporulated oocysts of *E. tenella* than BS1. The active anti-coccidial principles in the plants under study were characterized and contain betulinic and ursolic acids.Crude methanolic extracts of ten ethnoveterinary plants

frequently used by poultry farmers in Zaria; fractions and substances from two (*S. dasyphyllum* and *N. diderichii*) most active plant extracts had anti-coccidial efficacies.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 General Introduction

The plant world is extremely diverse and many plants have a lot of known benefits. Medicinal plants are plants in which one or more of their organs contain substance(s) that can be used for therapeutic purposes or precursors for pharmaceutical synthesis (Sofowora, 1984).

The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs being used in the treatment of diseases for revitalising body system in almost all ancient civilizations, the Egyptian, the Chinese and even Greek and Roman civilizations (Aftab and Sial, 1999). Herbs normally used are picorhiza, garlic, cloves, slippery elm, neem fruit and leaves, sophora flavescens, nutmeg, cinnamon, ginger, peppermint, sage, thyme, mustard and fenugreek. These plants are used as digestive stimulants, antidiarrhoic, antiseptic, anti-inflammatory, antiparasitic and appetite stimulants in human beings as well as animals (Tipu *et al*., 2006). A significant proportion of pharmaceutical products used currently are derived from plants (Cowan, 1999; Rankin *et al.,* 2002). For instance more than 140 compounds have been isolated from neem tree alone (Virginie, 2010). Different plant phytochemicals such as organic acids, tannins and essential oils seem to be candidates of interest as alternative to antibiotic growth promoters (Efterpi *et al*., 2012). These have been shown to reduce gastro intestinal infections and increase performance in birds (Aengwanish and Suttajit, 2010; Efterpi *et al.,* 2012).

Plants are used medicinally in different countries and are sources of many potent and powerful drugs (Lagu and Kayanja, 2010; Shivaramaiah *et al*., 2014).

Medicinal plants aid in healing of several diseases in poultry chickens such as diarrhoea, mange, coccidiosis, Newcastle diseases, coccidiosis, lice and tick infestation among others because the values of plant lies in some chemical substances called bioactive molecules or ingredients from plants extract (Okwu,1999; 2001; Olanipekun, 2011).

Poultry production is the most widespread in the world that forms an important component of small farmers' livelihoods as a tool for poverty alleviation (Dolberg, 2007). Birds are kept in a low input or low output system, with the available scavenging feed base supplemented with food scraps and grains (Sonaiya, 2007). Birds and their by-products are usually consumed by their owners, sold locally and used as gifts (FAO, 2005).

Poultry production though widespread is also faced with wide range of problems among which is infectious diseases. Poultry coccidiosis is the most studied of these infectious diseases as it has been considered a very harmful disease which affects growth and performance of birds in poultry (Bachaya *et al*., 2012).Coccidiosis is transmitted by direct or indirect contact with droppings of infected birds. New hosts are infected when they ingest sporulated oocysts in contaminated food or water supplies. This is referred to as faecal and or oral transmission (Guèye, 2003).

Coccidia parasite causes severe damage in chicken poultry production where chickens are reared in large numbers and high densities (Peek, 2010). Young individuals fall ill frequently, while older birds tend to be carriers (Shivaramaiah *et al*., 2014). Its causative agent is a single-celled protozoan belonging to genus *Eimeria* (Blake and Tomley, 2014; Chapman, 2014; Shivaramaiah *et al*., 2014).

Nine species of *Eimeria* are widely recognized to infect poultry chickens and each species has its own characteristics according to site of infection, immunogenicity and

pathogenicity. These include *E. tenella, E. hagani, E. mivati, E. necatrix, E. maxima, E. brunnetti, E. mitis, E. praecox* and *E. acervulina* (Taylor *et al.,* 1995). The two most common species that affect poultry production worldwide are *E. acervulina* and *E. tenella,* which are the causative agents of upper intestinal and caecal coccidiosis respectively (Virginie, 2010).The main problem with *Eimeria* infections is that they are caused by more than one species that attack different regions of the intestine. The use of several drugs, alone or in combination, has proven to be an effective alternative in the struggle against avian coccidiosis (Quiroz-Casta˜neda and Dant´an-Gonz´alez, 2015).

Coccidiosis causes heavy economic losses to commercial poultry farmers and is thought to be one of the most expensive infectious diseases of poultry (Masood *et al*., 2013; Chapman, 2014). An estimated $800 million is lost world wide in the poultry industry annually as a result of morbidity and mortality associated with coccidiosis (Oluyemi and Roberts, 2000). These include costs of prophylaxis, medications, losses of productivity due to mortality, morbidity and lowered feed conversion (Williams, 1998; Chapman, 2009).The disease is clinically characterized by bloody diarrhoea, poor feed conversion ratio, low growth rate or poor weight gain. This has also been considered a contributory factor in the pathogenesis of other diseases (Bachaya *et al*., 2012).

In external environment, farmers usually fight this disease by applying disinfectant compounds. Commonly used disinfectants include some phenolic products such as ammonia, methyl bromide and carbon disulphide. The toxic effects of these products represent a danger to the poultry staff and health of birds and therefore their use has been restricted (Hilbrich, 1975; Williams, 2004).

1.2 Statement of the Research Problem

The anti-coccidial efficacy and phytochemical properties of some ethno medicinal plants used in the treatment of poultry diseases in Zaria have not been scientifically evaluated and therefore not fully known.

1.3 Justification

The African environment is probably the least explored in terms of available plant resources that are not yet tapped (Cowan, 1999). The use of medicinal plants all over the world started before the introduction of antibiotics and other modern drugs in the African continent (Akinyemi *et al*., 2005).Due to the widespread of drug resistance constraint, residual effects of drugs in meat of birds and toxic effects of disinfectants, scientists all over the world are shifting towards alternative approaches for the control of parasitic problems (Akhter *et al*., 2014; Hamad *et al*., 2014).The use of medicinal plants in the treatment of diseases have generated renewed interest in recent times, as herbal preparations are increasingly used in health care systems to treat various animal diseases especially poultry (Xiao *et al*., 2014).

Local farmers in Nigeria and Zaria in particular use ethno veterinary medicine (EVM) to manage chicken diseases and parasites.Ethno-veterinary practices appear to work especially to the practitioners. The plant materials are available, cheap and easy to prepare in the home at no cost and administer. Herbal medicines are also known to be broad spectrum, some have potent properties and complex bioactivity (Zhang *et al*., 2012)and may be the future answer to resistance of pathogen to convectional drugs (Mwale *etal*., 2005).Over 300,000 species of flowering plants have been recorded worldwide. So far, less than 1% of them have been explored for use against

protozoanparasitic diseases and the African continent including Nigeria have record of the least exploitation of these plants (Thangarasu *et al*., 2016). The scientific basis for the claim of potential has not been verified and hence the study. It is necessary, therefore, to validate the usefulness of the practice of the use of plants by local and poor farmers to treat poultry diseases towards establishing a source of developing drugs for treating coccidiosis. This research work was therefore, carried out with the following aim and objectives as stated below.

1.4 Aim

To evaluate the anti-coccidial potentials of some crude extracts of some folkloric ethno medicinal plants used in the treatment of poultry diseases and to isolate and characterize some anti-coccidial constituents.

1.5 Objectives

This study was designed to determine:

i the folkloric plants used in the treatment of poultry diseases in Zaria;

ii the anti-coccidial activity of the methanolic crude extracts, fractions and substances of some selected folkloric plants;

iii the phytochemical constituents of the methanolic crude plant extracts;

iv isolate some of the active anti-coccidial principles in the plants and

v characterize some of the active anti-coccidial principles in the plants understudy.

1.6 Research Hypotheses

i There are no significant folkloric plants used in the treatment of poultry diseases in Zaria.

ii Methanolic crude plant extracts, fractions and isolated substances do not have significant anti-coccidial activity.

iii There is an insignificant difference in the phytochemical constituents of methanolic crude plant extracts.

iv There are insignificant active anti-coccidial principles in the plants selected.

v There are insignificant substances in the plants selected.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Management

Backyard poultry production is the most widespread form of poultry keeping in the world because it forms an important component of small farmers' livelihoods (Dolberg, 2007; Sonaiya, 2007). Birds are kept in a low input/low output system, with the available scavenging feed base supplemented with food scraps and grains. Birds and their by-products are usually consumed by their owners, sold locally and used as gifts (FAO, 2005). These practices represent favourable conditions that make poultry production more susceptible to receiving and spreading infectious diseases such as respiratory, gastrointestinal, external parasites and coccidiosis (Iqbal, 2009). Sick birds may be handled, sold, slaughtered and consumed without considering that the infections may also potentially be harmful to man (Iqbal, 2009).

Traditional medicine is preferred to commercial drugs because poultry farmers believe that the local medicines are also efficient, cost effective readily accessible and without eminent resistance. In addition, no transport costs are incurred, it is easy to administer, there are no known dosages, there is minimal risk of drug overdose and when prompt treatment was administered then the birds were healed and the birds benefited from supplementation from vitamins (Moreki, 2013).

The disadvantages experienced by the farmers following the use of medicinal plants include: the difficulty of getting plants during the dry season, delay in the recovery of the birds post herbal drug administration, difficulty in preservation of the herbal concoctions, ineffective concoctions unknown dosages therefore not standardized, may be harmful to persons preparing them, the tedious process of preparing herbal

concoctions for many birds. Therefore the diseases treated are unknown because of poor diagnosis (Sonaiya, 2007).

2.2 Diseases of Poultry

Poultry diseases of various kinds permanently occur in all corners of the world with some having regional impact, while others spread further (Sonaiya, 2007). The common diseases of poultry include Newcastle Disease (NCD), coccidiosis, salmonellosis, helminthosis or worm infections and bronchitis while the common parasites include mites and fleas (Gabanakgosi, 2012). Poultry diseases continue to represent a serious loss to poultry producers in all areas of the world (Okitoi *etal*., 2007). In our society today however, a large number of poultry chickens are affected with different types of sicknesses and diseases which affect different parts and organs in their body (Olanipekun, 2014) and the major constraints faced by family chicken rearers are disease outbreaks and parasites.

2.3 Historical Perspective of Coccidiosis

Eimeria, the causative agent of coccidiosis was named after the German Zoologist Theodore Emer. The oocysts of *Eimeria steidai* were first seen by Antonni van Leeuwenhoek in the bile of a rabbit in 1674 (Irvine, 1961). The life cycle of a coccidian parasite in birds was described by Fantham in 1910 who was working for an enquiry into diseases that were affecting the red grouse. The basic understanding of coccidiosis as a disease caused by several species of the protozoan *Eimeria* came from publications of two poultry pathologists between 1910 and 1938. The contributions of these scientists (W.T Johnson and E.E Tyzzer) laid the foundations for understanding coccidiosis as a disease complex involving several distinct species of coccidia (Reid, 1990). Despite the growing relevance of the poultry industry and the realization of the

importance of coccidiosis as a disease of fowls, little further work was carried out in the United Kingdom until 1940 (Chapman, 2003).

Approximately, 40 billion chickens are raised in the poultry industry yearly and coccidiosis is the most frequently reported disease of chickens all over the world (Williams *et al*., 1999). Coccidiosis is known to affect almost every species of animal on earth, but first attempts at treating this disease dated back to its outbreak in poultry in 1944 (Teixeira *et al*., 2004). The drug of choice for the treatment of coccidiosis (then) was sulphanimides based on availability. Through further experimentations, poultry producers found, in the late 1940s, that the most economical method of preventing and controlling the disease was by the continuous usage of sulphaquinoxalines included in the feed of chickens (Chapman, 2003). These chemicals reduced the mortality caused by the protozoan parasite and also lessened the morbidity of the disease in poultry. Since the 1950s, various types of anticoccidials have been produced from different drugs and chemicals. Amprolium and nicarbazine are some of the older chemicals that are still in use today. Most of the chemicals are no longer in use or allowed in various countries. This is because of proven toxicological findings or lack of efficacy due to development of resistance by the coccidian it was designed to destroy (Williams *et al*., 1999; Abbas *et al*., 2012).

A new class of antibiotics was discovered in the 1970s and was named ionophores which eventually replaced the earlier chemical compounds. Ionophores can permit a small amount of coccidia to survive and complete their life cycle within the intestines of the bird thus enabling the birds to develop certain levels of immunity. This allows a greater degree of protection against the parasite and is a very efficient method of control. Therefore, ionophores were considered unique (Shirley and Lillehoj, 2012).

However, these more recent development were still not able to address the issue of resistance, and soon enough, most species of coccidia had developed resistance to all the ionophores available in the poultry industry. Because of this problem of drug resistance, poultry producers all over the world are moving towards alternative control of coccidiosis (Abbas *et al.*, 2012).

However, coccidia were first studied and described during the 19th century and *Eimeria steidai* was the most important subject of investigation. Leuckast (cited according to Levine, 1973) distinguished liver and intestinal coccidian of rabbits and were named *Coccidium oviforme* (now *Eimeria steidai*) and *Coccidium perforans*. During the 20th century, many outstanding coccidiologists, such as Yakimoff, Pellerdy, Cheissin, Rose, Scholtyseck, Coudert, Norton, Gregory studied coccidiosis of rabbit at some periods of their life time (Levine, 1973).

2.4 Major Groups and Classification

Coccidiosis is caused by *Eimeria* species which are members of the Protista, phylum Apicomplexa, family Eimeriidae. All the members are monoxenous parasites that infect one or a few closely related vertebrate hosts (Fayer, 1980). In poultry, *Eimeria* infect various sites in the intestine and are highly specific. Nine species of *Eimeria* are widely recognized in chickens. These include *E. tenella, E. hagani, E. mivati, E. necatrix, E. maxima, E. brunnetti, E. mitis, E. praecox* and *E. acervulina* (Taylor *et al.,* 1995). Seven species of coccidian also affect turkeys which are *E. adenoides, E. dispersa, E. gallopavoni E. meleagrimitis, E. innova, E. meleagridis* and *E. subrotunda* (Levine, 1982).

A large number of specific coccidia have been reported in both wild and domestic ducks, but validity of some of the descriptions is questionable. Some species of coccidia

of domestic ducks are considered relatively non-pathogenic. In wild ducks, infrequent but dramatic outbreaks of coccidiosis occur in ducklings 2-4 week old (Shirley *et al.*, 2005). The causative organisms are classified according to certain characteristics which include possession of an environmentally resistant oocyst wall and also the number of sporocysts within an oocyst. The number of sporozoites that are developing within a sporocyst is also genus dependent (Lee *et al.*, 2000). Eimeriid coccidian contains a steidia body at the anterior pole of the sporocyst and a retractile body within sporozoites, while these features are not present within isosporoid coccidian (Barta, 2001), there is the possession of two polar rings within the apical complex (D'Haese *et al*., 1997). Microtubules exude from an outer polar ring but do not reach the posterior of the cell and their number is species dependent (D'Haese *et al*., 1997). Sporozoites and merozoites are surrounded by a tri-layered pellicle. The outer membrane is continuous over the entire parasites but the inner membranes are disrupted due to the presence of a microspore towards the anterior end (D'Haese *et al*., 1997). Sporozoites and merozoites contain an apical complex anterior to the cell. The cell contains a conoid structure which is made of tumbling fibers (Hu *et al*., 2002; Lee *et al.*, 2000).

Traditionally, coccidia include additional odd parasites from marine invertebrates and are classified into separate classes Protococcidiorida (Syn Ceolotrophida) or Agamoccocidiorida. This classification is based on absence of merogony and absence of both merogony and gamonts respectively. These are extremely obscure species and are classified into two suborders (suborders Adeleorina and Eimeriorina).

Suborder: Adeleorina, Leger, 1911 (Irvine, 1961).

Macrogametes and microgametes are usually associated with syzygy and microgamont produces small number (1-4) of microgametes. The sporozoites are enclosed in oocyst,

endodyogeny is absent, life cycle is either homoxenous or heteroxenous and about 500 species are named (Hu *et al., 2002*) and four families are recognized. These are Adeleidae, Hepatozoidae, Klossiellidae and Haemogregarinidae (Lee *et al.,* 2000).

Suborder: Eimeriorina, Leger, 1911 (Irvine, 1961).

Macrogamete and microgamete develop independently, syzygy absent and microgamont produces large number of microgametes. Zygote is not motile, sporozoites are enclosed in sporocyst, endodyogeny may be absent or present, the life cycle may be homoxenous or heteroxenous and about 2,500 species are named (Lee *et al*., 2000).

This suborder consists of up to eight families (Lee *et al.,* 2000) and four of which are Eimeriidae, Sarcocystidae, Aggregatidae and Lankesterellidae.

Majority of coccidian parasites belong to the suborder Eimeriorina (Lee *et al*., 2000). Together with the smaller suborder Adeleorina, they make up the order Eucoccidiorida (Levine, 1982) which is a collective group of coccidian known to parasitize vertebrates and invertebrates (Lee *et al.,* 2000). The suborder represents the majority of coccidian parasites of human and veterinary importance, which includes the families Eimeriidae and Sarcocystidae (Lee *et al.,* 2000; Barta, 2001).

2.5 Aetiology and Morphology

Coccidia are almost universally present in poultry-raising operations. However, clinical disease occurs only after susceptible birds ingest relatively large numbers of unsporulated oocysts (Chapman, 2003). Clinically infected as well as recovered birds shed oocysts in their droppings which contaminate feed, dust, water, litter and soil. The oocysts may be transmitted by direct or indirect i.e. mechanical carriers (such as equipment, clothing, insects, farm workers and other animals). Fresh oocysts are not infective until they sporulate under optimum conditions [70-90^{0}F (21-32^{0}C)] with adequate moisture and oxygen within 1-2 days and the prepatent period is 4-7 days.

Sporulated oocysts may survive for long periods, depending on environmental factors. Oocysts are resistant to some disinfectants commonly used around livestock but are killed by freezing or high environmental temperatures (Williams *et al*., 1999).

Pathogenicity is influenced by host genetics, nutritional factors, concurrent diseases and species of the coccidia. *Emeria necatrix* and *E. tenella* are the most pathogenic in chickens, because schizogony occurs in the lamina propria and crypts of Lieberkühn of the small intestine and caeca respectively thereby causing extensive haemorrhage. Most species develop in epithelial cells lining the villi. Protective immunity usually develops in response to moderate and continuing infection. True age-immunity does not occur, but older birds are usually more resistant than young birds because of earlier exposure to infection (Adene and Oluleye, 2004).

The coccidian parasites have three developmental stages known as schizonts, gamonts and oocysts (Woods *et al.,* 2000). Schizonts range in size depending on parasite species, location (predilection site) in the host and stage of majority. The parasites start as small basophilic rounded cells (mother meronts) which are located intracellularly within the cells of their hosts. The meronts form numerous daughter merozoites by endogenous division of the nucleus followed by cytokinesis (Lopez *et al.,* 2007). Mature schizonts appear as membrane bound clusters of small basophilic bodies (similar to bunches of grapes). Individual schizonts usually range in diameter from 10-100μm but some species form enormous megaloschizonts (up to 1mm in diameter) (Smith*et al*., 2002). Gamonts exhibit sexual differentiation that is microgamonts (♂) and macrogamonts (♀). Microgamonts are obvious (apparent) as multinucleate basophilic stages and shedding small biflagellated microgametes. Microgametes appear clear (evident) as uninucleate eosinophilic cells with a single ovoid nucleus (Woods *et al.,* 2000).

Developing oocysts contain numerous eosinophilic wall-forming bodies which give rise to the tough outer oocyst walls. Unsporulated oocysts contain a developing sporoblast which eventually sporulate to form sporocysts which contain the infective sporozoites. *Eimeria* oocysts exhibit a characteristic 1:4:2 configurations, that is, each oocyst contains 4 sporocysts and each sporocyst in turn contains 2 sporozoites. Oocysts are generally void to ellipsoid in shape and range from 10-40μm in length by 10-30μm in width. They may contain specialized structures such as polar caps, micropyles, residual and crystalline bodies (Reid and Long, 1979).

2.6 Distribution

Coccidiosis is one of the most prevalent protozoa infections in North America. *Eimeria* and *Isospora* are the two genera that are often referred to as coccidian. They contain a large number of species that infect different animals throughout the world. There is a higher concentration of the parasite in organically reared chicken than chickens given medicated food in France (Williams *et al*., 1999). A wide variety of mortality rate was recorded in United Kingdom flocks. These rates ranged from 2.5% to 30% in the first two weeks (Arczewska Wlosek and Swiat Kiewicz, 2012). In a Danish survey, severe problems of coccidiosis were observed in 3 out of every 24 organic table bird flocks (Smith *et al*., 2002). In Dutch organic layer flocks, only 0.01% was found to contain large numbers of coccidian oocysts (Woods *et al*., 2000).

In Nigeria, epidemiological studies have established the economic importance of coccidiosis as a major parasitic disease. It occurs throughout the year in northern Nigeria, with higher prevalence rates from May to September (Abdul, 2007). Outbreaks

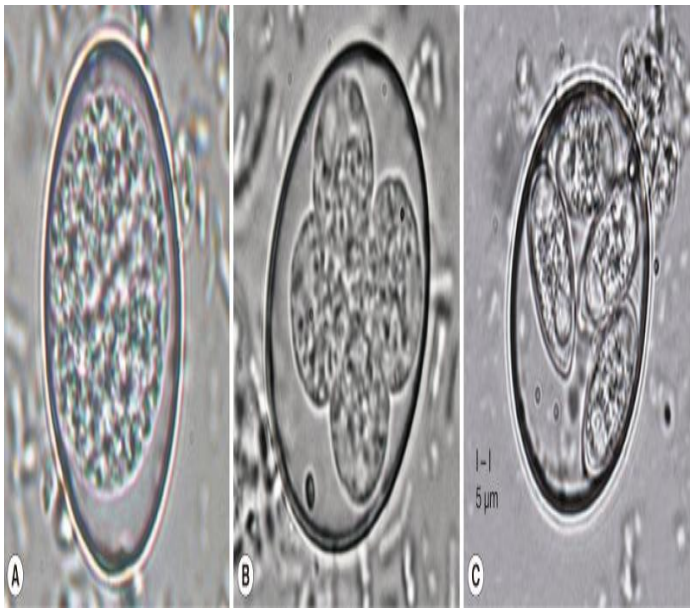
of up to 50% mortality were reported in a commercial poultry farm in Zaria, Nigeria (Musa *et al*., 2010).

In the United States, the most common *Eimeria* species in turkeys were recorded (Cervantes, 2006). Nine species of coccidian in cattle was identified in Japan, twelve species of coccidian in European cattle and *Eimeria* infection in first grazing season cattle in Sweden. Coccidiosis was also recorded in three localities in South Africa (Toulah, 2007).

Prevalence studies conducted in Benelux, France, Germany, Hungary, Italy, Poland, Portugal, Czech Republic and Spain showed high incidence of pathogenic coccidian species (*Eimeria bovis* and *E. zuernii*) in cattle (Nematollahi *et al*., 2009).

2.7 Disease Outbreaks

Coccidiosis outbreaks usually result in enormous economic losses due to the associated morbidity and mortality (Oluyemi and Roberts, 2000). Several factors affect the outbreaks of coccidiosis. Some of these factors include the parasite, the host and the environment.



Oocysts of *Eimeria tenella*.

A- Non- sporulated oocysts (×1,000); B-sporulating oocysts (×1,000); C- sporulated oocyst (×1,000) Bar = 5µm. Source: AlJumaili, 2013.

The hosts are the chickens selected for their zoo technical performance are particularly sensitive to coccidia. The intensively reared birds are more vulnerable to coccidiosis. All these assist in the outbreaks of coccidial infection (Virginie, 2010).

A flock of animals (birds) will develop a resistance to the exposed coccidia species after an outbreak of a specific species of coccidia. However, they remain susceptible to other infective species. This implies that a flock may experience several outbreaks of coccidiosis, each been caused by a different species of coccidian (Lompo *et al*., 2007).

An outbreak of coccidiosis can be economically devastating which is exacerbated by poor management practices and result in increased coccidiosis outbreaks. Weaning, drastic weather changes, rapid feed changes, transport and rough handlings are the factors that may induce outbreak of coccidiosis (Virginie, 2010). A severely contaminated environment, stress-related depression of immunity and pathogenicity (ability of the parasite to cause harm) of the coccidia species involved are the three most common factors that may contribute to coccidiosis outbreaks (Jackie, 2003).

2.8 Enzootic Stability

Eimeria and *Isospora* are the two genera that contain a large number of species that infect different animals throughout the world causing coccidiosis. This disease is one of the most prevalent protozoan infections in North American animals, second only to *Gardia* (Raymond and Patricia, 2000). All coccidia that infect mammals are considered to be host-specific but there are limited exceptions. Thirty-one species are known to occur in bats (*Chiroptera*) and two in turtles. Only two species of coccidia infect seals (i.e. *E. phocae*, and *E. weddelli*), five species (*E. alpacae, E. ivitaensis, E. lamae, E. macusaniensis and E. punonensis* infect *Ilamas* and alpacas while a hundred and thirty named species are known to infect fish. Also, a number of species infect rodents (*E.*

couesii, E. kinsellai, E. palustris, E. ojastii, and *E. oryzomysi*. *E. necatrix* and *E. tenella,* infect poultry, *E. steidai* infect rabbits (Donald *et al*., 2010).

The first known protozoan was coccidian when Leeuwenhoek observed oocysts in the liver of rabbit in 1674. This coccidium was named *Emeria steidai*. Eleven species infect rabbits. These are: *Emeria perforans*, *E. coecicola*, *E. exigua, E. flavescens, E. intestinalis, E. irresidua, E. magna, E. media, E. piriformis, E. vejdouskyi*, and *E. steidai. Emeria neoleporis* was described in cotton tail rabbits (*Sylvilagus floridanut*) but can be easily transferred to tame rabbits (*Oryctolagus cuniculus*) (Carpenter *et al*., 1984).

A wide variety of birds may harbour coccidia but disease is not common in free-ranging wild birds. Several epizootics due to *E. aythyae* have been reported among lesser scarp in the United States (Windingstad *et al*., 1980). The most common *Eimeria* species in turkeys are *E. meleagrimitis, E. dispersia* and *E. adenoeides* (Fitz-Coy, 2006). Various bovine coccidia species occur in cattle all over the world. Some clinically important bovine coccidia species are *Eimeria bovis, E. zuernii, E. alagamensis* and *E. ellipsoidalis* (Donald *et al*., 2010).

2.9 Transmission of Coccidiosis

Susceptible animals contact coccidiosis either by eating oocysts in vegetation or spread by insects, dust, wild birds and humans (from shoes and equipments) (Levine, 1982). Oocysts excreted with host faeces contaminate the external environment which must undergo internal sporulation (formation of sporozoites) before they become infective. This sporulation occurs within one to three days if the litter is warm and damp but can take much longer if the conditions are cool and dry. New hosts are infected when they

ingest sporulated oocysts from contaminated food or water supplies. This is known as faecal-oral transmission (Lopez *et al*., 2007). Oocysts and sporocysts excyst in the intestines of host following ingestion to release their sporozoites which involve host cells to begin merogony. Excystation is stimulated by appropriate post-gastric physicochemical conditions such as oxygen levels, pH, bile salts and pancreatic enzymes (Jatau *et al*., 2012).

2.10 Diagnosis of Coccidiosis

Clinical disease is not usually manifested until cumulative tissue damage associated with second or third generation schizogony is obtained. Moderately-affected animals may show progressive signs such as poor weight gain or loss, weakness and emaciation while severely affected individuals may die soon after the appearance of disease (Williams, 2002). Definite diagnosis is made from the microscopic examination of scrapings of the digestive tract and identification of the coccidian organisms. This is necessary because it is common for healthy birds to possess some coccidia. Thus, history of flock and lesions should be considered before making diagnosis and treatment recommendations (Chapman, 2003). Molecular techniques have been used by researchers to characterize genetic variation between and within parasite species but few techniques are suitable for routine diagnostic use (Shirley, 1986). Young animals are most susceptible to clinical disease, although survivors may develop strong specific protective immunity against subsequent infection and disease (Williams *et al*., 1999).

2.11 Pathogenesis and Pathology of Coccidiosis

Lesions of *E. maxima* comprises of multiple petechial (pin-point size) haemorrhages which are often seen from the outside of the mid-gut area. In addition, segmental ballooning or enlargement of the mid gut with presence of orange-tainted mucous may

be noted (Cervantes, 2002). However, lesions are more difficult to identify than those caused by *E. acervulina* and *E. tenella* unless the lesions are typical.

Most species of *Eimeria* are not significant pathogens and cause little or no disease but some species are highly pathogenic. These cause catarrhalic or haemorrhagic enteritis by severe erosion of the mucosal membranes through cell lyses which result in profuse watery-to-bloody diarrhoea (Arabkhazaeli *et al*., 2011). Pathogenicity depends on factors such as parasite species, viability, infectivity, virulence, tropism, host age, nutritional status, immunological competence prevailing environmental conditions (temperature moisture) and management practices.

Only seven out of nine species of coccidian that affects domestic chickens (*Gallus gallus domesticus*) are considered pathogenic to poultry. These are *E. acervulina, E. brunette, E. maxima, E. mitis, E. necatrix, E. praecox* and *E. tenella.* These pathogenic species are widely distributed worldwide and can cause infection simultaneously. Thus, disease (coccidiosis) caused by *Eimeria* species better represents a disease complex (Williams *et al*., 1999). The severity of coccidiosis in immunologically naive birds is directly related to the dose of infective stages that are ingested (Reid, 1990). Oocysts ingested in thousands by susceptible birds will produce clinical signs but oocysts ingested in hundreds may only produce subclinical infection (Reid, 1990). This relationship between number of oocysts ingested and consequent severity of infection occurs because coccidian parasites (*Eimeria* species) produce self-limiting infections with a genetically predetermined number of a sexual and pathogenic cycles (Cervantes, 2002). Pathogenicity is also related to the strain i.e. species of the parasite.

The most common form of the disease is caecal coccidiosis that is caused by *Eimeria tenella* which normally occurs between 4 and 6 weeks post hatching. A small but sudden rise in mortality may occur and dead birds will have an anaemic appearance. There is tissue damage and changes in intestinal tract as a result of infection. This may allow colonization by other harmful bacteria such as *Clostridium perfringens*, a causative agent of necrotic enteritis or *Salmonella typhimurium* (Kayla and Barta, 2010). Caecal coccidiosis may also produce a ballooning of the caecal pouches that is filled with free blood. A later stage is characterized by caeca that are filled with a material with cheesy consistency and being tinged with variable amounts of blood. Lesions of intestinal coccidiosis vary from rather mild enteritis to a severe necrotic or haemorrhagic type (Cervantes, 2006).

2.12Treatment and Control

It is almost impossible to remove or eliminate oocysts from a farm environment. Hence, control should focus on producing environmental conditions that would reduce the number of contaminating oocysts (Lopez *et al*., 2007). Sporulation of oocysts can be minimized by keeping litter dry but not dusty. The location of watering facilities is very critical in controlling the dampness of litter or kennel. Also, keeping age groups separate would assist in reducing the ingestion of oocysts. This is because older animals may produce oocysts that may be lethal when ingested by young animals (Adene and Oluleye, 2004). Coccidia oocysts can withstand disinfection but they are killed by noxious gases such as ammonia and methyl bromide (Shirley *et al*., 2005).

For commercial poultry, it is recommended that the land should be rested for two months in meat poultry and laying hens per year. However, this land resting period do not apply to farmers that keep small numbers of poultry such as less than 50 birds

(Williams, 2002). Outbreaks can generally be controlled by management practices based around improving hygiene, reducing crowding, removing contaminated litter and isolating infected individuals. Practical methods of management cannot prevent infection but combined approaches should be carried out. Poultry that are maintained at all times on wire floors to separate birds from droppings have less infections and clinical coccidiosis is very rare under such circumstances (Kayla and Barta, 2010).

Development of disease is so rapid that any curative (therapeutic) treatment may be too late. Thus, continuous in-food or in-water medication is often used for prophylactic (preventive) treatment in many intensive animal industries (Chapman *et al*., 2010). This controls the growth of coccidia in the digestive tract. Many coccidiostats are available commercially including those with coccidiostatic (reversible lethal) activity. However, coccidiostats should not be indiscriminately used and recommendations must be followed precisely because of parasite resistant to the drugs (Kayla and Barta, 2010).

An intake of plant-based exogenous anti-oxidant can assist to limit oxidative stress and also prevent the damage caused by free radicals on cell lipid and protein constituents and also on genetic material. This is done at the acute phase of primary coccidia infection (i.e. 5-7days post infection). This usually corresponds to oocysts shedding (Williams, 2002). Some natural compounds that possess anti-oxidant properties have been shown to be useful in the fight against coccidia. For example, grape seed proanthocyanidin used as protection against *E. tenella* showed positive effect on body weight, mortality and lesion scores (Virginie, 2010).

Coccidiosis is generally controlled by using anticoccidial drugs which are administered in feed of chickens (Blake and Tomley, 2014; Shivaramaiah *et al*., 2014). Some common anticoccidial drugs include Dufacox®, Pluricoccin®, Diclazuril®, Amprolium®,

(Haq *et al*., 2010). Success has been achieved by using these drugs but the main problem associated with their poor response is development of resistance in *Eimeria* species to the commonly available anticoccidial drugs (Abbas *et al*., 2012).

These constraints can be alleviated by the use of conventional drugs which are expensive and out-of-reach of rural farmers for the health management of chickens (Mwale *etal*., 2005). The existing conventional disease control programs also favour the high investment intensive systems of production with birds in confinement and not small-scale farmers (Ather, 2011). As a result, farmers use ethno veterinary medicine (EVM) to manage family chicken diseases and parasites. The wide use of traditional remedies by family poultry rearers is also attributed to lack of knowledge in the use of vaccines, lack of cooling facilities, unavailability of vaccines, and possible effectiveness of the remedies in curing some diseases in some parts of the world (Moreki, 2013).One of the advantages of using natural extracts is the lower risk of developing resistance, such as that observed with chemical drugs (Abbas *et al*., 2012). It is widely known that the availability of raw materials and the cost of production could be high in the development of natural extract alternatives. However, the cost is well worth it since these alternatives are believed to be friendly to the environment, producers, and consumers (Łowicki and Huczynski, 2013).Since ancient times, plants and plant parts have an indispensable source of medicine for indigenous poultry production systems (Hamilton-West *et al*., 2012).

2.13 Chemotherapy of Coccidiosis

The management of animals to allow natural immunity to develop is the best method of control. However, this requires high levels of vigilance and good management to avoid

any outbreaks of the disease. Any animal or group of animals that become infected can be treated therapeutically using drugs (anticoccidials) (Chapman, 2009). The main drug groups include sulphonamides (sulphanilamide, trimethoprim, and ethopabate), pyridinoles (clopidol, decoquinate), nitrobenzamides (zoalene), organic arsenicals (roxarsone), nitrofurans (furazolidone, Amprolium), quinazolinones (halofuginone), polyether ionophores antibiotics (monensin, laslocid, salinomycin, narasin), asymmetric (diclazuril) and symmetric (toltrazuril) triazines (Reid, 1990).

Regrettably, there are mounting problems being encountered with drug resistance amongst many coccidian species. This is common especially against synthetic drugs which tend to persist within parasite populations. Many industries recommend periodic rotation between different drug groups and the use of combination (cocktail) drugs to minimize the occurrence of resistance (Johnson and Reid, 1990).

2.14Symptoms and Clinical Signs

Young animals are most susceptible to clinical disease, although survivors may develop strong specific protective immunity against subsequent infection and disease (Williams *et al*., 1999). Clinical findings range from decreased growth rate to a high percentage of visibly sick birds, severe diarrhoea, and high mortality. Feed and water consumption are depressed (reduced), weight loss, development of culls and decreased egg production may accompany outbreaks. Mild infections (subclinical) of intestinal species may cause depigmentation (Cervantes, 2006).

The most easily recognized clinical sign of severe caecal coccidiosis is the presence of bloody droppings which may be accompanied by dehydration (Kayla and Barta, 2010). Caecal coccidiosis caused by *E. tenella* first becomes noticeable at about three days

after infection. Chickens droop, stop feeding, huddle together and by the fourth day, blood begins to appear in the droppings. The greatest amount of blood appears by day five or six, and by the eighth or ninth day, the bird is either dead or on the way to recovery. Mortality is highest between fourth and sixth days. Death may occur unexpectedly due to loss of excessive blood. Birds that recover may develop a chronic illness as a result of persistence caecal core. However, the core usually detaches itself by eight to ten days and is shed in the droppings (Jatau *et al*., 2012).

Drug resistance, especially to chemical anticoccidial drugs added to feed or feed mixing errors may lead to clinical coccidiosis. Birds with clinical cases of coccidiosis frequently display typical "sick bird" attitude with depression, prostration, huddling under the heat source as if chilled, soiled vents and watering or bloody droppings. One of the first signs of clinical and even subclinical coccidiosis may be paleness (Shirley, 1995).

Post mortem examination of intestinal tract and the caeca for the presence of gross lesions in dead hosts (such as birds) is necessary to ascertain coccidiosis. Gross lesions caused by *E. acervulina* are usually the most prevalent. These are usually confined to the upper part of the small intestine (duodenum) and sometimes may extend to the mid-gut (jejenum). The lesions have a unique appearance which consists of white patches or transverse white lines inside the gut that may readily be observed from the outside (Johnson and Reid, 1990). Thus, it is highly desirable to confirm its presence by identifying the presence of coccidial oocysts (eggs) in a scrapping from the mid gut under a microscope (Cervantes, 2006).

Gross lesions of *E. tenella* are confined to the caeca and consist of the presence of haemorrhages on the outside or inside of the wall of the caeca. There is also free blood or a chocolate-coloured fluid content inside the caeca with a thickening of its wall or the presence of a large core or cellular debris and blood. *E. tenella* can kill birds and dead birds in a flock or farm with increased mortality should always be examined for the presence of lesions compatible with *E. tenella* infection or caecal coccidiosis (Roespstorff and Nensen, 1998).

Eimeria necatrix causes a more chronic disease than *E. tenella* and produces less number of oocysts. Thus, it takes longer time for high levels of environmental contamination. Birds that are heavily infected with *E. necatrix* may die before any marked change is noticed in weight or before blood is found in the faeces (Majaro, 1981). *E. acervulina* is less pathogenic than *E. tenella* or *E. necatrix* and is responsible for sub-acute or chronic intestinal coccidiosis in broilers, older birds and chickens at the point of lay. The clinical signs consist of weight loss and watery, whitish diarrhoea. On post-mortem examination, greyish-white, pinpoint foci or transversely elongated areas are visible from the outer surface of the upper intestine. The foci consist of dense areas of oocysts and gamete production (Williams, 2002).

Eimeria maxima produces few marked changes in the small intestine until the fifth day after infection in severe infections, numerous small haemorrhages occur along with a marked production of thick mucus. The intestine loses tone and becomes flaccid and dilated. The inner surface is inflamed and the intestinal content consists of a pinkish mucous secretion (Kayla and Barta, 2010).

2.15 Immunology and Vaccination

A proven method for the control of coccidiosis involves the vaccination of birds using a low dose of the live parasites or live non-attenuated coccidiosis vaccines. This initiates the birds to acquire immunity. It was discovered that daily inoculations of small numbers of oocysts over twenty days produced a stronger immunity than when a large number of oocysts was given in a single dose (Reid, 1990). This method of vaccination was labelled trickle infection. The trickle infection suggests that for chickens, *Eimeria* species protective immunity is developed only after the bird has been infected by the parasite several times through cycling. It was generally found that the motile sporozoites played an important role in conferring an immune response while the gametogonic stages elicited little protective immunity (Beattie, 1998).

Protective immunity is generally regarded as the prevention of oocyst production and absence of clinical signs in birds challenged by the parasites (Beattie, 1998). Once protective immunity is developed for a certain *Eimeria* species, the bird is immuned to further infection with this parasite (Lightowlers, 1994). *Eimeria maxima* best demonstrates acquired immunity. Almost complete (>99.99%) immunity develops after a single infection with only a few oocysts (Smith *et al*., 2002). However, a lot of studies have shown that immunity against one strain pathogen will not necessarily protect the host against a different strain of the same species. This has been demonstrated with the antigenically variable *Eimeria maxima* as well as *E. acervulina* (Innes and Vermenlen, 2006; Martin *et al*., 1997; Lightowlers, 1994 and Smith *et al*., 2002).

The maximum natural challenge of oocysts to birds usually occurs between three and five weeks of age. It is therefore, necessary to initiate immunity before this challenge occurs (Chapman *et al*., 2005). The main objective of vaccination is to induce an

immune response that is adequate to enable birds to resist challenges with virulent parasites (Chapman *et al*., 2005). Newly hatched birds are not fully susceptible to coccidial infection because of high maternal antibodies (McDonald and Shirley, 2009; Beattie, 1998).

Furthermore, excystation of oocysts in new hatched birds is inefficient due to relatively lower total chymotrypsin activity and total bile salts in the intestinal tract (McDonald and Shirley, 2009; Beattie, 1998). Thus, a vaccine must be introduced early in the life of the animal so that cycling of oocysts can occur and protective immunity generated (Woods *et al*., 2000). Much research has been conducted on immunization and results have shown that vaccination with live attenuated *Eimeria* is effective while recombinant vaccines are still in development. However, immunization is species-specific and often associated with transient loss of performance and is very costly (Virginie, 2010).

Some compounds with indirect antiparasitic activity are able to assist the host organism to resist or fight infection or recover from tissue damages. Some plant-based polysaccharides exert an immune stimulating effect. They are able to do this by mimicking an infectious agent and some are able to trigger the non-specific immune response. For instance, it has been shown that immunoactive polysaccharides from mushrooms and other plants have a synergistic (greater) action with vaccination. Certain plant extracts with antioxidant or anti-inflammatory properties can have an immune modulating effect (Williams *et al*., 1999).

Coccidiosis leads to production and release of nitric oxide radicals (NO) by macrophages. This may result in disequilibrium between the body's endogenous anti-

oxidant defences and production of free radicals. If this occurs, the animal (host) is subjected to oxidative stress. This may lead to reduced performances and can also limit the response against infections if immune cells are damage (Virginie, 2010). Inflammation is a physiological mechanism which enables the recruitment of immune cells to the infection site. Over-reaction can lead to tissue damage at the site of infection (Berezin *et al*., 2008). Birds easily develop natural immunity to coccidian infection but this has declined because of the age (42 days) at which broilers are been generally slaughtered. At this age, their immune systems are still not fully developed. Young birds need to come into contact with low levels of several strains of coccidia parasites in order to acquire natural immunity (Williams *et al*., 1999).

2.16 Plants used as Anti-coccidial

Recently, plants have received great attention for the control and treatment of infectious diseases of animals including chickens (Pieri *et al*., 2014; Xiao *et al*., 2014). Coccidiosis is generally controlled by using anticoccidial drugs which are administered in feed of chickens (Blake & Tomley, 2014; Shivaramaiah *et al*., 2014). Success has been achieved by using these drugs but, main problem associated with their poor response is development of resistance in *Eimeria* species to the commonly available anticoccidial drugs (Abbas *et al*., 2012). In external environment, farmers usually fight against this disease applying disinfectant compounds. Commonly used disinfectants include some phenolic products such as ammonia, methyl bromide and carbon disulfide. Toxic effects of these products represent a danger to the staff and health of birds and therefore their use has been restricted (Hilbrich, 1975; Williams, 1998).

Many plants and herbal products have been found to have chemotherapeutic effect against coccidiosis in poultry and are being commercialized after a series of

experimental trials for their validation and economically cheaper approach for the control of coccidiosis (Abbas *et al*., 2012; Zaman *et al*., 2012). A number of *in vitro* experiments have also proved remarkable anticoccidial effects of different herbal extracts and essential oils on inhibition of sporulation of coccidian oocysts (Remmal *et al*., 2011).

The herbs especially *Azadirachta indica, Hobrrhena antidysentrica, Barberis aristata, Embelia ribes, Acorus calamus* and *Artemisia annua* have strong anticoccidial activity. Zycox, a herbal product of India containing *Hobrhena antidyseatria, Barberis aristata, Embelia ribes* and *Acorus calamus*, is used as a prophylactic measure against coccidiosis (Tipu *et al*., 2006). There has also been a lot of recent scientific publications which demonstrated the potential benefits of phytochemicals such as tannins against avian experimental coccidian infection (Youn and Noh, 2001; Arab *et al*., 2006; Jang *et al.,* 2007; Berezin *et al.,* 2008; Naidoo *et al.,* 2008; Wang *et al.,*2008; Nweze and Obiwulu, 2009). *Parkia biglobosa* has been reported to be of nutritional and medicinal purposes in West Africa (Sabiti and Cobbina, 1992; Mertz *et al.,* 2001). In the semi-arid zone of north eastern Nigeria, several plants, such as stem barks of *Vitellaria paradoxa, Khaya senegalensis* and *Anona senegalensis* are used by the natives to treat poultry diseases (Mbaya *et al*., 2007). The use of different plant extracts such as *Khaya senegalensis, Vitellaria paradoxa, Aloe spicata*, have been shown to have potential benefits in reducing coccidial infections (Jang *et al.,* 2007; Nweze and Obiwulu, 2009).

In some African countries such as Nigeria, Ghana, Mali and Zambia, the tradition of collecting, processing and applying plants and plant-based medication have been handed down from generation to generation over the years verbally or written

(Sofowora, 1993; Akinyemi *et al*., 2005). However, this is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture (Moreki, 2013). Herbal medicines are known to be broad spectrum and therefore may be a future answer to pathogen resistance to conventional drugs (Mwale *et al*., 2005).

Several plant species namely *Saccharum officinarum* (Fornazier *et al*., 2000), *Pinus radiata* (Wang *et al*., 2008; Molan *et al*., 2009) and *Aloe vera* (Molan *et al*., 2004; Narsih *et al*., 2012) have been shown to exhibit anticoccidial effect. Other plants with record of anticoccidial efficacy include green tea (Molan and Thomas, 2007; Molan and Faraj, 2015), *Moringa oleifera* (Ola-Fadunsin and Ademola, 2013), *Khaya senegalensis*, *Vitellaria paradoxa*, *Aloe spicata* (Jang *et al.*, 2007; Nweze and Obiwulu, 2009), *Vernoniaamygdalina* (Mohammed and Zakariya′u′, 2012; Oyagbemi and Adejinmi, 2012).

2.17 Ethno botanical Plants

2.17.1*Azadirachta indica* (General description)

Botanical Name: *Azadirachta indica*

Local Name: Neem (English)

Family: Meliaceae

The neem tree is a tropical evergreen tree which is native to India and also found in other Southeast countries. In Nigeria, the neem forms about 90% of the trees in the forestry plantations (Donli and Buahin, 1998). *A. indica* (Neem) has been known as the wonder tree of India for centuries. It is traditionally used in Ayurvedic remedies as an antiseptic to fight viruses and bacteria. It is also recommended for urinary disorders, diarrhoea, fever, skin diseases, burns and inflammatory diseases (Ezz-Din *et al*., 2011).

The bark of neem tree is used to manufacture herbal tea with curative properties. Neem barks possess insect repellent properties and are used as herbal pesticides, insecticides which help to retard the growth of insect and other pests (Pauliwal *et al*., 2005). Neem bark is used as an active ingredient in a number of tooth pastes and tooth powders because it contains anti-bacterial properties thus it is good for curing gum problems and maintaining dental health naturally. Bark powder and extracts are used in treating fevers and stomach problems. Neem bark is used as extract or powdered form in a number of herbal cosmetics. Acne treating and other skin creams use neem bark to cure pimples and improve the skin condition (Virginie, 2010).

Neem bark also contains a lot of tannins and so it is useful in tanning and dyeing a number of products (Ezz-Din *et al*., 2011). It is useful in curing tiredness, cough, and fever, loss of appetite and worm infestation. It heals wounds, vomiting, skin diseases, excessive thirst and diabetes. Neem leaves are reported to be beneficial for eye disorder as well as insect poisons and is an anti-leprotic. Its fruits are bitter, purgative, anti-haemorrhoids and antihelminthic (Donli and Buahin, 1998).

It is claimed that neem provides an answer to many incurable diseases. Traditionally, neem products have been used against a wide range of diseases such as heat-rash, boils, wounds, jaundice, leprosy, skin disorders, stomach ulcers, chicken pox etc. Modern research also confirms neem's curative powers in case of many diseases and provides much more cure (Koul *et al*., 2006). Polysaccharides in neem bark extracts is said to possess anti-tumor as well as anti-inflammatory properties. The bark of neem contains 3.43% proteins, 0.68% alkaloids and 4.16% minerals. Neem possesses limonoids, protolimonoids, tetranortriterpenoids, pentanortiterpenoids,

hexanortriterpenoids and some nonterpenoid (Koul *et al*., 2006).

2.17.2*Citrus sinensis (*General Description*)*

Botanical Name: *Citrus sinensis*

Local Name*:* Lemun tsami (Hausa)

Orombo/ osan wewe (Yoruba)

Family: Rutaceae

Citrussinensis is a genus of flowering plants in the family Rutaceae (orange family) and a common name for edible fruits of this genus and sometimes related genera. *Citrus* is likely the most widely planted fruit for direct human consumption in the world (Imade *et a*l., 2005). Lime is a small shrub-like tree ranging from 3.5 to 9 m in height and 2.5 to 7.5 m in width. The fruit is typically round, green to yellow in colour and about 3-6 cm in diameter (Moro and Basile, 2000).

Lime (*C. sinensis*) juice has been shown to have both medicinal and cosmetic values. Studies have shown that lime juice destroys both human immunodeficiency virus (HIV) and sperm cells (Preuss *et al.,* 2002). The high acidity of the lime juice is probably responsible for the destruction of the HIV and sperm cells (Bakare *et al*., 2012).

Lime juice is being used by women as a barrier contraceptive, and there is a long reported history of African women douching with lime juice, lemon juice, vinegar or acidic soft drinks in the belief that it may prevent pregnancy and/ or sexually transmitted diseases. In fact, lemon and lime juice are widely used for douches among women at high risk of HIV transmission in Central Nigeria (Imade *et a*l., 2005). Lemon and lime juices have been reported to exhibit antimicrobial activity against *Vibrio* strains and lime juice partially blocks ovulation. Thus, possesses an anti-fertility potential (Roger, 2000). The demonstration of *in vitro* effect of concentrated lime juice

extract on tumour cells suggests that the juice may have anti-proliferative effects on tumour cell lines (Preuss *et al*., 2002).

The fruits of *C. sinensis* are used in treating catarrh, stomach ache, sore throat, vomiting and intestinal parasites. The leaves are mixed with the root bark and included in feed of animals for the treatment and control of coccidiosis, trypanosomiasis as well as intestinal parasites (Dominguez-Carmona *et al*., 2010).

2.17.3 *Cissus populnea* Gill and Perr (General Description)

Botanical Name: *Cissus populnea*.

Local Names: Dafara (Hausa).

Okoho (Igala, Idomas and Igbo)

Ajara or Orogbolo (Yoruba)

Family: Amplidaceae (Vitaceae).

The plant is 2 to 3m high and a semi-climber. It grows in the savannah and is widely distributed in Senegal, Sudan, Uganda, Abyssinia and Nigeria (Kone *et al*., 2004). The fruits are edible in soups and the stem bark is also used in preparation of foods such as bean cake as well as soups. The roots or stems are used in building (Irvine, 1961 in: Musa *et al*., 2010). The extracts of various parts of the plants are used in ethno-medicine in the treatment of sore, indigestion, venereal disease, intestinal parasites, oedema and eye problems that result from attack of black cobra (Moody *et al*., 2003). The plant is also used as cathartic, aphrodisiac and antidotes to arrow wounds. The stem bark has been reported to contain carbohydrates, tannins, cyanogenic glycosides, anthraquinones, saponins, cardiac glycosides and flavonoids (Ibrahim, 1990; Ibrahim *et al*., 1993).

Cissus populnea is a plant associated with variety of medicinal uses in different parts of the world. Its extracts have been credited with antibacterial properties, antitrypanosomal plant and a source of gum powder as well as a component of a herbal anti-sickling Nigerian formula (Moody *et al*., 2003). In Benin Republic, it is used for its diuretic properties while in Ghana; it is used as a postharvest ethno botanical protectant. The aqueous extract of its stem bark is associated with aphrodisiac or fertility potential in South West Africa (Ojekale *et al*., 2006).

2.17.4 *Ficus platyphylla* Del (*G*eneral Description)

Botanical Name: *Ficus platyphylla*

Local Names: Gamji (Hausa)

Broad leaf fig (English)

Gutta percha tree (common name)

Family: Moracaec or Meliaceae

It is a Savannah tree of about 18m high, 6m in girth, with large widely spreading branches and broad crown. The bark is rusty red, flaking off in scattered branches and greys beneath and slashes pink. Branches are very stout with twigs, stipules and young foliage finely velvety. The leaves are 7-40cm long by 10-28cm broadly elliptic, round or blunt at the apex and narrowly cordate, thick, finely velvety or glabrous (Keay *et al*., 1989).

The extracts of *F*. *platyphylla* have been reported to inhibit gastrointestinal motility, possess analgesic, anti-inflammatory and anti-conceptive activities (Amos *et al.*, 2001; Amos *et al*., *2005* and Wakeel *et al*., 2004). The stem bark of *F*. *platyphylla* is used traditionally to treat malaria in Africa and in treating tuberculosis (Kubmarawa *et al*., 2009). *F*. *platyphylla* is highly reputed for its numerous medicinal uses. It has been

used by herbalist to treat insomnia, psychosis and depression (Audu, 1989; Chindo *et al*., 2003). The decoction of the stem bark leaves and seeds of *F. platyphylla* are used in combination as a medicine to promote fertility. Preliminary phytochemical screening of the stem bark revealed that it contains flavonoids, tannins and saponins (Amos *et al*., 2001). The scientific basis for the use of this plant in traditional medicine for the treatment of central nervous system (CNS) disorders have also been evaluated (Chindo *et al*., 2003).

2.17.5 *Khaya senegalensis* (Desr.) A. Juss (*G*eneral Description)

Botanical Name: *Khaya senegalensis* (Desr.)

Local Name: Africa mahogany or dry-zone mahogany

Family: Meliaceae

Khaya senegalensis is an important multipurpose tree in its natural range in sub-Sahara Africa. In its natural habitat, it is a medium-sized to large tree (up to 30m) with a wide crown. However, in cultivation as an exotic, it can grow up to more than 35m high and up to ½ mm diameter (Joker and Gamene, 2003). In its natural range, *K. senegalensis* provides fodder for cattle, food for human and cosmetic oils, medicinal products, shade and shelter in addition to providing fuel wood and valuable timber. In some West African countries such as Mali, Niger, Nigeria and parts of the Sahel, *K. senegalensis* is used to treat various ailments. The bark is useful for livestock to treat liver fluke, ulcers and internal ailments associated with mucous diarrhoea (Petit, 2004).

The bark of *K. senegalensis* is bitter and is regarded as a fever remedy, used as a vermifuge, taeniacide, depurative as well as for treating syphilis (Boffa, 1999). The ethanolic crude extract of the stem bark has been reported to possess free radical

scavenging activity (Lompo *et al*., 2007). An extract of the bark is useful for treating a range of maladies (infections) including jaundice, dermatoses, scorpion bites, allergies, infection of the gums, hookworms, disinfection of wounds and even constipation. In addition to the bark, both the seeds and leaves have also been used as medicine for treating fever and headache. Preparations made from the roots have also been used against syphilis, leprosy and as an aphrodisiac (Walter, 2011). The bark can also be used for tanning of leather as it is quite high in tannins (Boffa, 1999).

In some areas, *K. senegalensis* is also valued for the oils that it can provide. The oil is quite rich in oleic acid and is used in West Africa for cooking as well as cosmetics (Joker and Gamene, 2003). *K. senegalensis* is often planted by the roadsides for shade in countries like Nigeria, Australia, China, and Sri Lanka (Petit, 2004).

The timber of *K. senegalensis* is used for quality furniture and cabinet work, joinery, fixtures, flooring, boat building, decorative veneers, construction and form ring of household utensils (Walter, 2011). In Burkina Faso its timber is used in traditional handicrafts and in parts of West Africa, it is used as a source of fibre for pulp production. It is a popular timber in East Africa for lorry bodies, construction work and decking in boats (Petit, 2004).

2.17.6 Sarcocephalus diderichii: Nauclea diderichii (Wilderm) Nauclea diderichii (Current Name) (General Description)

Botanical Name: *Nauclea diderichii* (Merr.).

Local Names: Opepe (Trade Name)

African Peach (English)

Family: Rubiaceae.

Nauclea diderichii is an evergreen tree that may reach a height of 30-40m and a diameter of 0.9-1.5m. It is a bole cylindrical, slender, straight and branchless tree. The shining leaves are 15cm long and bigger when young, elliptic, acute at the ends and keeled towards the base. It is mostly deciduous except that the ends of shoots and the nodes are often occupied by ants. Flowers are small, green-white-yellow and tubular, in solitary terminal heads. The fruit is yellow, fleshy, in a globose head, (Agunu *et al*., 2005).

In Ghana, a part of these plants is used in palm soup. The fodder of the plant is eaten by elephants and other mammals. It is a commercial timber of West Africa where it is used for outdoor purposes (railway sleepers), buildings (carpentry, floors, indoor, and outdoor wood and for cabinet making). The wood is also suitable for fences, post and bridges because it is moderately termite resistant and resistant to fungi and marine borers. Decoction of the bark is prescribed for anaemia, stomach-ache and indigestion. It has also been used as part of an infusion for treating jaundice and gonorrhoea. A decoction of leaves is used as a wash for measles (Kubmarawa *et al*., 2009).

2.17.7(Sarcocephalus latifolius (Smith) Bruce) *Nauclea latifolia* (Current name)
(General Description)

Botanical Name: N*auclea latifolia* (Smith)

Local Names: Opepe (Trade Name)

Pin cushion tree, African Peach, Guinea Peach, Peach (English)

Tafashiya, tashiyaigia (Hausa)

Ubuluinu (Igbo)

Family: Rubiaceae

N. latifolia (Syn. *Sarcocephalus latifolius*) is an evergreen multi-stemmed shrub or a tree and can grow up to a height of 200m found in undisturbed fringing forest and closed savannah woodland (Emeje *et al*., 2005). It is widespread in the humid tropical rainforest zone or in savannah woodland of West Africa (Okoli and Iroegbu, 2004). The plant has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces and the fruit is syncarpous. The tree flowers from April to June and fruits ripen from July to September. The fruits are edible and usually consumed by baboons while livestock eat the shoot and leaves. N. *latifolia* is termite resistant and is used as live stakes in farms (Amos *et al*., 2005). This plant is used for medicinal purposes in folk medicines. The leaves and roots or its combination in equal proportions have local use in the treatment of diseases such as fever, malaria, gonorrhoea, wounds, coughs, stomach-aches, disorder of gastro intestinal tract and autonomic system, especially hypertension (Udoh, 1995; Abreu and Pereira, 1998).

Nauclea latifolia exhibits remarkable insecticidal, antimicrobial and anti-parasitic activities (Kela *et al*., 1989; Fakae *et al*., 2000). In West and South Africa, infusions and decoction of the leaves and bark are used for the treatment of diarrhoea, malaria, stomach-aches/pains as well as against parasites such as nematodes in humans and animals. In Kano (Nigeria), *N. latifolia* is used as a chewing stick and as a remedy against stomach ache and tuberculosis (Deeni and Husain, 1991).

2.17.8 *Parkia biglobosa (*General Description)

Botanical Name: *Parkia biglobosa*

Local Names: Dorawa (Hausa)

African locust bean (English)

Family: Leguminosae

It is a tree that is up to 20 meter high, bole slout, not buttressed, low branching and bear a large wide-spreading crown. The flowers are pendulous capital bearing pendulous large fruit pods (Soetan and Lasisi, 2008). *P. biglobosa* is a multipurpose tree of West Africa and is used for nutritional and medicinal purpose (Sabiti and Cobbina, 1992; Mertz *et al*., 2001). It is used as a remedy to treat different types of wounds and various ailments. The bark-infusion is taken as a tonic and anti-diarrheic in various West African countries such as Burkina Faso, Ivory Coast and Nigeria. The bark is commonly sold in herbalist shops in the Western part of Nigeria for the analgesic action it confers in mouthwashes and steam-inhalations for tooth ache (Roespstorff and Nansen, 1998). The bark is used for female sterility, baths and also administered in prescription with other drug-plants for skin infections and leprosy (Maphosa *et al*., 2010).

Fula and Tukular people of Senegal drink its decoction to treat *Schistosoma* infection. In Ivory Coast and Burkina Faso the pounded bark with lemon juice is applied to sore and ulcers. This plant is one of the 32 other plants in a complex prescription used in the Kenya region for leprosy (Soetan and Lasisi, 2008). In Nigeria and the Gambia, roots and leaves are pounded together in water to produce an eye-lotion while the leaves and bark are used in eye-lotion in Senegal. The crushed leaf is used to dab on the lips to dissipate 'fever spots'. They are made into steam inhalations in the Gambia for tooth ache. The leaflets are lightly heated, then crushed and applied to burns (Soetan and Lasisi, 2008).

It has been reported that *P. biglobosa* showed anti-diarrheal properties in mice. The hexane extract from the bark of this plant had some analgesic and anti-inflammatory

effects (Agunu *et al*., 2005). Methanol extract of *P*. *biglobosa* has shown significant protection against the neurotoxic, haemotoxic and cytotoxic effects of venoms of poisonous snakes (Asuzu and Harvey, 2003). Studies have shown that the petroleum ether extracts of the plant contains catechins and ferulates. These are antioxidants which may be responsible for some of the medicinal effect seen in this plant (Maphosa *et al*., 2010).

2.17.9*Solanum dasyphyllum*(General Description)

Botanical Name: *Solanum dasyphyllum* Schumach and Thonn.

Local Names: Gboma eggplant, African eggplant (English)

Gautar Kura (Hausa)

Family: Solanaceae

The genus *Solanum* comprises over 1000 species all over the world with shrubs or sub shrubs measuring up to 1.5m tall and stems are glabrous or with stellate hairs. The leaves are alternate, simple and without stipules. Inflorescences are lateral, racemose with 3-12 flowers (Bukenya and Carascos, 1994). *S. dasyphyllum* is a wild ancestor of *S. macrocarpon*. The domestication process of *S. dasyphyllum* has involved selection against hairs, prickles and deeply lobed leaves. Most leaf cultivars grow in high rainfall areas of West and Central Africa except a group of cultivars with small leaves and fruits found in semi-arid and savannah areas of Northern Ghana, Burkina Faso and neighbouring countries (Schippers, 2000). Fruit cultivars are restricted to the humid coastal areas from Cote d' Ivoir to Nigeria and also along the East Africa coast (Bonsu *et al*., 2002).

The young leaves and young fruits are cooked and consumed as vegetables. The leaves are eaten as a separate dish or in sauces together with other ingredients. The leaves are

either steamed as practiced in Uganda or fried in oil with onions. In West Africa both leaves and fruits are eaten (Bonsu *et al*., 2002). The leaves, fruits and roots have a variety of medicinal uses in different parts of the world. In Sierra Leone, heated leaves are chewed to treat throat troubles. In Nigeria, fruits are taken as a laxative and to treat cardiac diseases, while flowers and fruits are chewed to clean teeth. In Kenya, the juice of boiled root is drunk to get rid of hookworms, while crushed leaves are taken to treat stomach troubles (Schippers, 2000).

All *Solanum* species contain spirosolane alkaloids, including solanine and solanidine. However, these are bitter in taste and potentially poisonous when consumed frequently (Bonsu *et al*., 2002). The venereal disease, intestinal parasites, oedema and eye problems that result from attack of black cobra are treated using this plant (Moody *et al*., 2003). The plant is also used as cathartic, aphrodisiac and antidotes to arrow wounds. The stem bark has been reported to contain carbohydrates, tannins, cyanogenic glycosides, anthraquinones, saponins, cardiac glycosides and flavonoids (Ibrahim, 1990; Ibrahim *et al*., 1993).

2.17.10*Vitellaria paradoxa (*General Description*)*

Botanical Name: *Vitellaria paradoxa* (Formerly *Butryospermum parkii*)

Local Name: Shea tree or Shea butter tree (English)

Danya/Kadanya (Hausa)

Family: Sapotaceae

Vitellaria paradoxa is a tree that is indigenous to Africa which occurs in Mali, Cameroon, Congo, Cote d'Ivoir, Ghana, Guinea, Togo, Nigeria, Senegal, Sudan, Burkina Faso and Uganda. The tree is perennial and starts bearing its fruits when 10-15years old. The Shea tree is a traditional African food plant. It has been claimed to

have potential to improve nutrition, boost food supply in the annual hungry season foster rural development and support sustainable land care (Masters *et al*., 2010). It grows naturally in the dry savannah belt of West Africa. The Shea nut "butter" has many uses both in the refined and unrefined forms. It is used extensively for food and medicinal purposes throughout Africa. However, shea butter can be used for medicinal purposes only if it is raw and unrefined (Ibrahim, 1990).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Background Information

Ethno veterinary data for this research was collected over a seven-month period in 2011. The survey was conducted among poultry farmers in Zaria, Kaduna State. The medicinal plants reported in this research are based on such interviews (Moerman, 1998; Wynn and Marsden, 2003). Two visits and interactions with poultry farmers were conducted. During the first visit, poultry farmers in Zaria area were identified their consent was sought and participants were select for the interviews (Cheryl *et al*., 2007). During the second visit, the poultry farmers were interviewed on types of poultry diseases encountered and medicinal plants used in treating their poultry animals. Information such as local name of disease, the symptoms, name of plants used, parts of plants used and methods of preparation with the mode of administration were provided by the farmers. Other information included dosage, frequency and duration of treatment as well as solvents used in preparation of the plants. The medicinal uses of the plants were cross-checked and confirmed through repeated queries (Maikai *et al*., 2007). Plants claimed to be beneficial in the treatment of various poultry diseases were collected in the company of practitioners. Data obtained were collated and tabulated to give the botanical, family and local names, parts used, dosage, mode of administration and diseases treated. (Maikai *et al*., 2007). The percentage frequency of each plant was calculated.

Table 3.1: Common symptoms cited by poultry farmers, suspected diseases, plants used, preparation and mode of administration

Plants used	Symptoms described	Local name	Suspected disease	Preparation and mode of administration
Adansoniadigitata	i) Whitish, greenish, bloody faeces ii) Bloody diarrhoea	i) Zawo ii) Borin kaji	i) Diarrhoea ii) coccidiosis	i) leaves dried, pounded and put into drinking water for 2 – 3 days ii) stem bark dried, pounded and put into drinking
	iii) Watery faeces	iii) Zawo	iii) Fowl Cholera	water for 2 – 3 days
	iv) Feather picking and extreme sand bathing	iv) kwarikota da kaska	iv) lice and tick infestation	iii) Fruit broken and dipped in drinking water iv) wood ash
Allium cepa	Diarrhoea (foamy), coughing, unthrift ness and drooping wings	Susan ciki	Helminthiasis.	Fresh leaves cut into pieces and given to the chickens
Allium sativum L.	Watery faeces	Zawo	Dysentery	Bulbs cut into pieces and included in feed
Artemisia absinthium L.	i) Watery faeces	i) Zawo	i) Dysentery	i) Leaves and crushed garlic cloves are blended together and mixed with feed
	ii)Bloody diarrhoea	ii) Borin kaji	ii) Coccidiosis	ii) leaves ground into powder and added to drinking water
	iii) Feather picking and extreme sand bathing	iii) kwarikota	iii) lice infestation	iii) fresh leaves rubbed on skin of chickens
Azadirachta indica A. Juss	i) Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	i) Dried stem bark boiled in water together with cloves of garlic and added to drinking water for 3-5 days
	ii) Diarrhoea (foamy)	ii) susan ciki	ii) Internal parasites	ii) Fruits soaked in drinking water

Table 3.1: Continued

	i) Whitish diarrhoea that is associated with dry seasons, accompanied by high mortality ii) Bloody diarrhoea	i) Borin kaji	i) New castle disease	i) Leaves dried, grinded and put into drinking water for 2 – 3 days
Capsicum annum L		ii) Borin kaji	ii) Coccidiosis	ii) Fruits dried, grinded and added to drinking water for 2 – 3 days

Cassia occidentalis Linn	Noisy breathing and coughing, nasal discharge, watery eyes which spreads slowly within a flock	i) Muran kaji	Bronchitis	Fruits and leaves pulverized and small amount mixed with a little salt and added to drinking water
		ii) susan ciki	ii) helminthiasis	ii) Leaves dried, grinded and put into drinking water for 3– 7 days
		iii) Borin kaji	iii) coccidiosis	iii) Leaves dried, grinded and put into drinking water for 2 – 3 days
Cissus populnea	i)Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	i) stem bark, and leaves dried, pounded and put into drinking water for 2 – 3 days
	ii) Diarrhoea (foamy)	ii) susan ciki	ii) Worm infection	ii) fresh leaves soaked in water with juice of lime for 3-5 days
Citrus sinensis (Christm.) Swingle	i)Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	i) leaves dried, grinded and added to drinking water for 3-7 days
	ii) Diarrhoea (foamy)	ii) susan ciki	ii) Worm infection	ii) Mix juice with small red potash and added to drinking water
Euphorbia hirta Linn	i) Diarrhoea (foamy)	i) susan ciki	i) Intestinal parasites	i) latex given to the chickens orally
	ii) Bloody diarrhoea	ii) Borin kaji	ii) Coccidiosis	ii) Latex extracted, mixed with juice of lime and added to drinking water
Ficus platyphylla	i) Diarrhoea (foamy)		i) Intestinal parasites	i) Root soaked in drinking water
	ii) Bloody diarrhoea		ii) Coccidiosis	ii) Stem bark dried, pounded and put into drinking water for 2 – 3 days
Jatropha curcas Linn	i) Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	i) latex given to the chickens orally
	ii) Diarrhoea (foamy)	ii) susan ciki	ii) Helminthiasis	ii) Latex extracted and added to drinking water
Khaya senegalensis	Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	i) Mix soap with dried powered mahogany stem bark and add to drinking water for 2 days
	ii) Diarrhoea (foamy)	ii) susan ciki	ii) Helminthiasis	ii) Pound dried stem bark with red potash and given in drinking water for 2 – 3 days.

Table 3.1: Continued

Nauclea diderichii	i) Bloody diarrhoea	i) Borin kaji	i) Coccidiosis	Stem bark and root dried, pounded and added to drinking water for 5-7 days
	ii) Diarrhoea (foamy)	ii) Susan ciki	ii) Worm infection.	ii) fresh leaves grinded, squeezed, salt added and put in drinking water
Nauclea latifolia	Bloody diarrhoea	Borin kaji	Coccidiosis	Stem bark, root and leaves dried, pounded and mixed with feed
Solanum dasyphyllum (Scum et Thonn)	i) Bloody diarrhoea ii) Watery faeces	i) Borin kaji ii) Zawo	i) Coccidiosis ii) dysentery	i) Fruits broken and dipped in drinking water ii) Stem bark, root and leaves dried, pounded together and added to drinking water
Vernonia amygdalina Del	i) Bloody diarrhoea ii) Diarrhoea (foamy)	i) Borin kaji ii) Susan ciki	i) Coccidiosis ii) worm infection	i) Juice extracted from the fresh leaves and added to drinking water ii) leaves dried, pounded and added to drinking water
Vitellariaparadoxa	i) Bloody diarrhoea ii) Diarrhoea (foamy)	i) Borin kaji ii) Susan ciki	i) Coccidiosis ii) worm infection	Stem bark, root and leaves dried, pounded and put in drinking water ii) Fresh stem bark and fruits pounded and mixed with feed

3.2 Study Area

The study was carried out in Zaria ($11^{o}9'40''$ N; $7^{o}39'21''$E) with an area of 115.8 m^2 in Kaduna State, Nigeria. It is located at an elevation of 644 m (2112 ft.) above sea level. The inhabitants are predominantly farmers and practice mixed farming (Gihring, 1984).

3.3 Plant Collection and Identification

Ten most frequently used plant parts claimed to be beneficial in treating poultry diseases especially coccidiosis-like disease were collected from around Zaria between October, 2011and March, 2012. The plants were identified and authenticated by taxonomists at the Herbarium Unit, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria. Voucher number was given to each of the plants and samples were deposited in the Herbarium Unit.The detailed medicinal concoctions used in the management of poultry diseases are detailed in Table 2

3.4 Preparation of Plant Extracts

Collected plant parts were air dried in the laboratory at room temperature, (i.e. 24^{0}C - 28^{0}C) for two weeks. The parts of plants used include the parts most frequently used by poultry farmers in the study area. These are root bark, stem bark and leaves. The dried plant materials were pulverized using pestle and mortar (Kubmarawa *et al*., 2009). A weight of 1.5kg of each of the selected dried plant materials was extracted exhaustively by hot method of extraction using Soxhlet's apparatus at 80º C (Candeias *et al*., 2009). Repeated extractions were carried out in order to improve on the quantity of the crude extracts (Ndukwe *et al*., 2006). The respective extracts were concentrated *in vacuo* by evaporation using a rotary evaporator (Perkins, UK) under reduced pressure at

temperature below 50ºC. The resulting crude extracts were then dried, weighed and stored at room temperature (i.e. 24^0C -28^0C) until assayed (Vinoth *et al*., 2012).

3.5 Source and Maintenance of *Eimeria tenella*

Oocysts of *E. tenella* were obtained from the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. These were maintained in chickens purchased from Obasanjo Hatchery, Oluyole Estate, Ibadan, Oyo State, Nigeria. The oocysts were propagated in 5 two-week (14) day old chickens via oral inoculation. The birds were fed with a well standard broiler feed (anticoccidial free feed) which consists of corn, soybean, and groundnut cake and water provided *ad libitum* (Arabkhazaeli *et al*., 2011). The inoculated birds were sacrificed at day7 post inoculation. The caeca of each of the chicken were examined for lesions consistent with caecal coccidiosis (such as caecal lesions with prominent blood, swollen caecum), selected and oocysts harvested (Jatau *et al*., 2012).

The caecal contents of chickens were analysed for the presence of coccidial oocysts using a standard floatation technique (Lee *et al*., 2011). Following evisceration at post mortem, the caecal contents were washed into a beaker using tap water and oocysts were isolated using a flotation procedure (Permin and Hansen, 1998). Each sample was homogenized thoroughly in 50 ml preparation tubes (with sealing cap) with approximately 15 ml of concentrated sodium chloride solution (311 g/L, specific gravity 1.2). The suspension was sieved through a tea strainer into a second 30 ml centrifuge tube, filled almost entirely and centrifuged for 8–10 min at 2,000 revolutions per minute (rmp). Afterwards the tube was filled carefully with floatation solution (sodium chloride solution) to form a convex meniscus at the top. After 10 min a coverslip (22×22) mm

was placed cautiously in contact with the meniscus, lifted off and placed on a glass slide for microscopic examination. The cover glass was screened at X 100 magnification in a meandering pattern (Mehlhorn *et al*., 1998). The oocysts were aspirated from the surface of the saturated salt solution by pipetting and collecting into a separate test tube until no oocyst remained. Pasteur pipette was used to obtain one ml of the suspension, dropped on a glass slide, covered with a coverslip and observed under a microscope using the X10, X40 and X100 objectives to ensure that no oocysts remained. More floatation solution was added and aspirated when oocysts were found (Arabkhazaeli *et al*., 2011). After floatation, the coverslip was mounted on a slide and examined in its entirety for the presence of coccidial oocysts. The oocysts were collected and suspended in distilled water and sporulated within 72 h (Bowman, 2009).

Oocysts of *Eimeria tenella* were sporulated in Petri-dishes of about 6 mm thickness containing 2.5% potassium dichromate solution at 25-29º C and 60-80% humidity for 72 hrin the laboratory.The Petri dishes were partially covered to allow the passage of oxygen and the contents of the Petri dishes were stirred off and on to ensure the oxygenation. The sporulation of the oocysts was confirmed by examining sporocysts under inverted microscope at 40x (Pyziel and Demiaszkiewicz, 2015).The numbers of sporulated and non sporulated oocysts were counted as described in 3.5.1 below.

3.5.1 Counting of oocysts of *E*. t*enella*

Modified McMaster counting chamber of oocysts was employed. The oocysts suspension was homogenized and 2 ml of the suspension was transferred into a 30 ml centrifuge tube, filled with 8 ml of saturated sodium chloride solution (311 g/L, specific gravity 1.2). This suspension was used to fill two McMaster counting chambers (2×0.15 ml) allowed to stand for 5 min and examined under a microscope at 10 X 10

magnification. All oocysts within the engraved area of both chambers were counted and added together. The number of oocysts was calculated by multiplying the total number of oocysts obtained from the two McMaster chambers by 50(dilution factor) (AlJumaili, 2013).

3.5.2 Cleaning and sporulation of oocysts of *E. tenella*

The harvested oocyst culture was transferred into 30 ml plastic tubes. The tubes were balanced, corked carefully and centrifuged at 2,000 rpm for 10 min to pellet the oocysts (Mehlhorn *et al*., 1998). The supernatants were carefully poured into a beaker, and were checked microscopically for any oocyst that may have failed to pellet before discarding the suspension. If there were more oocysts in the supernatant it was centrifuged again until no oocyst remained in the supernatant (Soulsby, 1986).

The pellet was re-suspended in 10 ml of 3.8% hypochlorite in 30 ml tube for 10 min swirling intermittently. The 30 ml tube was topped up with tap water. This was mixed and centrifuged at 2000 rpm for 10 min to pellet the oocysts (Shirley, 1995). The supernatant was discarded after checking for lost oocysts microscopically. The oocysts pellet was re-suspended in saturated salt solution and mixed with a sterile Pasteur pipette. This is to ensure that the pellet is thoroughly re-suspended without lumps in a 30 ml centrifuge tube. The tube was filled to within 2 cm of the top and overlaid with distilled water. This was done by gently running the water down the side of the tube with a plastic wash bottle. This was centrifuged at 2000 rpm for 10 min and repeated thrice. The cleaned oocysts were re-suspended in a 30 ml of distilled water after the final wash (Shirley, 1995).

The oocysts contained in the sediment were allowed to sporulate in Petri-dishes of about 6 mm thickness containing 2.5% potassium dichromate solution at 25-29º C and 60-80% humidity for 72 hrin the laboratory; these Petri-dishes were aspirated with sterile Pasteur pipette frequently to ensure the oxygenation (Shirley, 1995). The sporulated oocysts were cleaned of potassium dichromate by centrifugation at 2,000 rpm for 5 min with 3.8% sodium hypochlorite solution and distilled water. The cleaned oocysts were stored in distilled water in a refrigerator until required for use (Chapman *et al*., 2013; Asghar *et al*., 2015).

3.5.3 Propagation and harvesting of oocysts of *E. tenella*

Ten certified - free fourteen (14) day old chickens were each orally inoculated with 1 ml of oocysts suspension containing 10,000 sporulated oocysts. The chickens were divided into two groups of five chickens per group. They were provided with a well standard broiler feed (anticoccidial free feed) which consists of corn, soybean, and groundnut cake and water available *ad libitum* (Arabkhazaeli *et al*., 2011). These birds were sacrificed on day 14 post inoculation. The caecum of each chicken from the groups of ten chickens inoculated with sporulated oocysts was examined at post mortem. Caeca with lesions consistent with caecal coccidiosis were selected. Each caecum was cut opened longitudinally and placed on glass plate. The edge of each caecum was held to carefully scrap the caecal content, mucosa and deeper muscle layer with a glass microscope slide. The scrappings were poured into a beaker containing 20 ml of phosphate buffer saline (PBS) and shaken vigorously for homogenization. 20 ml of 1.5% trypsin solution was added to the scrapings to break down any connective tissue. This was incubated in a water bath at 47^0C for 1hr 30 min swirling the tube occasionally (i.e. at 30 min intervals). The trypsin treated homogenate was strained through a double layer of muslin and centrifuged at 2000 rpm for 12 min. The supernatant was poured off

and replaced with PBS of p^H7.6 as a wash and was centrifuged at 2000 rpm for 12 min. These washes were repeated three times in tap water (Denis *et al*., 2014).

The harvested oocysts were microscopically enumerated using McMaster counting chamber (Taylor *et al*., 1995). The oocysts were sporulated in Petri-dishes of about 6 mm thickness containing 2.5% potassium dichromate solution at 27^0C and 60-80% humidity for 7 days with proper aeration (Mehlhorn *et al*., 1998). Sporulated oocysts were washed thrice to remove the potassium dichromate solution. Fresh potassium dichromate solution was added to clean oocysts which were stored at 40℃ until required for use (Chapman *et al*., 2013).

3.5.4 Identification of oocysts of *E. tenella*

The oocysts of *E. tenella* isolated from infected chickens were identified according to the morphology index after measuring 100 oocysts using calibrated ocular micrometre at ×40 magnification. This was done by comparing the calculated oocysts shape index values with the guides provided by Long and Reid (1982).

3.6 *In vitro* Anti-coccidial Effects of Methanolic Extracts of some Ethno MedicinalPlants

The *in vitro* anti-coccidial efficacy trials were conducted on ten ethno medicinal plants frequently used by poultry farmers to treat symptoms suspected to be coccidiosis in the study area (Zaria). The effect of these crude methanolic plant extracts was checked on sporulated oocysts of *Eimeria tenella*. The parameters observed include viable and population structure. Five different dilutions of the respective crude extracts of ten ethno medicinal plants and Amprolium® (1:25, 1:50, 1:100, 1:200, and 1:400) in distilled water were placed in separate Petri dishes labelled appropriately (Adriana *etal*.,

2008). Amprolium® (a universal anticoccidial) was purchased from a veterinary pharmacy shop in Zaria. 0.01ml of a suspension of freshly sporulated oocysts of *E. tenella* containing 500 oocysts was added to each Petri dish (Narsih *et al*., 2012). The set up was kept at room temperature (24^0C -28^0C), semi-covered with periodic homogenization. 0.1 ml suspension of each dilution was examined microscopically after 24 h, 48 h, 72 h and 96 h. The viability (oocysts with sporocysts and distorted walls) were viewed under the light microscope using $\times 10$, $\times 40$ and $\times 100$ objectives and photomicrographed (Abdul Lateef *et al*., 2009). The percent population of killed oocysts was estimated by counting the number of oocysts remaining in a total of 500 oocysts (Pieri *et al*., 2014).

3.7 Phytochemical Analyses of Methanolic Extracts of *Azadirachta indica*, *N. diderichii* and *S. dasyphyllum*

Qualitative analyses for plants' secondary metabolites were carried out on the three most effective plants obtained from *in vitro* anti-coccidial test. The compounds that were investigated in the crude plant extracts were alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids and tannins.

3.7.1 Test for alkaloids (Harborne, 1973; Trease and Evans, 1989)

The test for alkaloids was carried out by defatting about 0.5 g each of the three crude powered samples with 5% ethyl ether for 15 min. The defatted samples were extracted for 20 min with five millilitres (5 ml) of aqueous 1% HCl on a boiling water bath. The extracted samples were centrifuged for 10 min at 3000 rpm (revolution per minute). The solutions obtained were filtered and 1 ml of each filtrate was treated with few drops of Mayer's reagent (potassium mercuric iodide) and another 1 ml with Dragendroff's

reagent (bismuth potassium iodine solution) i.e. brick red coloration test. To another 1 ml of each filtrated, 2 drops of Hager's (saturated solution of picric acid) reagent was also added. The test tubes were observed for coloured precipitates.

3.7.2 Test for combined anthraquinones (Satheesh *et al.*, 2012)

To test for combined anthraquinones, 1 g of each of the crude powered extracts was boiled with 2 ml of 10% hydrochloric acid for 5 min. The mixtures of each of the samples were filtered while hot and filtrate was allowed to cool. Each of the cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using clean pipettes. Equal volume of 10% ammonia solution was added into each of the chloroform layer, shaken and allowed to separate. The separated aqueous layers were observed for any colour change.

3.7.3 Test for free anthraquinones

Free anthraquinones were tested for by adding 5 ml of chloroform to 0.5 g of each of powdered plant extract samples. Each of the resulting mixtures was shaken for 5 min and then filtered. Each filtrate was then shaken with equal volume of 10% ammonia solution and the appearance of bright pink colour in the aqueous layers was observed (Odebiyi and Sofowora, 1990).

3.7.4 Test for carbohydrates (Satheesh *et al.*, 2012)

One (1 ml) of distilled water was added to about 0.1 g of each sample in the test tube and the mixture boiled for 5 min to test for carbohydrates. The mixture was filtered while hot and then cooled. 5 ml of mixture or equal volumes of Fehling's solutions A

and B was added to 2 ml of the filtrate in a test tube. The resultant mixture was boiled for 2 min and colour type recorded

3.7.5 Test for cardiac glycosides

The test for cardiac glycosides was carried out by adding 5 ml of each extract and treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under laid with 1 ml of concentrated sulphuric acid (Harborne, 1983).

3.7.6 Test for flavonoids

The presence of flavonoids in the three plant samples was determined by adding 5 ml of dilute ammonia solution to a portion of the aqueous filtrate of each of the plant extracts. This was followed by addition of concentrated H_2SO_4. Few drops of 1% aluminium solution were added to a portion of each filtrate (Trease and Evans, 1989). A portion of each of the powered plant samples was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Colour change was recorded accordingly (Sofowora, 1993).

3.7.7 Test for saponins (Harborne, 1983)

Froth test was used to detect saponins in the three crude plant extracts. Each sample (1 g) was weighed into a conical flask; sterile distilled water (10 ml) was added and boiled for 5 min. The mixture was filtered and 2.5 ml of the filtrate was added to 10 ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for 30 sec. It was then allowed to stand for half an h. The appearance of honey comb froth was observed and recorded.

3.7.8 Test for steroids

Each portion of the sample (0.2 g) was dissolved in 2 ml of chloroform. 0.2 ml of concentrated H_2SO_4 was carefully added to form a layer. Characteristic colour change was recorded (Trease and Evans, 1989).

3.7.9 Test for terpenoids

Each extract (5 ml) was mixed in 2 ml of chloroform followed by the addition of 3 ml of concentrated H_2SO_4 to form a layer. The appearance of a reddish-brown precipitate colouration at the interface was recorded (Odebiyi and Sofowora, 1990).

3.7.10 Test for tannins

Each powdered sample (1 g) was separately boiled with 20 ml distilled water for 5 min in a water bath and filtered while hot. 1 ml of cooled filtrate was diluted to 5 ml with distilled water. A few drops of 10% ferric chloride was added and observed for any formation of precipitation and any colour change. The reaction mixture was observed and recorded (Satheesh *et al*., 2012).

3.8 Determination of Acute Toxicity of *A. indica, Nauclea diderichii* and *S*olanum *dasyphyllum*Methanolic Stem bark Extracts.

Oral acute toxicity tests of the three most effective plant extracts from the *in vitro* tests in 3.6 above were carried out in chicks in two phases as described by Lorke (1983). A pilot study was carried out with methanolic extract of *A. indica* in day old chicks by using nine chicks of three groups (three chicks per each group). Each chick in each group was administered with 10mg, 100mg and 1000mg respectively. The chicks were observed for signs of depression, limping and or death in the first twenty-four hours (Lorke, 1983; Hashemi *et al*., 2010). From the result of the pilot studies, suitable dose

interval was chosen (1,200 1600, 2900 and 5000) mg/kg body weight)and used to conduct the final toxicity of the plant extract in four chicks of four groups.They were observed for signs of toxicity (depression, limping and or death) for twenty-four hours. This was repeated for *Nauclea diderichii* and *Solanum dasyphyllum*methanolic stem bark extracts. The LD_{50} was then calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose (Lorke, 1983). This is the geometric mean of the consecutive doses for 0% and 100% survival rates recorded in the second phase. The oral lethal dose was calculated using the formula (Probit Analysis):

$$LD_{50} = \sqrt{Mimimum\ toxic\ dose\ \times maximum\ toxic\ dose}$$

3.9 *In vivo* Anti-coccidial Effect of Methanolic Extracts of *Azadirachtaindica, N*auclea*diderichii*and *S*olanum *dasyphyllum*

3.9.1 Chickens and housing

A total of 180 one-day-old apparently healthy broiler chicks weighing between 37 and 59 gm purchased from Obasanjo Hatchery, Oluyole Estate, Ibadan, Oyo State, Nigeria, were distributed into 18 different treatment groups (A_1 - F_1, A_2 – F_2 and A_3 – F_3) of ten chicks per group. The birds were raised in clean disinfected room under standard conditions that excluded *Eimeria* infection for two weeks. They were provided with a well standard broiler feed (anticoccidial free feed), (without antibiotics, anti-coccidial or growth promoters) which consisted of corn, soybean, and groundnut cake and water available *ad libitum* during the experiment (28days) (Arabkhazaeli *et al*., 2011).Temperature and lighting regiments were in accordance with the recommendation of the supplier. The initial temperature of 32 to 33^0C was reduced weekly by 10^0C to a final temperature of 28^0C (Ljijana *et al*., 2012).

3.9.2 Experimental infection and treatment (Ogbe *et al*., 2013)

Each bird in groups A_1 to E_1, A_2 to E_2 and A_3 to E_3 was inoculated with 15,000 sporulate oocysts of *E. tenella*. The oocysts were administered with 0.3 ml of distilled water using insulin syringe directly into their crop at two weeks of age. The negative control groups F_1, F_2 and F_3 were neither inoculated nor treated.

By day 3 post inoculation (Pi), the chickens in groups A1, A2 and A3 were treated with 100 mg/L of Amprolium (Nweze and Obiwulu, 2009). Chickens in groups $B_1 - B_3$, $C_1 - C_3$ and $D_1 - D_3$ were given 1000 mg/ml, 500 mg/ml and 250 mg/ml body weight of extracts of *Solanum dasyphyllum*, *Nauclea diderichii* and *Azadirachta indica* per day respectively for seven days. The drug and plant extracts were administered to the chickens via their drinking water. The medication with Amprolium and herbal extracts was carried out according to the following schedule:

Group A_1: Amprolium 100 mg/L/body weight per day,

Group B_1: *S. dasyphyllum* extract 1000 mg/kg/body weight per day,

Group C_1: *S. dasyphyllum* extract 500 mg/kg/body weight per day,

Group D_1: *S. dasyphyllum* extract 250 mg/kg/body weight per day,

Group E_1: infected untreated control and

Group F_1: uninfected, untreated control.

3.9.3 Examination of faeces for oocysts of *Eimeria tenella*

One gram (1 g) of faeces from each group (cage) were collected with the aid of polythene bags on day 4 post infection, days 3, 5 and 7 post treatments for oocyst counts and comparison (Oyagbemi and Adejinmi, 2012). Faeces from each group were thoroughly mixed in sterile plastic bottles with the aid of sterile spatulas and placed in

sterile bottles. Each sample was homogenized by mixing with 1 ml of floatation medium (sodium chloride) salt solution to make a suspension. This suspension was then mixed with 9 ml of the salt solution and sieved in a tea strainer. The solid matter was discarded and the filtrate collected in clean sterile plastic bottles. These were filled to the brim and a cover slip placed on top of each bottle carefully to exclude air bubbles. The bottles were allowed to stand upright for 15 min to enable the oocysts of coccidia float to the cover slips. The cover slips were then placed on clean glass slides and examined under a light microscope at ×10 and ×40 magnifications (Ogbe *et al.,* 2013).

Portions of the positive samples only were used to fill the McMaster counting chamber using sterile micropipettes. These were allowed to stand for about 15 min to enable oocysts to float and settle at the top of the chamber. This is to facilitate counting of the oocysts under the microscope using a differential counter (Ogbe *et al*., 2013). Output per chicken per group was calculated by dividing the total number of oocysts by the number of chickens per group (Denis *et al.*, 2014).

3.10 Chromatographic Procedure

3.10.1 Thin layer chromatography (TLC)

Slurry of Kieselgel Silica gel (60pf. 254-366) was made and poured evenly into the rectangular well of the hopper located on the end plate (placed over the spreader). This was then drawn steadily over the glass plate surface (20× 20cm). The coated plate was activated at 120^{O}C in the oven for about two hours. The loading of the sample was done by using a capillary tube and applying successive spots touching each other following a line drawn across one end of the plate (about 2 cm from the edge). The methanolic extract was chromatographed on the plates. Ethyl acetate was used to develop the plates.

The bands were observed under ultra-violet light (with different relative flow (RF) values) (Tijjani *et al*., 2012).

The components were removed from the plates by inscribing a line around the bands and then the desired areas were scraped off into a micro-column previously packed with a glass wool. The layers were eluted with aqueous methanol 30%, 60% and 100% respectively. The fraction obtained on elution with H_2O : MeOH (40: 60) was transferred on another column using Hexane/EtOAc ($0 \rightarrow 50\%$). Later, the Hexane : EtOAc (80 : 20) part was separated from the rich chlorophyll content and pigments by size exclusion chromatography on sephadex (LH-20) using Dichloromethane (DCM) / MeOH (1:2). Finally, fractions which showed violet spots on TLC with cerium sulphate reagent were selected and purified on recycling HPLC Jaigel-2H and 1H columns with chloroform as the mobile phase to yield two pure isolates (Csapi *et al*., 2010).

3.10.2 Chromatographic separation (Tijjani *et al*., 2012)

Each of the respective methanolic extracts (10 g) of *N. diderichii* and *S.* d*asyphyllum* was chromatographed over a silica gel packed column of dimension 75cm by 3.5 cm. The column was eluted continuously using neat n–hexane, hexane/ethyl acetate, chloroform/ethyl acetate and ethyl acetate/methanol at different ratio. 65 collections from *S. dasyphyllum* were pooled together based on their TLC profiles which produced 10 fractions. 75 collections from *N. diderichii* sample were pooled together based on their TLC profiles which produced 12 fractions. These fractions from both *S. dasyphyllum* and *N. diderichii* were pooled to six fractions respectively. These pooled fractions were tested for anti-coccidial activity on sporulated oocysts of *E. tenella* in comparison with Amprolium to obtain prominent fractions from each of the samples.

3.11 *In vitro* Anti-coccidial Effect of the Fractions of *Solanum dasyphyllum* and *Nauclea diderichii* (Kubmarawa *et al.*, 2009)

The *in vitro* anti-coccidial efficacy trials were carried out on six fractions of each of *S. dasyphyllum* and *N. diderichii* compared with Amprolium, a standard anti-coccidial drug. Five different dilutions of each of the fractions and Amprolium (1:25, 1:50, 1:100, 1:200 and 1:400) in distilled water were placed in separate Petri dishes. These were labelled appropriately. To each petri dish, 0.01 ml suspension containing 500 sporulated oocysts was added. These were kept at room temperature, semi-covered with periodic homogenization. 0.1 ml suspension of each dilution was examined microscopically after 24 h, 48 h, 72 h and 96 h for population structures as well as the viability of sporulated oocysts.

3.12 Preparative TLC of the Prominent Fractions (He and Lui, 2008)

One major spot and two minor spots obtained from the most active fractions (F6 and F9) from *S. dasyphyllum*. One major spot and three minor spots obtained from the most active fractions (F7 and F8) from *N. diderichii*. These were subjected to preparative TLC. One spot each was successfully removed as pure substance from each of these spots which were code named BS1 and BS2 respectively. These (BS1 and BS2) substances were tested on sporulated oocysts of *E. tenella* in comparison with Amprolium and were later subjected to spectroscopic analysis to elucidate the chemical structures. Proton (^{1}H) and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Joel Eclipse spectrophotometer at a frequency of 500 MHZ for ^{1}H and 125 MHZ for ^{13}C. These spectra were obtained on solutions of samples in deuterated chloroform ($CDCl_3$) with tetramethylsaline (TMS) as internal reference. Mass spectral were ran on a HRFAB mass spectrometer.

3.13 Data Analyses

Descriptive statistics such as frequencies and percentages were used in the analysis of the data obtained from ethno medicinal survey. Data obtained from *in-vitro* anti-coccidial tests were subjected to Analysis Of Variance (ANOVA). Least Significant Difference (LSD) was used to separate the means where significant difference occurs among the efficacies of the plant extracts. Student's t-test was used to compare the efficacy of the ten methanolic plants extracts on sporulated oocysts of *E*. *tenella*. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using SAS package (version 9.3) (Satheesh *et al*., 2012).

CHAPTER FOUR

4.0 RESULTS

4.1 Plant Collection and Identification

Results of plants collected and identified are presented as common plants used for treatment of poultry diseases including coccidiosis by local farmers and the percent of respondents are presented in Table 1. Common plants used and the percent of respondents are shown in Table 1. The frequency of use of *Khaya senegalensis* and *Azadirachta indica* was highest among the respondents (11.9% and 9.4%) respectively. Other plants that were frequently used by the poultry farmers in Zaria include *Ficus platyphylla*, *Nauclea diderichii* (7.5%), *N. latifolia*, *Solanum dasyphyllum* (6.9%), *Cissus populnea*, *Citrus sinensis*, *Vitellariaparadoxa* (6.3%) and *Parkia biglobosa* (5.0%). Medicinal plants such as *Capsicumannum* (4.4%), *Cassiaoccidentalis* (3.8%), *Artemisia absinthium*, *Euphorbia hirta* and *Jatropha curcas* (3.0%) were rarely used by poultry farmers in Zaria. *Adansoniadigitata*, *Allium cepa* and *Vernoniaamygdalina* (1.9%) were scarcely used in treating poultry diseases in the study area.Plant that was used by most of the farmers was *A. indica* and the least used plant was *J. carcus*. The medicinal plant parts used were stem bark and leaves while fruits and roots were seldom utilized. These plant parts were either used freshly or dry and all the medicinal plants were administered orally except for the treatment of ectoparasites. Water was the solvent used in preparation and mostly added to the birds' drinking water.

Symptoms described by most poultry farmers in the study area included bloody and foamy diarrhoea. Other suspected diseases encountered in the study area were fowl cholera, lice infestation, New castle disease, bronchitis and dysentery.

Table1: Medicinal plants used to treat poultry diseases in Zaria

S/N	Botanical name	Family	Common Name	Local name	Possible target diseases	Parts used	% of respond ence
1	*Adansonia digitata* Linn	Bombacaceae	Baobab	Kuka	Diarrhoea, coccidiosis, tick infestation and fowl cholera	Stem bark, leaves and fruits	2.5
2	*Allium cepa*	Liliaceae	Onion	Albasa	Helminthiasis.	Bulb and leaves (spring onion)	1.9
3	*Allium sativum* L.	Liliaceae	Garlic	Tafarnuwa	Dysentery	Bulb	2.5
4	*Artemisia absinthium* L.	Asteraceae	Worm Wood	Akujaadi	Dysentery, coccidiosis and lice	Leaves	3.0
5	*Azadirachta indica* A. Juss	Meliaceae	Neem	Dogon Yaro	Coccidiosis and external parasites	Root, stem bark, leaves and fruits	9.4
6	*Capsicum annum* L	Rutaceae	Pepper	Barkono/Tarshi	New castle disease, diarrhoea and coccidiosis	leaves and fruits	4.4
7	*Cassia occidentalis* Linn	Leguminoseae/ Caesalpiniaceae	Coffee weed	Tafassar masar	bronchitis, helminthiasis and coccidiosis	Leaves and fruits	3.8
8	*Cissus populnea*	Vitaceae	Food gum	Dafara	coccidiosis and intestinal parasites	Stem bark, leaves, root	6.3
9	*Citrus sinensis* (Christm.) Swingle	Rutaceae	Lime	Lemun tsami	coccidiosis and worm infestation	Leaves, dry peel and fruits.	6.3
10	*Euphorbia hirta* Linn	Euphorbiaceae	Goat weed	Nonon-kurchiya.	tick infestation and intestinal parasites	Latex	3.0

Table 1 Continued

11	*Ficus platyphylla*	Moraceae	Broad leaf fig	Gamji	Intestinal parasites and coccidiosis	Root and stem bark	7.5
12	*Jatropha curcas* Linn	Euphorbiaceae	purging nut	Birida zugu	Coccidiosis	Latex	3.0
13	*Khaya senegalensis*	Meliaceae	Mahogany	Madaci	Coccidiosis and helminthiasis.	Root, stem bark and leaves	11.1
14	*Nauclea diderichii*	Rubiaceae	Opepe	Tafashiya	Coccidiosis and worm infection.	Stem bark, root, leaves	7.5
15	*Nauclea latifolia*	Rubiaceae	African peach	Tajashi	Coccidiosis	Stem bark, root, leaves	6.9
16	*Parkia biglobosa*	Fabaceae	Locust bean	Dorawa	Coccidiosis	Leaves, stem bark, fruits and root	5.0
17	*Solanum dasyphyllum* (Schum et Thonn)	Solanaceae	Egg plant	Gautan kura	Amoebiasis and coccidiosis	Root, leaves, fruits and stem bark	6.9
18	*Vernonia amygdalina* Del	Compositae	Bitter leaf	Shuwaka	Coccidiosis and dysentery	Leaves	1.9
19	*Vitellariaparadoxa*	Sapotaceae	Shea tree	Kadanya	Coccidiosis and worm infection	Leaves, stem bark, fruits and root	6.3

4.2 *In vitro* Anti-coccidial Effect of crude Methanolic Extract of someEthno medicinal plants in Comparison with Amprolium

The *in vitro* anti-coccidial efficacy trials of ten ethnomedicinal plants frequently used by poultry farmers to treat symptoms suspected to be coccidiosis in Zaria showed some effects on sporulated oocysts of *E. tenella*. The various degrees of damaged cell walls, oocysts without cell walls containing free sporocysts observed after 72 and 96 h are shown in Plates I and II. These were compared with normal oocysts of *E. tenella* at different stages. Reduction in population of parasites was also obtained at various concentrations over a period of 96 h for each of the ten plant extracts compared with Amprolium (Figure I and Appendix I).

Most of the sporulated oocysts (89.8%) were killed in Amprolium over a 96 h period. The rate of lethality in *Azadirachta indica* extract was 73.0%, 67.0%, 61.0%, 58.0% and 53.0% in 1 mg/ 25 ml, 1 mg/ 50 ml, 1 mg/ 100 ml, 1 mg/200 ml and 1 mg/400 ml respectively after 96 h of exposure. Extract of *Citrus sinensis* killed 48.0%, 42.0%, 38.0%, 30.0% and 27.0% oocysts at different concentrations (1:25, 1:50, 1:100, 1:200 and1:400) after 96 h of exposure. 60.0% oocysts were killed by *Cissus populnea* extract at 1:25 concentration after 96 h. 52.0%, 28.0%, 20.0% and 14.0% oocysts were killed at concentration 1:50, 1:100, 1:200 and 1:400 respectively at 96 h. After 96 h, the rate of killed oocysts in *Ficus platyphylla* extract was 56.2%, 32.0%, 25.0%, 18.2% and 8.6% in 1:25, 1:50, 1:100, 1:200 and 1:400 concentrations respectively.

Khaya senegalensis extract killed 46.0%, 36.0%, 15.0%, 12.2% and 0.3% oocysts in the different concentrations after 96 h. In the case of *Nauclea diderichii*, at dilution 1:25 after 96 hrs, 92.0% oocysts were killed. For the second and third dilutions (1:50 and 1:100), 80.0% and 74.2% % sporulated oocysts were killed at 96 h respectively.

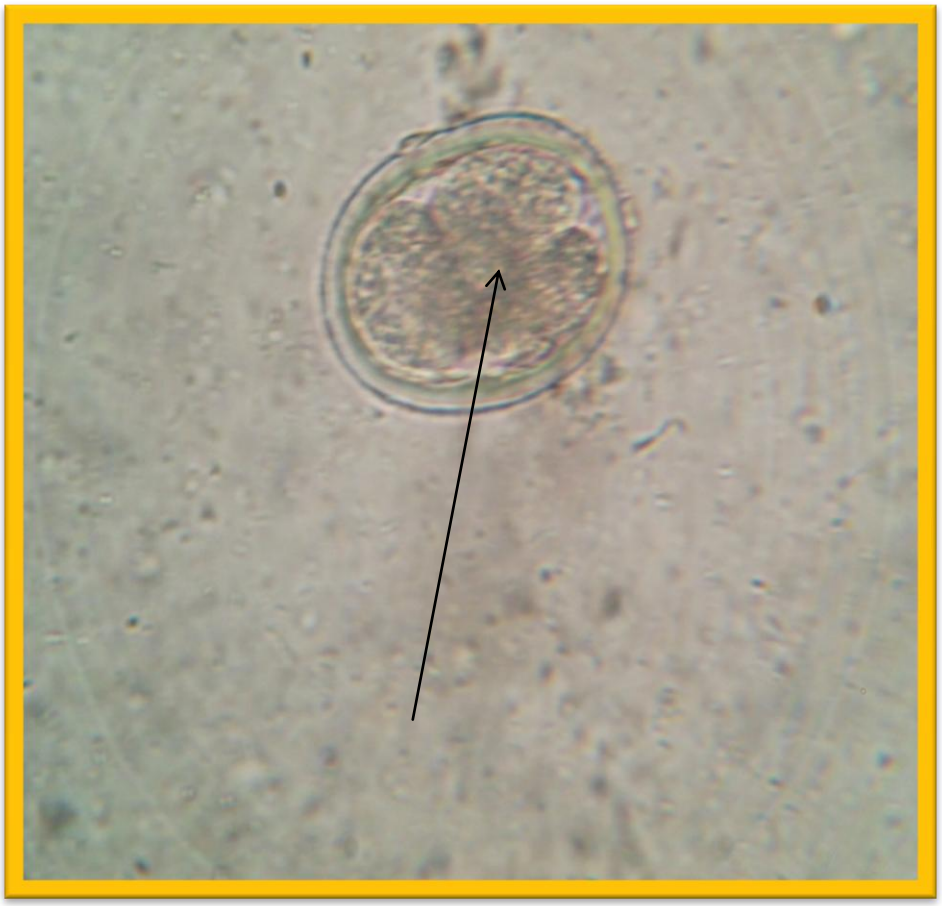


Plate I: Oocysts with destroyed sporocysts (Arrows show point of oocyst destruction)



Plate II: Oocysts with empty cell walls. (Arrows show empty spaces)

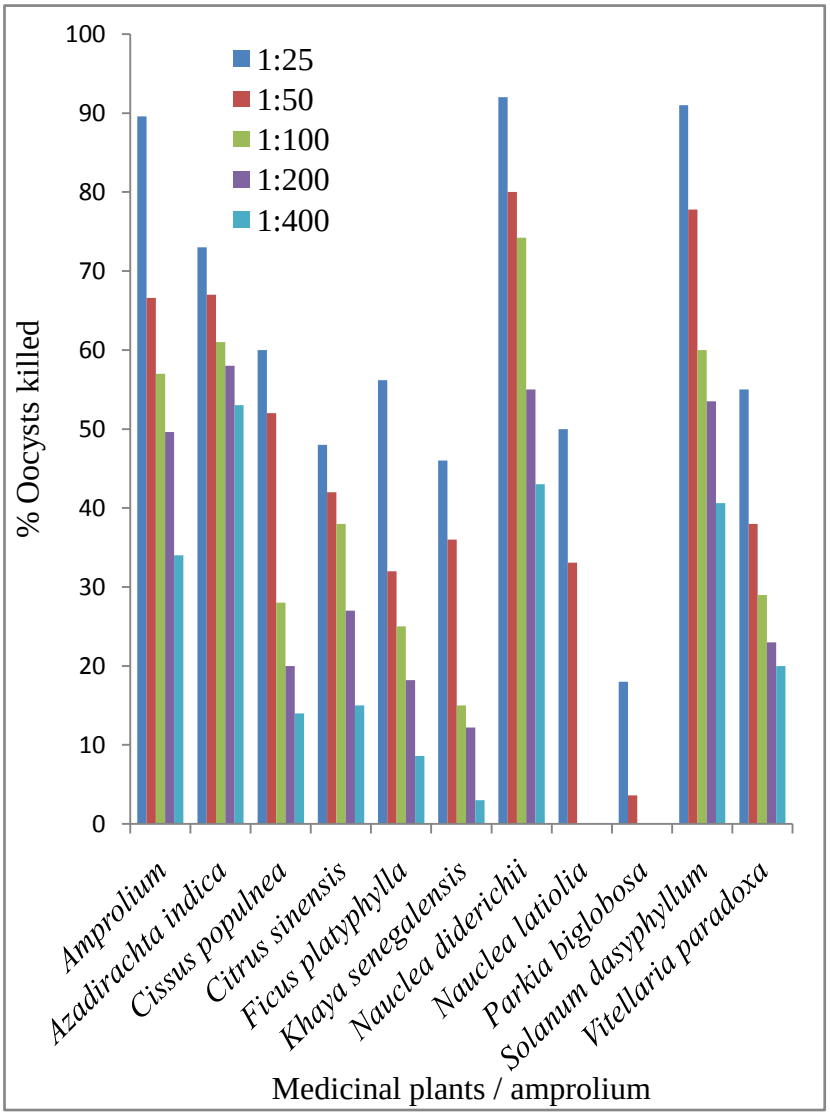


Figure 1: *In vitro* anti-coccidial effect of crude methanolic extracts of some ethnomedicinal plants on sporulated oocysts of *E. tenella* at different concentrations at 96 h

50.0% and 33.0% oocysts were killed after 96 h of exposure to *N. latifolia* extract in 1:25 and 1:50 concentrations respectively. All the oocysts in 1:100, 1:200 and 1:400 concentrations were not affected by the plant extract after 96 h of exposure. Above observation was also recorded in *Parkia biglobosa* extract where only18.0% and 02.0% oocysts were killed in 1:25 and 1:50 concentration respectively. For *Solanum dasyphyllum*, 91.0%, 77.8%, 60.0%, 53.4% and 40.6% oocysts were killed over a 96 h period of exposure at different concentrations of 1:25, 1:50, 1:100, 1:200 and 1:400 respectively. In the case of *Vitellaria paradoxa* extract, the rate of oocysts killed was 55.0%, 38.0%, 29.0%, 23.0% and 20.0% respectively after 96 h of exposure.

The efficacies of the different crude medicinal methanolic extracts as well as the universal drug (Amprolium®) were concentration dependent. It was shown that as the concentration of the plant methanolic extracts and Amprolium® increases, the anti-coccidial activity also increases (Figure 2 and Table 2).

There was a significant difference ($P< 0.05$) between the efficacy of the different crude methanolic plantextracts and Amprolium (Table 3 and Appendix II). *Naucleadiderichii*, *Solanumdasyphyllum* and *Azadirachtaindica* were the most effective of the ten ethnomedicinal methanolic extracts (Figure 1, Table 3and Appendix II). This informed the choice of the crude plant extracts for phytochemical, acute toxicity tests and *in-vivo* efficacy trials.

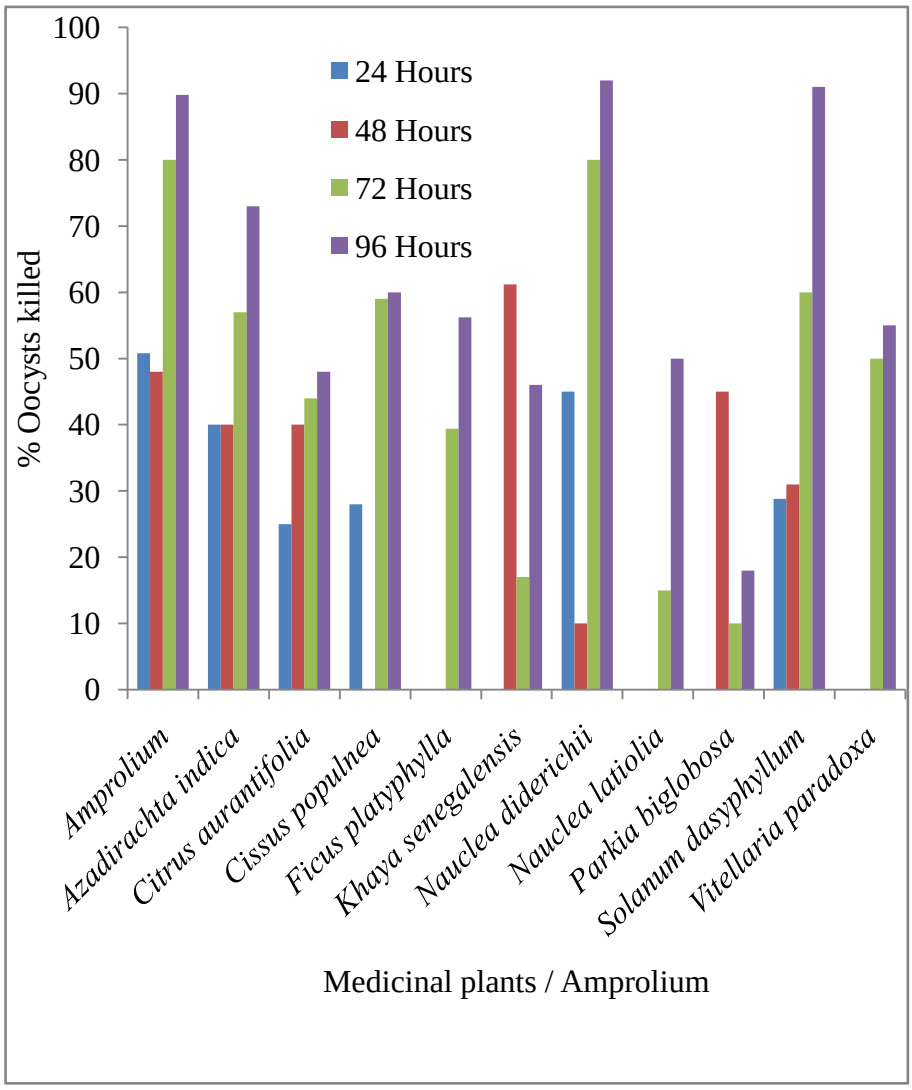


Figure 2: *In vitro* anti-coccidial effect of crude methanolic extracts of some ethno medicinal plants on sporulated oocysts of *E. tenella* at concentration 1:25 over a 96 h period

Table 2: *Invitro* anti-coccidial effect of some ethnomedicinal plant methanolic extracts (ANOVA) at p< 0.05 (concentration)

Treatment groups	Number of oocysts killed
Group 1 Amprolium positive control	297.6 ± 1.62
Group 2 1mg/25ml	306.9 ± 1.09[a]
Group 3 1mg/50ml	240.6 ± 1.09[b]
Group 4 1mg/100ml	176.0 ± 1.09[c]
Group 5 1mg/200ml	143.0 ± 1.09[d]
Group 6 1mg/400ml	104.7 ± 1.09[e]

Result in mean ± SEM (n =6) value in the same column with superscript are considered significant (p< 0.05) compared with group 1 (positive control)

Table 3: *Invitro* anti-coccidial effect of some ethnomedicinal plant methanolic extracts (ANOVA) at p< 0.05

Treatment groups	Number of oocysts killed
Group 1 Amprolium positive control	297.6 ± 1.62^{d}
Group 2 *Azadirachtaindica*	309.6 ± 1.62^{c}
Group 3 *Cissuspopulnea*	169.9 ± 1.62^{e}
Group 4 *Citrussinensis*	171.1 ± 1.62^{e}
Group 5 *Ficusplatyphylla*	138.6 ± 1.62^{g}
Group 6 *Khayasenegalensis*	112.6 ± 1.62^{h}
Group 7 *Naucleadiderichii*	344.7 ± 1.62^{a}
Group 8 *Nauclealatifolia*	84.0 ± 1.62^{i}
Group 9 *Parkiabiglobosa*	22.2 ± 1.62^{j}
Group 10 *Solanumdasyphyllum*	322.6 ± 1.62^{b}
Group 11 *Vitellariaparadoxa*	164.0 ± 1.62^{f}

Result in mean ± SEM (n =11) value in the same column with superscript are considered significant (p< 0.05) compared with group 1 (positive control)

4.3 Phytochemicals of crude Methanolic Extracts of *Azadirachta indica, N*auclea *diderichii* andSolanum*dasyphyllum*

The qualitative analyses of the phytochemical constituents of the three selected plant (*A. indica*, *N.diderichii* and *S. dasyphyllum*) extracts revealed that all the plant extracts contain alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. Combined anthraquinones and free anthraquinones are absent in all the plant extracts. Steroids were observed to be present in *S. dasyphyllum* extract only which are absent in the extracts of *A. indica* as well as *N. diderichii* (Table 4).

4.4 Acute Toxicity of crude Methanolic Extracts of *Azadirachtaindica, Nauclea diderichii* and *Solanumdasyphyllum*

Acute toxicity tests of *A. indica*, *N. diderichii* and *S. dasyphyllum* carried out on chickens show that the use of plant extracts did not show any hazardous symptoms that could lead to death. No chicken was lost or died on administration of 5000 mg/kg body weight of the plant extracts within the short-term outcome of the limit dose test (Tables 5 to 7).

4.5 Infection and Treatment of Chicks

On day 4 post-infection (dpi), all the birds experimentally infected with sporulated oocysts of *E. tenella* (Groups A-E) showed clinical signs of depression, weakness, ruffled feathers and bloody diarrhoea. Oocysts were detected in their faeces which indicated that infection of birds with *E. tenella* was successful. Prior to treatment of the birds, the oocysts output was 6,200 oocysts/g faeces (Group A1), 6,510 oocysts/g faeces (Group B1), 6,100 oocysts/g faeces (Group C1), 5,700 oocysts/g faeces (Group D1),7,500 oocysts/g faeces (Group E1), (Figure 3 and Appendix III).

Similarly, the oocyst output was 8,350 oocysts/g faeces (Group A2), 7,500 oocysts/g faeces (Group B2), 7,700 oocysts/g faeces (Group C2), 8,500 oocysts/g faeces (Group

D2) and 11,800 oocysts/g faeces (Group E2) before treatment (Figure 4 and Appendix IV). The oocyst output was 7,100 oocysts/g faeces (Group A3), 6,400 oocysts/g faeces (Group B3), 7,090 oocysts/g faeces (Group C3), 5,900 oocysts/g faeces (Group D3) and 6,070 oocysts/g faeces (Group E3), (Figure 5 and Appendix V).

The faeces of uninfected birds (Groups F1, F2 and F3) were free of coccidial oocysts, did not contain blood and the birds were alert throughout the experimental studies (Appendix III -V).

On the 3rd day after treatment with varying doses of *S. dasyphyllum,* the number of oocysts reduced drastically with (579 (8.9%); 756 (12.4%12.4%) and 1,210 (16.1%) oocysts/g faeces produced respectively as compared to the Amprolium- treated group A1, in which oocysts shedding reduced slightly with (1,800 (29.0%) oocysts/g faeces) produced. Birds in group E1, showed a significant increase ($p < 0.05$) in oocysts shedding (13,700 (91.3%) oocysts/g faeces) compared with the infected treated groups and uninfected group (Table 8 and Appendix III).

Similarly the birds treated with varying doses of *N.* d*iderichii* showed drastic reduction of oocysts in their faeces (100 (1.3%); 200 (2.6%) and 500 (5.9%)) oocysts /g faeces compared to the Amprolium-treated group A2 (2,472 (16.48%)) oocysts/g faeces. There was an increase in number of oocysts released by Group E2 birds (14,600 (97.3%) oocysts/g faeces) (Appendix IV). Oocysts/gram faeces shed significantly ($p < 0.05$) reduced in *N. diderichii*-treated group birds (Table 9). The oocysts detected in *A. indica* –treated birds reduced mildly in number (2,410 (37.7%,); 1,300 (18.3%) and 1,900 (31.3%)) oocysts/g faeces on the 3rd day after treatment in group B3, C3 and D3 respectively compared to Amprolium- treated group A3 (2,009 (28.3%) oocysts/g faeces). Group E3 birds showed a significant ($p<0.05$) increase in oocysts released (16,000 (>100%) oocysts/g faeces) (Table 10 and Appendix V).

Table 4: Phytochemical Constituents of *Azadirachta indica*, Nauclea *diderichii* and *Solanum dasyphyllum*Methanolic stem bark Extracts

S/N	Plant extracts	Phytochemical compounds									
		Alkaloids	Combined anthraquinones	Free anthraquinones	Carbohydrates	Cardiac glycosides	Flavonoids	Saponins	Steroids	Tannins	Terpenoids
1	*A. indica*	+	-	-	+	+	+	+	-	+	+
2	*N. diderichii*	+	-	-	+	+	+	+	-	+	+
3	*S. dasyphyllum*	+	-	-	+	+	+	+	+	+	+

Key: - = Absent; + = Present

Table 5: Acute Toxicity of *Azadirachtaindica* Methanolic stem bark Extract

Phase of Toxicity	Weight of chicks used	Dose (mg/kg body weight)	Number of death
Phase I	50g-70g	10	0/3
	50g-70g	100	0/3
	50g-70g	1000	0/3
Phase II	50g-70g	1,200	0/1
	50g-70g	1,600	0/1
	50g-70g	2,900	0/1
	50g-70g	5,000	0/1

$LD_{50} > 5{,}000mg/kg$

Key**:** 0/3 **=** none of the chickens died in the first phase.
0/1= none of the chickens died in the second phase.

Table 6: Acute Toxicity of *Nauclea diderichii* Methanolic stem bark Extract

Phase of Toxicity	Weight of chicks used	Dose (mg/kg body weight)	Number of death
Phase I	50g-70g	10	0/3
	50g-70g	100	0/3
	50g-70g	1000	0/3
Phase II	50g-70g	1,200	0/1
	50g-70g	1,600	0/1
	50g-70g	2,900	0/1
	50g-70g	5,000	0/1

$LD_{50} > 5{,}000mg/kg$

Key**:** 0/3 **=** none of the chickens died in the first phase.
0/1= none of the chickens died in the second phase.

Table 7: Acute Toxicity of *Solanum dasyphyllum* Methanolic stem bark Extract

Phase of Toxicity	Weight of chicks used	Dose (mg/kg body weight)	Number of death
Phase I	50g-70g	10	0/3
	50g-70g	100	0/3
	50g-70g	1000	0/3
Phase II	50g-70g	1,200	0/1
	50g-70g	1,600	0/1
	50g-70g	2,900	0/1
	50g-70g	5,000	0/1

$LD_{50} > 5{,}000mg/kg$

Key**:** 0/3 **=** none of the chickens died in the first phase.
0/1= none of the chickens died in the second phase.

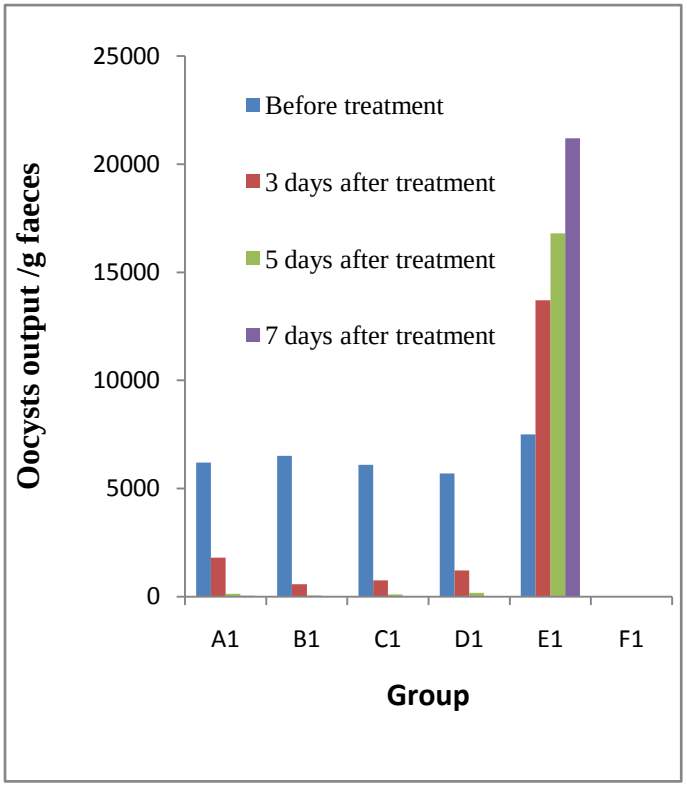


Figure 3: Faecal oocysts output of chickens treated with *S. dasyphyllum* methanolic extract (Group A1 infected and treated with 100 mg/L of Amprolium, B1, C1 and D1 infected and treated with 1,000, 500 and 250 (mg/kg) of *S.dasyphyllum* respectively, E1 infected/ not treated and F1 not infected, not treated)

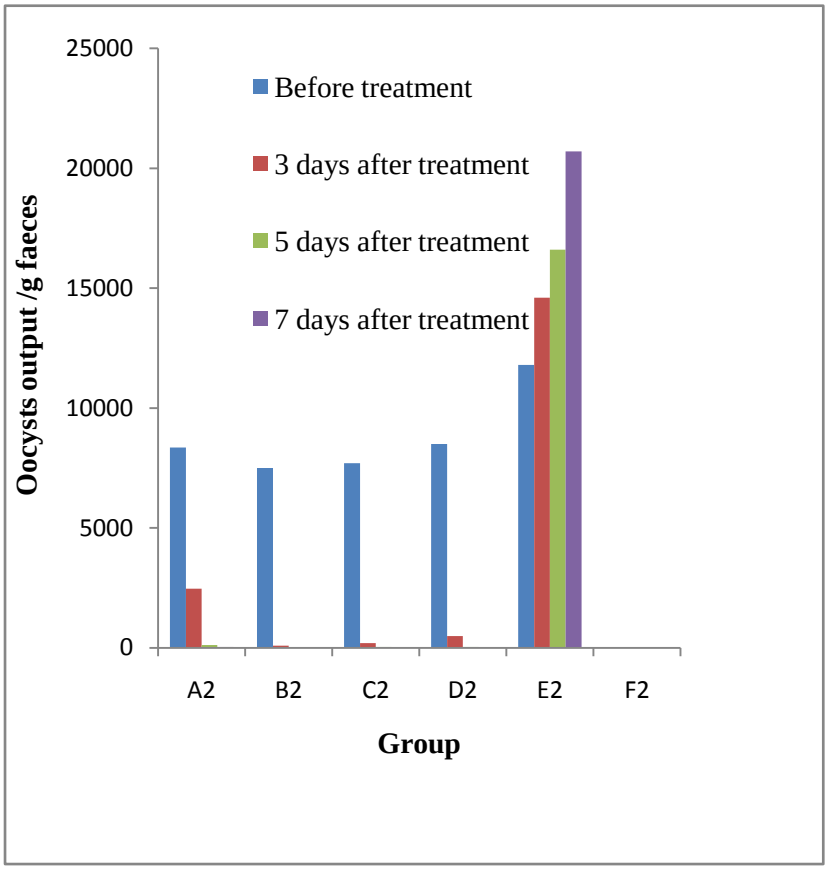


Figure 4: Faecal oocysts output of chickens treated with *N. diderichii* Methanolic extract (Group A2 infected and treated with 100 mg/L ofAmprolium, B2, C2 and D2 infected and treated with 1,000, 500 and 250 (mg/kg) of *N. diderichii* respectively, E2 infected/ not treated and F2 not infected, not treated)

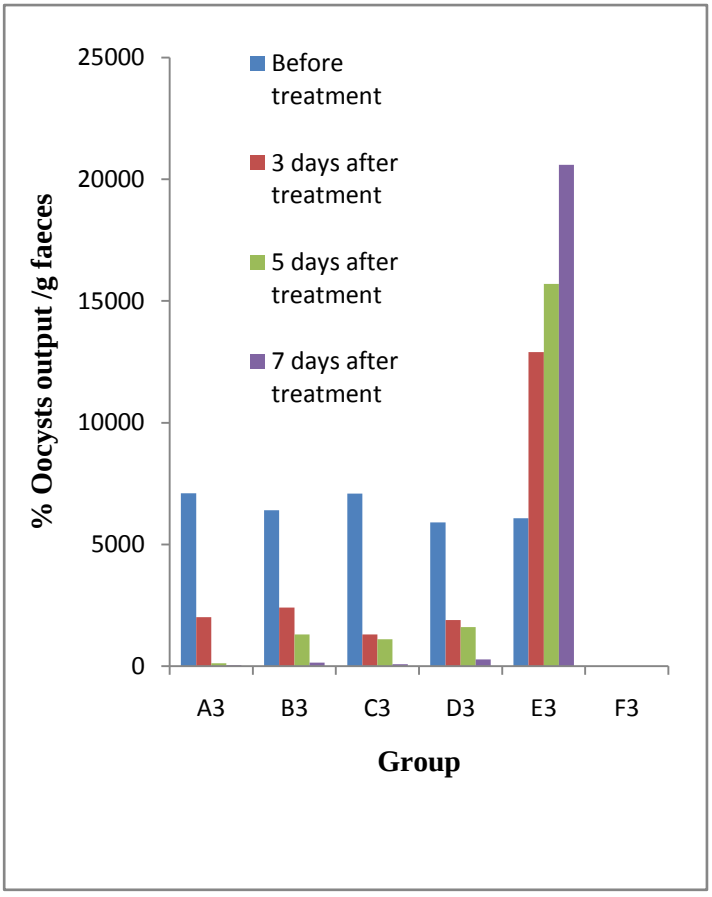


Figure 5: Faecal oocysts output of chickens treated with *A. indica* methanolic extract (Group A3 infected and treated with 100 mg/L ofAmprolium, B3, C3 and D3 infected and treated with 1,000, 500 and 250 (mg/kg) of *A. indica* respectively, E3 infected/ not treated and F3 not infected, not treated)

Table 8: *Invivo* anti-coccidial effect of *Solanum dasyphyllum* methanolic extract(ANOVA) at p< 0.05

Day	A1	B1	C1	D1	E1	F1
before treatment	6225.00 ± 1.32[a]	6,515.00 ± 2.61[a]	6,125.00 ± 4.12[a]	5,715.00 ± 2.80[a]	7,525.00 ± 125.04[d]	0[a]
3 days after treatment	1,815.00 ± 1.32[b]	574.50 ± 2.61[b]	746.00 ± 4.12[b]	1,205.00 ± 2.80[b]	13,720.00 ± 125.04[c]	0[a]
5 days after treatment	132.50 ± 1.32[c]	57.50 ± 2.61[c]	112.50 ± 4.12[c]	184.00 ± 2.80[c]	16,250.00 ± 125.04[b]	0[a]
7 days after treatment	40.00 ± 1.32[d]	0.00 ± 2.61[d]	0.00 ± 4.12[d]	0.00 ± 2.80[d]	21,150.00 ± 125.04[a]	0[a]

Result in mean ± SEM (n =4) value in the same column with superscript are considered significant (p< 0.05) compared with group A1 (positive control) F1 (negative control)

(Group A1 infected and treated with 100 mg/L of Amprolium, B1, C1 and D1 infected and treated with 1,000, 500 and 250 (mg/kg) of *S. dasyphyllum* respectively, E1 infected/ not treated and F1 not infected, not treated)

Table 9: *Invivo* anti-coccidial effect of *Nauclea diderichii* methanolic extract (ANOVA) at p< 0.05

Day	A2	B2	C2	D2	E2	F2
before treatment	8352.50 ±2.32[a]	7505.00 ± 2.81[a]	7710.00 ± 5.12[a]	8505.00 ± 2.80[a]	11805.00 ± 125.04[d]	0[a]
3 days after treatment	2472.00 ± 2.32[b]	100.00 ± 2.81[b]	200.00 ± 5.12[b]	500.00 ± 2.80[b]	20703.50 ± 125.04[c]	0[a]
5 days after treatment	122.50 ± 2.32[c]	22.50 ± 2.81[c]	37.500 ± 5.12[c]	47.500 ± 2.80[c]	16350.00 ± 125.04[b]	0[a]
7 days after treatment	45.00 ± 2.32[d]	0.00 ± 2.81[d]	0.00 ± 5.12[d]	0.00 ± 2.80[d]	20703.50 ± 125.04[a]	0[a]

Result in mean ± SEM (n =4) value in the same column with superscript are considered significant (p< 0.05) compared with group A1 (positive control) F1 (negative control)

(Group A2 infected and treated with 100 mg/L of Amprolium, B2, C2 and D2 infected and treated with 1,000, 500 and 250 (mg/kg) of *N. diderichii* respectively, E2 infected/ not treated and F1 not infected, not treated)

Table 10: *Invivo* anti-coccidial effect of *Azadirachta indica* methanolic extract(ANOVA) at p< 0.05

Day	A3	B3	C3	D3	E3	F3
before treatment	7,150.00 ± 1.32[a]	6,300 .00 ± 3.81[a]	7, 095.00 ± 6.12[a]	5,905.00 ± 1.80[a]	6,060.00 ± 120.04[d]	0[a]
3 days after treatment	2,050.00 ± 1.32[b]	2,415 .00 ± 3.81[b]	1,350.00 ± 6.12[b]	1,850.00 ± 1.80[c]	12, 800.00 ± 120.04[c]	0[a]
5 days after treatment	125.00 ± 1.32[c]	1,400.00 ± 3.81[c]	1,250.00 ± 6.12[c]	1,500.00 ± 1.80[c]	15,800.00 ± 120.04[b]	0[a]
7 days after treatment	40.00 ± 1.32[d]	155.00 ± 3.81[d]	85.00 ± 6.12[d]	285.00 ± 1.80[e]	20,650.00 ± 120.04[a]	0[a]

Result in mean ± SEM (n =4) value in the same column with superscript are considered significant (p< 0.05) compared with group A1 (positive control) F1 (negative control)

(Group A3 infected and treated with 100 mg/L of Amprolium, B3, C3 and D3 infected and treated with 1,000, 500 and 250 (mg/kg) of *A. indica* respectively, E3 infected/ not treated and F1 not infected, not treated)

By day 5 after treatment, the oocysts released in Amprolium–treated birds had reduced to 130,120 and 120oocysts/g faeces representing 2.1%, 1.4% and 1.4% in groups A1, A2 and A3 respectively (Appendix III -V). There was reduction in the oocysts released by birds in group B1, C1, and D1, had reduced to (55 (0.8 %,); 110 (1.8%) and 181(2.4%)) oocysts/g faeces respectively. The birds in groups B2, C2 and D2 also produced fewer number of oocysts (20 (0.13%); 35 (0.29%) and 45(0.3%)) oocysts/g faeces respectively.*A. indica*-treated birds produced moderate number of oocysts by day 5 after treatment (1,300 (20.3%); 1,400(15.5%) and1, 600 (26.4%)) oocysts/g faeces respectively. Groups E1, E2, and E3 continued to produce oocysts (16,900, 16, 600, 15,700) respectively.

The birds in *S. dasyphyllum*-treated group at 1000mg/kg body weight were negative and no oocysts were detected in their faeces after 7 days of treatment. However, the birds treated with 500mg/kg and 250mg/kg body weight produced very scanty oocysts (10 (0.06%) and 21 (0.14%)) in faeces at 7 day post treatment (Appendix III). In the case of birds treated with varying doses of *N.diderichii,* there were no oocysts discharged after 7 days of treatment (Appendix IV). The birds in groups B3, C3 and D3 produced oocysts but in small quantities (150, 80 and 280) (01%, 0.53% and 1.8%) oocysts/g faeces respectively (Appendix V). The oocysts in Amprolium-treated birds produced (38 (0.25%); 42 (0.28%) and 40 (0.26%))oocysts/g faeces respectively. Groups E1, E2 and E3 birds continued to discharge *E. tenella* oocysts up to 14 day post infection (21,200, 20,700 and 20,600) oocysts/g faeces respectively. The uninfected, control groups (negative control) F1, F2 and F3 did not pass any oocysts during the entire period of the experiment. Treatment of birds infected with *E. tenella* oocysts was best with methanolic extract of the stem bark of *S. dasyphyllum* and *N. diderichii* at different

doses (Tables 8 and 9). In the case of methanolic extract of the stem bark of *A. indica*, treatment was best at 500mg/kg body weight (Table 10).

4.6 *In vitro* Anti-coccidial effect of fractions of crude methanolic extracts of *S*olanum *dasyphyllum* and *N*aucle*adiderichii* and their substances

Result presented in Figure 6 and Appendix VI shows the *in* vi*tro* anti-coccidial activities of methanolic fractions as well as substances of *S. dasyphyllum* and*N. diderichii* compared with Amprolium (the universal anti-coccidial drug). There was a significant difference ($P < 0.05$) in the coccidial efficacy of fractions and substances of *S. dasyphyllum* and *N. diderichii* (Appendix VI).

Fractions 6 and 9 of methanolic extract of *S. dasyphyllum* were prominent and had high anti-coccidial activity. One spot was successfully removed as a pure substance and code named BS1. This was isolated as a brown solid substance. Fractions 7 and 8 of methanolic extract of N. *diderichii* were prominent and had high anti-coccidial activity. One spot was also successfully removed as a pure substance. This was code named BS2 which was isolated as a yellow amorphous solid substance.

The isolated substances BS1 and BS2 when tested on sporulated oocysts of *E. tenella* showed prominent activities and rate of reduction and damage of oocysts was concentration dependent (Figure 7).

Anticoccidial efficacy test of substance obtained from fractions of methanolic extract of *N. diderichii* and the substance obtained from fractions of methanolic extract of *S. dasyphyllum* showed that substance from fraction of methanolic extract of *N. diderichii* was more effective than the substance from fractions of methanolic extract of *S. dasyphyllum*. There was a significant difference ($P < 0.05$) between the activities of these substances (Appendix VI).

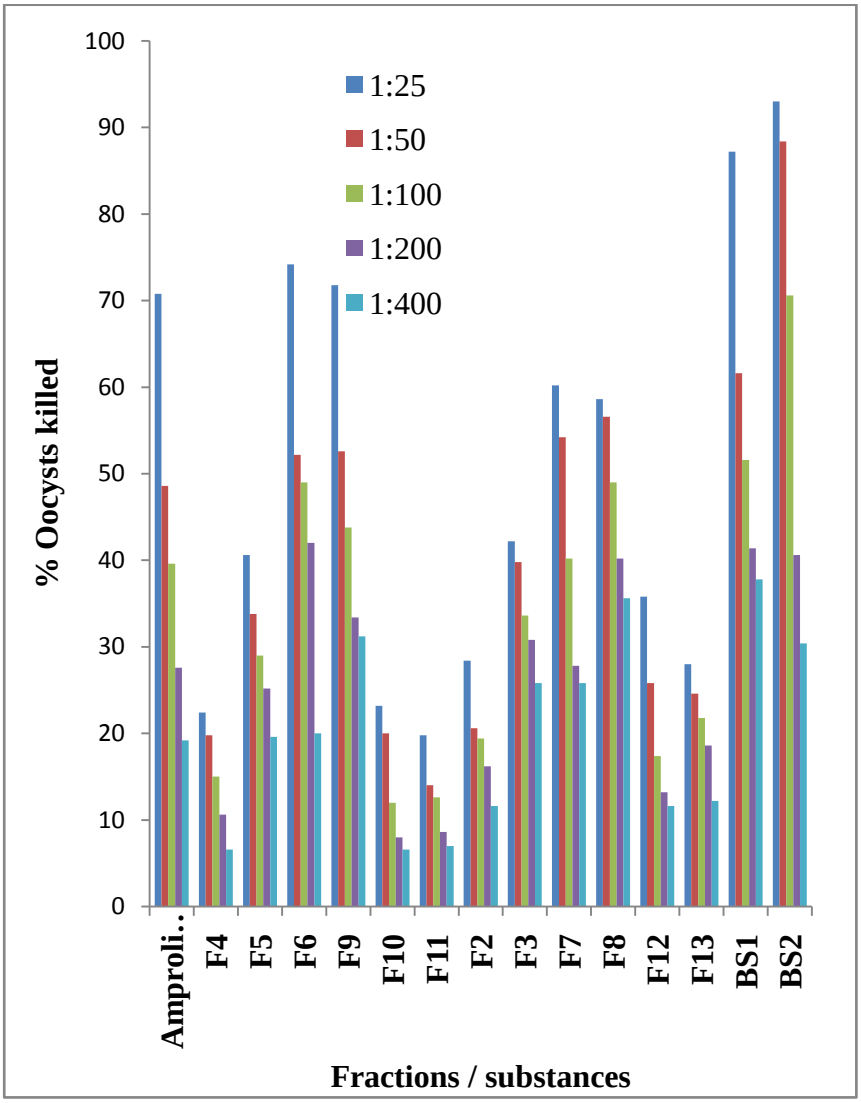


Figure 6: *In vitro* anti-coccidial effects of six fractions of *S.dasyphyllum*, *N. diderichii*and their substances on sporulated oocysts of *E. tenella* at different concentrations at 96 h

Note: F4, F5, F6, F9, F10 and F11 are fractions of methanolic extract of *S. dasyphyllum*
F2, F3, F7, F8, F12 and F13 are fractions of methanolic extract of *N. diderichii*
BS1 is the substance obtained from fractions of methanolic extract of *S.dasyphyllum*
BS2 is the substance obtained from fractions of methanolic extract of *N.diderichii*

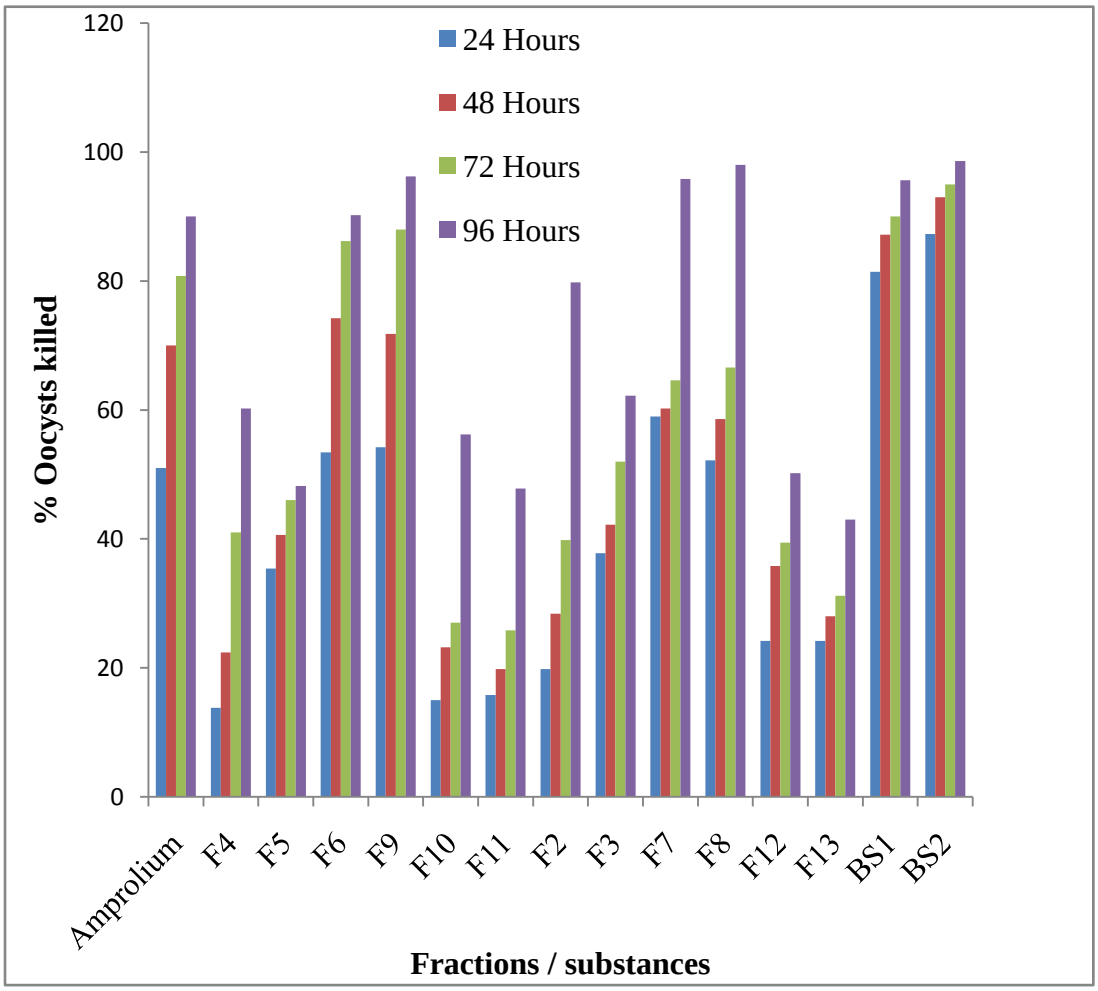


Figure 7: *In vitro* effects of six fractions of *S. dasyphyllum*, *N. diderichii* and their substances on sporulated oocysts of *E. tenella* at different time intervals at concentration 1:25 over a 96 h period

Note: F4, F5, F6, F9, F10 and F11 are fractions of methanolic extract of *S. dasyphyllum*
F2, F3, F7, F8, F12 and F13 are fractions of methanolic extract of *N. diderichii*
BS1 is the substance obtained from fractions of methanolic extract of *S. dasyphyllum*
BS2 is the substance obtained from fractions of methanolic extract of *N. diderichii*

4.7 The Isolated Substances

4.7.1 Substance BS2a (First Substance from Fractions of *Nauclea diderichii*Methanolic Extract)

Carbon Nuclear Magnetic Resonance (^{13}C NMR) spectrum indicated a total number of thirty carbon atoms (Figure 8 and Appendix IX) which were assigned by Distortionless Enhancement Polarization Transfer (DEPT) (Figure 9). This number indicates triterpenoidal nucleus. The proton Nuclear Magnetic Resonance (^{1}H NMR) spectrum has seven methyl groups at δ 4.35, δ 4.21, δ 1.56, δ 1.31, δ 1.20, δ 1.20, δ 0.56 and δ 0.37 ppm which are similar to the methyls attached to a triterpenoidal nucleus (Figure 10). Spectra data (IR and NMR) with the application of Correlation Spectroscopy (COSY) relates protons on adjacent carbon (Figure 11) while Nuclear Overhauser Enhancement Spectroscopy (NOESY) relates protons in space (Figure 12).

Heteronuclear Single Quantum Correlation (HSQC) (Figure 13) shows the relationship between ^{1}H - ^{13}C (J_{CH}) one bond away and Heteronuclear Multiple Bond Correlation (HMBC) (Figure 14) shows the relationship between proton-carbon two to three bonds away ($^{2}J_{CH}$ and $^{3}J_{CH}$) were used to assign 3 β-hydroxyurs-12-en-28-oic acid) (Appendix IX). The molecular ion peak (m/z 456.7g/mol) also suggested a triterpenoid (Figure 15).

The results for the proton Nuclear Magnetic Resonance (^{1}H NMR), Carbon Nuclear Magnetic Resonance (^{13}C NMR) and mass spectrometry are presented below:

Spectra data

^{1}H NMR (CDCL3, 500MHZ) δ 4.21 (H,d, H-29b), δ 4.35 (1H, d, H-29a), δ 1.56 (1H, d, J = 10.8, H- 23), δ 1.31 (1 H, d, J =10.8, H – 30), δ 1.20 (1H, dd, J = 5.3, H – 24), δ 1.20 (3H, s, H- 25), δ 0.56 (34, s, H- 26) and δ 0.37 (3H, s, H-27).

^{13} C NMR (CDCL3, 500MHZ) δ 178.2 (C-28), δ 150.4 (C-20), δ 109.7(C-29), δ 79.0 (C-3), δ56.3 (C-17), δ55.4 (C-5), δ50.61 (C-18), δ49.3 (C-19), δ 49.3 (C-9), δ 42.5 (C-14), δ40.7 (C-8), δ 38.9 (C-4), δ 38.8 (C-1), δ 38.4 (C-13), δ 37.2 (C-22), δ 37.0 (C-10), δ 33.7 (C-7), δ 33.2 (C-16), δ 31.9 (C-21), δ 30.6 (C-15), δ 29.1 (C-23), δ 27.9 (C-2), δ 25.5 (C-12), δ 20.9 (C-11), δ 19.3 (C-26), δ 19.2 (C-30), δ 18.3 (C-6), δ 16.0 (C-24), δ 16.0 (C-25), δ 14.7 (C-27); HRFABMS m/z 456.7.

4.7.2 Substance BS2b (Second Substance from Fractions of *Nauclea diderichii*Methanolic Extract)

Carbon Nuclear Magnetic Resonance (^{13} C NMR) spectrum indicated a total number of thirty carbon atoms (Figure 8 and Appendix X) which were assigned by Distortionless Enhancement Polarization Transfer (DEPT) (Figure 9).This number indicates triterpenoid. The ^{1}H NMR spectrum seven methyl groups at δ 1.24, δ 1.02, δ 0.99, δ 0.99, δ 0.97, δ0.97and δ 0.93 ppm which are similar to the methyls attached to a triterpenoidal nucleus) (Figure 10). Spectra data (IR and NMR) with the application of Correlation Spectroscopy (COSY) relates protons on adjacent carbon (Figure 11) while Nuclear Overhauser Enhancement Spectroscopy (NOESY) relates protons in space (Figure 12). Heteronuclear Single Quantum Correlation (HSQC) (Figure 13) shows the relationship between ^{1}H - ^{13}C (J_{CH}) one bond away and Heteronuclear Multiple Bond Correlation (HMBC) (Figure 14) shows the relationship between proton-carbon two to three bonds away ($^{2}J_{CH}$ and $^{3}J_{CH}$) were used to assign 3 β- hydroxyl lup-20 -en- 28-oic acid (Appendix X).The molecular ion peak (m/z 456.7g/mol) also suggested a triterpenoid (Figure 16).

The results for the proton NMR (1H NMR), ^{13} C NMR and mass spectrometry are presented below:

Spectra data

^{1} H NMR (CDCL3, 500MHZ) δ 1.24 (H, s, H-15), δ 1.02 (1H, s, H-26), δ 0.99 (1H, d, J = 6.1, H- 11), δ 0.99 (1 H, d, J =6.1, H – 30), δ 0.97 (1H, s, H- 26), δ 0.97 (1H, s, H-29), and δ 0.93 (1H, s, H-24).

^{13} C NMR (CDCL3,500 MHZ) δ 180.8 (C-28), δ 130.9 (C-13), δ 128.9 (C-12), δ 79.0 (C-3), δ55.4 (C-5), δ50.6 (C-18), δ46.9 (C-9), δ 46.9 (C17) δ42.5 (C-14), δ 40.7 (C-8), δ 38.9 (C-4), δ 38.9 (C-19), δ 38.9 (C-20), δ 38.8 (C-1), δ 37.2 (C-22), δ 37.0 (C-10), δ 33.7 (C-7), δ 30.6 (C-21), δ 29.2 (C-15), δ 28.0 (C-23), δ 27.4 (C-2) δ 24.7 (C-16), δ 24.7 (C-27) , δ 22.7 (C-11), δ 22.7 (C-30), δ 19.2 (C-6), δ 18.3 (C-26), δ 18.3 (C-29), δ 16.1 (C-24) and δ 15.4 (C-25) ; HRFABMS m/z 456.7.

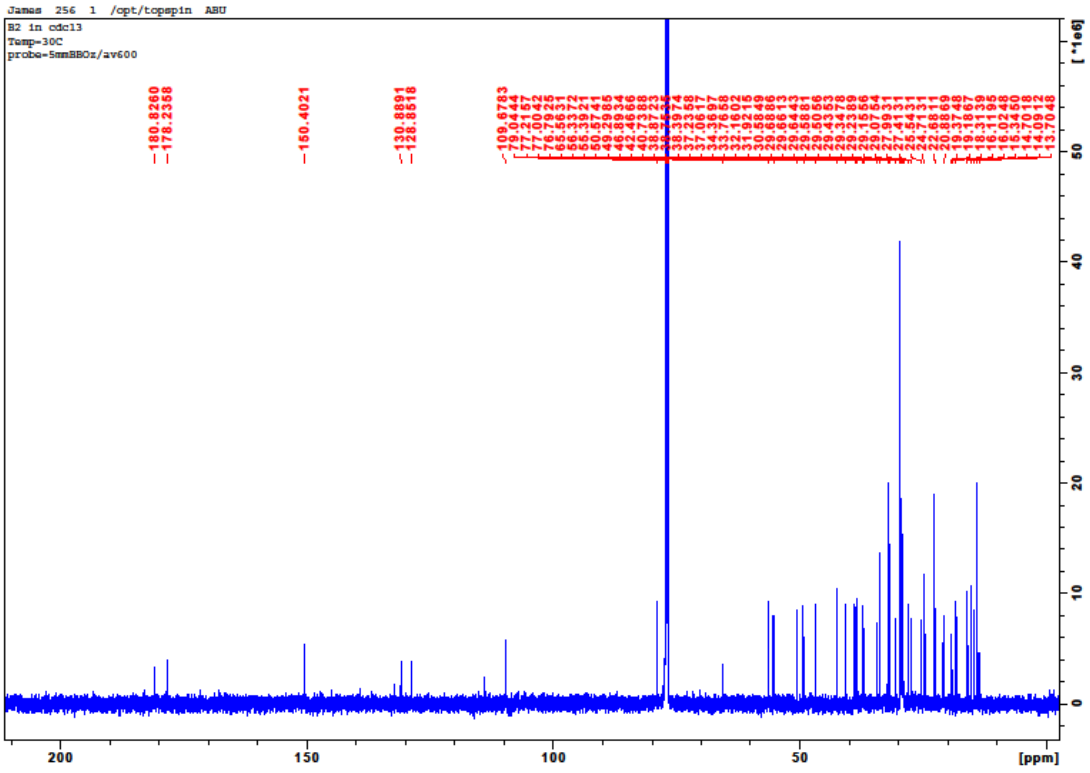


Figure 8: Carbon Nuclear Magnetic Resonance (^{13}C NMR) of BS2

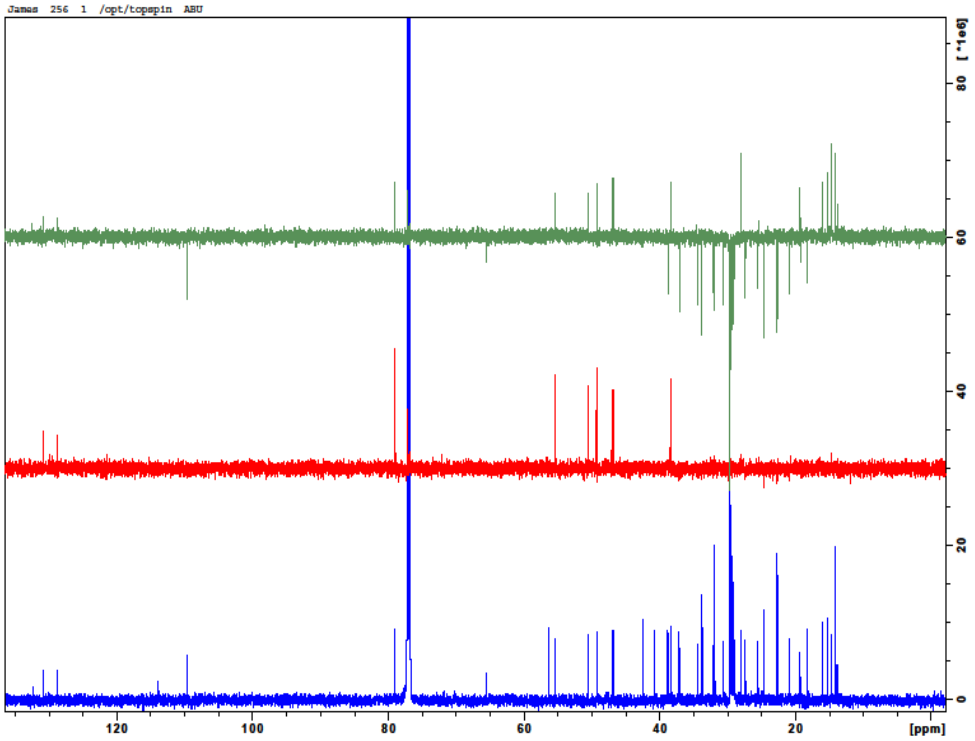


Figure 9: Distortionless Enhancement Polarization Transfer (DEPT) of BS2

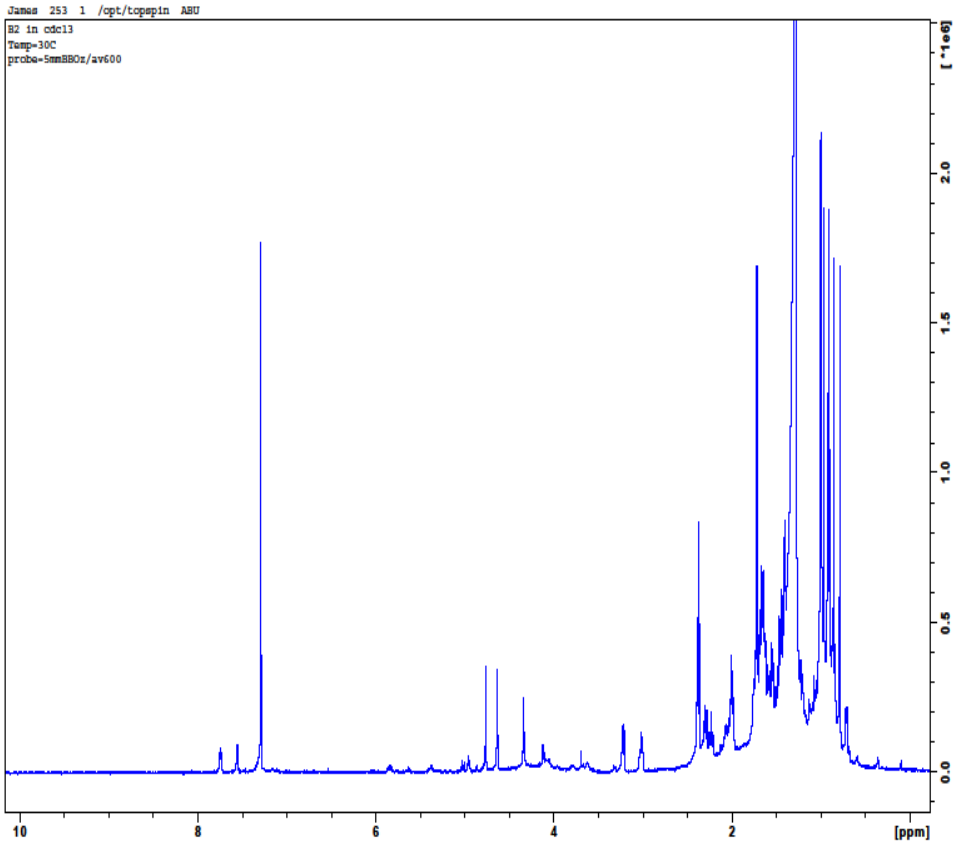


Figure 10: Proton Nuclear Magnetic Resonance (^{1}H NMR) of BS2

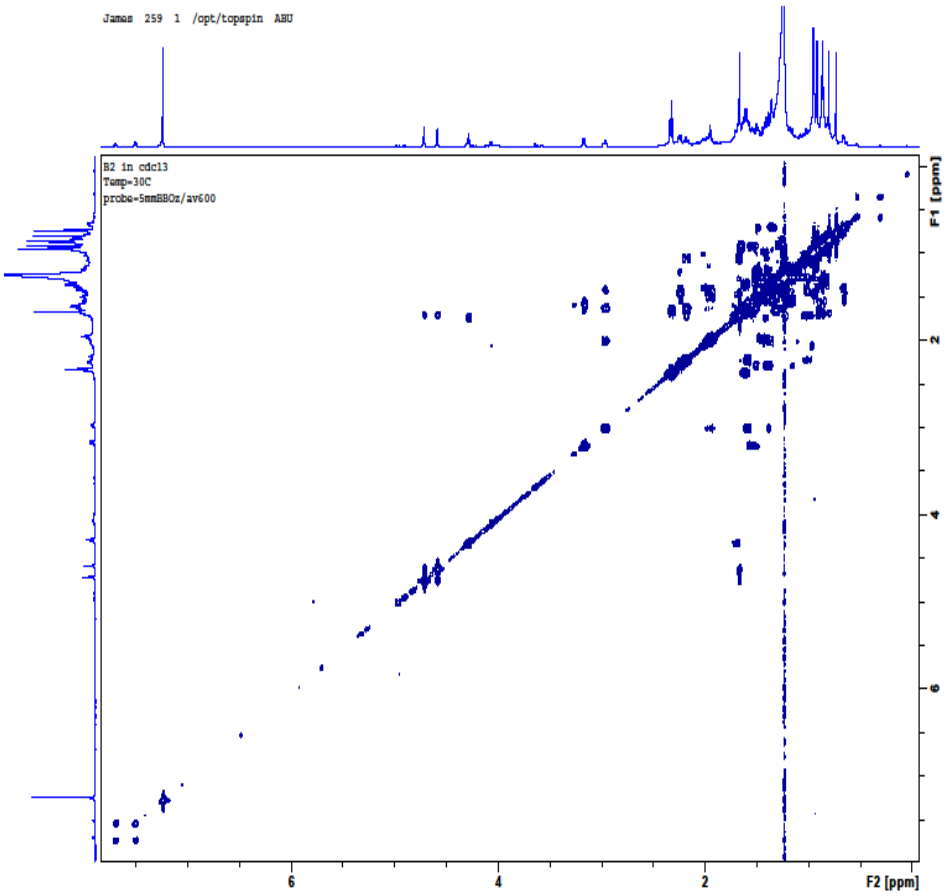


Figure 11: Correlation Spectroscopy (COSY) of BS2

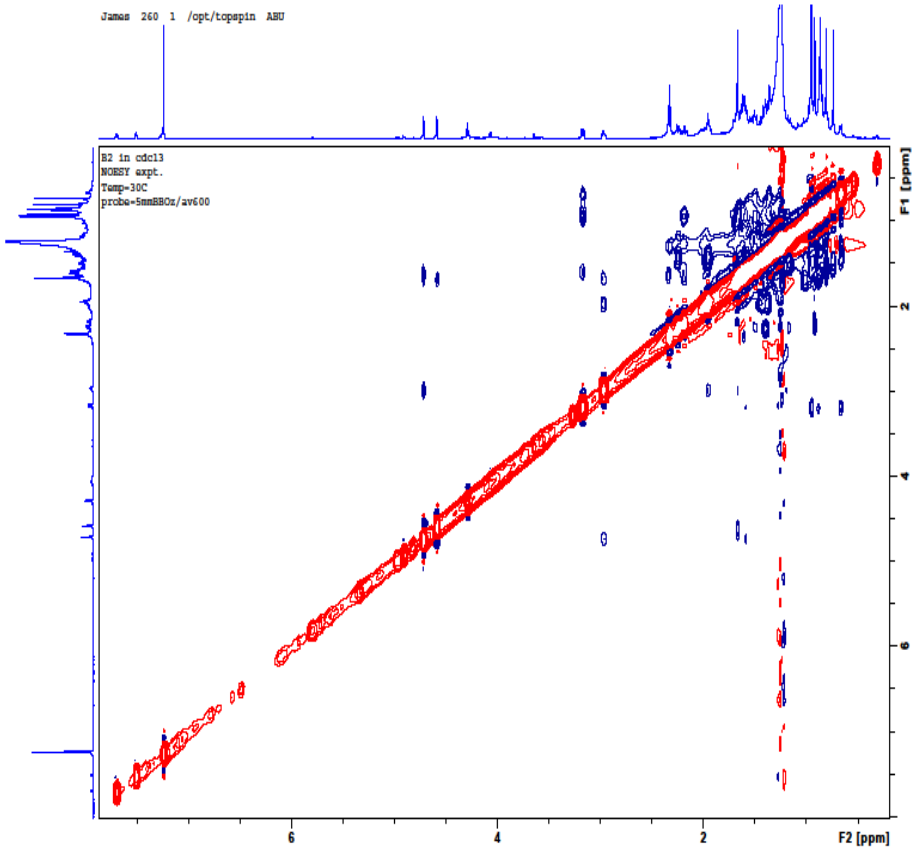


Figure 12: Nuclear Overhauser Enhancement Spectroscopy (NOESY) Experiment of BS2

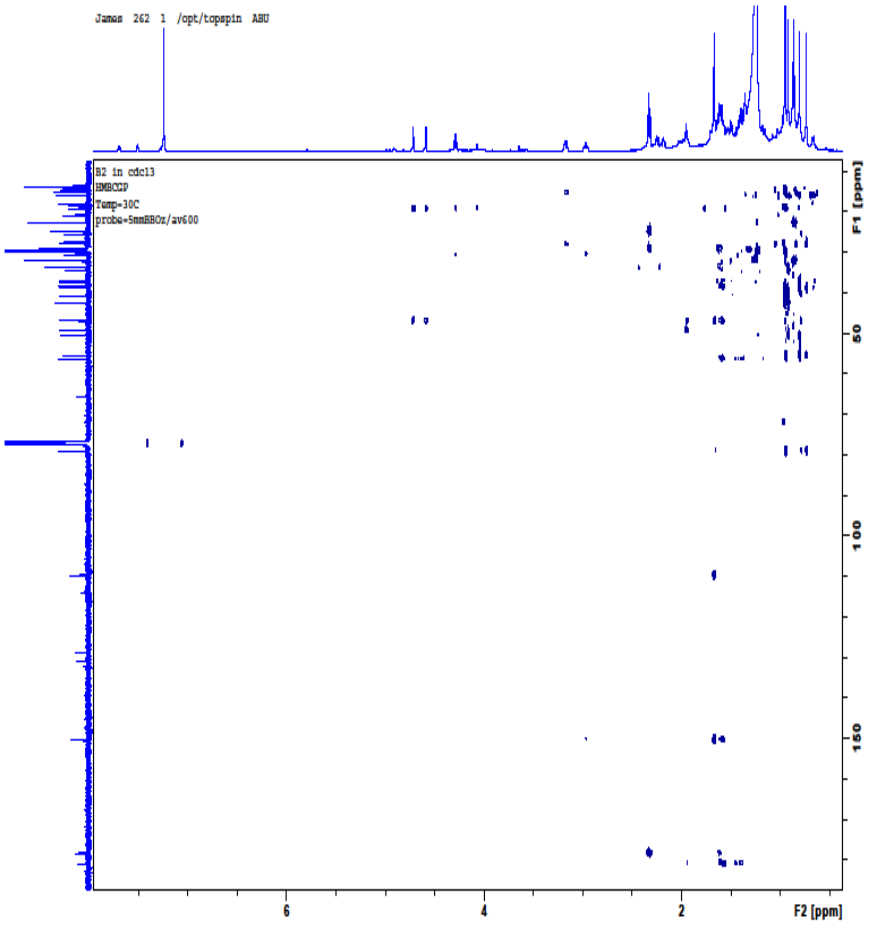


Fig 13: Heteronuclear Single Bond Quantum Correlation (HSQC) of BS2

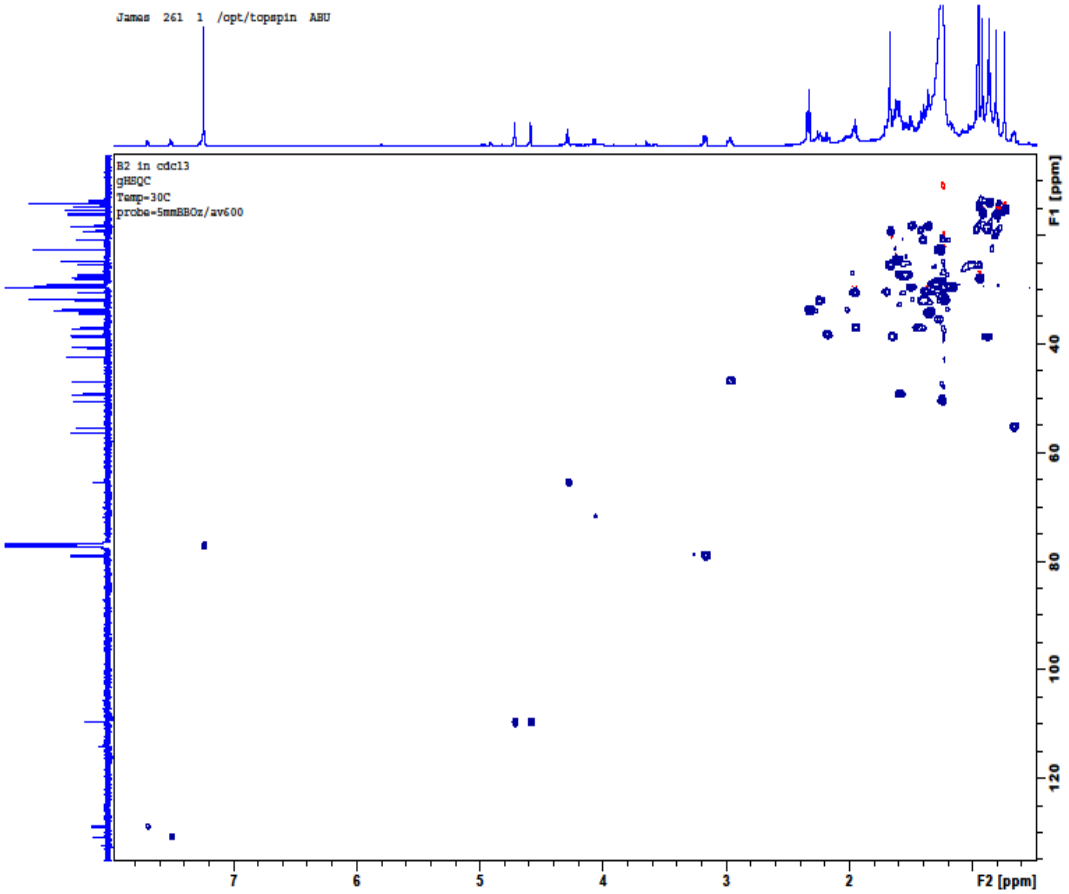


Figure14: Heteronuclear Multiple Bond Correlation (HMBC) of BS2

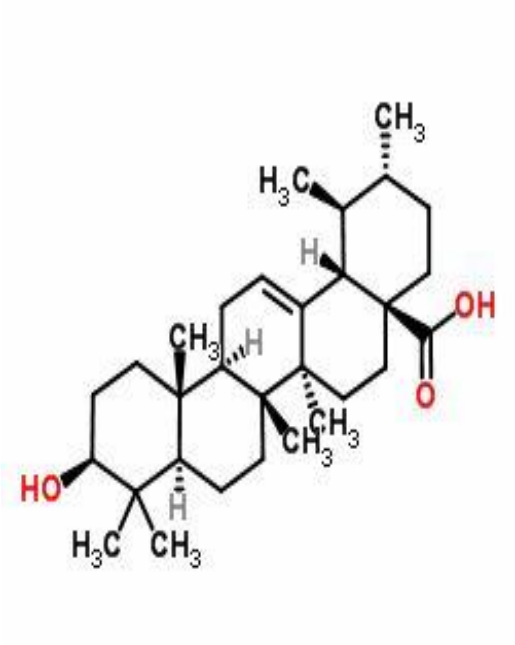


Figure 15: Ursolic acid (1) (456.7g/mol). ($C_{30}H_{48}O_3$)

Substance BS2a obtained from substance BS2

Substance BS2 was obtained from fractions of methanolic extract of *N. diderichii*

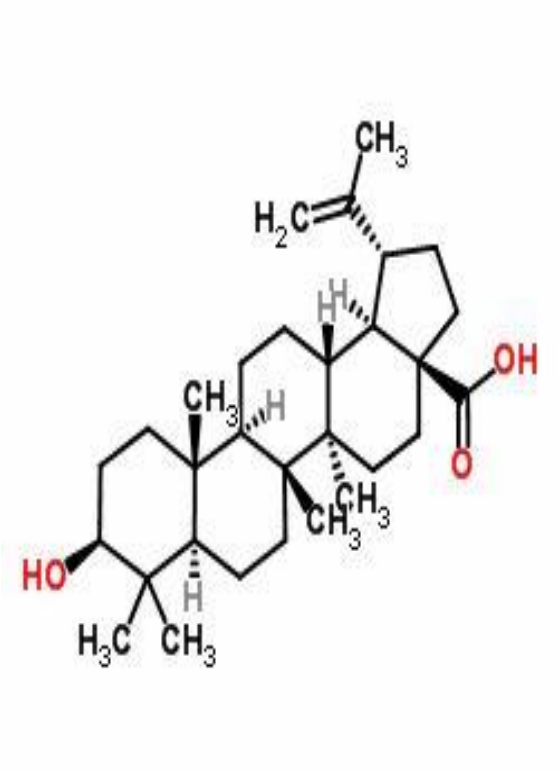


Figure16: Betulinic acid (2) 456.7g/mol)
Substance BS2b obtained from substance BS2

Substance BS2 was obtained from fractions of methanolic extract of *N. diderichii*

CHAPTER FIVE

5.0 DISCUSSION

The respondents identified nineteen medicinal plants that were used in the control and treatment of chicken diseases of which ten medicinal plants are frequently used by the poultry farmers. Ethnoveterinary medicine (EVM) is important in treatment and control of diseases and parasites of chickens in Zaria, as the majority of poultry rearers could not afford to purchase veterinary requisites such as vaccines, drugs, dips and chemical dusts and also lacked knowledge of vaccine handling and administration. In the study area, poultry farmers depended on medicinal plants as herbal remedies for their indigenous chicken flock health management and considered the use of ethnoveterinary medicine as sustainable, economical and culturally acceptable. Traditional medicine is preferred to commercial drugs because poultry farmers in the study area believe that the local medicines are also efficient, cost effective, readily accessible and without eminent resistance. In addition, no transport costs are incurred, it is easy to administer, there is minimal risk of drug overdose and when prompt treatment was administered, the birds were healed and the birds benefited from supplementation from vitamins.

In this study, various medicinal plants notably *Azadirachta indica*, *Khaya senegalensis*, *Parkia biglobosa*, *Cissus populnea*, V*itellaria paradox*a, *Solanum dasyphyllum*, *Citrus sinensis*, *Nauclea diderichii*, *N. latifolia* and F*icus platyphylla* were frequently used to treat various poultry diseases including symptoms that are coccidiosis-like in nature in the study area. This shows that medicinal plants were perceived as convenient, more reliable and cheaper sources of medication in the area. These plants are also very common in the northern parts of Nigeria where Zaria is located, and earlier works had published their application in diarrhoea, dysentery, expulsion of internal parasites (anthelminthic effect) and coccidiosis (Woolfe *et al*., 1977; De Caluwe *et al*., 2010; and

Ndjonka *et al*., 2010) which may justify their use in the management of poultry diseases.

The indigenous populationsindicated preference for the use of stem bark followed by leaves in the preparations of herbal medicines because it is more convenient collecting stem bark and leaves than root parts, flowers and fruits. Scientifically, leaves are actively involved in photosynthesis and the production of metabolites, thus, the numerous constituents found in leaves could explain their efficacy in the treatment of various ailments in both humans and animals. From the conservation point of view, collection of leaves for herbal preparations could be regarded as sustainable so long as some leaves are left on the parent plant. Removed stem bark can be recovered by plants within a short time. This is opposed to the collection of roots which could be a severe threat for rare and slowly producing plants. Fruits and roots were seldom utilized due to the fact that fruits are not available all year round. Also roots of plants are difficult to obtain and their frequent use may lead to destruction of the entire plants. This may lead to endangering or even extinction of the plants. However, some authors have reported that roots are more commonly collected plant parts in Ethnoveterinary practice (Tibuti *et al*., 2003; Hunde *et al*., 2004; Yinegar*et al*., 2007; Giday *et al*., 2009; Ayannar *et al*., 2011).

The methods of preparation before application to ensure their effectiveness was found ranging from grinding of plant parts to powdered, preparation of extracts from the plant parts, cooking of some fruits and storing of some leaves in the poultry house against the outbreak of ectoparasites. Plant parts to be used are usually prepared by soaking the fresh or dried plant parts in water and the extract administered through the drinking water of the chickens. In some cases, the plant materials were often prepared by

pounding either the fresh or dried parts of the plants followed by either soaking or boiling them in water and sometimes mixed with feed and/or potash to improve palatability and administered orally. This was earlier reported by Alawa *et al*., 2002 in sub humid zone of northern Nigeria; *Adua et al*., 2009 in Plateau State; Maikai *et al*., 2007 in Kaduna and Offiah *et al*., 2011 in Plateau State.

Plant products involved in the treatment of poultry diseases come from various botanical families such as Bombacaceae (*A. digitata*), Liliaceae (*Allium cepa and A. sativum*), Asteraceae (Artemisia absinthium), Meliaceae (*Azadirachta indica* and *Khaya senegalensis*), Rutaceae (*Capsicum annum* and *Citrus sinensis*), Leguminoseae (*Cassia occidentalis*), Vitaceae (*Cissus populnea*), Euphorbiaceae (*Euphorbia hirta* and *Jatropha curcas*), Rubiaceae (*Nauclea diderichii* and *N. latifolia*), Fabaceae (Parkia biglobosa), Solanaceae (Solanum dasyphyllum), Compositae (*Vernonia amygdalina*) and Sapotaceae (*Vitellaria paradoxa*). Similar results were reported by some authors in Nigeria (Gefu *et al*., 2000; Adedeji *et al*., 2006a, b; 2013).

A number of diseases and pests affect the farmer's poultry; this study has been able to document the above listed diseases that directly and indirectly affect the farmer's birds. This agreed with research findings conducted by (Illango *et al*., 2000). The common diseases observed by the respondents in their flock include Newcastle disease, fowl pox and coccidiosis. The indigenous ways of describing disease symptoms were identified and compared with the scientific ways of identification and naming of various diseases to confirm their authentication. This supports the report of (Bell *et al*., 1990; Bourzat and Saunders, 1990; Chrysostome *et al*., 1995; Yongolo, 1996; Musa *et al*., 2008; Adebayo *et al*., 2013; Olanipekun, 2014).

The *in vitro* anti-coccidial efficacy trials on ten ethno medicinal plants frequently used by poultry farmers to treat symptoms suspected to be coccidiosis in Zaria showed some effects on sporulated oocysts of E. *tenella*. Damaged oocysts and various degrees of damaged cell walls, oocysts without cell walls containing free sporocysts were obtained in the present study. The presence of damaged oocysts after 72 hr and 96 hr and in some cases even after 48 hr in the different concentrations of the crude plant extracts may be caused by anaerobic conditions from the Petri dishes. It may also be due to the active principles that are present in the plants extracts. Anaerobic digestion which is made up of series of microbial actions converts the organic content of the waste to biogas and has negative effects against pathogens. This is in agreement with the results of (Molan and Thomas, 2007; Adriana *et al.*, 2008; Abdullateef *et al*., 2009; Jang *et al.*, 2007 and Adnane *et al.*, 2013). They reported appearance of deformed oocysts with cracked walls and debris. The crude methanolic extracts of all the ethno medicinal plants produced similar effects at various degrees which compared favourably with that due to Amprolium, the universal anticoccidial drug (Plates II- IV). This result also correlates with the results of similar studies by Hilbrich (1975) and Guimaraes *et al* (2007) which revealed oocysticidal effect of essential oil components in some plants they tested on oocysts of coccidia.

The results revealed that the methanolic extracts from all the different plants have anticoccidial activities. In the recent years, botanicals have got great attention for the control and treatment of infectious diseases of poultry animals including coccidiosis (Pieri *et al*., 2014; Xiao *et al*., 2014). The result obtained in this study is in agreement with report of some authors who reported anticoccidial potential of some plants such as *Saccharum officinarum* (Fornazier *et al*., 2000; El-Abasy *et al*., 2003; Hikosaka *et al*., 2007; Asghar *etal*., 2015), *Pinus radiata* (Wang *et al*., 2008) and *Aloe vera* (Molan *et*

al., 2004; Narsih *et al*., 2012). Molan and Thomas (2007) reported similar *in vitro* effects of aqueous extracts from green tea on the sporulation of *E. tenella*, *E. acervulina* and *E. maxima* and found that addition of tea extracts to the incubations containing unsporulated oocysts resulted in a significant reduction in sporulation rate. Similar *in vitro* results were reported by Molan *et al*. (2009), who evaluated and found that the aqueous pine bark extract inhibited the sporulation of *Eimeria* oocysts. In a similar study, sugar cane (*Saccharum officinarum*) extract, a well-known natural immunostimulant, is reported to have protective effect against *E. tenella* infection in chickens (El-Abasy *et al*., 2003; Hikosaka *et al*., 2007).

The *in-vitro* anti-coccidial efficacy of the ethno medicinal plants was found to be concentration dependent. This suggests that the effect may be pharmacologically mediated indicating that the quantities of the bioactive principles may be higher in the plant extracts with lower dilutions (1:25 and 1:50). Similar result was obtained by Abdullateef *et al*. (2009) and Molan and Thomas (2007). Marizvikuru *et al*. (2006) and Séverin *et al*. (2012) also found that *Aloevera* and *Thonningia sanguinea* extracts exhibited a dose-dependent effect on oocysts of *Eimeria tenella*.

Some of the plant extracts tested (V. *paradoxa*, *N. latifolia* and *P. biglobosa*) had very low anti-coccidial efficacy. This may be as a result of absence or low quantities of bioactive compounds in them.

Contrary to the report by Nwosu *et al.* (2011) that there was no *in-vitro* anticoccidial activity of *K. senegalensis*, the present study has shown that the plant extracts exhibited coccidial action against the oocysts of *Eimeria tenella*. This may be as a result of penetration of the cell walls of the oocysts by the plant constituents causing damages to the cytoplasm, which was evidenced by the appearance of abnormal sporocysts in oocysts exposed to 1:25 and 1:50 dilutions in most of the plant extracts. This was

observed even in high dilutions of 1:100, 1:200 and 1:400 in *N. diderichii*, *S. dasyphyllum*, *A.* i*ndica* and *Cissus populnea* (Plates II to IV).

Among the ten plant extracts tested, that of *A. indica, N. diderichii* and *S. dasyphyllum* killed the highest percentage of theoocysts. Thus, further analyses (phytochemical screening, acute toxicity, *in vivo* anti-coccidial tests and characterization of bioactive principles) were carried out on these plant extracts.

From the findings of the qualitative preliminary phytochemical investigations, it was observed that alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, terpenoids and tannins were present in the three methanolic plant extracts screened.The presence of tannins in all the crude methanolic plant extracts confirms the astringent properties found in them. This makes the plants useful in the treatment of inflammatory skin eruption, wound healing and bowel condition and saponins also aid in wound healing and bleeding treatments. These phytochemicals are produced by plants as defence mechanism to stop attack by foreign pathogen and show tumour inhibiting activities on animals, thus making them to be natural antibiotics. These have been reported by many authors to be identified in many plants. These include Trease and Evans, 1989; Sofowora, 1993; Akindahunsi and Salawu, (2005); Edeoga *et al*. (2006); Iniaghe *et al*. (2009); Otoide and Kayode, (2011). Therefore, these plants have the potential when used and seem to justify their use for the treatment of coccidiosis-like and other poultry diseases. These phytochemicals have been found to exhibit antiprotozoal and antibiotic activities (Anyanwu and Dawet, 2005).

Studies have shown that saponins present in traditional medicine preparations cause hydrolysis of glycoside from terpenoids. This averts the toxicity associated with the intact molecule (Asl *et al*., 2008). Flavonoids obtained in the present study are potent

antioxidants as well as free radical scavengers and have been shown to protect cell membranes from damage (Noda *etal*., 2000). *Solanum* species are rich sources of precursors of steroid drugs which were obtained in the present study. These steroidal materials have been found useful as anti-inflammatory agents. *Solanum dasyphyllum* and *Nauclea diderichii* investigated in this study clearly fit into the World Health Organization description of plant as a plant with one or more organs which contain substances that can be used for therapeutic purposes and/or which are precursors for synthesis of useful drugs.

Several mechanisms of action could be suggested with regards to these groups of chemical compounds, particularly flavonoids and tannins which are present in the *N. diderichii* and *S. dasyphyllum* extracts. Tannins have the ability to form chelates with metal ions, particularly iron. This leads to the disruption of the walls of oocysts which could be one of the factors that contribute to its anticoccidial activity (Muhammed *et al.*, 2014). In addition, tannins, like tannic acid, have greater relative binding ability to iron and may act with iron from the medium to form chelates and deplete iron from microorganisms; it is well known that aerobic microorganisms require iron to perform a variety of functions like reduction of ribonucleotide and formation of haem (Chang *et al*., 1999). Tannins have also been shown to exhibit anticoccidial activity via mechanisms that involved damage to the cell wall. Tannins have been reported to have anticoccidial effect by showing reduction in sporulation of *E. tenella, E. maxima* and *E. acervulina* under laboratory conditions using pine extracts (Molan *et al*., 2009). Furthermore, Min and Hart (2003) also reported that tannins may form complexes with nutrients and inhibit their availability to the parasite for normal growth development and motility which may lead to decrease in the metabolism of parasite directly through inhibition of oxidative phosphorylation or electron transport (Newbold *et al*., 1997).

Tannins are water soluble plant polyphenols that precipitate proteins (Muhammed *et al.*, 2014). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et al*., 1991).The growth of many fungi, yeasts, bacteria, viruses and protozoa was inhibited by tannins (Chung *et al*., 1998). Phytotherapeutically, tannin containing plants are used to tract nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Westendary, 2006). In this study, the presence of tannins might have accounted for the efficacy of the methanolic plant extracts (*A. indica, S.dasyphyllum* and *N. diderichii*). Extracts of leaves of both *S. nigrum* L and *S. myriacanthus* Dunal have been reported to hasten the healing of wounds and inflamed mucous membrane (Okwu and Emenike, 2006).

Saponins have been found to have antiprotozoan activities (Wallace *et al*., 1994; Newbold *et al*., 1997) and this property has been exploited in the treatment of protozoan infections in other animals (Guo *et al*., 2003). It has also been reported that plant extracts with high saponins content are good sources of natural antimicrobial compounds. *A. indica*, *N. diderichii* and *S. dasyphyllum* used in the present study contain natural saponins that cause the inhibition of protozoan development. This is done by interacting with the cholesterol on the parasite cell membrane, thus resulting in death of parasite (Efterpi *et al*., 2012). This mechanism of action by which saponins work might be through their toxicity to protozoans which may be widespread and non-specific. It might also be as a result of their detergent effect on the cell membranes or walls (George Francis *et al*., 2002). Saponins are a special class of glycosides which have soapy characteristics (Fluck, 1973). It has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of

foams in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000; Okwu, 2004).

Saponins inhibit the development of protozoa by interacting with the cholesterol present on the parasite cell membrane, thus resulting in parasite death (Wang *et al*., 2008). Several studies with saponins have demonstrated their positive effects on improved nutrient absorption by increasing intestinal permeability via membrane depolarization (Oleszek *et al*., 1999). Based on the emulsifying properties (stabilizing water or oil emulsions) and the intense effect of making monoglycerides more soluble (Barla *et al*., 1979), dietary supplementation of saponins will result in the emulsification of oil fats, promoting their digestion. Saponins can also affect the absorption of vitamins and minerals (Efterpi *et al*., 2012).

Azadirachtaindica, *N. diderichii* and *S. dasyphyllum* were also found to contain alkaloids which were also reported to be present in *Solanum nigrum* L and *Solanum myriacanthus* Dunal (Pronob and Islam, 2012). Alkaloids have been associated with medicinal uses for centuries. In the present study, alkaloid content in *A. indica*, *N. diderichii* and *S. dasyphyllum* could be responsible for their observed much acclaimed medicinal values though the exact mode of action is poorly understood (Pronob and Islam, 2012). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria, viruses and protozoans to which *E. tenella* belongs. Alkaloids also possess anti-inflammatory properties with consequences of altered immunological status *in vivo* (Ganguly and Sainis, 2001; Staerk *et al*., 2002). Lilian *et al*. (2012) also reported that effects of alkaloids are elicited through neuropharmacological mechanism in oocysts of *E. tenella*.

Triterpenoids have been shown to possess numerous biological activities and display various pharmacological effects such as anti-inflammatory, cholesterol-lowering, wound-healing properties combined with relatively low toxicity (Seun *et al*., 2006; Akihisa *et al*., 2010; Bishayee *et al*., 2011). The multifunctionality of triterpenoids makes them promising multi-targeting agents in the treatment of certain inflammatory diseases such as coccidiosis (Yadav *et al*., 2010).

However, the findings of this study contradict the findings of Vinoth *et al*., 2012 who did not obtain alkaloids in the methanolic extract of neem (*A. indica*) they screened.

The quantities of these chemicals to be introduced to animals should be determined to enhance their safety and thus acute toxicity test was carried out. The results of acute toxicity tests show that the three plant (*A. indica*, *N. diderichii* and *S. dasyphyllum*) extracts are not toxic. No death of chickens was recorded on administration of 5000 mg/kg body weight within the short-term outcome of the limit dose test and there were also no hazardous symptoms. This implies that the plant extracts are relatively safe for the chicks. This was supported by Lorke (1983) that substances or plant extracts with LD_{50} ofabout 5000 mg/kg was considered relatively safe, thus these plants extracts have a wide margin of safety. This result is in agreement with the result of clinical manifestations of low toxicity in the birds treated with low concentrations (1,000mg/kg) of *K. senegalensis*, *B. paradoxa* and *Annona senegalensis* by Nwosu *et al*. (2011). However, their findings contradict the result of this work which showed no mortalities in birds but observed slight depression and in appetence 2-3h after treatment with 1,500 and 2,000 mg/kg concentrations of the plant extracts.

The result of this research correlates with report by (Harkonen, 1998; Hobbs, 1995; Hashemi *et al*., 2010; Guo *et al*., 2003; Elmusharaf *et al*., 2006) who observed that

mushrooms and some other plant extracts were not toxic even when used at high therapeutic doses.

From the results of the acute toxicity tests, varying doses of three crude methanolic extracts were selected for the treatment of *Eimeria tenella*-infected birds for seven days. In this study, the *Eimeria*-inoculated birds were successfully infected with *E. tenella* as indicated by the shedding of oocysts. Each of the birds had been inoculated with 15,000 sporulated oocysts. McDougald (2003) reported that inoculation with 1000 to 3000 sporulated oocysts would be sufficient to cause bloody droppings, and shedding of oocysts.

In the present study; the three crude methanolic extracts have demonstrated anticoccidial effect against *Eimeria tenella* in terms of lower oocysts shed by infected and treated chickens. The efficacies of these plant extractswere either comparable or even better than those of amprolium treated and/or uninfected unmedicated chicks. *Azadirachta indica* is well known for its anticoccidial activity against avian coccidiosis (Tipu *et al*., 2002; Abbas *et al*., 2006; Biu *et al*., 2006; Toulah *et al*., 2010). So far, the exact mechanism of action of *A. indica* against coccidian parasites is unknown, but the report by Biu *et al*. (2006) suggested that an aqueous leaf extract of *A. indica* when taken orally produced an increase in red blood cells, white blood cells and lymphocyte counts thus enhancing the cellular immune response, increasing antibody production. Thus most pathogens can be eliminated before they cause the ill effects associated with disease. The three plant extracts are also rich in alkaloids, carbohydrates, glycosides, tannins, flavonoids, saponins. These components are known to have antitumour, anti-inflammatory, anti-oxidant, anti-proliferative, anti-bacterial, anti-viral, and anti-parasitic properties which might be responsible for the anti-coccidial efficacies. Similar results

have been reported by other researchers (Crespy and Williamson, 2004; Fujiki, 2005; Isogai *et al*., 2001; Molan *et al*., 2003, 2004; Weber *et al*., 2003).

Active compounds such as alkaloids, tannins and flavonoids obtained from these plant extracts are known as anti-oxidants. Coccidian parasite-induced host cell destruction are associated with oxidative stress and lipid peroxidation, the anti-oxidants which have the ability to neutralize reactive oxygen species (ROS), are protective due to their ROS scavenging ability. This supports previous findings on the importance of these bioactive compounds in the management of coccidiosis (Allen *et al*., 1998). It was observed that bioactive principles that are anti-oxidant in plant extracts have benefits in treating and possibly preventing coccidial infection (Naidoo *et al*., 2008).

It iswell documented that coccidiosis in chickens usually lead to the generation of proinflammatory mediators, together with the oxidative and Nitrous Oxide (NO) species which contribute principally to inflammatory injury and diarrhoea (Allen, 1997; Sandoval-Chacon *et al*., 1998; Allen and Fetterer, 2002; Bodet *et al*., 2006; Brenes *et al*., 2008; Naidoo *et al*., 2008; Hamid *et al*., 2009; Allen-Hall *et al*., 2010). Therefore, substances that generate oxidative stress or possess anti-oxidant properties such as alkaloids, tannins and flavonoids obtained in these plant extracts demonstrated certain coccidiostatic effects. This is supported by some authors who obtained similar findings in their work (Kurkure *etal*., 2006; Oviedo-Rondon *etal*., 2006; Jang *etal*., 2007; Berenes *etal*., 2008; Wang *etal*., 2008; Betti *et al*., 2009).

It has also been reported that parasite invasion leads to production of free radicals, together with high levels of Nitrous Oxide (NO), which are the major factors that compromise the cellular anti-oxidant defense system. Compounds that are meeting the

demands of anti-oxidant defense system or directly interfere with free radicals, such as tannins, may restore the balance of oxidants/anti-oxidants, leading to improvement in intestinal integrity and performance during subclinical coccidiosis. Similar observation was reported by Allen, 1997; Bagchi *et al*., 2000; Allen and Fetterer, 2002; Naidoo *et al*., 2008. The anticoccidial effect of these tested plant extracts may be attributed to their antioxidant effect. Antioxidant compounds could hold promise for the control of *Eimeria* infections due to the association of coccidial infection with host cell destruction which is being associated with oxidative stress and lipid peroxidation of the intestinal mucosa. So, antioxidant-rich plants may be lethal to the parasites by inducing oxidative stress and neutralize reactive oxygen species and have potential benefits in treating coccidial infections. According to Allen *et al*. (1998), antioxidant compounds are known to reduce the severity of *Eimeria tenella* infections by ameliorating the degree of intestinal lipid peroxidation. Some of these compounds which are antioxidants may damage the parasite, thus preventing the infection. These observations were earlier reported by Hamad*et al*. (2014).

Reduction in oocysts count was better with increased concentration of crude methanolic extracts as well as Amprolium (universal drug) except for *A. indica*. The inability of the methanolic extract of *A. indica* to eliminate all the oocysts from infected chickens may be bio phasic as the extracts were administered via water due to their non-toxicity and to minimise systemic exposure (i.e. lower bioavailabiliy). With the coccidia parasite being intracellular, it will be necessary to have a degree of lipid solubility. It is possible that *A. indica* extract is lipid soluble and is likely to achieve a higher intracellular concentration than water-soluble compounds. Azadirachtin, the main compound in *A. indica* is also lipid soluble (Masood *et al*., 2013). Tipu *et al*. (2002). Abbas *et al*. (2006) reported an

anticoccidial effect of neem fruit against mixed *Eimeria* species in terms of reducing the oocyst per gram of faeces which were consistent with the present reports.

The findings in the current study are in consistent with the results of similar studies involving the use of different plant extracts which showed the potential benefits in reducing coccidial infections (Youn and Noh, 2001; Kurkure *et al*., 2006; Jang *et al*., 2007; Naidoo *et al*., 2008; Wang *et al*., 2008; Nweze and Obiwulu, 2009 and Nwosu *et al*., 2011).

It has been reported thatphytochemicals which possess many ecological and physiological roles are widely distributed as plant constituents in different concentrations/quantities (Abbas *et al*., 2010). Phytochemicals exhibit a wide range of biological effects as consequences of their concentration as observed in the present study which might be responsible for the concentration-dependent anti-coccidial efficacy of the crude methanolic extracts obtained. The dose-related nature of the manifestations of the extracts is variations in the concentrations of the various components in each plant extract. This concurs with the report of other authors Cumming, (1987); Murtaza *et al*. (2002); Nidaullah *et al*. (2010); Efterpi *et al*. (2012); Ljijana *et al*. (2012); Musthaq and Durrani (2007); Arczewska-Wlosek and Swiatkiewiez (2012); Oyagbemi and Adejinmi (2012).

There was observable and continuous increase in faecal oocyst shedding in infected-untreated group. This is due to the fact that this group of birds were not treated throughout the experimental period (four weeks) and were not reared up to table size of six weeks which may enable them to produce immunity against the oocysts. These results are consistent with other researchers studying the anti-coccidial activity of other

plants such as *Melia azedarach* (Akhtar and Rifaat, 1987), *Azadirachta indica* (Tipu *et al*., 2002; Shivaramaiah *et al*., 2014; Masood *et al*., 2013; Biu *et al*., 2006; Murtaza *et al*., 2002), mixture containing 5% dried leaves of *A. annua* (Allen *et al*. (1997).

The results obtained indicated the existence of anti-coccidial compounds in the crude methanolic extracts of these plants and the extracts of *N. diderichii* and *S. dasyphyllum* showed a good correlation between the reported uses of these plants in traditional medicine against poultry diseases including coccidiosis. For instance, the killing of the sporulated oocysts as well as their total clearance from *E. tenella*- infected and treated chickens has justified their use for the treatment of symptoms suspected to be coccidiosis in the traditional medicine.

Across the infected and treated groups, there was a progressive reduction in the number of oocysts in faeces with crude methanolic extract of *N. diderichii* and *S.dasyphyllum* groups having all the oocysts cleared from the birds. This was followed by Amprolium, the conventional drug group with few oocysts (Appendix III -V). This observation may be due to the decreasing sensitivity and increasing resistance of this parasite (*E. tenella*) to Amprolium because of continuous use. The plant compounds in the crude methanolic extracts of *N. diderichii* and *S. dasyphyllum* seem to have been responsible for the reduced gastrointestinal infections and increase performance. Similar result was reported by Idowu *et al*. (2006) where *A. indica* and *Allium cepa* extracts used to treat *Eimeria*-infected chickens were more effective than Amprolium. Also extracts of *N. diderichii* and *S. dasyphyllum* are new to the parasites and resistance has not yet been developed. In addition, the extracts contain phytochemicals such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, steroids, terpenoids and

tannins. These constituents have been reported to possess protozoal activity due to their antioxidant activity (Youn and Noh, 2001). Triterpenoids, steroids and saponins have been found to be detrimental to several infectious protozoans, one of which is *E. tenella* (Traore-Keita *et al.*, 2000). This report supports what was observed in this experiment both *in vitro* and *in vivo*. This might also be due to the lethal effect of tannins on the growth and proliferation of parasites as previously reported by Jang *et al*. (2007).Substantialaccumulations of vegetable tannins may occur in almost any part of a plant such as seeds, leaves, root, stem and wood. Tannins represent one of several categories of useful anti-coccidial phytochemicals and tannins of different plant species have specific physical and chemical properties and thus they have very different biological activities. Many scientists have reported similar findings of remarkable impact of these metabolites in health and disease (Shivaramaiah *et al*., 2014).

Results indicated reduction in population of sporulated oocysts as well as killing of oocysts of *E. tenella* by all methanolic fractions of *N. diderichii* and *S. dasyphyllum*. The identification of high percentage destruction of oocysts by F6 and F9 of *S. dasyphyllum* and F7 and F8 of *N. diderichii* when compared with Amprolium (the standard anticoccidial drug) showed that they were more potent among the fractions. They were also more effective than the universal drug (Amprolium). These fractions may contain secondary metabolites or bioactive compounds and also be good sources of lead anti-coccidial compounds responsible for the anticoccidial properties. The crude extracts were suspected to contain various types of compounds which included the other ineffective substances as well as other less effective fractions. These findings are in agreement with the *in vitro* activities of the fractions of stem bark of *N. diderichii* on *Plasmodium falciparum* reported by Lamidi *et al*. (1996). They reported high activity of

the fractions on *P. falciparum* (protozoa). The results of this research also correlate with the *in vitro* antimalarial activity of *N. latifolia* fractions reported by Traore -Keita *et al.* (2000).

The isolated substances BS1 and BS2 when tested on sporulated oocysts of *E. tenella* showed prominent activities (Appendix VI-VIII) and rate of reduction and killing of oocysts was concentration dependent (Figures 6-7). Anticoccidial efficacy test of substances obtained from *N. diderichii* and *S. dasyphyllum* shows that substance from *N. diderichii* was more effective than that of *S. dasyphyllum*. There was a significant difference ($P < 0.05$) between the activities of these substances. The result of *in-vitro* anticoccidial tests of the substance obtained from fractions of *N. diderichii* showed a higher destruction of sporulated oocysts (98.6%) compared to the substance obtained from *S. dasyphyllum* (95.6%). It was also more effective than the standard drug (Amprolium) (90.0%). The lowest (53.8%) destruction rate of sporulated oocysts of *E. tenella* by BS2 was recorded at concentration of 1: 400. This was also higher than the result (34.7%) observed for Amprolium. The *in- vitro* tests of substances showed that at concentrations of 1:25 and 1:50, rates of oocysts destruction were 98.6% and 94.2% respectively (Figure 6). The result of the study showed that the substance BS2 has greater potential as anticoccidial agent. These observations may be due to the fact that these substances are new to the parasites or contain lead anti-coccidial principles.

The combined spectra data analysis using Proton Nuclear Magnetic Resonance (^{1}H-NMR), Carbon Nuclear Magnetic Resonance (^{13}C- NMR), Distortionless Enhancement Polarization Transfer (DEPT), Correlation Spectroscopy (COSY) and Heteronuclear Single Bond Quantum Correlation (HSQC) show that substances from BS2 (BS2a and BS2b) were identical with those authenticated samples of betulinic acid and ursolic

acid. These were assigned the structures 3 β- hydroxyl lup-20 -en- 28-oic acid and 3 β-hydroxyurs-12-en-28-oic acid respectively.

A doublet of doublets was present at δ 3.43 ppm, which is a characteristic of an α-oriented hydrogen at C-3 of a 3 β- hydroxyl triterpene. The most downfield signal resonated at δ 180.8 is attributed to the carboxylic acid (C-28). The appearance of signals at δ 128.9 and δ 130.9 indicated the presence of a double bond in urs-12- ene triterpenoid. The seven degrees of unsaturation, ^{13} C- NMR and DEPT spectra data, suggested five rings plus one acidic carbonyl and one oleifinic group in the molecule. Doublets for germinal protons at δ 3 42 (brs, J = 10.8) inferring its α – and axial orientation δ of 2.52 (d, J =11-0 H2) (Figure 11). Another pair of doublets methyls at δ 0.99 and δ 0.97 which were characteristics for ursane skeleton (Vigar, 1994).

The ^{13} C – NMR and DEPT spectra confirmed the presence of thirty signals (carbons) consisting of four quaternary carbons, eleven methines, eight methylenes and seven methyls deduced from the DEPT experiments. The combined spectra data analysis using ^{1}H-, ^{13}C- NMR, DEPT, COSY and HSQC shows that BS2a is Ursolic acid (Figure 15) which is a pentacyclic triterpene (Gnoatto *et al*., 2008; Moghaddam *et al*., 2007; Mahato and Kundu, 1994 and Ogunkoya, 1981).

A doublet of doublets was present at δ 2.79 ppm, which is a characteristic of an α-oriented hydrogen at C-3 of a 3 β- hydroxyl triterpene. Doublets for germinal protons at δ 4.35 and δ 4.21 ppm, along with the methyl group at δ 1.31 ppm, suggests that Figure 15 was a lupeol-type triterpene derivative. Another pair of doublets at δ 1.56 and δ 1.31 ppm, rather than a seventh methyl singlet around δ 1.20 ppm confirms the presence of a second hydroxyl at C-28. The ^{13} C NMR spectrum further established Figure 16 as a

lupeol- type triterpene derivative. The characteristic pair sp2 carbons comprising the double bond of lupeol was observed as a shifts at δ 178.2 and δ 150.4 ppm (Reynolds *et al*., 1986). Oxygenated carbon shifts for C-3 and C-28 were observed at δ 79.2 and δ 178.2 ppm respectively. In all, the spectra revealed a compound with seven methyl groups, thirty carbon atoms (which is equivalent to the total number of carbon atoms in triterpenoid), a lupine-type triterpenoidal nucleus with two hydroxyl groups at C-3 and C-28 (a lupeol-type triterpene). Consequently, the compound BS2b was determined to be the known structure, 20(29)- lupine -3. 28-diol, more commonly known as betulin (Figure 16). Experimental NMR data was compared to that reported in the literature and all ^{13}C shifts were within ± 0.3 ppm (Siddiqui *et al*., 1988; Mahato and Kundu, 1994).

Ursolic acid is widely distributed in higher plants such as *Rosmarinus officinalis*, *Glechoma hederaceae*, *Ilex paraguariensis*, *Khnocarpus frutescens*, *Phoradendron juniperinum*, *Syzygiub claviflorum*, *Hyptis capitata*, sweetened, dried fruits, berries and apples (Pieri *et al*., 2014). Ursolic acid was isolated from root bark of *Uapaca nitida* (Euphorbiaceae), *Mitragyna inermis*, *Saturela parvifolia*, *Mmusops caffra* and *M. obtusifolia* (Pieri *et al*., 2014). Other sources include *Kleina odora*, *Spathodea campanulata* (Amusan *et al*., 1996).

In the present study, ursolic acid had high efficacy on sporulated oocysts of *E. tenella*. This is in agreement with other reports which reported ursolic acid to exhibit antiparasitic and antiplasmodial properties (Babalola *et al*., 2013). Other bioactivities of ursolic acid include antitumoral, antimycotic, anti-bacterial and antiviral (Simone, 2007). It has also been reported that ursolic acid has potentials against hepatitis B and C viruses (HBV and HCV respectively) (Dominguez-carmona *et al*., 2010). The anti-inflammatory activity of the pentacyclic triterpene has also been reported.

Betulinic acid has been largely derived from vegetable oils, vegetable foods, medicinal herbs, cereals and fruits. It has also been reported to be obtained from other plants such as birch tree (*Betula* spp.) (Betulaceae), *Ziziphus* spp. (Rhamnaceae), *Syzygium* spp. (Myrtaceae), *Diospyros* spp. (Ebeneceae) and *Paeonia* spp. (Paeoniaceae) (Chang *et al*., 1999; Chandramu *et al*., 2003; Alakurtti *et al*., 2006).

Betulinic acid has been reported to possess anti-protozoal activity as obtained in this study against *E. tenella* which is also a protozoan. Other uses include inhibitory activities against HIV-1 which makes it a possible potential therapeutic for HIV-1 (Alakurtti *et al*., 2006). Other bioactivities of Betulinic acid include anti-bacterial, cytotoxic, anti-malarial, trypanocidal, anti-obese and antinociceptive activities (Dominguez-carmona *et al*., 2010; Alakurtti *et al*., 2006).

The present study shows that substances obtained from *N. diderichii* had interesting results as they higher destruction of sporulated oocysts of *E. tenella*. This may be as a result of the compounds (BS2a and BS2b) which possess anti-inflammatory activities. This is in support of result obtained by Anamika *et al*. (2007), Zi *et al*. (2008) who reported that large number of pentacyclic triterpene possess anti-inflammatory properties. Among these are ursolic acid and the lupine-type triterpenes such as betulinic acid.

These two compounds have been tested both *in-vitro* and *in-vivo* models and shown to possess inhibitory activity. Betulinic acid possesses moderate *in-vitro* inhibitory activity and proliferation activities which are in agreement with the results of other authors (Rodriguez, 2010; Cichewicz and Kouzi, 2004; Anamika *et al*., 2007; Ayatollahi *et al*., 2011; Ibrahim and Francis, 2013).

CHAPTER SIX

6.0 SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This present study has demonstrated the relevance of traditional medicines in animal healthcare of poultry farmers. It has been found that traditional herbal medicines are still prevalent and used in the treatment of many poultry diseases including coccidiosis in Zaria, Nigeria.

Ten of the most commonly used plants extracts as anticoccidials had various degrees of efficacy on sporulated oocysts of *E. tenella*. Phytochemical screening of three of these plants revealed the presence of bioactive components. The extracts were also not toxic to chickens but eliminated oocysts from coccidia- infected chickens. The fractions of methanolic extracts of *N. diderichii* and *S. dasyphyllum* as well as substances obtained from them possess anticoccidial effects. Ursolic and Betulinic acids were obtained from the substances of methanolic extract of *N. diderichii*.

The results showed that the extracts of *N. diderichii* and *S. dasyphyllum* may be novel plant-based, environmentally friendly anticoccidial agents that do not possess health risks posed by chemical-based anti-coccidial agents currently in use. The health benefits of plant-based foods are attributed to bioactive phytochemicals, including triterpenoids and other groups of natural compounds, acting in an additive way (Akihisa *et al* ., 2010).

6.2 Conclusions

- Plants of ethno – veterinary importance used in the treatment of poultry disease were identified.

- Methanolic crude extracts of *Azadirachta indica*, *Citrus sinensis*, *Cissus populnea*, *Ficus platyphylla*, *Khaya senegalensis*,*Nauclea diderichii*, *N. latifolia*, *Parkia biglobosa*, *Solanum dasyphyllum* and *Vitellariaparadoxa*; fractions and substances from *S. dasyphyllum* and *N. diderichii* which are frequently used folkloric plants possessed anti-coccidial activities.
- *A. indica*, *N. diderichii* and *S. dasyphyllum* methanolic crude extracts screened contain phytochemical constituents.
- Active anti-coccidial principles were isolated from some of the ethno-veterinary plants.
- The active anti-coccidial principles in the plants under study were characterized and found to contain betulinic and ursolic acids.

6.3 Recommendations

- Efforts should be geared towards the use of plants and plant extracts in the control and prevention of coccidiosis.
- Conservation measures need to be applied to ensure the long term availability of the plants for future generation and continuity.
- It is important to note that the active plant constituents should be sufficiently lipid soluble to enable penetration of intracellular cells for effectiveness on the coccidian parasite that produce resistant oocysts.
- Active plant constituents should be further tested to determine fat or lipid soluble for ease of penetrating intracellular cells.
- There is the need to carry out chronic toxicity test of the plant extracts especially neem to determine the accurate dosage for the chickens.

- The effectiveness of the plant extracts, especially that of *N. diderichii* and *S. dasyphyllum* on oocysts of *E. tenella* provides a strong rationale for further evaluation of their anticoccidial efficacy in a larger study.
- These plants may also have merit in the prophylactic management of coccidiosis.
- Considering the numerous pharmacological activities of the betulinic and ursolic acids as well as their ubiquitous nature, there is clear need for further search on the bioavailability of these compounds and quantification in different plant parts.
- Betulinic and ursolic acids are not very toxic, thus further work is also required on the development of synthetic derivatives with lower toxic effect and higher therapeutic potential.
- Another virgin area is the mechanism of action of the therapeutic effects of these compounds as well as functional food factors.
- Due to low polarity and consequently poor aqueous solubility of triterpenoids occurring in free and ester forms, their real bioavailability has been questioned (Anosa and Okoro, 2011).
- Formulations based on nanotechnologies have been proposed to replace conventional dosage forms (Olowokudejo *et al*., 2008).
- Quantification of ursolic and betulinic acids present in *N. diderichii* and other plants is also recommended.

6.4 Contributions to Knowledge

1. The crude methanolic extracts of *Nauclea diderichii* killed 460 (92.0%), 400 (80.0%),371 (74.2%),275 (55.0%)and215 (43.0%) and *Solanum dasyphyllum* killed 455

(91.0%), 389 (77.8%), 300 (60.0%), 267 (53.5%) and 203 (40.6%) **s**porulated sporocysts at dilutions 1:25, 1:50, 1:100,1:200 and 1:400after 96 hr respectively.

2. Fraction 6 (F6) from *Solanum dasyphyllum* killed 451 (90.2%), 340 (68.0%), 300 (60.0%), 261 (52.2%) and 191 (38.2%) and Fraction 9 (F9) killed 481 (96.2%), 414 (82.8%), 375 (75.0%), 299 (59.8%) and 201 (40.2%) **s**porulated sporocysts at dilutions 1:25, 1:50, 1:100,1:200 and 1:400after 96 hr respectively.

3. Fraction 7 (F7) from *Nauclea diderichii* killed 479 (95.8%), 369 (73.8%), 310 (62.0%), 278 (55.6%) and 166 (33.2%) and Fraction 8 (F8) killed 490 (98.0%), 451 (90.2%),391 (78.2%), 305 (61.0%) and 219 (43.8%) **s**porulated sporocysts at dilutions 1:25, 1:50, 1:100,1:200 and 1:400after 96 hr respectively.

4. Substances from fractions of *S. dasyphyllum* (BS1) killed 478 (95.6%), 396 (79.2%), 333 (66.1%), 212 (42.4%) and 198 (39.9%)and*N. diderichi*i (BS2) killed 493 (98.6%), 471 (94.2%), 443 (88.6%), 332 (66.4%) and 269 (53.8%) sporulated oocysts at dilutions 1:25, 1:50, 1:100,1:200 and 1:400after 96 hr respectively.

5. Bioactive components isolated from *N. diderichii* were characterized and found to be betulinic and ursolic acids which are terpenoids and found to be anti-coccidial in action.

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Appendix I: *In vitro* Effect of Methanolic Extracts of some Ethnomedicinal plants on Sporulated oocysts of *Eimeria tenella* at different time Intervals in Comparison with Amprolium

			Time interval (hours)			
S/N	**Plants**	**Concentration (mg/ml)**	**24**	**48**	**72**	**96**
1	Amprolium	1:25	254 (50.8%)	352 (70.4%)	403 (80.6%)	449 (80.0%)
		1:50	204 (40.8%)	241 (48.4%)	263 (53.6%)	333 (66.6%)
		1:100	157 (31.0%)	197 (39.4%)	227 (45.4%)	285 (57.0%)
		1:200	101 (20.2%)	138 (27.6%)	163 (32.6%)	249 (49.6%)
		1:400	51 (10.2%)	94 (18.8%)	135 (27.0%)	170 (34.0%)
2	*Azadirachta indica*	1:25	200 (40.0%)	240 (48.0%)	285 (57.0%)	365 (73.0%)
		1:50	185 (37.0%)	200 (40.0%)	250 (50.0%)	335 (67.0%)
		1:100	145 (29.0%)	160 (32.0%)	225 (45.0%)	305 (61.0%)
		1:200	135 (27.0%)	145 (29.0%)	210 (42.0%)	290 (58.0%)
		1:400	100 (20.0%)	125 (25.0%)	180 (36.0%)	265 (53.0%)
3	*Citrus sinensis*	1:25	125 (25.0%)	200 (40.0%)	220 (44.0%)	240 (48.0%)
		1:50	0 (0.0%)	180 (36.0%)	200 (40.0%)	210 (42.0%)
		1:100	0 (0.0%)	115 (23.0%)	145 29.0%)	190 (38.0%)
		1:200	0 (0.0%)	70 (14.0%)	90 (18.0%)	135 (27.0%)
		1:400	0 (0.0%)	30 (06.0%)	65 (13.0%)	75 (15.0%)
4	*Cissuspopulnea*	1:25	140 (28.0%)	200 (40.0%)	295 (59.0%)	300 (60.0%)
		1:50	101 (20.2%)	106 (21.2%)	250 (50.0%)	260 (52.0%)
		1:100	24 (04.8%)	64 (12.8%)	85 (17.0%)	140 (28.0%)
		1:200	0 (0.0%)	28 (5.6%)	60 (12.0%)	100 (20.0%)
		1:400	0 (0.0%)	16 (3.2%)	35 (07.0%)	70 (14.0%)
5	*Ficus platyphylla*	1:25	0 (0.0%)	0 (0.0%)	197 (39.4%)	281 (56.2%)
		1:50	0 (0.0%)	0 (0.0%)	101 (20.2%)	160 (32.0%)
		1:100	0 (0.0%)	0 (0.0%)	90 (18.0%)	125 (25.0%)
		1:200	0 (0.0%)	0 (0.0%)	44 (08.0%)	91 (18.2%)
		1:400	0 (0.0%)	0 (0.0%)	16 (03.2%)	43 (08.6%)
6	*Khaya senegalensis*	1:25	0 (0.0%)	0 (0.0%)	85 (17.0%)	230 (46.0%)
		1:50	0 (0.0%)	0 (0.0%)	50 (10.0%)	180 (36.0%)

Appendix I Continued

		1:100	0 (0.0%)	0 (0.0%)	0 (0.0%)	75 (15.0%)
		1:200	0 (0.0%)	0 (0.0%)	0 (0.0%)	61 (12.2%)
		1:400	0 (0.0%)	0 (0.0%)	0 (0.0%)	15 (03.0%)
7	*Nauclea diderichii*	1:25	225 (45.0%)	306 (61.2%)	400 (80.0%)	460 (92.0%)
		1:50	177 (35.0%)	279 (55.8%)	375 (70.0%)	400 (80.0%)
		1:100	103 (20.6%)	250 (50.0%)	360 (72.0%)	371 (74.2%)
		1:200	91 (18.0%)	135 (27.0%)	200 (40.0%)	275 (55.0%)
		1:400	60 (14.4%)	116 (23.2%)	170 (34.0%)	215 (43.0%)
8	*Nauclea latifolia*	1:25	0 (0.0%)	50 (10.0%)	75 (15.0%)	250 (50.0%)
		1:50	0 (0.0%)	25 (05.0%)	45 (09.0%)	165 (33.06%)
		1:100	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		1:200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		1:400	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
9	*Parkia biglobosa*	1:25	0 (0.0%)	0 (0.0%)	50 (10.0%)	90 (18.0%)
		1:50	0 (0.0%)	0 (0.0%)	10 (02.0%)	18 (03.6%)
		1:100	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		1:200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
		1:400	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
10	*Solanum dasyphyllum*	1:25	144 (28.8%)	225 (45.0%)	306 (60.0%)	455 (91.0%)
		1:50	99 (19.8%)	158 (31.6%)	290 (58.0%)	389 (77.8%)
		1:100	40 (08.0%)	80 (16.0%)	165 (33.0%)	300 (60.0%)
		1:200	25 (05.0%)	50 (10.0%)	109 (21.8%)	267 (53.5%)
		1:400	16 (03.2%)	35 (07.0%)	80 (16.0%)	203 (40.6%)
11	*Vitellaria paradoxa*	1:25	0 (0.0%)	155 (31.0%)	250 (50%)	275 (55.0%)
		1:50	0 (0.0%)	100(20%)	135 (27.0%)	190 (38.0%)
		1:100	0 (0.0%)	90 (18.0%)	110 (22.0%)	145 29.0%)
		1:200	0 (0.0%)	0 (0.0%)	0 (0.0%)	115 (23.0%)
		1:400	0 (0.0%)	0 (0.0%)	0 (0.0%)	100 (20.0%)

Appendix II: *In vitro* anti-coccidial effect of some ethnomedicinal plant methanolic extracts (ANOVA) at p< 0.05

Source of variance	DF	MS
Between group	54	33756.15*
Within group	55	26.21
Total	109	

*Significant at $p < 0.05$

Appendix III: Oocyst Output of Chickens Infected with *E. tenella* and treated with varying doses of methanolic extract of *S. dasyphyllum*

	Group					
Oocyst output/g faeces	**A1**	**B1**	**C1**	**D1**	**E1**	**F1**
a. Before treatment	6,200	6,510	6,100	5,700	7,500	0
b. After 3 days of treatment	1,800	579	756	1,210	13,700	0
c. After 5 days of treatment	130	55	110	181	16,800	0
d. After 7 days of treatment	38	0	0	0	21,200	0

Appendix IV: Oocyst output of Chickens infected with *E. tenella* and treated with varying doses of methanolic extract of *N. diderichii*

	Group					
Oocyst output/g faeces	**A2**	**B2**	**C2**	**D2**	**E2**	**F2**
a. Before treatment	8,350	7,500	7,700	8,500	11,800	0
b. After 3 days of treatment	2,472	100	200	500	14,600	0
c. After 5 days of treatment	120	20	35	45	16,600	0
d. After 7 days of treatment	42	0	0	0	20,700	0

Appendix V: Oocyst output of Chickens infected with *E. tenella* and treated with varying doses of methanolic extract of *A. indica*

	Group and Clinical Observations					
Oocyst output/g faeces	**A3**	**B3**	**C3**	**D3**	**E3**	**F3**
a. Before treatment	7,100	6,400	7,090	5,900	6,070	0
b. After 3 days of treatment	2,009	2,410	1,300	1,900	12,900	0
c. After 5 days of treatment	120	1,300	1,100	1,600	15,700	0
d. After 7 days of treatment	40	150	80	280	20,600	0

Appendix VI: *In vitro* effects of some fractions of methanolic extract of *S. dasyphyllum, N. diderichii*and their substances on sporulated Oocysts of *E. tenella* at different time intervals

S/N	Fractions	Concentration (mg/ml)	Time interval (hours) 24	48	72	96
1	Amprolium	1:25	255(51.0%)	354 (70.8%)	404 (80.8%)	450 (90.0%)
		1:50	206 (41.2%)	243 (48.6%)	269 (53.8%)	335 (67.0%)
		1:100	158 (31.6%)	198 (39.6%)	228 (45.6%)	286 (57.2%)
		1:200	102 (20.4%)	138 (27.6%	165 (33.0%)	250 (50.0%)
		1:400	52 (10.4%)	96 (19.2%)	136 (27.2%)	172 (34.4%)
2	F4	1:25	69 (13.8%)	112 (22.4%)	202 (41.0%)	301 (60.2%)
		1:50	42 (08.4%)	99 (19.8%)	129 (25.8%)	221 (44.2%)
		1:100	31 (06.2%)	75 (15.0%)	95 (19.0%)	189 (37.8%)
		1:200	20 (04.0%)	53 (10.6%)	75 (15.0%)	152 (30.4%)
		1:400	15 (03.0%)	33 (06.6%)	58 (11.6%)	109 (21.8%)
3	F5	1:25	177 (35.4%)	203 (40.6%)	230 (46.0%)	241 (48.2%)
		1:50	159 (31.8%)	169 (33.8%)	189 (37.8%)	208 (41.6%)
		1:100	128 (25.6%)	145 (29.0 %)	160 (32.0%)	183 (36.6%)
		1:200	108 (21.6%)	126 (25.2%)	148 (29.6%)	172 (34.4%)

Appendix VI Continued

		1:400	79 (15.8%)	98 (19.6%)	105 (21.0%)	109 (21.8%)
4	F6	1:25	267 (53.4%)	371 (74.2%)	431 (86.2%)	451 (90.2%)
		1:50	221 (44.2%)	266 (52.2%)	307 (61.4%)	340 (68.0%)
		1:100	189 (37.8%)	245 (49.0%)	277 (55.4%)	300 (60.0%)
		1:200	141 (19.8%)	210 (42.0%)	243 (48.6%)	261 (52.2%)
		1:400	76 (15.2%)	100 (20.0%)	79 (35.8%)	191 (38.2%)
5	F9	1:25	271 (54.2%)	359 (71.8%)	440 (88.0%)	481 (96.2%)
		1:50	231 (46.2%)	263 (52.6%)	301 (60.2%)	414 (82.8%)
		1:100	173 (34.6%)	219 (43.8%)	297 (59.4%)	375 (75.0%)
		1:200	153 (30.6%)	167 (33.4%)	191 (38.2%)	299 (59.8%)
		1:400	144 (28.8%)	156 (31.2%)	177 (35.4%)	201 (40.2%)
6	F11	1:25	95 (15.0%)	116 (23.2%)	135 (27.0%)	281 (56.2%)
		1:50	62 (12.4%)	100 (20.0%)	117 (23.4%)	225 (45.0%)
		1:100	34 (06.8%)	60 (12.0%)	81 (16.2%)	201 (40.2%)
		1:200	28 (05.6%)	44 (08.0%)	61 (12.2%)	150 (30.0%)
		1:400	15 (03.0%)	33 (06.6%)	51 (10.2%)	131 (26.2%)
	F13	1:25	79 (15.8%)	99 (19.8%)	129 (25.8%)	239 (47.8%)

Appendix VI Continued

	1:50	62 (12.4%)	70 (14.0%)	101 (20.2%)	251 (50.2)
	1:100	54 (10.8%)	63 (12.6%)	79 (15.8%)	222 (44.4%)
	1:200	32 (06.4%)	40 (08.6%)	66 (33.0%)	179 (35.8%)
	1:400	28 (05.6%)	35 (07.0%)	53 (10.6%)	116 (23.2%)
F2	1:25	99 (19.8%)	142 (28.4%)	199 (39.8%)	399 (79.8%)
	1:50	87 (17.4%)	103 (20.6%)	188 (37.6%)	280 (56.0%)
	1:100	66 (13.2%)	97 (19.4%)	115 (23.0%)	196 (39.2%)
	1:200	45 (09.0%)	81 (16.2%)	98 (18.0%)	107 (21.4%)
	1:400	32 (06.4%)	58 (11.6%)	69 (13.8%)	93 (18.6%)
F3	1:25	189 (37.8%)	211 (42.2%)	260 (52.0%)	311 (62.2%)
	1:50	166 (33.2%)	199 (39.8%)	213 (42.6%)	221 (44.2%)
	1:100	141 (28.2%)	168 (33.6%)	199 (39.8%)	216 (43.2%)
	1:200	121 (24.2%)	154 (30.8%)	187 (37.4%)	193 (38.6%)
	1:400	103 (20.6%)	129 (25.8%)	143 (28.6%)	166 (33.2%)
F7	1:25	295 (59.0%)	301 (60.2%)	323 (64.6%)	479 (95.8%)
	1:50	205 (41.0%)	271 (54.2%)	289 (57.8%)	369 (73.8%)

Appendix VI Continued

	1:100	163 (32.6%)	201 (40.2%)	222 (44.4%)	310 (62.0%)
	1:200	124 (24.8%)	139 (27.8%)	166 (33.2%)	278 (55.6%)
	1:400	118 (23.6%)	129 (25.8%)	151 (30.2%)	166 (33.2%)
F8	1:25	261 (52.2%)	293(58.6%)	333 (66.6%)	490 (98.0%)
	1:50	251 (50.2%)	283 (56.6%)	301 (60.2%)	451 (90.2%)
	1:100	207 (41.4%)	245 (49.0%)	276 (55.2%)	391 (78.2%)
	1:200	191 (38.2%)	201 (40.2%)	222 (44.4%)	305 (61.0%)
	1:400	166 (33.2%)	178 (35.6%)	199 (39.8%)	219 (43.8%)
F12	1:25	121 (24.2%)	179 (35.8%)	197 (39.4%)	251 (50.2%)
	1:50	99 (18.0%)	129 (25.8%)	141 (28.2%)	234 (46.8%)
Appendix VI Continued	1:100	86 (17.2%)	87 (17.4%)	99 (19.8%)	179 (35.8%)
	1:200	58 (11.6%)	66 (13.2%)	78 (15.6%)	156 (31.2%)
	1:400	39 (07.8%)	58(11.6%)	69 (13.8%)	98 (19.6%)
F13	1:25	121 (24.2%)	140 (28.0%)	156 (31.2%)	217 (43.0%)
	1:50	99 (18.0%)	123 (24.6%)	135 (27.0%)	167 (33.4%)
	1:100	86 (17.2%)	109 (21.8%)	121 (24.2%)	141(28.2%)

	1:200	58 (11.6%)	93 (18.6%)	104 (20.8%)	133 (26.6%)
	1:400	39 (07.8%)	61 (12.2%)	77 (15.4%)	89 (17.8%)
BS1	1:25	407 (81.4%)	436 (87.2%)	450 (90.0%)	478 (95.6%)
	1:50	222 (44.4%)	308 (61.6%)	340 (68.0%)	396 (79.2%)
	1:100	198 (39.6%)	258 (51.6%)	291 (58.2%)	333 (66.1%)
	1:200	152 (30.4%)	207 (41.4%)	224 (44.8%)	212 (42.4%)
	1:400	139 (27.8%)	189 (37.8%)	202 (40.4%)	198 (39.9%)
BS2	1:25	437 (87.3%)	465 (93.0%)	447 (95.0%)	493 (98.6%)
	1:50	379 (75.8%)	442 (88.4%)	457 (91.4%)	471 (94.2%)
Appendix VI Continued	1:100	300(60.0%)	345 (70.6%)	416 (83.2%)	443 (88.6%)
	1:200	166 (33.2%)	203 (40.6%)	302 (60.4%)	332 (66.4%)
	1:400	91 (18.2%)	152 (30.4%)	227 (45.4%)	269 (53.8%)

Note: F4, F5, F6, F7, F8 and F9 are fractions of S. dasyphyllum; F2, F3, F7, F8, F12 and F13 are fractions of N. diderichii; BS1 is substance from fractions of S. dasyphyllum and BS2 is substance from fractions of N. diderichii

Appendix VII: *In vitro* anti-coccidial effect of six fractions of *Solanum dasyphyllum* methanolic extract (ANOVA) at p< 0.05

Source of variance	DF	MS
Between group	6	64872.78*
Within group	24	300.59
Total	30	

*Significant at $p < 0.05$

Appendix VIII: *In vitro* anti-coccidial effect of six fractions of *Nauclea diderichii* methanolic extract (ANOVA) at p< 0.05

Source of variance	DF	MS
Between group	34	25653.4*
Within group	35	9.51
Total	69	

*Significant at p< 0.05

Appendix IX: MeanEffect of some Fractions of crude Methanolic Extracts of *S. dasyphyllum*, N. *diderichii* and their substances on Sporulated Oocysts of *E. tenella* at Different Time Intervals in Comparison with Amprolium

	Time Intervals (Hours)			
Fractions	**24**	**48**	**72**	**96**
F4	7.0800000^{d}	14.8800000^{c}	22.4800000^{c}	38.8800000^{c}
F5	26.0400000^{c}	29.6400000^{b}	33.2800000^{b}	36.5200000^{c}
F6	34.0800000^{ab}	47.4800000^{a}	57.4800000^{a}	61.7200000^{b}
F9	38.8800000^{a}	46.5600000^{a}	56.2400000^{a}	70.8000000^{a}
F10	8.5600000^{d}	13.9600000^{c}	67.8000000^{c}	39.5200000^{c}
F11	10.2000000^{d}	12.4000000^{c}	21.0800000^{c}	40.2800000^{c}
F2	13.1600000^{d}	19.2400000^{c}	26.4765837^{c}	43.00000^{c}
F3	28.8000000^{c}	34.4400000^{b}	40.0800000^{b}	44.2800000^{c}
F7	36.2000000^{b}	41.6400000^{ab}	46.0400000^{ab}	64.0800000^{b}
F8	43.0400000^{a}	48.0000000^{a}	53.2400000^{a}	74.2400000^{a}
F12	15.7600000^{d}	20.7600000^{c}	23.3600000^{c}	36.7200000^{cd}
F13	15.7600000^{d}	21.0400000^{c}	23.7200000^{c}	29.800000^{d}

Appendix IX Continued

BS1	44.720[a]	55.920[a]	60.280[b]	64.640[b]
BS2	54.900[a]	64.600[a]	75.080[a]	80.320[a]
Amprolium	30.9200000[bc]	41.1600000[a]	48.0800000[a]	59.7200000[b]
SEM ±	2.48	3.10	3.68	2.78

Means followed by the same letter(s) in the same column are not significantly different (P≥ 0.05) from each other using Student's t-test. Note: F4, F5, F6, F7, F8 and F9 are fractions of S. dasyphyllum; F2, F3, F7, F8, F12 and F13 are fractions of N. diderichii; BS1 is substance from fractions of S. dasyphyllum and BS2 is substance from fractions of N. diderichii

Appendix X: ^{13}C- NMR Data of Ursolic acid

Position	Ursolic acid(ppm)	Literature, (Hamad *et al*., 2014) (ppm)
C1	38.8	38.7
C2	27.4	27.9
C3	79.0	78.0
C4	38.9	39.2
C5	55.4	55.7
C6	19.2	18.6
C7	33.7	33.4
C8	40.7	39.8
C9	46.9	47.9
C10	37.0	37.3
C11	22.7	23.5
C12	128.9	125.5
C13	130.9	139.1
C14	42.5	42.3
C15	29.2	28.5
C16	24.7	24.8
C17	46.9	47.9
C18	50.6	53.4
C19	38.9	39.2
C20	38.9	39.3
C21	30.6	30.9
C22	37.2	37.1
C23	28.0	28.7
C24	16.1	16.4
C25	15.4	15.8
C26	18.3	17.3
C27	24.7	23.8
C28	180.8	179.7
C29	18.3	17.4
C30	22.7	21.3

Appendix XI: ^{13}C- NMR Data of Betulinic acid

Position	Betulinic acid (ppm)	Literature (Hamad *et al*., 2014) (ppm)
C1	38.8	39.1
C2	27.9	28.1
C3	79.0	78.0
C4	38.9	39.3
C5	55.4	55.7
C6	18.3	18.6
C7	33.7	34.7
C8	40.7	40.9
C9	49.3	50.8
C10	37.0	37.3
C11	20.9	21.0
C12	25.5	25.9
C13	38.4	38.4
C14	42.5	42.7
C15	30.6	30.1
C16	32.2	32.7
C17	56.3	56.4
C18	50.6	50.8
C19	49.3	49.6
C20	150.4	151.1
C21	31.9	31.0
C22	37.2	37.4
C23	29.1	28.5
C24	16.0	16.2
C25	16.0	16.2
C26	19.3	19.3
C27	14.7	14.7
C28	178.2	178.7
C29	109.7	109.8
C30	19.2	19.3